State of Hawaii
Department of Agriculture
Agricultural Loan Division

August 23, 2022

Board of Agriculture
Honolulu, Hawaii

Subject: Request for Approval to Activate and Set Parameters for DOA's Emergency Loan Program to Provide Relief Due to Overpopulation of the Axis Deer in the County of Maui

Governor David Y. Ige issued a proclamation on March 23, 2022, declaring a state of emergency with a relief period until May 20, 2022. According to data from the U.S. Department of Agriculture for the year 2022, Maui County continues to be designated as a primary natural disaster area due to drought conditions and despite ongoing efforts the axis deer have propagated to approximately 60,000 or more, which cannot currently be sustained by the environment in Maui County. Supplementary proclamations were issued by Governor Ige on May 23, 2022, and July 22, 2022, to extend the relief period until September 20, 2022.

The Governor's proclamations allow for the activation of section 155-9(e), Hawaii Revised Statutes (HRS), which provides for emergency loans to provide relief and rehabilitation to qualified farmers without limit as to purpose who have suffered great economic losses in the stricken areas. Senator Lynn DeCoite and others have informed the Department that the residents of Maui County are facing threats to the health, safety, and welfare and the members of the agricultural community are experiencing severe economic impacts due to overpopulation of Axis deer to approximately 60,000, or more. This crisis has been exacerbated due to persistent drought conditions which have significantly reduced vegetation and pasture and forced wildlife, in particular Axis deer, to migrate into agricultural and developed areas seeking food and water. In addition to the decimation of agricultural crops and threats to public safety, the expansion of deer into these areas pose a significant risk of spreading disease in the environment.

Section 155-9(e), HRS, requires that "the maximum amounts and period for such loans shall be determined by the board of agriculture; provided that the board shall require that any settlement or moneys received by qualified farmers as a result of an emergency declared under this section shall first be applied to the repayment of an emergency loan made under this chapter."

In this regard, the following loan amounts, terms, and parameters are recommended for board approval:

1. Maximum loan amount: $150,000.00

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2. Terms to be determined on a case-by-case basis as needed. Consideration will be based on historical farm performance and projected cash flow based on reasonable assumptions of revenue and expenses.

3. Interest rate: 3%

4. The credit elsewhere requirement shall be waived for loans $100,000 or less. Loans above $100,000.00 shall require one (1) credit denial.

5. The 3-year residency requirement for U.S. Citizens and permanent resident aliens shall not apply.

6. Collateral requirements may be modified or waived, as necessary, on a case-by-case basis. Wherever possible, the provisions of Section 155-11, Security for Loans, should be followed.

7. Emergency Loan Applications can be accepted until December 31, 2022.

It is hereby requested that the Board of Agriculture approve activation of the emergency loan program with the above recommendations.

Morris M. Atta
Acting Agricultural Loan Administrator

APPROVED FOR SUBMISSION:

Phyllis Shimabukuro-Geiser
Chairperson, Board of Agriculture

OFFICE OF THE GOVERNOR
STATE OF HAWAI'I

PROCLAMATION

By the authority vested in me by the Constitution and laws of the State of Hawai'i, in order to provide relief for disaster damages, losses, and suffering, and to protect the health, safety, and welfare of the people, I, DAVID Y. IGE, Governor of the State of Hawai'i, hereby determine, designate and proclaim as follows:

WHEREAS, pursuant to Chapter 127A, Hawaii Revised Statutes (HRS), emergency powers are conferred on the Governor of the State of Hawai'i to respond to disasters or emergencies, to maintain the strength, resources, and economic life of the community, and to protect the public health, safety, and welfare; and

WHEREAS, despite ongoing efforts, axis deer have propagated to approximately 60,000 or more, which cannot currently be sustained by the environment in Maui County; and

WHEREAS, the axis deer population in Maui County has not been sufficiently reduced through hunting efforts alone; and

WHEREAS, the large number of axis deer in Maui County have devastated pasture forage and much of the vegetation that is already scarce due to persistent drought conditions; and

WHEREAS, the devastation of vegetation has forced wildlife, in particular axis deer in Maui County, to migrate into agricultural and developed areas seeking food and water; and

WHEREAS, the increased numbers of axis deer are foraging in urbanized areas; and

WHEREAS, immediate measures to appreciably reduce and control axis deer populations in Maui County and to implement deer management strategies, including but not limited to, corralling of axis deer, culling of axis deer to sustainable levels, clearing vegetation along fence lines, and erecting and/or reinforcing or repairing fence lines to keep axis deer away from roadways, airports, and runways are needed to protect the health and welfare of the community; and

WHEREAS, the current threat to the health, safety, and welfare of the people of Maui County caused from the axis deer overpopulation constitutes an emergency under section 127A-14, HRS, and warrants preemptive and protective actions;

NOW, THEREFORE, I, DAVID Y. IGE, Governor of the State of Hawai'i, hereby determine that an emergency or disaster contemplated by section 127A-14, HRS, has occurred in the County of Maui, State of Hawai'i, and do hereby authorize and invoke the following emergency provisions which are expressly invoked, if not already in effect upon this declaration of an emergency:

I. Invocation of Laws

Section 127A-12(b)(13), HRS, requiring each public utility, or any person owning, controlling, or operating a critical infrastructure, to protect and safeguard its or the person's property, or to provide for the protection and safeguarding thereof; and provide for the protection and safeguarding of all critical infrastructure and key resources; provided that without prejudice to the generality of the foregoing two clauses, the protecting or safeguarding may include the regulation or prohibition of public entry thereon, or the permission of the entry upon terms and conditions as I may prescribe.

Section 127A-12(b)(16), HRS, directing all state agencies and officers to cooperate and extend their services, materials, and facilities as may be required to assist in emergency response efforts.

Section 127A-16, HRS, by activating the Major Disaster Fund.

II. Deer Control

Pursuant to sections 127A-12 and 127A-13, HRS, the county and state agencies are to provide emergency relief and engage in emergency management functions as defined in section 127A-2, HRS, to enable planning and implementation of deer management strategies, including but not limited to, creating buffers and to erect, reinforce, or repair fence lines to keep the deer away from roadways, airports, and runways, taking action to immediately cull axis deer, and reducing the herds of axis deer to sustainable numbers, so as to provide protection and relief from damages, losses, and suffering caused by the emergency.

III. Suspension of Laws

The following specific provisions of law are suspended, as allowed by federal law, pursuant to sections 127A-12(b)(8) and 127A-13(a)(3), HRS, to the extent that the law impedes or tends to impede or be detrimental to the expeditious and efficient execution of, or to conflict with, emergency functions, including laws which by this chapter specifically are made applicable to emergency personnel:

Chapter 6E, HRS, **historic preservation**, to the extent that compliance requires additional time detrimental to the expeditious and efficient execution of emergency actions.

Section 37-41, HRS, **appropriations to revert to state treasury; exceptions**, to the extent that appropriations lapse at the end of the fiscal year prior to completion of the emergency actions.

Section 37-74(d), HRS, **program execution**, except for sub-sections 37-74(d)(2) and 37-74(d)(3), HRS, and any such transfers or changes considered to be authorized transfers or changes for purposes of section 34-74(d)(1) for legislative reporting requirements, to the extent that legislative authorization would likely delay appropriation transfers or changes between programs to provide necessary funding to complete the emergency actions.

Section 40-66, HRS, **lapsing of appropriations**, to the extent that the timing of the procurement of the construction of the emergency permanent repairs may occur in the fiscal year following the original emergency proclamation.

Chapter 46, HRS, **county organization and administration**, as any county ordinance, rule, regulation, law, or provision in any form applies to any county permitting, licensing, zoning, variance, processes, procedures, fees, or any other requirements that hinder, delay, or impede efforts to implement deer management strategies, including, but not limited to clearing vegetation from fence lines to create a buffer against the axis deer under this Proclamation, to the extent that compliance results in any delays involved in securing County permits. These would include but not be limited to chapter 20.08, Maui County Code, **soil erosion and sedimentation control,** chapter 12-302, Rules for the Molokai Planning Commission, **special**

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management area rules, chapter 12-402 Rules for the Lanai Planning Commission, **special management area rules**, and chapter 12-202, Rules of the Maui Planning Commission, **special management area rules**.

Chapter 89, HRS, **collective bargaining in public employment,** to the extent that compliance with this chapter requires additional time detrimental to the expeditious and efficient execution of emergency actions.

Chapter 89C, HRS, **public officers and employees excluded from collective bargaining,** to the extent that compliance with this chapter requires additional time detrimental to the expeditious and efficient execution of emergency actions.

Section 103-2, HRS, **general fund**, to the extent that compliance results in any additional delays.

Section 103-53, HRS, **contracts with the State or counties; tax clearances, assignments**, only to the extent necessary to waive the Internal Revenue Service (IRS) tax clearance requirement.

Section 103-55, HRS, **wages, hours, and working conditions of employees of contractors performing services**, to the extent that compliance results in any additional delays.

Chapter 103D, HRS, **Hawaii public procurement code**, to the extent that compliance results in any additional delays involved in meeting procurement requirements for selecting contractors in a timely manner to respond to emergency situations.

Chapter 104, HRS, **wages and hours of employees on public works**, to the extent that compliance with this chapter requires additional time detrimental to the expeditious and efficient execution of emergency actions.

Sections 105-1 to 105-10, HRS, **use of government vehicles, limitations,** to the extent that compliance with this chapter requires additional time detrimental to the expeditious and efficient execution of emergency actions.

Section 127A-30, HRS, **rental or sale of essential commodities during a state of emergency; prohibition against price increases**, for the reason that the automatic invocation of this provision during an emergency is not needed for this emergency.

Chapter 183D, HRS, **wildlife**, and chapter 13-124, Hawaii Administrative Rules (HAR), **indigenous wildlife, endangered and threatened wildlife and introduced wild birds,** to the extent that compliance results in any delays involved in implementation of axis deer management planning or activities or requires additional time detrimental to the expeditious and efficient execution of emergency actions.

Chapter 205A, Part II, HRS, **coastal zone management**, to the extent that compliance results in any additional delays involved with securing approvals from the counties or the Department of Land and Natural Resources for work within the special management area.

Chapter 342D, HRS, **water pollution**, and chapters 11-54, **water quality standards**, and 11-55, HAR, **water pollution control**, to the extent that compliance requires additional time detrimental to the expeditious and efficient execution of emergency actions.

Chapter 342H, HRS, **solid waste pollution**, and chapter 11-58.1, HAR, **solid waste management control**, to the extent that compliance requires additional time detrimental to the expeditious and efficient execution of emergency actions.

Chapter 343, HRS, **environmental impact statements,** and chapter 11-200.1, HAR, **environmental impact statement rules**, to the extent that compliance results in any additional delays involved with the environmental review process.

IV. Severability

If any provision of this Proclamation is rendered or declared illegal for any reason, or shall be invalid or unenforceable, such provision shall be modified or deleted, and the remainder of this Proclamation and the application of such provision to other persons or circumstances shall not be affected thereby but shall be enforced to the greatest extent permitted by applicable law.

V. Enforcement

No provision of this Proclamation, or any rule or regulation hereunder, shall be construed as authorizing any private right of action to enforce any requirement of this Proclamation, or of any rule or regulation. Unless the Governor, Director of Emergency Management, or their designee issues an

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express order to a non-judicial public officer, no provision of this Proclamation, or any rule or regulation hereunder, shall be construed as imposing any ministerial duty upon any non-judicial public officer and shall not bind the officer to any specific course of action or planning in response to the emergency or interfere with the officer's authority to utilize his or her discretion.

I FURTHER DECLARE that the disaster emergency relief period shall commence immediately and continue through May 20, 2022, unless terminated or superseded by separate proclamation, whichever shall occur first.

Done at the State Capitol, this
23rd day of March 2022

DAVID Y. IGE,
Governor of Hawai`i

APPROVED:

Holly T. Shikada
Attorney General
State of Hawai`i

STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANGEMENT DIVISION
HONOLULU, HAWAII

August 23, 2022

Board of Agriculture
Honolulu, Hawaii

Subject:	REQUEST FOR APPROVAL TO AWARD LEASES TO VARIOUS AWARDEES AND BACKUP BIDDER POSITIONS; TMK NOS. (2) 2-2-004:001, 002, 029, 031, AND 066, AND (2) 2-2-005:047 AND 053, KEOKEA, KULA, MAKAWAO, ISLAND OF MAUI, HAWAII
Authority:	Section 166E-8, Hawaii Revised Statutes (HRS), and Section 4-158-24 and 29, Hawaii Administrative Rules (HAR)
Tax Map Keys:	(2) 2-2-004:001, 002, 029, 031, and 066 (Kula, Island of Maui) (2) 2-2-005:047 and 053 (Kula, Island of Maui)
Land Area:	(2) 2-2-004:001 22 gross acres (2) 2-2-004:002 13 gross acres (2) 2-2-004:029 20.980 gross acres (2) 2-2-004:031 139.280 gross acres (2) 2-2-004:066 149.030 gross acres (2) 2-2-005:047 19.100 gross acres (2) 2-2-005:053 223.290 gross acres
Land Status:	Properties set aside to the Department of Agriculture (DOA) by Governor's Executive Order 4625 dated March 6, 2020
Lease Term:	35 years each, commencing upon the completion of pre-requisite requirements and before or upon expiration of the Right-of-Entry term of 6 months
Base Annual Rental:	Various - per qualified applicant bid
Character of Use:	Pasture

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BACKGROUND:

The Agricultural Resource Management Division received the Kula, Maui parcels from the Department of Land and Natural Resources via Governor's Executive Order No. 4625 signed on March 6, 2020 transferring TMK Nos.: (2) 2-2-004:001, 002, 029, 031, and 066, and (2) 2-2-005:047 and 053.

In accordance with §166E-8, HRS, and §4-158-24 and 29, HAR, a public notice of public auction was published on April 9, 2022 announcing the subject parcels available for lease by public auction. The division received a total of six (6) applications for the vacant parcels, of which four (4) applicants qualified to bid in accordance with the 4-158-1 and 27, HAR. Staff determined that each applicant qualified as a bona fide rancher with more than two years of years of ranching experience and meets eligibility residency requirements of the Non-Agricultural Park Lands Program.

Exhibit "A" attached hereto, lists the applicants, their status and respective bids. Exhibit "B" is a map that reflects the locations of the parcels.

In addition to the highest bid awardees, staff identified three backup bidder proposals for TMK: (2) 2-2-004:001, two backup bidder proposals for TMK: (2) 2-2-004:066, and one backup bidder proposal for TMK: (2) 2-2-004:002.

RECOMMENDATIONS:

That the Board of Agriculture approve:

1. Issuance of the appropriate Right-of-Entry document to the successful awardees for the lots, and subsequently issue the appropriate general leases subject to the completion of lease pre-requisites.

2. Backup bidder proposals per lot as alternative awardees in the event the highest bidder awardees fail to complete the lease pre-requisites.

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All related documents are subject to the review and approval as to form by the Department of the Attorney General, and such other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State.

Respectfully submitted,

BRIAN KAU, P.E.
Administrator and Chief Engineer
Agricultural Resource Management Division

Attachments: Exhibits "A" and "B"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

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EXHIBIT "A"

MAUI NON-AGRICULTURAL PARK LANDS PUBLIC AUCTION
Keokea, Kula, Makawao, Island of Maui
July 28, 2022

Applicant Name	**TMK: (2) 2-2-004:001 bid amount**	
Diamond B Ranch, LLC	$6,100.00	highest bid
Cowboy Built, Inc.	$6,010.00	1st backup
Adam Kaleolani Wong	$3,710.00	2nd backup
William G. Jacintho	$200.00	3rd backup

Applicant Name	**TMK: (2) 2-2-004:002 bid amount**	
Cowboy Built, Inc.	$2,610.00	highest bid
Diamond B Ranch, LLC	$2,600.00	1st backup

Applicant Name	**TMK: (2) 2-2-004:029 bid amount**	
Diamond B Ranch, LLC	$170.00	highest bid

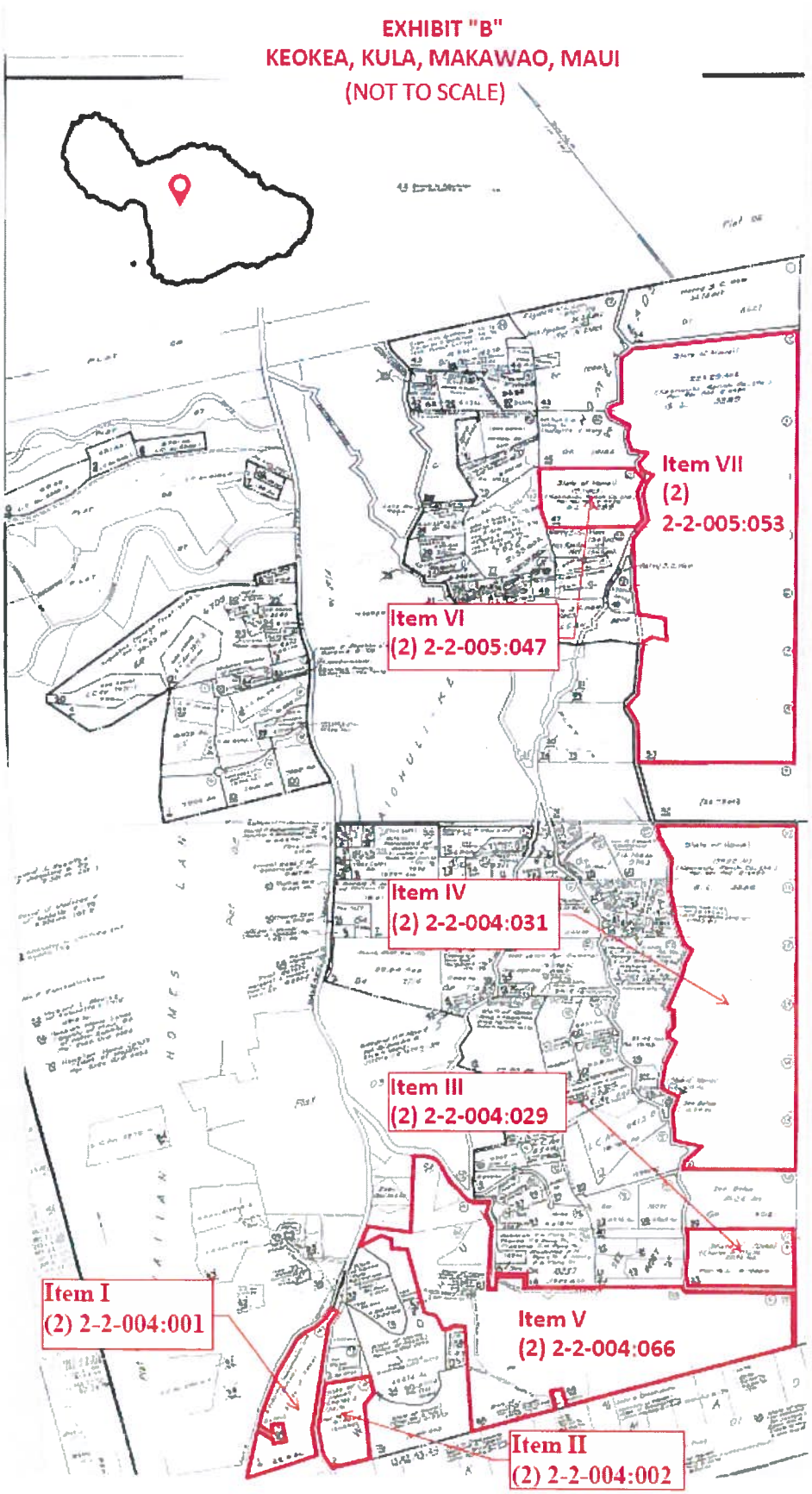
Applicant Name	**TMK: (2) 2-2-004:031 bid amount**	
Diamond B Ranch, LLC	$1,120.00	highest bid

Applicant Name	**TMK: (2) 2-2-004:066 bid amount**	
Diamond B Ranch, LLC	$5,500.00	highest bid
Adam Kaleolani Wong	$5,100.00	1st backup
William G. Jacintho	$2,100.00	2nd backup

Applicant Name	**TMK: (2) 2-2-005:047 bid amount**	
Adam Kaleolani Wong	$310.00	highest bid

Applicant Name	**TMK: (2) 2-2-005:053 bid amount**	
Adam Kaleolani Wong	$1,790.00	highest bid

EXHIBIT "B"
KEOKEA, KULA, MAKAWAO, MAUI
(NOT TO SCALE)



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STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANAGEMENT DIVISION
HONOLULU, HAWAII

August 23, 2022

Board of Agriculture
Honolulu, Hawaii

Subject:	REQUEST FOR (1) ACCEPTANCE FOR ANNUAL RENEWAL OF REVOCABLE PERMITS FOR TMK NOS.: (1) 9-4-002:080; (1) 4-1-008:071 & 072; (2) 1-1-003:028; (3) 3-1-004:001; (3) 4-6-002:001; (3) 4-7-004:009; (3) 4-9-011:002; (3) 5-5-007:011; (4) 1-9-002:019; (4) 1-9-003:006; (4) 1-9-003:010; (4) 1-9-012:011; (4) 4-1-001:007; (4) 4-1-001:012; and (4) 4-1-009:005 & 006; AND (2) RESERVATION AND DELEGATION TO THE CHAIRPERSON THE RIGHT AND AUTHORITY AT ANY TIME TO REVIEW AND ADJUST THE RENTAL CHARGES FOR ANY OF THE REVOCABLE PERMITS
Authority:	Section 166E-6, Hawaii Revised Statutes (HRS), and Section 4-158-2(a)(8), Hawaii Administrative Rules (HAR)
Revocable Permit:	See Exhibit "A"
Permittee:	See Exhibit "A"
Land Status:	Properties set aside to the Department of Agriculture by various Governor's Executive Orders
Character of Use:	See Exhibit "A"

BACKGROUND:

At the end of each calendar year, staff reviews its list of current revocable permits issued statewide and determines which ones to recommend to the Board of Agriculture (Board) for renewal for the upcoming year. Generally, those revocable permits in good standing will be recommended for renewal, unless the Board has approved a different disposition for the land covered by a particular permit.

REMARKS:

The list of revocable permits statewide that staff recommends be renewed for 2023 is attached as Exhibit "A". The exhibit is in the table format with information that includes tax map key, revocable permit number, land area, original commencement date of the permit, annual

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rent, and character of use. A location map of the revocable permits to be renewed is attached as Exhibit "B". Staff recommends no rent increases for 2023 due to the economic downturn associated with the COVID-19 pandemic.

RECOMMENDATION:

That the Board:

1. Approve the continuation of the revocable permits listed in Exhibit "A" on a month to month basis effective January 1, 2023 for a one-year period through December 31, 2023 except for permits that are in arrears of rental payment for more than 60 days and/or have been approved for forfeiture by a separate Board action. Permits in arrears of rental for 60 days or more and/or approved by the Board for forfeiture shall not be renewed; and

2. Reserve and delegate to the Chairperson the right and authority at any time to review and adjust the rental charges for any of the revocable permits listed in Exhibit "A" any time from and after January 1, 2023, where such adjustments will best serve the interests of the State.

Respectfully submitted,

BRIAN KAU, P.E.
Administrator and Chief Engineer
Agricultural Resource Management Division

Attachments - Exhibits "A" & "B"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

EXHIBIT "A"

REVOCABLE PERMIT LIST 2022

Tax Map Key	Permit No.	Acres	Permit From	2022 Annual Rent	Proposed 2023 Rent	Character of Use
ISLAND OF OAHU						
(1) 9-4-002:080	RP-26	150.000	1/20/2005	$17,895.00	$17,895.00	Diversified Agriculture
(1) 4-1-008:071 & 072	RP-7889	14.387	7/1/2016	$1,670.64	$1,670.64	Diversified Agriculture
ISLAND OF MAUI						
(2) 1-1-003:028	RP-5932	1.100	7/1/1982	$156.00	$156.00	Diversified Agriculture
ISLAND OF HAWAII						
(3) 3-1-004:001	RP-7775	489.670	10/1/2003	$996.00	$996.00	Pasture
(3) 4-6-002:001	RP-2001	90.800	1/22/1999	$1,480.00	$1,480.00	Pasture
(3) 4-7-004:009	RP-3131	35.500	2/1/2018	$3,600.00	$3,600.00	Pasture
(3) 4-9-011:002	RP-7839	11.600	10/1/2005	$1,716.00	$1,716.00	Diversified Agriculture
(3) 5-5-007:011	RP-7732	77.400	7/1/2002	$756.00	$756.00	Pasture
ISLAND OF KAUAI						
(4) 1-9-002:019	RP-7317	1.140	3/27/2002	$608.00	$608.00	Diversified Agriculture
(4) 1-9-003:006	RP-7045	7.826	8/31/1995	$2,235.00	$2,235.00	Diversified Agriculture
(4) 1-9-003:010	RP-7794	4.037	9/1/2011	$1,275.00	$1,275.00	Diversified Agriculture
(4) 1-9-012:011	RP-7321	0.950	11/20/2002	$723.96	$723.96	Diversified Agriculture
(4) 4-1-001:007	RP-7738	19.980	9/1/2011	$320.00	$320.00	Pasture
(4) 4-1-001:012	RP-7771	6.130	9/1/2011	$608.00	$608.00	Diversified Agriculture
(4) 4-1-009:005 & 006	RP-2102	10.444	9/19/2017	$170.00	$170.00	Pasture

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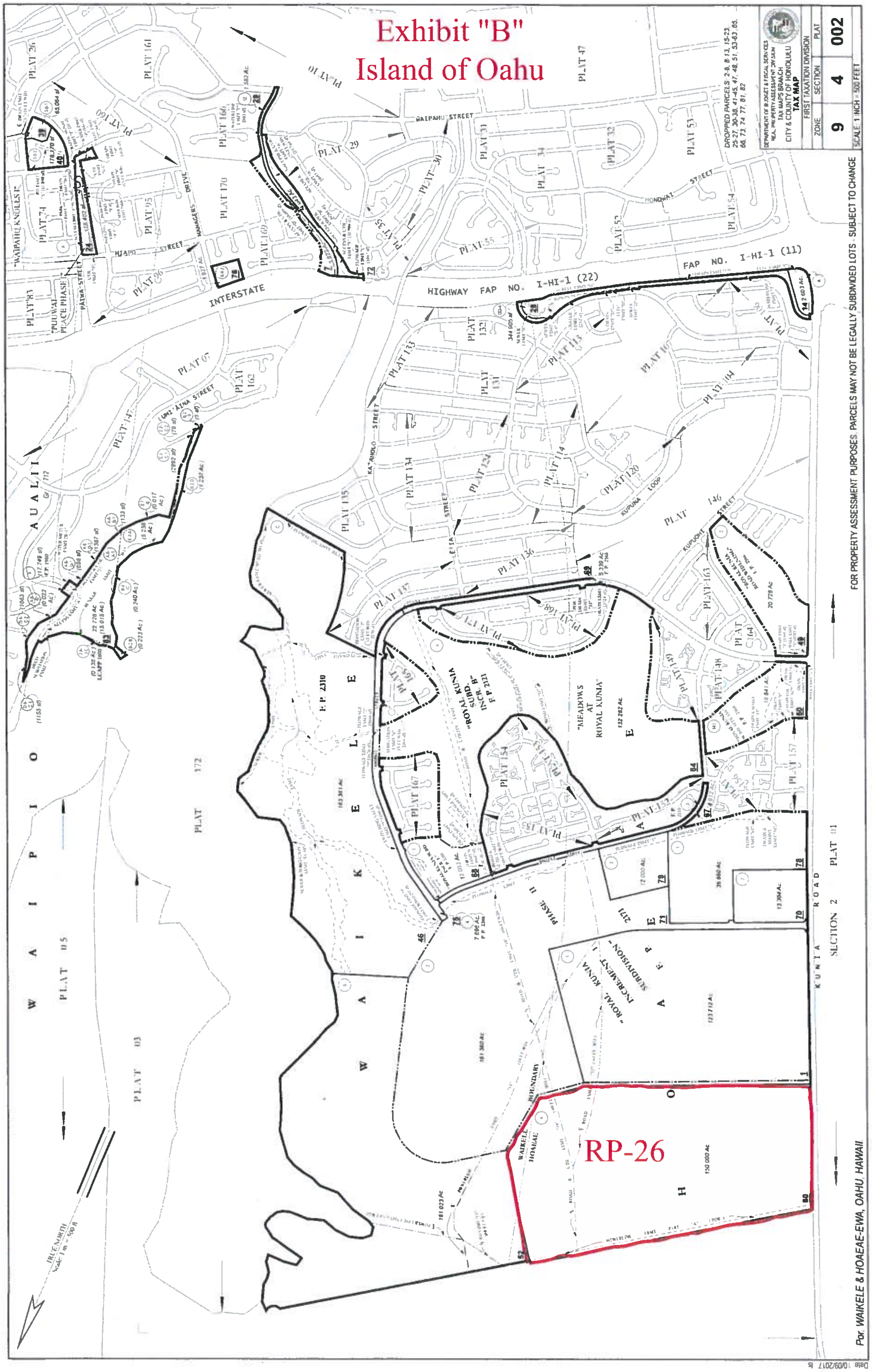

Exhibit "B"
Island of Oahu
RP-26
DEPARTMENT OF BUDGET & FISCAL SERVICES
REAL PROPERTY ASSESSMENT DIVISION
TAX MAPS BRANCH
CITY & COUNTY OF HONOLULU
TAX MAP
FIRST TAXATION DIVISION
ZONE 9
SECTION 4
PLAT 002
SCALE 1 INCH = 500 FEET
FOR PROPERTY ASSESSMENT PURPOSES. PARCELS MAY NOT BE LEGALLY SUBDIVIDED LOTS - SUBJECT TO CHANGE
Por. WAIKELE & HOAEAE-EWA, OAHU, HAWAII
HIGHWAY FAP NO. I-HI-1 (22)
FAP NO. I-HI-1 (11)
INTERSTATE
WAIPAHU STREET
KUNIA ROAD
WAIPIO
WAIKELE
HOAEAE
"MEADOWS AT ROYAL KUNIA"
"ROYAL KUNIA SUBD. INCR. B"
Date 10/09/2017

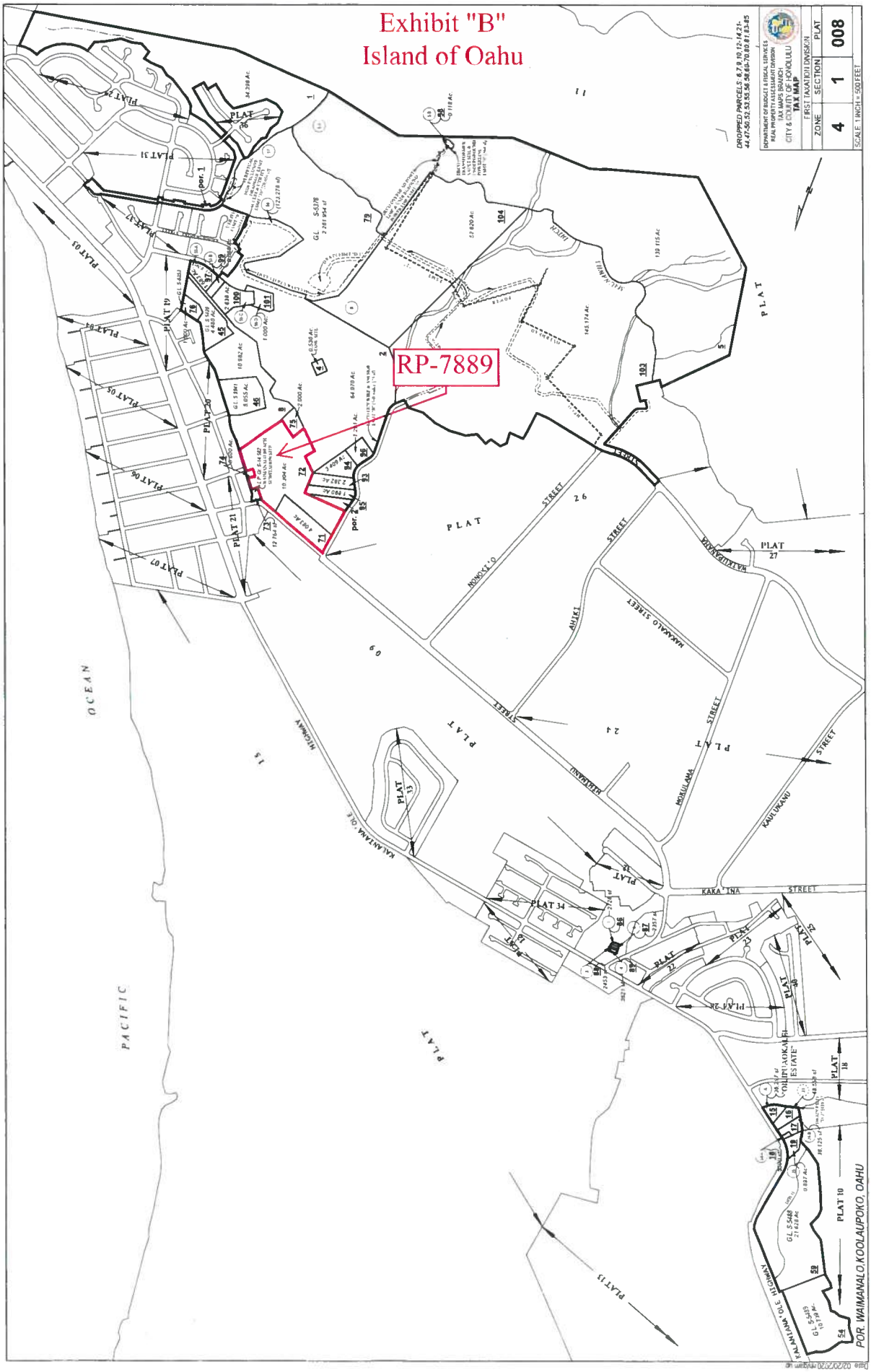
Exhibit "B"
Island of Oahu
RP-7889
PACIFIC OCEAN
POR. WAIMANALO,KOOLAUPOKO, OAHU
DEPARTMENT OF BUDGET & FISCAL SERVICES
REAL PROPERTY ASSESSMENT DIVISION
TAX MAP
CITY & COUNTY OF HONOLULU
FIRST TAXATION DIVISION
ZONE 4
SECTION 1
PLAT 008
SCALE 1 INCH = 600 FEET

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Exhibit "B"
Island of Maui

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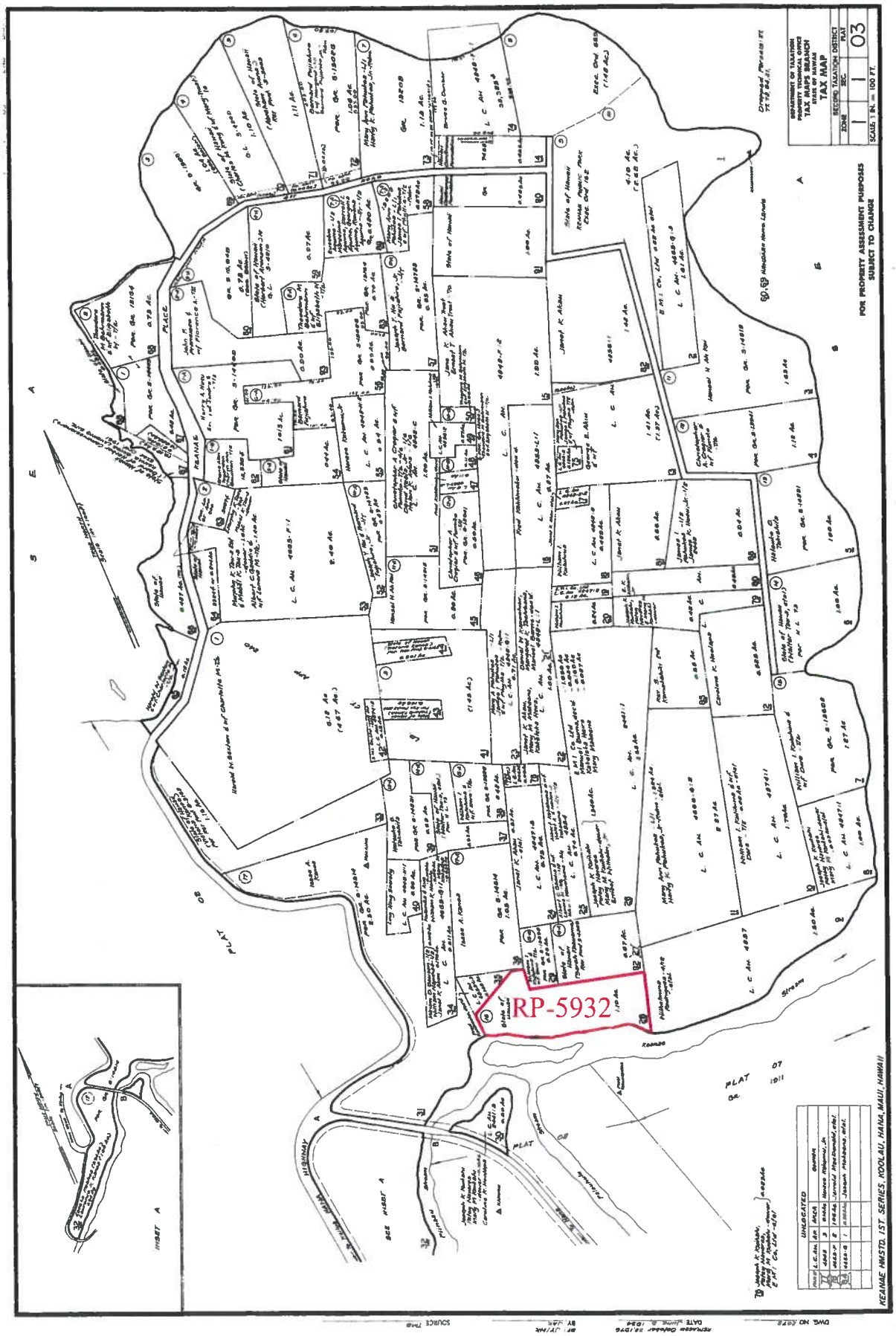

Exhibit "B"
Island of Hawaii

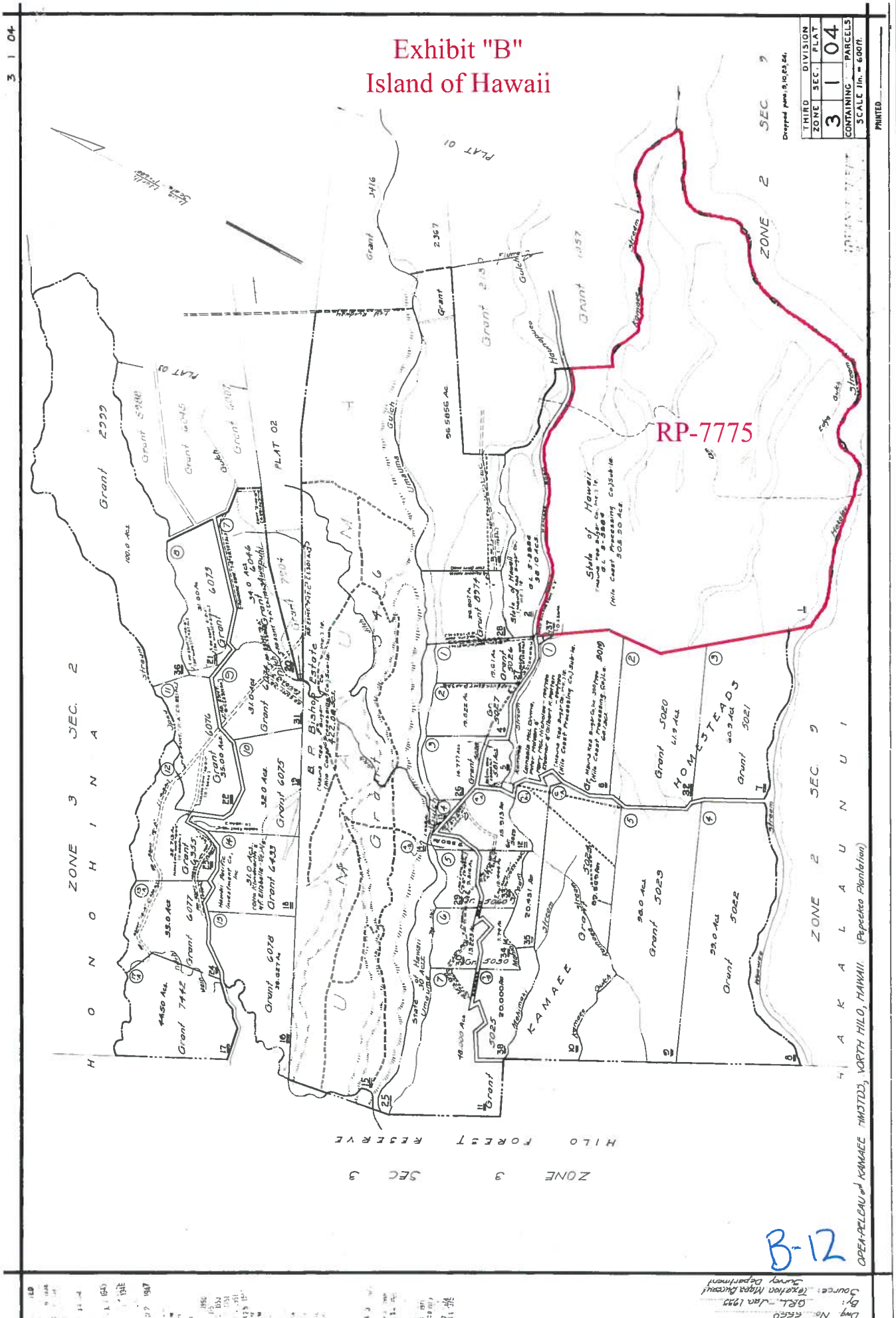

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Exhibit "B"
Island of Hawaii

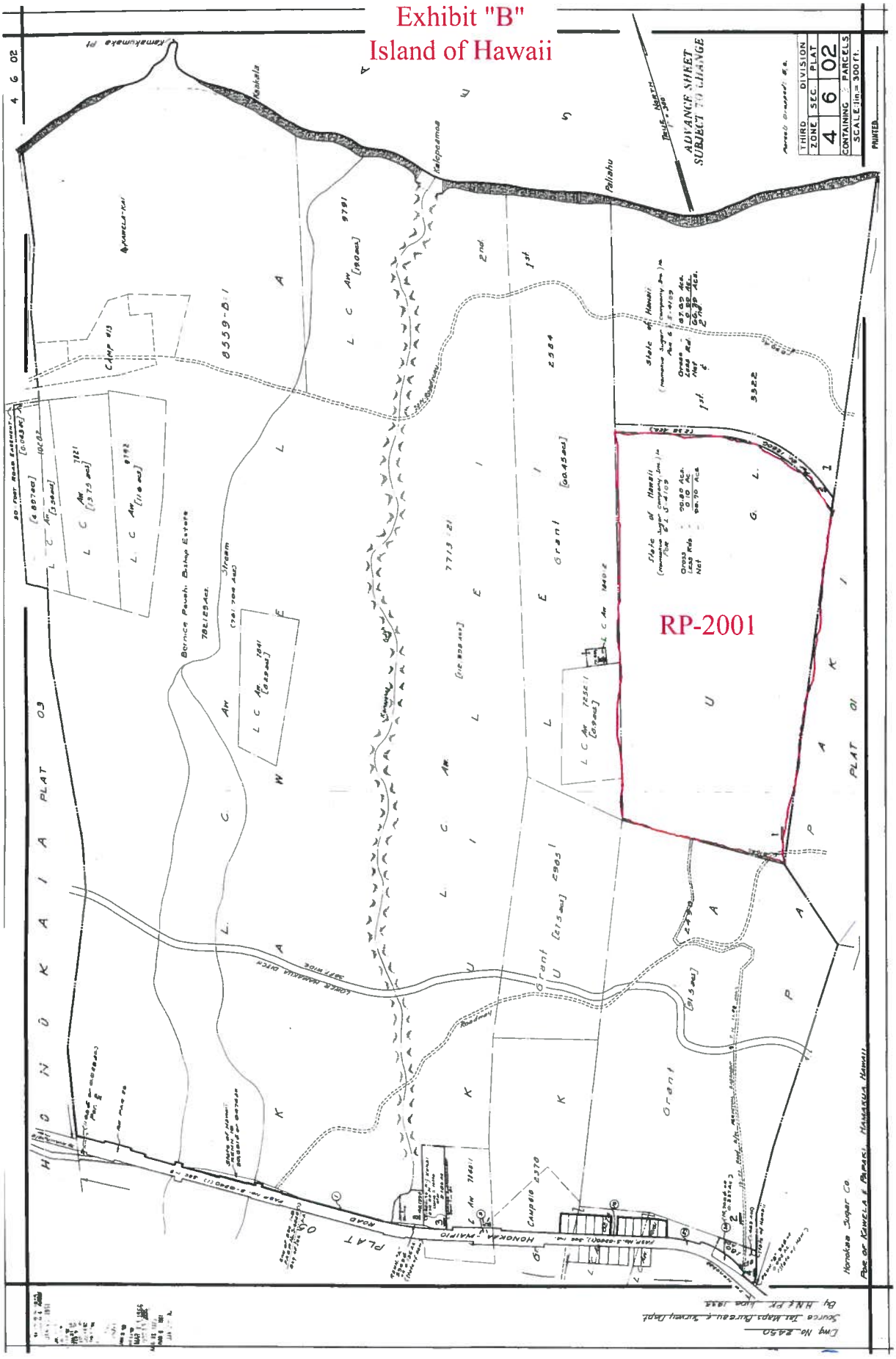

Exhibit "B"
Island of Hawaii

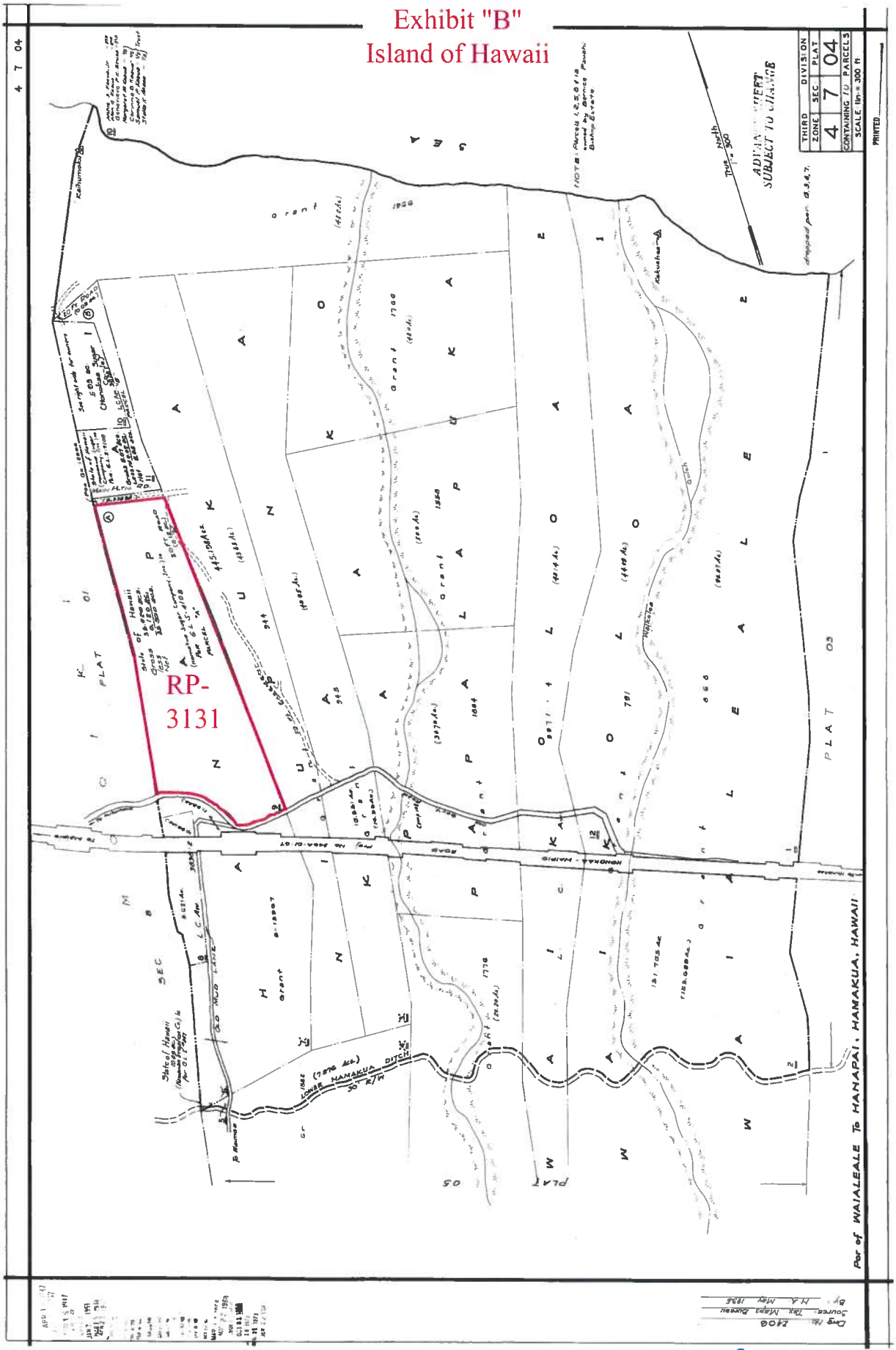

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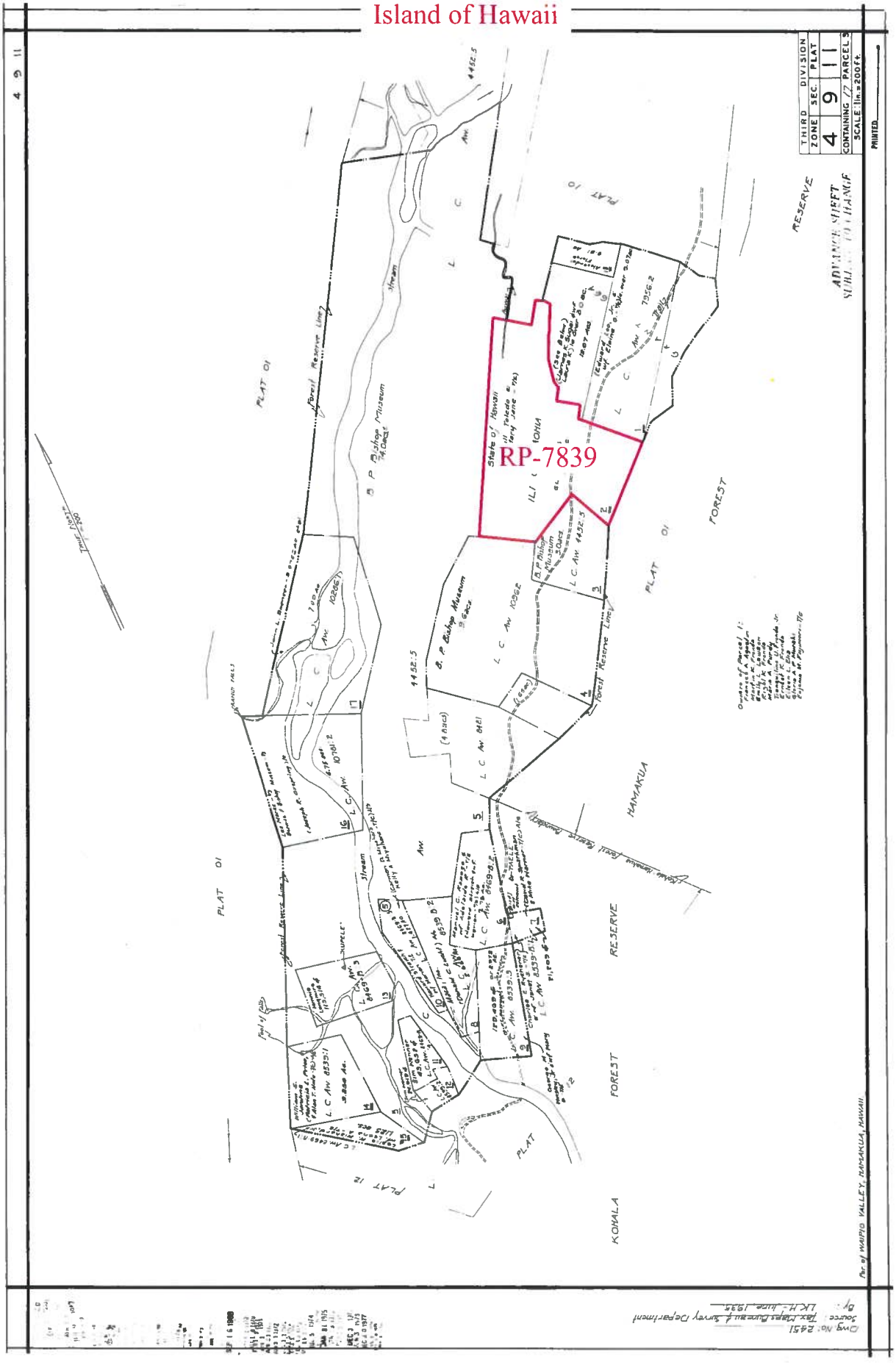
Exhibit "B"
Island of Hawaii



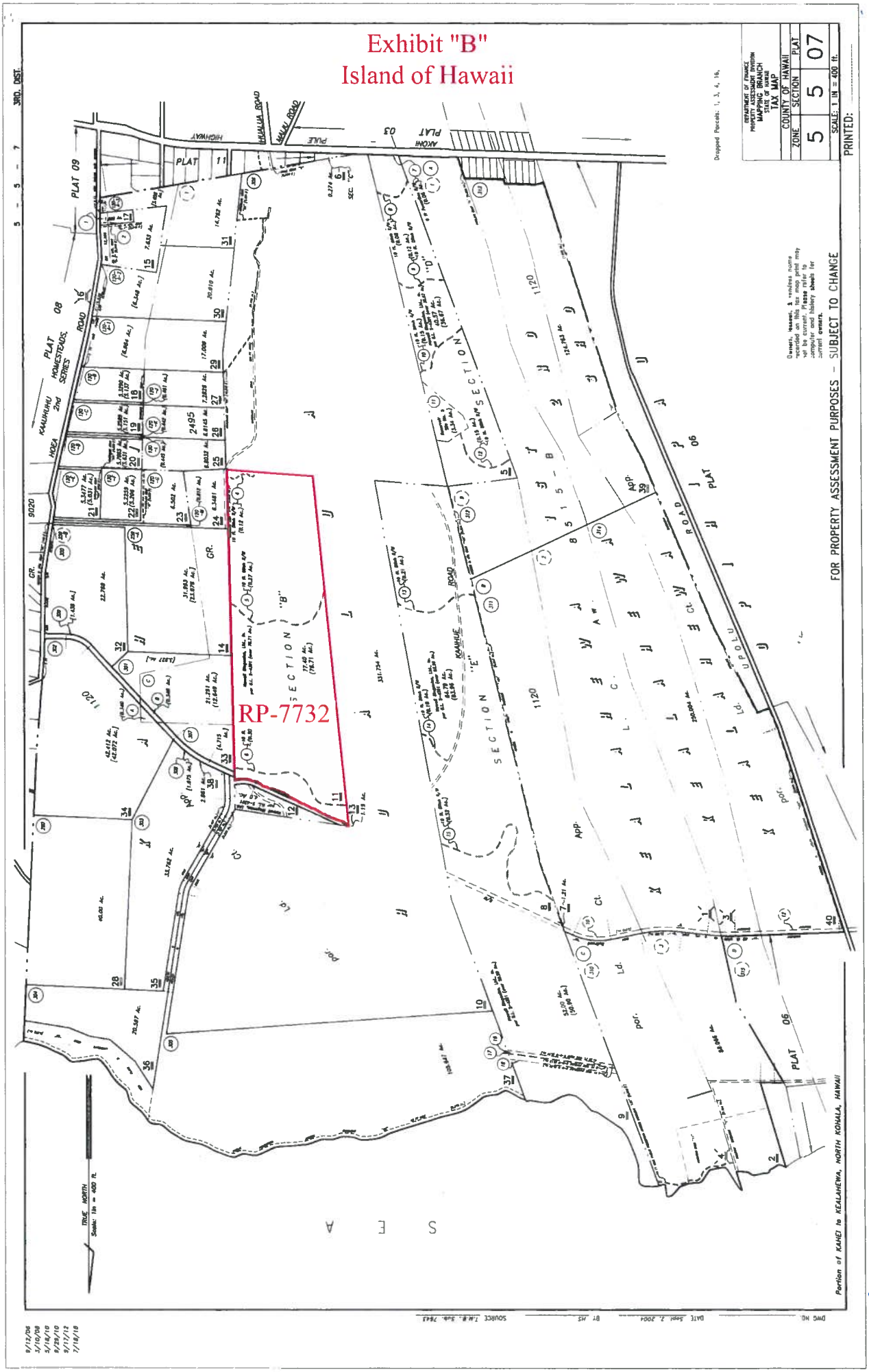


Exhibit "B"
Island of Hawaii
RP-7732
"B"
SECTION
FOR PROPERTY ASSESSMENT PURPOSES – SUBJECT TO CHANGE
COUNTY OF HAWAII
ZONE 5 SECTION 5 PLAT 07
SCALE: 1" = 400 ft.
PRINTED:
TAX MAP
Portion of KAHEI to KEALAHEWA, NORTH KONALA, HAWAII

B-16

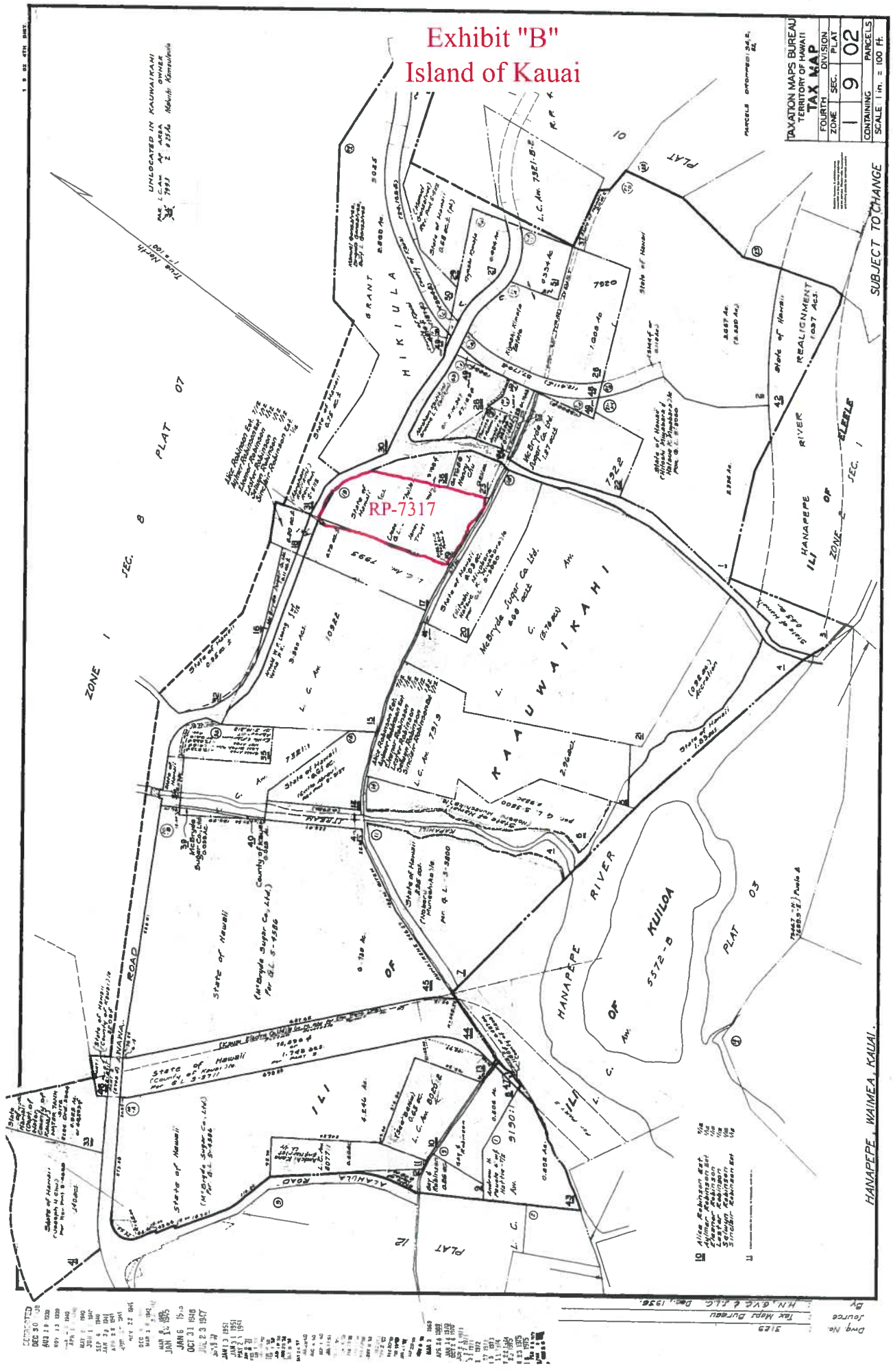


Exhibit "B"
Island of Kauai
RP-7317
SUBJECT TO CHANGE
TAXATION MAPS BUREAU
TERRITORY OF HAWAII
TAX MAP
FOURTH DIVISION
ZONE 1 SEC. 9 PLAT 02
CONTAINING PARCELS
SCALE 1 in. = 100 Ft.
PRINTED
HANAPEPE, WAIMEA, KAUAI.
KAUAWAIKAHI
HIKIULA
ILI
KUILOA
ALAHULA ROAD
ANAHA ROAD
REALIGNMENT 1937 Act.
HANAPEPE RIVER
ZONE 1 SEC. 1
PLAT 07
PLAT 03
PLAT 12
PLAT 10

B-17

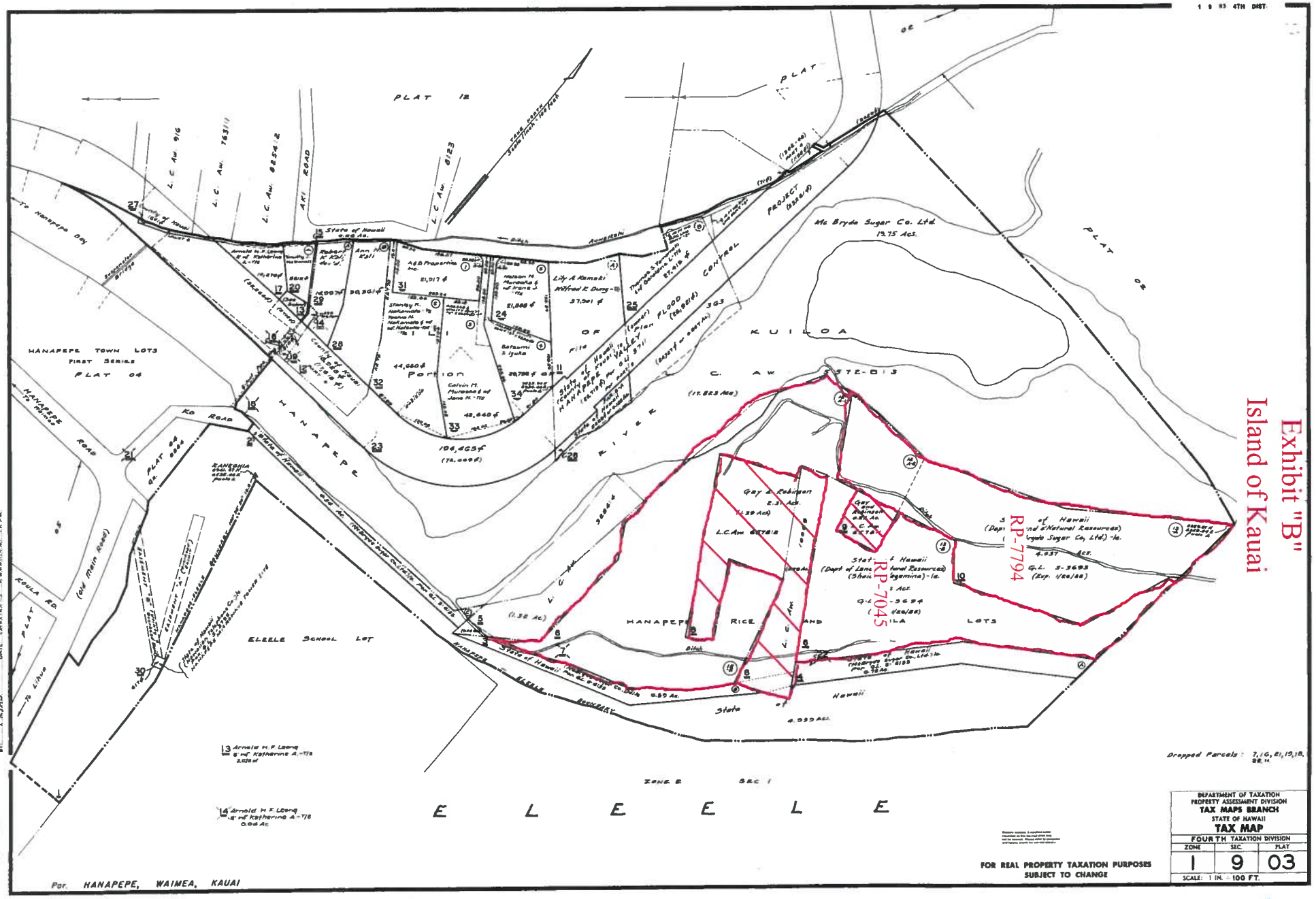


Exhibit "B"
Island of Kauai
RP-7794
RP-7045

B-18

Exhibit "B"
Island of Kauai

B-19

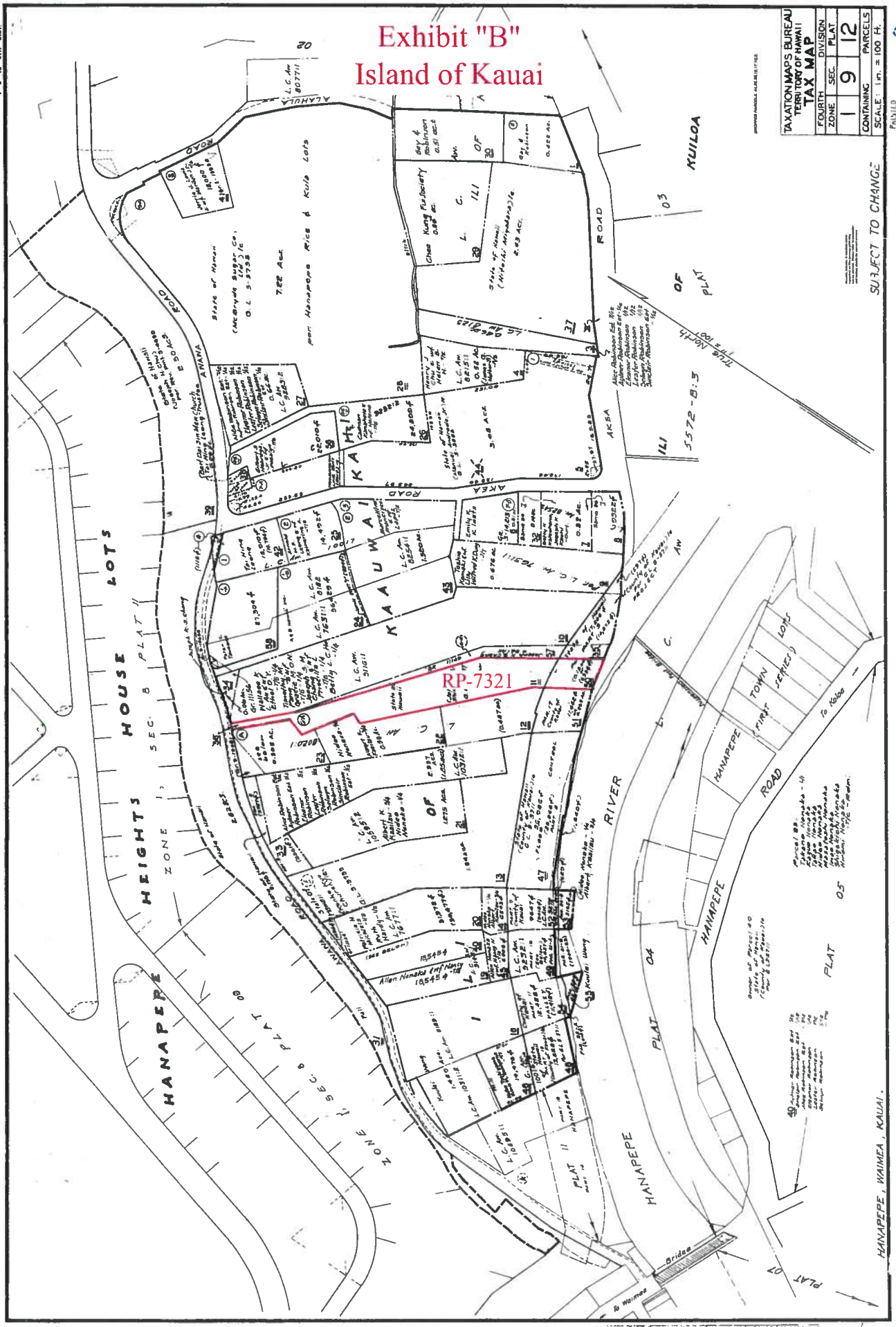
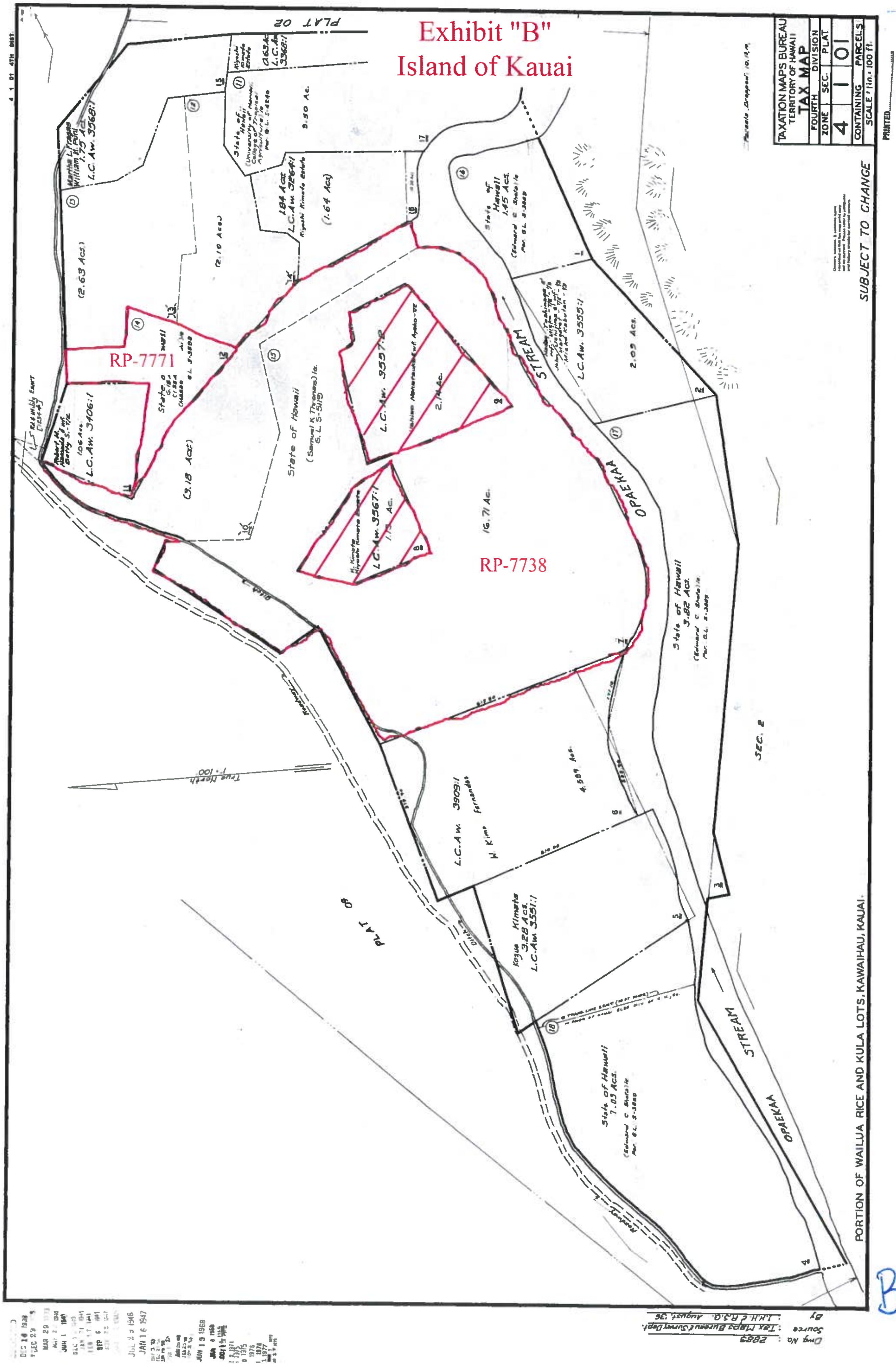


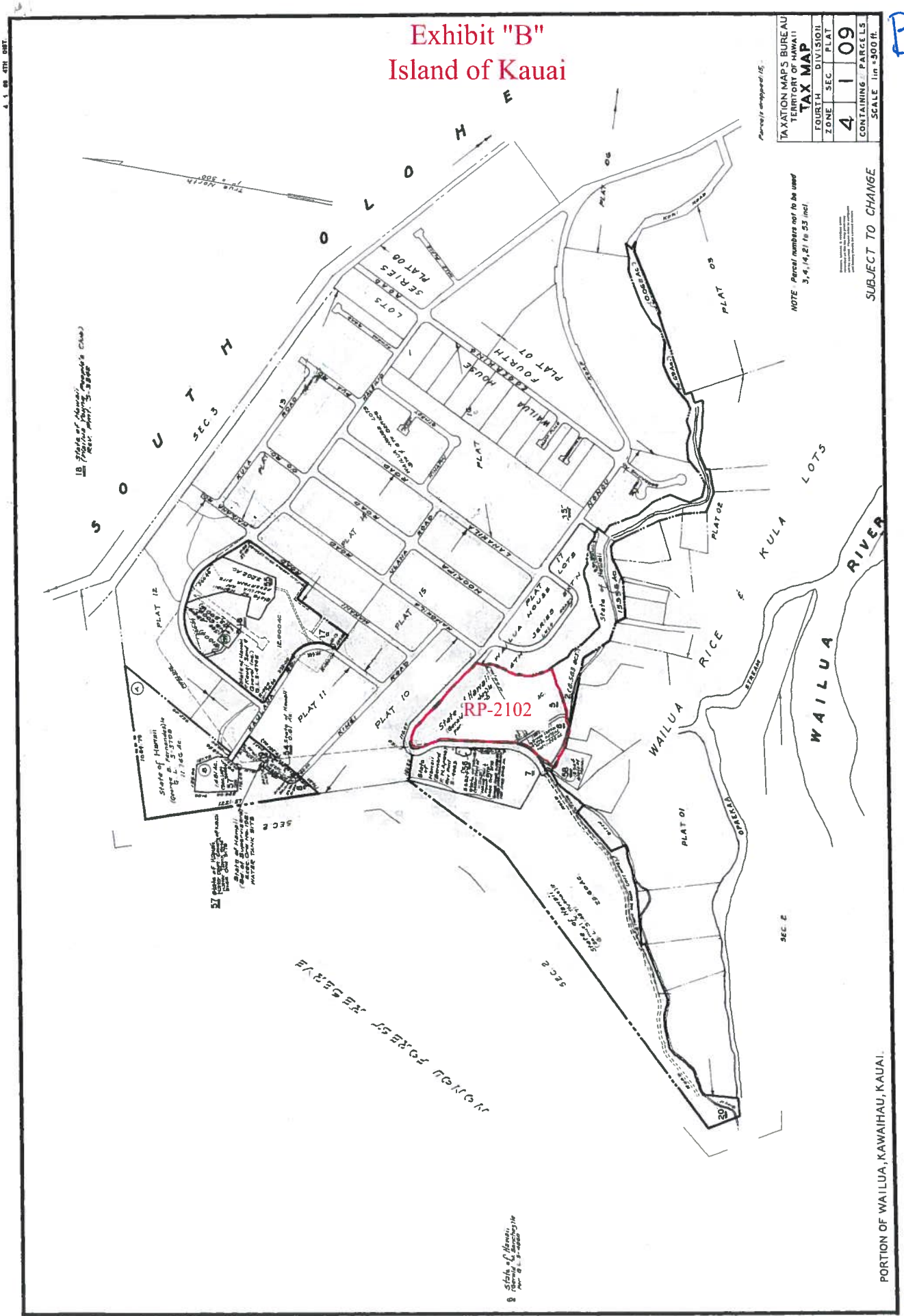
Exhibit "B"
Island of Kauai



B-20

Exhibit "B"
Island of Kauai

B-21



PORTION OF WAILUA, KAWAIHAU, KAUAI.

STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANAGEMENT DIVISION
HONOLULU, HAWAII

August 23, 2022

Board of Agriculture
Honolulu, Hawaii

Subject:	REQUEST FOR APPROVAL FOR FARM DWELLING; GENERAL LEASE NO. S-6020; FONG SOURIVONG, LESSEE; TMK: (1) 5-6-006:048; KAHUKU AGRICULTURAL PARK, LOT 20, KOOLAULOA, KAHUKU, ISLAND OF OAHU, HAWAII
Authority:	Section 166-9, Hawaii Revised Statutes, (HRS), and Section 4-153-32(c), Hawaii Administrative Rules (HAR)
Lessee:	Fong Sourivong
Land Area:	9.808 gross acres
Tax Map Key:	(1) 5-6-006:048 (see Exhibit "A")
Land Status:	Encumbered by Governor's Executive Order No. 3867 to the Department of Agriculture for Agricultural Park purposes dated April 26, 2001
Annual Base Rent:	$4,270.00 per year until rental re-opening (April 1, 2024)
Lease Term:	45 years, April 1, 1999 to March 31, 2044
Character of Use:	Diversified agriculture purposes

BACKGROUND:

Fong Sourivong acquired General Lease No. S-6020 through public drawing in 1999 as husband of Khamsy Sourivong, tenant-in-severalty. Mr. Sourivong and his family have developed the lot into a successful farm that produces dragon fruit, banana and bitter melon.

There is an existing farm dwelling on the premises for which final building permits were obtained from the City and County of Honolulu in 2007. Corresponding information documenting the permits for this dwelling are in the lessee's file. Board approval has yet to be obtained and the lessee is requesting after-the-fact Board approval. Staff reviewed the construction plans and dwelling for suitability of the improvement for appropriate agricultural use and recommends after-the-fact approval pursuant to Section 4-153-32(c), HAR, and lease

B-22

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provision paragraph "14. Dwelling restrictions." The dwelling is occupied by the lessee's family to provide security for crops, supplies and equipment on the premises. There have been numerous incidences of trespassing and theft of crops, farm tools and equipment from the premises. Other lessees of the Kahuku Agricultural Park have reported similar incidences of theft, vandalism and illegal trespassing.

The lessee is in compliance with the terms and conditions of General Lease No. S-6020.

RECOMMENDATION:

Staff recommends that the Board of Agriculture approve Lessee, Fong Sourivong's, request for after-the-fact approval of a farm dwelling on the premises of General Lease S-6020, subject to other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State, and subject to the following condition: The Lessee shall indemnify, defend and hold harmless the Lessor from and against any claim or demand for loss, liability, or damage including claims for property damage, personal injury, or wrongful death, arising out of Lessee's use of said improvements and appurtenances.

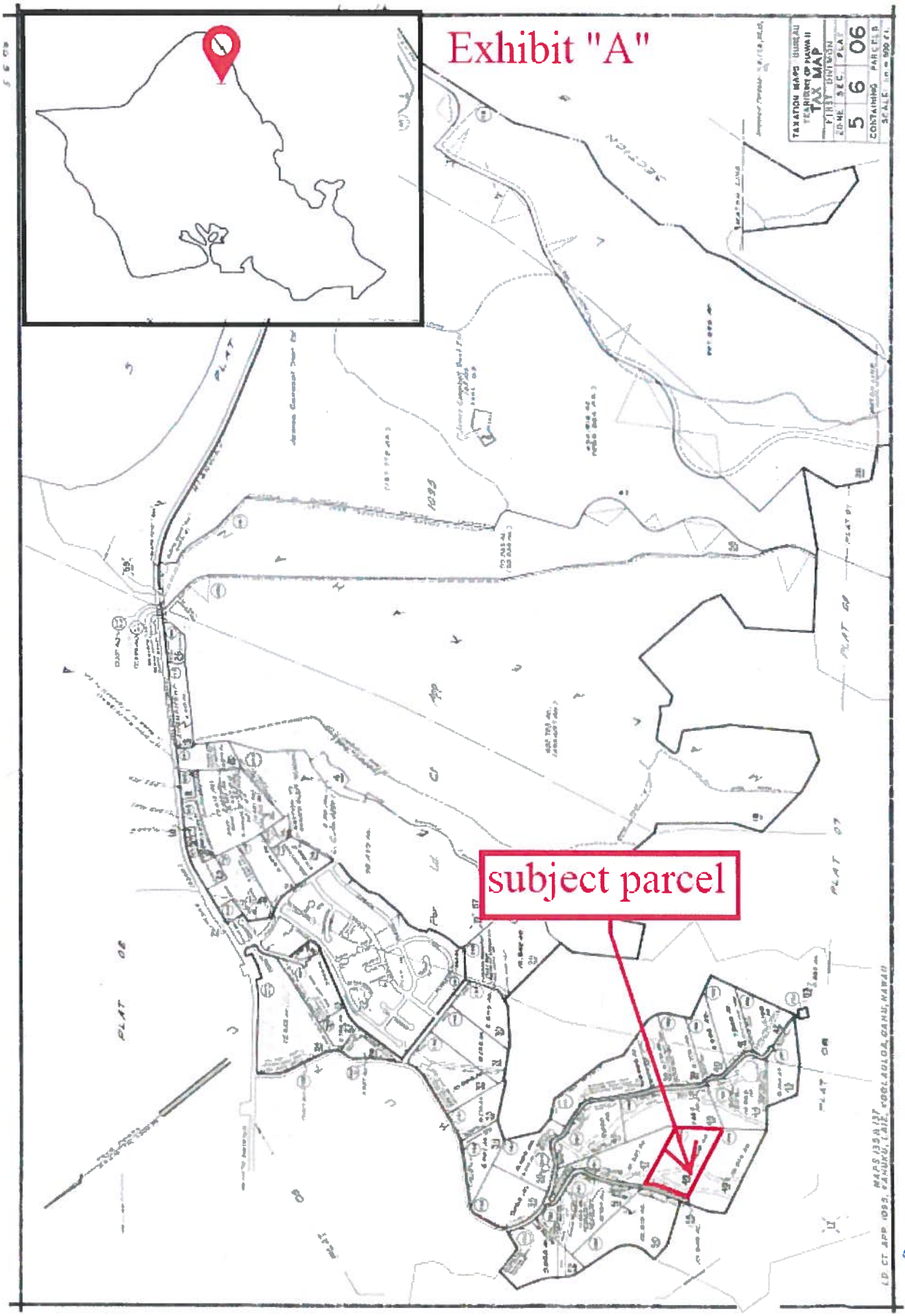
Respectfully submitted,

BRIAN KAU, P.E.
Administrator and Chief Engineer,
Agricultural Resource Management Division

Attachment – Exhibit "A"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture



B-24

STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANAGEMENT DIVISION
HONOLULU, HAWAII

August 23, 2022

Board of Agriculture
Honolulu, Hawaii

Subject:	REQUEST FOR APPROVAL FOR FARM DWELLING, GENERAL LEASE NO. S-6021; HI KOKO FARMS LLC, LESSEE; TMK: (1) 5-6-006:049; KAHUKU AGRICULTURAL PARK, LOT 21, KOOLAULOA, KAHUKU, ISLAND OF OAHU, HAWAII
Authority:	Section 166-9, Hawaii Revised Statutes, (HRS), and Section 4-153-32(c), Hawaii Administrative Rules (HAR)
Lessee:	HI Koko Farms LLC
Land Area:	10.913 gross acres
Tax Map Key:	(1) 5-6-006:049 (see Exhibit "A")
Land Status:	Encumbered by Governor's Executive Order No. 3867 to the Department of Agriculture for agricultural park purposes dated April 26, 2001
Annual Base Rent:	$3,260.00 per year until rental re-opening (April 1, 2024)
Lease Term:	45 years, April 1, 1999 to March 31, 2044
Character of Use:	Diversified agriculture purposes

BACKGROUND:

John Amphone acquired General Lease No. S-6021 through public drawing in 1999 as husband of Chanhouane Amphonephong, tenant-in-severalty. On July 28, 2015, the Board of Agriculture (BOA) consented to the assignment of General Lease No. S-6021 from John Amphone to himself and his sons, Bounlay Amphonephong and Sountone Amphonephong. On June 20, 2017, the BOA consented to the assignment of General Lease No. S-6021 to HI Koko Farms LLC, which is wholly owned by Charles Crismon. Mr. Crismon grows cacao on the subject lot for the production and marketing of chocolate.

B-25

B-26

There is an existing farm dwelling on the premises for which final building permits were obtained from the City and County of Honolulu in 2007. Corresponding information documenting the permits for this dwelling are in the lessee's file. Board approval has yet to be obtained and the lessee is requesting after-the-fact Board approval. Staff reviewed the construction plans and dwelling for suitability of the improvement for appropriate agricultural use and recommends after-the-fact approval pursuant to Section 4-153-32(c), HAR, and lease provision paragraph "14. Dwelling restrictions." The dwelling will be occupied by the lessee's family to provide security for crops, supplies and equipment on the premises. There have been numerous incidences of trespassing and theft of crops, farm tools and equipment from the premises. Other lessees of the Kahuku Agricultural Park have reported similar incidences of theft, vandalism and illegal trespassing.

The lessee is in compliance with the terms and conditions of General Lease No. S-6021.

RECOMMENDATION:

Staff recommends that the Board of Agriculture approve Lessee, HI Koko Farms LLC's, request for after-the-fact approval of a farm dwelling on the premises of General Lease S-6021, subject to other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State, and subject to the following condition: The Lessee shall indemnify, defend and hold harmless the Lessor from and against any claim or demand for loss, liability, or damage including claims for property damage, personal injury, or wrongful death, arising out of Lessee's use of said improvements and appurtenances.

Respectfully submitted,

BRIAN KAU, P.E.
Administrator and Chief Engineer,
Agricultural Resource Management Division

Attachment – Exhibit "A"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

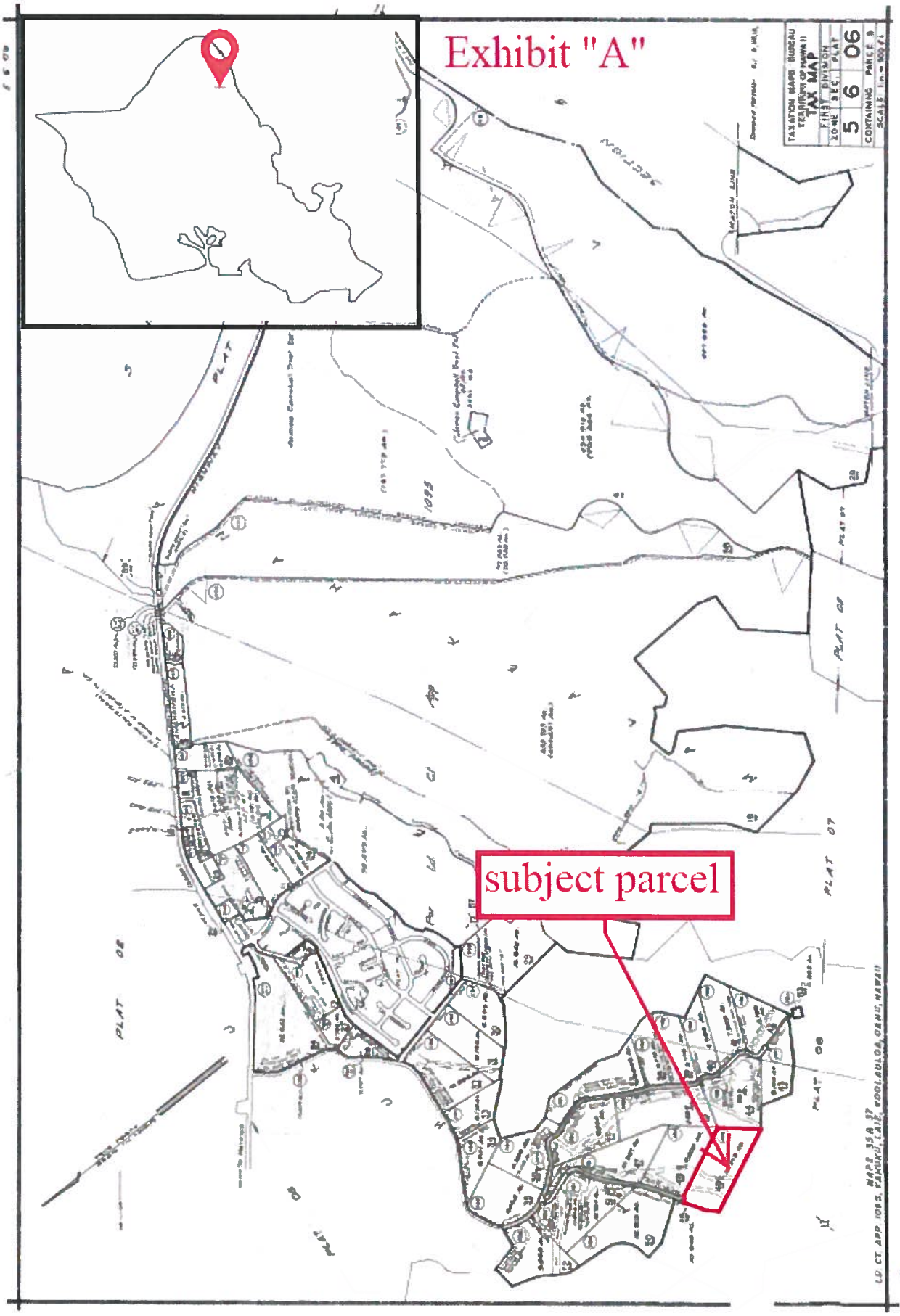


Exhibit "A"
subject parcel

STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANAGEMENT DIVISION
HONOLULU, HAWAII

August 23, 2022

Board of Agriculture
Honolulu, Hawaii

Subject:	REQUEST FOR APPROVAL FOR FARM DWELLING; GENERAL LEASE NO. S-6024; KHAMPHOU KHOUTHONG, LESSEE; TMK: (1) 5-6-006:052; KAHUKU AGRICULTURAL PARK, LOT 24, KOOLAULOA, KAHUKU, ISLAND OF OAHU, HAWAII
Authority:	Section 166-9, Hawaii Revised Statutes, (HRS), and Section 4-153-32(c), Hawaii Administrative Rules (HAR)
Lessee:	Khamphou Khouthong
Land Area:	9.865 gross acres
Tax Map Key:	(1) 5-6-006:052 (see Exhibit "A")
Land Status:	Encumbered by Governor's Executive Order No. 3867 to the Department of Agriculture for agricultural park purposes dated April 26, 2001
Annual Base Rent:	$4,530.00 per year until rental re-opening (April 1, 2024)
Lease Term:	45 years, April 1, 1999 to March 31, 2044
Character of Use:	Diversified agriculture purposes

BACKGROUND:

General Lease No. S-6024 was originally awarded to Douglas Anamizu and Carol Anamizu in 1999 by way of public drawing. On August 25, 2009, the Board of Agriculture (BOA) consented to the assignment of General Lease No. S-6024 from Carol Anamizu, widow of Douglas Anamizu, to Fong Sourivong. On October 22, 2019, the BOA consented the assignment of General Lease No. S-6024 from Mr. Sourivong to his nephew Khamphou Khouthong. Mr. Khouthong and his family have developed the lot into a successful farm that produces taro, cucumbers, papayas, coconut, and banana.

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There is an existing farm dwelling on the premises for which final building permits were obtained from the City and County of Honolulu in 2014. Corresponding information documenting the permits for this dwelling are in the lessee's file. Board approval has yet to be obtained and the lessee is requesting after-the-fact Board approval. Staff reviewed the construction plans and dwelling for suitability of the improvement for appropriate agricultural use and recommends after-the-fact approval pursuant to Section 4-153-32(c), HAR, and lease provision paragraph "14. Dwelling restrictions." The dwelling will be occupied by the lessee's family to provide security for crops, supplies and equipment on the premises. There have been numerous incidences of trespassing and theft of crops, farm tools and equipment from the premises. Other lessees of the Kahuku Agricultural Park have reported similar incidences of theft, vandalism and illegal trespassing.

The lessee is in compliance with the terms and conditions of General Lease No. S-6024.

RECOMMENDATION:

Staff recommends that the Board of Agriculture approve Lessee, Khamphou Khouthong's, request for after-the-fact approval of a farm dwelling on the premises of General Lease S-6024, subject to other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State, and subject to the following condition: The Lessee shall indemnify, defend and hold harmless the Lessor from and against any claim or demand for loss, liability, or damage including claims for property damage, personal injury, or wrongful death, arising out of Lessee's use of said improvements and appurtenances.

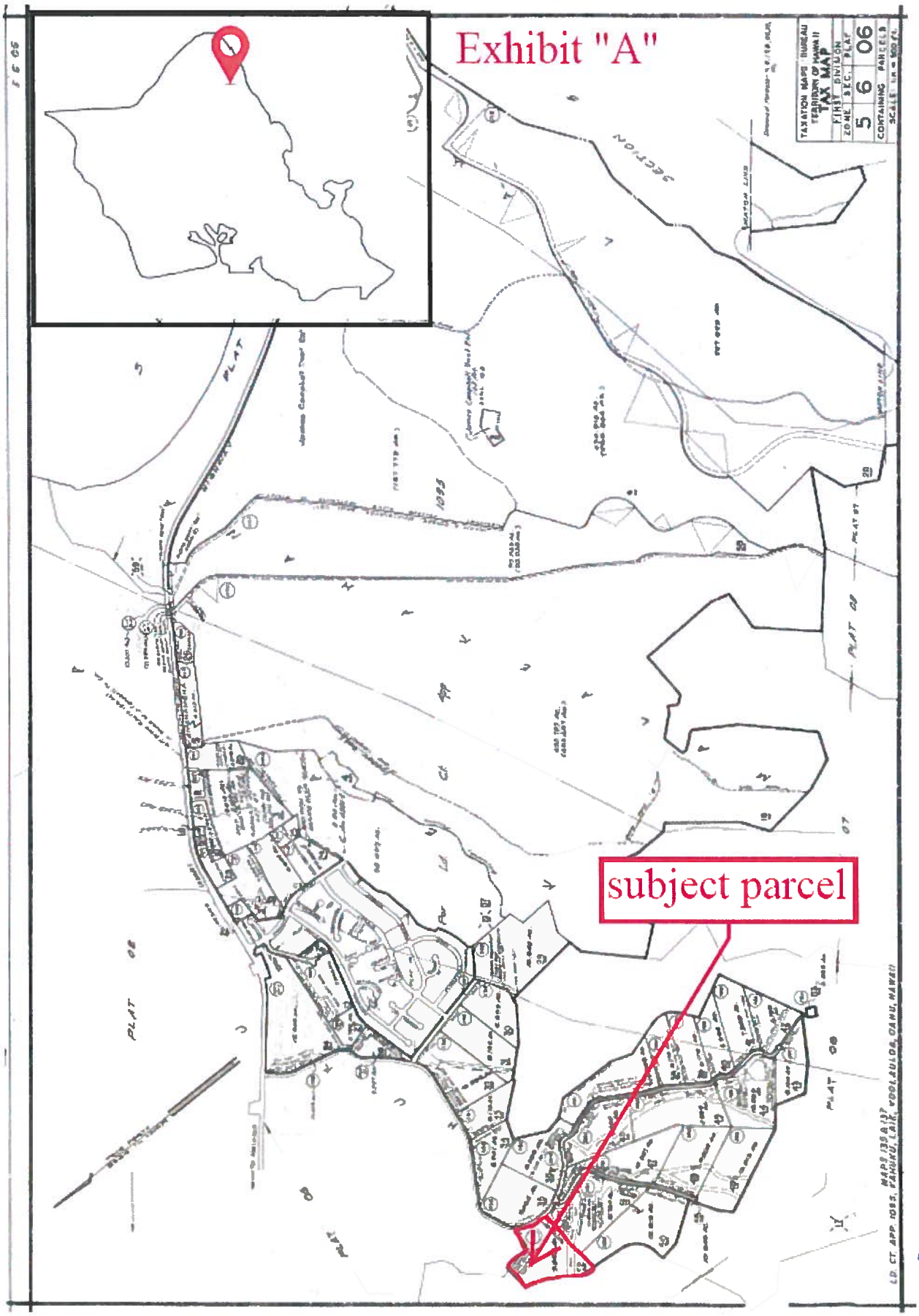
Respectfully submitted,

BRIAN KAU, P.E.
Administrator and Chief Engineer,
Agricultural Resource Management Division

Attachment – Exhibit "A"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture



B-30

0011
STATE OF HAWAII
DEPARTMENT OF AGRICULTURE
AGRICULTURAL RESOURCE MANAGEMENT DIVISION
HONOLULU, HAWAII

August 23, 2022

Board of Agriculture
Honolulu, Hawaii

Subject: REQUEST FOR APPROVAL TO SUBLEASE BETWEEN THE HAMAKUA AGRICULTURAL COOPERATIVE, LESSEE/SUBLESSOR, AND HAMAKUA LANDSCAPING, LLC, SUBLESSEE; GENERAL LEASE NO. S-5551, TMK: (3) 4-6-003:001, 002 and 020 (por), LOT NO 17, HONOKAIA, HAMAKUA, ISLAND OF HAWAII, HAWAII

Authority:	Section 166E-6 Hawaii Revised Statutes, (HRS), and Section 4-158-19(a)(6), Hawaii Administrative Rules (HAR)
Lessee/Sublessor:	Hamakua Agricultural Cooperative
Sublessee:	Hamakua Landscaping, LLC
Land Area:	Approximately 3.67 acres
Tax Map Key:	3rd Div/4-6-003:001, 002 and 020 (por) (Exhibit "A")
Land Status:	The Hamakua lands were transferred to the Department of Agriculture by Governor's Executive Order No. 4250, dated October 22, 2008, pursuant to Act 90, SLH 2003
Lease Term:	35-years, June 30, 1998 through June 29, 2033
Sub-Lease Term:	August 7, 2022 through June 29, 2033
Annual Base Rent:	$4,940/year
Character of Use:	General Agricultural Purposes in accordance with a Plan of Utilization and Development approved by the Department

REMARKS:

Hamakaua Landscaping, LLC, is solely owned and operated by Charles Oldfather. Hamakua Landscaping, LLC is requesting approval to sublease Lot No. 17, consisting of approximately 3.67 acres, located in Honokaia. Hamakua Landscaping, LLC will utilize the subject property to grow a variety of tropical fruits, ornamental potted plants, cut flowers and nursery stock. Their goal is to supply plant materials for their landscaping business and to expand their production to include the sale of agricultural products.

B-31

Charles Oldfather is the son of the late Stephen Oldfather, who previously sub-leased the subject property. Together they developed the subject property and grew a variety of fruit trees and flowers.

Hamakua Landscaping, LLC qualifies as a bona fide farmer with more than 2 years of full-time farming experience and meets the application and eligibility requirements in accordance with sections 4-158-1 and 27, HAR.

RECOMMENDATION:

That the Board of Agriculture approve the Sublease between the Hamakua Agricultural Cooperative, Lessee/Sublessor, and Hamakua Landscaping, LLC, Sublessee, for Lot No. 17 in Honokaia, under General Lease S-5551, through the expiration date of June 29, 2033, and further subject to the review and approval as to form of the consent document by the Department of the Attorney General, and such other terms and conditions as may be prescribed by the Chairperson to best serve the interests of the State.

Respectfully submitted,

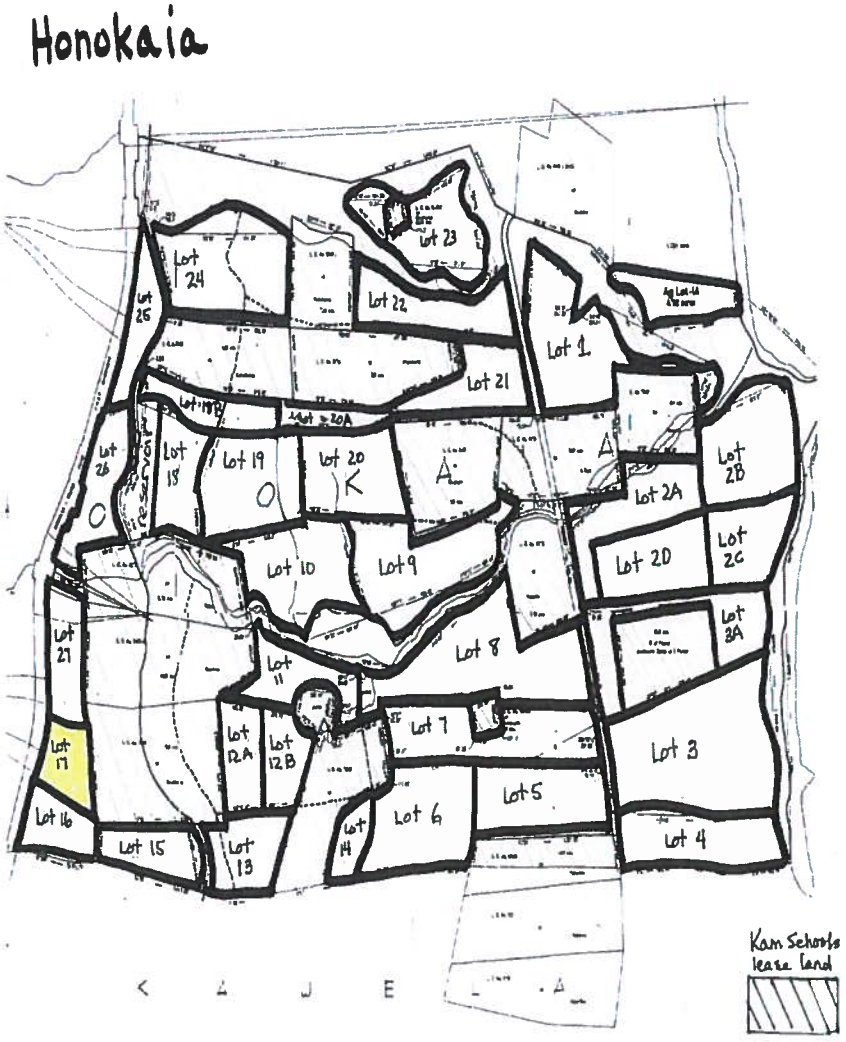
BRIAN KAU, P.E.
Administrator and Chief Engineer,
Agricultural Resource Management Division

Attachments - Exhibit "A"

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

EXHIBIT A



B-33

State of Hawaii
Department of Agriculture
Plant Industry Division
Plant Quarantine Branch
Honolulu, Hawaii

August 23, 2022

Board of Agriculture
Honolulu, Hawaii

SUBJECT: Request to: (1) Preliminarily Review the Currently Unlisted Beetle, *Syphraea uberabensis* (Coleoptera: Chrysomelidae) for Future Placement on the List of Restricted Animals (Part A) As a Biocontrol Agent of *Tibouchina herbacea* and Other Related Species in the Family Melastomataceae, by the United States Department of Agriculture Forest Service (USDA FS);

(2) Determine If the Release of the Beetle *Syphraea uberabensis* as a Biocontrol Agent of *Tibouchina herbacea* and other related species in the family Melastomataceae, by the USDA FS Poses No Significant Impact on the Environment;

(3) Provided the Beetle Syphraea uberabensis is Placed on the List of Restricted Animals (Part A), Allow the Release from Laboratory Quarantine of the beetle *Syphraea uberabensis*, by Permit, For Biocontrol of *Tibouchina herbacea* and Other Related Species In the Family Melastomataceae by USDA FS;

(4) Provided the Beetle *Syphraea uberabensis* is Placed on the List of Restricted Animals (Part A), Allow the Importation and Release of the Beetle *Syphraea uberabensis*, by Permit, For Biocontrol of *Tibouchina herbacea* and Other Related Species in the Family Melastomataceae, by the USDA FS;

(5) Provided the Beetle *Syphraea uberabensis* is Placed on the List of Restricted Animals (Part A), Establish Permit Conditions For the Importation and Release of the Beetle *Syphraea uberabensis* As a Biocontrol Agent of *Tibouchina herbacea* and Other Related Species in the Family Melastomataceae, by the USDA FS; and

(6) Provided the Beetle *Syphraea uberabensis* is Placed on the List of Restricted Animals (Part A), Authorize the Chairperson to Schedule a

C1

C2

Public Hearing and Appoint a Hearing Officer in Connection with the Proposed Amendments to Chapter 4-71, HAR.

I. Summary Description of the Request

PQB NOTES: *The Plant Quarantine Branch (PQB) submittal for requests for import or possession permits, as revised, distinguishes information provided by the applicant, Dr. Matthew Tracy Johnson, from procedural information and advisory comment and evaluation presented by PQB. With the exception of PQB notes, hereafter "PQB NOTES," the text shown below in section III from page 4 through page 9 of the submittal was taken directly from the applicant's application and subsequent written communications provided by the applicant. For instance, the statements on pages 6 through 8 regarding effects on the environment are the applicant's statements in response to standard PQB questions and are not PQB's statements. This approach for PQB submittals aims for greater applicant participation in presenting import requests in order to move these requests to the Board of Agriculture (Board) more quickly, while distinguishing applicant provided information from PQB information. The portion of the submittal prepared by PQB, including the procedural background, environmental assessment and proposed permit conditions, are identified as sections II, IV and V of the submittal, which start at pages 3, 9, and 20 respectively.*

COMMODITY: Various shipments of the beetle, *Syphraea uberabensis* (Coleoptera, Chrysomelidae, Galerucinae, Alticini).

SHIPPER: M. Vitorino
Universidade Regional de Blumenau
Rua Antonio da Veiga, 140 89012-570 Blumenau
Santa Catarina, Brazil

IMPORTER: Dr. Matthew Tracy Johnson
USDA Forest Service
Hawaii Volcanoes National Park Quarantine Facility
Kilauea Research Station, Building 34
Volcano, HI 96718

CATEGORY: *Syphraea uberabensis* is currently an unlisted animal. Animals not found on any list are considered prohibited until placed on a list. Additionally, Chapter 4-71, Hawaii Administrative Rules (HAR), allows importation of unlisted animals into Hawaii under special permit for the purpose of remediating medical emergencies or ecological disasters, or conducting scientific research that is not detrimental to agriculture, the

environment, or humans by special permit, on a case-by-case basis, as approved by the Board.

PQB NOTES: *The applicant is requesting that the Board place Syphraea uberabensis on the List of Restricted Animals (Part A) for import and release for biological control of Tibouchina herbacea and other related weed species in the family Melastomataceae.*

Syphraea uberabensis was originally brought into the Hawaii Volcanoes National Park Quarantine Facility from Brazil in July 2005 for biocontrol research and host range testing. The applicant is not currently requesting a special permit at this time.

In January 2020, a draft environmental assessment was submitted to the Office of Environmental Quality Control (OEQC) with an Anticipated Finding of No Significant Impact. The draft was published in OEQC's Environmental Notice on January 23, 2020 (See Attachment 2).

II. Procedural Background

USDA FS has requested that one of the lists in Chapter 4-71, Hawaii Administrative Rules (HAR), be amended to include the beetle, *Syphraea uberabensis.* The species may be placed on the List of Conditionally Approved Animals, List of Restricted Animals (Part A or B), or the Prohibited List. Species on the Restricted and Conditionally Approved Lists may enter the State of Hawaii under permits with conditions approved by the Board. Until placement on a list, species are considered prohibited except as provided by Section 150A-6.2(c), Hawaii Revised Statutes (HRS).

Species on the List of Restricted Animals (Part A) are available for research by universities and government agencies, exhibition in municipal zoos and government-affiliated aquariums, and for other institutions for medical and scientific purposes as determined by the Board. All species listed for import require a permit for entry into the State. Based on the Board's decision, species preliminarily reviewed for future list placement on a specific list will be compiled in-house for a future rule amendment. The Board's action to preliminarily place a species on a list has no legal effect until the rule has been changed. This procedure is solely for administrative ease in preparation for amendments to the various lists.

Provided the Board acts favorably on this request for future list placement, at a future date, the proposed amendments will be brought to the Board for preliminary approval to go to public hearings. A species is listed in the rules only after: (1) following Chapter 91, HRS, rulemaking procedures, which include the public hearing process, Board adoption, and Governor's approval: or (2) alternatively, the expedited amendment procedure through Board orders, which involves an abbreviated process available in certain circumstances. Generally speaking, once a species has been placed on a respective list, it is eligible for import and/or possession. PQB can then process a

C4

permit application by having the Board approve the future importation and establishment of appropriate permit conditions for the organism and proposed purpose.

III. Information Provided by the Applicant in Support of the Application

PURPOSE:

Syphraea uberabensis, a small beetle native to Brazil, has been selected and evaluated as a new biological control for managing invasive melastome weeds in Hawaii. It is a narrowly host-specific leaf-feeding beetle intended for statewide field release to cause suppression of non-native plants in the genera of *Tibouchina*, *Melastoma* (designated as noxious weeds), and *Pterolepis*. The beetle is expected to cause severe defoliation of targeted weeds, without affecting any native or otherwise valued plants. Suppression of these weeds will benefit forest watersheds statewide.

DISCUSSION:

1. Person Responsible:

Dr. Matthew Tracy Johnson, Institute of Pacific Island Forestry, USDA FS, Pacific Southwest Research Station, Mailing address: P.O. Box 236, Volcano, HI 96785

2. Safeguard Facility and Practices:

Initial quarantine will be at USDA Forest Service, Hawaii Volcanoes National Park Quarantine Facility, Kilauea Research Station, Bldg. 34. The *Syphraea uberabensis* colony will originate from insects collected from southern Brazil and shipped under USDA Plant Protection and Quarantine permit P526P-20-02009 to the Volcano quarantine, for rearing and screening to eliminate associated natural enemies. Tracy Johnson will positively identify the insects and determine them to be free of natural enemies in preparation for release.

3. Method of Disposition:

Any unused material will be autoclaved within the quarantine facility. Roughly 30 insects at a time will be removed from quarantine as newly pupated adult beetles, independent of host plant material and other potential contaminants. Adults will be used to establish colonies reared in petri dishes at USDA and Hawaii Department of Agriculture insectaries in Volcano, Hilo, and Honolulu. Offspring from rearing colonies will be used for environmental releases at selected locations statewide[.]

4. Abstract of Organism:

Syphraea uberabensis is a small beetle that has been evaluated in its native Brazil between 1993 and 2009 and in containment in Hawaii between 2005 and 2015. Adults and larvae feed externally on foliage and soft stems of *Tibouchina herbacea*, causing enough damage to kill small plants. *Syphraea uberabensis* is host specific to a subset of species within the melastome family, which contains no native taxa in Hawaii.

Taxonomy:
Syphraea uberabensis Bechyné is a flea beetle, classified under the tribe Alticini and the leaf beetle family Chrysomelidae. Flea beetles are similar to other leaf beetles but are characterized by having enlarged hind legs, which afford them the ability to leap/spring when disturbed, hence the common name. Flea beetles are herbivores that feed on various parts of the plant; some flea beetle species are important agricultural pests. They do not bite humans or animals. The genus *Syphraea* Baly (1876) includes more than 100 species and is found throughout South and Central America (Scherer 1983).

Description of Adults:
Body elongated, slightly broader posteriorly; robust legs; thorax, abdomen, legs and antennae covered with fine short hairs; coloration deep metallic blue, females 3.3 mm and males 3.0 mm in length, on average (Souder 2008).

Description of Larvae:
Mature larva. Length: 4.4–6.30 mm; width of pronotum: 0.75–1.41 mm. Eruciform, general integument cream/yellowish with brown head; antennae, maxillae and legs partially membranous; thorax and abdomen with setous sclerotized plates or setous sclerotized tubercles, brown or yellowish-brown, clearer to apex direction; ventral tubercles clearer than dorsal. Segments separated by transverse grooves forming plicae. Setae club-like, whitish, wide with widened apex; ventral setae narrower than dorsal (Casari and Teixeira 2011).

Distribution:
Syphraea uberabensis is native to southern Brazil. The distributional range of the species is not well studied.

Life History:
A life history study conducted in the quarantine facility in Hawaii showed that *S. uberabensis* reared on *T. herbacea* had an adult life span ranging from 2 to 127 days and averaged 78.2 days. *Syphraea uberabensis* samples of the quarantine colony had a sex ratio close to 1:1. Males and females developed and emerged at similar rates (Souder 2008).

Survival and development of *S. uberabensis* was evaluated in the laboratory at five constant temperatures ranging from 12 to 28 °C. No egg or larval development occurred below 16 °C. Complete development to adulthood was only seen at 20 and 24°C. Mean time for development from egg to adult was 50.5 days at 20°C

and 31.5 days at 24°C, fitting the expected pattern for insects in general: faster development at increasing temperatures. Although development was slightly faster at 28°C than at 24°C, beetle survivorship was reduced and no adults developed at 28°C. Reduced development and increased mortality of beetle larvae at 16 and 28°C is an indication that the minimum and maximum temperature thresholds were being approached (Souder 2008).

Habitat/Ecology:
Syphraea uberabensis is tolerant of cool and moderate temperatures and is not expected to be restricted in range by temperatures in Hawai'i, except perhaps in exceptionally warm habitats (Souder 2008). However, the potential of *S. uberabensis* as a biological control could be limited by humidity at the microhabitat level. In Brazil, *S. uberabensis* is found with its melastome hosts in boggy soils, similar to the areas where Tibouchina and Pterolepis thrive in Hawaii. On the other hand, Melastoma in Hawai'i can grow in relatively drier areas, such as young lava flows. *S. uberabensis* could be less effective against Melastoma in the drier parts of its range, because its externally feeding larvae appear to be susceptible to drying out (Raboin et al. 2009).

Natural Enemy:
There is very little information regarding the natural enemies of *S. uberabensis*. Two unidentified generalist Hemipterans were observed attacking the adult insects in its native range (Wikler and Souza 2008). Under laboratory conditions, larvae and pupae were reported to succumb to a ubiquitous entomopathogenic fungus, *Beauveria bassiana*.

Effect on Target Weed:
Syphraea uberabensis was selected to be used in the control of *T. herbacea* due [to] the extensive damage it caused to the target plant in Brazil. Both larvae and adults feed on the leaves as well as the soft exterior of young stems. *Tibouchina herbacea* demonstrated little regenerating capacity after attack of *S. uberabensis*, drying after a period of 2 weeks of insect feeding, both in the field and in the laboratory. The leaves were skeletonized, leaving only the stem and vein structures. Plant growth was reduced, and flowering and consequently seed production were prevented (Wikler and Souza 2008).

5. Potential Effects on the Environment and Health:

Specificity tests indicated the host range of *Syphraea uberabensis* is restricted to a few melastome species, all non-native and considered invasive in Hawaii. The results of no-choice starvation tests and multi-choice testing consistently identified the potential Hawaiian hosts as: *Tibouchina herbacea, Tibouchina longifolia, Pterolepis glomerata, Melastoma septemnervium* and *Melastoma sanguineum*.

Potential host preferences were evaluated on a total of 58 plant species in 30 families. Test plants were selected based on the centrifugal phylogenetic method proposed by Wapshere (1974). The test list included six plant species requested by the U.S. Fish and Wildlife Service because of their ecological importance, as well as a variety of species with economic significance in Hawaii (see attached host specificity results for plant species lists and more information).

Testing revealed *Syphraea uberabensis* to be narrowly host-specific within the family Melastomataceae and able to complete development on only the five plant species listed above. Larvae and naïve adults showed a somewhat broader range of feeding compared to mature adults in tests lasting a few days, however low levels of feeding outside the normal host range is a common result of no-choice tests, in which insects are unable to seek out preferred hosts (Heard 2002). Longer test periods demonstrated that only a few melastome species support survival to maturity and oviposition. Choice tests demonstrated the same few melastome species to be highly preferred over other related plants.

The preferred melastome hosts of *S. uberabensis* are all considered serious weeds in Hawaii (HDOA 1992, Jacobi and Warshauer 1992, Almasi 2000, Motooka et al. 2003). Of these plants, *T. longifolia* has the most limited distribution and appears least likely to have significant ecological interaction with the potential biocontrol agent. If *T. herbacea* and *M. septemnervium* can maintain substantial populations of *S. uberabensis*, these might help suppress *T. longifolia* and prevent it from spreading. The species *T. herbacea* and *M. septemnervium* overlap geographically across large areas, which could facilitate establishment and impacts of *S. uberabensis* generally. *M. sanguineum* is ecologically similar to *M. sanguineum* but less widely distributed. Impacts of biocontrol by *S. uberabensis* would likely be swifter and more severe on *T. herbacea* than *M. septemnervium* and *M. sanguineum*, which grow to large woody shrubs. Increased herbivory of *M. septemnervium*, which has been targeted but not adequately impacted by past introductions of other biocontrols (Conant et al. 2013), would have potential benefit to extensive forest watersheds in Hawaii (Jacobi and Warshauer 1992). The final host, *P. glomerata*, is a less prominent invader but broadly distributed in wet forests and pastures, including mountain areas on the island of Oahu where it has limited overlap with the other melastome hosts. Although *P. glomerata* appears to be equally suitable as a host for *S. uberabensis*, longer development times on this plant might delay the impacts of biocontrol (Souder 2008).

S. uberabensis is tolerant of cool and moderate temperatures and is not expected to be restricted in range by temperatures in Hawaii, except perhaps in exceptionally warm habitats (Souder 2008). However, the potential of *S. uberabensis* as a biological control could be limited by humidity at the microhabitat level. In Brazil, *Syphraea* is found with its melastome hosts in boggy soils, similar to the areas where *Tibouchina* and *Pterolepis* thrive in Hawaii, so these hosts should be highly susceptible. On the other hand, *Melastoma* in Hawaii can grow in

relatively drier areas – such as young lava flows. *S. uberabensis* could be less effective against *Melastoma* in drier habitats, because its eggs and larvae appear to be susceptible to drying when humidity is not high.

In conclusion, our testing indicates that *S. uberabensis* is narrowly host specific and will not feed or survive on any native or otherwise important plants in Hawaii. Given that Melastomataceae are entirely alien to Hawaii, and the host range of *S. uberabensis* includes only five weedy melastome species here, this flea beetle appears to hold great potential benefit and minimal environmental risk as a future biological control agent.

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IV. Environmental Assessment (EA):

Pursuant to a May 2008 Hawai'i Intermediate Court of Appeals decision ('Ohana Pale Ke Ao v. Board of Agriculture, 118 Haw. 247 (Haw. App. 2008), the Department of Agriculture's (Department's) import permit process is subject to the requirements of the Hawai'i Environmental Protection Act, chapter 343, Hawai'i Revised Statutes (HRS). Under this decision, the requirement for an EA as a condition of the import permit or related authorization applies in those circumstances where the underlying permit activity for the importation initiates a "program or project" and where the use of state or county funds or state or county lands is involved. When those circumstances are present, as they appear to be when a new organism is used in a new program or project located at a facility located at the University of Hawaii (state lands), an EA is required to determine whether the proposed project or program is likely to have a significant impact on the environment. However, certain activities may be eligible for "exemption" under provisions established through the Environmental Advisory Council, provided that the project or program is determined to have little or no impact on the environment.

Analysis of Application re EA: Under the above-cited court decision, the EA requirement is triggered under certain circumstances, including when an applicant proposes an action on state lands that requires agency approval and is not specifically exempted under Chapter 343, HRS. That is the case here. The applicant's request in this instance involves the field-release of *Syphraea uberabensis* for research and biocontrol of *Tibouchina herbacea* and related species in the family Melastomaceae in the environment. So, agency approval is required for the applicant's proposed action/activity on state lands or sensitive habitats. As PQB understands the court's analysis in the 'Ohana Pale decision, the activity proposed under this permit application would initiate a project that may use state lands and/or sensitive habitats, initially triggering the EA requirement.

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Dr. Johnson has submitted a Draft EA prepared by the Hawaii Department of Land and Natural Resources with an Anticipated Finding of No Significant Impact, published in the Office of Environmental Quality Control's Environmental Notice on January 23, 2020 (See Attachment 2).

V. Advisory Review

ADVISORY SUBCOMMITTEE REVIEW: This request was submitted to the Advisory Subcommittee on Entomology for its review and recommendation. Advisory Subcommittee recommendations and comments are as follows:

1. **I recommend Approval ___/___Disapproval of future placement of the unlisted beetle, *Syphraea uberabensis* (Coleoptera: Chrysomelidae) on the List of Restricted Animals (Part A) as a biocontrol agent for the noxious weed *Tibouchina herbacea* and related weed species in the family Melastomataceae.**

   Dr. Peter Follett: Recommends Approval

   Comments: This flea beetle is highly specific to several melastomes, all of which are invasive and weedy, and should pose no risk to the environment if released. The benefits of release may be significant if the target weed and relatives are controlled or suppressed. The risk of nontarget or negative environmental effects is negligible.

   Dr. Daniel Rubinoff: Recommends Approval

   Comments: This is a badly needed biocontrol agent that has been well researched and poses no threat to Hawaiian ecosystems and agriculture.

   Mr. Darcy Oishi: Recommends Approval

   Comments: For full disclosure, the *Syphraea* project is a partner project of the Plant Pest Control Branch (PPC) and the US Forest Service (FS) per the existing MOU between the two agencies. As such, comments to the subcommittee, advisory committee on plants and animals and the Board of Agriculture by myself or the entomologists of PPC should be viewed as full partners of the project.

   I recommend approval for future placement of *Syphraea uberabensis* on the List if Restricted Animals part A. Evaluations done in the native range and in containment by the USDA FS indicate placement on the restricted list is prudent and will be a welcome tool in the management of Melastomes in Hawaii.

   Dr. Mark Wright: Recommends Approval

Dr. Jesse Eiben: Recommends Approval

Comments: Listing the taxon for restricted importation is the appropriate action to ensure no subsequent possibly contaminated new individuals of this species are imported.

Dr. Francis Howarth: No Response

2. **I Agree___/___Disagree that the release of *Syphraea uberabensis* as a biocontrol agent of *Tibouchina herbacea* and related weed species in the family Melastomataceae by the USDA FS poses no significant negative impact on the environment.**

Dr. Peter Follett: Agrees

Comments: This flea beetle is highly specific to several melastomes, all of which are invasive and weedy, and should pose no risk to the environment if released. The benefits of release may be significant if the target weed and relatives are controlled or suppressed. Overall, this is a good piece of research.

Dr. Daniel Rubinoff: Agrees

Comments: This is a very good bet to at least help limit the spread of some serious weeds at very little risk.

Mr. Darcy Oishi: Agrees

Comments: After reviewing the documentation supplied by the applicant, there is no significant concerns with the statewide release of this organism.

Dr. Mark Wright: Agrees

Comments: Convincing data indicates extremely narrow host range is presented.

Dr. Jesse Eiben: Agrees

Commenrts: Host specificity tests were appropriate. Attack of Melastome plants is an ecological benefit.

Dr. Francis Howarth: No Response

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CIZ

3. **Provided *Syphraea uberabensis* is placed on the List of Restricted Animals (Part A), I recommend Approval___/___Disapproval to Allow the importation and release of *Syphraea uberabensis*, by permit, for biological control of *Tibouchina herbacea* and related weed species in the family Melastomataceae by USDA FS.**

Dr. Peter Follett: Recommends Approval

Comments: It should be made clear if new beetles will be imported or if the releases will be existing beetles used in host specificity testing. In the latter case, beetles will have passed through more than two genereations and should have no contaminants.

***PQB Note*:** Permit condition #5 addresses Dr. Follett's concern.

Dr. Daniel Rubinoff: Recommends Approval

Comments: As long as imported material is confirmed to be free of parasitoids and other organisms, the importation of additional *Syphraea uberabensis* material would be important to ensure adequate genetic diversity in the biocontrol agent.

Mr. Darcy Oishi: Recommends Approval

Comments: As a partner project, I recommend this species for importation and release. Dr. Johnson has a well-established track record for the prudent and careful evaluation of insects for release as a potential biocontrol agent. Exploration by PPC Staff identified this species as a viable agent for the control of *Tibouchina*. Non-target testing and evaluation is well thought out and considered and is comprehensive in nature. I similarly concur that it is unlikely that unanticipated non-target impacts will occur with this species. As such I recommend approval for importation and release.

Dr. Mark Wright: Recommends Approval

Comments: This insect appears to be a host-specific natural enemy of a significant invasive plant species.

Dr. Jesse Eiben: Recommends Approval

Dr. Francis Howarth: No Response

4. **Provided *Syphraea uberabensis* is placed on the List of Restricted Animals (Part A), I recommend Approval___/___Disapproval to establish permit conditions for the import and release of *Syphraea uberabensis* as a**

biocontrol agent of *Tibouchina herbacea* and related weed species by USDA FS.

Dr. Peter Follett: Recommends Approval

Comments: The researchers should be encouraged to write up the host range testing data and submit to a scientific journal for peer-review. Souder (2008) is a Master's thesis from UH but otherwise unpublished; likewise Raboin (2009) is unpublished. USFS (2013) is unpublished. Reviewers with weed biocontrol expertise may see ways to improve the methodology, host list, and discussion of the literature. For example, a reference should be provided for the Centrifugal Phylogenetic Method. Certain ecological aspects are not discussed such as the seed bank, e.g. how long are the target's seeds viable in the soil. This may directly impact the overall success of the biocontrol agent. Does the target weed exhibit compensatory growth after feeding by the flea beetle? Etc. These are the types of comments that might surface during peer-review.

> Dr. Johnson's Response: I intend to publish these *Syphraea* studies in the scientific literature. Regarding Dr. Follett's question about how long *T. herbacea* seeds are viable in soil, I don't know of any studies on seed longevity of *T. herbacea* or its close relatives. There has also not been any extended studies of impacts of *Syphraea uberabensis* herbivory on whole plants. Only a brief study with caged plants lasting a few weeks showing that severe defoliation is possible. Given that a mature plant typically dies back to near the ground in an annual cycle (especially in the native range), addressing compensatory growth experimentally is very challenging. Since the plant is adapted for annual regrowth, we can expect that long term suppression by biocontrol will depend on repeated severe defoliations year after year.

Dr. Daniel Rubinoff: Recommends Approval.

Comments: The research on this agent has been long and thorough and it's ready to be released.

Mr. Darcy Oishi: Recommends Approval

Comments: The permit conditions presented here are consistent with permit conditions for a restricted article that is being imported and shipped from a source outside of Hawaii not with how biological control agents for classical biological control exist within the quarantine framework of Hawaii. Per 150A-5.5(b), addresses what constitutes importation. The language states that importation of "articles quarantined in the biocontrol containment facilities of the department or of other government agencies engaged in joint projects... may be released upon issuance of a permit approved by the board." This statement

therefore states IMPORTATION occurs when articles are removed from the biocontrol containment facilities with a permit from the Board of Agriculture. As such, this creates a conflict with permit condition 5 which states screening will occur after importation. This means the insect will be outside of the bounds of the containment facility therefore negating the protection these facilities inherently offer to prevent unintentional impacts. This permit condition should be changed and reflect the need for screening prior to importation or release from the containment facility. Suggested language is "Upon entry into the state, the restricted article(s) shall be screened for other species, predators, parasites, parasitoids, or hyperparasitoids for a minimum of two generations in the USDA approved Insect Containment Facility, USDA FS, Hawaii Volcanoes National Park Quarantine Facility, Kilauea Research Station, Building 34, Volcano, HI 96718 prior to release from containment. A report shall be submitted to PQB detailing the discovery of any organisms found other than the restricted article(s)" Note: as written, this will only allow screening to occur at the Volcano facility and does not include the potential to use the King St. Facility for screening and ultimately release.

Similarly, permit condition 11 is fraught with issues. HRS §150A-5(10) refers to specific ports by which entry into the state can be made. From a regulatory standpoint, biological control agents are inspected by APHIS PPQ as the first port of entry in the United States. Material is inspected by USDA at a Plant Inspection Station under a permit. For Hawaii, this port of entry is at the Port of Honolulu. There can be exceptions if the first port of US entry is NOT Honolulu. However, permit condition 11 requires importation to be the port of Honolulu. Entrance into the state and importation are two separate issues. Importation of a biocontrol agent could be removal from an approved containment facility or importation of material from other sources under permit which would mean importation and entrance would be the same. Limiting importation to the port of Honolulu creates a situation that is impractical and does not reflect reality. Requiring all shipments to ENTER through the port of Honolulu is do-able. The permit condition should be reframed to state: "All parcels containing the restricted article(s) shall be subject to inspection by the PQB prior to entering the State. Entry should be through the port of Honolulu as designated by the Board. Entry into Hawaii through another port is prohibited". This permit condition should also be listed as permit condition 5 as entrance occurs prior to importation and release.

PQB NOTE: *PQB has consulted with legal counsel and it has been determined there is no requirement for Syphraea uberabensis to be transported back to Honolulu after the issuance of a permit if the Board so approves.*

Permit condition #11 has been amended to comply with Chap. 150A-5.5(b).

Dr. Mark Wright: Recommends Approval

Comments: As indicated above, I believe the petitioner has provided convincing evidence that *Syphraea uberabensis* does not pose threats to native Hawaiian plant species and shows promise a biological control agent of *T. herbacea*.

Dr. Jesse Eiben: Recommends Approval

Comments: As always, it is nice to see the specimens imported will be reared for potential parasitoids or other natural enemies before release from the lab colonies.

Dr. Francis Howarth: No Response

ADVISORY COMMITTEE REVIEW: This request was submitted to the Advisory Committee on Plants and Animals (Advisory Committee) at its meeting on May 20, 2022, held online via Zoom.

PQB Entomologist Christopher Kishimoto provided a synopsis of the request.

Chairperson Darcy Oishi said he would like to recuse himself from voting noting he was notified that he would be serving as the Chairperson of this meeting after he provided commentary as a member of the Advisory Subcommittee on Entomology. Chairperson Oishi noted the Plant Pest Control Branch (PPC) normally **does not** comment on biocontrol submittals from the U.S. Department of Agriculture Forest Service (USDA FS) as this proposal is deemed as a partner project as it relates to an existing MOU between the USDA FS and HDOA.

Committee member Ken Matsui expressed concern that he may have to recuse himself. He said he presented his situation to the ethics commission, and Keith Campbell said that he probably did not need to recuse himself. He said his basis for concern is that this beetle attacks the leaves of the Catappa plant (*Terminalia catappa*). He said the Catappa plant is found around Oahu especially Waimanalo, noting it surrounds the baseball field at the Waimanalo Park. He said it is in his friend's yard along the ocean in Waimanalo and this tree protects her yard from being consumed by the ocean. Mr. Matsui also said that he uses the Catappa leaves as a water conditioner for aquarium fish.

Chairperson Oishi asked for a motion.

Committee member Robert Hauff wanted to ask a couple of questions to PQB and the applicant. Mr. Hauff asked about the requirement in permit condition 5 to rear *Syphraea uberabensis* out two generations. He wanted to know if it is a standard

condition or is it being applied to this specific organism because of its biology. Mr. Kishimoto said it is a standard condition with biocontrol agents that go into quarantine, but it could also vary on the biology of the organism. He said close attention is paid to an organism's life cycle and PQB will try to alter that permit condition if required.

Committee member Hauff had a question about permit condition 1 with the intention that this project will eventually be implemented statewide. He didn't know how well this organism is going to disburse on its own and there will likely need additional efforts made to ensure it hits populations of all the target species. Mr. Hauff was concerned the way permit condition 1 was written, it sounded like the applicant would have to do all those releases and they would not be able to hand it off to partners. He said it's clear the initial releases will be conducted by Dr. Johnson, but as it becomes operational and could be used as a management tool, what does an agency like DLNR, or a partner, or non-partner that might want to help to control a target weed need to do to be able to acquire the agent and release it?

Mr. Kishimoto said for permit condition 1, because HDOA is a partner agency and also has a quarantine facility of their own in Honolulu, they can help with the release. Regarding transfers to other partners like DLNR or non-government agencies, we might have to ask our DAG for clarification as it wasn't requested in this permit request. He said maybe it is something that we can try and address before it gets to the Board. Mr. Hauff stated he was thinking about the long-term implementation – what will be done if this passes and with the board approval.

Mr. Kishimoto said in the future, once the existing population is at a level where it can be field collected, then partner agencies outside of USDA FS and HDOA wouldn't be prohibited from collecting and redistributing *Syphraea uberabensis* throughout the state. He said HDOA has facilities statewide on all the islands and initial releases can be done by HDOA staff.

Committee member Thomas Eisen said the condition says that the transfer can be approved by the Board, so the Board would need to allow for it either at a future point or maybe as part of the current review to factor in the transition to allow the transfer of *Syphraea uberabensis* to other entities for release. Chairperson Oishi said the permit application is for the actual release into the environment and those releases will be conducted both by the USDA FS and HDOA PPC. He said the intent of the application is for field release into the natural environment and the permit is only governing the activities up until establishment. He noted this is the traditional interpretation of this permit condition for our prior biocontrol releases.

Committee member Matsui asked, once it becomes established, would it be considered on the conditionally approved list? Mr. Kishimoto said it will always be on the restricted list. Chairperson Oishi said restricted list placement means any new attempts to import this species would trigger this review process and ensures that not anyone can import

this. Mr. Matsui asked if he picked a leaf that has a beetle on it could he not use that leaf or would he have to leave it as it is? Chairperson Oishi said once it is considered established, it can be moved around. PQB Inspection and Compliance Section Chief Mr. Jonathan Ho said Mr. Kishimoto is correct that placement of *Syphraea uberabensis* will be for the Restricted A list and the intent is for release and once established it is still on the Restricted A list. Placement on the restricted list prohibits individual possession, however PQB does not take action against possession of organisms that are established and widespread. He said the intent is to manage things that are controllable, with the focus on introduction and spread. This process is controlling how that it is being done. Committee member Hauff said that was helpful.

Mr. Ho noted that Mr. Matsui may have been disconnected. Mr. Ho commented that the high restriction level of *Syphraea uberabensis* is placed to manage risk and distribution to agencies whose mission is to do that field release work. He said once establishment occurs and the organism is widespread, then additional agencies are free to do what needs to be done.

Chairperson Oishi asked if the permit condition also relates to what Dr. Johnson can do with it prior to establishment, which is he cannot give it or transfer it or conduct any of these activities without express permission from the Board of Agriculture? Mr. Ho agreed.

Chairperson Oishi asked if there were any other questions?

Mr. Hauff indicated he wanted to ask questions of the applicant, Dr. Tracy Johnson. Dr. Johnson introduced himself to the Committee members.

Committee member Hauff asked Dr. Johnson if he felt the permit conditions were workable for implementing the project as they sounded like reasonable precautions to take but he wanted to make sure if this project is able to be implemented. Dr. Johnson noted that they were rearing a couple of generations and that there were corrections on whether it needed to go through Honolulu or not. He said he didn't think he had any problems with the permit conditions PQB had created.

Dr. Johnson commented on Mr. Matsui's comments about Catappa. He said Catappa is a common coastal tree and is not a viable host plant for *Syphraea uberabensis*. He said in the testing there was some really minor feeding and when he put the insects on the plants for an extended period without offering them anything else to eat, they can't survive.

Chairperson Oishi asked for additional questions from the committee for the applicant or PQB. He noted that Mr. Matsui returned and asked him if he had additional questions.

Committee member Matsui asked to what extent will the leaves of the Catappa tree plant be removed by the beetle. Dr. Johnson replied his expectation is zero removal of any leaf material by this insect on Catappa. Mr. Matsui asked why the report showed

that the beetle damaged leaves. Dr. Johnson said the report shows when you put insects in a small cage or container with a variety of different plants, sometimes the insects will take a bite to figure out if they can eat it, so you get a little leaf damage but that's common in this kind of testing. Dr. Johnson said the damage isn't sustained because the insects don't recognize the plant as one they can eat and they don't survive on it to be able to live a long time and reproduce.

Committee member Matsui asked to what extent will the Catappa plant be decimated by their absence of leaves because the beetles are eating them. Dr. Johnson responded that basically the beetles are not going to eat the Catappa at all. Mr. Matsui said that is not what the research showed.

Chairperson Oishi explained that this type of testing is called a no choice test which basically determines will an insect feed and survive on a particular plant, or will it die. He said in some of these tests, sometimes the organisms will taste the leaves, that counts as feeding damage, but it's not impacting the plant and the insect will not actually carry out its life cycle. He noted in the natural environment, *Syphraea uberabensis* would be laying its eggs on proper hosts, not necessarily Catappa Mr. Hauff noted the range of Catappa and the target host plants do not overlap so you are not going to find these plants together, anyway.

Chairperson Oishi asked if there are any other question and concerns. Hearing none, asked if anyone would make a motion. Committee member Hauff made a motion to recommend that the Board approval of all parts of USDA FS's request. Committee member Haws seconded the motion.

Chairperson Oishi asked for any comments or questions from the public. Ms. Christy Martin, representing the Coordinating Group on Alien Pest Species, said she is in strong support of this proposal. She said Dr. Johnson's work has been stellar and his work and tests over the past several years show no non-target impacts from the release of *Syphraea uberabensis*. She said that *Tibouchina* and other host species will continue to exist in the forest but at a lower and less vigorous level. This would give our native species some chance to compete. She urged the Committee to approve this request.

Chairperson Oishi asked for any other public testimony or further discussion by the Committee. Hearing none, he called for a vote. He reiterated that he would be abstaining from the vote.

Vote: 5 approvals, with 1 abstention (Oishi)

Motion Carries.

VI. Proposed Permit Conditions

1. The restricted article(s), *Syphraea uberabensis*, which includes progeny, shall be used for field release and research, a purpose approved by the Board of Agriculture (Board), and shall not be sold, given away, or transferred in Hawaii, except as approved by the Board.

2. The permittee, Dr. Matthew Tracy Johnson, U.S. Department of Agriculture (USDA) Forest Service (FS), Hawaii Volcanoes National Park Quarantine Facility, Kilauea Research Station, Building 34, Volcano, HI 96718, shall be responsible and accountable for all restricted article(s) imported, from the time of their arrival until their final disposition.

3. The restricted article(s) shall be safeguarded and maintained at the USDA approved Insect Containment Facility, USDA FS, Hawaii Volcanoes National Park Quarantine Facility, Kilauea Research Station, Building 34, Volcano, HI 96718 or the Hawaii Department of Agriculture Plant Pest Control Branch Containment Facility, 1428 South King Street, Honolulu, Hawaii 96814, sites approved by the Plant Quarantine Branch (PQB), by trained or certified personnel designated by the permittee.

4. Upon request by the PQB, the permittee shall submit samples of the restricted article(s) prior to importation to the PQB.

5. Upon entry into a PQB approved containment facility, the restricted article(s) shall be screened for other species, predators, parasites, parasitoids or hyperparasitoids for a minimum of two generations in the USDA approved Insect Containment Facility, USDA FS, Hawaii Volcanoes National Park Quarantine Facility, Kilauea Research Station, Building 34, Volcano, HI 96718 or the Hawaii Department of Agriculture Plant Pest Control Branch Containment Facility, 1428 South King Street, Honolulu, Hawaii 96814. A report shall be submitted to PQB detailing the discovery of any organisms found other than the restricted article(s).

6. In the event the restricted article(s) become parasitized or infected by disease, the permittee shall:

   a. Destroy the entire lot of the restricted article(s) by freezing;

   b. Autoclave all insects, dietary and oviposition media; and

   c. Subject all used cages and equipment to autoclaving or treatment with a bleach solution containing at least 0.5% sodium hypochlorite concentration.

7. At least 48 hours prior to shipping any parcel containing the restricted article(s), the permittee shall notify the PQB Chief in writing and provide the following information:

a. Expected arrival date;

b. Waybill, bill of lading, and/or tracking number;

c. Name and address of the shipper;

d. Name and address of the importer or importer's agent in the State of Hawaii;

e. Number of packages;

f. Description of contents of each package (including scientific name); and

g. Port of entry into the State.

8. At least four sides of all parcels containing the restricted article(s) imported into the State shall be clearly and legibly marked: "**This parcel may be opened and delayed for agricultural inspection in Hawaii**" in 2-inch minimum sized font.

9. The restricted article(s) shall be shipped in sturdy PQB-approved containers designed to be escape-proof and leak-proof.

10. Each shipment of the restricted article(s) shall be accompanied by a complete copy of the PQB permit for the restricted article(s) and an invoice, packing list or other similar PQB-approved document listing the scientific and common names of the restricted article(s), the quantity of the restricted article(s), the shipper, and the permittee(s) for the restricted article(s).

11. All parcels containing the restricted article(s) shall be subject to inspection by the PQB prior to entering the State and shall be imported through the port of Honolulu except as designated by the Board. Entry into Hawaii through another port is prohibited unless designated by the Board.

12. The approved site, restricted article(s), progeny, records, and any other document pertaining to the restricted article(s) and progeny under this permit, may be subject to post-entry inspections by the HDOA, PQB. The permittee shall make the site, restricted article(s), progeny, and records pertaining to the restricted article(s) available for inspection upon request by a PQB inspector.

13. It is the responsibility of the permittee to comply with any applicable requirements of municipal, state, or federal law pertaining to the restricted article(s).

14. The permittee shall submit to the PQB Chief a copy of all valid licenses, permits, certificates or their equivalent required for the restricted article(s) or for their import, possession, movement, or transfer. The permit issued by the PQB Chief

may be cancelled upon revocation, suspension, or termination of any of the aforementioned documents.

15. The permittee shall submit an annual report to the PQB no later than January 31st of the following year, of the results of post release monitoring programs, and shall include the following:

    a. Amount of the restricted article(s) released and number of releases for the year;

    b. Establishment and current field populations of the restricted article(s);

    c. Effect of the restricted article(s)on *Tibouchina herbacea* and other species in the family Melastomataceae; and

    d. Effect of the restricted article(s) on native plant and animal species.

16. The permittee shall adhere to the use, facility, equipment, procedures, and safeguards described in the permit application, and as approved by the Board and the PQB Chief.

17. The permittee shall have a biosecurity manual available for review and approval by the PQB, at the time of the initial site inspection and any subsequent post-entry inspection(s), which identifies the practices and procedures to be adhered to by the permittee to minimize or eliminate the risk of theft, escape, or accidental release of the restricted article(s), including the risk of introduction and spread of diseases and pests associated with the restricted article(s) to the environment. The permittee shall adhere to all practices and procedures as stated in this biosecurity manual.

18. The permittee shall immediately notify the PQB Chief verbally and in writing under the following circumstances:

    a. If any escape, theft, accidental release, parasitoid, hyperparasitoid, or other pest or disease outbreaks involving the restricted article(s) under this permit occurs.

    b. Prior to any changes to the approved site, facility and/or procedures regarding the restricted article(s) being made, the permittee shall also submit a written report documenting the specific changes to the PQB Chief for approval.

    c. If a shipment of the restricted article(s) is delivered to the permittee without a PQB "Passed" stamp, tag or label affixed to the article, container, or delivery order that indicates that the shipment has passed inspection and is allowed entry into the State, then the permittee shall not open or tamper with the

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shipment and shall secure, as evidence, all restricted article(s), shipping container(s), shipping document(s) and packing material(s) for PQB inspection.

d. If the permittee will no longer import or possess the restricted article(s) authorized under this permit.

19. The permittee shall be responsible for all costs, charges, or expenses incident to the inspection, treatment, or destruction of the restricted article(s) under this permit, as provided in Act 173, Session Laws of Hawaii 2010, section 13, including, if applicable, charges for overtime wages, fixed charges for personnel services, and meals.

20. Any violation of the permit conditions may result in citation, permit cancelation, and enforcement of any or all of the penalties set forth in HRS §150A-14.

21. A cancelled permit is invalid and upon written notification from the PQB Chief, all restricted article(s) listed on the permit shall not be imported. In the event of permit cancelation, any restricted article(s) imported under permit may be moved, seized, treated, quarantined, destroyed, or sent out of State at the discretion of the PQB Chief. Any expense or loss in connection therewith shall be borne by the permittee.
22. This permit or conditions of this permit are subject to cancellation or amendment at any time due to changes in administrative rules restricting or disallowing import of the restricted article(s) or due to Board action disallowing a previously permitted use of the restricted article(s).

23. These permit conditions are subject to amendment by the PQB Chief in the following circumstances:

    a. To require disease screening, quarantine measures, and/or to place restrictions on the intrastate movement of the restricted article(s), as appropriate, based on scientifically validated risks associated with the restricted article(s), as determined by the PQB Chief, to prevent the introduction or spread of disease(s) and/or pests associated with the restricted article(s); or

    b. To conform to more recent Board approved permit conditions for the restricted article(s), as necessary to address scientifically validated risks associated with the restricted article(s).

24. The permittee(s) shall agree in advance to defend and indemnify the State of Hawaii, its officers, agents and employees for any and all claims against the State of Hawaii, its officers, agents, employees, or Board of Agriculture members that may arise from or be attributable to any of the restricted article(s) that are

introduced under this permit. This permit condition shall not apply to a permittee that is a federal or State of Hawaii entity or employee, provided that the State or federal employee is a permittee in the employee's official capacity.

STAFF RECOMMENDATION: Based on the recommendations and comments of the Advisory Subcommittee on Entomology and the Advisory Committee's (5-0 with 1 abstention) recommendation to approve this request, the Plant Quarantine Branch recommends approval with the proposed permit conditions.

Respectfully Submitted,

BECKY L. AZAMA
Acting Manager, Plant Quarantine Branch

CONCURRED:

HELMUTH W. ROGG
Administrator, Plant Industry Division

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

C23

ATTACHMENT 1

PQ-7 (01/04)

State of Hawaii
Department of Agriculture
PLANT QUARANTINE BRANCH
1849 Auiki Street, Honolulu, HI 96819-3100
Phone: (808) 832-0566, FAX: (808) 832-0584

For Office Use Only

Fee: $_____ Receipt No. _____

☐ Approve Permit No._____ Date: _____

☐ Disapprove ☐Other _____

Processed by: _____ Date: _____

PERMIT APPLICATION FOR RESTRICTED COMMODITIES INTO HAWAII

Date: *April 30, 2021*

In accordance with the provision of Chapter 342 , Hawaii Administrative Rules of the Division of Plant Industry, Department of Agriculture, a permit is requested for the following commodities:

Please type or print clearly.

Quantity	Commodity	Scientific Name
300	flea beetle for biological control of melastome weeds	Syphraea uberabensis (Coleoptera; Chrysomelidae)

Name and address of shipper: USDA Forest Service, Hawaii Volcanoes National Park Quarantine Facility

Original source: M. Vitorino, Universidade Regional de Blumenau, Santa Catarina, Brazil

(Mainland or Foreign address)

Approximate date of arrival: _____

Mode of Shipment: ☐ Mail ☐ Air Freight ☐ Boat

Type of Permit:

--- Import
☐ one time only ☑ multi-shipments

--- Intrastate shipment
☐ one time only ☑ multi-shipments

☐ Possession

Object of importation:

☐ Kept caged at all time
☑ Used for propagation
☐ Imported for exhibition
☑ Imported for liberation
☐ Other purposes - specify field release of biocontrol agent from quarantine facility

Please type or print clearly.

Applicant's Name M. Tracy Johnson

Company Name USDA Forest Service (if applicable)

Hawaii Mailing Address PO Box 236
Volcano HI 96785

Telephone number 808-967-7122

Facsimile number 808-967-7158

Fee Amount Enclosed (cash, check or mail order) $_____

(complete reverse side)

PLEASE COMPLETE THE FOLLOWING INFORMATION (attach extra sheet if necessary)

1. State in detail the reasons for introduction (include use or purpose).

Syphraea uberabensis from Brazil has been selected and evaluated as a new biological control for managing invasive melastome weeds in Hawaii. It is a narrowly host-specific leaf-feeding beetle intended for statewide field release to cause suppression of alien Tibouchina, Melastoma (designated as noxious weeds) and Pterolepis species. The beetle is expected to cause severe defoliation of targeted weeds, without affecting any native or otherwise valued plants. Suppression of these weeds will benefit forest watersheds statewide. See attached results.

2. Person responsible for the organism (include name, address and phone number).

Dr. M. Tracy Johnson
Institute of Pacific Islands Forestry
USDA Forest Service, Pacific Southwest Research Station
P.O. Box 236
Volcano, HI 96785
tel: 808-967-7122

3. Location(s) where the organism will be kept and used (include address, contact and phone number).

USDA Forest Service, Hawaii Volcanoes National Park, Magma House, Bldg 34
M. Tracy Johnson 808-967-7122

Hawaii Dept of Agriculture, Plant Pest Control Branch, Biocontrol Section
16 E. Lanikaula Street, Hilo; 1428 S. King Street, Honolulu
Stacey Chun 808-974-4140; Darcy Oishi 808-973-9524

4. Method of disposition.

Syphraea uberabensis will be removed from a source colony maintained at the Hawaii Volcanoes National Park Quarantine Facility. This colony will originate from insects collected from southern Brazil and screened in quarantine to eliminate associated natural enemies. Roughly 30 insects at a time will be removed from quarantine as newly pupated adult beetles, independent of host plant material and other potential contaminants. Adults will be used to establish colonies reared in petri dishes at USDA and HDOA insectaries in Volcano, Hilo and Honolulu. Offspring from rearing colonies will be used for environmental releases at selected locations statewide.

5. Give an abstract of the organism with particular reference to potential impact on the environment of Hawaii (include impact to plants, animals and humans).

Syphraea uberabensis (Coleoptera: Chrysomelidae), a flea beetle native to Brazil, is proposed for biological control of invasive weeds in the family Melastomataceae. Adult beetles, 3-4 mm long, feed and lay eggs on leaves and soft stems of host plants. Larvae also feed on leaves. The host range of S. uberabensis is restricted to species in the tribe Melastomeae, all non-native and invasive in Hawaii. Testing consistently identified the potential Hawaiian hosts as: Tibouchina herbacea, Tibouchina longifolia, Pterolepis glomerata, Melastoma septemnervium and Melastoma sanguineum. This beetle thrives in moist habitats, similar to the areas where Tibouchina and Pterolepis occur in Hawaii, which should allow for maximal impact, including severe defoliation. Impacts may be lower on Melastoma, which can occur in drier habitats, where S. uberabensis eggs and larvae may be susceptible to drying.

****

I request permission to import the articles as listed on the permit application and further, request that the articles be examined by an authorized agent of the Department of Agriculture upon arrival in Hawaii.

I agree that I, as the importer, will be responsible for all costs, charges or expenses incident to the inspection or treatment of the imported articles.

I further agree that damages or losses incident to the inspection or the fumigation, disinfection, quarantine, or destruction of the articles, by an authorized agent of the Department of Agriculture, shall not be the basis of a claim against the department or the inspectors for the damage or loss incurred.

Signature _____ **Date** May 4 2021
(Applicant)

ATTACHMENT 2

FILE COPY

JAN 23 2020

DAVID Y. IGE
Governor

JOSH GREEN
Lt. Governor

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

MORRIS M. ATTA
Deputy to the Chairperson

State of Hawaii
DEPARTMENT OF AGRICULTURE
1428 South King Street
Honolulu, Hawaii 96814-2512
Phone: (808) 973-9600 FAX: (808) 973-9613

January 13, 2020

Director
Office of Environmental Quality Control
Department of Health, State of Hawaii
235 S. Beretania Street, Room 702
Honolulu, Hawaii 96813

Dear Director:

With this letter, the Hawaii Department Agriculture hereby transmits the Draft Environmental Assessment and Anticipated Finding of No Significant Impact (DEA-AFNSI) for the Proposed Statewide Field Release of the Brazilian Beetle *Syphraea uberabensis* for Biological Control of the Noxious Weed Cane Tibouchina *Tibouchina herbacea* and Related Weeds for publication in the next available edition of The Environmental Notice.

Enclosed is a completed OEQC Publication Form, two copies of the DEA-AFNSI, an Adobe Acrobat PDF file of the same, and an electronic copy of the publication form in MS Word. Simultaneous with this letter, we have submitted the summary of the action in a text file by electronic mail to your office.

If there are any questions, please contact Christopher Kishimoto, Plant Quarantine Branch Entomologist at: (808) 832-0581 or Christopher.M.Kishimoto@hawaii.gov.

Sincerely,

Jonathan Ho
Acting Manager
Plant Quarantine Branch

Enclosures:

1. OEQC Publication Form (Agency)
2. Draft Environmental Assessment for the Proposed Statewide Field Release of the Brazilian Beetle *Syphraea uberabensis* for Biological Control of the Noxious Weed Cane Tibouchina *Tibouchina herbacea* and Related Weeds

Island Fresh

20-184

AGENCY
PUBLICATION FORM

Project Name:	Proposed Statewide Field Release of the Brazilian Beetle *Syphraea uberabensis* for Biological Control of the Noxious Weed Cane Tibouchina *Tibouchina herbacea* and Related Weeds
Project Short Name:	Tibouchina Biological Control DEA
HRS §343-5 Trigger(s):	(1) Propose the use of state or county lands or the use of state or county funds
Island(s):	Statewide
Judicial District(s):	Statewide
TMK(s):	N/A
Permit(s)/Approval(s):	USDA-APHIS-PPQ and Board of Agriculture (HDOA Plant Quarantine Branch)
Proposing/Determining Agency:	Department of Agriculture (DOA), State of Hawai'i
Contact Name, Email, Telephone, Address	Christopher Kishimoto; christopher.m.kishimoto@hawaii.gov; (808) 832-0581; 1849 Auiki Street, Honolulu, Hawai'i 96819
Accepting Authority:	(for EIS submittals only)
Contact Name, Email, Telephone, Address	
Consultant:	Garcia and Associates; please use Proposing Agency contact for any questions
Contact Name, Email, Telephone, Address	Huang-Chi Kuo; kuo@garciaandassociates.com; 146 Hekili St., Suite 101, Kailua, Hawai'i 96734

Status (select one)	**Submittal Requirements**
__X__ DEA-AFNSI	Submit 1) the proposing agency notice of determination/transmittal letter on agency letterhead, 2) this completed OEQC publication form as a Word file, 3) a hard copy of the DEA, and 4) a searchable PDF of the DEA; a 30-day comment period follows from the date of publication in the Notice.
____ FEA-FONSI	Submit 1) the proposing agency notice of determination/transmittal letter on agency letterhead, 2) this completed OEQC publication form as a Word file, 3) a hard copy of the FEA, and 4) a searchable PDF of the FEA; no comment period follows from publication in the Notice.
____ FEA-EISPN	Submit 1) the proposing agency notice of determination/transmittal letter on agency letterhead, 2) this completed OEQC publication form as a Word file, 3) a hard copy of the FEA, and 4) a searchable PDF of the FEA; a 30-day comment period follows from the date of publication in the Notice.
____ Act 172-12 EISPN ("Direct to EIS")	Submit 1) the proposing agency notice of determination letter on agency letterhead and 2) this completed OEQC publication form as a Word file; no EA is required and a 30-day comment period follows from the date of publication in the Notice.
____ DEIS	Submit 1) a transmittal letter to the OEQC and to the accepting authority, 2) this completed OEQC publication form as a Word file, 3) a hard copy of the DEIS, 4) a searchable PDF of the DEIS, and 5) a searchable PDF of the distribution list; a 45-day comment period follows from the date of publication in the Notice.
____ FEIS	Submit 1) a transmittal letter to the OEQC and to the accepting authority, 2) this completed OEQC publication form as a Word file, 3) a hard copy of the FEIS, 4) a searchable PDF of the FEIS, and 5) a searchable PDF of the distribution list; no comment period follows from publication in the Notice.
____ FEIS Acceptance Determination	The accepting authority simultaneously transmits to both the OEQC and the proposing agency a letter of its determination of acceptance or nonacceptance (pursuant to Section 11-200-23, HAR) of the FEIS; no comment period ensues upon publication in the Notice.
FEIS Statutory Acceptance	Timely statutory acceptance of the FEIS under Section 343-5(c), HRS, is not applicable to agency actions.
____ Supplemental EIS Determination	The accepting authority simultaneously transmits its notice to both the proposing agency and the OEQC that it has reviewed (pursuant to Section 11-200-27, HAR) the previously accepted FEIS and determines that a supplemental EIS is or is not required; no EA is required and no comment period ensues upon publication in the Notice.

____ Withdrawal — Identify the specific document(s) to withdraw and explain in the project summary section.

____ Other — Contact the OEQC if your action is not one of the above items.

Project Summary
Provide a description of the proposed action and purpose and need in 200 words or less.

The HDOA, in collaboration with the Hawai'i Department of Land and Natural Resources, proposes the release of a beetle from Brazil, *Syphraea uberabensis*, for biocontrol of invasive cane tibouchina, *Tibouchina herbacea*, and related weeds. Tibouchina and its relatives are noxious weeds in Hawai'i, where they form dense stands in pastures and forests, outcompeting native species.

Syphraea uberabensis is a small beetle whose adults and larvae feed on cane tibouchina in its native region of Brazil, causing extensive damage to the leaves as well as the soft exterior of young stems. Heavy feeding is expected to reduce plant density and prevent reproduction and spread to new areas, benefiting native ecosystems in Hawai'i.

This Draft Environmental Assessment supports the release of the biocontrol agent, *Syphraea uberabensis*, to control cane tibouchina and related weeds. Observations in Brazil and extensive testing in Brazil and Hawai'i have shown that *S. uberabensis* is narrowly host-specific to cane tibouchina and a few closely related plants that are also weeds in Hawai'i.

Draft Environmental Assessment

Statewide Field Release of the Brazilian Beetle *Syphraea uberabensis* for Biological Control of the Noxious Weed Cane Tibouchina *Tibouchina herbacea* and Related Weeds

Prepared For:

Department of Land and Natural Resources
Division of Forestry and Wildlife
1151 Punchbowl St., Room 325
Honolulu, Hawai'i 96813

Prepared By:

Garcia and Associates
146 Hekili St., Suite 101
Kailua, Hawai'i 96734

GANDA Report No. 2327-1

GANDA

January 2020

PROJECT SUMMARY

Project Name: Statewide Field Release of the Brazilian Beetle *Syphraea uberabensis* for Biological Control of the Noxious Weed Cane Tibouchina *Tibouchina herbacea* and Related Weeds

Proposing Agency: Department of Agriculture
State of Hawai'i

Project Location: Statewide

Property Owner: State of Hawai'i

State Land Use Classification: Not Applicable

Agency Determination: Anticipated Finding of No Significant Impact (AFNSI)

Agencies, Organizations, and Other Stakeholders Consulted:

FEDERAL AGENCIES

- US House of Representatives, Representative Tulsi Gabbard
- US House of Representatives, Representative Colleen Hanabusa
- US Senate, Senator Mazie Hirono
- US Senate, Senator Brian Schatz
- National Park Service, Hawai'i Volcanoes National Park
- National Park Service, Haleakalā National Park
- Natural Resources Conservation Service, Pacific Islands Area
- US Army Garrison, Commander Col. Stephen E. Dawson
- US Army Garrison, Environmental Division
- US Army Garrison, Natural Resource Section
- US Fish & Wildlife Service
- US Fish & Wildlife Service, O'ahu National Wildlife Refuge Complex
- US Geological Survey, Pacific Island Ecosystems Research Center

STATE AGENCIES

- Aha Moku Councils
- BLNR O'ahu Member
- Department of Business, Economic Development & Tourism
- Department of Hawaiian Homelands
- Department of Health
- Department of Health, Office of Environmental Quality Control

- DLNR Division of Forestry & Wildlife
- DLNR Division of State Parks
- DLNR Land Division
- DLNR Office of Conservation & Coastal Lands
- DLNR State Historic Preservation Administration
- DLNR Watershed Partnership Program
- Land Use Commission
- Natural Area Reserves System Commission
- Office of the Governor
- Office of Hawaiian Affairs
- University of Hawai'i, College of Tropical Agriculture and Human Resources
- University of Hawai'i, Environmental Center
- University of Hawai'i, Pacific Cooperative Studies Unit

CITY AND COUNTY AGENCIES

- Honolulu City Council
- City & County of Honolulu, Office of the Mayor
- City & County of Honolulu, Board of Water Supply
- City & County of Honolulu, Planning Department
- Hawai'i County Council
- Hawai'i County, Office of the Mayor
- Hawai'i County, Department of Water Supply
- Hawai'i County, Department of Planning
- Kaua'i County Council
- Kaua'i County, Office of the Mayor
- Kaua'i County, Department of Planning
- Kaua'i County, Department of Water Supply
- Maui County Council
- Maui County Office of the Mayor
- Maui County, Department of Planning
- Maui County, Department of Water Supply

ORGANIZATIONS

- Big Island Invasive Species Committee
- Bishop Museum
- Conservation Council of Hawai'i

- Environment Hawai'i Inc.
- Hawai'i Audubon Society
- Hawai'i Cattlemen's Council
- Hawai'i Conservation Alliance
- Hawai'i Forest and Trail
- Hawai'i Forest Industry Association
- Hawaiian Botanical Society
- Hawaiian Trail and Mountain Club
- KAHEA
- Kamehameha Schools
- Kaua'i Invasive Species Committee
- Ko'olau Mountains Watershed Partnership
- Maui Invasive Species Committee
- Moloka'i Invasive Species Committee
- Native Hawaiian Advisory Council
- Native Hawaiian Legal Corporation
- O'ahu Invasive Species Committee
- Pig Hunters Association of O'ahu
- Plant Extinction Prevention Program
- Sierra Club, O'ahu Chapter
- The Nature Conservancy of Hawai'i

CONTENTS

FIGURES

TABLES

PROJECT SUMMARY DESCRIPTION

The Hawai'i Department of Agriculture and the Hawai'i Department of Land and Natural Resources propose the field release on State lands in Hawai'i of a beetle from Brazil, *Syphraea uberabensis* (Coleoptera, Chrysomelidae, Galerucinae, Alticini), for biological control of cane tibouchina, *Tibouchina herbacea* (Melastomataceae).

Tibouchina herbacea is a noxious weed native to Southern Brazil, Uruguay, and Paraguay. In Hawai'i, it naturalized and is locally abundant in disturbed mesic to wet forest on the islands of Hawai'i, Lāna'i, Maui, Moloka'i, and O'ahu. It is able to invade native forest through abundant production of tiny, easily dispersed seeds. Once established it forms dense stands and displaces native vegetation.

Syphraea uberabensis is a natural herbivore of *T. herbacea* in the plant's native range in Brazil. Of the potential natural control agents evaluated in Brazil, *S. uberabensis* demonstrated the most potential for successful control of cane tibouchina. Further testing has shown that *S. uberabensis* is narrowly host-specific to *T. herbacea* and a few closely related plants that are also weeds in Hawai'i.

Release of the biocontrol agent is currently proposed on State lands on all islands where *T. herbacea* has naturalized. Populations of *S. uberabensis* are expected to increase to effective levels on the target plant within a few years at release sites. Spread of the insect from the initial release sites will occur naturally and artificially via redistribution efforts by state and federal agencies involved in management of cane tibouchina and related weeds. Within several years of initial release, *S. uberabensis* is expected to range statewide in all areas infested by cane tibouchina and four related weed species. The state and federal agencies responsible for biocontrol introductions and weed management will closely monitor the establishment of the beetle and its effectiveness for long term weed control.

The proposed action requires Plant Protection and Quarantine permits from the US Department of Agriculture, Animal and Plant Health Inspection Service; a permit for import and liberation of restricted organisms from the Hawai'i Department of Agriculture, Plant Quarantine Branch; and a permit for release and monitoring of the insect on State forest land from the Hawai'i Department of Land and Natural Resources, Division of Forestry and Wildlife.

An alternative to the proposed action considered in this assessment is no action. Under this alternative *S. uberabensis* would not be released on State forest land, and management of cane tibouchina would be limited to mechanical and chemical controls, solutions which are applicable only to relatively small areas.

Because *S. uberabensis* is specialized on a few species of melastomes, all of which are invasive, the environmental consequences of its release are expected to be beneficial to the native forests and agricultural economy of Hawai'i, and adverse effects are expected to be negligible. Therefore, the anticipated determination from this Draft Environmental Assessment is an Anticipated Finding of No Significant Impact (AFNSI).

1.0 INTRODUCTION

This Draft Environmental Assessment (DEA) supports a proposed field release of a small beetle, *Syphraea uberabensis*, in the State of Hawai'i for biocontrol of *Tibouchina herbacea* and related weeds in the melastome family. The proposing agency for this program is the State of Hawai'i Department of Agriculture (HDOA).

The proposed action of releasing the biological control agent has the potential to impact the local environment and involves the use of state and federal funds and approval of permits. Therefore, in accordance with the Hawai'i Revised Statutes (HRS) Chapter 343, Hawai'i Environmental Policy Act, and the National

Environmental Policy Act, the proposing agencies are conducting an Environmental Assessment (EA) of the proposed project.

This Environmental Assessment identifies proposed and alternative actions of the project; describes the affected physical, biological, cultural, and socioeconomic environments; and analyzes potential environmental impacts to the existing environment resulting from the proposed action.

1.1 Purpose and Need

The Hawai'i Department of Agriculture defines "noxious weeds" in HRS Chapter 152 as "any plant species which is, or which may be likely to become, injurious, harmful, or deleterious to the agricultural, horticultural, aquacultural, or livestock industry of the State and to forest and recreational areas and conservation districts of the State, as determined and designated by the department from time to time." The criteria for designating noxious weeds, and the list of species currently designated as such, are available in Hawai'i Administrative Rules (HAR) Chapter 68.

The Hawai'i Department of Agriculture's Plant Pest Control Branch is responsible for limiting plant pest populations that have the potential to cause significant economic damage in the state. This is achieved through statewide programs using chemical, mechanical, biological, and integrated control measures to eradicate or control plant pests, including insects and mites, molluscs, weeds, and plant pathogens.

1.1.1 Biocontrol

Biological control, or biocontrol, has a long history of managing pests. Classical biocontrol involves the use of natural enemies that act as herbivore, predator, pathogen, or parasite of pests in order contain, reduce, or otherwise suppress the pests' populations and their negative impacts. There are three basic types of biological pest control strategies: conservation, augmentation, and importation. Conservation involves taking measures, such as providing food or improving habitats, to increase naturally occurring natural enemies. Augmentation involves breeding and releasing locally available natural enemies to improve control. Importation (also known as classical biological control) involves the importation and release of an organism outside its natural range for controlling a pest species. The current proposed biocontrol is through importing a natural enemy from the invasive weed's native range.

When introduced to a new location, a species often arrives without the natural enemies that controlled it in its native range. Lack of top-down control from the natural enemies can contribute to the successful colonization and unusually high population size of invasive species. The Enemy Release Hypothesis has been used to explain the success of invasive plants (Keane and Crawley 2002). Because natural enemies evolved with the pests in their native range, they can be among the most specific and effective ways of controlling the pests.

The use of biocontrol agents for invasive weeds in natural areas has important advantages over mechanical or chemical control. Mechanical and chemical controls are often less selective and tend to cause unintended impact to the environment. In contrast, biocontrol agents can be selected to target a very specific set of pests. While mechanical and chemical control methods may be cost prohibitive for remote or large areas, biocontrol can provide a long-term, cost-effective, and environmentally-friendly solution (Howarth 1991; Mack et al. 2000).

The major concern for biological control is the potential adverse effects on non-target species. If care is not taken, it can have significant and irreversible adverse effects, perhaps even leading to biological extirpation (Howarth 1991; Simberloff and Stiling 1996). The risks of non-target effects from biocontrol can be minimized by extensive testing of host specificity and selecting agents and targets that have the least environmental risk and the most predicted effectiveness (Markin et al. 1992; Louda et al. 2003).

1.2 Primary Target Species: *Tibouchina herbacea* - Cane Tibouchina

Figure 1. Cane tibouchina (*Tibouchina herbacea*); Photo by Forest & Kim Starr

Taxonomy: *Tibouchina herbacea* (DC.) Cogn. (Synonyms: *Arthrostemma herbacea* DC.; *Arthrostemma hirsutissimum* DC.; *Pterolepis herbacea* (DC.) Triana) belongs to the pantropical melastome family (Melastomataceae). *Tibouchina* Aubl. is a genus containing about 350 species ranging from Mexico, West Indies, to northern Argentina. The center of diversity is in southeastern Brazil. *Tibouchina* is classified in the tribe Melastomeae, which contains several related genera (e.g. *Arthrostemma*, *Dissotis*, *Melastoma*, and *Pterolepis*) that also have naturalized in Hawai'i (Wagner et al. 1999). A phylogenetic study indicates that *Tibouchina* is a well-supported phylogenetic group (clade), although several derived genera nest within the clade (Michelangeli et al. 2012).

Description: *Tibouchina herbacea* is a semi-woody upright shrub (Figure 1 and Figure 2). Young stems angled, hairy. Leaves opposite, 3 inches long by 1.4 inches wide, hairy, with 5–7 prominent veins. Flowers pink, 4 petals, bright yellow anthers. Fruit cuplike, small, 0.2 inches long by 0.2 inches wide. Seeds very small, numerous (Motooka et al. 2003). Many of the hairs covering leaves, stems and fruits are gland-tipped, so that plants leave an oily, scented residue when touched. The growth form is notably different between the populations in Brazil and Hawai'i. In Brazil, it rarely grows above l m in height and dies back each year. In Hawai'i, it can grow up to 3–4 m and the previous year's stems can survive the dormant period forming rank sprawling stems from which new shoots arise the following year. It forms dense thickets that are difficult to traverse and smother adjacent vegetation, gradually increasing the size of the infestation (Almasi 2000; Smith 2002).

Distribution: *Tibouchina herbacea* is native to South America, including Brazil, Argentina, Paraguay, and Uruguay. *Tibouchina herbacea* was introduced to Hawai'i as an ornamental (Motooka et al. 2003) and was first collected in Hawai'i Island in 1977. It subsequently colonized Maui by 1982. It is widely established on Hawai'i and Maui and has been found on Lāna'i, Moloka'i, and O'ahu (Wagner et al. 1999; Wysong et al. 2007; Imada 2012). Attempts at eradication have continued since its discovery in 2008 at Poamoho on O'ahu (Neville 2020).

Habitat: *Tibouchina herbacea* is found in swamps, meadows, and forests in its native range (Wagner et al. 1999). It naturalized in mesic and wet areas between 100 m and 1600 m in Hawai'i (SPREP 2000). A habitat modeling study in Kohala Mountain indicates that *T. herbacea* is most frequently found in partially-shaded wet forests above 300 m and is positively associated with feral pig disturbance (Purell 2006).

Reproduction and Dispersal: This invasive plant spreads by prolific production of seeds that are the size of grains of sand, as well as vegetatively. Each multi-stemmed plant can produce hundreds of 5-mm wide seed capsules (fruiting hypanthia), with each capsule producing up to 700 seeds that fall or blow distances up to several meters (Almasi 2000). The tiny seeds can be transported by birds, rats, pigs, water, and human foot and vehicular traffic. Plants also can reproduce vegetatively by growing roots along leaf nodes or producing new shoots from rhizomes (Almasi 2000). Rats and birds are claimed to be dispersers in Hawai'i, despite the fact that the plant does not produce fleshy fruit (Almasi 2000; Motooka et al. 2003). Pigs likely spread the seeds externally and could conceivably spread stem fragments, as areas disturbed by pigs are often completely taken over by this plant (Buddenhagen 2013).

Impact: *Tibouchina herbacea* invades wet and mesic forests that are disturbed (especially by pigs and landslides), though it can grow in shaded areas. It forms dense stands in pastures and disturbed forests, out-competing native species. It is listed amongst the invasive plants that are considered the most serious habitat modifying species (Medeiros and Loope 2013). Along with other *Tibouchina* species, it has been placed on the Hawaii State Noxious Weed List (HAR 68), and it has a Weed Risk Assessment rating of 24. Visit http://www.hpwra.org for more information on Weed Risk Assessments.

Figure 2. *Tibouchina herbacea* growing along Waihe'e Ridge Trail, Maui; Photo by Forest & Kim Starr

Management: Various herbicide applications have been reported to control *T. herbacea*. These include application of 1) undiluted triclopyr ester to the stem base; 2) triclopyr amine in foliar sprays with a surfactant and in cut-stump treatments; 3) glyphosate at 2% product in water in foliar spray; and 4) 10% Garlon 3A as a foliar spray. Based on work with other melastomes, *T. herbacea* is probably sensitive to 2,4-D, dicamba, triclopyr, and metsulfuron (Motooka et al. 2003; Loh et al. 2014). Mechanical removal is not effective as the cut plants will sprout and the broken pieces can root and form new plants if left in place. Because of its wide distribution and ability to invade remote areas, the use of chemical and mechanical controls is economically prohibitive for controlling advanced infestation, therefore biocontrol is considered the only sustainable control method at the landscape scale.

Natural Enemies: Exploration for potential biological control agents was conducted in the native range of *T. herbacea* in southeastern Brazil. Surveys in the 1990s yielded several insects and plant diseases that were considered in initial screening for potential biocontrol agents. Plant diseases found to infect *T. herbacea* include *Cryphonectria cubensis*, a canker disease affecting a wide range of hosts including *Eucalyptus* spp. (Seixas et

al. 2004); and leaf spots caused by cercopsoroid fungi (asexual stage of Mycosphaerellaceae), including *Cercospora apii*, *Passalora tibouchinae*, *Pseudocercospora subsynnematosa*, *Pseudocercospora tamonae*, *Pseudocercospora tibouchina-herbaceae*, and *Pseudocercospora tibouchinicola* (Killgore 2002; Parreira et al. 2014). Insects found to feed on *T. herbacea* include a flea beetle, *Syphraea uberabensis* (Coleoptera, Chrysomelidae, Alticini); a weevil, *Anthonomus partiarius* (Coleoptera, Curculionidae); a moth, *Schreckensteinia* sp. (Lepidoptera: Schreckensteiniidae); and another flea beetle, *Margaridisa* sp. (Coleoptera: Chrysomelidae). The proposed biological control agent *Syphraea uberabensis* is considered the most suitable after extensive studies of its effectiveness and its potential host range in Hawai'i.

1.3 Biocontrol Agent: *Syphraea uberabensis*

Syphraea uberabensis is the insect that is proposed for release for biocontrol of *T. herbacea* and related weeds in Hawai'i. *Syphraea uberabensis* is a small beetle that has been evaluated in its native Brazil between 1993 and 2009 and in containment in Hawai'i between 2005 and 2015. Adults and larvae feed externally on foliage and soft stems of *T. herbacea.*, causing enough damage to kill small plants. *Syphraea uberabensis* is host specific to a subset of species within the melastome family, which contains no native taxa in Hawai'i.

Taxonomy: *Syphraea uberabensis* Bechyné is a flea beetle, classified under the tribe Alticini and the leaf beetle family Chrysomelidae. Flea beetles are similar to other leaf beetles but are characterized by having enlarged hind legs, which afford them the ability to leap/spring when disturbed, hence the common name. Flea beetles are herbivores that feed on various parts of the plant; some flea beetle species are important agricultural pests. They do not bite humans or animals. The genus *Syphraea* Baly (1876) includes more than 100 species and is found throughout South and Central America (Scherer 1983).

Description of Adults: Body elongated, slightly broader posteriorly; robust legs; thorax, abdomen, legs and antennae relatively covered with fine short hairs; coloration deep metallic blue, females 3.3 mm and males 3.0 mm in length, on average (Souder 2008).

Description of Larvae: Mature larva. Length: 4.4–6.30 mm; width of pronotum: 0.75–1.41 mm. Eruciform, general integument cream/yellowish with head brown; antennae, maxillae and legs partially membranous; thorax and abdomen with setous sclerotized plates or setous sclerotized tubercles, brown or yellowish-brown, clearer to apex direction; ventral tubercles clearer than dorsal. Segments separated by transverse grooves forming plicae. Setae club-like, whitish, wide with widened apex; ventral setae narrower than dorsal (Casari and Teixeira 2011).

Distribution: *Syphraea uberabensis* is native to southern Brazil. The distributional range of the species is not well studied.

Life History: A life history study conducted in the quarantine facility in Hawai'i showed that *S. uberabensis* reared on *T. herbacea* have an adult life span ranging from 2 days to 127 days and averaged 78.2 days. *Syphraea uberabensis* samples of the quarantine colony had a sex ratio close to 1:1. Males and females developed and emerged at similar rates (Souder 2008).

Survival and development of *S. uberabensis* was evaluated in the laboratory at five constant temperatures ranging from 12 to 28 °C. No egg or larval development occurred below 16 °C. Complete development to adulthood was only seen at 20 and 24°C. Mean time for development from egg to adult was 50.5 days at 20°C and 31.5 days at 24°C, fitting the expected pattern for insects in general: faster development at increasing temperatures. Although development was slightly faster at 28°C than at 24°C, beetle survivorship was reduced and no adults developed at 28°C. Reduced development and increased mortality of beetle larvae at 16 and 28°C is an indication that the minimum and maximum temperature thresholds were being approached (Souder 2008).

Habitat/Ecology: *Syphraea uberabensis* is tolerant of cool and moderate temperatures and is not expected to be restricted in range by temperatures in Hawai'i, except perhaps in exceptionally warm habitats. (Souder 2008). However, the potential of *S. uberabensis* as a biological control could be limited by humidity at the microhabitat level. In Brazil, *S. uberabensis* is found with its melastome hosts in boggy soils, similar to the areas where *Tibouchina* and *Pterolepis* thrive in Hawai'i. On the other hand, *Melastoma* in Hawai'i can grow in relatively drier areas, such as young lava flows. *S. uberabensis* could be less effective against *Melastoma* in the drier parts of its range, because externally feeding larvae appear to be susceptible to drying (Raboin et al. 2009).

Natural Enemy: There is very little information regarding the natural enemies of *S. uberabensis*. Two unidentified generalist Hemipterans were observed attacking the adult insects in its native range (Wikler and Souza 2008). Under laboratory conditions, larvae and pupae were reported to succumb to a ubiquitous entomopathogenic fungus, *Beauveria bassiana*.

Effect on Target Weed: *Syphraea uberabensis* was selected to be used in the control of *T. herbacea* due the extensive damage it caused to the target plant in Brazil. Both larvae and adults feed on the leaves as well as the soft exterior of young stems. *Tibouchina herbacea* demonstrated little regenerating capacity after attack of *S. uberabensis*, drying after a period of 2 weeks of insect feeding, both in the field and in the laboratory. The leaves were skeletonized, leaving only the stem and vein structures (Figure 3). Plant growth was reduced, and flowering and consequently seed production were prevented. (Wikler and Souza 2008)

Figure 3. Adults and larvae of *Syphraea uberabensis* feeding on *Tibouchina herbacea*

1.3.1 Host Specificity

Understanding host specificity is critical for identifying potential direct effects of a candidate biocontrol agent on non-target species. Host specificity depends upon acceptability and suitability of plants to insects. Acceptability can be evaluated in terms of willingness of larvae and adult beetles to feed and deposit eggs on test plant species. Suitability of potential host plants can be evaluated by the ability of larvae to survive and develop to adulthood, and adults to survive and reproduce.

Host specificity of *S. uberabensis* has been tested on a wide variety of native and non-native plants both in Brazil and in Hawai'i to identify its ability to feed and reproduce on potential target and non-target plants. The Centrifugal Phylogenetic Method was used for selecting the plants to be tested. This method is based on the knowledge that host specificity usually correlates with phylogenetic affinity/proximity. In other words, a plant that is closely related to a known host is more likely to be a suitable host than a distantly related plant. Using this

method, sampling of potential hosts starts from closely related species, usually within the same genus, then centrifugally expanding to higher taxonomic ranks, for example species in the same family, order, etc.

Results of host specificity studies indicate that *S. uberabensis* does not have the capacity to colonize native or economic plants in Hawai'i, and the host range is limited to *T. herbacea* and several melastomes in the tribe Melastomeae in the melastome family, specifically *Tibouchina longifolia, Pterolepis glomerata, Melastoma septemnervium*, and *Melastoma sanguineum*. All *Tibouchina* and *Melastoma* species are listed as noxious weeds in the state, and *Pterolepis glomerata* has invaded native habitats and been targeted for eradication or control in conservation areas. Results of the host specificity studies are summarized below; more information can be found in the cited literature.

Wikler and Souza (2008): Tests were conducted on 20 plant species across ten families in Brazil, including two *Tibouchina* species in the Melastomataceae, eight species from another three families in the order Myrtales, and ten more species outside the Myrtales, including a monocot. The results showed that among the 20 species tested *S. uberabensis* only fed and reproduced on the two *Tibouchina* species (*T. herbacea* and *T. cerastifolia*).

Souder (2008): Host specificity tests were carried out in the quarantine facility in Hawai'i. No-choice tests (also known as starvation tests) were conducted on 35 plant species found in Hawai'i, including 12 native species that are considered significant components of native plant communities. Feeding by beetles was mainly, but not completely, restricted to the family Melastomataceae (Figure 4 and Figure 5). Larvae and young adult beetles fed at very low levels on a few introduced non-melastomes, mainly *Terminalia catappa* (Combretaceae) and *Cuphea* species (Lythraceae). Persistence of beetle populations on these plants did not appear to be possible, because they did not support larval development to adulthood, and they were not accepted by mature beetles for oviposition (

Table 1 and Figure 5). High levels of mature beetle feeding and oviposition occurred only on four melastomes: *Tibouchina herbacea*, *Melastoma septemnervium* (syn. *M. candidum*), *Tibouchina longifolia*, and *Pterolepis glomerata*. Less suitable potential hosts (all belonging to melastome family) were *Heterocentron subtriplinervium*, *Dissotis rotundifolia*, and *Tetrazygia bicolor*. When exposed over a long period, *S. uberabensis* did not persist on these four melastomes. Although occasional non-target feeding may occur on some non-melastomes, no plants outside this family are expected to experience significant damage from this insect. Native and endemic plants appear very unlikely to experience direct adverse effects from *S. uberabensis*.

Raboin et al. (2009): Multi-choice testing with *S. uberabensis* adults began in early 2009 as a follow-up to the Souder (2008) study. Multi-choice tests used a subset of 12 plants from Souder's tests to determine the relative preferences in a setting that better resembles the composition of the natural environment. The results indicate that *S. uberabensis* is unlikely to impact the weeds *Tibouchina urvilleana*, *Miconia calvescens*, and *Clidemia hirta*, and showed significant preferences for feeding and egg laying on *Tibouchina herbacea, T. longifolia*, *Pterolepis glomerata*, and *Melastoma septemnervium*, all of which are invasive weeds in Hawai'i (Figure 6).

Additional no-choice testing conducted by USFS in 2013 with leaves exposed for two days to adult *S. uberabensis* in 10 cm petri dishes included *Tibouchina herbacea, Melastoma sanguineum, Melastoma septemnervium*, *Heterocentron subtriplinervium*, and 24 other common Hawaiian plants, most of which were not previously tested. Results again demonstrated high specificity of *S. uberabensis* in feeding and egg-laying for *Tibouchina* and *Melastoma* species (Figure 7).

Extensive host specificity testing of *S. uberabensis* for the biological control of *T. herbacea* has been performed to ensure that it poses minimal risk to other plants in Hawai'i. The above studies demonstrated that *S. uberabensis* is host-specific to a subset of melastomes. It is highly unlikely to attack native and introduced plants outside of the melastome family.

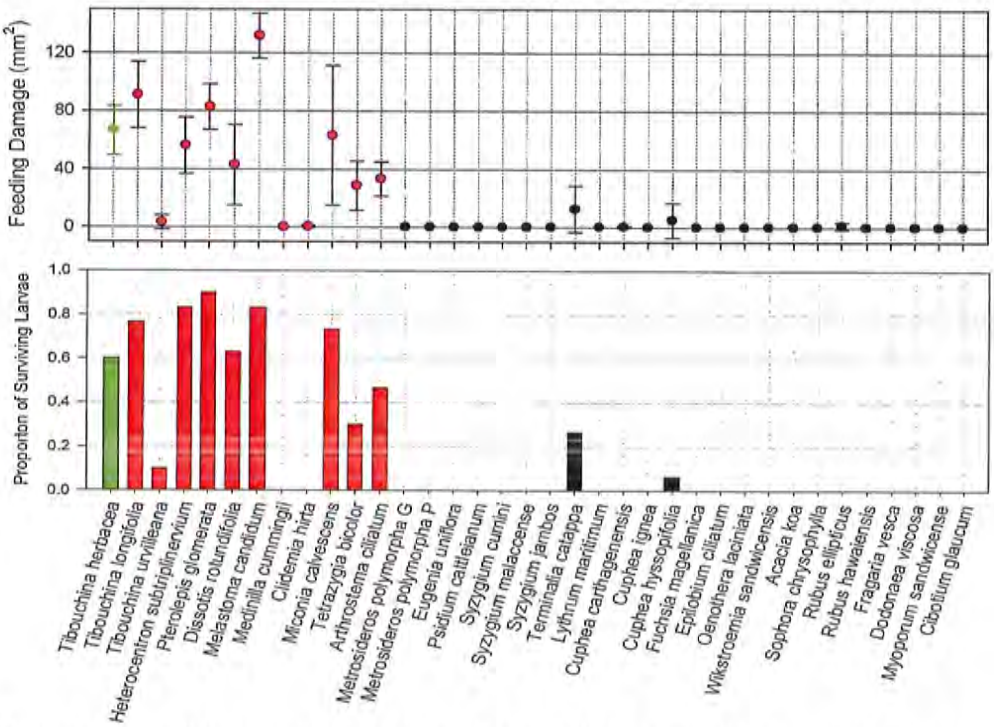

Figure 4. Feeding damage and survival of young larvae after 7 days of no-choice test. Green plot represents the target weed and red plots represent members of the family Melastomataceae. Phylogenetic relationship to the target weed decreases from left to right. Two forms of *Metrosideros polymorpha* were tested: G for glabrous, P for pubescent (Souder 2008).

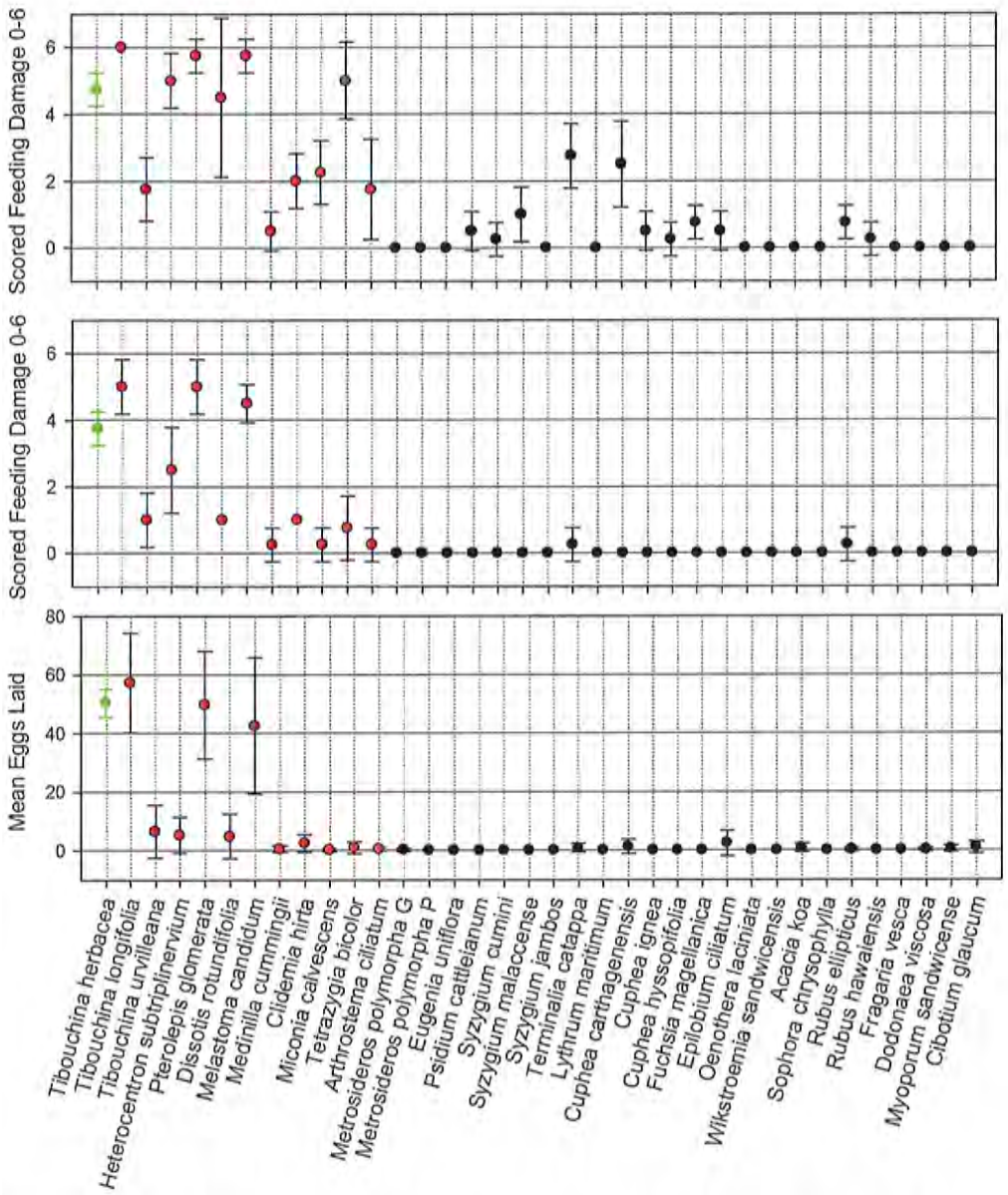

Figure 5. Results of specificity tests with adult *Syphraea uberabensis*. Feeding damage was assessed for young adults (upper graph) and mature adults (middle graph) on a scale of 0 (no damage) to 6 (>4 cm² of leaf area damaged). Oviposition tests recorded number of eggs laid by two mature females in 4 days (Souder 2008).

Table 1. Survival on Test Plant Species that Experienced Feeding Damage in No-Choice Larval Test*

	Number Alive				
Test Plant	**1st Instar**	**2nd Instar**	**3rd Instar**	**Pupa**	**Adult**
Tibouchina herbacea	40	32	28	27	23
Tibouchina longifolia	40	33	31	30	25
Tibouchina urvilleana	40	0	-	-	-
Heterocentron subtriplinervium	40	20	12	10	6
Pterolepis glomerata	40	36	34	32	27
Dissotis rotundifolia	40	17	11	7	5
Melastoma candidum	40	33	30	27	25
Medillina cummingii	40	0	-	-	-
Clidemia hirta	40	0	-	-	-
Miconia calvescens	40	15	9	0	-
Tetrazygia bicolor	40	13	7	4	0
Arthrostema ciliatum	40	0	-	-	-
Terminalia catappa	40	6	0	-	-
Cuphea carthagenensis	40	0	-	-	-
Cuphea hyssopifolia	40	0	-	-	-

* Larvae were evaluated in 100 x 100 x 15 mm petri with leaf cuttings. This test was replicated four times with 10 beetles each replicate (Souder 2008).

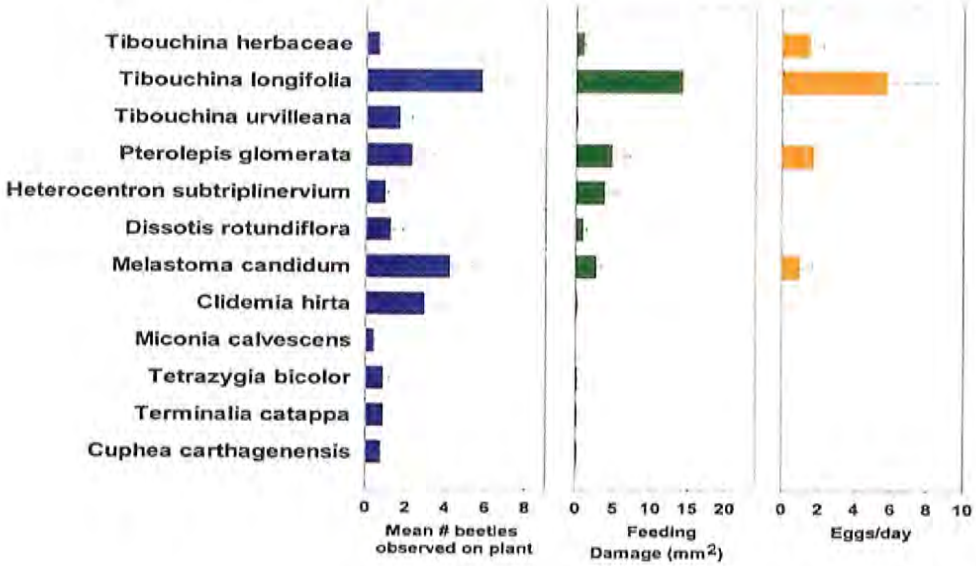

Figure 6. Results of multi-choice host preference tests with adult *Syphraea uberabensis* (Raboin et al. 2009).

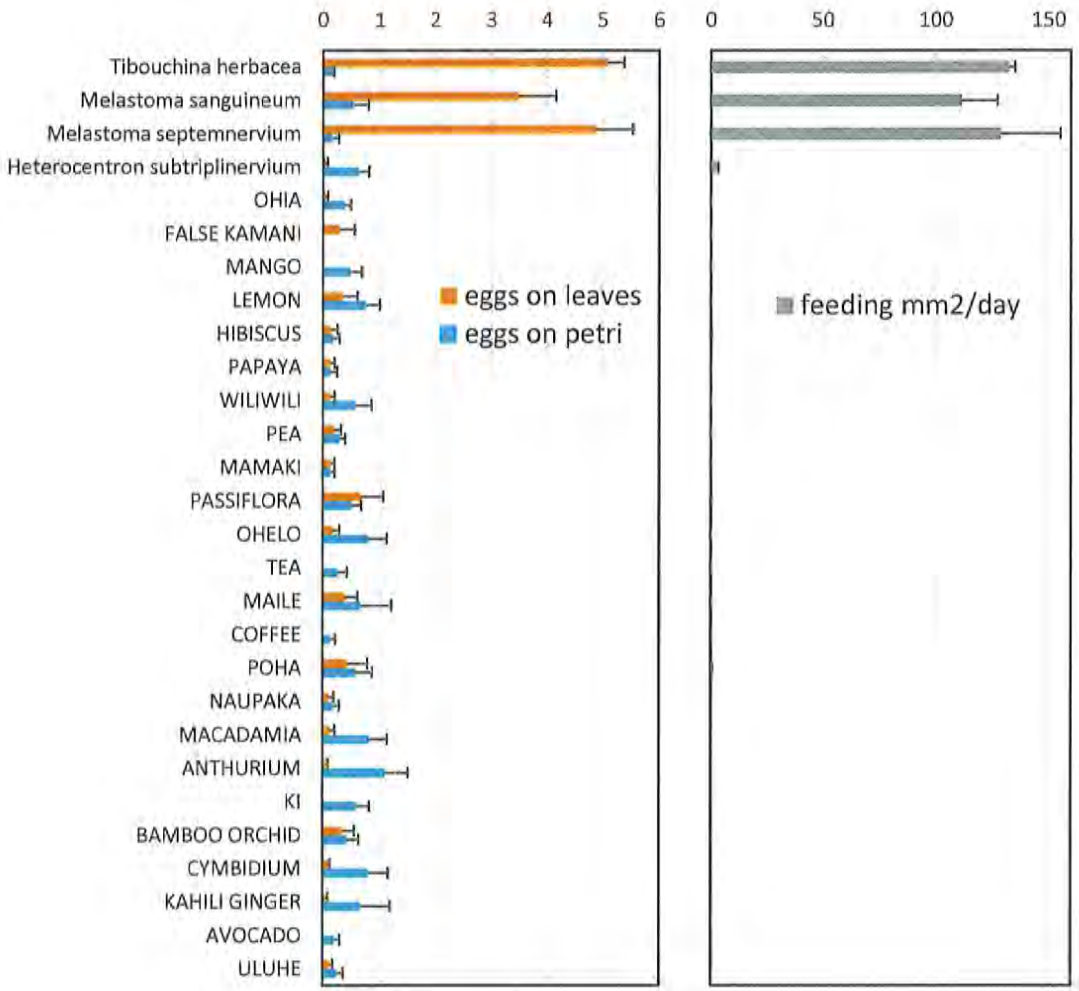

Figure 7. Results of no-choice specificity tests with adult *Syphraea uberabensis* exposed to leaves in small petri dishes for two days. Tests were replicated 4 times per plant species. Egg laying on all but three host plants occurred at negligible levels below or near the rate of egg laying on petri dish surfaces. The same three host plants were accepted equally for feeding, while non-hosts were consistently rejected (USFS unpublished data).

1.4 Secondary Target Species: Related Weeds in Melastomataceae

During host specificity tests, it was found that *S. uberabensis* fed and successfully developed and reproduced on several invasive melastomes that are suitable targets for the proposed release of *S. uberabensis* (Souder 2009; Raboin et al. 2009). These include *Tibouchina longifolia*, *Pterolepis glomerata*, *Melastoma sanguineum*, and *Melastoma septemnervium*, all of which have invaded native wet forest habitats in Hawai'i. *Melastoma septemnervium*, in particular, is widely distributed on Hawai'i Island, where it has been recognized as a threat for many years (Jacobi and Warshauer 1992). Each of these melastome species is likely to increase in population and expand in range in the absence of additional management attempts such as biocontrol by *S. uberabensis*.

1.4.1 *Melastoma septemnervium* - Asian melastome

Figure 8. Asian melastome (*Melastoma septemnervium*); Photo by Forest & Kim Starr

Taxonomy: *Melastoma septemnervium* Lour. belongs to the tribe Melastomeae and the genus *Melastoma* L., which comprises 22 species centered in Southeast Asia and extending to India, South China, Japan, northern Australia, and Oceania. *Melastoma septemnervium* was previously known in Hawaii by the synonyms *Melastoma candidum* D. Don and *Melastoma malabathricum* auct. non L.: Sims.

Description: Shrubs or small trees 2-5 m tall; young branches quadrangular, densely covered with appressed brown scales (Figure 8). Leaves elliptic to ovate, 4–11 by 2–6 cm, 7 nerved but marginal nerves sometimes inconspicuous, upper surface rough with bristly hairs, lower surface with fine hairs but also with scales on the nerves like those of the young branches, margins entire, apex acute, base obtuse to rounded, petioles 5-12 mm long. Inflorescences 2-7 flowered, petals usually 5, purple to pink, 2.5-3.2 cm long, 1.5-2.3 cm wide; anthers of larger stamens 10-11 mm long, anthers of smaller stamens 8.5-10 mm long; fruit a bell-shaped, 5-celled, fleshy capsule, 8–12 by 7–10 mm, densely covered with scales. (Wagner et al. 1999; Meyer 2001).

Distribution: Native to northern Vietnam, southern China, and Taiwan (Meyer 2001). In Hawai'i, it is naturalized on Kaua'i (Wahiawa Bog), O'ahu (Kalihi, Maunawili Valleys), and Hawai'i Islands. One individual was found on the island of Maui in 2002 and removed (Penniman et al. 2011).

Reproduction and Dispersal: The fruit is a bell-shaped fleshy capsule roughly 1 cm in diameter, which ruptures at maturity, exposing red-black pulp and yellow seeds (Meyer 2001). Fruits are dispersed by birds (Smith 1985).

Impact: *Melastoma septemnervium* was cultivated and is now naturalized in mesic to wet areas and bog margins from sea level to 700 m in Hawai'i. (Wagner et al. 1999). It forms dense stands up to 2 m tall shading out understory (Smith 1985; Jacobi and Warshauer 1992)

Management: Sensitive to hormone-type herbicides 2,4-D, dicamba, and triclopyr at 1 lb./acre, and to metsulfuron at 0.45 oz./acre. Sensitive to basal bark and stump bark applications of 2,4-D and triclopyr at 4% product in diesel (Motooka et al. 2003). The HDOA conducted a biological control program on *M. septemnervium* in 1957–1965. Three moth species were released; two of which became established: *Ategumia*)

(=*Bocchoris*) *fatualis* (Lederer) (Crambidae) and *Rhynchopalpus brunellus* Hampson (= *Selca brunella*) (Noctuidae) (Krauss 1965; Conant and Hirayama 2001). *Rhynchopalpus burnellus* is considered partially effective, occasionally causing severe damage to the plant (Conant and Hirayama 2001).

1.4.2 *Melastoma sanguineum* - fox-tongued melastoma

Figure 9. Fox-tongued melastoma (*Melastoma sanguineum*); Photo by Forest & Kim Starr

Taxonomy: *Melastoma sanguineum* Sims has three recognized varieties: *M. sanguineum* var. *sanguineum*, var. *laevifolium,* and var. *ranauense* (Meyer 2001). *Melastoma sanguineum* var. *sanguineum* is known to hybridize with *M. candidum* in southeastern China (Liu et al. 2014).

Description: Shrubs or small trees 2-4 (up to 8) m tall; quadrangular young branches and petioles sparsely covered with spreading, smooth hairs 5-15 mm long, and appressed, smooth, awl-shaped hairs approximately 1 mm long; leaves lanceolate-elliptic, 10-20 cm long, 2-6 cm wide, surface rough or smooth; nerves 5 or 7, the marginal nerves inconspicuous, covered with appressed or semi-erect scales, nerves often red; petiole 10-30 mm long, with red bristles, 5–9 mm long, margins entire, apex tapering to a point, base obtuse to rounded (Figure 9). Inflorescences 2-7-flowered, petals usually 6, purplish pink, 2.5-4.7 cm long, 2.7-3.5 cm wide; anthers of larger stamens 12-15 mm long, anthers of smaller stamens 9-11 mm long; fruits bell-shaped, 6-celled, fleshy capsules, 8–19 by 8–18 mm, covered with spreading or incurved, basally flattened hairs. (Wagner et al. 1999; Meyer 2001).

Reproduction and Dispersal: Like *M. septemnervium*, the fruit is a fleshy capsule which splits open exposing yellow pulp with orange seeds, which are bird-dispersed.

Distribution: In China, it occurs on open slopes, thickets, grasslands, woodland margins on low hills, trailside; below 400 m (Chen and Renner 2007). In Hawai'i, it was once cultivated and has naturalized since at least 1957, occurring on the Island of Hawai'i in Keaukaha and along the highway between Volcano and Hilo. One individual was found on the island of Maui in 2004 and removed (Penniman et al. 2011).

Impact: Although *M. sanguineum* has not dispersed on the same scale as *M. septemnervium,* it is thought to have similar potential to form dense monotypic thickets and crowd out native vegetation (Penniman et al. 2011).

1.4.3 *Pterolepis glomerata* - false meadowbeauty

Figure 10. False meadowbeauty (*Pterolepis glomerata*); Photo by Gerald Crank

Taxonomy: *Pterolepis glomerata* (Rottb.) Miq. belongs to a genus of 15 herbs and small shrubs with center of diversity in Brazil (Renner 1994; Almeda and Martins 2015). Taxonomic treatment of the Hawaiian population of *P. glomerata* by Wagner et al. did not include sub-specific ranking, which the authors considered weakly defined (Wagner et al. 1999). *Pterolepis* is closely related to the old world Melastomeae, which diverged around 11–12 million years ago (Renner and Meyer 2001).

Description: Erect, basally woody herbs or subshrubs up to 0.5 m tall; young branches somewhat squared, with stiff hairs (Figure 10). Leaves ovate to elliptic, 1.4–4.5 cm long, 0.6–1.6 cm wide, 3-nerved, both surfaces sparsely to moderately bristled, petioles 1–5 mm long. Flowers usually 3–5 in terminal tight clusters; 4 petals white, pink or violet, 10–15 mm long, 10–14 mm wide; larger anthers pink, 3–4 mm long, smaller anthers yellow, 2.5–3.5 mm long. Fruiting hypanthium 4–6 mm long, 2–5 mm wide, covered with simple and branched hairs. Seeds ca. 0.5 mm long (Wagner et al. 1999).

Distribution: *Pterolepis glomerata* occurs from the Dominican Republic (Hispaniola) and Puerto Rico over the Lesser Antilles and Trinidad to Venezuela, the Guianas, and south to Santa Catarina in Brazil; reaching adjacent Paraguay and Bolivia (Renner 1994; Wagner et al. 1999). In Hawai'i, it naturalizes on Kaua'i, O'ahu, Moloka'i, Lāna'i, and Hawai'i Islands (Imada 2012). It was first collected on O'ahu in 1949 (Wagner et al. 1999).

Reproduction and Dispersal: *Pterolepis glomerata* reproduces by seeds and vegetative fragmentation. About 500 seeds can be found in a capsule. The seeds are dispersed by birds and water (Ramirez and Brito 1988; Wagner et al. 1999).

Habitat/Ecology: In Hawai'i, the species is not cultivated, but weedy and locally naturalized in mesic to wet disturbed sites and trail margins (Wagner et al. 1999). It is considered among the invasive plants that threaten many endangered plants on O'ahu (USFWS 2012).

Management: Control efforts in the Waianae Mountains of O'ahu were carried out by the O'ahu Army Natural Resources Program. It was suggested that a pre-emergent herbicide, such as 'Oust', should be used to achieve eradication (OANRP 2010).

Natural Enemies: *Rhynchopalpus brunellus*, a moth introduced to Hawai'i from Malaysia for biocontrol of *Melastoma septemnervium*, is known to feed on *P. glomerata.* Foliar damage to the population of *P. glomerata* in the observed site (Waiakea Timber Management Area in the Waiakea Forest Reserve off of Stainback Highway, Island of Hawai'i) was light overall, but heavy on certain plants (Conant and Hirayama 2001).

1.4.4 *Tibouchina longifolia*

Figure 11. *Tibouchina longifolia*; Photo by Forest & Kim Starr

Taxonomy: *Tibouchina longifolia* (Vahl) Baill. ex Cogn. (Synonyms: *Rhexia longifolia* Vahl.) belongs to the pantropical melastome family (Melastomataceae). *Tibouchina* Aubl. is a genus containing about 350 species ranging from Mexico, West Indies, to northern Argentina (Wagner et al. 1999). The center of diversity is in southeastern Brazil. *Tibouchina* is classified in the tribe Melastomeae, which contains several related genera (e.g. *Arthrostemma*, *Dissotis*, *Melastoma*, and *Pterolepis*) that also have naturalized in Hawai'i (Wagner et al. 1999). A phylogenetic study indicates that *Tibouchina* is a well-supported phylogenetic group (clade), although several derived genera nest within the clade (Michelangeli et al. 2012).

Description: *Tibouchina longifolia* is a weedy shrub 0.5-2 m tall (Figure 11). Leaves are narrowly elliptic to lanceolate with dense smooth hairs, 3.5-11.5 cm long and 1-3 cm wide. Flowers are white and approximately 0.5 inches in diameter with 5 petals 5-7 mm long and 2.5-4 mm wide. Anthers 1.5-2 mm long, fruiting hypanthium 4-4.5 mm long and 3-4 mm wide. Seeds are very small, typically 0.25-0.5 mm long (Wagner et al. 1999).

Distribution: *Tibouchina longifolia* is native to the Neotropics and widespread from Mexico and the West Indies to Bolivia and Brazil (Wagner et al. 1999). It was first collected in Hawai'i in 1983 in the Puna District and is now established in the wild (Wagner et al. 1999).

Reproduction and Dispersal: In Hawai'i, *T. longifolia* is now naturalized in native 'ōhi'a forests on Hawai'i Island. It has been propagated by cuttings and cultivated by humans in the past, however it is now recognized as

a noxious weed. Mechanisms for natural dispersal are not documented but are likely the same as for related species. (USGS, 2003).

Management: Methods for control of *T. longifolia* are not documented. Its distribution appears to be limited with no active spread beyond some locations in East Hawaii (USGS 2003). It has not been the target of active management.

1.5 Proposed Action

The HDOA Plant Pest Control Branch will submit an application to the HDOA Plant Quarantine Branch for a permit to release a beetle species, *Syphraea uberabensis* (Coleoptera: Chrysomelidae: Alticini), into the environment of the State of Hawai'i under the provisions of HRS Chapter 141, Department of Agriculture, and Chapter 150A, Plant and Non-Domestic Animal Quarantine. *Syphraea uberabensis* will be released into the environment to control infestations of *Tibouchina herbacea* and related weeds (*Melastoma sanguineum, M. septemnervium, Tibouchina longifolia* and *Pterolepis glomerata*) in the melastome family.

The US Department of Agriculture (USDA) Forest Service has planned detailed monitoring of the impacts of the biocontrol after establishment. This effort will focus on selected sites, following up on pre-release measurements of invasive weeds already obtained in collaboration with the University of Hawai'i.

1.5.1 Project Cost

Although rearing of *S. uberabensis* requires specialized knowledge, the costs for distributing the insect for management will be relatively low after it is approved for release. Facilities, equipment, and personnel needed for rearing the insect are simple and minimal. Establishing self-sustaining populations in field sites statewide likely can be accomplished within one year with a few staff working only part-time (estimate: $40,000 for 1 FTE technician over one year). Agencies contributing to this effort are expected to include the USDA Forest Service, HDOA, and State of Hawai'i Department of Land and Natural Resources. Invasive species committees, watershed partnerships, and others involved in weed management are expected to be active partners in identifying release sites and helping to monitor initial establishment at some release sites.

The pre-release study was conducted over two years with $75,000 of Forest Service funding. A similar investment will likely cover costs of post-release monitoring. Long-term monitoring of the status of the targeted weeds, to determine whether the biocontrol is ultimately successful, will likely require a partnership of researchers and managers. The potential to utilize remote sensing technology for this purpose is high, although it has not yet been applied to this project's target weeds.

1.6 Affected Area

The proposed release of *S. uberabensis* will be statewide. Although initial release of the beetle will focus on locations of high-density infestation, the beetle has the potential to expand its range throughout the state in suitable environments where the target weeds occur.

The first stage of release will focus on the locations of *T. herbacea* infestations on Maui and Hawai'i, as well as locations of *P. glomerata* infestation on O'ahu, where that host plant is most abundant. Once successfully established, the beetle may expand its range to other locations or islands both naturally and by additional releases.

1.7 Sources of Primary Environmental Impact

Primary impacts are defined in HAR §11-200-1 as "effects which are caused by the action and occur at the same time and place." Primary impacts from the release of a biocontrol agent are the damages directly caused by the biocontrol agent; for example, feeding damages on non-target species. The potential impacts of this action are analyzed in Section 2.

1.8 Sources of Secondary Environmental Impact

Secondary impacts are defined in HAR §11-200-1 as "effects which are caused by the action and are later in time or farther removed in distance, but are still reasonably foreseeable." The principal sources of secondary impact may include the long-term and indirect effects such as change of vegetation composition after successful control of *T. herbacea*.

1.9 Agency Identification

The Hawai'i Department of Agriculture is the proposing agency assuming responsibility for the proposed action in accordance with HRS Chapter 343 and the National Environmental Protection Act.

1.10 Required Approvals

The proposed action requires the following permits and approvals:

- Plant Protection and Quarantine permit from the USDA, Animal and Plant Health Inspection Service;
- a permit for import and liberation of restricted organisms from the HDOA Plant Quarantine Branch upon review and approval by the Hawai'i Board of Agriculture; and
- a permit for access for release and monitoring of the insect on State forest land from the State of Hawai'i Department of Land and Natural Resources (DLNR) Division of Forestry and Wildlife (DOFAW).

1.11 Alternatives Considered

The no action alternative and preferred alternative (proposed action) are discussed below. Table 2 summarizes the advantages and disadvantages of each alternative.

1.11.1 No Action Alternative

No action alternative is not to issue permits for the release of *S. uberabensis* in the State of Hawai'i for biocontrol of *Tibouchina herbacea* and the four related weeds (*Melastoma sanguineum, M. septemnervium, Tibouchina longifolia*, and *Pterolepis glomerata*) in the melastome family.

Under the no action alternative, *S. uberabensis* will not be released for biocontrol of the target weeds. Control of the target weeds will be limited to mechanical and chemical control methods. For incipient infestations that are easily accessible and limited in size, mechanical or chemical control can be a preferred method as these have the advantage of short response time and minimal initial resource investment required. However, for infestations in large areas or remote locations, mechanical and chemical controls are infeasible or economically prohibitive, and likely will lead to continued population increase and range expansion of the target weeds (Helen Spafford personal communication).

Environmental impacts associated with mechanical and chemical controls may include impacts on native biota, soil, and water quality. Given the current extent of infestation, the environmental impacts required to achieve adequate control of the target weeds will be unacceptable. For the No Action Alternative, the environmental impacts caused by the target weeds will continue and likely increase, as the weeds will continue to invade suitable habitats and islands that are not currently colonized. The main environmental consequence of the No Action alternative is continued degradation of the native forests, which harbors large numbers of native plants and animals, including threatened and endangered species that rely on the ecosystem to survive and recover.

The "No Action" alternative is considered undesirable for this project.

1.11.2 Proposed Action (Preferred Alternative)

Proposed action is to issue permits for the release of a beetle species, *Syphraea uberabensis*, in the State of Hawai'i for biocontrol of *Tibouchina herbacea* and the four related weeds (*Melastoma sanguineum, M. septemnervium, Tibouchina longifolia,* and *Pterolepis glomerata*) in the melastome family.

The preferred alternative has the advantage of providing long-term control of the target weeds and is the only economically sustainable option for controlling the target weeds at a landscape scale. Although the initial investment in research and development is often high for biological control, as compared to conventional mechanical and chemical controls, the costs in this case have been invested in the past few decades and are ready for use. Benefits of successful biocontrol can accrue for many decades into the future, with benefits amounting to many times the cost. For example, estimates of benefit:cost over 100 years of weed biocontrol efforts averaged 23:1 including all projects, even those that were not successful. (McFadyen 2008)

Although field release will be permanent and there is risk of non-target effects, the extensive host range tests have shown that the biocontrol agent has a very limited host range within the Melastome family, of which all naturalized species in Hawai'i are considered noxious weeds.

Table 2. Summary of Alternatives Considered and Their Associated Advantages/Disadvantages Compared to the Proposed Action

	Actions	Advantages	Disadvantages
No Action	Not releasing *S. uberabensis*; Management of *T. herbacea* and the related weeds will rely on mechanical and chemical controls.	1. Effective for incipient infestations if response is timely. 2. Low developmental investment required. 3. Short-term negative effects are likely reversible.	1. Only provide short-term control; continual efforts required. 2. Economically prohibitive for widespread infestation. 3. Not able to reach inaccessible areas. 4. Given the resources available, the environmental impact of the invasive plants will worsen.
Proposed Action	Field release of the beetle *Syphraea uberabensis* in the State of Hawai'i for biocontrol of *Tibouchina herbacea* and the related weeds in the melastome family.	1. Provide long-term control. 2. Ecological and economic benefits accrue permanently. 3. Able to reach areas that are infeasible by mechanical and chemical controls.	1. Require significant investment in research and monitoring. 2. Irreversible once established. 3. Risk of non-target effects exist.

2.0 Affected Environment and Impact Assessment

This section presents an overview of baseline physical, biological, socio-economic, and cultural environments that the project may affect and the assessment of potential impacts and mitigation measures, when negative impacts are anticipated.

2.1 Biological Environment

The proposed action will have its foremost effect on the biological environment. The biological environment affected by the proposed action is expected to include all ecosystems that are currently occupied by the target weeds.

The introduction of a natural enemy to control target weeds involves direct interaction between the biological control agent and the target weeds. In addition to the direct effects, complex indirect interactions

between other biological and physical components of the environment will both affect and be affected by the direct effects of the proposed action.

Due to these complexities, the end outcome of a biological control release is often difficult to predict, but would fall between no effect (if the biological control agent fails to establish) and widespread suppression of the target species. There is risk for a biological agent to affect non-target species, however, rigorous tests on the host range can minimize this risk.

2.1.1 Direct Effect on the Target Species

The direct effect on the target weeds is the reduction of abundance through herbivory. *Syphraea uberabensis* feeding has the potential to significantly reduce the abundance and distributional range of the target weeds wherever the insect and the plants interact. The level of control, however, will likely depend on the physical and biological environments and is expected to vary by location.

If *S. uberabensis* successfully establishes at release sites, it is expected to disperse and expand its range throughout each island over time. Unaided dispersal between islands is unlikely, however, human-mediated dispersal of *S. uberabensis*, especially as eggs or larvae along with the host plants, is possible. Therefore, the effect is expected to occur on all the main Hawaiian Islands.

2.1.2 Direct Effect on Non-Target Species

Extensive studies have demonstrated that the host range of *S. uberabensis* is limited to a subset of genera (*Tibouchina*, *Melastoma*, and *Pterolepis*) within the melastome family. *Syphraea uberabensis* is not expected to attack plants outside of the melastome family. Because there are no native melastomes and all naturalized melastome species are considered noxious weeds in Hawai'i, non-target plant use is unlikely to directly affect any native or economically important plants of Hawai'i.

2.1.3 Indirect Effect on Flora

If *S. uberabensis* successfully controls the target species, the sites previously occupied can become available to other plants. In the less degraded wet forest, native plants may benefit from the natural resources previously occupied by the target species. In more degraded plant communities, the target species are more likely be replaced by other non-native species present nearby. Controlling existing populations of *T. herbacea* will help to prevent spread to new locations and between islands. If biological control is successful, its effects are likely to develop gradually over a period of years, allowing time for appropriate management responses.

2.1.4 Indirect Effect on Fauna

Native fauna is expected to benefit from the proposed action after the successful control of the target species, which pose threats to the remaining native ecosystems. There is no evidence that native fauna use the target species to an appreciable degree. A small number of native fauna might be indirectly affected by the proposed action if the target weeds are utilized for food or shelter. However, the effect is expected to be insignificant, as the native fauna that adapted to use the introduced species would be generalists, capable of using alternative plant species. Successful control or elimination of the target weeds will not threaten the existence of these generalist species.

The release of *S. uberabensis* has the potential to affect predator or pathogen populations and indirectly affect alternative prey or host species. However, the effect is expected to be insignificant. The family of insects to which *S. uberabensis* belongs, Chrysomelidae, is not native to Hawai'i and is represented by relatively few introduced species. Although there are a few pest chrysomelids in Hawai'i, they have not been actively targeted for biocontrol. Therefore, there is not a known threat of specialized natural enemies affecting *S. uberabensis*. Its populations can be expected to be subject to predation by some generalist predators and diseases that affect

beetles broadly. These natural enemies may increase in abundance where populations of *S. uberabensis* grow large, but such interactions are expected to be localized and temporary given the fluctuating nature of the beetle populations on their host plants.

Indirect effects on pollinating insects is a potential concern, in the event that biocontrol successfully reduces target weeds serving as a food source for pollinators. Native yellow-faced bees in the genus *Hylaeus* (Hymenoptera: Colletidae) can be found across the state, in sea level to sub-alpine habitats that include the invasive plants targeted for biocontrol with *S. uberabensis*. *Hylaeus* species are adapted to forage on pollen and nectar resources from a diversity of native plants, and rarely use non-native floral forage (Daly et al. 2003). Native yellow-faced bees have not been observed to forage on invasive melastomes, and any use of the targeted plants would be peripheral to their primary foraging on native species (K. Magnacca, personal communication). The seven *Hylaeus* species which are currently listed as T/E are known from dry to mesic forest habitats. Their range does not overlap significantly with the range of *Tibouchina herbacea* or other targeted melastomes, which are invasive predominantly in wet to mesic forests. Controlling the spread of invasive melastomes is likely to benefit rare, but yet unlisted, yellow-faced bees which inhabit wet forests, as they are known to suppress the growth of native plants that the bees prefer, and homogenize the composition of native wet forest habitat. The effect of the proposed action is expected to be beneficial for native pollinators.

2.1.5 Uncertainty of Non-Target Effect

There is no action that has consequences that are completely predictable, and thus there is uncertainty associated with any proposed action, including this one. Uncertainty must be weighed against potential benefits of an action and adverse impacts that are likely to occur if an action is not undertaken. In this case, there is a consensus among biologists in Hawai'i that tibouchina and related melastomes are deleterious to local ecosystems and that the severity of ecosystem damage is continually increasing. The uncertainty associated with this biocontrol introduction appears to be low due to the rigorous testing of this biocontrol agent and the general success of biocontrol projects in Hawai'i. Balanced against the certainty of the damage posed by the continued spread of tibouchina and related melastomes, the magnitude of their threat to Hawai'i's endangered species and ecosystems, and the urgent need for more effective methods for protecting these resources at risk, the levels of uncertainty associated with the proposed action appear acceptable.

2.2 Physical Environment

In general, a biological control program would have minimal impact on the physical environment as the action is based on the herbivore-host interaction between the biological control agent and the target species and not directly on the physical environment. The proposed action will have no or negligible effects on geology and topography, air-quality, noise, hazardous substance, and natural hazards. The results of the biocontrol, however, may indirectly affect the physical environment by altering the ecological functions that may affect the physical environment. Most importantly, successful biological control of invasive plants can change composition of the vegetational communities, which consequently can alter local microclimate, transpiration rate, and soil characteristics. The following assesses potential impacts on the elements of physical environment that may be affected by the proposed action.

2.2.1 Climate

The proposed action will have no to negligible effect on long-term or regional climate patterns. The proposed action may affect microclimates that are influenced by the invasive vegetation. Successful control of the invasive weeds is expected to enable the native vegetation to recolonize the invaded area, which will reduce the negative effect of the invasive weeds on the microclimates and should be beneficial to native biota.

2.2.2 Hydrology

Although the proposed action will not directly affect hydrology, the successful control of the target weeds has the potential to indirectly affect hydrology. The successful control of the invasive weed is expected to benefit watershed function of the invaded wet forests which plays an important role in the hydrological cycle. Specifically, forest composition can affect evaporation-transpiration rates and water input from interception of mist and fog.

A study conducted in a lowland wet forest in Hawai'i demonstrated that native trees are more conservative in overall water use than invasive trees (Cavaleri et al. 2014). This study involves the most dominant native wet forest species, 'ōhi'a lehua (*Metrosideros polymorpha*), and one of the target weeds, *Melastoma septemnervium.* The study shows that the wet forest sites dominated by 'ōhi'a lehua that are mixed with invasive species has higher transpiration rates (i.e., water loss) compared to the sites where invasive species were removed.

2.2.3 Soils

Soil erosion is not expected due to the slow acting nature of biocontrol and the ability of other native and non-native plants to fill in areas where *T. herbacea* cover might be reduced. The successful establishment of *S. uberabensis* and control of *T. herbacea* and other melastomes is expected to decrease the abundance of the invasive weeds. In the mesic to wet environments where the target weeds occur, other plant species are expected to grow rapidly to replace their decreasing densities. The proposed action, therefore, will not have significant impact on soils.

2.2.4 Wildland Fires

The proposed action is expected to have negligible effects on wildland fire. The biocontrol has the potential to create small amounts of dead biomass of *T. herbacea* or related melastomes. However, the affected area is usually in mesic to wet environments, where the biomass is expected to decompose at a high rate and fire hazard is generally low. The proposed action is unlikely to significantly increase wildland fire hazard.

2.3 Cultural Resources

ASM Affiliates Hawai'i, a Heritage and Cultural Resource Management firm, prepared a Cultural Impact Assessment (CIA) for the proposed action, which is attached as Appendix B and summarized below. The CIA report was prepared in adherence with the Office of Environmental Quality Control (OEQC) *Guidelines for Assessing Cultural Impacts*, adopted by the Environmental Council, State of Hawai'i, on November 19, 1997 and pursuant to Act 50, approved by the Governor on April 26, 2000.

In general, CIA studies are intended to inform environmental studies that are conducted in compliance with HRS Chapter 343. The purpose of a CIA is to gather information about the practices and beliefs of a particular cultural or ethnic group or groups that may be affected by the actions subject to HRS Chapter 343.

The primary focus of the report is on understanding the cultural and historical context of *T. herbacea* and other weedy melastomes with respect to Hawai'i's host culture. It includes a cultural-historical context of the settlement of the Hawaiian Islands by early Polynesian settlers and the transformation of their beliefs and practices associated with the land following western contact, an overview of the history of biocontrol in Hawai'i, and a discussion of the introduction of *T. herbacea* to the Hawaiian Islands. It also includes a discussion of potential impacts as well as appropriate actions and strategies to mitigate such impacts.

2.3.1 Location

Conventional CIAs assess the potential impacts on cultural practices and features within a geographically defined "project area," which are often defined by an established Tax Map Key number or numbers. However,

CIAs conducted for biocontrol projects differ in that the assessment must consider statewide impacts with emphasis on areas where the target species can be found in abundance. In Hawai'i, *T. herbacea* and related melastomes are naturalized and locally abundant in disturbed mesic to wet forest on the islands of Hawai'i, Lāna'i, Maui, Moloka'i, and O'ahu.

2.3.2 Consultation

As stated in the OEQC Guidelines for Assessing Cultural Impacts, the goal of the oral interview process is to identify potential cultural resources, practices, and beliefs associated with *Tibouchina* and related melastomes and the habitats they occupy. Gathering input from community members with genealogical ties and long-standing residency or relationships to the anticipated area of impact or to the target species is vital to the process of assessing potential cultural impacts on resources, practices, and beliefs.

In an effort to identify individuals knowledgeable about traditional cultural practices and/or uses associated with the subject affected environment, a public notice was submitted by ASM Affiliates to the Office of Hawaiian Affairs (OHA) for publication in the May 2019 issue of their monthly newspaper, *Ka Wai Ola.* While no responses were received from the public notice, 45 individuals were contacted via email and/or phone regarding the preparation of the CIA report. A list of those individuals is available upon request. Of the 45 individuals contacted, 20 responded to the request with either brief comments, referrals, or acceptance of the interview request (see Table 3). ASM Affiliates conducted a total of eight interviews, the summaries of which can be found in the CIA.

The interviewees were asked a series of questions regarding their background, and their experience and knowledge of the target species. Additional questions focused on any known cultural uses, traditions, or beliefs associated with any of the target species. The interviewees were then asked about their thoughts on the cultural appropriateness of using biocontrol agents and whether they were aware of any potential cultural impacts that could result from the use of biocontrol and whether they had any recommendations to mitigate any identified cultural impacts or any other thoughts about the proposed action.

Table 3. Persons that responded to request for consultation.

Name	*Affiliation, Island*	*Initial Contact Date*	*Comments*
Shalan Crysdale	The Nature Conservancy, Ka'ū Preserve, Hawai'i	3/6/2019	See summary in CIA
John Repogle	Retired from The Nature Conservancy, Ka'ū Preserve, Hawai'i	3/6/2019	See summary in CIA
Nohealani Ka'awa	The Nature Conservancy, Ka'ū Preserve, Hawai'i	3/6/2019	See summary in CIA

Arthur Medeiros	Auwahi Forest Restoration Project, Maui	3/7/2019	Responded via email on March 11, 2019, stating "Thank you for your valuable work supporting this essential action to attempt to slow the loss of Hawaiian biota."
Jen Lawson	Waikōloa Dry Forest Initiative, Hawai'i	4/3/2019	See summary in CIA
Robert Yagi	Waikōloa Dry Forest Initiative, Hawai'i	4/3/2019	See summary in CIA
Wilds Brawner	Ho'ola Ka Manaka'ā at Ka'ūpūlehu, Hawai'i	4/9/2019	See summary in CIA
Sam 'Ohu Gon III	The Nature Conservancy, O'ahu	4/22/2019	Responded to interview request but was unable to provide input on this project.
Mike DeMotta	National Tropical Botanical Gardens, Kaua'i	4/22/2019	See summary in CIA
Wili Garnett	Cultural practitioner, Moloka'i	5/7/2019	Responded via email stating "I have mostly been involved with Erythrina gall wasp parasite release and monitoring, but experience watching *Tibouchina* and *Schinus* degrade watershed on many islands, including Molokai and even cultural resources at Kalaupapa."
Emily Grave	Laukahi Network, O'ahu	5/7/2019	Responded via email stating that she was not aware of cultural uses of this plant.
Kim Starr	Starr Environmental, Maui	5/9/2019	See summary in CIA
Forest Starr	Starr Environmental, Maui	5/9/2019	See summary in CIA
Manaiakalani Kalua	Cultural practitioner, Hawai'i	5/30/2019	See summary in CIA
Talia Porter	Honolulu Botanical Gardens, O'ahu	6/3/2019	Responded to interview request but was unable to secure an interview.

Robert Keano Ka'upu	Cultural practitioner, O'ahu	6/16/2019	Responded via phone that he has been interested in learning about the cultural uses of *wiliwili* but was not aware of any uses or of anyone else who used the wood for cultural purposes. Did not address *T. herbacea*
Hinaleimoana Wong-Kalu	Cultural practitioner, O'ahu	7/16/2019	Responded to interview request but was unable to secure an interview.
Pelehonuamea Harman	Cultural practitioner, Hawai'i	7/31/2019	Referred ASM staff to Dennis Kana'e Keawe.
Dennis Kana'e Keawe	Cultural practitioner, Hawai'i	8/12/2019	See summary in CIA
Iliahi Anthony	Cultural practitioner, Hawai'i	8/30/2019	See summary in CIA

2.3.2 Summary of Findings, Identification of Cultural Impacts, and Proposed Mitigative Measures

A review of the cultural-historical background in addition to the consultation efforts has yielded no reported cultural use for *T. herbacea* nor is there any historical evidence to suggest that this plant is crucial to any particular ethnic groups' cultural history, identity, practices, or beliefs, nor does it meet any of the significance criteria outlined in the CIA. Although *T. herbacea* does not meet any of the significance criteria, what is culturally significant is the wet forest habitat in which it thrives. Hawai'i's wet forest habitat could be considered significant as a traditional cultural property under Criterion E, as it contains many culturally important indigenous and endemic taxa, which are still utilized in certain Hawaiian cultural practices. Some of these wet forest resources are also associated with certain Hawaiian cultural beliefs.

Based on the information derived from the cultural-historical background and from the insight shared by the consulted parties, it is the assessment of this study that the release of the proposed biocontrol agent, *Syphraea uberabensis*, will not result in impacts to any valued cultural, historical, or natural resources. Conversely, if no action is taken to further reduce remaining populations of *T. herbacea* and other highly invasive melastomes from claiming more of Hawai'i's wet forest habitat, impacts to this valued resource would be anticipated.

While no specific cultural impacts were identified through the CIA, the consulted parties shared valuable insight, concerns, and recommendations that could reduce the potential for any future impacts and improve public transparency regarding the effectiveness of biocontrol as a conservation management strategy. Several key themes emerged from the consultation efforts, all of which are further described in the CIA:

1) maintain stringent pre and post-release testing and monitoring;
2) improved community transparency and input;
3) active and ongoing public outreach and education;
4) improve efforts to limit the introduction of potentially harmful invasive species.

While the consulted parties did not explicitly oppose the use of biocontrol, especially to aid in the recovery of Hawai'i's native forest habitat, they all shared a sense of concern and spoke about the risks inherent in biocontrol activities.

The CIA recommends that conducting background research, consulting with community members, and taking steps toward mitigating any potential cultural impacts is done in the spirit of *Aloha 'Āina*, a contemporary movement founded on traditional practices and beliefs that emphasize the intimate relationship that exists between Native Hawaiians and the *'āina* (land).

2.4 Socio-economic Environment

The release of the any biocontrol agent poses a risk to socioeconomic environment when the biocontrol agent causes negative effects on non-target species that are socio-economically important. This may be caused by direct predation, competition, or secondarily when the results of the action cause socio-economic impact.

The action is not expected to negatively affect the socio-economic environment. The successful control of invasive weeds will benefit the environment and can release the resources used in chemical and mechanical control efforts for other purposes.

2.4.1 Population

The proposed action is expected to have negligible effect on population. The target species are of minimum economic value and the locations of the biocontrol are largely uninhabited natural areas with no existent population. The successful control of the invasive weeds is not expected to cause significant socio-economic changes that would affect population.

2.4.2 Existing Land Use

The proposed locations of biocontrol release will largely consist of conservation areas that are mainly used for watershed protection, conservation of native flora and fauna, and public recreation. A small part of the affected areas may be used for agriculture or the harvest of forest resources. The proposed action will not significantly change the land use of the affected areas. The successful control of the invasive weeds, however, is expected to benefit the intended uses. The results of successful control of the invasive weeds would improve the integrity of the native forest, which is crucial to the conservation of biodiversity as well as watershed value.

2.4.3 Recreation

Recreational use of the affected area is expected to benefit from the proposed action. The target species are environmental weeds that can degrade the recreational value of natural areas. The invasive weeds colonize areas including trails and forests, which can decrease the value of the natural areas for recreational use. Therefore, the proposed action is expected to benefit recreation.

2.4.4 Scenic and Visual Resources

The proposed action is expected to have negligible effect on scenic and visual resources. The effect of successful biocontrol will take place gradually over the span of years to decades. The change in scenic or visual value of the invaded area, therefore, will not dramatically change in a short time period. The areas of infestation are expected to be replaced by other vegetation and have minimal visual change at landscape level. The proposed action will have insignificant effect in scenic value and visual resources.

2.4.5 Household Nuisance

Syphraea uberabensis lives and feeds on its host plants as adults and larvae and pupates in the soil under these host plants. Although populations of the insects may grow large, these populations are expected to remain localized on and near the host plants, and populations will decline as the leaves of their host plants are consumed. Due to this intimate association with its host plants, which are not cultivated and grow mainly in wild environments and unmanaged areas, humans are unlikely to come into contact with *S. uberabensis*. This insect and its relatives are not known to be a nuisance elsewhere, for example, by exhibiting attraction to lights or mass migration or aggregation. *S. uberabensis* is unlikely to become nuisance to residents and visitors.

2.5 Consistency with Government Plans and Policies

The proposed action is consistent with all government plans and policies, especially those that call for conservation of natural resources.

2.5.1 Hawai'i State Plan

The *Hawai'i State Plan* was adopted in 1978. It was revised in 1986 and again in 1991 (HRS Chapter 226, as amended). The Plan establishes a set of goals, objectives, and policies that are meant to guide the State's long-run growth and development activities. The proposed project is consistent with State goals and objectives that call for increases in employment, income and job choices, and a growing, diversified economic base extending to the neighbor islands.

Chapter 226-4 sets forth goals associated with the *Hawai'i State Plan*:

1. A strong, viable economy, characterized by stability, diversity, and growth, that enables the fulfillment of the needs and expectations of Hawai'i's present and future generations.

2. A desired physical environment, characterized by beauty, cleanliness, quiet, stable natural systems, and uniqueness, that enhances the mental and physical well-being of the people.

3. Physical, social, and economic well-being, for individuals and families in Hawai'i, that nourishes a sense of community responsibility, of caring, and of participation in community life.

The aspects of the plan most pertinent to the proposed classification are the following:

Chapter 226-11 *Objectives and policies for the physical environment—land-based, shoreline, and marine resources*. Planning for the State's physical environment with regard to land-based, shoreline, and marine resources shall be directed towards achievement of prudent use of Hawai'i's land-based, shoreline, and marine resources and effective protection of Hawai'i's unique and fragile environmental resources. To achieve the land-based, shoreline, and marine resource objectives, it shall be the policy of the State to:

- Exercise an overall conservation ethic in the use of Hawai'i's natural resources.
- Ensure compatibility between land-based and water-based activities and natural resources and ecological systems.
- Take into account the physical attributes of areas when planning and designing activities and facilities.
- Manage natural resources and environs to encourage their beneficial and multiple uses without generating costly or irreparable environmental damage.
- Consider multiple uses in watershed areas, provided such uses do not detrimentally affect water quality and recharge functions.
- Encourage the protection of rare or endangered plant and animal species and habitats native to Hawai'i.
- Pursue compatible relationships among activities, facilities, and natural resources.
- Promote increased accessibility and prudent use of inland and shoreline areas for public recreational, educational, and scientific purposes.

The proposed action is consistent with the goals, objectives and policies of the *Hawai'i State Plan*. Specifically, it will encourage the protection of rare or endangered plant and animal species and habitats through the control of the invasive weeds.

2.5.2 Hawai'i County General Plan

The County of Hawai'i's General Plan is the policy document expressing the broad goals and policies for the long-range development of the Island of Hawai'i. The plan was adopted by ordinance in 1989 and amended in 2005. The chapter of Natural Resources and Shoreline are the most relevant to the proposed project and include the following goals and policies.

Natural Resources and Shoreline – Goals:

- Protect and conserve the natural resources from undue exploitation, encroachment, and damage.
- Protect rare or endangered species and habitats native to Hawai'i.
- Protect and effectively manage Hawai'i's open space, watersheds, shoreline, and natural areas.

Natural Resources and Shoreline – Policies:

- Coordinate programs to protect natural resources with other government agencies.
- Encourage public and private agencies to manage the natural resources in a manner that avoids or minimizes adverse effects on the environment and depletion of energy and natural resources to the fullest extent.
- Encourage an overall conservation ethic in the use of Hawai'i's resources by protecting, preserving, and conserving the critical and significant natural resources of the County of Hawai'i.
- Encourage the protection of watersheds, forest, brush, and grassland from destructive agents and uses.
- Work with the appropriate State, Federal agencies, and private landowners to establish a program to manage and protect identified watersheds.

The proposed action would help to protect and conserve native species and habitats and is consistent with the policies for encouraging conservation ethics, watershed protection, and interagency coordination for the management of natural resources.

2.5.3 Kaua'i County General Plan

The General Plan for the County of Kaua'i is the document expressing the broad goals and policies for the long-range development and resource management for the Island of Kaua'i. First adopted in 1971, the Plan was revised in 1984 and 2000. The General Plan is thematically arranged, discussing issues including management of public facilities, preservation of rural character, and caring for land, water, and culture, among others. The General Plan also includes a chapter entitled "*Vision for Kaua'i 2020*" that states:

> In 2020, management of development, agriculture, and other activities on Kaua'i is based on the related principles of ahupua'a and watershed. Land is developed and used in ways that conserve natural streams and streamflows; conserve habitat for native species of plants and animals, both on land and in the ocean; and preserve sandy beaches and coral reefs. Best management practices used by government agencies, agricultural companies, other businesses, and individuals are effective in avoiding increases in floodwaters downstream; preventing beach loss; and minimizing pollution of ocean waters. All of Kaua'i's waters are fishable and swimmable.

The proposed action is consistent with the vision of the Kaua'i County General Plan, specifically the successful control of the target weeds would contribute to conserving habitat for native plants and animals.

2.5.4 Maui County General Plan

The Maui County General Plan is a long-term, comprehensive blueprint for the physical, economic, environmental development, and cultural identity of the county. The Countywide Policy Plan, adopted on March 24, 2010, provides broad goals, objectives, policies, and implementing actions that portray the desired direction of the County's future. Furthermore, this Countywide Policy Plan provides the policy framework for the development of the Maui Island Plan and nine Community Plans. The Countywide Policy Plan is the outgrowth of and includes the elements of the earlier General Plans of 1980 and 1990. The portions of the plan pertaining to the Protection of the Natural Environment are the most relevant to the proposed project and include the following goals and objective.

Goals: Maui County's natural environment and distinctive open spaces will be preserved, managed, and cared for in perpetuity.

Objective: Improve the opportunity to experience the natural beauty and native biodiversity of the islands for present and future generations. Policies to achieve the objective include:

- Perpetuate native Hawaiian biodiversity by preventing the introduction of invasive species, containing or eliminating existing noxious pests, and protecting critical habitat areas.
- Preserve and reestablish indigenous and endemic species' habitats and their connectivity.
- Restore and protect forests, wetlands, watersheds, and stream flows, and guard against wildfires, flooding, and erosion.
- Expand coordination with the State and nonprofit agencies and their volunteers to reduce invasive species, replant indigenous species, and identify critical habitat.

The proposed action is consistent with the goal, objective, and policies of the Maui County General Plan for the protection of natural environment through the control of the target weeds to conserve and restore native ecosystems and watersheds.

2.5.5 City and County of Honolulu General Plan

The City and County of Honolulu General Plan (1992 edition, amended in 2002) is a comprehensive statement of objectives and policies which sets forth the long-range aspirations of O'ahu's residents and the strategies of actions to achieve them. It is the focal point of a comprehensive planning process that addresses physical, social, economic, and environmental concerns affecting the City and County of Honolulu. This planning process serves as the coordinative means by which the City and County government provides for the future growth of the metropolitan area of Honolulu.

The policies most relevant to the proposed action are in the section of Natural Environment with the objective to protect and preserve O'ahu's natural environment including:

- Seek the restoration of environmentally damaged areas and natural resources.
- Protect plants, birds, and other animals that are unique to the State of Hawai'i and the Island of O'ahu.
- Increase public awareness and appreciation of O'ahu's land, air, and water resources.

The proposed action is consistent with the objective and policies concerning the natural environment of the plan. Specifically, the proposed action would contribute to the restoration of natural environment and protection of native plants and animals through the control of the invasive weeds.

2.5.6 Hawai'i's State Wildlife Action Plan

The 2015 edition of Hawai'i's State Wildlife Action Plan (SWAP) details the strategy and plans of the Department of Land and Natural Resources and its partners to address the conservation needs of over 10,000 species native to Hawai'i. This is an update of the Comprehensive Wildlife Conservation Strategy 2005 plan and outlines a statewide strategy for conserving native wildlife species.

The SWAP identified the major threats to Hawai'i's native wildlife which include:

- Loss and degradation of habitat resulting from human development, alteration of hydrology, wildfire, recreational overuse, natural disaster, and other factors;
- Invasive species (e.g., habitat-modifiers, including weeds, ungulates, algae and corals, predators, competitors, disease carriers, and disease);
- Ecological consequences of climate change;
- Limited information and insufficient information management;
- Uneven compliance with existing conservation laws, rules, and regulations;
- Overharvesting and excessive extractive use;
- Management constraints; and
- Inadequate funding.

The SWAP sets goals to guide conservation efforts across the state to ensure protection of Hawai'i's Species of Greatest Conservation Need and the diverse habitats that support them. The following seven objectives have been identified as elements necessary for the long-term conservation of Hawai'i's native wildlife:

- Maintain, protect, manage, and restore native species and habitats in sufficient quantity and quality to allow native species to thrive;
- Combat invasive species through a three-tiered approach combining prevention and interdiction, early detection and rapid response, and ongoing control or eradication;
- Develop and implement programs to obtain, manage, and disseminate information needed to guide conservation management and recovery programs;
- Strengthen existing and create new partnerships and cooperative efforts;
- Expand and strengthen outreach and education to improve understanding of our native wildlife resources among the people of Hawai'i;
- Support policy changes aimed at improving and protecting native species and habitats; and
- Enhance funding opportunities to implement needed conservation actions.

The target weeds of the proposed biological control are invasive plants that pose threats to the native ecosystem. The proposed project will address the threat of invasive species and provide a tool for the resource managers to combat invasive species that would otherwise not be feasible due to management constraints and inadequate funding. The proposed project is consistent with the goals of SWAP by providing a cost-effective tool for resource managers to combat the invasive weeds targeted by the project. The project will also contribute to maintain, protect, manage, and restore native species and habitats.

2.5.7 Hawai'i's Interagency Biosecurity Plan

The 2017-2027 Hawai'i Interagency Biosecurity Plan (HIBP) is the State's first multi-agency, comprehensive biosecurity plan that includes coordinated strategies to protect Hawaii's agriculture, environment, economy and health from invasive species. The HIBP identifies gaps in the current biosecurity system which consists of a

network of state agencies and partners working within the areas of preborder, border, and postborder as well as public engagement. The plan creates a shared path forward to address these gaps through 147 actions.

This project is consistent with the actions identified in the HIBP related to biological control which is an essential tool to address widespread invasive species that are difficult to control through conventional methods. Those actions are:

- Increase funding and staffing for Hawai'i's biological control programs;
- Hiring a biological control program coordinator, doubling the size of HDOA's Biological Control Section Staff; and
- Building state-of-the-art biocontrol facilities equipped to develop effective biocontrol for high-impact target species.

2.5.8 Hawai'i Forest Action Plan

The 2016 Hawai'i Forest Action Plan (FAP) is an update to the original assessment and strategy produced in 2010 called the Hawai'i Statewide Assessment of Forest Conditions and Trends. The Department of Land and Natural Resource Division of Forestry and Wildlife is the lead agency in the development of the FAP, which covers all forest land ownerships (state, private, and federal) and enables DOFAW to continue to seek funding for landscape-scale management and to integrate the many programs the division administers through one planning document. The plan identifies nine priority areas for Hawai'i's forests including:

- Water quality and quantity;
- Forest health, invasive species, insects and disease;
- Wildfire;
- Urban and community forestry;
- Climate change and sea level rise;
- Conservation of native biodiversity;
- Hunting
- Nature-based recreation; and
- Tourism.

The target weeds of the proposed biological control are invasive plant species and pose threats to other priority areas such as water quality and quantity and conservation of native biodiversity. The FAP identifies plants that are non-native, invasive, and habitat-modifying as one of the current, most pervasive threats to native biodiversity in Hawai'i and discusses the negative impacts that invasive plants can have on the hydrological processes of forested watersheds.

The proposed project in consistent with the goals of the FAP, which supports and suggests a substantial increase in resources for biocontrol as a necessary tool in invasive species management and identifies biocontrol as one of the management approaches in the FAP.

3.0 ANTICIPATED DETERMINATION

Section 11-200-12 of the HAR sets forth the criteria by which the significance of environmental impacts shall be evaluated. The following discussion restates these criteria individually and evaluates the project's relation to each.

1. *The project will not involve an irrevocable commitment or loss or destruction of any natural or cultural resources.*

The proposed action involves specific interactions between the biological control agent and the target weeds and is not expected to involve irrevocable commitment or loss or destruction of any natural or cultural resources.

2. *The project will not curtail the range of beneficial uses of the environment.*

The proposed action involves specific interactions between the biological control agent and the target weeds and is not expected to curtail any beneficial uses of the environment.

3. *The project will not conflict with the State's long-term environmental policies.*

The proposed action is expected to benefit the environment by reducing the negative impact caused by the target weeds. This is in line with the State's long-term environmental policies.

4. *The project will not substantially affect the economic or social welfare of the community or State.*

The proposed action involves specific interactions between the biological control agent and the targeted noxious weeds. The proposed action is not expected to affect the economic or social welfare of the community or State.

5. *The project does not substantially affect public health in any detrimental way.*

The proposed action involves specific interactions between the biological control agent and the targeted noxious weeds, both are not public health concerns.

6. *The project will not involve substantial secondary impacts, such as population changes or effects on public facilities.*

The proposed action involves specific interactions between the biological control agent and the targeted noxious weeds and is not expected to cause substantial secondary impacts.

7. *The project will not involve a substantial degradation of environmental quality.*

The proposed action involves specific interactions between the biological control agent and the target weeds and is expected to improve environmental quality by reducing the negative impact caused by the noxious weeds.

8. *The project will not substantially affect any rare, threatened, or endangered species of flora or fauna or habitat.*

The proposed action is expected to benefit many rare, threatened, or endangered species of flora or fauna by reducing the negative impact caused by the noxious weeds to the ecosystems.

9. *The project is not one which is individually limited but cumulatively may have considerable effect upon the environment or involves a commitment for larger actions.*

The proposed action does not involve a commitment for larger actions. The cumulative effect is expected to be beneficial by reducing the overall impact of invasive species to the native ecosystems.

10. The project will not detrimentally affect air or water quality or ambient noise levels.

The proposed action involves specific interactions between the biological control agent and the target weeds and is not expected to affect air or ambient noise levels. Although the proposed action has the potential to reduce vegetation cover and affect water quality, the effect is expected to be temporary and off-set by reducing the long-term impact on watershed integrity caused by the noxious weeds.

11. The project will not affect or will not likely be damaged by being located within an environmentally sensitive area such as flood plains, tsunami zones, erosion-prone areas, geologically hazardous lands, estuaries, fresh waters or coastal waters.

The proposed action involves specific interactions between the biological control agent and the target weeds. In some cases these interactions may take place within environmentally sensitive areas, however impacts in these areas are expected to be beneficial, decreasing the detrimental effects of invasive plants, and not subject to damage by being located within these areas.

12. The project will not substantially affect scenic vistas and viewplanes identified in county or state plans or studies.

The proposed action may temporarily reduce vegetation cover in natural areas but is not expected to substantially affect scenic vistas and viewplanes.

13. The project will not require substantial energy consumption.

The proposed action involves specific interactions between the biological control agent and the target weeds and will not require substantial energy consumption.

3.1 Conclusion

For the reasons above, and in consideration of comments received during early consultation, the State of Hawai'i Department of Agriculture, with support from the State of Hawai'i Department of Land and Natural Resources, Division of Forestry and Wildlife, has concluded that the proposed project will not have a significant impact in the context of HRS Chapter 343 and Section 11-200-12 of the HAR, and has determined an Anticipated Finding of No Significant Impact with the Draft Environmental Assessment.

4.0 Agencies, Organizations, and Individuals Consulted

The following legislators, agencies, advisory commissions, and educational institutes received a letter inviting their participation in the preparation of the Draft Environmental Assessment. The information and issues raised were considered and included in the Draft Environmental Assessment. Comments received during early consultation are provided in Appendix A.

Federal Agencies

- US House of Representatives, Representative Tulsi Gabbard
- US House of Representatives, Representative Colleen Hanabusa
- US Senate, Senator Mazie Hirono
- US Senate, Senator Brian Schatz
- National Park Service, Hawai'i Volcanoes National Park
- National Park Service, Haleakala National Park

- Natural Resources Conservation Service, Pacific Islands Area
- US Army Garrison, Commander Col. Stephen E. Dawson
- US Army Garrison, Environmental Division
- US Army Garrison, Natural Resource Section
- US Fish & Wildlife Service
- US Fish & Wildlife Service, O'ahu National Wildlife Refuge Complex
- US Geological Survey, Pacific Island Ecosystems Research Center

State Agencies

- Aha Moku Councils
- BLNR O'ahu Member
- Department of Business, Economic Development & Tourism
- Department of Hawaiian Homelands
- Department of Health
- Department of Health, Office of Environmental Quality Control
- DLNR Division of Forestry & Wildlife
- DLNR Division of State Parks
- DLNR Land Division
- DLNR Office of Conservation & Coastal Lands
- DLNR State Historic Preservation Administration
- DLNR Watershed Partnership Program
- Land Use Commission
- Natural Area Reserves System Commission
- Office of the Governor
- Office of Hawaiian Affairs
- University of Hawai'i, College of Tropical Agriculture and Human Resources
- University of Hawai'i, Environmental Center
- University of Hawai'i, Pacific Cooperative Studies Unit

City and County Agencies

- Honolulu City Council
- City & County of Honolulu, Office of the Mayor
- City & County of Honolulu, Board of Water Supply
- City & County of Honolulu, Planning Department
- Hawai'i County Council
- Hawai'i County, Office of the Mayor
- Hawai'i County, Department of Water Supply
- Hawai'i County, Department of Planning

- Kaua'i County Council
- Kaua'i County, Office of the Mayor
- Kaua'i County, Department of Planning
- Kaua'i County, Department of Water Supply
- Maui County Council
- Maui County Office of the Mayor
- Maui County, Department of Planning
- Maui County, Department of Water Supply

Organizations

- Big Island Invasive Species Committee
- Bishop Museum
- Conservation Council of Hawai'i
- Environment Hawai'i Inc.
- Hawai'i Audubon Society
- Hawai'i Cattlemen's Council
- Hawai'i Conservation Alliance
- Hawai'i Forest and Trail
- Hawai'i Forest Industry Association
- Hawaiian Botanical Society
- Hawaiian Trail and Mountain Club
- KAHEA
- Kamehameha Schools
- Kaua'i Invasive Species Committee
- Ko'olau Mountains Watershed Partnership
- Maui Invasive Species Committee
- Moloka'i Invasive Species Committee
- Native Hawaiian Advisory Council
- Native Hawaiian Legal Corporation
- O'ahu Invasive Species Committee
- Pig Hunters Association of O'ahu
- Plant Extinction Prevention Program
- Sierra Club, O'ahu Chapter
- The Nature Conservancy of Hawai'i

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Wysong, M., G. Hughes, and K.R. Wood
2007 New Hawaiian plant records for the island of Moloka'i. In *Records of the Hawaii Biological Survey for* 2006. Bishop Museum Occasional Papers No. 96, pp.1–8.

APPENDIX A: COMMENTS RECEIVED DURING EARLY CONSULTATION

17913

DAVID Y. IGE
GOVERNOR OF HAWAII

SUZANNE D. CASE
CHAIRPERSON
BOARD OF LAND AND NATURAL RESOURCES
COMMISSION ON WATER RESOURCE MANAGEMENT

ROBERT K. MASUDA
FIRST DEPUTY

JEFFREY T. PEARSON, P.E.
DEPUTY DIRECTOR - WATER

AQUATIC RESOURCES
BOATING AND OCEAN RECREATION
BUREAU OF CONVEYANCES
COMMISSION ON WATER RESOURCE MANAGEMENT
CONSERVATION AND COASTAL LANDS
CONSERVATION AND RESOURCES ENFORCEMENT
ENGINEERING
FORESTRY AND WILDLIFE
HISTORIC PRESERVATION
KAHOOLAWE ISLAND RESERVE COMMISSION
LAND
STATE PARKS

UHM/College of Tropical Agriculture & Human Resources

DEC 26 2017

Office of the Dean and Director

STATE OF HAWAII
DEPARTMENT OF LAND AND NATURAL RESOURCES
DIVISION OF FORESTRY AND WILDLIFE
1151 PUNCHBOWL STREET, ROOM 325
HONOLULU, HAWAII 96813

December 13, 2017

ATTN: **Interested Agencies and Organizations**

RE: **Early Consultation on Environmental Assessment for the state-wide release of the flea beetle *Syphraea uberabensis* for biological control of the noxious weed *Tibouchina herbacea* and related weeds**

The co-proposing agencies, Hawaii State Department of Agriculture (HDOA) and Hawai'i State Department of Land and Natural Resources (DLNR), are preparing an Environmental Assessment (EA) in support of the field release of the flea beetle *Syphraea uberabensis* in the state of Hawai'i for biological control of the noxious weed *Tibouchina herbacea*. This letter is to share information about the project and to solicit your input regarding potential environmental impacts that may be associated with proposed project actions.

Overview

Cane tibouchina (*Tibouchina herbacea*) is an herbaceous plant in the melastome family (Melastomataceae) and aggressively spreads in mesic and wet areas in Hawai'i. It is widely established on Hawai'i and Maui islands and is also naturalized on Lāna'i, Moloka'i, and O'ahu. This invasive plant spreads by prolific production of bird-dispersed seeds, as well as vegetatively. It forms dense stands in pastures and undisturbed forests, out-competing other species. The entire genus of *Tibouchina* is listed as noxious weed in the state.

Syphraea uberabensis is a small South American beetle (Chrysomelidae; Alticini) whose adults and larvae feed externally on foliage and soft stems of *Tibouchina spp.*, causing enough damage to kill small plants. *S. uberabensis* has been evaluated in containment facilities in Hawai'i as a potential biological control agent for *T. herbacea* with encouraging results. Tests have been conducted on a variety of native and non-native plants to identify the beetle's potential host range. Results indicate that it does not have the capacity to impact native or economic plants in Hawai'i and the host range is limited to *T. herbacea* and closely related weeds within the melastome family.

The proposed action of releasing the biological control agent involves the use of state land and funds as well as approval of permits. Therefore, in accordance with the Hawai'i Revised Statutes Chapter 343 or Hawaii Environmental Policy Act (HEPA) the proposing agencies are conducting an Environmental Assessment of the proposed project to evaluate potential environmental impacts.

Project Actions

State-wide release of the *S. uberabensis* for *T. herbacea* biocontrol will be the primary action considered in the Environmental Assessment. Activities associated with the project include:

1. State-wide field release of *S. uberabensis* on state lands where infestation of *T. herbacea* and related weeds in the melastome family (*Pterolepis glomerata, Melastoma septemnervium and M. sanguineum*) occurs.
2. Monitoring of *S. uberabensis* populations and the impact on *T. herbacea* population in selected release sites.

Public Input Needed

The EA will address topics including but not limited to: biological resources; cultural resources; and socioeconomic impacts. If you would like to contribute input regarding this project, or would like clarification on any aspect, please contact me at Robert.D.Hauff@hawaii.gov. Please send your comments on the project to me by **Friday, January 12th, 2018**. Thank you very much for your time.

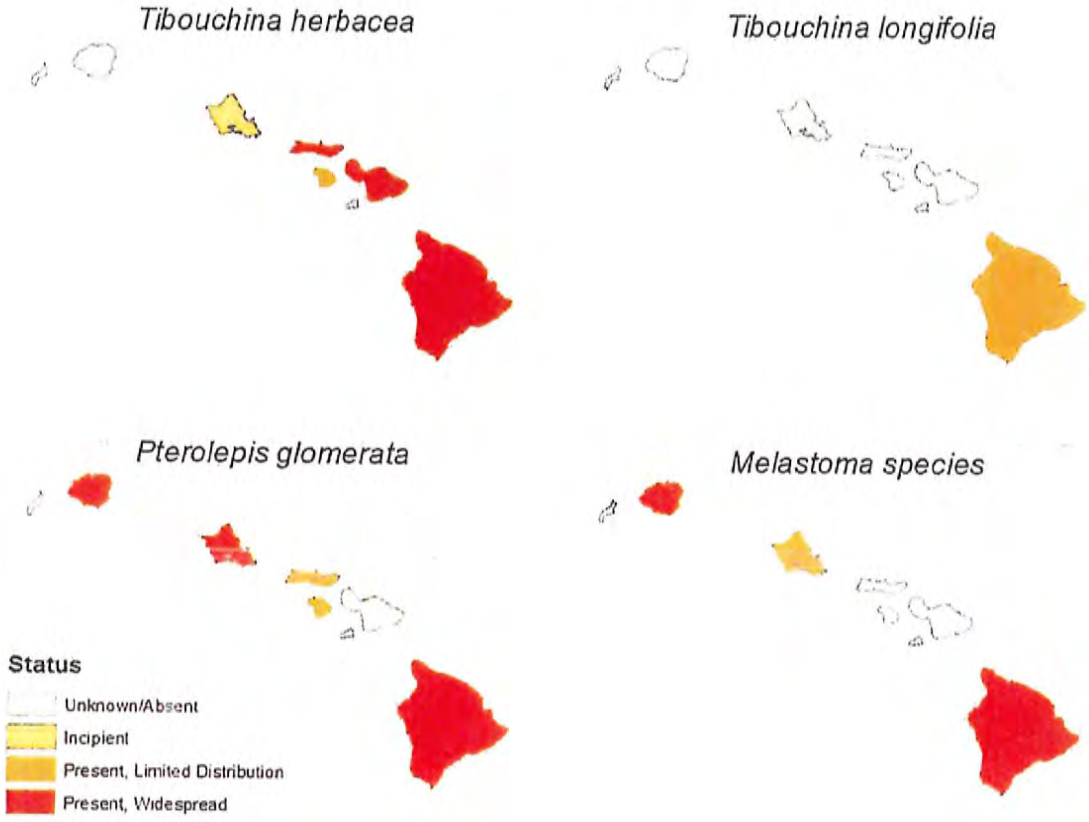
Sincerely,

Robert Hauff

State Protection Forester

Cane Tibouchina Growing in Hawaii

Status of invasion by *Tibouchina herbacea* and relatives across the State of Hawaii



Feeding damage by adults and larvae of *Syphraea uberabensis* on the host plant *Tibouchina herbacea*.

O'AHU INVASIVE SPECIES COMMITTEE

Robert Hauff
State Protection Forester
Department of Land and Natural Resources/Division of Forestry and Wildlife
1151 Punchbowl Street Rm. 325
Honolulu, HI 96813

December 27, 2017

Dear Mr. Hauff,

The O'ahu Invasive Species Committee (OISC) strongly supports the field release of the flea beetle *Syphraea uberabensis* as a natural enemy of the ecosystem-changing weed *Tibouchina herbacea.* OISC, the Ko'olau Mountain Watershed Partnership and DLNR/DOFAW's Native Ecosystems Protection & Management, have been attempting to eradicate this species from the Poamoho summit, where an isolated population was introduced into intact native forest. However, the challenges of finding this weed in thick underbrush over extremely steep terrain has made this difficult to accomplish, despite the species' relatively small footprint.

Unlike many invasive plants, *T. herbacea* does not require prior disturbance to establish in native forests. A study done in Hawai'i in 2000 found that *T. herbacea* can germinate and grow even in dense native underbrush. Once germinated, it grows quickly and outcompetes native plants, including tree seedlings. These traits give *T. herbacea* the ability to convert a forest of native trees into a carpet of alien weeds.

T. herbacea currently occurs along a fork of the Helemano stream and around the summit of the Poamoho trail. However, two immature plants were found along the 'Aiea Ridge Trail in 2015 and 2016 and OISC removed a single immature plant from Halawa in 2007. All these sites were surveyed thoroughly, but no additional plants were found. Our data suggest that Poamoho is the only place on the island with reproductive *T. herbacea,* but this species' history on O'ahu shows that it can jump watersheds and islands. Releasing the flea beetle will reduce the damage that *T. herbacea* can do if it moves into new areas.

Climate change in Hawai'i may cause hotter, drier summers and wetter winters with less rainfall that will be delivered during intense storm events according to a 2014 University of Hawai'i report. Healthy forests that can direct that rainfall into the aquifer and prevent erosion will be a crucial part of Hawai'i's ability to withstand these climate shifts. Reducing the threat of invasive weeds using a species' natural enemies will help keep Hawai'i's forest healthy.

T. herbacea is one of the most damaging invasive weeds in Hawai'i's forests. Reducing the density of *T. herbacea* and limiting the damage it does to native forests will help Hawai'i stay resilient to climate change. Letting the flea beetle destroy plants that field crews would otherwise have to will free up funds for other invasive species projects. For these reasons, we support the field release of this natural enemy. Mahalo for the opportunity to comment.

Sincerely,

Rachel Neville

Rachel Neville
OISC Manager

743 Ulukahiki Street • Kailua, Hawaii 96734 • Ph: (808) 266-7994 Fax: (808) 266-7995
www.oahuisc.org

United States Department of the Interior

NATIONAL PARK SERVICE
Hawai'i Volcanoes National Park
Post Office Box 52
Hawai'i National Park, Hawai'i, 96718

IN REPLY REFER TO:

HAVO 1.D. (L7619)

December 28, 2017

Mr. Robert Hauff
Dept. of Land & Natural Resources
Division of Forestry and Wildlife
1151 Punchbowl Street, Room 325
Honolulu, Hawaii 96813

Dear Mr. Hauff,

The National Park Service (NPS), Hawai'i Volcanoes National Park, received your letter requesting input regarding the proposed state-wide release of the flea beetle *Syphraea uberabensis* for biological control of the noxious weed *Tibouchina herbacea* and related weeds. Hawai'i Volcanoes National Park also has some populations of *T. herbacea* that we control in our Special Ecological Areas. We are supportive of safe effective methods to control invasive plants and look forward to further details in the environmental assessment.

We appreciate the opportunity to provide input during early consultation. Please include us on the Environmental Assessment distribution list. If you have any questions, please contact Danielle Foster, Environmental Protection Specialist at danielle_foster@nps.gov or 808-985-6073.

Sincerely,

Cynthia L. Orlando
Superintendent

cc: Robert.D.Hauff@hawaii.gov

Huang-Chi Kuo

From: Hauff, Robert D <robert.d.hauff@hawaii.gov>
Sent: Friday, January 19, 2018 12:53 PM
To: Huang-Chi Kuo
Subject: FW: Proposed release of biological control agent for Tibouchina

From: Helen Spafford [mailto:hspaffor@hawaii.edu]
Sent: Wednesday, January 03, 2018 12:41 PM
To: Hauff, Robert D <robert.d.hauff@hawaii.gov>
Cc: CTAHR Dean <dean@ctahr.hawaii.edu>
Subject: Proposed release of biological control agent for Tibouchina

Dear Rob,

A graduate student and I have been evaluating the population of Tibouchina herbacea in Hawaii over the last two years. We found the numbers and size of plants to be increasing at all locations and across all elevations on two islands. This plant, and its relatives, are significant weeds. Given the accessibility issues related to the current and expanding areas of infestation, biological control of tibouchina is the only reasonable option for management.

The proposed agent is not host-specific, i.e. it does not feed only on Tibouchina herbacea. However, its host range is limited to melastomes all of which are weeds in Hawaii. If there is any non-target feeding in Hawaii it will be on another weed. This is actually a positive outcome and will ensure that populations of the agent will be sustained over time and can disperse to new patches of the invasive plants.

I highly support the release of the biological control agent.

The sites that we have been monitoring over the last two years could also be used as release sites. The data we have collected can be used for assessment of post-release impact and effectiveness of the biological control agent, should it establlsh.

--
Regards,

Helen Spafford, Ph.D.
Associate Professor of Applied Entomology
Department of Plant and Environmental Protection Sciences
University of Hawaii, Manoa

Website

Huang-Chi Kuo

From: Hauff, Robert D <robert.d.hauff@hawaii.gov>
Sent: Friday, January 19, 2018 12:53 PM
To: Huang-Chi Kuo
Subject: FW: DEADLINE ITEM: Early Consultation on Environmental Assessment
Attachments: D000260 DLNR Early Consultation on Environmental Assessment re Release of Flea Beetle.pdf

From: Daniel Rubinoff [mailto:rubinoff@hawaii.edu]
Sent: Thursday, January 04, 2018 11:30 AM
To: Hauff, Robert D <robert.d.hauff@hawaii.gov>
Subject: Fwd: DEADLINE ITEM: Early Consultation on Environmental Assessment

Hi Rob,
I am writing in strong support of the release. It's overdue and badly needed. If there comes a time that a letter like that from me would be helpful, please just let me know!

Aloha,

Dan
---------- Forwarded message ----------
From: **Koon-Hui Wang** <koonhui@hawaii.edu>
Date: Tue, Jan 2, 2018 at 4:24 PM
Subject: Fwd: DEADLINE ITEM: Early Consultation on Environmental Assessment
To: "Pulakkatu-Thodi, Ishakh" <ishakpt@gmail.com>, "Gutierrez, Rosemary" <gr6@hawaii.edu>, Ethel M Villalobos <emv@hawaii.edu>, Paul Krushelnycky <pauldk@hawaii.edu>, "Borth, Wayne" <borth@hawaii.edu>, Julian Dupuis <jrdupuis@hawaii.edu>, Meng Mao <mengm@hawaii.edu>, Shizu Watanabe <shizuw@gmail.com>, Mohammad Arif <arif@hawaii.edu>, Zhiqiang Cheng <cheng241@hawaii.edu>, Steve Ferreira <stephenf@hawaii.edu>, "Hamasaki, Randall" <rth@hawaii.edu>, John Hu <johnhu@hawaii.edu>, "'Michael Kawate' (mkawate@hawaii.edu)" <mkawate@hawaii.edu>, Mike Melzer <melzer@hawaii.edu>, Daniel Rubinoff <rubinoff@hawaii.edu>, "Shimabuku, Robin" <ShimabukuR@ctahr.hawaii.edu>, Brent Sipes <sipes@hawaii.edu>, "Spafford, Helen" <helen.spafford@hawaii.edu>, "Sugano, Jari" <SuganoJ@ctahr.hawaii.edu>, Miaoying Tian <mtian@hawaii.edu>, Janice Y Uchida <juchida@hawaii.edu>, "Valenzuela, Hector" <hector@hawaii.edu>, Koon-Hui Wang <koonhui@hawaii.edu>, "Mark G. Wright" <markwrig@hawaii.edu>, "Graham, Jason" <jrgraham@hawaii.edu>, Camiel Doorenweerd <cdoorenw@hawaii.edu>, Christina Mogren <cmogren@hawaii.edu>, "Comerford, Nicholas" <ComerfordN@ctahr.hawaii.edu>

Dear all,

Please see an Early consultation for environmental assessment of a new biological control agent to be released for weed management from HDOA. Please send your comments if you have to Robert Hauff and Dean Comerford by Jan 12.

Thanks
Koon-Hui

---------- Forwarded message ----------
From: **Debbie Wong** <wongdebo@hawaii.edu>
Date: Tue, Jan 2, 2018 at 3:02 PM

Subject: DEADLINE ITEM: Early Consultation on Environmental Assessment
To: Catherine Chan-Halbrendt <chanhalb@hawaii.edu>, Koon-Hui Wang <koonhui@hawaii.edu>

Good afternoon Cathy & Koon-Hui,

The attached is being forwarded on behalf of Dean Comerford as you and your faculty may wish to email comment by Jan. 12, 2018 to Robert Hauff (Robert.D.Hauff@hawaii.gov). Please cc the Dean (dean@ctahr.hawaii.edu) on all comments submitted.

Thank you!

Debbie

Deborah Wong, Secretary
Office of the Dean and Director for Research and Cooperative Extension
College of Tropical Agriculture & Human Resources
3050 Maile Way, Gilmore Hall 202
University of Hawai`i at Mānoa
Honolulu, HI 96822

Telephone: (808) 956-8234

--

Koon-Hui Wang, Associate Professor
University of Hawaii
CTAHR Dept. Plant and Environmental Protection Sciences
http://www.ctahr.hawaii.edu/WangKH/index.html

Huang-Chi Kuo

From: Hauff, Robert D <robert.d.hauff@hawaii.gov>
Sent: Friday, January 19, 2018 12:52 PM
To: Huang-Chi Kuo
Subject: FW: Early Consultation on EA for the state wide release of the flea beetle

From: Susan A. Foley [mailto:Susan.Foley@mauicounty.us]
Sent: Friday, January 05, 2018 1:37 PM
To: Hauff, Robert D <robert.d.hauff@hawaii.gov>
Subject: RE: Early Consultation on EA for the state wide release of the flea beetle

Aloha Robert,

Thank you for sending the correspondence regarding the proposal to release the flea Beetle Syphraea uberabensis in the State of Hawai'i for biological control of the noxious weed Tibouchina Herbacea to Kelly King's County Council office.

We have a few questions:

- Are there other successful examples of this project that you could share with us?
- Are we right to understand that as of this date there have only been studies in containment facilities and any not open air tests?
- What are the known negative side-effects of introducing the flea beetle into a new environment, if any?
- How much will the project cost?

Mahalo for your time and consideration,

Thanks,
Susan

Susan Foley
Executive Assistant
808.270.7108
susan.foley@mauicounty.us

Huang-Chi Kuo

From: Hauff, Robert D <robert.d.hauff@hawaii.gov>
Sent: Friday, January 19, 2018 12:50 PM
To: Huang-Chi Kuo
Subject: FW: DEADLINE ITEM: Early Consultation on Environmental Assessment

From: Christina Mogren [mailto:cmogren@hawaii.edu]
Sent: Friday, January 05, 2018 4:48 PM
To: Hauff, Robert D <robert.d.hauff@hawaii.gov>
Cc: dean@ctahr.hawaii.edu; Koon-Hui Wang <koonhui@hawaii.edu>
Subject: Re: DEADLINE ITEM: Early Consultation on Environmental Assessment

Robert,

I just wanted to share some thoughts on your EA for the *Tibouchina herbaceae weed*. As a pollinator ecologist, a concern that comes to mind is that widespread removal of this flowering plant may impact pollinator communities, despite it's weedy and noxious status. I would be less concerned about honey bees (since they are also introduced and capable of foraging elsewhere), but more concerned about potential impacts to native *Hylaeus*.

It may be useful to document any visitation to the flowers of *T. herbaceae* by native bees, and have a plan in place to replace stands with native flowering plants that are also utilized by these bees, if needed. An alternative could be that death of these plants results in new nesting habitat in dried out stems, and thus killed stands should be left in place. These types of plant-pollinator interactions are unfortunately not well understood in the state, so a study to see if any native pollinators are impacted would be beneficial on multiple fronts.

If these plants were originally introduced as ornamentals, then it is likely homeowners throughout the state may have them on their property. A campaign to educate citizens and landscaping companies about voluntary removal could help reduce or eliminate reintroduction, particularly in suburban areas.

I hope these comments are helpful. If you have questions, please do not hesitate to reach out!

Dr. Chrissy Mogren, PhD
Assistant Researcher/Professor
University of Hawaii, Mānoa
College of Tropical Agriculture and Human Resources
Plant and Environmental Protection Sciences
3050 Maile Way, Gilmore 310
Honolulu, HI 96822

Office: Gilmore 608

cmogren@hawaii.edu
408-421-5747 (cell)
808-956-6745 (office)

On Tue, Jan 2, 2018 at 4:24 PM, Koon-Hui Wang <koonhui@hawaii.edu> wrote:

Dear all,

Please see an Early consultation for environmental assessment of a new biological control agent to be released for weed management from HDOA. Please send your comments if you have to Robert Hauff and Dean Comerford by Jan 12.

Thanks
Koon-Hui

---------- Forwarded message ----------
From: **Debbie Wong** <wongdebo@hawaii.edu>
Date: Tue, Jan 2, 2018 at 3:02 PM
Subject: DEADLINE ITEM: Early Consultation on Environmental Assessment
To: Catherine Chan-Halbrendt <chanhalb@hawaii.edu>, Koon-Hui Wang <koonhui@hawaii.edu>

Good afternoon Cathy & Koon-Hui,

The attached is being forwarded on behalf of Dean Comerford as you and your faculty may wish to email comment by Jan. 12, 2018 to Robert Hauff (Robert.D.Hauff@hawaii.gov). Please cc the Dean (dean@ctahr.hawaii.edu) on all comments submitted.

Thank you!

Debbie

Deborah Wong, Secretary
Office of the Dean and Director for Research and Cooperative Extension
College of Tropical Agriculture & Human Resources
3050 Maile Way, Gilmore Hall 202
University of Hawai`i at Mānoa
Honolulu, HI 96822

Telephone: (808) 956-8234

--

Koon-Hui Wang, Associate Professor
University of Hawaii
CTAHR Dept. Plant and Environmental Protection Sciences
http://www.ctahr.hawaii.edu/WangKH/index.html

DAVID Y. IGE
GOVERNOR OF HAWAII

SUZANNE D. CASE
CHAIRPERSON
BOARD OF LAND AND NATURAL RESOURCES
COMMISSION ON WATER RESOURCE
MANAGEMENT

STATE OF HAWAII
DEPARTMENT OF LAND AND NATURAL RESOURCES
LAND DIVISION

POST OFFICE BOX 621
HONOLULU, HAWAII 96809

January 10, 2018

State of Hawaii
Department of Land and Natural Resources
Division of Forestry and Wildlife
Attention: Mr. Robert Hauff
1151 Punchbowl Street, Room 325
Honolulu, Hawaii 96813

via email: Robert.D.Hauff@hawaii.gov

Dear Mr. Hauff:

SUBJECT: Early Consultation on Environmental Assessment for the state-wide release of the flea beetle *Syphraea uberabensis* for biological control of the noxious weed *Tibouchina herbacea* and related weeds

Thank you for the opportunity to review and comment on the subject matter. The Department of Land and Natural Resources' (DLNR) Land Division distributed or made available a copy of your report pertaining to the subject matter to DLNR Divisions for their review and comments.

At this time, enclosed are comments from the (a) Engineering Division and (b) Land Divisions – Oahu District and Hawaii District on the subject matter. Should you have any questions, please feel free to call Lydia Morikawa at 587-0410. Thank you.

Sincerely,

Russell Y. Tsuji
Land Administrator

Enclosure(s)
cc: Central Files

DAVID Y. IGE
GOVERNOR OF HAWAII

SUZANNE D. CASE
CHAIRPERSON
BOARD OF LAND AND NATURAL RESOURCES
COMMISSION ON WATER RESOURCE MANAGEMENT

STATE OF HAWAII
DEPARTMENT OF LAND AND NATURAL RESOURCES
LAND DIVISION

POST OFFICE BOX 621
HONOLULU, HAWAII 96809

December 20, 2017

'17 DEC 20 AM 11:49 ENGINEERING

MEMORANDUM

TO:

DLNR Agencies:
___Div. of Aquatic Resources
___Div. of Boating & Ocean Recreation
X Engineering Division
___Div. of Forestry & Wildlife
___Div. of State Parks
X Commission on Water Resource Management
___Office of Conservation & Coastal Lands
X Land Division – ODLO/HDLO/MDLO/KDLO
X Historic Preservation

FROM

FROM: TO
SUBJECT: Russell Y. Tsuji, Land Administrator
Early Consultation on Environmental Assessment for the state-wide release of the **flea bettle *Syphraea uberabensis*** for biological control of the **noxious weed *Tibouchina herbacea*** and related weeds
LOCATION: State-wide
APPLICANT: State Departments of Agriculture and Land and Natural Resources

Transmitted for your review and comment is information on the above-referenced project. We would appreciate your comments by **January 10, 2018.**

If no response is received by this date, we will assume your agency has no comments. If you have any questions about this request, please contact Lydia Morikawa at 587-0410. Thank you.

( ) We have no objections.
(✓) We have no comments.
( ) Comments are attached.

Signed: _____

Print Name: Carty S. Chang, Chief Engineer
Date: 1/2/18

Attachments
cc: Central Files

DAVID Y. IGE
GOVERNOR OF HAWAII

SUZANNE D. CASE
CHAIRPERSON
BOARD OF LAND AND NATURAL RESOURCES
COMMISSION ON WATER RESOURCE MANAGEMENT

STATE OF HAWAII
DEPARTMENT OF LAND AND NATURAL RESOURCES
LAND DIVISION

POST OFFICE BOX 621
HONOLULU, HAWAII 96809

December 20, 2017

MEMORANDUM

TO: **DLNR Agencies:**
___Div. of Aquatic Resources
___Div. of Boating & Ocean Recreation
_X_ Engineering Division
___Div. of Forestry & Wildlife
___Div. of State Parks
_X_ Commission on Water Resource Management
___Office of Conservation & Coastal Lands
_X_ Land Division – ODLO/HDLO/MDLO/KDLO
_X_ Historic Preservation

FROM: Russell Y. Tsuji, Land Administrator
SUBJECT: Early Consultation on Environmental Assessment for the state-wide release of the **flea bettle *Syphraea uberabensis*** for biological control of the **noxious weed *Tibouchina herbacea*** and related weeds
LOCATION: State-wide
APPLICANT: State Departments of Agriculture and Land and Natural Resources

Transmitted for your review and comment is information on the above-referenced project. We would appreciate your comments by **January 10, 2018.**

If no response is received by this date, we will assume your agency has no comments. If you have any questions about this request, please contact Lydia Morikawa at 587-0410. Thank you.

(✓) We have no objections.
( ) We have no comments.
( ) Comments are attached.

Signed: Darlene Bryant-Takamatsu

Print Name: Darlene Bryant-Takamatsu
Date: 12/21/17

Attachments
cc: Central Files

DAVID Y. IGE
GOVERNOR OF HAWAII

SUZANNE D. CASE
CHAIRPERSON
BOARD OF LAND AND NATURAL RESOURCES
COMMISSION ON WATER RESOURCE
MANAGEMENT

STATE OF HAWAII
DEPARTMENT OF LAND AND NATURAL RESOURCES
LAND DIVISION

POST OFFICE BOX 621
HONOLULU, HAWAII 96809

December 20, 2017

MEMORANDUM

TO: **DLNR Agencies:**
___Div. of Aquatic Resources
___Div. of Boating & Ocean Recreation
_X_ Engineering Division
___Div. of Forestry & Wildlife
___Div. of State Parks
_X_ Commission on Water Resource Management
___Office of Conservation & Coastal Lands
_X_ Land Division – ODLO/HDLO/MDLO/KDLO
_X_ Historic Preservation

FROM: Russell Y. Tsuji, Land Administrator
SUBJECT: Early Consultation on Environmental Assessment for the state-wide release of the **flea bettle *Syphraea uberabensis*** for biological control of the **noxious weed *Tibouchina herbacea*** and related weeds
LOCATION: State-wide
APPLICANT: State Departments of Agriculture and Land and Natural Resources

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(✓) We have no objections.
( ) We have no comments.
( ) Comments are attached.

Signed: _____

Print Name: GORDON C. HEIT
Date: 1/4/18

Attachments
cc: Central Files

DEPARTMENT OF PLANNING AND PERMITTING
CITY AND COUNTY OF HONOLULU
650 SOUTH KING STREET, 7TH FLOOR • HONOLULU, HAWAII 96813
PHONE: (808) 768-8000 • FAX: (808) 768-6041
DEPT. WEB SITE: www.honoluludpp.org • CITY WEB SITE: www.honolulu.gov

FILE
scanned

KIRK CALDWELL
MAYOR

KATHY K. SOKUGAWA
ACTING DIRECTOR

TIMOTHY F. T. HIU
DEPUTY DIRECTOR

2017/ELOG-2574 (mw)
1548785

January 10, 2018

Suzanne D. Case
Chairperson
Board of Land and Natural Resources
1151 Punchbowl Street, Room 130
Honolulu, Hawaii 96813
Attn: Robert Huff, Division of Forestry
and Wildlife

Dear Chairperson Case:

Thank you for your letter dated December 13, 2017, regarding "Early Consultation on Environmental Assessment for the state-wide release of the flea beetle...". We have reviewed the project and have the following comments:

1. The environmental assessment (EA) should fully explain how damaging the noxious weed cane tibouchina is, compared to other invasive species such as the water plant salvinia.

2. The EA should also fully disclose your findings and expectations on your ability to control the flea beetle population.

3. The EA should discuss both State and County policies on controlling invasive species. The General Plan of the City and County of Honolulu has two partially relevant policies under its Natural Environment chapter: "Protect plants, birds, and other animals that are unique to the State of Hawaii and the Island of Oahu", and "Seek the restoration of environmentally damaged areas and natural resources." (Objective A, Policies 8 and 2).

Should you have any questions, please contact Mike Watkins, of our staff, at 768-8044.

Very truly yours,

Eugene H. Takahashi
Acting Division Chief
Planning Division

EHT:bkg

DAVID Y. IGE
GOVERNOR OF HAWAII

VIRGINIA PRESSLER, M.D.
DIRECTOR OF HEALTH

LORRIN W. PANG, M.D., M.P.H.
DISTRICT HEALTH OFFICER

STATE OF HAWAII
DEPARTMENT OF HEALTH
MAUI DISTRICT HEALTH OFFICE
54 HIGH STREET
WAILUKU, HAWAII 96793-3378

January 11, 2018

Mr. Robert Hauff
State Protection Forester
Department of Land & Natural Resources
Division of Forestry & Wildlife
1151 Punchbowl Street, Room 325
Honolulu, Hawaii 96813

Dear Mr. Hauff:

Subject: Early consultation on Environmental Assessment for the statewide release of the flea beetle Syphraea uberabensis for biological control of the noxious weed Tibouchina herbacea and related weeds.

Thank you for the opportunity to review this project. We have no comments to offer. Should you have any questions, please contact me at 808 984-8230 or email me at patricia.kitkowski@doh.hawaii.gov.

Sincerely,

Patti Kitkowski

Patti Kitkowski
District Environmental Health Program Chief

c EPO

Huang-Chi Kuo

From: Hauff, Robert D <robert.d.hauff@hawaii.gov>
Sent: Friday, January 19, 2018 12:51 PM
To: Huang-Chi Kuo
Subject: FW: Syphraea uberabensis

From: Clifford Smith [mailto:cliff@hawaii.edu]
Sent: Thursday, January 11, 2018 1:01 PM
To: Hauff, Robert D <robert.d.hauff@hawaii.gov>
Cc: Joby <jobyrohrer@gmail.com>; Jane Beachy <beachy@hawaii.edu>; Smith, Paul F IV CIV USARMY IMCOM PACIFIC (US) <paul.f.smith133.civ@mail.mil>
Subject: Syphraea uberabensis

State-wide release of *Syphraea uberabensis* for biological control of *Tibouchina herbacea* and related species.

OANRP welcomes the preparation of an Environmental Assessment supporting the release of *Syphraea uberabensis* and would be willing to assist in monitoring the release and its impacts on *Pterolepis glomerata* in particular. UH's PCSU sponsored the earlier surveys for control agents against *Tibouchina herbacea* in Parana State, Brazil in the early 1990s as well as the life history studies by Dr. Charles Wikler at the University of Irati, Parana.

Tibouchina herbacea. The negative impacts of this species were documented on West Maui initially, which led to sponsorship of the biological investigations in Brazil. It was later found on East Maui and Hawaii. Though only an incipient infestation occurs in one valley in the Koolau range, it does not reach the stature that it attains on Maui and Hawaii. It is not a major weed needing control in Army lands at present though it could soon threaten the endangered *Gardenia mannii* habitat in Poamoho in the next few years. *Syphraea,* once established, should keep this species under control on Oahu.

Tibouchina longifolia. Essentially confined to the Big Island. However, some seedlings were found on a load of cinder from the Big Island used in our horticulture program at Schofield. Its potential to spread to the other islands is high.

Pterolepis glomerata. This species is widespread in the Koolau range. We are finding it increasingly in the Waianae range particularly along trails and fencelines. It is spreading

out from there. Its preference for disturbed areas means that it will likely spread significantly in years to come. It is considered more a nuisance and generally overgrown by shrubs and trees. Knocking it back and preventing further spread by *Syphraea* would be welcome as it appears to exacerbate pig damage by colonizing wallows.

Melastoma species. If the insect attacks any of the other established *Melastoma* species it will be welcomed by the conservation community as an important component of the fight against members of the family.

Cliff Smith

January 12, 2018

Mr. Robert Hauff, State Protection Forester
State of Hawaii
Department of Land and Natural Resources
Division of Forestry and Wildlife
1151 Punchbowl Street, Room 325
Honolulu, Hawaii 96813

Dear Mr. Hauff:

SUBJECT: EARLY CONSULTATION COMMENTS IN PREPARATION OF A DRAFT ENVIRONMENTAL ASSESSMENT (EA) FOR THE PROPOSED STATEWIDE RELEASE OF THE FLEA BEETLE SYPHRAIA UBERABENSIS FOR BILOGICAL CONTROL OF THE NOXIOUS WEED TIBOUCHINA HERBACEA AND RELATED WEEDS ON ISLAND OF MAUI, MOLOKAI AND LANAI, HAWAII (RFC 2017/0124)

The County of Maui Department of Planning (Department) is in receipt of the above-referenced document for early consultation on an EA to consider the release of the Flea Beetle, *Syphraea Uberabensis*, to control the noxious weed, *Tibouchina Herbacea,* and related weeds throughout the State of Hawaii. The Department understands the proposed action includes the following:

> Co-proposing agencies, the Hawaii State Department of Agriculture (HDOA) and Hawaii State Department of Land and Natural Resources (DLNR), are planning the field release of the Flea Beetle, *Syphraea Uberabensis,* in the State of Hawaii in geographic areas where infestation of the noxious weed, *Tibouchina Herbacea,* and related weeds in the melastome family (*Pterolepis glomerata, Melastoma septemnervium, and M. sanguineeum)* occurs and are currently soliciting early consultation from Maui County regarding the project action's potential environmental impacts. Monitoring of *Syphraea Uberabensis* populations and the impact on *Tibouchina Herbacea* populations in selected release sites will also occur.

Based on the foregoing, the Department provides the following comments in preparation of the Draft EA:

1. The project area includes selected sites where infestation has occurred within the entire State of Hawaii. The Department has jurisdiction over actions affecting the islands of Maui County, which includes Maui, Lanai, Molokai, Kahoolawe, and Molokini islet. We will constrain our analysis to

these geographic boundaries but will exclude Kalawao County over which Maui County does not have jurisdiction. Maui County also does not have jurisdiction over the State Conservation District; however, we note that the proposed action is regional in nature and thus may affect areas that cross over from the State Conservation District into the State Agriculture, Rural, or Urban Land Use District boundaries.

As such, please define the geographic location(s) of the initial release and subsequent beetle releases and provide a digital copy of the boundaries of the release sites to our office. Please thoroughly discuss all phases of the project including the project's scope, scale, timing, and phases.

2. The Draft EA should include a discussion of how the proposed action will address the relevant sections of Section 11-200-17, HAR, and the regulatory and policy framework of the State Land Use Districts, Maui County General Plan, Title 19 of the Maui County Code (MCC), the Coastal Zone Management Act, and the Special Management Areas (SMA) of Maui County. The Draft EA should address:

   a. State Land Use Districts

      - Agriculture
      - Rural
      - Urban

   b. Countywide Policy Plan

      Please include a discussion on how the project will address the goals, objectives, policies and implementation actions of the Countywide Policy Plan.

   c. Maui Island Plan

      Please include a thorough discussion on how the project will address the goals, objectives, policies and implementation actions of the Maui Island Plan with particular attention given to:

      - Chapter 2, Heritage Resources (Section 2 through Section 5);
      - Chapter 4, Economic Development;
      - Chapter 6, Infrastructure and Public Facilities;
      - Chapter 7, Land Use;
      - Chapter 8, Directed Growth;
        The potential impacts to the Maui Island's Sensitive Lands (please see Table 8-2 on page 8-5) and the Protected Areas described within each community plan district; and

- Chapter 9, Monitoring and Evaluation
  Provide indicators such as those found in Table 9-2 on pages 9-5 to 9-8 of the Maui Island Plan that can be useful over time to assess the effect and success of the proposed action.

d. Community Plans

Please address how the project will implement the goals, objectives, policies and implementation actions of the Community Plans of Maui County. Please also discuss how the project conflicts with any goals, objectives, policies and implementation actions of the Community Plans and how the Applicant intends to resolve or mitigate the conflicts.

e. County Zoning

Please include a discussion on how the project will comply with Title 19 of the MCC.

f. SMA

Please include a discussion of the project's potential effects upon the Special Management Areas of each of Maui County's islands and the measures the Applicant will consider in mitigating any negative effects.

3. Please discuss the proposed strategy and methods for how the Flea Beetle, *Syphraea Uberabensis,* will effectively biologically control and/or eradicate the noxious weed, *Tibouchina Herbacea,* and related weeds.

4. Please provide relevant scientific research and technical studies that have been used to determine all potential, beneficial, and adverse impacts of the project and that your offices are relying upon to determine the viability of the project. Please discuss the rationale for proceeding with the project and the effect of not proceeding with the project. Please include a discussion of all potential adverse effects, particularly effects that are irreversible.

5. Please provide a discussion of all alternatives being considered that could attain the objectives of the action, regardless of cost, in sufficient detail to determine the basis for evaluating the best alternative to pursue. Please include a thorough alternative analysis and research that has been completed or relied upon to determine any and all potential unintended consequences, and a description of all irreversible and irretrievable commitments of resources. Please identify unavoidable impacts.

6. Please include a thorough discussion on the anticipated population growth of the Flea Beetle, *Syphraea Uberabensis*, and how population growth or unintended proliferation of the biocontrol will be managed.

7. Please include a thorough discussion of the impacts that the biocontrol will have biological resources, including animal and plant populations, including sensitive, rare, threatened, or endangered species, or their habitats.

8. Please include a thorough discussion of the predators of the Flea Beetle, *Syphraea Uberabensis*, and how the associated predatory populations will be affected and any related effects of these changes as a result of the introduction of the biocontrol.

9. Please include a thorough discussion of how the Flea Beetle may migrate into habitable areas of Maui County, and the extent to which the Flea Beetle may be a nuisance and can be controlled by residents and visitors.

10. Please discuss how the populations of the biocontrol will be managed by HDOA and DLNR. Please discuss measures that will be implemented to prevent any anticipated negative impacts.

Thank you for the opportunity to comment. Please include the Department on the distribution list of the Draft EA or Draft Environmental Impact Statement (EIS). Should you require further clarification, please contact Staff Planner Simone Bosco, by email at simone.bosco@mauicounty.gov or by phone at 808-270-5780.

Sincerely,

WILLIAM SPENCE
Planning Director

xc: Clayton Yoshida, AICP, Planning Program Administrator (PDF)
Jeff P. Dack, Current Planning Supervisor (PDF)
Simone Bosco, Staff Planner (PDF)
Robert Hauff, DLNR-Division of Forestry & Wildlife (PDF)
Project File

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STATE OF HAWAII
DEPARTMENT OF HAWAIIAN HOME LANDS
P. O. BOX 1879
HONOLULU, HAWAII 96805

January 19, 2018

Robert Hauff, State Protection Forester
Department of Land and Natural Resources
Division of Forestry and Wildlife
1151 Punchbowl Street, Rm. 325
Honolulu, Hawaii 96813

Dear Mr. Hauff:

Subject: Early Consultation on Environmental Assessment for the state-wide release of the flea beetle *Syphraea uberabensis* for biological control of the noxious weed *Tibouchina herbacea* and related weeds

Mahalo for the notice and opportunity for early consultation on this matter.

Tibouchina herbacea and other invasive plants in the Melastome family are rapidly invading native ecosystems and replacing native flora across Hawaii. Melastomes tend to have shallow root systems that do not adequately prevent erosion and soil loss which has a negative effect on water quality in Hawaiian streams and rivers. Member of this plant family are difficult to control because of their prolific, precocious seed production and ease of dispersal.

A biological control agent has the potential to be a cost effective, long term solution for invasive plant control that reduces reliance on chemical herbicides as well as mechanical and manual control methods. To be effective and safe to use, adequate studies must confirm that 1.) the biological control agent effectively controls the target specie(s), and 2.) The biological control agent will not inadvertently spread to and negatively affect non-target species such as indigenous or endemic Hawaiian plants and important agricultural crops.

We look forward to reviewing the Draft Environmental Assessment for this biological control release and commend the

Hawaii State Department of Agriculture (HDOA) and the Department of Land and Natural Resources (DLNR) for being proactive in investigating solutions to the continued spread of invasive plant species. If the proposed biological control agent is found to be effective and safe to use, it will benefit many land owners and resource managers including the Department of Hawaiian Homes Lands and improve the health and resilience of our native forests, streams and nearshore waters.

If you have any questions, please contact Kualii Camara, at 808.933.3480 or via email at joseph.k.camara@hawaii.gov.

Aloha,

Jobie M. K. Masagatani
Chairman
Hawaiian Homes Commission

APPENDIX B: CULTURAL IMPACT ASSESSMENT FOR THE PROPOSED STATEWIDE RELEASE OF A BEETLE (*SYPHRAEA UBERABENSIS*) AS BIOCONTROL FOR *TIBOUCHINA HERBACEA* (MELASTOMATACEAE) AND RELATED WEEDS

A Cultural Impact Assessment for the Proposed Statewide Release of a Beetle (*Syphraea uberabensis*) as Biocontrol for *Tibouchina herbacea* (Melastomataceae)& Related Weeds

FINAL

State of Hawai'i

Photo courtesy of Forest and Kim Starr

Prepared By:
Lokelani Brandt, M.A.

Prepared For:

Department of Land and Natural Resources, Division of Forestry and Wildlife
1151 Punchbowl Street, #325
Honolulu, HI 96813

October 2019

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ASM Project Number 31270.00

A Cultural Impact Assessment for the Proposed Statewide Release of a Beetle (*Syphraea uberabensis*) as Biocontrol for *Tibouchina herbacea* (Melastomataceae)

State of Hawai'i

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1. INTRODUCTION

At the request of the Department of Land and Natural Resources (DLNR), Division of Forestry and Wildlife (DOFAW) and Hawai'i Department of Agriculture (HDOA), referred to hereafter as the State of Hawai'i, ASM Affiliates (ASM) has prepared this Cultural Impact Assessment (CIA) for the proposed statewide release of a small beetle (*Syphraea uberabensis*) native to South America as a biocontrol agent targeting cane tibouchina (*Tibouchina herbacea*) as well as other weedy Melastomes (Melastomataceae) including *T. longifolia, Pterolepis glomerata, Melastoma sanguineum*, and *M. septemnervium*. Native to portions of South America, *T. herbacea* was first discovered on Saddle Road on Hawai'i Island in 1977. Since then it has spread to Maui, Moloka'i, Lāna'i, and O'ahu. In 1992, under Hawai'i Administrative Rules, Chapter 68, *T. herbacea* along with other highly invasive species of the Melastome (Melastomataceae) family were officially listed as a noxious weed in the State of Hawai'i and since then efforts to limit its spread have been undertaken (Medeiros et al. 1997). In the State of Hawai'i the term "invasive species" is any "alien species whose introduction does or is likely to cause economic or environmental harm or harm to human health" (Invasive Species Advisory Committee 2006:1). To control the spread of *T. herbacea*, the State of Hawai'i is proposing to release a natural enemy, a small beetle (*S. uberabensis*).

The current CIA is intended to supplement an Environmental Assessment (EA) conducted in compliance with Hawai'i Revised Statutes (HRS) Chapter 343. This CIA was prepared in adherence with the Office of Environmental Quality Control (OEQC) *Guidelines for Assessing Cultural Impact*, adopted by the Environmental Council, State of Hawai'i, on November 19, 1997. As stated in Act 50, which was proposed and passed as Hawai'i State House of Representatives Bill No. 2895 and signed into law by the Governor on April 26, 2000, "environmental assessments . . . should identify and address effects on Hawaii's culture, and traditional and customary rights . . . native Hawaiian culture plays a vital role in preserving and advancing the unique quality of life and the 'aloha spirit' in Hawai'i. Articles IX and XII of the state constitution, other state laws, and the courts of the State impose on governmental agencies a duty to promote and protect cultural beliefs, practices, and resources of native Hawaiians as well as other ethnic groups."

The primary focus of this report is on understanding the cultural and historical context of *T. herbacea* with respect to Hawai'i's host culture. This CIA is divided into four main sections, beginning with an introduction of the proposed action followed by a physical description of *T. herbacea* and the proposed biocontrol agent *S. uberabensis*. Section two of this report provides a cultural-historical context of the settlement of the Hawaiian Islands by early Polynesian settlers and the transformation of their beliefs and practices associated with the land following Western contact. An overview of the history of biocontrol in Hawai'i is also provided, and this section concludes with a detailed discussion of the introduction of *T. herbacea* into the Hawaiian Islands; all of which combine to provide a geographical and cultural context in which to assess the proposed action. The results from the consultation process are then presented, along with a discussion of potential impacts as well as appropriate actions and strategies to mitigate any such impacts.

PROPOSED ACTION

DOFAW has been working cooperatively with HDOA and the United States Forest Service (USFS) to control the harmful impacts of certain widespread invasive plant or pest species through the use of biological control (also referred to as biocontrol). Biocontrol is the strategy of using an invasive species' natural enemies from its native range to reduce the impacts of the invasive species. Biocontrol projects typically require years of research and survey work to find potential candidates that are subjected to a host of tests. Only those candidates that are host-specific, meaning they can only complete their life cycle on their intended invasive species host and shown to only negatively impact the growth and abundance of the target invasive species are considered for release. Once testing has been successfully completed, agencies must comply with national and state regulatory requirements for the release of the biocontrol agent. As such, the proposed action involves the use of state lands and funds, which necessitates compliance with Hawai'i Revised Statutes (HRS) Chapter 343, also known as the Hawai'i Environmental Policy Act (HEPA). The proposing agencies are conducting an Environmental Assessment (EA) of the proposed action to evaluate potential environmental impacts and this CIA is an essential component of the EA to ensure compliance with HRS Chapter 343.

TIBOUCHINA HERBACEA AND THE PROPOSED BIOLOGICAL CONTROL AGENT

Native to the tropical and subtropical regions of South America, *T. herbacea* and other weedy Melastomes thrive in wet to mesic forests, wetlands, wet pastures, and disturbed areas (Figures 1 and 2). In its native range, *T. herbacea* is variable and typically grows to a height of 1.5 meters, however, in Hawai'i, *T. herbacea* can reach heights of four meters and flowers after a year of being established (Almasi 2000). *T. herbacea* produces viable seeds which are spread by avian populations and rodents and is known to "reproduce vegetatively by growing roots along its leaf nodes, or by producing new shoots from rhizomes" (ibid.:220). It is also known to grow epiphytically on tree ferns (CABI 2018). The young branches of *T. herbacea* are square-shaped and typically covered with gland-tipped hairs, which can be a skin irritant (Figure 3). The leaves are oval-shaped and measure 3.0-7.5 centimeters long and 1.3-3.5 centimeters wide and contain 5-7 parallel veins (see Figure 3). The inflorescences extend from 10-20 centimeters long with fruiting capsules that measure 4-5 millimeters long and 3.5-5 millimeters wide (Figure 4) (CABI 2018). A distinguishing feature of this species is its purple-pink four-petaled flower with large yellow anthers that emerge from the flower's center (ibid.) (Figure 5). While the other species of Melastomes (i.e. *T. longifolia, Pterolepis glomerata, Melastoma sanguineum*, and *M. septemnervium*; Figures 7, 8, and 9) share similar attributes with *T. herbacea*, particularly the leaf veination, they differ in growth with the latter two typically forming bush like thickets.

T. herbacea is one of several species of the Neotropical Melastome family that "are among the most aggressive invaders of the Hawaiian and other Pacific islands" (Baruch et al. 2000:107). This shrub germinates easily in the shade and can quickly establish significant populations in forests with an intact canopy (CABI 2018). Although this plant dies back annually, new sprouts will emerge from the old roots which can create thickets that evenrually consume habitat for native species (Figure 6) (Strohecker 2018). *T. herbacea* as with other species of the Melastome family are known to clog waterways and infest wet forests and upland pastures (ibid.). The reproductive vigor, small seed size, dispersion capacity, and lack of natural predators have contributed to the rapid spread of this highly invasive plant in Hawai'i (Baruch et al. 2000; Wikler and Souza 2008). In 1992, under HRS Chapter 68, *T. herbacea*, along with other highly invasive species of the Melastome family, was officially listed as a noxious weed in the State of Hawai'i (Medeiros et al. 1997). Since 1998, a biological research program to combat *T. herbacea* has developed in southern Brazil, which has led to the identification and evaluation of potential biocontrol agents. Among the identified biocontrol agents for *T. herbacea* was a flea beetle, *Syphraea uberabensis*, native to South America. The adults and larvae of *S. uberabensis* were observed feeding externally on foliage and soft stems of certain *Tibouchina spp.* in Brazil, in some cases causing enough damage to kill small plants. Wikler and Souza describe the characteristics of *S. uberabensis* as:

> ...oval, compact, small black or blue-black flea beetles...[that] are 3-4mm in length and have a dark blue color. The antennas have robust articles from the base to the apex compared with the anterior tibia; the elytra have simple and very fine punctuations. (Wikler and Souza 2008:340)

On July 15, 2005, specimens of *S. uberabensis* were exported from Brazil and received at the Volcano quarantine facility, where a colony was maintained and studied by Steven Souder (Johnson 2006). *S. uberabensis* has been evaluated in containment facilities in Hawai'i as a potential biological control agent for *T. herbacea*. Tests have been conducted on a variety of native and non-native plants to identify the beetle's potential host range. Results from these studies indicate that the host range is limited to *T. herbacea* and other closely related weeds within the Melastome family, and . *S. uberabensis* does not have the capacity to impact native or economically important plants in Hawai'i.

Figure 1. Growth of *T. herbacea* at the end of the Waihe'e Ridge Trail, Maui Island. Photo courtesy of Forest and Kim Starr.

Figure 2. *T. herbacea* growing through a thicket of *uluhe* (*Dicranopteris linearis*) along the Waihe'e Ridge Trail, Maui Island. Photo courtesy of Forest and Kim Starr.

Figure 3. Close up of leaves and stem of *T. herbacea* in Kahikinui, Maui Island covered in fine gland-tipped hairs. Photo courtesy of Forest and Kim Starr.

Figure 4. Flowers and seed pods of *T. herbacea* found in West Maui. Photo courtesy of Forest and Kim Starr.

Figure 5. Close up of *T. herbacea* flower with large yellow anthers. Photo courtesy of Forest and Kim Starr.

Figure 6. New growth of *T. herbacea* at Kapunakea Preserve in West Maui emerging from former roots. Photo courtesy of Forest and Kim Starr.

Figure 7. *Tibouchina longifolia*. Photo courtesy of Forest and Kim Starr.

Figure 8. *Melastoma sanguineum*. Photo courtesy of Forest and Kim Starr.

Figure 9. *Melastoma septemnervium*. Photo courtesy of Forest and Kim Starr.

2. BACKGROUND

The following section contains a cultural-historical context of the settlement of the Hawaiian Islands by early Polynesian settlers and the transformation of their beliefs and practices associated with the land following Western contact. An overview of the history of biocontrol in Hawai'i is also provided and this section concludes with a detailed discussion of the introduction of *T. herbacea* to the Hawaiian Islands and its impacts to Hawai'i's wet forests.

GEOGRAPHICAL AND CULTURAL CONTEXT OF HAWAI'I

The Hawaiian Islands are located within the vast and remote Pacific Ocean, situated more than 3,200 kilometers (2,000 miles) from the nearest continent (Juvik and Juvik 1998). The 16,640 square kilometers (6,425 square miles) of land consists of eight main large volcanic islands, Hawai'i, Maui, Kaho'olawe, Lāna'i, Moloka'i, O'ahu, Kaua'i, and Ni'ihau and 124 smaller islands, reefs, and shoals (ibid.) (Figures 10 and 11). Due to its geographical placement in the middle of the vast Pacific Ocean, coupled with its diverse climatic conditions, the Hawaiian Islands boasts the highest levels of endemism in both native plants and animals, with over 10,000 species found nowhere else in the world (Cannarella 2010).

While the question of the timing of the first settlement of Hawai'i by Polynesians remains unanswered, several theories have been offered that derive from various sources of information (i.e., archaeological, genealogical, mythological, oral-historical, radiometric). However, none of these theories are today universally accepted. What is more widely accepted is the answer to the question of where Hawaiian populations came from and the transformations they went through on their way to establish a uniquely Hawaiian culture. More recently, with advances in palynology and radiocarbon dating techniques, Kirch (2011) and others (Athens et al. 2014; Wilmshurst et al. 2011) have convincingly argued that Polynesians arrived in the Hawaiian Islands, sometime between A.D. 1000 and A.D. 1200 and expanded rapidly thereafter (c.f., Kirch 2011). The initial migration to Hawai'i is believed to have occurred from Kahiki (the ancestral homelands of Hawaiian gods and people) with long distance voyages occurring fairly regularly through at least the 13th century. It has been generally reported that the sources of the early Hawaiian populations originated from the southern Marquesas Islands (Emory in Tatar 1982). In these early times, Hawai'i's inhabitants were primarily engaged in subsistence-level agriculture and fishing (Handy and Handy 1991). This was a period of

great exploitation and environmental modification when early Hawaiian farmers developed new subsistence strategies by adapting their familiar patterns and traditional tools to their new environment (Kirch 1985; Pogue 1978). According to Fornander (1969), the Hawaiians brought from their homeland certain Polynesian customs and belief: the major gods Kāne, Kū, Lono, and Kanaloa; the *kapu* system of law and order; the *pu'uhonua* (places of refuge), the *'aumakua* concept, and the concept of *mana*.

For generations following initial settlement, communities were clustered along the watered, windward (*Ko'olau*) shores of the Hawaiian Islands. Along the *ko'olau* shores, streams flowed and rainfall was abundant, and agricultural production became established. The *ko'olau* region also offered sheltered bays from which deep-sea fisheries could be easily accessed, and nearshore fisheries, enriched by nutrients carried in the fresh water, could be maintained in fishponds and coastal waters. It was around these bays that clusters of houses where families lived could be found (McEldowney 1979). In these early times, Hawai'i's inhabitants were primarily engaged in subsistence-level agriculture and fishing (Handy and Handy 1972). Following the initial settlement period, areas with the richest natural resources became populated and perhaps crowded, and by about A.D. 1200, the population began expanding to the Kona (leeward side) and more remote regions of the island (Cordy 2000).

As the population continued to expand so did social stratification, which was accompanied by major socioeconomic changes and intensive land modification. Most of the ecologically favorable zones of the windward and coastal regions of all major islands were settled and the more marginal leeward areas were being developed. During this expansion period, additional migrations to Hawai'i occurred from Tahiti in the Society Islands. Rosendahl (1972) has proposed that settlement at this time was related to the seasonal, recurrent occupation in which coastal sites were occupied in the summer to exploit marine resources, and upland sites were occupied during the winter months, with a focus on agriculture. An increasing reliance on agricultural products may have caused a shift in social networks as well; as Hommon (1976) argues, kinship links between coastal settlements disintegrated as those links within the *mauka-makai* settlements expanded to accommodate the exchange of agricultural products for marine resources. This shift is believed to have resulted in the establishment of the *ahupua'a* system sometime during the A.D. 1400s (Kirch 1985), which added another component to an already well-stratified society. The implications of this model include a shift in residential patterns from seasonal, temporary occupation, to the permanent dispersed occupation of both coastal and upland areas.

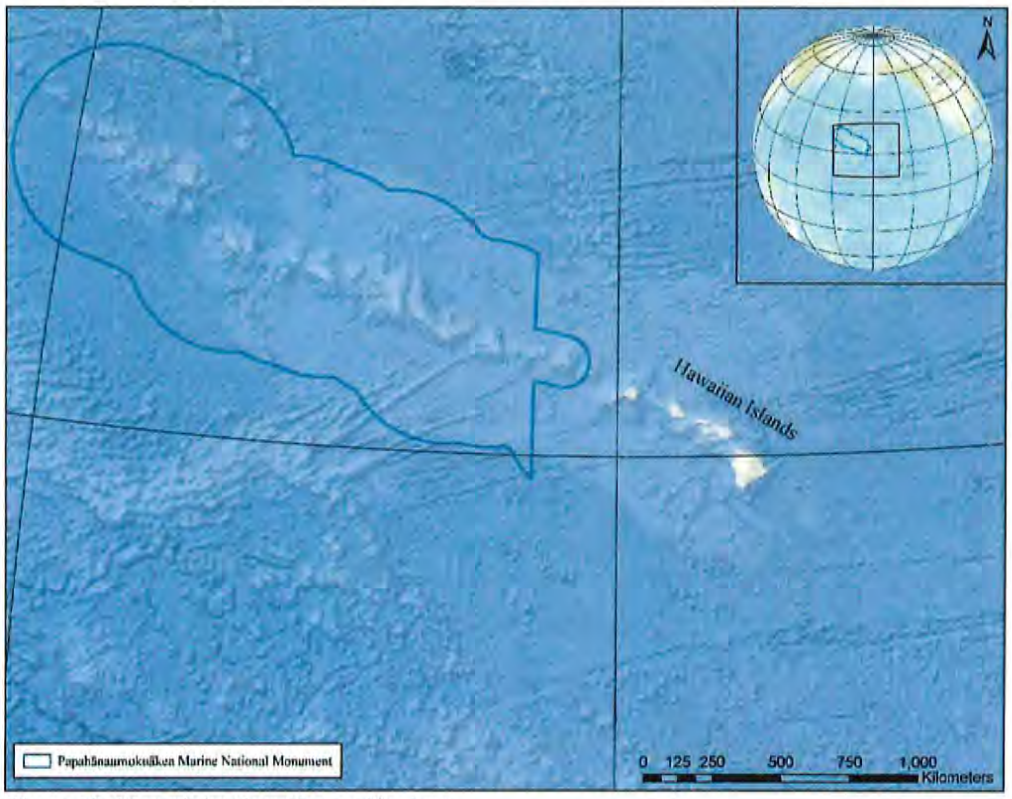

Figure 10. Map of the Hawaiian archipelago.

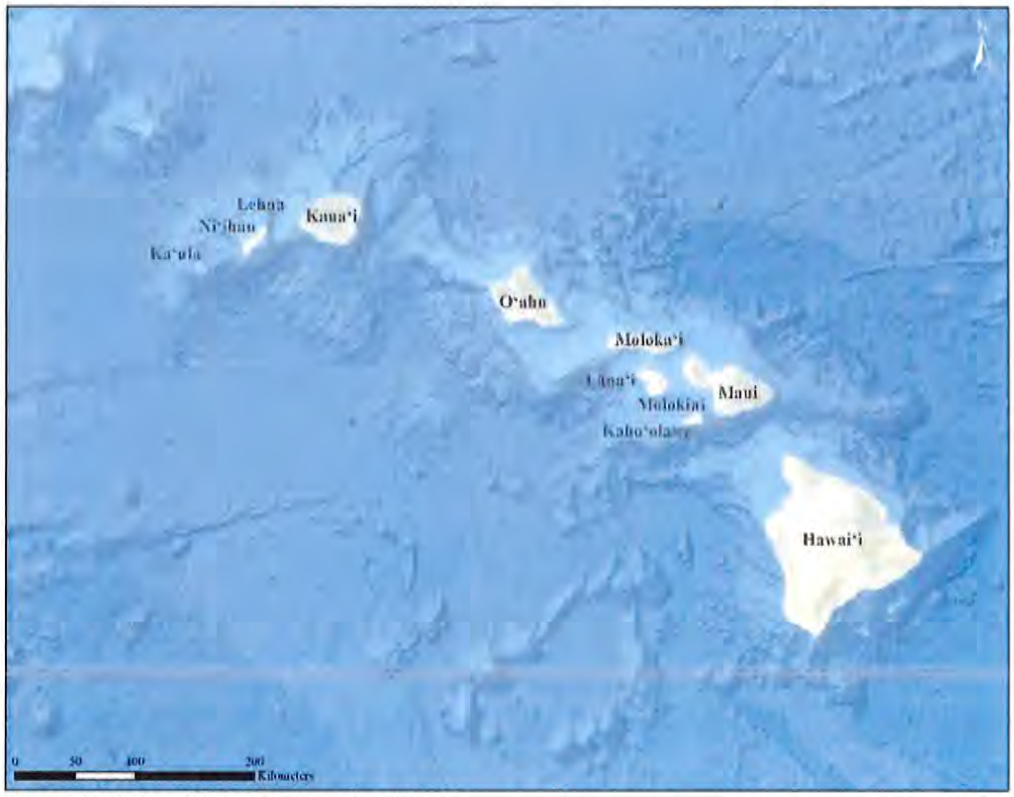

Figure 11. Map of the main Hawaiian Islands.

Adding to an already highly-complex society was the development of the traditional land division system, which included the *ahupua'a*—the principle land division that functioned for both taxation purposes and furnished its residents with nearly all of the fundamental necessities. *Ahupua'a* are land divisions that typically incorporated all of the eco-zones from the mountains to the sea and for several hundred yards beyond the shore, assuring a diverse subsistence resource base (Hommon 1986). Although the *ahupua'a* land division typically incorporated all of the eco-zones, their size and shape varied greatly (Cannelora 1974). The *hoa'āina* (native tenants) and *'ohana* (families) who lived on the land had rights to the gather resources for subsistence and for tribute (Jokiel et al. 2011). As part of these rights, the *ahupua'a* residents were also required to supply resources and labor that supported the royal community of regional and/or island kingdoms. The *ahupua'a* became the equivalent of a local community, with its own social, economic, and political significance and served as the taxable land division during the annual *Makahiki* procession (Kelly 1956). During this annual procession, the highest chief of the land sent select members of his retinue to collect *ho'okupu* (tribute and offerings) in the form of goods from each *ahupua'a*. The *hoa'āina* (native tenants) who resided in the *ahupua'a* brought their share of *ho'okupu* to an *ahu* (altar) that was symbolically marked with the image of a *pua'a* (pig). *Ahupua'a* were ruled by *ali'i 'ai ahupua'a* or chiefs who controlled the *ahupua'a* resources; who, for the most part, had complete autonomy over this generally economically self-supporting piece of land (Malo 1951). *Ahupua'a* residents were not bound to the land nor were they considered the property of the *ali'i*. If the living conditions under a particular *ahupua'a* chief were deemed unsuitable, the residents could move freely in pursuit of more favorable conditions (Lam 1985). This structure safeguarded the well-being of the people and the overall productivity of the land, lest the chief loses the principle support and loyalty of his or her supporters. *Ahupua'a* lands were in turn, managed by an appointed *konohiki* or lesser chief-landlord, who oversaw and coordinated stewardship of an area's natural resources (ibid.). In some places, the *po'o lawai'a* (head fisherman) held the same responsibilities as the *konohiki* (Jokiel et al. 2011). When necessary, the *konohiki* took the liberty of implementing *kapu* (restrictions and prohibitions) to protect the *mana* of the area's resources from physical and spiritual depletion.

Many *ahupua'a* were further divided into smaller land units termed *'ili* and *'ili kūpono* (often shortened to *'ili kū*). *'Ili* were created for the convenience of the *ahupua'a* chief and served as the basic land unit to which the *hoa'āina*, retained for often long periods of time (Jokiel et al. 2011; MacKenzie 2015). As the *'ili* themselves were typically passed down in families, so too were the *kuleana* (responsibilities, privileges) that were associated with it. The right

to use and cultivate *'ili* was maintained within the *'ohana*, regardless of any change in title of the *ahupua'a* chief (Handy and Handy 1991). Malo (1951), recorded several types of *'ili*: the *'ili pa'a,* a single intact parcel and the *'ili lele*, a discontinuous parcel dispersed across an area. Whether dispersed or wholly intact, the *'ili* land division required a cross section of available resources, and for the *hoa'āina*, this generally included access to agriculturally fertile lands and coastal fisheries. While much of the same resource principles applied to the *'ili kūpono*, these land units were politically independent of the *ahupua'a* chief. This designation was applied to specific areas containing resources that were highly valued by the ruling chiefs, such as fishponds (Handy and Handy 1991).

The *ali'i* who presided over the *ahupua'a* (*ali'i-'ai-ahupua'a*), in turn, answered to an *ali'i 'ai moku* (chief who claimed the abundance of the entire *moku or* district) (Malo 1951). Although *moku* (districts) were comprised of multiple *ahupua'a*, they were considered geographical subdivisions with no explicit reference to rights in the land (Cannelora 1974). This form of district subdividing was integral to Hawaiian life and was the product of resource management planning that was strictly adhered to. As knowledge of place developed over the centuries and passed down intergenerationally by direct teaching and experience, detailed information of an area's natural cycles and resources were retained and well-understood. Decisions were based on generations worth of highly informed knowledge and sustainably adapted to meet the needs of a growing population. This highly-complex land management system mirrors the unique Hawaiian culture that coevolved with these islands.

Evolution of Hawaiian Land Stewardship Practices and the Impacts on Hawai'i's Native Forests

Ancient and ingrained philosophy of life tied Hawaiians to their environment and helped to maintain both natural, spiritual, and social order. In describing the intimate relationship that exists between Hawaiians and *'āina* (land), Hawaiian historian and cultural specialist, Kepā Maly writes:

> In the Hawaiian context, these values—the "sense of place"—have developed over hundreds of generations of evolving "cultural attachment" to the natural, physical, and spiritual environments. In any culturally sensitive discussion on land use in Hawai'i, one must understand that Hawaiian culture evolved in close partnership with its' natural environment. Thus, Hawaiian culture does not have a clear dividing line of where culture and nature begins.
>
> In a traditional Hawaiian context, nature and culture are one in the same, there is no division between the two. The wealth and limitations of the land and ocean resources gave birth to, and shaped the Hawaiian world view. The *'āina* (land), *wai* (water), *kai* (ocean), and *lewa* (sky) were the foundation of life and the source of the spiritual relationship between people and their environs. (Maly 2001:1)

The Hawaiian *'ōlelo no'eau* (proverbial saying) "*Hānau ka 'āina, hānau ke ali'i, hānau ke kanaka*" (Born was the land, born were the chiefs, born were the commoners), conveys the belief that all things of the land including *kanaka* (humans) were literally born (*hānau*), and are thus connected through kinship links that extend beyond the immediate family (Pukui 1983:57). *'Āina* or land, was perhaps most revered, as another *'ōlelo no'eau* notes, "*He ali'i ka 'āina; he kauwā ke kanaka,*" which has been translated by Pukui (1983:62) as "The land is a chief; man is its servant." The lifeways of early Hawaiians, which were derived entirely from the finite natural resources of these islands, necessitated the development of sustainable resource management practices. Over time, what developed was an adaptable management system that integrated the watershed, freshwater, nearshore fisheries, all of which are connected through the many unique ecosystems that extend from the mountains to the sea (Jokiel et al. 2011).

Kilo or astute observation of the natural world became one of the most fundamental stewardship tools used by the ancient Hawaiians. The vast knowledge acquired through the practice of *kilo* enabled them to observe and record the subtlest changes, distinctions, and correlations in their natural world. Examples of their keen observations are evident in Hawaiian nomenclature, where numerous types of rains, clouds, winds, stones, environments, flora, and fauna, many of which are geographically unique, have been named and recorded in centuries-old traditions such as *oli* (chants), *mele* (songs), *pule* (prayers), *inoa 'āina* (place names), *'ōlelo no'eau* (proverbial sayings), all of which were transmitted orally through the ages. Other traditional Hawaiian arts and practices including, (but not limited to) *hula* (traditional dance), *lapa'au* (traditional healing), *lawai'a* (fishing), *mahi'ai* (farming) further reinforced knowledge of and connection to the natural environment.

Their exclusive dependency on a thriving natural environment led Hawaiians to develop a sophisticated and comprehensive system of land stewardship that was reinforced through the strict adherence to practices that maintained and enhanced the *kapu* and *mana* of all things in the Hawaiian world. In Hawaiian belief, all things natural, places, and even people, especially those of high rank, possesses a certain degree of *mana* or "divine power" (Pukui et al. 1972; Pukui and Elbert 1986:235). *Mana* is believed to be derived from the plethora of Hawaiian gods (*kini akua*) who were embodied in elemental forces and natural resources, such as the land, mountains, plants, animals, water and certain material objects and persons (Crabbe et al. 2017). Buck (1993) expanded on this concept noting that *mana* was

associated with "the well-being of a community, in human knowledge and skills (canoe building, harvesting) and in nature (crop fertility, weather, etc.)" (in Else 2004:244). Hawaiian cultural practitioner and conservation biologist, Sam Gon III adds that this belief "imposes familial responsibilities on people, and engenders respect and care for native plants and animals" (Gon III 2010:1–2)

To ensure the *mana* of the resources, certain places, and people remained protected from over-exploitation and defilement, *kapu* of various kinds were implemented and strictly enforced. According to Elbert and Pukui (1986:132) *kapu* are defined as "taboo, prohibitions; special privilege or exemption..." Kepelino (1932) notes that *kapu* associated with the gods applied to all social classes, while the *kapu* associated with the chiefs were applied to the people. As the laws of *kapu* dictated social relationships, it also provided "environmental rules and controls that were essential for a subsistence economy" (Else 2004:246). Juxtaposed to the concept of *kapu* was *noa*, translated as "freed of taboo, released from restrictions, profane, freedom" (Pukui and Elbert 1986:268). Some *kapu*, particularly those associated with maintaining social hierarchy and gender differentiation were unremitting, while those *kapu* placed on natural resources were applied and enforced according to seasonal changes. The application of *kapu* to natural resources ensured that such were resources remained unspoiled and available for future use. When the *ali'i* or the lesser chiefs (including *konohiki* and *po'o lawai'a*) determined that a particular resource was to be made available to the people, a decree was proclaimed indicating that *kapu* had been lifted, thereby making it *noa*. Although transitioning a resource from a state of *kapu* to *noa* allowed for its use, people were still expected to practice sustainable harvesting methods and pay tribute to the ruling chief and the gods and goddesses associated with that resource. *Kapu* were strictly enforced and violators faced serious consequences including death (Jokiel et al. 2011). Violators who managed to escape death sought refuge at a *pu'uhonua*, a designated place of refuge or sometimes were freed by the word of certain chiefs (Kamakau 1992). After completing the proper rituals, the violator was absolved of his or her crime and allowed to reintegrate back into society.

This ancient and ingrained way of life underwent serious transformations following the arrival of Captain James Cook in 1778. This year marks the end of what is often referred to as Hawai'i's Precontact Period and the beginning of the Historic Period. While this time mark signifies an important date in Hawaiian history, it is vital to note that throughout the early Historic Period, even with Western influences, the Hawaiian chiefs still held outright rule over the land and its resources and maintained strict adherence to the *kapu* system—the very system from which their power was derived. For many Hawaiian historians, the abrogation of the *kapu* system in 1819, also marked significant socio-religious changes. Some scholars have argued that the abolishment of the *kapu* system undermined the very foundation upon which traditional Hawaiian society was built, ultimately altering the relationship between the chiefs and the people as well as their relationship to the land (Else 2004; Kame'eleihiwa 1992). At the outset of the Historic Period, there was a continued trend toward craft and status specialization, intensification of agriculture, *ali'i* controlled aquaculture, the establishment of upland residential sites, and the enhancement of traditional oral history. The veneration of traditional gods and the strict observation of the *kapu* system were at their peaks (Kent 1983; Kirch 1985). With the influx of foreigners, many of whom were quick to introduce the idea of trade for profit, Hawai'i's traditional culture, and the socio-political economy began to shift to meet the growing demands of the foreign populations.

The Arrival of Foreign Plants and Animals and the Transformation of the *Kapu* System

By the time Kamehameha had conquered O'ahu, Maui, and Moloka'i, in 1795, Hawai'i saw the beginnings of a market system economy and the work of the native tenants shifted from subsistence agriculture to the production of foods and goods that could be traded with early explorers and whalers (Kent 1983). Introduced fruit trees and garden vegetables, often grown for trade with Westerners included yams, coffee, melons, Irish potatoes, Indian corn, beans, figs, oranges, guavas, and grapes (Wilkes 1845). Animals such as goats, sheep, pigs, cattle, horses, and turkeys that were left by Cook and other early visitors between 1778 and 1803 were allowed to roam freely (Kuykendall 1938). Of all the foreign introductions, cattle had the most profound impact. Setting the foundations of Hawai'i's livestock industry, in 1793, Captain George Vancouver, who had visited the islands during Cook's 1778 voyage, gifted the first cattle to Kamehameha. The lack of quality cattle feed proved to be detrimental to the animals. To combat this, Kamehameha, at the demand of Captain George Vancouver, enforced a *kapu*, which lasted until the 1830s that prohibited the killing of the animals (Bergin 2004; Kuykendall 1938). The first head of steer and sheep that were gifted by Vancouver were driven into the upland plains of Waimea on Hawai'i Island and allowed to roam and multiply (Barrera 1983). The unrestrained populations of cattle had increased significantly and by the 1830s had become a nuisance to native farmers. Additionally, the environmental degradation of the native forests had become apparent to Kamehameha's sons and heirs who began to take steps to control the ravenous cattle population. In an effort to protect their crops, and to reduce the risk of encountering the large and often dangerous animals, native farmers began constructing taller enclosures to prevent the animals from plundering their gardens and destroying their homes. On Hawai'i Island, where

cattle populations are said to have numbered in the tens of thousands, tall rock walls that stretched for miles were built around the more densely populated areas (Bergin 2004). While the introduced plants and animals contributed to the development of Hawai'i's early market economy, the exportation of native hardwoods, particularly *'iliahi* or sandalwood compounded the preexisting environmental degradation and wreaked havoc on the native lifeways.

The *'iliahi* or sandalwood (*Santalum ellipticum*) trade established by Euro-Americans in 1790 quickly turned into a viable commercial enterprise (Oliver 1961). By 1810, and with the backing of Kamehameha and other chiefs, this industry flourished, as farmers and fishermen were ordered into the mountains of their district to cut sandalwood and carry it to the coast. Although the laborers were compensated with *kapa* (material), food and fish (Kamakau 1992), the neglect of their personal subsistent duties lead to food shortages and famine. The harsh working conditions coupled with lack of nutrition severely degraded the health and *mana* of the native people, ultimately contributing to a population decline. This industry also began to erode the relationship between the *ali'i* and the common people (Else 2004). Kamakau (ibid.:204) described the collapse of a traditional subsistence system and the industry's detrimental effects on the people: "...this rush of labor to the mountains brought about a scarcity of cultivated food . . . The people were forced to eat herbs and tree ferns, thus the famine [was] called Hi-laulele, Haha-pilau, Laulele, Pualele, 'Ama'u, or Hapu'u, from the wild plants resorted to." Once Kamehameha realized the dire effects this industry on his people, he "declared all the sandalwood the property of the government and ordered the people to devote only part of their time to its cutting and return to the cultivation of the land" (ibid.: 1992:204). Kamehameha also proclaimed sustainable harvesting strategies as noted by Kamakau, who wrote, "He ordered the sandalwood cutters to spare the young trees and, not to let the felled trees fall on the saplings" (ibid.:209-210).

On May 8th, 1819, Kamehameha, who had seen the onset of impacts brought about by foreign introductions, died at his royal residence at Kamakahonu in Kailua-Kona and named his son 'Iolani Liholiho heir to his kingdom (Kamakau 1992). By May 21st 'Iolani Liholiho (Kamehameha II) at the age of twenty-one began his rule. As traditional custom dictated and to allow for all people to rightfully mourn the loss of their chief, all *kapu* were relaxed following the death of a chief (ibid.). It was the responsibility of the new ruler to conduct the proper rituals and ceremonies to reinstate all *kapu.* However, Liholiho's attempts to reinstate the long-standing *kapu* system was futile and the future of the *kapu* system stood in a state of uncertainty. *Kuhina Nui* (Premier), Ka'ahumanu (the wife of Kamehameha and the *hānai* (adopted) mother of Liholiho) and his biological mother Keōpūolani lured the young chief back to Kona and the *kapu* system was symbolically abolished when Liholiho ate in the presence of his mothers. While Liholiho, his mothers and other chiefs favored the complete abolishment of the *kapu* system, others including Kekuaokalani and his followers prepared to wage war, determined to have the ancient laws reinstated. After several failed attempts at negotiation, Liloliho's army led by Kalaimoku went head-to-head against the forces of Kekuaokalani in the Battle of Kuamo'o (Fornander 1918–1919). Western weaponry had already permeated traditional Hawaiian warfare and Kekuaokalani, who stood behind the ancient laws of the land was killed by gunfire on the battlefield alongside his wife Manono, thereby extinguishing the last public display of resistance. The abolishment of the *kapu* system in 1819, began to undermine the very foundations upon which traditional Hawaiian culture was formed. Adding to an already socio-politically fractured society was the arrival of Protestant missionaries who sought to fill the spiritual void of the Hawaiian people.

In October of 1819, just five months after the death of Kamehameha, the first American Protestant missionaries aboard the Brig. *Thaddeus* left Boston, Massachusetts and by March 30th, 1820, sailed to Kawaihae on the northwest coast of Hawai'i Island (Hawaiian Mission Children's Society 1901). Having heard of the overturning of the ancient *kapu* system, these early missionaries formed close alliances with some of Hawai'i's royalty, including Ka'ahumanu who held a tremendous amount of political power. Starting in 1823, these early missionaries, one of which included William Ellis (1917) set out into the remote parts of the islands in search of suitable locations for future mission stations and within a few short years, mission stations were being constructed outside of the main town centers. Christian beliefs quickly spread and soon established a firm foothold in the islands. The missionaries quickly discovered that many Hawaiians were selective about what aspects of Christianity they were willing to adopt. In striving for complete conversion, the missionaries with the help of the *ali'i* implemented laws that enforced Euro-American beliefs on the Hawaiian people. To an extent, this furthered the efforts of the missionaries. Despite these massive cultural changes, many Hawaiians continued to hold to their ancient beliefs, especially those associated with their relationship to the land. Throughout the remainder of the 19th century, introduced diseases and global economic forces continued to degrade the traditional life-ways of the Hawaiian people.

Private Property and Its Effects on Traditional Concepts of Land and Land Use Practices

By the mid-19th century, the ever-growing population of Westerners in the Hawaiian Islands forced socioeconomic and demographic changes that promoted the establishment of a Euro-American style of land ownership. By 1840, the first Hawaiian constitution had been drafted and the Hawaiian Kingdom shifted from an absolute monarchy into a

constitutional government. Convinced that the feudal system of land tenure previously practiced was not compatible with a constitutional government, the *Mō'ī* Kauikeaouli and his high-ranking chiefs decided to separate and define the ownership of all lands in the Kingdom (King n.d.). The change in land tenure was further endorsed by missionaries and Western businessmen in the islands who were generally hesitant to enter business deals on leasehold lands that could be revoked from them at any time. The push for exclusive private property rights culminated in the *Māhele 'Āina* of 1848 and the subsequent *Kuleana* Act or Enabling Act of 1850.

While the formalization of private property rights was a success for many Westerners, this ultimately led to the displacement of many Hawaiians from their ancestral lands—lands that they had come to know so intimately. In general, although many Hawaiians were awarded lands during this period, it was realized that the parcels they were awarded were insufficient to sustain their traditional subsistence lifestyles. Additionally, access to resources that were once a part of the now fragmented *ahupua'a* system further curtailed traditional subsistence activities. As many Hawaiian continued to migrate to the populated centers around the islands and even elsewhere, large tracts of land that were once dotted with small communities and extensive traditional agricultural fields were being prospected for large scale commercial agriculture and ranching. Although these industries added to the cultural tapestry of the islands, such operations required vast amounts of land and water. The mass acquisition of land and the diversion of water from their natural courses during the 19th and 20th centuries resulted in numerous court battles between Western businessmen competing to increase their operations and native Hawaiians who willfully held to their traditional lifeways. Such issues continue to be vetted in Hawai'i courtrooms.

Formerly forested lands were being grazed down and, in some places, planted with introduced species of grass and various shrubs to form natural fencing and to be used as livestock feed (Henke 1929). In the drier leeward area of Hawai'i, the planting of *kiawe* or algaroba (*Prosopis robusto*) proved to be useful for the cattle and apiary industry (ibid.). By the mid-19th century, the apparent destruction of native forest habitat had severely diminished the water supply of islands, ultimately prompting action by the Hawaiian Kingdom government. In 1876, the Kingdom legislature under the administration of King David Kalākaua passed "An Act for the Protection and Preservation of Woods and Forests" (Planters' Labor and Supply Company 1887:438)." Between 1876-1910, uncoordinated efforts between the government and various agricultural sectors were undertaken to remedy the loss of native forests and to increase water supply (Cannarella 2010). Wild ungulates were removed from some native forests habitats—an effort that began in the 1830s—and efforts to fence off sections of intact forests set the foundation for Hawai'i's forest reserves. To replenish severely degraded forests, a large number of non-native species were experimentally planted, including, *paina* or ironwood (*Casuarina equisitifolia*), silver oak (*Grevillea robusto*), wind acacia, sour plum, and a number of other species (Henke 1929). Efforts to diversify the Kingdom's economy and the long-standing trend of introducing exotic plant and animal species to the islands continued to mount.

The introduction of large-scale planting of sugar cane during the mid- to late-19th century resulted in massive land clearing efforts around the islands. The success and growth of the sugar industry within the more arid parts of the islands was highly dependent upon an ample supply of irrigation water (Wilcox 1996). Occasional wildfires and pests such as the leafhopper threatened the burgeoning sugar industry (Campbell and Ogburn 1990). To ensure economic prosperity, these sugar companies invested in experimental agriculture. New varieties of cane collected from various parts of the world were introduced without restraint and tested to meet the climatic challenges of growing cane in Hawai'i. By the 1890s, under the administration of King David Kalākaua, efforts to regulate plant and animal imports, many of which carried pests that were unknown to the islands, had become a priority for the Hawaiian Kingdom government.

HISTORY OF BIOCONTROL IN THE HAWAIIAN ISLANDS

The use of classical biocontrol, "the suppression of pest populations by introduction and liberation of natural enemies," has been actively undertaken in the Hawaiian Islands for roughly 130 years with varying degrees of success (Funasaki et al. 1988:105; Lai 1988). Throughout the latter half of the 19th century, as the Hawaiian Islands became an agricultural hotspot for sugarcane and other crops, many new plant species, some carrying insect pests, were introduced without restraint. In 1890, the Hawaiian Kingdom Government, under the administration of King David Kalākaua established the Commissioners of Agriculture to prevent unwanted immigrant pests from entering the islands, and to control those that had already been introduced. The duties of the Commissioners were detailed in Chapter II of *Session Laws of 1890*. Chapter II titled "An Act Relating to the Suppression of Plant Disease, Blight, and Insect Pests" reads:

> SECTION 2. It shall be the duty of such Commissioners to seek to prevent the introduction into this Kingdom of any plant disease, blight, or insect pests injurious to any tree or trees, plant or plants,

> or vegetation; and to seek to exterminate any such diseases, blight or insect pests now existing or hereafter introduced.
>
> They shall have the power to enter upon any premises where they have reason to believe there is any tree, plant, or vegetation affected with any disease, blight, or insect pest; and to take all reasonable and proper steps to prevent the spread of any such disease, blight or insect pest, and if after due trial (such trial to be not longer than ten days) it is found by said Commissioners, or one of them, that the trees, plants or vegetation cannot be cured, or the blight destroyed, that then an in such case he or they may order the same destroyed. (Kalakaua 1890:4–5)

The initiation of the 1890 laws was in response to unregulated efforts to control pests—an act that prior to 1890 was being initiated by private citizens. The earliest accounts of the unregulated use of biocontrol can be traced back to 1865, when Dr. William Hillebrand, a physician, and naturalist brought the mynah bird (*Acridotheres tristis*) from India to Hawai'i to control armyworms that were infesting Hawai'i's pastures (Funasaki et al. 1988). Because of the mynah bird's appetite for rotting and decomposed things, and for its use of garbage as nesting material, the bird was given the Hawaiians name of "*manu-'ai-pilau,*" which can be translated as the bird that consumes rotten things (Pukui and Elbert 1986:486). The mynah bird is also known in Hawaiian as "*piha'ekelo*", literally translated as "full of 'ekelo sound," a name given because of its raucous nature (ibid.:326). The debate over whether the introduction of the mynah bird was successful in controlling army worms spilled over into local newspapers. Proponents of the mynah bird emphasized its success, however, others alleged that such comments poorly represented the birds' impacts to agriculture and to the people. An article published in *The Pacific Commercial Advertiser* in 1876 challenged some of the alleged successes:

> THOSE CATERPILLARS.—The *Gazette* says that owing to the large increase of *mynah birds*, "not a caterpillar is to be seen in this regions," (Honolulu) while at points outside of this favored range of the birds the grass has been destroyed. This would be a very pretty and pleasing statement in favor of the usefulness of the *mynahs*, if it were true, as unfortunately it is not. Right here and now, in the immediate neighborhood of the city, on the plains and elsewhere the birds abound, caterpillars do much more abound,—in such immense quantities that it would be simply impossible for the former to make any perceptible impressions on the mass. No doubt the *mynah* would not refuse a fat caterpillar now and again; but we don't believe they prefer them as a regular diet, for the bird is something of an epicure and delights to range from stolen beefsteak to a nest of pigeon's or dove's eggs. Chickens are very good at destroying the vermin, so far as their capacities go; and turkeys are better. But the plague is usually of but brief duration. (The Pacific Commercial Advertiser 1876:3)

Complaints of the mynah bird attacking people and livestock filled the local newspapers throughout the late 19th century. The noisy mynah bird had become such a nuisance to the residents of Honolulu that some people took to the city with guns to exterminate the birds. The mynah bird proponents fired back and proposed a law that would prevent the killing of the birds. An article written in the November 9th, 1894, issue of *The Hawaiian Star* blamed the mynah bird and the dove for aiding in the spread of another noxious introduction, *Lantana camara*, which was brought to the islands from "tropical America in the year 1858" (The Hawaiian Star 1894:3).

During Hawai'i's sugar plantation era, rats had become a serious pestilence to sugar plantation owners and considerable attempts to bring Hawai'i's rat population under control were being actualized. An article published in the March 31, 1883, edition of *The Pacific Commercial Advertiser* details the proposed introduction of the infamous mongoose (*Herpestes javanicus*), a native of India to Hawai'i's cane fields:

> THE Planters' Monthly has lately been proposing the introduction of a little animal from India called the mongoose, as a destroyer of rats. He is a famous ratter, surpassing the cat or the ferret. He is described as a lively little urchin, about the size of a weasel, as having a snaky body, vicious looking claws, a sharp nose, a villainous eye and looks like "murder incarnate." In speaking of his action in capturing rats, it is said that he crawls sinuously up to his victim until within easy distance for a rush, and then strikes with unerring aim, snapping rats just at the base of the brain. The rat has not time even to squeak, so sudden and deadly is the onslaught. Wherever the rat can enter the mongoose can follow. Thus as a ratter this lively little Indian is incomparable, but the trouble is he will not confine his operations to what is deemed his legitimate business. Some writers have endeavored to save his credit as a poultry destroyer, but a naturalist, who has carefully observed his characteristics, says that he is a general destroyer, not only of everything under, but of many creatures over his size. When in a cage the sight of a small living creature made him frantic and whenever he escaped, as he sometimes did, he made a sensation in the poultry house. The mongoose is not content with marauding forays in the yard, but he seems to pervade the house when domesticated...The rat is

> unquestionably a great pest of the cane and rice planter and grain cultivator in all parts of the world. The rat pest was deemed so serious here some fifty years ago that an enlightened and enterprising Commissioner of the Hawaiian Government, sent inquest of Chinese...to procure a species of snake famed as a destroyer of rats; but the Hawaiian people, whose sacred soil had been kept free from snakes and toads by some patron saint equal in influence to St. Patrick, conceived a holy terror of the snake, notwithstanding his possible utilities, and passed a decree that Hawaii would have no snake in her plantations. The destruction of rats in the cane-fields was hardly deemed a sufficient compensation to the Hawaiian mind for the probable presence every now and then of his snakeship in the thatch of the Hawaiian *hale pili*...(The Pacific Commercial Advertiser 1883:2)

By September of 1883, Mr. William H. Purvis, a plant collector and investor in the Pacific Sugar Mill at Kukuihaele on Hawai'i Island, imported seven mongooses, fowls, and exotic plants from Australian colonies (Daily Honolulu Press 1883). The imported mongooses were "...intended for the damp lands of the Kukuihaele plantation at Hamakua..." (ibid.:4). A number of *'iole manakuke* or mongooses, were liberated in the cane fields of both Hilo and Hāmākua (Funasaki et al. 1988; Pukui and Elbert 1986). Subsequently, in 1885, mongooses were released on Maui, Moloka'i, O'ahu, and Kaua'i. While mongoose populations had quickly established themselves on Maui, Moloka'i, and O'ahu, to date, the mongoose has not established itself on Kaua'i. Both introductions rapidly multiplied and spread beyond their intended target species. While the introduction of the mongoose appears to have some success in combatting the rodents, their impacts were highlighted in newspaper editorials as early as 1886, from writers complaining that the mongooses were becoming a pest in their own. One such article read:

> The mongoose is a useful little creature for the destruction of rats. He was brought here for that purpose, and, we believe, had done his work thoroughly well on several plantations. But the mongoose does not confine himself to rats, and complaints come from some quarters that ducks and chickens are being destroyed by wholesale. The mongoose may ultimately prove to be a greater nuisance than a benefit. (The Daily Bulletin 1886:2)

By the late 19th-century, the mongoose had become a sort of cultural symbol. A review of newspaper articles published in Hawai'i during this period reveals that the mongoose was often used metaphorically to refer to people or things that exhibited wild behavior and for people who came to the islands without having any intent to leave. However useful these introductions were in controlling its intended target, over time, their unintended impacts had become obvious. In its wake, the mongoose destroyed livestock, the eggs of native bird species, and the noisy mynah bird is associated with aiding in the proliferation of the noxious weed, *Lantana camara* (Funasaki et al. 1988). These early and poorly thought out introductions are what Funasaki et al. (1988:106) described as a classic example of "biological control gone astray." Funasaki et al. (ibid.) emphasize that:

> However, it must be realized that prior to 1890, planning and evaluation before the introduction of any organism were nonexistent simply because they were not required. There were no laws or regulations restricting or prohibiting the importation of any plant or animal from other geographical areas into Hawaii.

While these early introductions appear to have been a practical solution to a growing problem, ultimately, the lack of regulation, adequate pre-release testing protocols, and post-release monitoring created even more problems for Hawai'i's environment and people. In response to these ill-fated early and unregulated releases, Hawai'i's government leaders began to formalize a plan that would limit the introduction of unwanted pest species and control those that had already been introduced.

Regulated Efforts to Control Unwanted Pest in Hawai'i

By the late 19th century, efforts to study the natural enemies of unwanted pests that were impacting Hawai'i's agricultural industry were being formalized. In 1893, the year of the unlawful overthrow of Queen Lydia Lili'uokalani, the provisional government of the Republic of Hawai'i appointed Albert Koebele as the entomologist to biologically control the many species of immigrant pests (Funasaki et al. 1988). Koebele is credited with being "one of the first, if not the very first entomologist, to engage in the introduction of natural enemies as a method of combating insect pests" (Giffard et al. 1925:340). Between 1893 and 1910, Koebele spent much of his time traveling to places like Australia, Fiji, Japan, China, Ceylon (modern-day Sri Lanka), Mexico, and California where he studied various insects that he thought would be beneficial to combat pests that were introduced to the islands. In 1893, Koebele successfully used biocontrol to combat the cottony cushion scale (*Icerya purchasi*) . In summarizing Koebele's biological introductions to the Hawaiian Islands, Giffard et al. (1925:342) remarked:

> He made the beginning in this line of work, and much of the time was working alone, yet seventeen species of lady beetles were successfully introduced by him and have become valuable factors in

> keeping reduced such pests as scale insects, mealybugs, plant lice and leaf mites. At least six other lady beetles were introduced and became established, but after a few years disappeared. The eight lantana insects were introduced by him, and about the same number of miscellaneous parasites of Diptera and Lepidoptera, etc. Following Mr. Koebele in this line of work, the other entomologists have introduced a larger number of beneficial insects, and some of them have produced more spectacular and valuable results, but this should not in any way lessen the credit to be given to him who was the pioneer in Hawaii in this important branch of entomological work.

Encouraged by Koebele's successes, in 1903, the Territorial Government (formalized in 1898), enacted laws to create the Board of Commissioners of Agriculture and Forestry (the precursor to the Hawaii Department of Agriculture (HDOA)). These early laws provided for facilities and materials "to obtain, propagate, study, and distribute beneficial species of insects to control pest species of insects and weeds" (Funasaki et al. 1988:107). Additionally, a quarantine system to prevent new immigrant pests from entering the islands was also created. Another early organization responsible for the release of a number of biological control agents was the Hawaiian Sugar Planters' Association (HSPA), founded in 1895. In 1904, HSPA instituted an Entomology branch and from its founding to about 1942, this branch aided in combatting a variety of pests that were plaguing Hawai'i's cane fields and threatening the economic promise of the sugar industry (ibid.). Throughout the early to mid-20th century, as Hawai'i's agricultural interest grew to include pineapple and other tropical fruit, additional institutions were organized to study and combat its share of pests. Such organizations included the United States Bureau of Entomology and Plant Quarantine's Fruit Fly Laboratory (now U.S. Department of Agriculture's Tropical Fruit and Vegetable Research Laboratory), Experiment Station of the Pineapple Producers Cooperative Associations, HSPA's Experiment Station, Hawaii Agricultural Experiment Station of the University of Hawaii's College of Tropical Agriculture, the California Agricultural Experiment Station of the University of California, and the Hawaii Department of Health (ibid.). By the 1940s and 1950s, the creation and introduction of chemical pest control had become the favored alternative (Howarth 1983). While chemical pest control still maintains its place in managing unwanted pests, the environmental and health risks associated with its use has led to the adoption of stricter regulations and a push towards finding more natural and low-cost alternatives (ibid.).

Collectively, the laws passed in 1890 to regulate unwanted immigrant pests set the foundation for what is known today as Hawai'i Revised Statutes (HRS) Chapter 141, which governs the State of Hawai'i, Department of Agriculture (HDOA)—the state agency responsible for protecting and diversifying Hawai'i's agricultural industry. HDOA's Plant Industry Division maintains three branches: Pesticides Branch, Plant Pest Control Branch, and the Plant Quarantine Branch that collectively work "to protect Hawaii's agricultural industries, environment, and [the] general public by preventing the introduction and establishment of harmful insects, diseases, illegal non-domestic animals, and other pests..." (Department of Agriculture 2016). In 2003, under Hawai'i Revised Statutes (HRS), Chapter 194, the State of Hawai'i legislature authorized the creation of the Hawai'i Invasive Species Council (HISC), the agency responsible for coordinating efforts between various local, state, federal, and international agencies and organizations to stop the introduction and spread of invasive species in the islands (State of Hawai'i 2005). Since the creation of the HISC, millions of dollars have been allocated to various local councils and government departments and programs to combat invasive species. Efforts have been directed at prevention, response and control, research and technology, and outreach (ibid.). There are four invasive species committees that represent each of the four counties (Kaua'i, O'ahu, Maui, and Hawai'i Island) in addition to an aquatic invasive species team (ibid.).

Historically, Hawai'i's biological control programs were aimed at controlling weeds and pests that were adversely impacting the agricultural industry. During the 1970s and 1980s, the heightened interest in native and endemic taxa, fueled by the passing of federal legislation to protect endangered plants coupled with the growth of native-plant organizations has led to greater consideration of the potential risk of introduced biological control agents on endemic taxa (Pemberton 2004). Hawai'i as a "hub for tourism, trade, and military transport" and the state's continued reliance on globally imported goods perpetuates the ongoing assault of introduced foreign species (Messing and Wright 2006). Funasaki et al. (1988:108) report that "more biological control projects against immigrant species of insect pests have been conducted in Hawaii than anywhere else in the world" and nearly a third of the introduced species (roughly 200 pest species) are known to be established. Reimer (2002:86) reports that "many of these introductions appear to have been successful in that the pest populations eventually did drop to acceptable levels, although scientific evaluations of the effectiveness of these introductions have been virtually non-existent." The lack of natural enemies to combat such pests has propelled state agencies, namely HDOA to continue to identify the pests' natural enemies and to develop stringent host-range testing protocols for the study and release of such agents. Although the application of classical biocontrol in Hawai'i has, at times proven to be economically successful, it is recognized that environmental risks are inherent in biological control programs (Holland et al. 2008; Howarth 1983; Pemberton 2004).

Historically, several individuals and agencies have participated in the study and release of biocontrol agents in the Hawaiian Islands. Today, the U.S. Department of Agriculture-Animal Plant Health Inspection Service-Plant Pest Quarantine (USDA-APHIS-PPQ) and the HDOA regulates the importation of biocontrol agents (Reimer 2002). While these agencies have distinct mandates and jurisdictions, there is some overlap with respect to the regulated use of biocontrol. Efforts to improve pre-release testing has resulted in a federal and state permitting process which includes an environmental review. In summarizing this process, Reimer (ibid.:87) writes:

> All biocontrol agents imported for weed control attack plants and are by definition plant pests. They are, therefore, regulated by USDA.
>
> The USDA requires separate permits for
>
> 1) Importation of a plant pest into the U.S.;
>
> 2) Movement of a plant pest between States; and
>
> 3) Release of a plant pest into the environment.
>
> The federal permitting process requires the submission of PPQ Form 526 (Application for Release) that is forwarded to the HDOA for review and recommendations. All applications to date, for which HDOA has recommended rejection, have also been denied by the USDA. If approval is recommended by HDOA, USDA then reviews the application. This process usually involves review by the Technical Advisory Group; however, Hawai'i applications are exempt from TAG review due to the thoroughness of the HDOA review process. A draft environmental assessment (EA) is requested from the applicant for any requests for the release of weed biocontrol agents. The USDA prepares the final EA. If endangered or threatened species potentially are affected by the release of a biocontrol agent then the application is sent to the U.S. Fish and Wildlife Service for review. A release permit is issued if the evaluation of the EA produces a finding of no significant impact (FONSI).

While there are some similarities between the federal and state process, Chapter 150A of the Hawai'i Revised Statutes (HRS) regulates the importation of any plant or animal into the State of Hawai'i whether or not it is a plant pest (Reimer 2002). HRS 150A strictly prohibits the importation of all non-domestic animals and microorganisms unless approval is obtained by the Board of Agriculture. The review process for a state importation permit application involves six steps. Reimer (ibid.:88-89) provides a synthesis of the six-step process:

> First, the application is submitted to the HDOA with all of the required and pertinent information, including information on host specificity, distribution, preferred habitat, temperature requirements, etc. Host specificity studies may be carried out either in the country of origin or in one of the three approved containment facilities in Hawai'i. The Advisory Subcommittee then reviews the application. The recommendations from this subcommittee are passed on to the Plants and Animals Committee for their recommendations to the BOA. The BOA either approves or disapproves the application. If approved, the application is submitted to a public hearing process. Comments from the public are brought back to the BOA for discussion, followed by final approval or disapproval of the application. If approved, a State permit is issued. The organism may be imported and released if both State and Federal permits have been issued and permit conditions are met by the importers.
>
> The HDOA review process for the introduction of biocontrol agents has evolved into an effective system that screens agents for host specificity and potential negative impacts on other species. None of the agents introduced since the review process was initiated in 1975 have attacked any native or beneficial plant or animal species. This was not the case before 1975.

Additionally, efforts to improve public transparency following the decision rendered by the Hawai'i Intermediate Court of Appeals (*Ohana Pale Ke Ao v. Board of Agriculture, State of Hawaii*, 118 Hawaii 247, 249-50, 188 P.3d 761, 763-64 [Hawaii Ct. App. 2008]) has made the HDOA recognize that such biocontrol activities are subject to Chapter 343, Hawai'i Revised Statutes (Hawai'i Environmental Policy Act, HEPA) (Holland et al. 2008). Between 1890 and 1999, a total of 708 natural enemies have been released in Hawai'i, of which 286 have become established and the majority (237) of the introduced agents have contributed to the control of the target pest species (Reimer 2002). Prior to 1944 (before the formalization of the BOA), only 54% of the introduced agents were host-specific. This percentage has increased over the years with 77% host specificity being reported between the years 1944-1975. Since 1975, host specificity for all released biocontrol agents increased to 100% (ibid.). While stricter regulations have been adopted and modified over the years to reduce the environmental risk associated with the use of biological control agents, continued field research and open dialogue remains as a critical component to improving our understanding and mitigating the environmental, economic, and cultural risks associated with such actions.

INTRODUCTION OF *TIBOUCHINA HERBACEA* TO THE HAWAIIAN ISLANDS

While it is not known whether *T. herbacea* was intentionally or accidentally introduced to the islands, it was recorded first in 1977, growing on Saddle Road on Hawai'i Island—an important route connecting east and west Hawai'i. In 1982, the first specimens were collected at Lanilili in West Maui and at the Ko'olau Forest Reserve in East Maui (Almasi 2000). Infestations of *T. herbacea* were also found in Kīpahulu Valley between the 600-5,500 foot elevation. Nearly ten years later, populations of *T. herbacea* were reported on Lāna'i Island, and in 2003, this plant was observed at Hīpuapua Falls in Hālawa Valley on the east end of the island of Moloka'i. In 2008, a few plants were discovered by the O'ahu Army Natural Resources Program at Poamoho in the Waialua District along the leeward side of the Ko'olau Mountain Range on the island of O'ahu (Frohlic and Lau 2007). Several plants were also found growing above the H-3 tunnel in Hālawa Valley, "which was apparently landscaped after construction of the tunnels" (ibid.:10). It is believed that seeds of *T. herbacea* arrived on infested *hāpu'u* (*Cibotium spp.*) ferns that were transported from an off-island area, which were used to landscape the tunnel entrance (ibid.). These plants were removed after their discovery. Of the five islands in which this plant is known, it has become naturalized on the islands of Hawai'i and Maui where it forms dense thickets and is now beyond the scope of eradication (O'ahu Invasive Species Committee 2016).

Ecological and Cultural Impacts of *T. herbacea*

T. herbacea is known to threaten critical watershed habitat where numerous endemic and highly vulnerable plants and animals are found. On the islands of Maui and Hawai'i, this highly invasive plant is known to form dense thickets that crowd out and suppress native plant growth, including the *'ōhi'a* (*Metrosideros polymorpha*) (O'ahu Invasive Species Committee 2016). On the island of Maui, *T. herbacea* is scattered through some 50,000 acres of ecologically important watershed land in West Maui (Strohecker 2018). It can be found from sea level to the summit of Pu'u Kukui and thrives in the wet windward regions between 2,000-4,000 feet elevation (ibid.). The steep and treacherous terrain has made control of this plant nearly impossible on Maui (ibid.). At Poamoho in the northern Ko'olau Mountains Range of O'ahu, where populations of *T. herbacea* remain somewhat manageable, this plant continues to threaten many animals and plants many of which have a federal protection status. In their 2016 report, the O'ahu Invasive Species Committee (OISC) informed that *T. herbacea*:

> ...poses a major threat to Ko'olau forests, especially the near-pristine summit regions, as it thrives in wet forest conditions, produced hundreds of tiny seeds and is spread by broken stems or via wind, birds, and pigs. We suspect that the population at Poamoho was accidentally introduced by hikers that had recently been hiking on Maui or Hawai'i Island. Plant material capable of reproducing can be carried on shoes, clothes, and backpacks. (O'ahu Invasive Species Committee 2016:1)

Since its discovery near the summit area of Poamoho, continued monitoring led to the discovery of this plant's spread downstream from its known historical point. In 2015, with additional funds, OISC was able to increase its control efforts at Poamoho. With the increased manpower to survey and control populations of *T. herbacea* at Poamoho, the OISC field crew has discovered more plants in the Punalu'u watershed area. The steep terrain of this area, however, makes access and control of this plant very difficult. The OISC attributes the continued spread of this plant to hikers who may be inadvertently spreading seeds. OISC has more recently begun to undertake aerial surveys using helicopters to identify naturalized populations of *T. herbacea*. Although a significant amount of land can be surveyed using helicopters in comparison to pedestrian surveys, the cost associated with renting a helicopter means fewer surveys can be undertaken in a year (ibid.). The OISC continues to rely on ground surveys to monitor and control populations of *T. herbacea*.

Aerial and ground-level monitoring continue to play an important role in helping to manage existing infestations and detecting new populations of *T. herbacea*. However, despite these long-standing efforts, concerted attempts to educate the public about limiting the spread of invasive species has been a critical component in managing Hawai'i's invasive species problem. As part of the public outreach efforts, the four invasive species councils emphasize the importance of thoroughly washing and cleaning hiking boots and gear between hikes. Efforts to increase public knowledge in the identification of invasive species have also been ramped up in recent decades and access to this information has been streamlined through virtual media. The invasive species councils on Kaua'i, O'ahu, Maui, and Hawai'i all depend on the public to report new infestations. Hiking and trails groups across the state have also contributed to these management efforts by leading organized hikes focused on the removal of invasive species.

The spread of *T. herbacea* throughout the native wet forest habitat in the Hawaiian Islands is both an ecological and cultural concern. Hawai'i's wet forest habitat, which is a culturally valued resource has maintained a significant role in perpetuating the life-ways and traditions of the Hawaiian people. Continued encroachment upon this habitat

by highly invasive species such as *T. herbacea* and other Melastomes poses an ecological threat that has significant cultural ramifications.

Cultural Uses of Native Wet Forest Habitat in Hawai'i

The use of native wet forest plants in traditional Hawaiian culture is both extensive and well-documented (see Abbott 1992; Buck 1957; Krauss 1993). The flowers, fruits, woods, roots, and bark of many native plants found in the wet forests of the Hawaiian Islands have been and continue to be extensively used in many Hawaiian cultural practices. Although plants were held in high esteem and celebrated in traditional lore, plants were also valued as a collective whole for its ability to attract diverse wildlife, such as birds and insects. Endemic Hawaiian birds were highly valued for their colorful plumages which were extensively used in creating spectacular feathered garbs, headdresses, *lei*, and other insignia that were worn or displayed traditionally by Hawaiian nobility. The task of collecting birds was undertaken by the *po'e kia manu* (bird catchers), who held a profound understanding of avian behavior and the forest resources, including what plants to use to attract and capture the birds.

The plethora of plants found in Hawai'i's wet forest was and remains an integral component of many traditional Hawaiian cultural practices. Large trees provided a variety of hardwoods from which canoes, houses, *ki'i* (carved images), fishing accessories, and various utilitarian and recreational implements were made. Aerial roots of the climbing *'ie'ie* (*Freycinetia arborea*) were harvested and plaited together to form tightly stitched *'ie* (baskets). Ferns were collected from the forest floor and woven into *lei* or tucked into *kapa* (bark cloth) as a scenting agent. Flowers and fruits were collected for *lei*, natural dyes, and sometimes mixed together with other plants to make medicinal concoctions. Additionally, plots in the wet forests were cleared to cultivate *olonā* (*Touchardia latifolia*), an endemic plant that was purposefully grown and from which cordage of the finest quality was made. Hawaiian ethnobotanist, Beatrice Krauss notes:

> The finest cordage made by the ancient Hawaiian—in fact, the finest cordage made in the Pacific basin—was made from *olonā*. *Olonā* was cultivated in patches of two or three acres primarily in wet, upland areas. Young shoots or layered cuttings were used for planting material; the latter were obtained by bending down a branch and covering the portion touching the ground with soil so that roots emerged from it. The rooted section, with its terminal leaves, was severed and this became a rooted cutting. Planting was close to prevent side branches from growing. *Olonā* patches were kept free of weeds, especially fom [*sic*] creeping vines, which were abundant in surrounding areas; these would otherwise have choked the *olonā* plants. The stalks were ready for harvest at the end of a year or eighteen months. (Krauss 1993:27–28)

The forest itself also holds profound spiritual implications as various plants found in the wet forest were considered *kinolau* (embodiments) of named deities, many of whom took specific plant forms of the deity Kū. Such examples include but are not limited to Kūka'ōhi'alaka, Kūpulupulu, Kūmokuhāli'i, and Kūalanawao (Fornander 1919–1920; Handy and Handy 1991; Kamakau 1976). While Kū is considered the activating energy associated with the forest, other deities are also recognized including Kāne, who is embodied in the sun and in freshwater; Lono who is connected to winds, storms, and fertility; and Laka who is associated with transpiration (Edith Kanaka'ole Foundation n.d.). Therefore, the Hawaiian forest, at a minimum, represents the dynamic interplay between Hawaiian deities.

These forested spaces also filled an important spiritual and utilitarian need for Hawaiian *hula* dancers, healing practitioners, and artisans, all of whom rely heavily on Hawai'i's forest resources (Stewart 2003). *Hula* practitioners have long valued Hawai'i's rich forest, which continues to be extensively used in making adornments, implements, and in furnishing the *kuahu* (altars). In describing the *kuahu*'s association with the forest, Emerson (1909:19) explained that "the wildwoods of Hawaii furnished in great abundance and variety small poles for the framework of the kuahu, the altar, that holy place of the halau, and sweet-scented leaves and flowers suitable for its decoration." In detailing the thoughtful process of greening a *kuahu*, Emerson adds:

> It was necessary to bear in mind that when one deflowered the woods of their fronds of *ie-ie* and fern or tore the trailings lengths of *maile*—albeit in honor of Laka herself—the body of the goddess was being despoiled, and the despoiling must be done with all tactful grace and etiquette.
>
> It must not be gathered from this that the occasion was made solemn and oppressive with weight of ceremony, as when a temple was erected or as when a tabu chief walked abroad, and all men lay with their mouths in the dust. On the contrary, it was a time of joy and decorous exultation, a time when in prayer-song and ascriptions of praise the poet ransacked all nature for figures and allusions to be used in caressing the deity. (Emerson 1909:16)

Other plants utilized in greening a *kuahu* included *'ie'ie* (*Freycinetia arborea*), *halapepe* (*Pleomele sp.*), *'ōhi'a lehua* (*Metrosideros polymorpha*), *'ekaha* (*Asplenium nidus*), *ma'o hau hele* (*Hibiscus brackenridgei*), *hau* (*Hibiscus tiliaceus*), *kī* (*Cordyline fruticosa*), *'ilima* (*Sida fallax*), and *lama* (*Diospyros sandwicensis*) (Emerson 1909).

While historical literature enumerates many different types of *kahuna* (esteemed and highly specialized experts), the *kahuna* whose practice involved the extensive use of both cultivated and wild plants was the *kahuna lā'au lapa'au*. These *kahuna* treated the sick using highly tailored plant-based recipes that were accompanied by rituals and ceremonies. With the change in landscape and the arrival of non-native plants to the islands, Krauss (ibid) notes that many "Precontact prescriptions have been altered by addition or substitution of postcontact-introduced plants." Krauss provides a succinct summary of the meticulous preparation of traditional plant-based medicines:

> Different parts of a plant were used for medicine: roots, stems, leaves, flowers, bark, fruits, and seeds. These were prepared for use by brewing, pounding and extracting the juice or sap, pounding and making an infusion, or the part to be used was chewed and swallowed without any preparation. Plant material was pounded in special stone mortars with stone pestles made for this purpose only. In cases where leaves were used, dosages consisted of a specific number of leaves; specific handfuls of leaves; or the quantity of leaves that, when rolled together, fitted within the circle formed when the tips of the thumb and forefinger were joined. When bark was used, a strip of a designated width and length was prescribed. For berries, flowers, flower buds, and the like specific numbers determined the dosage. The "magic" numbers in prescribing dosages, times and, duration of treatment were one, three, and five; four and five; five and six; or five only, according to different sources. Pounded material was strained through or squeezed out with cleaned fabriclike sheath at the base of coconut fronds (*'a'a niu*) or with the fibers of the native sedge *makaloa.* Medicinal herbs were usually administered in formulations that almost always included salt and red clay, *'alaea.* (Krauss 1993:101)

The adaption of cultural traditions is an important aspect of any living culture. While many artisans continue to utilize Hawai'i's forest plants in a more traditional manner, it is common today to see many Native Hawaiian (and non-Hawaiian) artisans incorporate or draw inspiration from native plants to create contemporary clothing, home furnishings, musical implements, accessories, art, and many other utilitarian and decorative items. The restoration and revitalization of native plant habitat is crucial to sustaining Hawaiian traditions, beliefs, cultural practices well into the future whether that be in a traditional or more contemporary manner.

3. CONSULTATION

Gathering input from community members with genealogical ties and long-standing residency or relationships to the study area is vital to the process of assessing potential cultural impacts to resources, practices, and beliefs. It is precisely these individuals that ascribe meaning and value to traditional resources and practices. Community members often possess traditional knowledge and in-depth understanding that are unavailable elsewhere in the historical or cultural record of a place. As stated in the OEQC Guidelines for Assessing Cultural Impacts, the goal of the oral interview process is to identify potential cultural resources, practices, and beliefs associated with the affected project area. It is the present authors' further contention that the oral interviews should also be used to augment the process of assessing the significance of any identified traditional cultural properties. Thus, it is the researcher's responsibility to use the gathered information to identify and describe potential cultural impacts and propose appropriate mitigation as necessary.

INTERVIEW METHODOLOGY

In an effort to identify individuals knowledgeable about traditional cultural practices and/or uses associated with *T. herbacea* or the habitat in which this plant is found, a public notice was submitted to the Office of Hawaiian Affairs (OHA) for publication in their monthly newspaper, *Ka Wai Ola*. The notice was submitted via email on April 9th and was subsequently published in the May 2019 issue of *Ka Wai Ola* (2019:21) (Appendix A). As of the date of the current report, no responses have been received from the public notice. Although no responses were received as a result of the *Ka Wai Ola* publication, ASM staff contacted forty-five individuals/organizations via email and/or telephone regarding the preparation of the current CIA. These individuals/organizations were selected because they were either recognized cultural practitioners, plant experts, or Native Hawaiian organizations who utilize Hawai'i's forest resources for cultural purposes or were believed to have cultural knowledge about the target species or other plants found within the target species habitat. Of the forty-five individuals contacted, twenty individuals responded to our request with either brief comments, referrals, or accepted the interview request. The names and affiliation of these twenty individuals are listed in Table 1 below. Of the twenty respondents, ASM staff successfully conducted

interviews with nine individuals (see summaries below). A complete list of all persons contacted for consultation is available upon request.

The interviewees were asked a series of questions regarding their background, and their experience and knowledge of the target species. Additional questions focused on any known cultural uses, traditions, or beliefs associated with any of the target species. The interviewees were then asked about their thoughts on the cultural appropriateness of using biocontrol control agents and whether they were aware of any potential cultural impacts that could result from the use of biocontrol control. The interviewees were then asked whether they had any recommendations to mitigate any identified cultural impacts as well as share any additional thoughts about the proposed action.

As part of the interview process and with the consent of the interviewees, some of the interviews were audio-recorded for note-taking purposes only (audio files not available). Where audio recordings were not permitted, ASM staff recorded notes throughout the interview process. Upon completion of the interview, ASM staff prepared an interview summary, which was emailed to the interviewees for review. The interviewees were given the opportunity to review the summary for accuracy and allowed to make any necessary edits. With the approval of the interviewees, the finalized version of the summaries is presented below.

Table 1. Persons contacted for consultation.

Name	*Affiliation, Island*	*Initial Contact Date*	*Comments*
Shalan Crysdale	The Nature Conservancy, Ka'ū Preserve, Hawai'i	3/6/2019	See summary below
John Repogle	Retired from The Nature Conservancy, Ka'ū Preserve, Hawai'i	3/6/2019	See summary below
Nohealani Ka'awa	The Nature Conservancy, Ka'ū Preserve, Hawai'i	3/6/2019	See summary below
Arthur Medeiros	Auwahi Forest Restoration Project, Maui	3/7/2019	Responded via email on March 11, 2019, stating "Thank you for your valuable work supporting this essential action to attempt to slow the loss of Hawaiian biota."
Jen Lawson	Waikōloa Dry Forest Initiative, Hawai'i	4/3/2019	See summary below
Robert Yagi	Waikōloa Dry Forest Initiative, Hawai'i	4/3/2019	See summary below
Wilds Brawner	Ho'ola Ka Manaka'ā at Ka'ūpūlehu, Hawai'i	4/9/2019	See summary below
Sam 'Ohu Gon III	The Nature Conservancy, O'ahu	4/22/2019	Responded to interview request but was unable to provide input on this project.
Mike DeMotta	National Tropical Botanical Gardens, Kaua'i	4/22/2019	See summary below
Wili Garnett	Cultural practitioner, Moloka'i	5/7/2019	Responded via email stating "I have mostly been involved with Erythrina gall wasp parasite release and monitoring, but experience watching *Tibouchina* and *Schinus* degrade watershed on many islands, including Molokai and even cultural resources at Kalaupapa."

Table 1 continues on next page

Table 2. continued.

Name	*Affiliation, Island*	*Initial Contact Date*	*Comments*
Emily Grave	Laukahi Network, O'ahu	5/7/2019	Responded via email stating that she was not aware of cultural uses of this plant.
Kim Starr	Starr Environmental, Maui	5/9/2019	See summary below
Forest Starr	Starr Environmental, Maui	5/9/2019	See summary below
Manaiakalani Kalua	Cultural practitioner, Hawai'i	5/30/2019	See summary below
Talia Porter	Honolulu Botanical Gardens, O'ahu	6/3/2019	Responded to interview request but was unable to secure an interview.
Robert Keano Ka'upu	Cultural practitioner, O'ahu	6/16/2019	Responded via phone that he has been interested in learning about the cultural uses of *wiliwili* but was not aware of any uses or of anyone else who used this wood for cultural purposes.
Hinaleimoana Wong-Kalu	Cultural practitioner, O'ahu	7/16/2019	Responded to interview request but was unable to secure an interview.
Pelehonuamea Harman	Cultural practitioner, Hawai'i	7/31/2019	Referred ASM staff to Dennis Kana'e Keawe
Dennis Kana'e Keawe	Cultural practitioner, Hawai'i	8/12/2019	See summary below
Iliahi Anthony	Cultural practitioner, Hawai'i	8/30/2019	See summary below

End of Table 1

SHALAN CRYSDALE, JOHN REPLOGLE, AND NOHEA LANI KA'AWA

On March 6th, 2019, Lokelani Brandt and Matt Clark interviewed Shalan Crysdale, John Replogle (retired from the Nature Conservancy), and Nohea Ka'awa of The Nature Conservancy (TNC) Ka'ū Preserve regarding DOFAW's proposed action and to gather any known cultural knowledge of *T. herbacea*. The crew from TNC indicated that they were not aware of any known cultural uses of *T. herbacea*, but commented that this plant is widespread in portions of the TNC Ka'ū preserve. Shalan described past efforts to control *T. herbacea* but noted that the manpower and chemicals needed were costly, time-consuming, and not entirely effective at managing this highly invasive plant. Shalan explained that *T. herbacea* is effective at shading out native understory species. Both Shalan and John have observed an abundance of *T. herbacea* growing along the forest preserve fence lines. Based on their observations, Shalan and John firmly believe that birds have aided in the widespread dispersal of this plant, especially along the length of the fence lines where the canopy cover is less abundant and where birds frequent. Shalan believes that if *T. herbacea* is removed, it may lend to the recovery of many native understory species.

While Shalan and John were not entirely against the use of biological control agents, they did share some of their concerns. Shalan, John, and Nohea stressed the importance of trial testing to ensure that the release of any proposed biological control agent does not adversely impact other native species as well as other valued crops. They spoke about the limitations of laboratory trial testing that may not account for all the variables that are present in the natural habitat. They strongly recommended that extensive trial testing be conducted prior to any proposed field release and they hope to see more post-release field monitoring to safeguard against the spread beyond the intended target species.

WILDS PIHANUI BRAWNER

Wilds Brawner, Site Manager of the non-profit organization, Ho'ōla Ka Makana'a at Ka'ūpūlehu Dryland Forest, was interviewed by Lokelani Brandt on April 18th, 2019. Since 2008, Wilds has worked at the 70-acre Ka'ūpūlehu Dryland Forest preserve performing a variety of duties including management and education.

When asked about his knowledge of *T. herbacea*, Wilds indicated that in his years of work, he has not encountered *T. herbacea* populations in the leeward side of Hawai'i Island, but was aware of its impacts to the wet forest of Hawai'i Island and elsewhere. Wilds indicated that he was not aware of any known past cultural uses of this plant.

When asked about any potential cultural impacts that could result from the use of biocontrol, Wilds emphasized that utilizing biocontrol has "great potential" and that it may be a solution to help manage unwanted pests under the condition that there has been extensive research, lab and field testing, and controlled releases. He emphasized that extensive research should consider every possible factor that could potentially result in negative impacts, especially to other endemic taxa. He also stressed that public education should be a key component in this process, as it will create opportunities for the public to learn and provide input. He believes that public input can help assess the possible risks and identify steps to manage those risks. Wilds strongly recommended that all future biological control efforts integrate public input and that it should move towards a community-based resource management structure. Wilds suggested that ways to promote biocontrol are through responsible action, extensive and evidence-based testing and research, and if these pre-release efforts are successful, biocontrol "can be the silver bullet" to managing pests. He concluded that although the process has the potential to control invasive species, the idea and use of the word "control," as opposed to "management," is very loaded and attaches unrealistic expectations to the effort. As with any forest, Wilds believes that with proper "management", the results will net a positive cultural impact. New forest growth produces more flowers and seed and ultimately creates more opportunities for people to interact with these forests through place-based learning. He emphasized that when people interact and participate in caring for our "beloved" resources and when the *mo'olelo* of these resources are shared, it can then become a living cultural resource for the people.

MIKE DEMOTTA

On April 24th, 2019, Lokelani Brandt conducted an interview with Mike DeMotta, the Head Curator of the living collections for the National Tropical Botanical Gardens (NTBG) on Kaua'i. Mike manages the center's plant inventory database, which includes a large collection of native plants. He has also been tasked with developing ways to improve their native plant populations by creating spaces for a thriving living collection. Through his work, Mike has been heavily involved with native plant restoration from the coastal dry areas on Lehua Island to the pristine native forests in Limahuli Valley on Kaua'i's north shore.

When asked about any traditional cultural uses of *T. herbacea*, Mike stated that he was unaware of any cultural importance or uses for any part of this plant. While no specific information about any known past or current cultural uses of this plant was shared he did offer insights into the proposed use of biological control to aid in conservation efforts. Mike believes that with proper research, biocontrol could preserve or rescue native forests. With his strong involvement with restoration, Mike strongly believes biocontrol will assist in opening up spaces for the regeneration of native forests and proposed that drastic measures are imperative to control or eradicate the aggressive nature of invasive species. Although he is genuinely concerned about the possibility of a collateral loss of one or two native species, Mike reasoned that the overwhelming threat to native forests from invasive species had lent to his advocacy for biocontrol. He argued that the manpower needed to control these threats are not feasible and are unrealistic. He is particularly pleased that the focus has shifted to conservation and that there is a growing awareness that we are losing pristine forests to these invasive species.

JEN LAWSON AND ROBERT YAGI

On April 26, 2019, Lokelani Brandt and Aoloa Santos met with Executive Director, Jen Lawson and Preserve Manager, Robert Yagi of the Waikoloa Dry Forest Initiative. The Waikoloa Dry Forest Initiative manages 275 acres of dryland forest located near the Waikoloa community. When asked about any known cultural uses of *T. herbacea,* Jen and Robert were not aware of any known past or current uses of this plant. While no specific information about *T. herbacea* was obtained, they did offer their insights into the proposed use of biological control to aid in management strategies.

Although Jen is a proponent of biocontrol, she explained that the proper research must be conducted, and that dissemination of that research should be provided to the affected communities. She expressed that one of the main challenges will be garnering public support for the proposed action because of preconceived notions that are heavily influenced by the historical and unsuccessful application of biocontrol. Although Jen was aware of the extensive research that is conducted prior to the release of any biocontrol agent, she remarked that such research is not always effectively shared with the communities. She added that the lack of public information and transparency only exacerbates misconceptions thereby making community support difficult to establish. In light of this, Jen recommended that DOFAW and other associated agencies restructure informational public meetings to be engaging

and inclusive of community input as she believes this may improve trust between the affected communities and the agencies. Additionally, she strongly advocates for a more collaborative partnership between the DOFAW and its agencies as a way to promote a more open dialogue between the agencies and community groups who work closely with some of these invasive species. Jen and Robert also recommended that more consistent post-release monitoring be conducted and that such efforts should be done in conjunction with established community groups.

FOREST AND KIM STARR

On May 31st, 2019, Lokelani Brandt and Aoloa Santos met with Forest and Kim Starr at their home in Olinda, Maui. Born and raised on Maui, Forest always enjoyed nature. He later moved to New York to attend Cornell University and in 1992 met his now wife and business partner, Kim, who is of Hawaiian descent but was *hānai* (adopted and raised) by a Japanese-Italian family. Since then they have done numerous volunteer and contract work in the conservation field. They currently co-own Starr Environmental and serve as biologists and environmental consultants for developers and federal and state agencies. Forest and Kim have extensive experience in botanical and environmental restoration work in the Hawaiian Islands. Forest shared that they have assisted in prior biocontrol releases but they primarily focus on the early detection of introduced species.

When asked about any known cultural uses for *T. herbacea*, Forest and Kim stated they are not aware of any cultural uses of this plant. They both expressed that this plant is considered rare in its homeland because of its numerous threats but is highly invasive in Hawai'i because it has no natural predators. Forest stated that in West Maui, specifically at Kapilau ridge and Waikapū, *T. herbacea* is widespread.

Forest described much of the vegetation that dominates the islands as a "rag-tag assemblage of pantropical invasive species" and opined that this sort of global homogenization of the islands' plant life is exacerbating the spread of really aggressive species. Adding to this, Forest expressed that changes in the environment are inevitable and noted that these changes are difficult for many to accept. Forest and Kim believe that biocontrol is a method that can help mitigate or slow the growth of species but "it never eradicates, it just reduces the numbers" and cited the example of the Erythrina Gall Wasp and the panini cactus (*Opuntia ficus-indica*) which have had biocontrol agents released against them. Both Forest and Kim explained that over the course of many years they have seen limited success where biocontrol has resulted in complete eradication.

When asked about their thoughts on the cultural appropriateness of biocontrol, Forest and Kim shared that they have witnessed the culture and traditions of these islands evolve within an inevitable changing environment. Forest emphasized that the mixed-culture of Hawai'i has been able to co-exist with the changing environment and they have seen various cultures including Hawaiian culture utilize introduced plants in place of rare or extinct native plants in order to perpetuate their traditional cultural practices. In spite of these cultural adaptations, they feel that biocontrol can be useful in protecting native plant habitats which are both ecologically and culturally important and remain open-minded to these types of undertakings.

Based on their knowledge of the efficacy of former biocontrol efforts, Forest and Kim shared that generally, the way a biocontrol agent is introduced is not very effective and that for the most part, in order for the biocontrol to be entirely successful a large number of biocontrol agents must be introduced. Kim stated that although the purpose of biocontrol is to introduce an organism that is specific to a target plant, the efficacy is oftentimes underwhelming and as a result, there have been a few unintentional consequences. Kim shared that although biocontrol agents are introduced with good intentions, "the unknown," meaning its potential to cause unforeseen impacts to a non-target species is the main factor that contributes to the general resistance to implement biocontrol. Additionally, Forest and Kim both stated that once a biocontrol agent is released there is very limited and often times no follow-up by the agencies that have invested in the pre-release studies. In light of this, Forest and Kim recommended that post-release monitoring should be held to the same standard as the pre-release of a biocontrol agent. Forest described that "mother nature is so crafty" and that changes are often muted or other factors become more significant than the release, therefore on-going post-release monitoring is a crucial component to this process. Forest also stated that misinformation has been detrimental to these biocontrol efforts and believes that more should be done to effectively communicate these types of undertakings to the public.

MANAIAKALANI KALUA

On June 6th, 2019, Lokelani Brandt conducted an interview with Manaiakalani "Manai" Kalua, a *kumu hula* and life-long Hawaiian cultural practitioner. Born and raised in the Hawaiian homestead community of Keaukaha, Manai has dedicated his life to *hula* and because of this, he has had extensive interactions with Hawai'i's native plant life, which is a fundamental element to traditional *hula* practices.

When asked about any known cultural uses for *T. herbacea*, Manai was not aware of any known traditional cultural uses of this plant but recalled seeing it when gathering foliage for *hula* and for other ceremonies. Manai, however, spoke at length about the ways in which invasive species are changing traditional cultural practices specific to *hula*. He explained that within his *hula hālau* he teaches about the proper way to harvest plants in addition to practices that will help limit the spread of invasive species. He now stresses the importance of cleaning all clothing, equipment, and cars after every visit to the forest. He stated that invasive species are a serious problem that has major environmental and cultural implications and cited the example of Rapid ʻŌhiʻa Death (ROD), which has significantly impacted *hula* practices. He noted that culturally, *ʻōhiʻa* is an important part of *hula* adornments and rituals, since becoming aware of ROD, he no longer gathers *ʻōhiʻa* nor does he condone the gathering of this plant. He explained that not being able to utilize *ʻōhiʻa* has required him to be more creative with his cultural practices.

When asked about his thoughts on the cultural appropriateness of utilizing biocontrol, Manai explained that historically we have a long history of unsuccessfully utilizing biocontrol and cited examples including the introduction of the mongoose to control rats and the scale insect to control strawberry guava. Manai expressed concern for the idea of introducing other foreign insects which may adversely impact its intended target but whose impacts are somewhat unknown to the many other species that grow in the same habitat as the target species. He questioned, what will happen to the introduced biocontrol once the target species is eliminated, and what are the long-term impacts of utilizing biocontrol? He noted that we are still living with the repercussion of previous biocontrol choices that we still cannot manage. Although Manai is not a proponent of utilizing biocontrol, he understands that the shift to use biocontrol suggests that all other methods for controlling these invasive species have been exhausted. He was aware that utilizing biocontrol is a much slower process and stated that the government does not have the means to manually eradicate Hawaiʻi's invasive species. He stated that there are also risks associated with the manual removal of invasive species.

While Manai remains skeptical of the effectiveness of biocontrol, he believes that the government must develop stricter laws and policies to stop the introduction of invasive species. He noted that in his travels to other parts of the world, including Japan and New Zealand, their customs process is far more thorough and intensive. He believes that these countries and exemplary models where the emphasis is placed on stopping the introduction instead of trying to combat its spread. He also advocates for a more rapid response to known invasive species and cited the example of the coqui frog, which on Hawaiʻi Island is now so widespread and nearly unmanageable. He believes that rapidly responding to invasive species, especially when populations are far more contained, could be far more effective.

DENNIS KANAʻE KEAWE

On August 13, 2019, Aoloa Santos conducted an interview with Dennis "Kanaʻe" Keawe, a retired Commercial Services Consultant for Hawaiian Electric Light Company (HELCO) and former lecturer at the University of Hawaiʻi at Hilo (UH Hilo). Born and raised on Oʻahu, Kanaʻe moved to Hawaiʻi Island in November of 1974, to help his father with his coffee farm in Hōnaunau, Kona. Following his retirement from HELCO at age 55, he was asked to teach a Hawaiian studies ethnobotany course at the UH Hilo. Kanaʻe stated that when he was asked to teach the course, his botanical vocabulary and knowledge was appropriate for teaching young children and therefore acknowledged that in order to instruct at the university level, he needed to expand and develop his botanical nomenclature. Through this process, Kanaʻe learned that many varieties of Hawaiʻi's native plants "exists within the tropical belt around the world" and by having in-depth knowledge of scientific names and identifiers allowed him to effectively communicate with people well-versed in similar plants of those regions. Additionally, Kanaʻe is a renowned Hawaiian artisan and cultural practitioner endearingly referred to by many as "the all-around guy." He has been recognized for his expert-crafted oeuvres, such as *hula pahu* (drum), *kapa* (bark cloth), *iʻe kuku* (*kapa* beater), and feather crafts. As a result of his artisanship, he has been afforded opportunities and invitations to visit communities and institutions around the world, notably the Smithsonian Museum, an institution that houses a large collection of Hawaiian antiquities.

When asked about any traditional cultural uses of the *T. herbacea*, Kanaʻe stated that he was unaware of any cultural importance or uses for any part of this plant but suggests that it perhaps may have medicinal properties and noted that this claim would have to be substantiated with proper research. While no specific information about any known past or current cultural uses of this plant was shared, he did offer thoughts on the use of biocontrol. Kanaʻe expressed his support of its use and did not foresee any major cultural impacts if extensive study and testing is done prior to its release. He added that although there are unknown variables to this method, humans can only do so much, especially in the current state of our environment and the rapid growth of invasive species.

ILIAHI ANTHONY

On September 3rd, 2019, Lokelani Brandt interviewed Iliahi "Ili" Anthony, a *hula* dancer, *lauhala* weaver, *lei* maker, and natural dye expert. Ili is also an art teacher at Ka ʻUmeke Kāʻeo Hawaiian Immersion Public Charter School and

has a background in designing furniture and exhibit spaces. Ili grew up in the community of Keaukaha and has been dancing *hula* since the age of four. As a life-long *hula* dancer for Hālau O Kekuhi, Ili explained that her knowledge of Hawai'i plant life comes from years of gathering foliage (primarily indigenous and endemic species) and other natural resources for their *'a'ahu* (costume), *lei*, and *hula* implements. Ili recalled as a child being accompanied by her *kumu hula* and family members into their gathering areas where they taught her about the Hawaiian cultural significance of the plants, gathering protocols, how to identify them in the forest, and how to sustainably gather and prepare them to be used in the context of *hula*. She emphasized that as a small kid, she learned about these practices by watching and listening to her *kumu* and relatives and stated that when you are that young, you're not keenly aware of what it is they are teaching you, but as an adult, those teachings remain and are better understood. Ili openly stated that although she is not of Hawaiian ancestry, she has been raised by native Hawaiians and has learned about many of the traditional practices and customs. She expressed that although she chooses to remain respectful when it comes to Hawaiian issues and matters, she is willing to share her knowledge when asked and feels that she has something to offer.

Ili explained that as a *hula* dancer, she has learned to depend on other cultural practices to help her with gathering certain natural resources needed in *hula*. She described going on expeditions with her brother, who is a hunter, to gather *maile*. Ili explained that her brother knows the trails very well and is very particular about how they cut *maile*, and how much they take from any one plant. She added that although her brother is not necessarily a *lei* maker, he knows this plant and forest resources very well. She explained that she also relies on her father who is a woodcarver to help her make certain *hula* implements. Ili also described gathering with other *hula* dancers, some of whom have a background in native plants and botany, and shared that when she gathers with them, they often teach her about the names and can point out the subtleties that are not obvious to her. Ili believes that this demonstrates the interconnectedness of cultural practices and stated that even people who we think may not use plants, such as hunters and fishers, do often know a lot about native plant life. She stressed that as a *hula* practitioner and in terms of plant resources, she relies greatly on other practices that are not necessarily defined as *hula*.

With respect to learning about and identifying plants, whether native or non-native, Ili shared that unless someone shares that knowledge with her, then she would most likely not know about it. She expressed that when she has gone to get gathering permits from DLNR, she recalled seeing various informational posters in their office which she finds useful for learning about Hawai'i's plant life and invasive pests.

When asked about her knowledge regarding any cultural uses for *T. herbacea*, Ili stated that she was not aware of this plant nor of any cultural uses. While Ili supports the removal of invasive species, especially if they are directly impacting native plants or native plant habitat, she cautioned that some plants that have been dubbed "invasive" are utilized for various traditional and contemporary cultural purposes. Ili opined that today, people utilize various "rubbish plants" to make adornments such as *lei* and that such plants if properly arranged can be turned into something beautiful and wearable. She also noted that weedy plants such as *laukahi* (*Plantago major*) and the introduced guava (*Psidium guajava*) have become incorporated into Hawaiian *lā'au lapa'au* (plant healing) practices. While she believes that finding a cultural purpose for an invasive plant is not a strong reason to halt invasive species management efforts, she cautioned that people have come to rely on certain invasive species to perpetuate select cultural practices because they are easily accessible and abundant. Adding to this, Ili expressed that people have and will continue to adapt to living with invasive species. Ili also worries that if invasive species, particularly those that are used for cultural purposes become less abundant and available, then people will likely have to find a more readily available substitute, which could result in people gathering indigenous or endemic species. She stated that people tend to use invasive species because they are abundant and easily accessible.

Ili shared that over the years she has observed an increasing number of pests on native plants and made specific reference to *'a'ali'i* (*Dodonaea viscosa*), which now seems to be infested with spiders. She shared that as a *lei* maker, she often brings these plants into her home and disposes of her *hakina* (scrap pieces) in her yard. Although she has not seen those spiders move onto the plants at her home, Ili expressed a sense of uncertainty with gathering and possibly transporting unknown pest.

Ili also spoke about the need to improve our understanding of the ecological relationships that may exist between native and non-native species. She shared that some native plants such as *'iliahi* (sandalwood; *Santalum ellipticum*) is semi-parasitic and relies on a host plant to thrive. She added that we know that native plants have adapted to each other and wonders if native species may have adapted or are adapting to living amongst non-native species as well. She pondered on the idea of removing invasive species and the possibility of causing indirect impacts to native species that have come to rely on them for some life-giving element.

When asked about her thoughts on the cultural appropriateness of using biocontrol, Ili opined that this is a difficult question to answer and lightheartedly stated that "basically, you're introducing another culture into the culture." She

asked, what things have we introduced in the past that actually worked? Ili added that she feels there have been more things in the past that have been introduced that haven't worked in comparison to those that have actually worked. Ili stated that introducing more foreign species to the islands is a scary thought and wondered what the future would look like. She asked, will we have to continually introduce more foreign species to combat those we previously introduced? Additionally, she wondered what would take the place of these invasives once they are removed?

When asked about her thoughts and recommendations about the proposed action, Ili believes the state could do more in terms of educating the public about identifying invasive species and the ways in which everyone can help limit the spread. She stated that there is a general lack of awareness and believes that providing more information to those who are obtaining gathering permits may be one way to improve awareness. She stressed that the information needs to be presented in a reasonable manner that would not deter people from obtaining a gathering permit. Ili shared that since the events taking place on Mauna Kea, she believes there is growing alertness amongst the people about land and culture-related issues. She has noticed an increasing awareness in schools where teachers are working with students to better understand and to seek solutions to these issues. She believes that the state should improve support to the schools so that the information is more accessible to students and teachers. Ili explained that many teachers want to do more of these kinds of projects with their students but there are many challenges that hinder their ability to execute such projects, including accessibility, funding, time, and finding a good resource person that can connect them to specific places and resources. She expressed that teachers can only guide and facilitate these kinds of projects, but they are not plant experts. She believes that education can be a key component in improving public awareness. She also added that while there may be a robust amount of scientific information about the potentially positive aspects of biocontrol, it needs to be condensed and expressed in layman's terms to that the general population can actually understand and connect to what scientists are discovering. She lamented that otherwise, people won't listen or hear what is being said because they can't connect to or understand what the scientists are saying. Ili made reference to the tremendous educational efforts that were put into improving public awareness about Rapid ʻŌhiʻa Death and noted that their outreach team was doing big and small things such as community talks, stickers, hats, and being present at various local community events. She believes that more of these kinds of efforts could be undertaken for other invasive species.

Ili also shared that many scientists are not practitioners and opined that these two groups, although they may share an affinity for preserving plants, both have two completely different relationships with the resource. She believes that the relationship between scientists and practitioners should also be improved because both groups can help to elevate and improve each other's practices if they are willing to work collaboratively. While she feels that this dynamic has been changing, she thinks its especially important as we move towards the possibility of using biocontrol in native plant habitats.

4. IDENTIFICATION AND MITIGATION OF POTENTIAL CULTURAL IMPACTS

The OEQC guidelines for assessing cultural impacts identify several possible types of cultural practices and beliefs that are subject to assessment. These include subsistence, commercial, residential, agricultural, access-related, recreational, and religious and spiritual customs. The guidelines also identify the types of potential cultural resources associated with cultural practices and beliefs that are subject to assessment, which "may include traditional cultural properties or other types of historic sites, both man made and natural, including submerged cultural resources"(Office of Environmental Quality Control (OEQC) 1997:1). The origin of the concept of traditional cultural property is found in National Register Bulletin 38 published by the U.S. Department of Interior-National Park Service (Parker and King 1998). A traditional cultural property can be generally defined as:

> ...one that is eligible for inclusion in the National Register because of its association with cultural practices and beliefs of a living community that (a) are rooted in that community's history, and (b) are important in maintaining the continuing cultural identity of the community. (Parker and King 1998:1)

This definition also implies that any identified traditional practices and beliefs of an ethnic community, or members of that community, exceeds fifty years. "Traditional" as defined in the National Register Bulletin 38 "refers to those beliefs, customs, and practices of a living community of people that have been passed down through the generations, usually orally or through practices (ibid.). Whereas, "Culture" refers to "a system of behaviors, values, ideologies, and social arrangements" in addition to "tools and expressive elements such as graphic arts" (ibid.). The

use of the term "Property" defines this category of resource as an identifiable place. Traditional cultural properties are not intangible, they must have some kind of boundary; and are subject to the same kind of evaluation as any other historic resource, with one very important exception. By definition, the significance of traditional cultural properties should be determined by the community that values them.

It is however with the definition of "Property" wherein there lies an inherent contradiction and corresponding difficulty in the process of identification and evaluation of potential Hawaiian traditional cultural properties because it is precisely the concept of boundaries that runs counter to the traditional Hawaiian belief system. The sacredness of a particular landscape feature is often cosmologically tied to the rest of the landscape as well as to other features on it. To limit a property to a specifically defined area may actually partition it from what makes it significant in the first place. However offensive the concept of boundaries may be, it is nonetheless the regulatory benchmark for defining and assessing traditional cultural properties. As the OEQC guidelines do not contain criteria for assessing the significance for traditional cultural properties, this study will adopt the state criteria for evaluating the significance of historic properties, of which traditional cultural properties are a subset. To be significant the potential historic property or traditional cultural property must possess integrity of location, design, setting, materials, workmanship, feeling, and association and meet one or more of the following criteria:

a Be associated with events that have made an important contribution to the broad patterns of our history;

b Be associated with the lives of persons important in our past;

c Embody the distinctive characteristics of a type, period, or method of construction; represent the work of a master; or possess high artistic value;

d Have yielded, or is likely to yield, information important for research on prehistory or history;

e Have an important value to the native Hawaiian people or to another ethnic group of the state due to associations with cultural practices once carried out, or still carried out, at the property or due to associations with traditional beliefs, events or oral accounts—these associations being important to the group's history and cultural identity.

While it is the practice of the DLNR-SHPD to consider most historic properties significant under Criterion d at a minimum, it is clear that traditional cultural properties by definition would also be significant under Criterion e. A further analytical framework for addressing the preservation and protection of customary and traditional native practices specific to Hawaiian communities resulted from the *Ka Pa'akai O Ka 'Āina* v Land Use Commission court case. The court decision established a three-part process relative to evaluating such potential impacts: first, to identify whether any valued cultural, historical, or natural resources are present; and identify the extent to which any traditional and customary native Hawaiian rights are exercised; second, to identify the extent to which those resources and rights will be affected or impaired; and third, specify any mitigative actions to be taken to reasonably protect native Hawaiian rights if they are found to exist.

Summary of Findings, Identification of Cultural Impacts, and Proposed Mitigative Measures

A review of the culture-historical background information reveals that *T. herbacea* was first discovered in 1977, growing along the Saddle Road on Hawai'i Island and by 1982, specimens were found at locations in both east and west Maui. By the 1990s, *T. herbacea* was discovered on the island of Lāna'i and in the 2000s, it was found growing in Hālawa Valley in east Moloka'i and at several locations on the island of O'ahu. It is now naturalized on both the islands of Maui and Hawai'i. A review of the culture-historical background in addition to the consultation efforts has yielded no reported cultural use for this plant nor is there any historical evidence to suggest that *T. herbacea* is crucial to any particular ethnic groups' cultural history, identity, practices, or beliefs, nor does it meet any of the significance criteria outlined above. Although *T. herbacea* does not meet any of the significance criteria, what is culturally significant is the wet forest habitat in which it thrives. Hawai'i's wet forest habitat could be considered significant as a traditional cultural property under Criterion e, as it contains many culturally important indigenous and endemic taxa, which are still utilized in certain Hawaiian cultural practices. Some of these wet forest resources are also associated with certain Hawaiian cultural beliefs.

Based on the information presented in the culture-historical background and from the insights shared by the consulted parties, it is the assessment of this study that the release of the proposed biological control agent, *Syphraea uberabensis* will not result in impacts to any valued cultural, historical, or natural resources. Conversely, if no action is taken to further reduce remaining populations of *T. herbacea* and other highly invasive Melastomes from claiming more of Hawai'i's wet forest habitat, then impacts to this valued resource would be anticipated.

While no specific cultural impacts have been identified, the consulted parties shared valuable insight, concerns, and recommendations that could reduce the potential for any future impacts and improve public transparency regarding the effectiveness of biocontrol as a conservation management strategy. Several key themes emerged from the consultation efforts, all of which are further described below:

1) maintain stringent pre and post-release testing and monitoring;
2) improved community transparency and input;
3) active and ongoing public outreach and education;
4) improve efforts to limit the introduction of potentially harmful invasive species.

While the consulted parties did not explicitly oppose the use of biocontrol, especially to aid in the recovery of Hawai'i's native forest habitat, they all shared a sense of concern and spoke about the risks inherent in biocontrol activities. While they were all aware of the extensive studies that are conducted prior to the release of any biocontrol agent, they all spoke about the uncertainty of introducing another foreign insect to Hawai'i's fragile ecosystems. Several of the consulted parties noted that although pre-release host specificity test helps with the screening process, they shared that laboratory testing cannot account for all the variables found in nature. The generally held belief is that field release is merely another screening and testing procedure. Despite this element of uncertainty, all of the consulted parties agreed that some sort of action is necessary to limit the growth and spread of *T. herbacea* and other weedy Melastomes. Nearly all of the consulted parties stressed the importance of thorough controlled pre-release studies to safeguard against the potential for the collateral loss of other endemic taxa or economically valuable crops. Several of the consulted parties also stressed the importance of conducting on-going and consistent post-release monitoring to ensure that the biocontrol agent does not spread beyond its intended target. These individuals noted that consistent post-release monitoring will help with early detection if it is found that the proposed biocontrol agent has unintentionally spread beyond the host plant. Wild Brawner suggested the concept of integrated pest management, particularly for native plants, where natural and cultural management practices are employed concurrently. Examples of this include, timing weed removal and planting companion plants to attract active pollinators or insects that may combat other invasive insects.

In looking to future biocontrol efforts, nearly all of the consulted parties expressed the need to integrate more public input and stressed the importance of moving towards a community-based resource management structure. Based on the past public meetings held by HDOA for biocontrol, Jen Lawson felt that the public meetings held by the HDOA should be restructured so that they are engaging and inclusive of community input as she believes this may improve trust between the affected communities and the agencies. Jen Lawson and Iliahi Anthony believe that supporting biocontrol research must be clearly and effectively communicated to the public using various media forms. Iliahi Anthony noted that education and outreach are key components to improve the public's understanding of biocontrol and empowering them with the knowledge and tools to help limit the spread of invasive species. Both Jen Lawson and Iliahi Anthony expressed that improving the public's understanding of the risk and benefits of biocontrol may help to build public transparency and hopefully resolve some of the misconceptions associated with biocontrol. Jen Lawson encourages the responsible agencies to consider partnering with conservation-focused non-profit organizations and community groups, especially during the field release monitoring phase as these groups are working directly with these target species daily. As noted by Kim and Forest Starr, the conventional biocontrol release methods that have been used in the past typically yields results that are underwhelming. Perhaps, the additional support from non-profit organizations could potentially improve the efficacy of biocontrol.

All of the consulted parties spoke about the many misconceptions associated with biocontrol, many of which are based on failed historical examples. While testing and screening procedures have improved significantly since the late 19th century, many people today remain resistant and skeptical to implement biocontrol. It is the author's contention and as described by some of the consulted parties that this widely held belief stems from the agencies' lack of public outreach and education. In light of this, it is imperative that DLNR, DOFAW, and HDOA make serious efforts to participate in public outreach events and to educate the public so that these misconceptions, some of which are rooted in a historical context, can be better understood. Public outreach and education efforts should also demonstrate the potential effectiveness of biocontrol as a conservation management strategy. Iliahi Anthony spoke about the effectiveness of the Rapid 'Ōhi'a Death (ROD) community outreach efforts and believes that this could be an exemplary model. Iliahi Anthony noted that the ROD outreach team has been actively disseminating information using various media forms.

While combatting existing populations of invasive species is a critical step in managing Hawai'i's natural resources, it was noted by Manaiakalani Kalua that the State of Hawai'i must also ramp up their efforts to prevent the arrival and introduction of unwanted pest species. Manaiakalani Kalua believes that current policies and laws must be revised and strengthened. Both Manaiakalani Kalua and Iliahi Anthony noted that in their travels to other countries

their customs entry process is far more rigorous and thorough. Manaiakalani Kalua believes that the State should look to other countries such as New Zealand and Japan as models to prevent the arrival of unwanted pests.

In summary, the recommendations provided above are intended to ensure that the release of *S. uberabensis* as a biocontrol agent for *T. herbacea* and other Melastomes considers the culture-historical context and the concerns and thoughts shared by the consulted parties. While none of the consulted parties explicitly opposed the use of biocontrol, the concerns, and recommendations offered above are intended to support the State of Hawai'i, specifically DLNR, DOFAW, and HDOA in being mindful of the cultural, social, and environmental uniqueness of Hawai'i. Conducting background research, consulting with community members, and taking steps towards mitigating any potential cultural impacts is done so in the spirit and practice of *Aloha 'Āina*, a contemporary movement founded on traditional practices and beliefs that emphasize the intimate relationship that exists between Native Hawaiians and the *'āina* (land). If DLNR, DOFAW, and HDOA assume ownership of their right and responsibility to release a biocontrol agent, we recommend it be done so in that same spirit and practice. Attention to and implementation of the above-described issues and measures will help to ensure that no such resources, practices, or beliefs will be adversely affected by the proposed release of *S. uberabensis*.

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APPENDIX A.
KA WAI OLA PUBLIC NOTICE

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PUBLIC NOTICE

ASM Affiliates is preparing a Cultural Impact Assessment (CIA) in advance of a proposed statewide release of four (4) biological control (biocontrol) agents for four target invasive species. In brief, DOFAW is seeking to conduct a statewide field release of four (4) separate biocontrol agents on four target species:

- introduction of a wasp parasitoid *(Aprostocetus nitens)* to further control the erythrina gall wasp *(Quadradstichus erythrinae)*, which has been impacting the native *wiliwili (Erythrina sandwicensis)*;
- introduction of a small beetle *(Syphraea uberabensis)* to control weedy melastomes *(Tibouchina spp.)*;
- introduction of a thrips insect *(Pseudophilothrips ichini)* to control Christmas berry *(Schinus terebinthifolia)*;
- introduction of a butterfly *(Euselasia chrysippe)* to control miconia *(Miconia calvescens)*.

We are seeking consultation with any community members that might have knowledge of traditional cultural uses or who are involved in any ongoing cultural practices associated with the target species (i.e. *wiliwili*, melastones, Christmas berry, and miconia). If you have and can share any such information please contact Lokelani Brandt lbrandt@asmaffiliates.com, or Aoloa Santos asantos@asmaffiliates.com, phone (808) 969-6066, mailing address ASM Affiliates 507A E. Lanikaula Street, Hilo, HI 96720.

(Ka Wai Ola 2019:21)

ATTACHMENT 3

Host specificity of *Syphraea uberabensis* (Coleoptera: Chrysomelidae), a proposed biological control agent for invasive melastomes in Hawaii

Summary

The South American flea beetle, *Syphraea uberabensis* (Coleoptera: Chrysomelidae), was evaluated as a potential biological control agent for the invasive weed, *Tibouchina herbacea* and its relatives (Melastomataceae), in Hawaii. Adult beetles, 3-4 mm in length, feed and lay eggs on leaves and soft stems of their host plants. Larvae feed externally on leaves as well. Specificity tests indicated the host range of *Syphraea uberabensis* is restricted to a few melastome species, all non-native and considered invasive in Hawaii. The results of no-choice starvation tests and multi-choice testing consistently identified the potential Hawaiian hosts as: *Tibouchina herbacea, Tibouchina longifolia, Pterolepis glomerata, Melastoma septemnervium* and *Melastoma sanguineum*.

In no-choice tests, substantial feeding by *Syphraea* beetles was observed on no more than seven melastome species, and egg laying was further restricted to the five mentioned species, all in the tribe Melastomeae, which includes American and Asian species. In no-choice tests with larvae, these same melastomes supported high rates of survival, while a few other melastomes supported lower rates of survival, and other plants did not support survival beyond the second instar.

Multi-choice testing with adult beetles revealed strong preferences for feeding and oviposition in the same species identified as probable hosts during no-choice trials. Feeding within tribe Melastomeae occurred at significantly higher levels than in other tribes ($p < 0.01$). When the preferred hosts were excluded in reduced multi-choice tests, adult insect feeding decreased dramatically. In the absence of the preferred hosts, oviposition increased slightly on other species within family Melastomataceae, with the greatest increase in oviposition occurring on *Tibouchina urvilleana*. Although closely related to preferred host plants, this weedy shrub was rarely accepted by *Syphraea* for feeding or egg laying. It appears to be an unlikely host because its leaves are well protected by dense hairs.

The Hawaiian ranges of *T. herbacea, T. longifolia, P. glomerata, M. septemnervium* and *M. sanguineum* overlap considerably. Although *Syphraea* showed a clear preference for *T. longifolia* in laboratory tests, it is unlikely that this preference will have a significant impact in

the Hawaiian environment because *T. longifolia* is so scarce compared to *T. herbacea* and other potential hosts. A more likely scenario is that *Syphraea* will negatively impact widespread *T. herbacea*, while perhaps helping prevent *T. longifolia* from spreading.

Syphraea is tolerant of cool and moderate temperatures, and is not expected to be restricted in range by temperatures in Hawaii, except perhaps in exceptionally warm habitats (Souder 2008). However, the potential of *Syphraea* as a biological control could be limited by humidity at the microhabitat level. In Brazil, *Syphraea* is found with its melastome hosts in boggy soils, similar to the areas where *Tibouchina* and *Pterolepis* thrive in Hawaii, so these hosts should be highly susceptible. On the other hand, *Melastoma* in Hawaii can grow in relatively drier areas – such as young lava flows. *Syphraea* could be less effective against *Melastoma* in drier habitats, because its eggs and larvae appear to be susceptible to drying when humidity is not high.

Feeding damage by adults and larvae of *Syphraea uberabensis* on the host plant *Tibouchina herbacea*.

Testing Methods

Insect rearing: *Syphraea uberabensis* eggs and larvae on *T. herbacea* cuttings were shipped in July 2005 from Universidade Estadual Centro-Oeste in Irati, Parana State, Brazil to the Hawaii Volcanoes National Park Quarantine Facility (HVNPQF). The shipment resulted in an initial colony of approximately 50 adult flea beetles. Abnormal growth of potted *Tibouchina*

plants in HVNPQF limited rearing the flea beetle on live plants. Therefore, *T. herbacea* cuttings collected around Glenwood and Volcano, Hawaii (700-1200 m) were used to maintain colony insects. In HVNPQF, the environmental conditions ranged from 18-24° C, 20-95% relative humidity (RH), with a natural photoperiod (approximately12-12h light:dark). Flea beetles were reared on fresh leaf cuttings of *T. herbacea* over moistened paper towel in 150mm x 25mm circular petri dishes. The moistened towel maintained a level of humidity inside the petri dish that kept plant material turgid. Each dish was filled with 30-40 newly emerged adults (roughly 1:1 sex ratio). Deteriorating and heavily damaged leaves were removed and replaced with new cuttings every other day, and each petri dish was changed completely approximately twice per week. When adults began to lay eggs, the egg bearing leaves were removed and recombined in equal proportions from different source dishes to maintain a diverse genetic pool. Larvae were reared on fresh leaf cuttings in large petri dishes, similar to adults. Large third instars were transferred to petri dishes with moistened vermiculite to simulate soil for pupation. Beetles completed a full generation cycle in approximately two months.

Test Plants: Potential host preferences were evaluated on a total of 58 plant species in 30 families. Test plants were selected based on the centrifugal phylogenetic method proposed by Wapshere (1974). The test list included six plant species requested by the U.S. Fish and Wildlife Service because of their ecological importance, as well as a variety of species with economic significance in Hawaii (Table 1). Potted plants were grown with a standard medium of half potting soil and half cinder under automated irrigation and either direct sunlight (1200 m elevation at HVNPQF) or 73% shade cloth (300 m elevation at Waiakea Experiment Station, University of Hawaii College of Tropical Agriculture and Human Resources, Hilo). Cuttings were made from wild plants growing in the vicinities of Volcano and Hilo, Hawaii. Two common forms of Hawaii's dominant forest tree, *Metrosideros polymorpha*, were tested: with glabrous and pubescent leaves. All plants and cuttings were maintained without pesticides and were inspected and cleaned to remove pests and previous damage before testing.

Table 1. Plant species used for *Syphraea uberabensis* host specificity testing, listed in order of phylogenetic relation to the target weed, *Tibouchina herbacea*.

Order	Family	Tribe	Species	Common name
Myrtales	Melastomataceae	Melastomeae	*Tibouchina herbacea*	cane tibouchina
			Tibouchina longifolia	
			Tibouchina urvilleana	glorybush
			Pterolepis glomerata	
			Heterocentron subtriplinervium	pearl flower
			Melastoma septemnervium	
			Melastoma sanguineum	
			Dissotis rotundifolia	
		Microlicieae	*Arthrostemma ciliatum*	
		Dissochaeteae	*Medinilla cumingii*	
		Miconieae	*Clidemia hirta*	Koster's curse
			Miconia calvescens	miconia
			Tetrazygia bicolor	
	Myrtaceae		*Metrosideros polymorpha* *	ohia lehua
			Syzygium cumini	Java plum
			Syzygium malaccense	mountain apple
			Syzygium jambos	rose apple
			Psidium cattleianum	strawberry guava
			Eugenia uniflora	surinam cherry

Table 1 (continued).

Order	Family	Tribe	Species	Common name
Myrtales	Lythraceae		*Lythrum maritimum*	
			Cuphea carthagenensis	
			Cuphea ignea	cigar flower
			Cuphea hyssopifolia	false heather
	Onagraceae		*Fuchsia magellanica*	fuchsia
			Epilobium ciliatum	
			Oenothera laciniata	evening primrose
	Combretaceae		*Terminalia catappa*	tropical almond, false kamani
Sapindales	Anacardiaceae		*Mangifera indica*	mango
	Rutaceae		*Citrus limon*	lemon
	Sapindaceae		*Dodonaea viscosa* *	‘a’ali’i
Malvales	Malvaceae		*Hibiscus arnottianus*	hibiscus
	Thymelaeaceae		*Wikstroemia sandwicensis*	akia
Brassicales	Caricaceae		*Carica papaya*	papaya
Fabales	Fabaceae		*Acacia koa* *	koa
			Erythrina sandwicensis	wiliwili
			Pisum sativum	pea
			Sophora chrysophylla *	mamane
Rosales	Rosaceae		*Rubus ellipticus*	Himalayan raspberry
			Rubus hawaiensis	akala
			Fragaria vesca	strawberry
	Urticaceae		*Pipturus albidus*	mamaki
Malpighiales	Passifloraceae		*Passiflora* spp.	passion flower
Ericales	Ericaceae		*Vaccinium calycinum*	ohelo
	Theaceae		*Camellia sinensis*	tea
Gentianales	Apocynaceae		*Alyxia stellata*	maile
	Rubiaceae		*Coffea arabica*	coffee
Solanales	Solanaceae		*Physalis peruviana*	poha
Lamiales	Myoporaceae		*Myoporum sandwicense* *	naio
Asterales	Goodeniaceae		*Scaevola chamissoniana*	naupaka
Proteales	Proteaceae		*Macadamia integrifolia*	macadamia
Alismatales	Araceae		*Anthurium* sp.	anthurium
Asparagales	Asparagaceae		*Cordyline fruticosa*	ki
	Orchidaceae		*Arundina graminifolia*	bamboo orchid
			Cymbidium sp.	cymbidium
Zingiberales	Zingiberaceae		*Hedychium gardnerianum*	kahili ginger
Laurales	Lauraceae		*Persea americana*	avocado
Gleicheniales	Gleicheniaceae		*Dicranopteris linearis*	uluhe
Polypodiales	Dicksoniaceae		*Cibotium glaucum* *	hapuu pulu

*Ecologically significant native species tested on request of U.S. Fish and Wildlife Service

Results: Larval Feeding and Survival

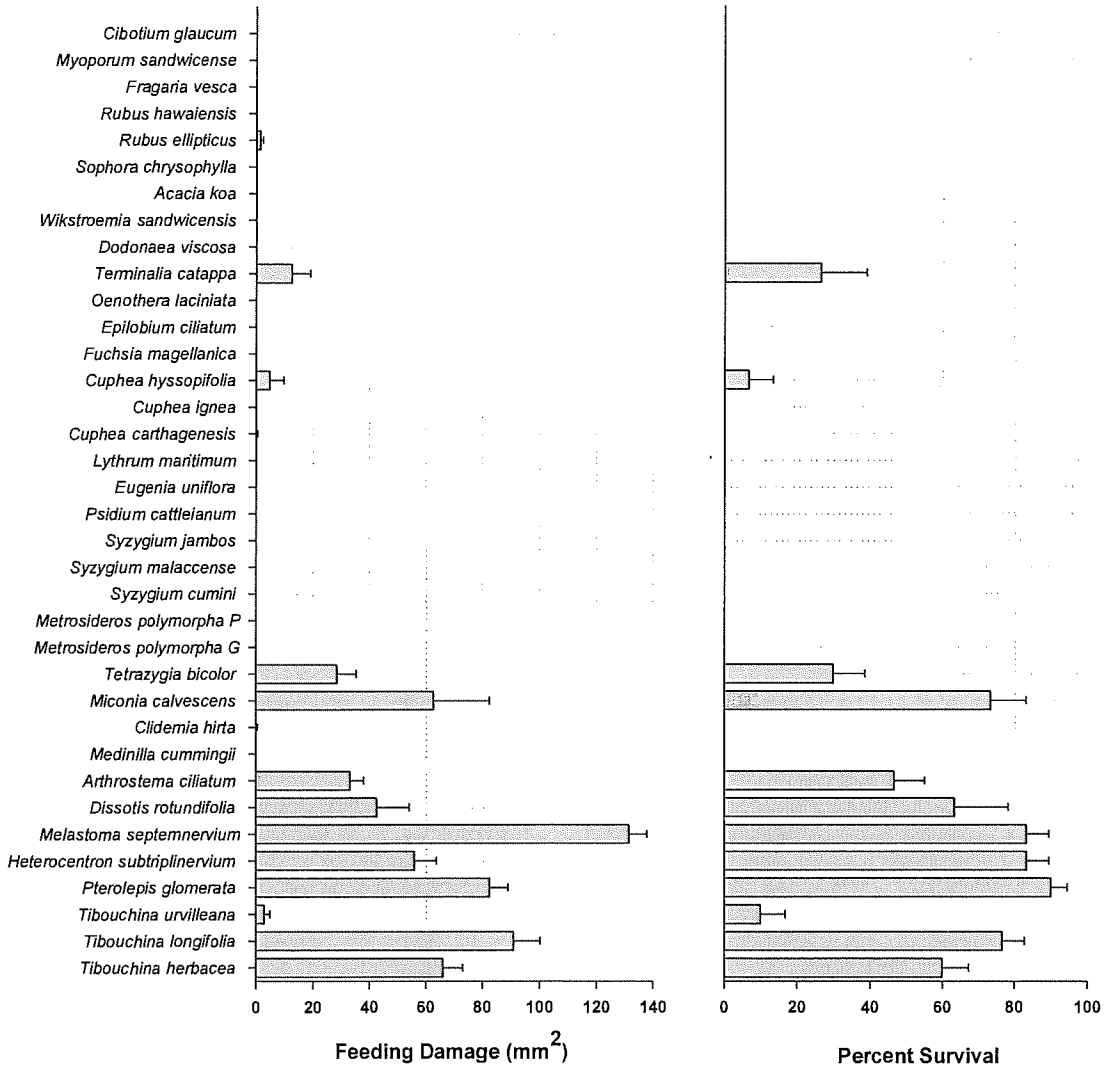

Figure 1. Feeding and survival of newly hatched *S. uberabensis* larvae after 7 days on potted plants under no-choice conditions (means ± standard errors; 6 replicates, 5 larvae per replicate). Genetic relationship to *Tibouchina herbacea* increases from top to bottom. Two leaf forms of *Metrosideros polymorpha* were tested: glabrous (G), and pubescent (P).

Results: Larval Survival and Development

Table 2. Survival of *S. uberabensis* from newly hatched first instars to successive developmental stages on fresh plant cuttings under no-choice conditions (mean percentage ± standard error; 4 replicates 10 larvae per replicate). Plant species were selected based on occurrence of at least minor levels of larval feeding in 7 day tests.

Test Plant	2nd Instar	3rd Instar	Pupa	Adult
Tibouchina herbacea	80.0 ± 4.1	70.0 ± 4.1	67.5 ± 4.8	62.5 ± 4.8
Tibouchina longifolia	82.5 ± 2.5	77.5 ± 4.8	75.0 ± 2.9	62.5 ± 2.5
Tibouchina urvilleana	0	-	-	-
Pterolepis glomerata	90.0 ± 4.1	85.0 ± 2.9	80.0 ± 4.1	67.5 ± 2.5
Heterocentron subtriplinervium	50.0 ± 4.1	30.0 ± 7.1	25.0 ± 6.5	15.0 ± 6.5
Melastoma septemnervium	82.5 ± 8.5	75.0 ± 5.0	67.5 ± 2.5	62.5 ± 2.5
Dissotis rotundifolia	42.5 ± 11.1	27.5 ± 4.8	17.5 ± 7.5	12.5 ± 4.8
Arthrostema ciliatum	0	-	-	-
Medinilla cummingii	0	-	-	-
Clidemia hirta	0	-	-	-
Miconia calvescens	37.5 ± 8.5	22.5 ± 9.5	0	-
Tetrazygia bicolor	32.5 ± 8.5	17.5 ± 2.5	10.0 ± 4.1	0
Cuphea carthagenensis	0	-	-	-
Cuphea hyssopifolia	5.0 ± 2.9	0	-	-
Terminalia catappa	15.0 ± 6.5	0	-	-

Results: Young Adult Feeding and Survival

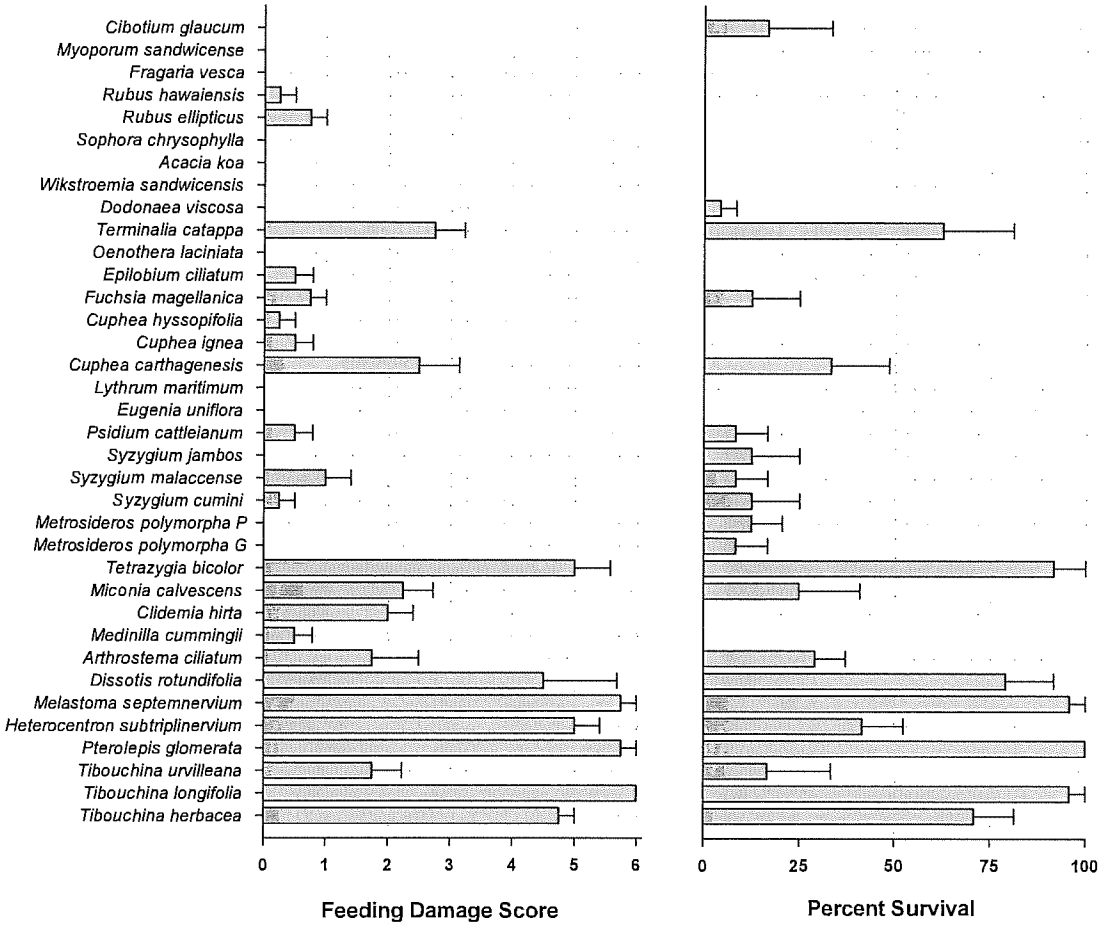

Figure 2. Feeding and survival of naïve adult *S. uberabensis* after 7 days on potted plants under no-choice conditions (means ± standard errors; 4 replicates). Naïve adults (3 male and 3 female per replicate) were newly emerged from pupation in vermiculite (<12 hours old) and had not been exposed to any plant material prior to testing. Feeding score: 0 = no damage, 1 = fewer than 10 pinholes, 2 = less than 1 cm^2 damaged, 3 = 1-2 cm^2, 4 = 2-3 cm^2, 5 = 3-4 cm^2, 6 = greater than 4 cm^2 damaged.

Results: Mature Adult Feeding and Survival

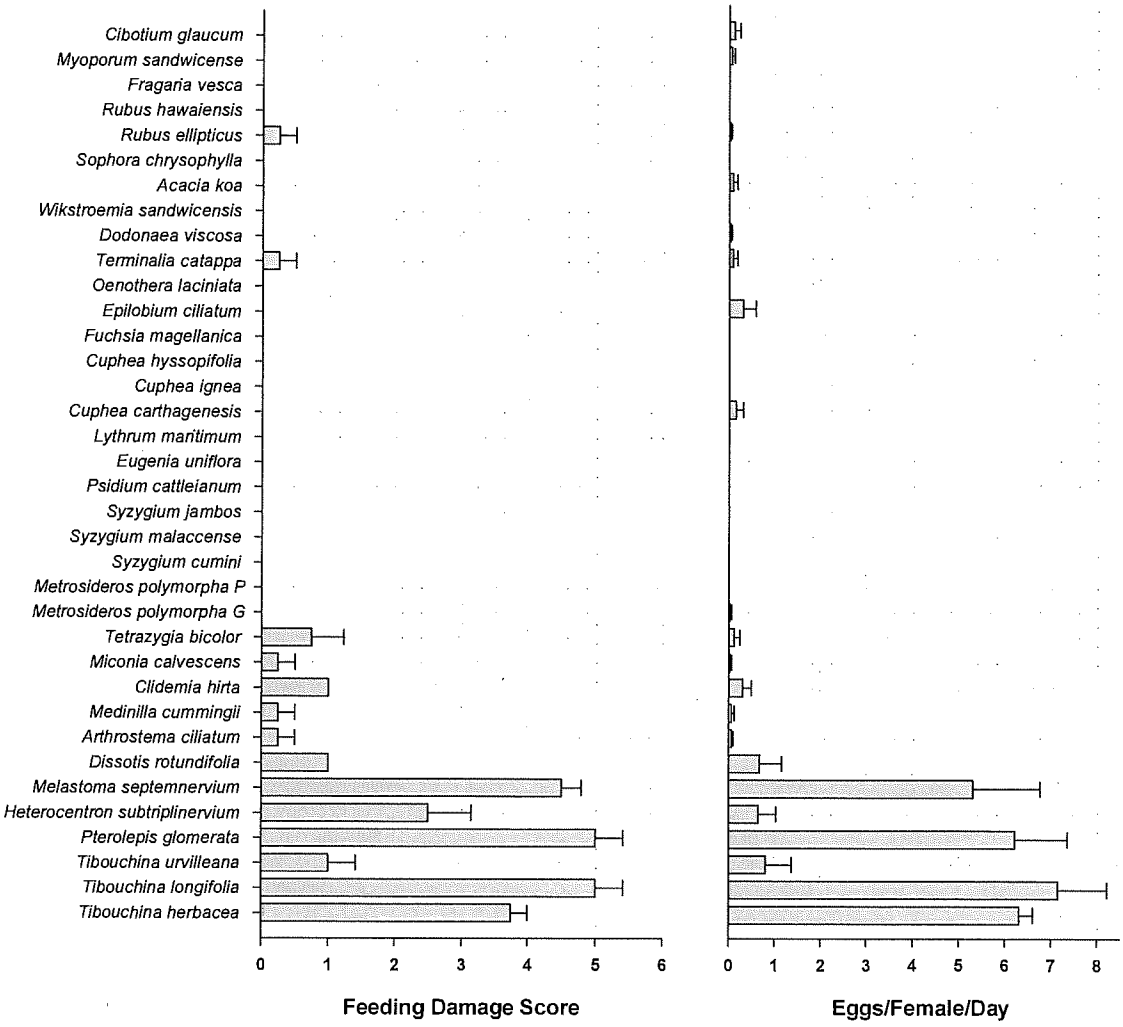

Figure 3. Feeding and oviposition by mature adult *S. uberabensis* after 4 days on potted plants under no-choice conditions (means ± standard errors; 4 replicates). Before testing, adults were reared in petri dishes on *T. herbacea* cuttings for 30 days, removed from food for 24 hours, and then transferred as mating pairs into enclosures on potted plants (2 males and 2 females per replicate). Mature beetles fed more selectively than naïve adults which had no prior feeding experience on *T. herbacea* (Fig. 2). However, testing naïve adults for longer periods showed that only a few melastome species support survival to maturity and oviposition (Table 3).

Results: Fecundity and Development

Table 3. Female lifespan, pre-oviposition period, and total fecundity (means ± standard errors), and survival of offspring for *S. uberabensis* male-female pairs fed fresh plant cuttings under no-choice conditions.

Test Plant	Number females	Life Span (d)	Pre-oviposition (d)	Eggs per female	F1 Survival egg to adult (n=eggs collected)	F2 Survival egg to 3rd instar (n=eggs collected)
Tibouchina herbacea	12	50.3 ± 3.4	23.4 ± 0.5	173 ± 38	38% (94)	67% (100)
Tibouchina longifolia	10	70.1 ± 8.0	40.3 ± 3.3	175 ± 36	43% (90)	72% (100)
Tibouchina urvilleana	7	51.4 ± 4.9	31.6 ± 1.5	36 ± 13	0% (50*)	-
Pterolepis glomerata	14	98.8 ± 7.4	58.6 ± 6.5	220 ± 31	51% (93)	79% (100)
Heterocentron subtriplinervium	10	25.8 ± 3.8	23.0 ± 0	6 ± 6	0% (11*)	-
Melastoma septemnervium	11	63.6 ± 7.5	29.6 ± 2.5	207 ± 37	42% (91)	71% (100)
Tetrazygia bicolor	11	52.9 ± 5.6	45.3 ± 6.9	17 ± 12	0% (40*)	-

* Collected every egg laid by females on this test plant

This test was initiated with naïve adults caged on foliage of a potted plants of 13 species (30 beetles per plant). After 14 days surviving individuals were separated into single male-female pairs and placed in petri dishes with plant cuttings. Only four plants of 13 species tested sustained naïve adults to maturation, oviposition, and development of F1 generation adult beetles: *T. herbacea*, *T. longifolia*, *P. glomerata*, and *M. septemnervium*. F1 progeny were reared on each of these four plant species, producing viable F2 eggs and larvae that development to third instars before testing was terminated. Plant species *T. urvilleana, H. subtriplinervium,* and *T. bicolor* supported survival of beetles to maturation and egg-laying, but larvae did not survive. No beetles survived beyond 14 days on plants species *A. ciliatum*, *C. hirta*, *M. calvescens*, *C. carthagenesis*, and *T. catappa*; some beetles survived on *D. rotundifolia* but did not produce any eggs. (For these reasons, results for these six plant species are not shown above).

There were significant differences between *T. herbacea*, *T. longifolia*, *P. glomerata*, and *M. septemnervium* in female lifespan ($H = 17.90$, $df = 3$, $P \geq 0.001$) and pre-oviposition time ($H = 17.74$, $df = 3$, $P \geq 0.001$), but no significant differences in the total number of eggs laid ($H = 1.46$, $df = 3$, $P = 0.691$) or in daily oviposition rates ($H = 1.73$, $df = 3$, $P = 0.631$).

Results: Additional Tests with Mature Adults

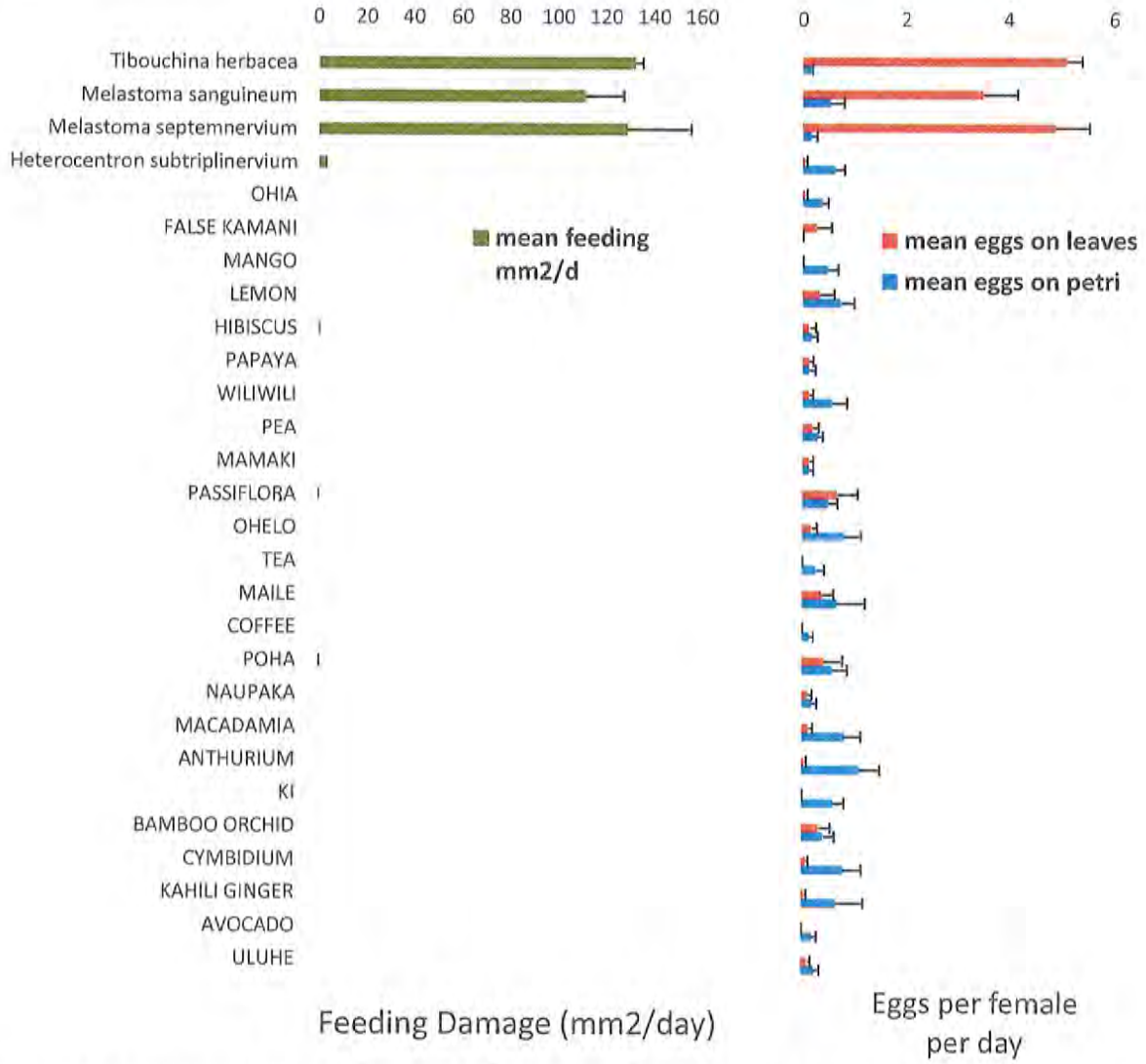

Figure 4. Feeding and egg-laying by mature adults exposed to plant leaves in petri dishes (10cm x 1cm) for 2 days under no-choice conditions; 3 females and 3 males per test (means ± standard errors; 4 replicates). This test utilzed a variety of plants not closely related to the family Melastomataceae. It was common for females to occasionally lay a few eggs in petris with non-host plants, typically on a paper towel rather than on a leaf. "Egg dumping" is not unusual in this kind of confined testing (Heard 2002, Papaj 2000, Wang & Horng 2004).

Results: Adult Choice Tests

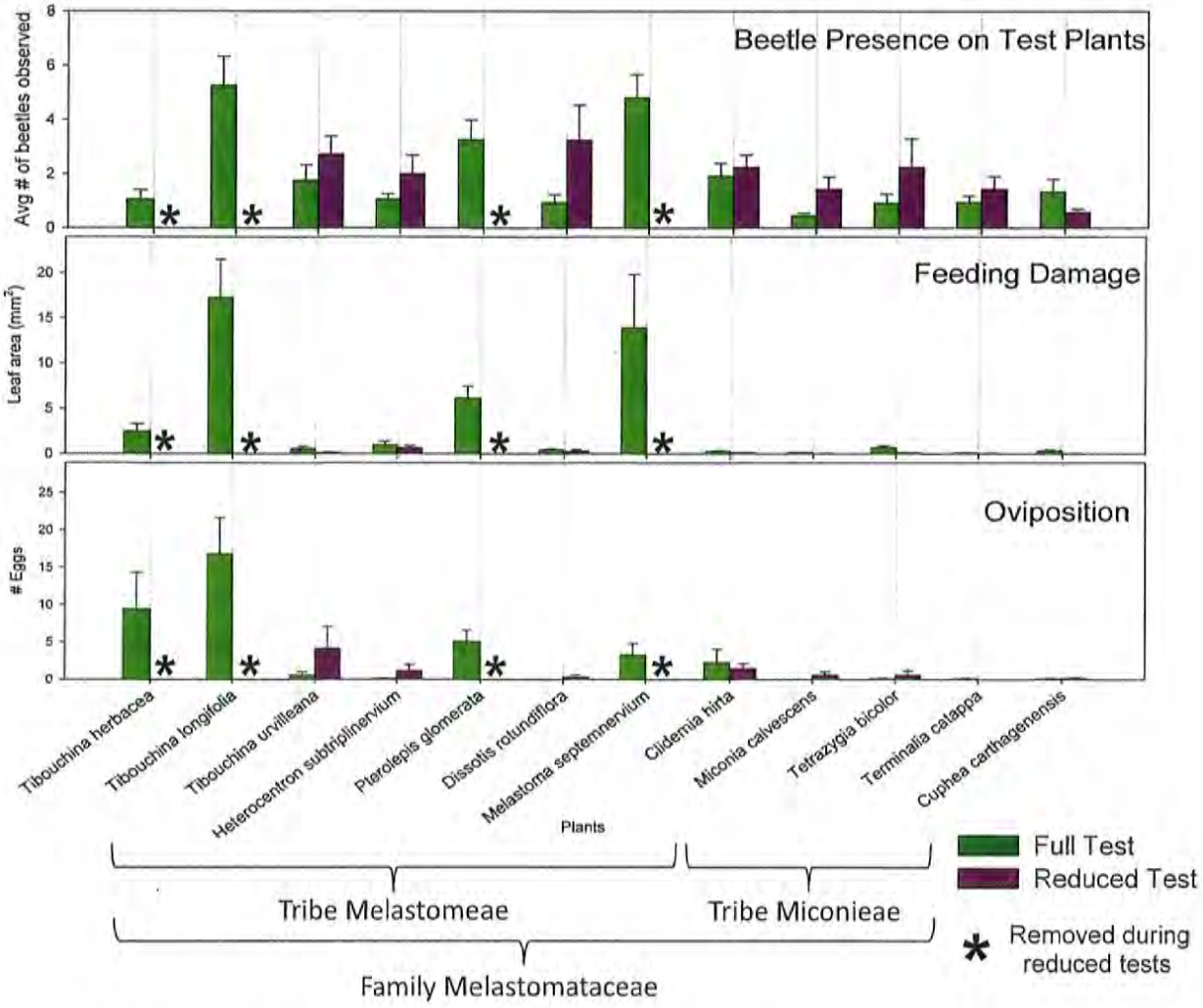

Figure 5. Location, feeding, and oviposition of *Syphraea uberabensis* in multi-choice testing over 3 days in an arena (40x40x40 cm) with cut stems of several plant species (means ± standard errors). Plant species are listed from left to right in order of decreasing genetic relationship to *Tibouchina herbacea*. Green bars represent Full tests (12 replicates, 12 test plants), and purple bars represent Reduced tests, for which the highly preferred host plants were removed (7 replicates, 8 test plants). Feeding and egg laying decreased greatly overall when preferred host plants were removed, and egg laying increased only slightly on the non-preferred melastomes, mainly *Tibouchina urvilleana*.

Conclusions

Testing revealed *Syphraea uberabensis* to be narrowly host-specific within the family Melastomataceae and able to complete development on only five plant species in Hawaii. Larvae and naïve adults showed a somewhat broader range of feeding compared to mature adults in tests lasting a few days, however low levels of feeding outside the normal host range is a common result of no-choice tests, in which insects are unable to seek out preferred hosts (Heard 2002). Longer test periods demonstrated that only a few melastome species support survival to maturity and oviposition. Choice tests demonstrated the same few melastome species to be highly preferred over other related plants.

Egg laying was negligible on all plants tested except *Tibouchina herbacea*, *Tibouchina longifolia*, *Pterolepis glomerata*, *Melastoma septemnervium* and *Melastoma sanguineum*. Furthermore, these species were the only plants that supported the complete life cycle of *S. uberabensis*. Eggs laid in very low numbers on other species may have been a result of egg dumping, which occurs with some insects when a female's egg load exceeds a maximum threshold (Papaj 2000, Wang & Horng 2004). Feeding and minor egg laying suggested that a few Melastomataceae (*T. urvilleana*, *T. bicolor*, *H. subtriplinervium* and *D. rotundifolia*) might be marginal hosts, however longer development tests showed that these plants are unlikely to sustain populations of *S. uberabensis*. If introduced to Hawaii, it is possible that *S. uberabensis* could be found in association with these plants where they grow in proximity to hosts that support complete development. Additional association could be observed on the non-melastome *Terminalia catappa*, which experienced minor feeding damage in host specificity tests. However, no sustained development occurred during long-term larval and adult tests on this plant, and in Hawaii *T. catappa* typically occurs at coastal sites where the preferred melastome hosts are not common. Feeding observed in no-choice testing on plants like *T. catappa* is less likely to occur when flea beetles can move to a preferred host (Heard 2002). Choice tests confirmed this, showing negligible feeding and egg laying by *S. uberabensis* on *T. catappa*, regardless of presence or absence of highly preferred hosts.

It is interesting to note that two suitable hosts of *S. uberabensis*, *Melastoma septemnervium* and *Melastoma sanguineum*, originate from Asia, and that ancestors of this plant genus likely diverged from neotropical ancestral hosts of *Syphraea* an estimated 11-12 million

years ago (Renner and Meyer 2001). Molecular analyses place the three genera, *Melastoma*, *Pterolepis* and *Tibouchina*, all in the same clade (Clausing & Renner 2001). Thus our host range results are consistent with a long coevolutionary relationship between *Syphraea* and members of these taxa. Plant secondary chemistry of melastomes has received limited study (Yoshida et al 2005), but would likely shed additional light on relationships between these three genera and host preference of *S. uberabensis*.

The preferred melastome hosts of *S. uberabensis* are all considered serious weeds in Hawaii (HDOA 1992, Jacobi and Warshauer 1992, Almasi 2000, Motooka et al. 2003). Of these plants, *T. longifolia* has the most limited distribution and appears least likely to have significant ecological interaction with the potential biocontrol agent. If *T. herbacea* and *M. septemnervium* can maintain substantial populations of *S. uberabensis*, these might help suppress *T. longifolia* and prevent it from spreading. The species *T. herbacea* and *M. septemnervium* overlap geographically across large areas, which could facilitate establishment and impacts of *S. uberabensis* generally. *M. sanguineum* is ecologically similar to *M. sanguineum* but less widely distributed. Impacts of biocontrol by *S. uberabensis* would likely be swifter and more severe on *T. herbacea* than *M. septemnervium* and *M. sanguineum*, which grow to large woody shrubs. Increased herbivory of *M. septemnervium*, which has been targeted but not adequately impacted by past introductions of other biocontrols (Conant et al. 2013), would have potential benefit to extensive forest watersheds in Hawaii (Jacobi and Warshauer 1992). The final host, *P. glomerata*, is a less prominent invader but broadly distributed in wet forests and pastures, including mountain areas on the island of Oahu where it has limited overlap with the other melastome hosts. Although *P. glomerata* appears to be equally suitable as a host for *S. uberabensis*, longer development times on this plant might delay the impacts of biocontrol (Souder 2008).

Syphraea is tolerant of cool and moderate temperatures, and it is not expected to be restricted in range by temperatures in Hawaii, except perhaps in exceptionally warm habitats (Souder 2008). However, its potential as a biological control could be limited by humidity at the microhabitat level. In Brazil, *S. uberabensis* is found with its melastome hosts in boggy soils, similar to the areas where *T. herbacea* and *P. glomerata* thrive in Hawaii, so these hosts should be highly susceptible. On the other hand, *Melastoma* spp. can grow in drier areas – such as

young lava flows. *Syphraea* could be less effective against *Melastoma* in dry habitats, because its eggs and larvae appear to be susceptible to drying when humidity is not high.

In conclusion, our testing indicates that *S. uberabensis* is narrowly host specific and will not feed or survive on any native or otherwise important plants in Hawaii. Given that Melastomataceae are entirely alien to Hawaii, and the host range of *S. uberabensis* includes only five weedy melastome species here, this flea beetle appears to hold great potential benefit and minimal environmental risk as a future biological control agent.

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USDA United States Department of Agriculture

ATTACHMENT 4

United States Department of Agriculture
Animal and Plant Health Inspection Service
Plant Protection & Quarantine
4700 River Road
Riverdale, MD 20737

Permit to Move Live Plant Pests, Noxious Weeds, and Soil

Importation
Regulated by 7 CFR 330

This permit was generated electronically via the ePermits system

PERMITTEE NAME:	Matthew Johnson	**PERMIT NUMBER:**	P526P-20-02009
ORGANIZATION:	USDA Forest Service	**APPLICATION NUMBER:**	P526-190826-015
ADDRESS:	Hawaii Volcanoes National Park Quarantine Facility Kilauea Research Station, Building 34 Volcano, HI 96718	**FACILITY NUMBER:**	22
MAILING ADDRESS:	P.O. Box 236 Volcano, HI 96785	**HAND CARRY:**	No
		DATE ISSUED:	04/21/2020
PHONE:	808-967-7122		
FAX:	808-967-7158	**EXPIRES:**	**04/21/2023**
DESTINATION:	HI		
DESIGNATED PORTS:	HI, Honolulu		

Under the conditions specified, this permit authorizes the following:

Regulated Article	Life Stage(s)	Intended Use	Shipment Origins	Originally Collected	Culture Designation
Allorhogas clidemiae	Any	Research - Lab	Central America, South America	Originally Collected from Outside the U.S. and Territories	
Allorhogas granivorus	Any	Research - Lab	Central America, South America	Originally Collected from Outside the U.S. and Territories	
Anthonomus monostigma	Any	Research - Lab	Central America, South America	Originally Collected from Outside the U.S. and Territories	
Diclidophlebia lucens	Any	Research - Lab	Central America, South America	Originally Collected from Outside the U.S. and Territories	
Euselasia bettina	Any	Research - Lab	Central America, South America	Originally Collected from Outside the U.S. and Territories	
Euselasia chrysippe	Any	Research - Lab	Central America, South America	Originally Collected from Outside the U.S. and Territories	
Syphraea uberabensis	Any	Research - Lab	Central America, South America	Originally Collected from Outside the U.S. and Territories	

SPECIAL INSTRUCTIONS TO INSPECTORS

See permit conditions below

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS. Robert Pfannenstiel	DATE 04/21/2020

DHS CBP INSPECTORS - SHIPMENT BY BONDED CARRIER

1) Confirm that the carrier of the shipment imported under this USDA PPQ 526 permit is commercially bonded.

2) Confirm that the imported shipment has a valid USDA PPQ Form 599 Red/White label attached to the exterior for routing to a USDA APHIS PPQ Inspection Station or other "Designated Port" as stated on the Permit. A valid label will have the permit number, expiration date, label number, and address of a USDA APHIS PPQ Plant Inspection Station/Designated Port. PLEASE NOTE: In the event of a shipment of bulk container with discrete units, a single PPQ Form 599 Red/White label may be used.

3) Validate the permit in ePermits using the CBP search feature.

4) If a valid PPQ Form 599 Red/White label is not attached to the exterior of the package or the label has been covered or is otherwise not legible, then forward to the nearest USDA APHIS PPQ Plant Inspection Station.

5) If the address on the airway bill does not match the address on the PPQ Form 599 Red/White label then forward the package to the nearest USDA APHIS PPQ Plant Inspection Station/designated port shown on the PPQ Form 599 label. All costs associated with rerouting misaddressed packages will be assumed by the permit holder.

APHIS PPQ INSPECTORS at PIS -High-Risk Invertebrates

Follow the instructions in the Plant Inspection Station Manual for High-Risk Invertebrates Red and White Labeled Packages (must be opened in a sleeved cage; see procedures for handling on page 3-7-39). For questions or concerns, contact the USDA APHIS PPQ Pest Permit Branch in Riverdale, MD, at 301-851-2046, toll free 866-524-5421.

PERMIT GUIDANCE

1) Receipt or use of foreign isolates or samples from countries under sanctions requires specific permission from the U.S. Department of Treasury; please refer to https://www.treasury.gov/resource-center/sanctions/Programs/Pages/Programs.aspx

2) This permit does not authorize movement or release into the environment of genetically engineered organisms produced with the regulated organisms described in this permit. Importation, interstate movement, and environmental release of genetically engineered plant pests require a different permit issued under regulations at 7 CFR part 340. Any unauthorized interstate movement or environmental release, including accidental release, of a regulated GE organism would be a violation of those regulations. Additional guidance and contact information for APHIS Biotechnology Regulatory Services, can be found at: https://www.aphis.usda.gov/aphis/ourfocus/biotechnology.

3) If an animal pathogen is identified in your shipment, to ensure appropriate safeguarding, please refer to http://www.aphis.usda.gov/import_export/animals/animal_import/animal_imports_anproducts.shtml

4) If a human pathogen is identified, please refer to the CDC Etiologic Agent Import Permit Program at http://www.cdc.gov/od/eaipp/

5) This permit does not fulfill the requirements of other federal or state regulatory authorities. Please contact the appropriate agencies, such as the U.S. Environmental Protection Agency, the U.S. Fish and

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS.	DATE
Robert Pfannenstiel	04/21/2020

Wildlife Service, the U.S. Food and Drug Administration, the Centers for Disease Control and Prevention, the APHIS Veterinary Services unit, the APHIS Biotechnology Regulatory Services, or your State's Department of Agriculture to ensure proper permitting.
6) If you are considering renewal of this permit, an application should be submitted at least 90 days prior to the expiration date of this permit to ensure continued coverage. Permits requiring containment facilities may take a longer period of time to process.

PERMIT CONDITIONS

USDA-APHIS issues this permit to Matthew Johnson, USDA Forest Service, Hawaii Valcanoes National Park, Quarantine Facility, Kilauea Research Station, Volcano, HI 96718. This permit authorizes the importation of any life stages of the various taxa shown under Regulated Article above, collected in/from Central and South American countries, and observed to feed on or be associated with Miconia calvescens,(the target/host plant), to the permit holder Dr. Matthew Johnson, USDA Forest Service, Hawaii Volcanoes National Park, to be received into the USDA APHIS approved containment facility at that address (CF #22).

The imported material may contain various host plant parts of Miconia calvescens, including roots, leaves and stems.

This permit authorizes the possession and rearing of any species imported under this permit for research in the USDA APHIS inspected containment facility (Facility #22) at USDA Forest Service, Hawaii Volcanoes National Park, Kilauea Research Station, Quarantine Facility, Building 34, Volcano, HI 96718, subject to the conditions below.

1. • This permit is issued by the United States Department of Agriculture's Animal and Plant Health Inspection Service (APHIS). It conveys APHIS regulations and requirements for the material(s) listed on this permit. It does not reduce or eliminate your legal duty and responsibility to comply with all other applicable Federal and State regulatory requirements.

   • The permit number or a copy of the permit must accompany the shipment.

   • You must be an individual at least 18 years old, or legal entity such as partnership, corporation, association, or joint venture.

   • You are legally responsible for complying with all permit requirements and permit conditions.

   • The regulated material and shipping container(s) are subject to inspection by officials of Custom and Border Protection (CBP) and APHIS. CBP or APHIS officials may require the shipment to be treated, seized, re-exported, or destroyed (in part or whole). You will be responsible for expenses.

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS.	DATE
Robert Pfannenstiel	04/21/2020

- If you violate any applicable laws associated with this permit, you may face substantial civil or criminal penalties. We may cancel all current permits and deny future permit applications.
- Without prior notice and during reasonable hours, authorized Federal and State Regulators must be allowed to inspect the conditions associated with the regulated materials/organisms authorized under this permit.

2. The permit holder must:

- maintain a valid PPQ526 permit so long as the regulated materials/organisms are alive or viable,
- not assign or transfer this permit to other persons without APHIS PPQ authorization,
- maintain an official permanent work assignment, residence, or affiliation at the address on this permit,
- notify the Pest Permit Staff as soon as possible of any change in the permit holder's work assignment, residence, or affiliation,
- notify the Pest Permit Staff of the receipt of unauthorized and/or misdirected shipments of regulated materials/organisms,
- adequately mitigate environmental impacts resulting from unauthorized release of regulated materials/organisms and notify the Pest Permit staff immediately if one occurs,
- notify the Pest Permit Staff if the facility is damaged/destroyed or if you wish to decommission the facility,
- destroy all regulated materials/organisms prior to departure from the organization unless other arrangements are confirmed by the Pest Permit Staff.

Notifications to the Pest Permit Staff must be made via 866-524-5421 or pest.permits@usda.gov within one business day of the event triggering a notification.

Permit Number P526P-20-02009

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS.	DATE
Robert Pfannenstiel	04/21/2020

WARNING: Any alteration, forgery or unauthorized use of this Federal Form is subject to civil penalties of up to $250,000 (7 U.S.C.s 7734(b)) or punishable by a fine of not more than $10,000, or imprisonment of not more than 5 years, or both (18 U.S.C.s 1001)

3. All packages for transport must minimally consist of both inner/primary and outer/secondary packages securely sealed so that both are effective barriers to escape or unauthorized dissemination of the listed materials/organisms. The inner/primary package(s) will contain all regulated materials/organisms and must be cushioned and sealed in such a way that it remains sealed during shock, impact, and pressure changes that may occur. The outer/secondary shipping container must be rigid and strong enough to withstand typical shipping conditions (dropping, stacking, impact from other freight, etc.) without opening.
4. After PPQ issues this 526 permit, you will need to request Red/White labels (PPQ Form 599) at least 5 days in advance of your shipping date. If you applied for your permit online using ePermits, you may request the labels using the My Shipments/Labels feature. Otherwise, send your request to Redandwhitelabelrequest@usda.gov. All email requests must come from the permit holder or designee. If requested by the designee, the permit holder must be copied on all requests. Specify the approved port as listed on the permit and the total number of labels needed. You may request additional labels the same way.
   Packages without labels on the exterior may be refused entry.

   Review label instructions at:
   https://www.aphis.usda.gov/aphis/ourfocus/planthealth/import-information/permits/plant-pes ts/or ganisms-shipping-requirements

   You are responsible for instructing your shipper to carefully follow these instructions. You are responsible for each import shipping label issued under this permit.
5. Upon receipt, open the package only in the approved containment facility identified above. Depending on the organism(s) or developmental stage, it may be necessary to open the package inside a cage (glove box or sleeve cage) or use other appropriate means that must prevent the organisms from escaping.
6. After separation of organisms regulated under this permit, along with any necessary host organisms and host plant parts, all other foreign biological material and substrate, including soil, and foreign plant material, if any, must be properly disposed of or destroyed immediately.

   Only authorized/permitted organisms may be retained as live organisms, plus any hosts and plant parts as needed for continued rearing and culture of the regulated organisms until transfer to lab-sourced material. Upon completion of isolations/transfers from imported material (i.e., soil, hosts) these imported materials must likewise be properly disposed of or destroyed immediately, as described above.

   Only secondary containers and packing materials suitable for re-use (such as coolers and icepacks) may be reused, and only after sterilization by autoclave, or with bleach or alcohol, etc., as per protocols established in the SOP's for this facility.

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS.	DATE
Robert Pfannenstiel	04/21/2020

7. This permit authorizes the importation and possession of live organisms of only those taxa/species listed under "Regulated Article" above, and not authorized under this permit are live cultures of other taxonomic groups from other hosts, or are from other source countries/continents, or received by way of any other permit, except as described below.

   In addition, this permit authorizes continued possession/continued curation of only the live organisms (identified and unidentified) cultured or stored by the permit holder which were imported as authorized on previous permits, of which this is a "renewal". All other such live regulated organisms must be kept under separate USDA APHIS permit, or devitalized.

8. The regulated organisms authorized for import under this permit are to be maintained only in the laboratory area approved for containment at the address indicated under the "Authorizations" above on this permit (CF 22). Any distribution or other removal of live organisms regulated under this permit from the designated area of Containment Facility Forest Service requires a separate prior authorization from APHIS PPQ.

   This permit does not authorize field release, interstate transport, field research, greenhouse work, or any other activities with the regulated organisms authorized for import under this permit outside of the containment facility.

9. All operations must be consistent with information submitted in association with this Containment Facility (CF #22) including the most recent Standard Operating Procedures (SOP's) submitted for the Facility, and any information submitted in association with the inspection of this Containment Facility. This includes, minimally, maintenance of restricted access to unauthorized persons of building and or approved containment areas (key, key card or code), and/or restricted access to unauthorized persons of growth chambers and other equipment (for example by lock) where organisms will be kept, as well as proper/prescribed maintenance of the Autoclave and/or other equipment used to devitalize or sterilize waste.

   The permit holder must insure that all persons working with these regulated organisms
   a) are trained in the importance of approved containment practices;
   b) follow the Standard Operating Procedures (SOP) established for the facility and filed with the USDA APHIS Pest Permit Evaluation Unit at the time of facility inspection; and
   c) are informed of these permit conditions and understand the requirement to adhere to these conditions and the SOP.

   The permit holder shall document such training or familiarization with these permit conditions and the SOP's for the facility, by having copies of both dated and signed/initialed by all persons handing the regulated articles, and have such documentation made available to USDA APHIS upon request.

Permit Number P526P-20-02009

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS.	DATE
Robert Pfannenstiel	04/21/2020

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10. A separate authorization from USDA APHIS (a new PPQ 526 permit) is required for possession/maintenance of live regulated organisms received under this permit beyond the expiration of this permit. Otherwise, all regulated organisms received under this permit must be devitalized prior to expiration of this permit.

END OF PERMIT CONDITIONS

Permit Number P526P-20-02009

THIS PERMIT HAS BEEN APPROVED ELECTRONICALLY BY THE FOLLOWING PPQ HEADQUARTER OFFICIAL VIA EPERMITS.	DATE
Robert Pfannenstiel	04/21/2020

ATTACHMENT 5

M. TRACY JOHNSON

Institute of Pacific Islands Forestry
Pacific Southwest Research Station
USDA Forest Service

P.O. Box 236, Volcano, Hawaii 96785
tel: 808-967-7122
email: tracy.johnson@usda.gov

Education

Ph.D., 1995, Entomology, North Carolina State University
Thesis: The role of natural enemies in ecology and evolution of *Heliothis virescens* on transgenic plants.

M.S., 1990, Entomology, North Carolina State University
Thesis: Combined effects of genetically engineered host plant resistance and natural enemies on *Heliothis* populations in tobacco.

A.B., 1984, Biology, University of California - Berkeley

Work Experience

Research Entomologist, Aug 2000-Present, USDA Forest Service, PSW, Institute of Pacific Islands Forestry
Biological control of weeds in Hawaiian forests, Insect ecology, Post-release monitoring of biocontrol, Non-target impacts of biocontrol, Plant-herbivore-enemy interactions

Junior Researcher, Mar-Aug 2000, Department of Zoology, University of Hawaii – Manoa
Examining population dynamics of the agricultural pest *Nezara viridula* under sublethal biological control by an introduced parasitoid.

Junior Researcher, Dec 1997-Feb 2000, Dept. Entomology, University of Hawaii - Manoa
Quantifying the off-target effects of biological control on the native Hawaiian koa bug, and surveying parasitism of an alien leafhopper invading native forests.

Fulbright Fellow, Oct 1996-Sep 1997, Internatl Centre of Insect Physiology and Ecology, Kenya
Assessing risk of African maize stemborers evolving resistance to transgenic maize expressing toxins of *Bacillus thuringiensis*.

Technician, May 1984 – Dec 1986, Biological Control of Weeds Lab, USDA-ARS, Albany CA
Field studies of native thistles and insects to measure nontarget impact of weevil introduced for biocontrol of weedy thistles; quarantine study of insects shipped from Greece in search for biocontrol agents against thistles.

Recent Publications

Alfaro-Alpízar MA, Koster SJC, Johnson MT, and Badenes-Pérez FR. 2020. Description, biology, and impact of the fruit-feeding moth, *Mompha luteofascia* sp. n. (Lepidoptera: Momphidae), on *Miconia calvescens* (Melastomataceae) in Costa Rica. *Annals of the Entomological Society of America* 113: 30-39.

Pejchar L, Lepczyk CA, Lepczyk- Fantle J, Hess SC, Johnson MT, Leopold CR, Marchetti M, McClure KM, Shiels AB. 2020. Hawaii as a microcosm: advancing the science and practice of managing introduced and invasive species. *BioScience*

Mayfield AE, Seybold SJ, Haag WR, Johnson MT, Kerns BK, Kilgo JC, Larkin DJ, Lucardi RD, Moltzan BD, Pearson DE, Rothlisberger JD, Schardt JD, Schwartz MK, and Young MK. CHAPTER 2: Impacts of Invasive Species in Terrestrial and Aquatic Systems in the USA, *In* Poland, T.M., Patel-Weynand, T., Finch, D., Miniat, C. F., and Lopez, V. (eds). 2019. Invasive Species in Forests and Grasslands of the United States: A Comprehensive Science Synthesis for the United States Forest Sector. Springer Verlag.

Horvitz CC, Denslow JS, Johnson T, Gaoue O, Uowolo A. 2018. Unexplained variability among spatial replicates in transient elasticity: implications for evolutionary ecology and management of invasive species. *Population Ecology* 60: 61-75.

Barbosa, J. M.; Asner, G. P.; Hughes, R. F.; Johnson, M. T. 2017. Landscape-scale GPP and carbon density inform patterns and impacts of an invasive tree across wet forests of Hawaii. *Ecological Applications* 1-13

Barbosa, J.M.; Asner, G.P.; Martin, R.E.; Baldeck, C.A.; Hughes, F.; Johnson, T. 2016. Determining subcanopy *Psidium cattleianum* invasion in Hawaiian forests using imaging spectroscopy. *Remote Sensing* 8, 33

Johnson, M.T. 2016. Managing conflict over biological control: the case of strawberry guava in Hawaii, pp. 264-276. *In*: Integrating Biological Control into Conservation Practice; Van Driesche, R.G.; Simberloff, D.; Blossey, B.; Causton, C.; Hoddle, M.S.; Wagner, D.L.; Marks, C.O.; Heinz, K.M.; Warner, K.D. (eds). Wiley.

Castillo, A., Johnson, M.T., and Badenes-Perez, F.R. 2014. Biology, behavior, and larval morphology of *Salbia lotanalis*, a potential biological control agent of *Miconia calvescens* from Costa Rica. *Annals of the Entomological Society of America* 107: 1094-1101.

Badenes-Perez, F.R., Castillo, A., and Johnson, M.T. 2014. Damage to *Miconia calvescens* and Seasonal Abundance of *Salbia lotanalis* (Lepidoptera: Crambidae) in Costa Rica. *Environmental Entomology* 43: 877-882.

Hughes, R.F., M.T. Johnson and A. Uowolo. 2013. The invasive alien tree *Falcataria moluccana*: Its impacts and management. Pp 218-223 *in* Wu, Y., T. Johnson, S. Sing, S. Raghu, G. Wheeler, P. Pratt, K. Warner, T. Center, J. Goolsby and R. Reardon (eds), Proceedings of the XIII International Symposium on Biological Control of Weeds.

Conant, P., J.N. Garcia, M.T. Johnson, W.T. Nagamine, C.K. Hirayama, G.P. Markin and R.L. Hill. 2013. Releases of natural enemies in Hawaii since 1980 for classical biological control of weeds. Pp. 230-242 *in* Wu, Y., T. Johnson, S. Sing, S. Raghu, G. Wheeler, P. Pratt, K. Warner, T. Center, J. Goolsby and R. Reardon (eds), Proceedings of the XIII International Symposium on Biological Control of Weeds.

Chacón-Madrigal, E., M.T. Johnson, and P. Hanson. 2012. The life history and immature stages of the weevil *Anthonomus monostigma* Champion (Coleoptera: Curculionidae) on *Miconia calvescens* DC (Melastomataceae). *Proceedings of the Entomological Society of Washington* 114: 173-185.

Ramadan, M.M., K.T. Murai, T. Johnson. 2011. Host range of *Secusio extensa* (Lepidoptera: Arctiidae), and potential for biological control of *Senecio madagascariensis* (Asteraceae). *Journal of Applied Entomology* 135: 269-284.

Badenes-Pérez, F.R., M.A. Alfaro-Alpízar, and M.T. Johnson. 2010. Diversity, ecology and herbivory of hairstreak butterflies (Theclinae) associated with the velvet tree, *Miconia calvescens* in Costa Rica. *Journal of Insect Science* 10, 209

Reichert, E., M.T. Johnson, E. Chacón, R.S. Anderson, and T.A. Wheeler. 2010. Biology and host preferences of *Cryptorhynchus melastomae* (Coleoptera: Curculionidae), a possible biocontrol agent for *Miconia calvescens* (Melastomataceae) in Hawaii. *Environmental Entomology* 39: 1848-1857.

Hanson, P., K. Nishida, P. Allen, E. Chacón, B. Reichert, A. Castillo, M. Alfaro, L. Madrigal, E. Rojas, F. Badenes-Perez, and T. Johnson. 2010. Insects that feed on *Miconia calvescens* in Costa Rica. *In*: Loope, L.L., J.-Y. Meyer, B.D. Hardesty and C.W. Smith (eds.), Proceedings of the International Miconia Conference, Keanae, Maui, Hawaii, May 4-7, 2009, Maui Invasive Species Committee and Pacific Cooperative Studies Unit, University of Hawaii at Manoa. www.hear.org/conferences/miconia2009/proceedings/

Johnson, M.T. 2010. Miconia biocontrol: Where are we going and when will we get there? *In*: Loope, L.L., J.-Y. Meyer, B.D. Hardesty and C.W. Smith (eds.), Proceedings of the International Miconia Conference, Keanae, Maui, Hawaii, May 4-7, 2009, Maui Invasive Species Committee and Pacific Cooperative Studies Unit, University of Hawaii at Manoa. www.hear.org/conferences/miconia2009/proceedings/

Badenes-Perez, F.R., M.A. Alfaro-Alpizar, A. Castillo-Castillo, and M.T. Johnson. 2008. Biological control of *Miconia calvescens* with a suite of insect herbivores from Costa Rica and Brazil. *In* Proceedings of the XII International Symposium on Biological Control of Weeds. Julien MH, Sforza R, Bon MC, Evans HC, Hatcher PE, Hinz HL, Rector BG, editors. CAB International, Wallingford, UK., Montpellier, France. 129-132.

Badenes-Perez, F.R., and M.T. Johnson. 2008. Biology, herbivory, and host specificity of *Antiblemma leucocyma* (Lepidoptera: Noctuidae) on *Miconia calvescens* DC. (Melastomataceae) in Brazil. *Biocontrol Science and Technology* 18: 183-192.

Badenes-Perez, F.R., and M.T. Johnson. 2007. Ecology and impact of *Allorhogas* sp. (Hymenoptera: Braconidae) and *Apion* sp. (Coleoptera: Curculionoidea) on fruits of *Miconia calvescens* DC (Melastomataceae) in Brazil. *Biological Control* 43: 317-322.

ATTACHMENT 6

RESTRICTED ANIMAL LIST (Part A) §4-71-6.5

SCIENTIFIC NAME	COMMON NAME
CLASS Insecta	
ORDER Coleoptera	
FAMILY Apionidae	
Apion scutellare	biocontrol agent, gorse
FAMILY Buprestidae	
Lius poseidon	biocontrol agent, clidemia
FAMILY Chrysomelidae	
Chlamisus gibbosa	biocontrol agent, blackberry
Syphraea uberabensis	biocontrol agent, Tibouchina herbacea
FAMILY Coccinellidae	
Delphastus pusillus	predator, spiraling whitefly
Hippodamia convergens	beetle, convergent lady
Nephaspis oculatus	predator, spiraling whitefly
Nephaspis bicolor	predator, spiraling whitefly
Stethorus nigripes	predator, spider mites
Stethorus picipes	predator, spider mites
FAMILY Curculionidae	
Acythopeus sp. 1	biocontrol agent, ivy gourd
Acythopeus sp. 2	biocontrol agent, ivy gourd
Acythopeus sp. 3	biocontrol agent, ivy gourd
Auletobius convexifrons	biocontrol agent, firetree
Gymnaetron tetrum	biocontrol agent, common mullein
FAMILY Scarabaeidae	
Euoniticellus intermedius	predator, hornfly
Onitis vanderkelleni	predator, horn fly
ORDER Diptera	
FAMILY Chamaemyiidae	
Leucopis (all species in subgenus)	predator
FAMILY Drosophilidae	
Drosophila (all species in genus)	flies, pomace
Zapriothrica sp.	biocontrol agent, banana poka

State of Hawaii
Department of Agriculture
Plant Industry Division
Plant Quarantine Branch
Honolulu, Hawaii

August 23, 2022

Board of Agriculture
Honolulu, Hawaii

SUBJECT: Request to: (1) Allow the Importation of Four (4) African Black-Footed Penguins, *Spheniscus demersus*, an Animal on the List of Restricted Animals (Part B), by Permit, for Exhibition, by Hyatt Regency Maui Resort and Spa; and (2) Update Permit Conditions for the Importation of Four (4) African Black-Footed Penguins, *Spheniscus demersus,* an Animal on the List of Restricted Animals (Part B), for Exhibition, by Hyatt Regency Maui Resort and Spa.

I. Summary Description of the Request

PQB NOTES: *The Plant Quarantine Branch (PQB) submittal for requests for import or possession permits, as revised, distinguishes information provided by the applicant from procedural information and advisory comment and evaluation presented by PQB. With the exception of PQB notes, hereafter "PQB NOTES," the text shown below in Section II from page 2 through page 8 of the submittal was taken directly from the Hyatt Regency Maui Resort and Spa's application and subsequent written communications provided by the applicant, Mrs. Povi Carisa-Abney. For instance, the statements beginning on page 7 regarding effects on the environment are the applicant's statements in response to standard PQB questions and are not PQB's statements. This approach for PQB submittals aims for greater applicant participation in presenting import requests in order to move these requests to the Board of Agriculture (Board) more quickly, while distinguishing applicant provided information from PQB information. The portion of the submittal prepared by PQB, including the Advisory Subcommittee Review, Advisory Committee Review, and Proposed Permit Conditions is identified as Sections III, IV, and V of the submittal, which starts at pages 9, 11, and 17, respectively.*

We have a request to review the following:

COMMODITY: Four (4) African Black-Footed Penguins, *Spheniscus demersus* (Refer to Appendix A for Permit Application).

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SHIPPERS: Holly Hunt & Kara Campbell, International Animal Exchange, Inc. 25600 Woodward Avenue, Suite 110, Royal Oak, Michigan 48067. Phone No.: (248) 545-4125.

IMPORTER: Povi Carisa-Abney, Wildlife Supervisor, Hyatt Regency Maui Resort and Spa, 200 Nohea Kai Drive, Lahaina, Hawaii 96761. Phone No.: (808) 250-1030. Fax No.: (808) 667-4717. (Refer to Appendix B for resume).

PQB NOTES: *The PQB has previously approved Import Permits for Patricia Lonick, Hyatt Regency-Maui, on November 10, 1992 and December 1, 1992 to import Black-Footed Penguins, Spheniscus demersus and Magellanic Penguins, Spheniscus magellanicus. (Refer to Attachments 1 and 2).*

CATEGORY: African Black-Footed Penguins, *Spheniscus demersus,* are on the List of Restricted Animals (Part B). Pursuant to Hawaii Administrative Rules (HAR), Chapter 4-71, all species in the family Spheniscidae (*Spheniscus demersus),* may be imported into Hawaii for government use, or private and commercial use, including research, zoological parks, or aquaculture production.

II. Information Provided by the Applicant in Support of the Application

PROJECT: Currently, our facility can no longer produce viable offspring as they are too closely related. We are seeking to obtain 4 penguins of a different bloodline to ensure genetic vitality. We plan on registering all of our penguins to Association of Zoos and Aquariums (AZA) Species Survival Plan via their regional studbook, as they are one of the most reputable organizations and we have worked with them in the past. The purpose of the AZA Regional Studbook is to document the pedigree and entire demographic history of each animal within a managed population. These collective histories, compiled and maintained by an AZA Regional Studbook Keeper, are known as the population's genetic and demographic identity and are valuable tools to track and manage each individual as part of a single *ex situ* population. Refer to Attachment 3 for the AZA Species Survival Plan Program Handbook).

PQB NOTES: *The Hyatt Regency Maui Resort and Spa has applied for a Captive-Bred Wildlife Registration, Endangered Species Act (ESA) from the United States Fish & Wildlife Service on 11-10-2021. If this request is approved, PQB would obtain any required licenses prior to the issuance of an import permit.*

OBJECTIVE: We are also seeking to obtain 4 new penguins in order to comply with AZA's minimum population standard recommendation of 10 penguins for an animal enclosure of our size. (Refer to Attachment 4 for the AZA

Penguin (Spheniscidae) Care Manual). With the introduction of a new bloodline, it is our hope that our penguins will produce healthy offspring to continue the future of our colony. If we enter a situation of having surplus penguins, we will look to AZA to see if there is a need at other facilities for African Penguins, and follow all recommendations, rules and regulations regarding this species.

PROCEDURE: Our penguin habitat was designed to house 12-16 penguins and we now only have 6 penguins. Should we have surplus penguins, our habitat can be restructured to house up to 20 penguins. We have had a very successful colony of penguins at the Hyatt Regency Maui for 36 years. Our 3 "founding" penguins all lived past their expected lifespan and have produced 6 healthy offspring.

DISCUSSION:

1. **Person Responsible:** Povi Carisa-Abney, Wildlife Supervisor,
Hyatt Regency Maui, 200 Nohea Drive, Lahaina Hawaii 96761.
Phone No.: (808) 250-1030.
Email Address: povi.carisa-abney@hyatt.com

I am the wildlife supervisor at the Hyatt Regency Maui and have worked with animals for over 30 years, caring for these penguins over the last 3.5 years. I will use my experience with these animals and other experts in the field to provide these penguins with the best care possible. I am an active member of AZA and have connections at several zoos and aquariums that work with African Penguins. I work with a very skilled team of wildlife technicians, and an exotic animal's veterinarian who provide amazing care to our penguins. They include:
Rogelio Yasana, 12 years with African Penguins

Lisa Braun, 1.5 years with African Penguins, 10 years animal experience Dr. Paul McCurty: Veterinarian (contracted). 16 years wildlife medicine/10 years as a vet.

2. **Safeguard Facility and Practices:** Povi Carisa-Abney, Wildlife Supervisor,
Hyatt Regency Maui, 200 Nohea Drive, Lahaina Hawaii 96761.
Phone No.: (808) 250-1030.
Email Address: povi.carisa-abney@hyatt.com

(Refer to Attachment 5 for aerial map of facility).

The exact location of penguin habitat. Penguin habitat inside the Hyatt Regency Resort Maui (200 Nohea Drive, Lahaina, Hawaii 96761). I have provided a photo and a map of our penguin enclosure below. This habitat is safeguarded by a lava rock wall to keep penguins inside and to protect from elements. We have 24-hour security on property, and water features that monitor's the penguins' water quality. We also have a quarantine space inside for severe weather, training and minor

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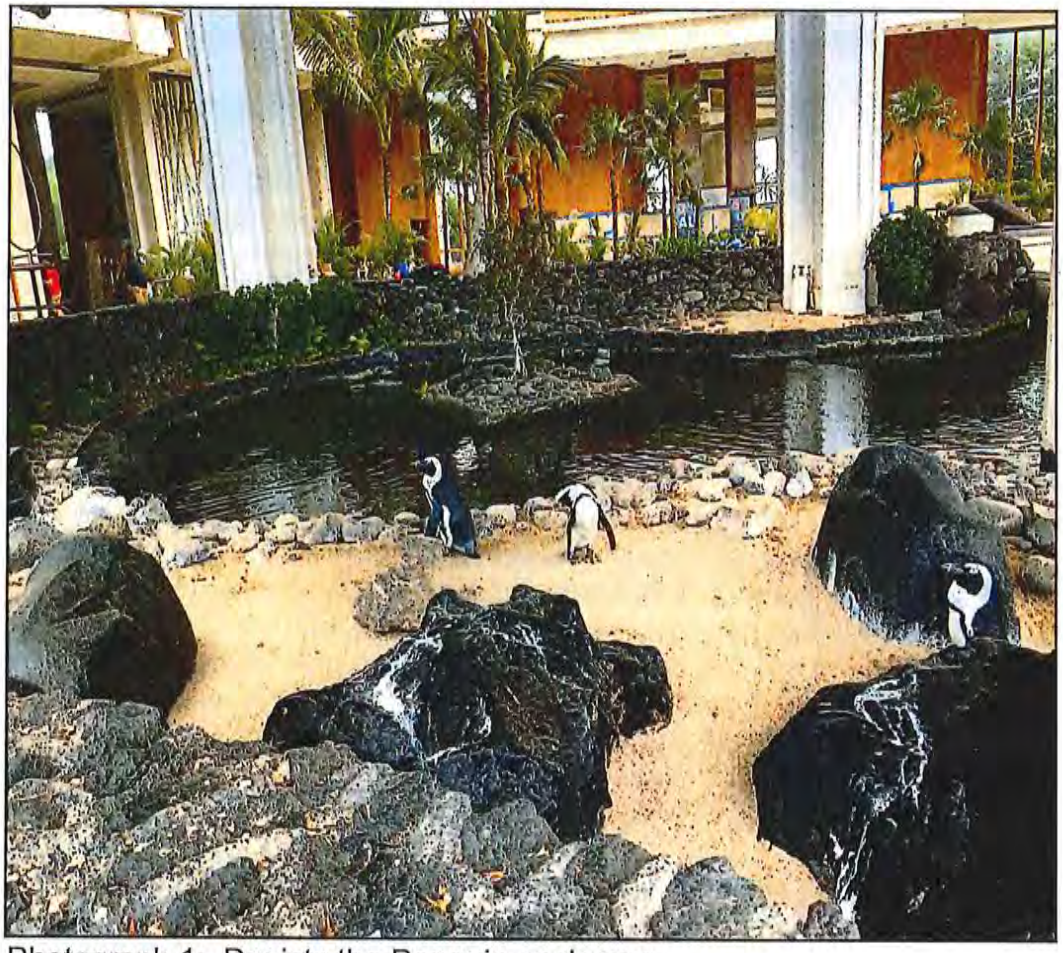
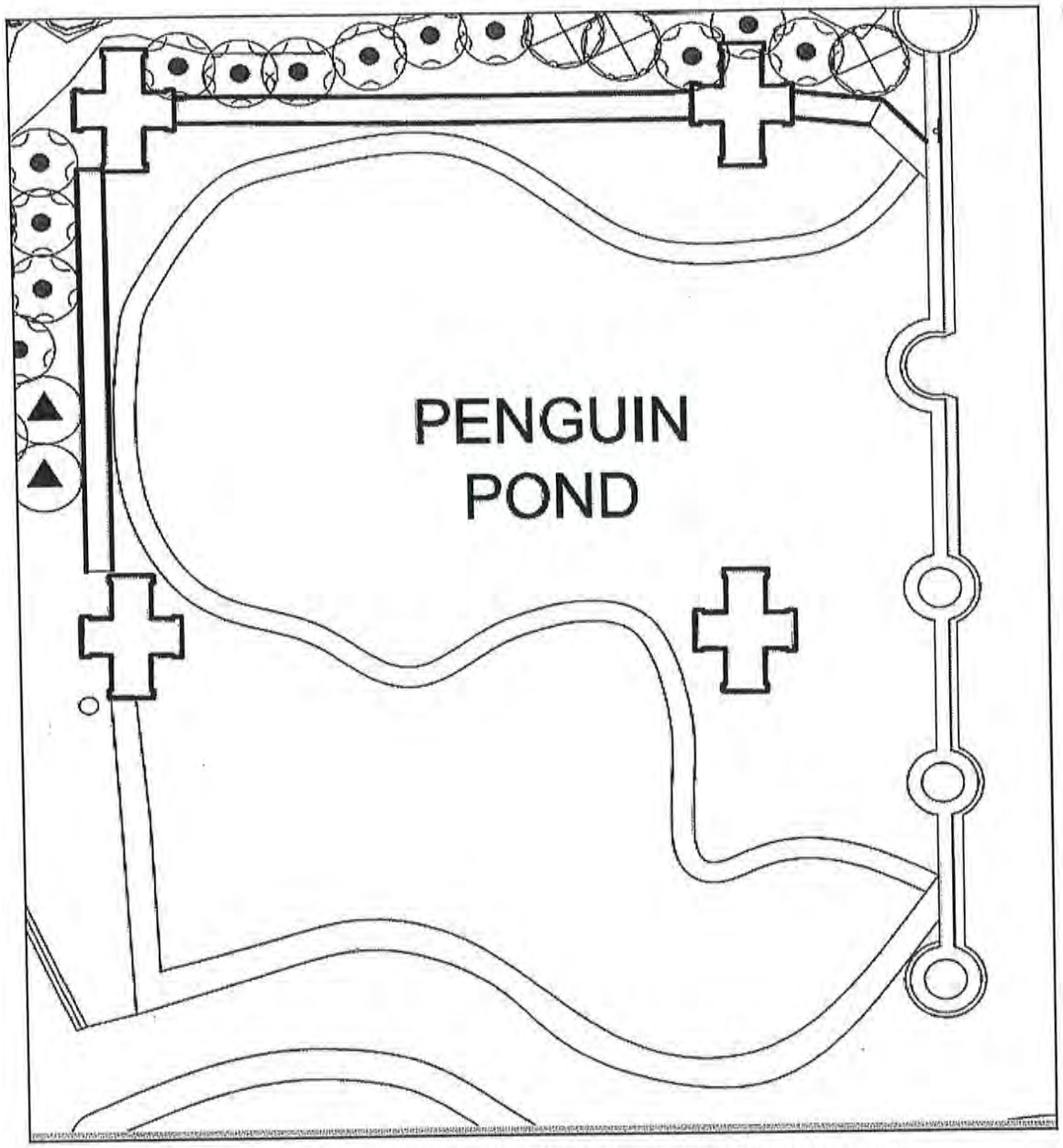
medical procedures. Major illness/injuries would take place at South Maui Animal Clinic in Kihei, Maui. We follow AZA guidelines for penguin welfare and the Hawaii Department of Agriculture (HDOA) provides yearly inspections. (Refer to Attachment 4 for the AZA Penguin (Spheniscidae) Care Manual).



Photograph 1: Depicts the Penguin enclosure.

Our penguin habitat is located in the center of our main lobby. It is made up of a sand beach and walkway, a rock beach, hard plastic igloo-style penguin houses and a waterway with a plastic liner. We have plants growing in all the corners and trees that provide shade. The habitat is kept enclosed by a lava-rock wall and concrete bridge. (Refer to Attachment 6 for additional photographs). We have 3 occupied houses, 3 empty houses with space for at least 2 more homes, and each igloo typically houses 2 penguins.



Photograph 2: Depicts the Schematic Diagram of the Penguin Pond.

3. **Method of Disposition:** We are looking to acquire penguins to keep our colony going for generations to come. We have no plans to end this project, but should our penguins have to be re-homed, we would contact AZA to find a suitable home for them. When a penguin dies, we send the body in for a necropsy to better understand the cause of death, then would dispose of the body through cremation or burial (as individual case would dictate).

We keep our penguins enclosed and they are monitored through our 24-hour security team on property. This is not a species that could establish a wild colony

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in Hawaii due to lack of food (they eat cold-water schooling fish not found in Hawaii).

4. Abstract of Organisms:

a. **African Black-Footed Penguin also known as Jackass Penguin and African Penguin** *(Spheniscus demersus).*

b. They have no close relatives in Hawaii.

c. The African penguin averages 2 ft. tall and weighs up to 8 lbs. Eggs are laid in pairs and both parents help incubate and feed offspring. After 2-4 years, the chicks will mature and lay their own eggs. Reproductively mature at 4-6, they typically live 10-15 years in the wild but longer in captivity. They tolerate extreme temperatures of 40-100 degrees, regulate heat well, and do well in our moderate climate.

d. Their habitats require shelter, a dry substrate like sand, rock beaches, water and shade.

e. Native range is South Africa.

f. This is not a species that could establish a wild colony in Hawaii due to lack of this species' food (they eat cold-water schooling fish not found in Hawaii).

g. African Penguins have not established a viable population anywhere except Africa.

h. African Penguins feed on cold water schooling fish like sardines, anchovies and capelin (not found in Hawaii). This species is not considered a pest or invasive as it does not inhabit anywhere except Africa.

i. This species is not considered domesticated, nor is it cultivated for commercial purposes.

j. This species is declining in its natural environment, mainly due to overfishing, loss of habitat, global warming and human impact.

k. Illnesses and bacteria: salmonellosis, clostridiosis, and the polymicrobial contribution to penguin diphtheria, avian malaria and aspergillosis.

l. Not a threat to local wildlife through disease or parasites as they are in an enclosed area and contained water system.

We have a very successful colony of penguins at the Hyatt Regency Maui for 36 years. Our 3 "founding" penguins all lived past their expected lifespan and have produced 6 healthy offspring. We have come to a point where our penguins are too closely related to produce offspring of genetic vitality, and our current penguins will begin to age out of their healthiest reproductive years. Our enclosure can support the additional penguins and we feel it is important to contribute to this population of endangered species. We have an excellent support team, connections with several other zoos and aquariums, an active role in the AZA, a strong working relationship with our veterinarian, as well as the HDOA Quarantine department. It is our goal to provide all of our penguins with the best care and welfare possible, and to work with AZA and US Fish and Wildlife to help revive a declining species.

The last of our original penguins passed away this year at the age of 36, and the other two lived to 25 and 27. We are currently housing their 6 offspring.

- Number of years we have had African Penguins: 36
- Successful births in the last 5 years: 0
- Mortalities in the last 5 years: 1 mortality due to cancer/advanced age- 36 years old.

We will continue to give all of our penguins the best nutrition, habitat, medical care, enrichment, social opportunities and overall welfare to ensure our penguins live long, healthy lives.

5. Effects on the Environment:

There should be very little environmental impact regarding our penguins, as they are kept in a closed system. All water is treated and kept inside the enclosure, not dumped anywhere on property. All penguins waste and debris is disposed of in sealed plastic garbage bags and placed in our dumpster. The penguins do not have any contact with animals or people outside the enclosure.

a. Our penguins do not have contact with any animals or people outside their enclosure. Our staff washes hands and/or uses proper PPEs before working with any other species on our property. Penguins in other zoo facilities have been affected by Avian Malaria, but that is a very low risk as we stock Mosquitofish (*Gambusia affinis)* in our habitat as a natural way to get rid of mosquitos. Aspergillosis is considered one of the most common causes of respiratory disease in pet birds. It is caused by infection with a fungus of the genus Aspergillus. This can be avoided by keeping our

penguin houses and enclosure clean, so we have implemented a daily cleaning schedule for our penguins.

b. There is very little risks regarding importing this species into Hawaii. We do not have any wild penguin colonies in Hawaii to be impacted. Our penguins never leave their enclosure, therefore do not pose any risks to endemic species, agricultural industries, natural resources, the economy and human/animal health risks. Nobody besides wildlife staff and our vet will be handling our penguins. Their food source is shipped in from the North Atlantic, and therefore harvesting their food will not impact any local species.

Biosecurity:

We have a 3-foot-tall rock wall that completely surrounds our penguin exhibit, as well as a locked gate and bridge over the water area. We have over 150 Avigilon security cameras, 24-hour staff to prevent escape or harassment of the animals. All penguins have their own weatherproof igloo houses to protect from the elements. Disease and pest exposure is minimal due to the design of the habitat, and that our penguins are not in contact with other animals or people outside their enclosure.

Our most athletic penguin can jump almost 1 foot into the air. The waterway is closed off by an underwater mesh netting under our bridge. All structures are kept away from the wall to prevent a penguin from using such item as a springboard. To my knowledge, in 36 years, we have never had a penguin escape or go missing from our enclosure. Our security team monitors all of our security cameras 24 hours a day as well as patrols the property 24 hours a day.

The Hyatt Maui has over 150 Avigilon security cameras installed across the lobby and around the property. Our security team patrols the lobby 18 times a day. We have security, engineering, housekeeping and front desk personnel who are on duty 24 hours a day to help monitor.
The Hyatt Regency Maui Resort & Spa has provided a Biosecurity Manual for their facility that is based of the National Zoo Biosecurity Manual/Guidelines. (Refer to Attachment 7 for the National Zoo Biosecurity Manual).

6. **Alternatives:**

We will not be seeking an alternate species, as we want them to be compatible with our existing penguins. If International Animal Exchange does not have African penguins available, we may look at other zoos and aquariums and will update you accordingly

7. **References:** (Refer to Attachment 3 for the AZA Species Survival Plan Program Handbook and Attachment 4 for the AZA Penguin (Spheniscidae) Care Manual).

III. Advisory Subcommittee Review

This request was submitted to the Advisory Subcommittee on Land Vertebrates for their review and recommendations. Their recommendations and comments are as follows:

1. **I recommend approval ___ / ___ disapproval to allow the importation of four (4) African Black-Footed Penguins, *Spheniscus demersus*, an animal on the List of Restricted Animals (Part B), by permit, for exhibition, by the Hyatt Regency Maui Resort and Spa.**

Dr. Allen Allison, Vice President/Assistant Director, Research and Scholarly Studies, Bernice Pauahi Bishop Museum: Recommends approval.

> Comments: "Request is reasonable; environmental risk is extremely low and track record of maintaining a colony of penguins is impressive."

Dr. Isaac Maeda, DVM, State Veterinarian, HDOA-Animal Industry Division: Recommends approval.

> Comments: No comments.

Mr. Tom May: No response.

Dr. Carolyn McKinnie, DVM, Supervisory Veterinary Medical Officer, USDA, APHIS-Animal Care: Recommends approval.

> Comments: "Birds are regulated under the AWA though no standards have been set as yet. There is currently a proposed rule published in the Federal Register on bird standards so in the future these animals will be regulated and inspected by USDA. **The estimated date that the final rule will be in place is February 2023**. The Hyatt will be required to have an active USDA license at that time. Although the standards are still being promulgated, the licensee would need to comply with all the standards, as the penguins habitat is in the middle of the lobby area, a suitable barrier from the public would be required thought the lava rock wall may be sufficient. It is likely an attendant would need to be present or in the near vicinity. The security team and 24/hour cameras the applicant uses is a proactive measure to protecting the animals. USDA APHIS recommends that the applicant go to our website and review the proposed bird rule and review all the associated materials.
> https://www.aphis.usda.gov/aphis/ourfocus/animalwelfare/proposed-awa-standards-for-birds/aphis-2020-0068"

Dr. Robert Reed, Deputy Director of the United States Geological Survey, Pacific Island Ecosystems Research Center, Hawaii Volcanoes National Park: Recommends approval.

> Comments: "**My approval is conditional on the importer providing definitive evidence that the penguins are free from internal and external parasites.** I've attached two papers to my response demonstrating that penguins in general are known to harbor a wide range of blood parasites, and that S. demersus in particular is known to harbor subclinical avian malaria infections both in mainland zoos and collections and in wild populations in South Africa. Introducing a new strain of Plasmodium relictum (the avian malaria parasite) or a new species such as Plasmodium elongatum would greatly complicate current efforts by State of Hawai'i and a wide range of partners to control avian malaria that is causing extinctions of native Hawaiian birds (these impacts would certainly end up costing many millions of dollars in order to develop and implement new mosquito control tools for these new parasites). Moreover, given the potential for infection with Babesia and Borrelia that are tick transmitted and because chewing lice can also transmit filarial nematodes, I recommend that the birds be screened and treated for ectoparasites. The hotel's comment about controlling mosquitoes with Gambusia is not very credible unless they are able to provide evidence to support that claim. I consulted with Dr. Carter Atkinson, perhaps the best qualified expert in Hawai'i for assessing this risk. Here's his recommendation for testing and prophylactic treatment for the malarial parasites: [I would suggest multiple PCR tests (from at least three blood samples collected several days apart) plus prophylactic treatment with chloroquine and primaquine to try to eliminate any cryptic blood or tissue stages of the parasites that don't show up by PCR. If the birds are originating from a screened facility with no prior reports of malaria, then that would be even better.] A bird-focused veterinarian could suggest a proven treatment regimen for external parasites."

PQB NOTES: *Refer to Attachments 8 and 9 for the published research papers provided for review by Dr. Reed demonstrating the known risks for this species. Dr. Maeda was consulted on the information presented.*

2. I recommend approval ___ / ___ disapproval to update permit conditions for the importation of four (4) African Black-Footed Penguins, *Spheniscus demersus*, an animal on the List of Restricted Animals (Part B), by permit, for exhibition, by the Hyatt Regency Maui Resort and Spa.

Dr. Allen Allison, Vice President/Assistant Director, Research and Scholarly Studies, Bernice Pauahi Bishop Museum: Recommends approval.

> Comments: "Permit conditions are reasonable."

Dr. Isaac Maeda, DVM, State Veterinarian, HDOA-Animal Industry Division: Recommends approval.

> Comments: "Include negative test for Newcastle disease and avian influenza within 14 days of import as a requirement."

Mr. Tom May: No response.

Dr. Carolyn McKinnie, DVM, Supervisory Veterinary Medical Officer, USDA, APHIS-Animal Care: Recommends approval.

> Comments: "From the information submitted, the facility has a good record of maintaining penguins in captivity. In reviewing this material from an Animal Welfare Act lens, it appears that the facility would meet the requirements using the AWA subpart F requirements. However, once the bird standards are in place, the facility would need to follow the AWA regulations and standards and have a valid license."

Dr. Robert Reed, Deputy Director of the United States Geological Survey, Pacific Island Ecosystems Research Center, Hawaii Volcanoes National Park: Recommends approval.

> Comments: "Approval is conditional on providing evidence that animals are free of parasites, as discussed above."

IV. Advisory Committee Review:

This request was submitted to the Advisory Committee on Plants and Animals (Advisory Committee) at its meeting on May 20, 2022 via a Zoom virtual meeting. PQB Land Vertebrate Specialist Noni Putnam provided a synopsis of the request.

Chairperson Darcy Oishi asked the Committee if they have any questions or concerns for Ms. Putnam or the applicant. Committee member Thomas Eisen had one point of clarification on the two last PQB notes that were referred to on page 13 that spoke to how the conditions were amended. He said the notes refer to condition #14 but looks like it's condition #12. He wanted to make sure that its correct or clear what is going on. Ms. Putnam acknowledged the mistake indicated Mr. Eisen was correct, referring to be #12a and #12b, not #14a or #14b as on the submittal.

Committee member Robert Hauff said he had a question for the applicant. The applicant, Ms. Povi Carisa-Abney introduced herself to the members. Committee member Hauff asked about the origin of the birds. Ms. Carisa-Abney said we have three original penguins that we received from the International Animal Exchange about 37 years ago. She said those were our three founding penguins, one lived to 25, one lived

to the age of 27, and one lived to age 36. They had six offspring between the three of them and those are the current residents. Committee member Hauff clarified that he was asking about the origin of the imported penguins being covered by this permit. He asked what the origin of the penguins being brought to Hawaii, noting the application doesn't say anything about the origin of the imported animals. Ms. Carisa-Abney said we are not 100% sure at this time. She said all facilities spoken to required having the permit in place before they are willing to take the next step. She indicated contact with International Animal Exchange where the original three penguins were from, and also a facility in Florida, as well as SeaWorld in San Diego. She said the latter two facilities have an excess number of penguins that were born in captivity.

Committee member Hauff said that when the Committee weighs these issues, they often look at the risk, which can be difficult to really define in some the cases. He asked what are the benefits of bringing the penguins into the State, for the State of Hawaii? Ms. Carisa-Abney said the penguins 36 years ago were not considered to be an endangered species, but since then, the wild population has taken a nosedive. She said it's a fantastic opportunity, from an education and conservation standpoint, to be able to explain to our guests what issues are occurring outside Hawaii and within our facility. She said the goal is to keep this colony healthy for generations to come and to maintain the mental and social wellbeing of the penguins that we currently have. She noted a lot of these penguins we have are trying very hard on being parents, but they are all too closely related, so they just don't have the opportunity.

Committee member Hauff noted that in other applications regarding sanctuary for animals that might be threatened, the question has been asked, why can't these animals be protected somewhere else and is Hawaii an appropriate place for protected colonies of endangered ones? Ms. Carisa-Abney said because we have a similar landscape to the colonies back in South Africa, it provides a perfect well habitat for them. She noted the average life span in the wild is about 15-20 years and they have been able to keep penguins for 36 years, so all three penguins are well past their life expectancy. She said they also contribute, as far as science and research goes, by working closely with the Association of Zoos and Aquariums and other facilities.

Committee member Hauff asked if the applicant's educational efforts entail explaining to people that penguins are not native to Hawaii, the native bird species typically found in the habitats are extinct or endangered, and what do you think about native species? Ms. Carisa-Abney confirmed they do, and it provides an opportunity to explain how delicate our ecosystem is in Hawaii by talking about how delicate their ecosystem is in South Africa. She said it is a "full circle" moment because it's such an unexpected animal to see in Hawaii and so does gain a lot of interest. She said they have a lot of people that come up and ask questions.
Committee member Hauff asked about the practicability of testing these birds for avian malaria following the comments from the Subcommittee as far as multiple PCRs test to

ensure the birds aren't infected and also prophylactic treatment. Ms. Carisa-Abney said Dr. Reed's questions are very good and it is something that we could have the facility do before they are shipped out. She said they have their own contract Veterinarian and if this is something you would like in yearly reports, they can continue the testing. She said the goal is to be completely compliant and to protect any birds outside from their facility.

Committee member Hauff noted restricted List B use is for research through zoological parks and asked if there is a definition or requirement for a facility to be considered a zoo? Ms. Putnam said she what not aware of one, but noted pursuant to HAR chapter 4-71, these can be for private and commercial use as well.

Chairperson Oishi said it is clear the applicant intends to breed these penguins. He said he sees some issues with the permit conditions that could make it difficult for the applicant to allow them to breed the penguins. Ms. Putnam confirmed the applicant is intending to breed and asked which conditions were being referenced. Chairperson Oishi said permit conditions 6 and 12. He said it appears that importation and entrance are being used interchangeably where importation is, by definition in HRS §150A-2, shipment to the state from any point outside of the state, and this becomes problematic because permit condition 12 is basically stating progeny are required to follow any of the pre- and post- entry requirements for the adult. He said he didn't see how the progeny can be checked 10 days in advanced of being imported into the state. He said this needs to be cleared up and the simplest way would be to indicate the progeny have a different set of conditions that apply to them and do not have to adhere to the same prescreening entry requirements that the imported animals have to undergo. Ms. Putnam said it makes sense and normally the conditions are for the animals coming in and the progeny. We can try to make a condition that is. Chairperson Oishi asked how you could engage in health checks before importation if they are being born within the Hyatt facility. Ms. Putnam recommend possibly adding another condition that just talks about the progeny.

Chairperson Oishi referenced condition 8, noting these are birds which lay eggs, and you can't use an individualize marker that will stay with it since you're marking the shell. He asked why we're limiting the language to marking as his understanding is typically birds are banded to give them a unique identifier. Ms. Putnam said the condition is to mark with a unique identification code that is approved by the PQB chief. She noted there are different types of identifications that are approved, such as microchip, band, rings, etc. and the identification that is approved can vary for different birds.

Ms. Putnam asked if the addition of another condition indicating the requirements for progeny is needed. Chairperson Oishi said yes, because it makes the most sense because the screening requirements are for health issues of the parents and if the

screening processes are appropriate and thorough enough the progeny should not have any issues. He said the requirements should be communicated to the applicant.

Mr. Ho said historically, PQB has the import conditions manage the progeny as opposed to creating two sets of conditions because two sets of conditions are normally used for transferring animals as opposed to a single facility having two sets of conditions, one for the imported animals, and one for progeny. He said condition 12 does seem to conflict with reality because the progeny are here before import, therefore to comply with the 10-day screening process prior to entry is impossible. He said the intent is for the imported ones. He said the progeny are not subjected to this because that is detailed with possession in and of itself. He said striking the reference to progeny makes it very clear this is for imported animals; not for those that are just in their possession. Mr. Ho then spoke about the idea of banding and issue with eggs. He said the potential movement of eggs is there for any bird species, but this requirement is gauged for the adults. He said if some random birds popped up in their facility without the required mark, they were either smuggled into the state or illegally transferred from another permittee that has the birds. He said there is also an inventory that allows PQB to follow up on this. He said the intent is for the permittee to import and maintain, but they can't transfer or give them away, at least within the State, and if they wanted to transfer, would have to go to the Board and go through the review process to do that. He said PQB can go through the conditions because the term progeny is used throughout and make corrections on the clear conflicts for importation versus the possession.

Chairperson Oishi asked if there were a requirement anywhere, whether it be the SOPS or biosecurity plan, that the applicant has to notify PQB if there are eggs and how do you track the eggs since they could "walk away". Ms. Putnam said condition 21 states the applicant submits semi-annual reports including the counts of restricted animals with the progeny. She said normally if there is some type of birth or death, she is notified through email. Chairperson Oishi asked what defines birth for a penguin, laying of the egg or hatching of the egg? Ms. Putnam said if eggs were present, they need to notify me. She said the applicant has mentioned when they are born they have unique spots which can also be used to identify them as well. Ms. Carisa-Abney recommended after hatching as being when a penguin would be counted. She said it is simpler because their penguins have laid infertile eggs because they are closely related. She said they try to take an egg to see if there is any life inside and noted some of the eggs don't meet maturity. She said they typically leave it up to parental care and the babies will be accepted in the colony if the parents raise them rather, than if they incubate the eggs, they are raised separately. She said as far as marking or moving the eggs, they try not to do either because it stresses the parents and generally, they only lay two eggs at a time, so it is easy to track. She said they have unique markings and generally stay in the same place in the nest so it is something they can keep an eye on and differentiate.

Committee member Kenneth Matsui asked if the current birds are banded and if there are infection problems related to the band. Ms. Carisa-Abney said they do not currently band their penguins because they have identifying spot markers, which is how they can differentiate them. She said the spots are very distinct so it's easy to tell them apart. She said there are other facilities that band their birds and have had different successes, but generally the colonies when they band them are 40-50 animals, so it is harder to differentiate. She indicated they only have six.

Committee member Matsui said his observation when bird's legs are banded, like canaries, the rate of infections at that point of banding seem to be higher. He said he wasn't sure if it would necessarily be appropriate for an endangered species to be put at additional risk by requiring banding. Ms. Carisa-Abney agreed with Mr. Matsui and noted the majority of birds are not banded because they don't want to add any risk to their health or mobility.

Committee member Matsui said he didn't agree with the idea that the penguins will starve to death if they were to escape. He said as a practical matter, penguins eat squid and crustations and we have those here. He didn't see where the penguins would necessarily suffer from that same problem because they are not in the cold water. Ms. Carisa-Abney asked if the question was directed to her. Mr. Matsui said it was because the submittal states the penguins would starve to death because they only eat cold water fish, but the documentation shows they eat squid and crustations as well. He said he had doubts they will eat only cold-water fish and surmised they could survive on local fish. Ms. Carisa-Abney said every penguin we have has been born at the Hyatt, have only been fed certain kinds of fish, mostly sardines, and have never been introduced to squid or any other fish that we have available. She noted they never have gone after live bait and didn't believe they would survive in the wild based on their habits. She said they have live mosquito fish that are in the water and the penguins will just chase them but never eat them. She said they have tried introducing a variety of fish but they are extremely picky and refused them, noting they are spoiled and also haven't had the opportunity to swim in the ocean to forage on their own. She said she has worked with dolphins that have been given the same opportunities and they don't readily adapt to foraging on their own and trying new fish.

Committee member Matsui said the 6 penguins appear to be very human-friendly and are the new imports going to be similarly imprinted? Ms. Carisa-Abney assumed so because the facilities they are working with are similar in nature where they do education and training programs. She said the training programs are really helpful as far as just taking care of the animals without stressing them out. She said they would never consider taking anything from the wild since their numbers are low. Mr. Matsui said if they did escape, they are not as likely to be hard to recapture because they will be friendly to their handlers. Ms. Carisa-Abney said correct, and noted they rarely take them out of their habitat. She said during shut down, they were taken out of their habitat and were very uncomfortable. They are very familiar with their surroundings and are not

animals that really try to escape because everything they want is inside their habitat, their friends, their food. She said outside their habitat they do more of a freeze then a flight response and are not animlas that startle or run, when they are nervous they just stop. She did not think escape is an issue because there is a large number of people at the facility 24 hours a day, they have over 150 security cameras and have staff literally 10 steps away from the enclosure. She said the security monitors the entire property, 24 hours a day and she is confident that they can keep them safely.

Committee member Matsui asked if accepting pictures of the birds with their unique markings would be an acceptable alternative to leg banding. Ms. Putnam said that would be okay. Mr. Matsui said this is preferable because we don't want an infection problem with banding these birds when they are endangered.

Chairperson Oishi asked if there are other organizations that have the same species of penguins? Ms. Putnam said off the top of her head there may be two facilities that have the penguins. Ms. Carisa-Abney said the Honolulu Zoo has the same species and Sea Life Park has a different species. Chairperson Oishi asked if there were any other questions or concerns or comments from the public. He noted there was no submitted testimony. Without comments or testimony, Chair Oishi asked for a motion?

Committee member Hauff said he didn't necessarily want to make a motion but would support this with reservations. He said his main concern is avian malaria and if a condition was added that incorporated Dr. Atkinson's recommendations for the testing and prophylactic treatment, he would support a recommendation with reservations. Committee member Matsui asked if avian malaria was already in the state. Chairperson Oishi said "correct". Committee member Hauff said "yes" but the concern is different strains and genotypes. He noted that there was no way to guarantee that transmission wouldn't happen if they had an infection to another bird species. Mr. Matsui noted wild birds would probably fly in.

Chairperson Oishi asked Mr. Hauff if a modification or addition to permit condition 12 is needed. Mr. Hauff said wherever PQB staff thinks it would best fit, probably condition 12. Ms. Putnam asked for verification to include avian malaria, prophylactic treatment, and multiple testing on multiple days of the birds, as recommended by Dr. Atkinson. Ms. Carisa-Abney said she would support a testing program on behalf of the Hyatt and whatever testing program should be put into place, they are more than happy to make the modifications. She said they can regularly test our penguins and any other birds you feel may be at risk and they have a fantastic vet. She said whatever standards you put into place they will 100% follow because they want to keep our birds safe and want to keep all of Hawaii's birds safe.

Committee member Matsui asked Mr. Hauff regarding avian malaria, if the sampling is concentrated in certain areas of the bird, for example, in other diseases it seems to be concentrated in the joints between the bones. Committee member Hauff said he was

unable to answer that question. Mr. Matsui said in testing chickens they can miss because they are testing the blood and most often it should be found in the joints. Ms. Putnam said PQB can work with Dr. Maeda and will include as the conditions prior to presenting it to the board.

Chairperson Oishi asked for a motion. Hearing none, Chairperson Oishi recommend approval of this submittal contingent upon modifications of the permit conditions to reflect the creation of separate permit conditions for progeny, cleaning up of the language throughout the permit conditions in the use of progeny to indicate that progeny will be held to different standards then the imported articles, changes to permit condition 12 to encompass Avian Malaria testing, and the housekeeping areas that Mr. Eisen noted relating to the permit condition. Mr. Matsui mentioned recording of the markings as opposed to banding if needed. Mr. Hauff asked if the prophylactic treatment along with the testing was included. Mr. Oishi agreed. Committee member Matsui seconded the motion.

Chairperson Oishi asked if there were any discussion on the motion? Committee member Hauff said he found the applicant's education goal admirable; but questioned whether a child from the Midwest who comes to Hawaii on vacation and returns home, is asked by their teacher about their favorite thing in Hawaii and the response is seeing penguins at the resort. He said that was erasure of sense of place and found it a little bit concerning. Chairperson Oishi asked for any other comments or questions. Hearing none, called for a vote.

Vote: Approved 6/0

Motion carries.

V. Proposed Import Permit Conditions

1. The restricted article(s), four (4) African Black-Footed Penguins, *Spheniscus demersus*, including progeny, shall be used for exhibition, a purpose approved by the Hawaii Department of Agriculture (HDOA), Board of Agriculture (Board), and shall not be given, sold, and/or transferred in Hawaii unless approved by the Board. Release of the restricted article(s) into the environment is prohibited.

2. The permittee, Povi Carisa-Abney, Hyatt Regency Maui Resort and Spa, 200 Nohea Drive, Lahaina, Hawaii 96761, shall be responsible and accountable for the restricted article(s) imported, including progeny, from the time of their arrival to their final disposition.

3. The restricted article(s), including progeny, shall be safeguarded at Hyatt Regency Maui Resort and Spa, 200 Nohea Drive, Lahaina, Hawaii 96761, a site inspected and approved by the Plant Quarantine Branch (PQB) prior to

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importation. Prior to the removal of the restricted article(s) or progeny to another site, a site inspection and approval by the PQB Chief is required.

4. The restricted article(s) shall be maintained by the responsible person, Povi Carisa-Abney, Hyatt Regency Maui Resort and Spa, 200 Nohea Drive, Lahaina, Hawaii 96761, or by trained or certified personnel designated by the permittee.

5. The restricted article(s) shall be imported only through the port of Honolulu, as approved by the Board. Entry into Hawaii through another port is prohibited.

6. The permittee shall provide the HDOA, PQB and Animal Industry Division (AID) with the confirmed arrival date, time, mode of transportation, and any other required information for the arrival of the restricted article(s) at least 48 hours prior to arrival. The permittee shall immediately notify the HDOA, PQB and AID of any changes to this information.

7. Each shipment shall be accompanied by a complete copy of the PQB permit for the restricted article(s) and an invoice, packing list, or other similar PQB approved document listing the scientific and common names of the restricted article(s), the quantity of the restricted article(s), the shipper, and the permittee for the restricted article(s).

8. The restricted article(s) and progeny shall be permanently marked with a unique identification code that is approved by the PQB Chief.

9. At least four sides of each parcel containing the restricted article(s) shall be clearly labeled with "Live Animals" and "This Parcel May be Opened and Delayed for Agriculture Inspection" in 1/2-inch minimum sized font.

10. Water used to transport the restricted article(s) shall be disinfected with a solution of 50 mg chlorine/L (50 ppm), for a duration of 30 minutes, then neutralized with sodium thiosulfate, another approved neutralizing agent, or by holding the solution for 48 hours, prior to disposal into an individual wastewater system, municipal sewer system or other PQB approved system.

11. All bedding used to transport the restricted article(s) and fecal material from the restricted article(s) shall be bagged and disposed of directly into the municipal landfill.

12. The restricted article(s) shall comply with all pre-entry animal heath requirements of the AID, Chapter 4-28, Hawaii Administrative Rules, (Ph: (808) 837-8092).

PQB NOTES: *Condition No. 12 was amended as a result of the Committee's discussion to clarify that progeny are not subject to pre-entry AID requirements.*

a. Imported restricted article(s) shall be accompanied by a Poultry and Bird

Import Permit and a valid original health certificate issued by a Category II Accredited Veterinarian within ten (10) days prior to importation. The health certificate shall include a declaration indicating the restricted article(s) are free from diseases designated by the HDOA State Veterinarian, free of external parasites and a declaration indicating the restricted article(s) received a negative test for Newcastle disease and avian influenza within fourteen (14) days prior to importation.

PQB NOTES: *Condition No. 12(a.) was amended as a result of comments made by Dr. Maeda. He was consulted on the language of this condition as presented.*

b. Prior to importation, restricted article(s) shall be isolated at a veterinary clinic in a mosquito-free/proof enclosure for West Nile Virus under the direct supervision of a Category II Accredited Veterinarian. The isolation shall be a minimum of seven (7) days (168 hours), and the restricted article(s) shall enter the State within thirty-six (36) hours of completion of the isolation.

PQB NOTES: *Condition No. 12(b.) was inserted as a result of comments and correspondence with Dr. Maeda. He was consulted on the language of this condition as presented.*

c. Prior to importation, restricted article(s) shall receive multiple negative Polymerase Chain Reaction diagnostic tests for parasites from at least three blood samples collected several days apart and a prophylactic treatment with chloroquine and primaquine, as approved by the State Veterinarian.

PQB NOTES: *Condition No. 12(c.) was inserted as a result of comments and correspondence that were made by Dr. Robert Reed and Mr. Hauff. Dr. Maeda was consulted on the language of this condition as presented.*

d. Upon arrival at the port of Honolulu, the restricted article(s) must be issued a permit to ship (form DC-8), by the HDOA State Veterinarian or authorized representative, prior to transport to the approved inspection site, if movement is allowed prior to inspection.

13. The restricted article(s), shall be subject to inspection by the HDOA, PQB, and the AID prior to entering the State. It is the responsibility of the permittee to provide any restraint(s), including chemical restraint(s), deemed necessary by the AID to conduct a proper inspection. The permittee shall be responsible for ensuring an inspection is conducted.

PQB NOTES: *Condition No. 13 was amended as a result of the Committee's discussion to clarify that progeny are not subject to inspection prior to entry into the State.*

14. The approved site, restricted article(s), including progeny, records, and any other document pertaining to the restricted article(s) and progeny under this permit, may be subject to post-entry inspections by the HDOA, PQB, and the AID. The permittee shall make the site, restricted article(s), including progeny, and records pertaining to the restricted article(s) and progeny available for inspection upon request by a PQB inspector.

15. The permittee shall adhere to the use, facility, equipment, procedures, and safeguards described in the permit application and as approved by the PQB Chief and Board.

16. Effluent from the permittee's system shall be sufficiently treated, as determined by the PQB Chief, to prevent the accidental release of any potential parasites and/or pathogens associated with the restricted article(s), prior to disposal into any individual wastewater system, municipal sewer system or other PQB approved system. Effluent from the permittee's system shall not be discharged to or have a direct connection to the ocean or any other body of water, such as ponds, estuaries, reservoirs, rivers and/or streams.

17. The permittee shall have a biosecurity manual available for review and approval by the PQB, at the time of the initial site inspection and any subsequent post-entry inspection(s), which identifies the practices and procedures to be adhered to by the permittee to minimize or eliminate the risk of theft, escape, or accidental release of the restricted article(s), including the risk of introduction and spread of diseases and pests associated with the restricted article(s) to the environment. The permittee shall adhere to all practices and procedures as stated in this biosecurity manual.

18. The permittee shall immediately notify the PQB Chief verbally and in writing under the following circumstances:

    a. If any escape, theft, release, disease outbreaks, pest emergence and/or mortality involving the restricted article(s) or progeny under this permit occurs. If the restricted article(s) or progeny escape or are found to be free from confinement, the HDOA may confiscate or capture the restricted article(s) or progeny at the expense of the permittee, pursuant to the Hawaii Revised Statutes (HRS), §150A-7(c). The AID shall also be notified of any sign or occurrence of disease.

    b. If any changes to the approved site, facility, and/or procedures regarding the restricted article(s) or progeny occur or are to be made, the permittee shall obtain written approval from the PQB Chief as soon as practicable (if unplanned) or prior to implementation (if planned). Also, the permittee shall submit a written report documenting the specific changes to the PQB Chief.

c. If a shipment of the restricted article(s) is delivered to the permittee without a PQB "Passed" stamp, tag or label affixed to the article, container, or delivery order that indicates that the shipment has passed inspection and is allowed entry into the State, then the permittee shall not open or tamper with the shipment and shall secure, as evidence, all restricted article(s), shipping container(s), shipping document(s) and packing material(s) for PQB inspection.

d. If the permittee will no longer import or possess the restricted article(s) or progeny authorized under this permit, then the permittee shall submit a written report to the PQB Chief stating the name and address of the individual to whom the restricted article(s) will be transferred to. If the restricted article(s) or progeny will be transferred within the State, a PQB possession permit shall be obtained by the new owner prior to transfer. Once the transfer is complete, this permit shall be cancelled.

e. If the restricted article(s) or progeny reproduce, the permittee shall submit a written report to the PQB Chief indicating the number of offspring and any other information deemed necessary by the PQB Chief.

PQB NOTES: *Condition No. 18 was amended as a result of the Committee's discussion to clarify that progeny are not subject to inspection prior to entry into the State.*

f. If the restricted article(s) or progeny expires, the permittee shall submit a written report to the PQB Chief that details the circumstances surrounding the death of the restricted article(s) or progeny, the cause of death of the restricted article(s) or progeny, and any other information deemed necessary by the PQB Chief. The permittee shall also submit a necropsy report from a U.S. Department of Agriculture accredited veterinarian within thirty (30) days post-mortem.

19. The permittee shall submit a copy of all valid licenses, permits, certificates or other similar documents required by other agencies for the restricted article(s) to the PQB Chief. The permittee shall immediately notify the PQB Chief in writing when any of the required documents are suspended, revoked, or terminated. This permit may be amended, suspended, or canceled by the PQB Chief upon suspension, revocation, or termination of any license, permit, certificate, or similar documents required for the restricted article(s).

20. It is the responsibility of the permittee to comply with all applicable requirements of municipal, state, or federal law pertaining to the restricted article(s) and progeny.

21. The permittee shall submit a semi-annual report to the PQB Chief in January and

CHH

July of all restricted articles(s) and progeny imported or possessed. The report shall be in a format approved by the PQB Chief and include the following information for the prior 6-month period:

a. The permit number, quantity, scientific name of each restricted article(s) and progeny;

b. The status of the use and possession of the restricted article(s) and progeny;

c. A summary of any significant changes to the permittee's operation, personnel, and/or procedures; and

d. Any significant events that occurred at the permittee's site.

22. Any violation of the permit conditions may result in citation, permit cancelation, and enforcement of any or all of the penalties set forth in HRS §150A-14.

23. The permittee is responsible for costs, charges, or expenses incident to the inspection, treatment, or destruction of the restricted article(s), including progeny, as provided in Act 173, Session Laws of Hawaii 2010, Section 13, including, if applicable, charges for overtime wages, fixed charges for personnel services, and meals.

24. A canceled permit is invalid and upon written notification from the PQB Chief, all restricted article(s) listed on the permit shall not be imported. In the event of permit cancelation, any restricted article(s) imported under permit, including progeny, may be moved, seized, treated, quarantined, destroyed, or sent out of State at the discretion of the PQB Chief. Any expense or loss in connection therewith shall be borne by the permittee.

25. The permit conditions are subject to cancelation or amendment at any time due to changes in statute or administrative rules restricting or disallowing import of the restricted article(s) or due to Board action disallowing a previously permitted use of the restricted article(s).

26. These permit conditions are subject to amendment by the PQB Chief in the following circumstances:

a. To require disease screening, quarantine measures, and/or to place restrictions on the intrastate movement of the restricted article(s), as appropriate, based on scientifically validated risks associated with the restricted article(s), as determined by the PQB Chief, to prevent the introduction or spread of disease(s) and/or pests associated with the restricted article(s).

b. To conform to more recent Board approved permit conditions for the restricted article(s), as necessary to address scientifically validated risks associated with the restricted article(s).

27. The permittee shall agree in advance to defend and indemnify the State of Hawaii, its officers, agents, employees and the Board of Agriculture members for any and all claims against the State of Hawaii, its officers, agents, employees or Board of Agriculture members that may arise from or be attributable to any of the restricted article(s) that are introduced under this permit. This permit condition shall not apply to a permittee that is a federal or State of Hawaii entity or employee, provided that the state or federal employee is a permittee in the employee's official capacity.

STAFF RECOMMENDATION: Based on the recommendations and comments of the Advisory Subcommittee on Land Vertebrates and unanimous recommendation to approve by the Advisory Committee on Plants and Animals, the PQB is recommending that the Board approve this request and establish permit conditions for this request.

Respectfully Submitted,

BECKY AZAMA
Acting Manager, Plant Quarantine Branch

CONCURRED:

HELMUTH W. ROGG, Ph.D.
Administrator, Plant Industry Division

APPROVED FOR SUBMISSION:

PHYLLIS SHIMABUKURO-GEISER
Chairperson, Board of Agriculture

C46

PQ-7 (01/04)

State of Hawaii
Department of Agriculture
PLANT QUARANTINE BRANCH
1849 Auiki Street, Honolulu, HI 96819-3100
Phone: (808) 832-0566, FAX: (808) 832-0584

For Office Use Only

Fee: $_____ Receipt No. _____

☐ Approve Permit No. _____ Date: _____

☐ Disapprove ☐ Other _____

Processed by: _____ Date: _____

PERMIT APPLICATION FOR RESTRICTED COMMODITIES INTO HAWAII

Date: *10/08/2021*

In accordance with the provision of Chapter 71, Hawaii Administrative Rules of the Division of Plant Industry, Department of Agriculture, a permit is requested for the following commodities:

Please type or print clearly.

Quantity	Commodity	Scientific Name
4	African Black-Footed Penguins	Spheniscus demersus

RECEIVED
NOV 05 2021
PLANT QUARANTINE BRANCH

PAID
Amount: $50 Chk: 30009858
Date: 11/5/21 Initial: TSy

Name and address of shipper: International Animal Exchange 25600 Woodward Ave #110, Royal Oak, MI 4806

(Mainland or Foreign address)

Approximate date of arrival: TBD. Goal is 6-12 month from now.

Mode of Shipment: ☐ Mail ☑ Air Freight ☐ Boat

Type of Permit:
- --- Import
  - ☑ one time only ☐ multi-shipments
- --- Intrastate shipment
  - ☑ one time only ☐ multi-shipments
- ☐ Possession

Object of importation:
- ☐ Kept caged at all time
- ☐ Used for propagation
- ☑ Imported for exhibition
- ☐ Imported for liberation
- ☐ Other purposes - specify Povi Carisa-Abney

Please type or print clearly.

Applicant's Name Povi Carisa-Abney

Company Name Hyatt Regency Maui Resort and Spa
(if applicable)

Hawaii Mailing Address 200 Nohea Kai Drive
Lahaina, Hawaii 96761

Telephone number 808-250-1030

Facsimile number 808-667-4717

Fee Amount Enclosed (cash, check or mail order) $ $50

Povi Carisa-Abney

(complete reverse side)

PLEASE COMPLETE THE FOLLOWING INFORMATION (attach extra sheet if necessary)

1. State in detail the reasons for introduction (include use or purpose).

We have a very successful colony of penguins at the Hyatt Regency Maui for 36 years. Our 3 founding penguins all lived past their expected lifespan and have produced 6 healthy offspring. We have come to a point where our penguins are too closely related to produce offspring of genetic vitality, and our current penguins will begin to age out of their healthiest reproductive years. Our enclosure can support the additional penguins and we feel it is important to contribute to this population of endangered species. We will provide all of our penguins with the best care and welfare possible, and to work with Dept. of Agriculture to help revive a declining species.

2. Person responsible for the organism (include name, address and phone number).

Povi Carisa-Abney (Wildlife Supervisor at the Hyatt Regency Maui)
37 Polohina Lane #8, Lahaina HI 96761
(808) 250-1030
povi.carisa-abney@hyatt.com
I have worked with animals for over 30 years and have cared for these penguins over 3 years. I will use my experience with these animals and other experts in the field to provide these penguins with the best care possible.

3. Location(s) where the organism will be kept and used (include address, contact and phone number).

The penguins will be kept in the penguin habitat located in the center of our lobby at the Hyatt Regency Resort and Spa. 200 Nohea Drive, Lahaina Hawaii 96761. Povi Carisa-Abney (wildlife supervisor) will be contact person and can be reached at 808-250-1030 or by email at povi.carisa-abney@hyatt.com

4. Method of disposition.

We are looking to acquire penguins to keep our colony going for generations to come. We have no plans to end this project, but should our penguins have to be re-homed, we would contact AZA to find a suitable home for them. When a penguin dies, we send the body in for a necropsy to better understand the cause of death, then would dispose of the body through cremation or burial (as individual case would dictate).

5. Give an abstract of the organism with particular reference to potential impact on the environment of Hawaii (include impact to plants, animals and humans).

a. Spheniscus demersus, African Black-Footed Penguin
b. The African penguin averages 2 ft. tall and weighs up to 8 lbs. Eggs are laid in pairs and both parents help incubate and feed offspring. After 2-4 years, the chicks will mature and lay their own eggs. Reproductively mature at 4-6, they typically live 10-15 years in the wild but longer in captivity. They tolerate extreme temperatures of 40-100 degrees, regulate heat well, and do well in our moderate climate.
c. Their habitats require shelter, a dry substrate like sand, rock beaches, water and shade.
d-k. Native range is South Africa, no colonies in Hawaii unlike to establish here or elsewhere due to lack of food (small schooling fish like sardines). Not a threat to local wildlife, through disease or parasites (enclosed area).

****

I request permission to import the articles as listed on the permit application and further, request that the articles be examined by an authorized agent of the Department of Agriculture upon arrival in Hawaii.

I agree that I, as the importer, will be responsible for all costs, charges or expenses incident to the inspection or treatment of the imported articles.

I further agree that damages or losses incident to the inspection or the fumigation, disinfection, quarantine, or destruction of the articles, by an authorized agent of the Department of Agriculture, shall not be the basis of a claim against the department or the inspectors for the damage or loss incurred.

Signature Povi Carisa-Abney Povi Carisa-Abney **Date** 10-8-2021
(Applicant)

Povi Carisa-Abney
(808) 250-1030
37 Polohina Ave Lahaina, HI 96761
alohapovi@mac.com

Education: Southern Oregon University. Ashland, OR. Bachelor of Science Degree in Psychology. Graduated with Honors.

Certifications:
*Professional Level Member AZA
*USCG License- 100 Ton Master Boat Captain
*NAUI Master Scuba Diver
*Red Cross Lifeguarding, CPR and 1st Aid Certifications
*Hyatt Regency and Ritz Carleton Hospitality Training Course
*Pacific Whale Foundation Marine Naturalist Training Courses

Work Experience:
Hyatt Regency- Wildlife Supervisor
*Supervisor. Overseas all aspects of animal welfare and wildlife staff. Leads guest encounters, talks and public feedings animals on property. Care and feeding of penguins, parrots, cranes, flamingos, swans and exotic ducks. My focus is to help our guests create amazing experiences and nurture a love of wildlife.

Ritz-Carlton- Ambassadors of the Environment
*Naturalist Leading ecology focused tours to guests at the Ritz Carleton, including hikes, snorkels, whalewatches and kids programs. My position requires extensive knowledge of Hawaiian history, culture, plants and animals, as well as guest interaction, photography and computer skills.

Ultimate Rafting/Ultimate Whale Watching
*Public Relations/Naturalist As a naturalist I provide interactive talks on humpback whales and other marine species, as well as guide snorkel trips for onboard guests.

Pacific Whale Foundation
*Naturalist/1st Mate/ Cruise Ship Coordinator: Managing boat operations on a 149 passenger boat. Creating Powerpoint presentations and managing guest lecturers on board visiting cruise ships. Providing interactive talks on humpback whales and other marine species, as well as leading snorkel trips to onboard passengers.

Dolphin Research Center
*Animal Care and Training Intern: Assisted trainers in modifying dolphin and sea lion behaviors, as well as serving as a medical back-up for veterinary care. This position allowed me the opportunity to work with the Marine Mammal Stranding Network for the Florida Keys in the rescue and rehabilitation of injured and stranded animals.

References:

Alex Bonilla	Ken Keidan	Krystle Alcain
Hyatt Engineering Manager	Hyatt Engineering Supervisor	Hyatt Marketing Manager
808-280-6975	410-456-7574	808-280-8501

Attachment 1

PQ-8b
5/87

Permit No. 11-93-M-7397

Date Nov. 10, 1992

State of Hawaii
DEPARTMENT OF AGRICULTURE
Plant Quarantine Branch
701 Ilalo Street
Honolulu, Hawaii 96813-5524

IMPORT PERMIT

(Valid for one shipment(s) within one year(s) from date)

Permission is hereby granted to introduce the following, in accordance with Chapter 71, Rules of the Division of Plant Industry, Department of Agriculture, and the conditions listed below. *(Each lot must be inspected by a Plant Quarantine Inspector upon arrival before release.)*

Quantity	Commodity	Scientific Name
5	Black-Footed Penguins See attached condition Conditions: Must be certified in accordance with ~~Reg. 11~~ Chap. 19 attached and must be certified as to progeny of captive populations or have been held in captivity for a period of one year immediately prior to importation or have been specifically approved for importation by the board. (NO SUBSTITUTIONS ALLOWED)	Spheniscus demersus

INSTRUCTION To Shipper: One copy of permit to accompany shipment to Hawaii.

Conditions or Object of Importation:
- ☒ To be kept in captivity at all times. (Caged)
- ☐ For propagation
- ☐ Other

Conditions: It is the responsibility of the named importer to personally contact the Federal Government as to their requirements which are contingent to this permit.

Name and Address of Shipper: International Animal Exchange, 130 E. Nine Mile, Ferndale, MI 48220

Name and Address of Importer: Hyatt Regency-Maui, Patricia Lonick, 200 Nohea Kai Dr., Lahaina, HI 96761 Phone: 661-1234

Tony R. Nakahara
CHIEF PLANT INSPECTOR

Yukio Kitagawa
CHAIRPERSON, BOARD OF AGRICULTURE

FOR OFFICIAL USE ONLY

PORT _____ ARRIVAL DATE _____ FLIGHT/SHIP _____

WAYBILL NO. _____ INSPECTION DATE/TIME _____ INSPECTOR _____

REMARKS _____

07/89

Conditions applicable to birds imported for Display:

1. Each lot of birds shall be inspected by a State Veterinarian upon arrival and all dead birds shall be returned to the Department of Agriculture for necropsy. The owner shall keep a record of all introduced birds and progenies for the inspection of State officials.

2. The following birds shall be pinioned:

   Flamingoes
   Swans
   Spoonbills
   Ducks
   Ibis
   Geese
   Cranes

   All birds shall be certified by a veterinarian as pinioned and subject to inspection upon arrival by a State Veterinarian.

3. All birds for exhibition out of cages shall be pinioned prior to entry into Hawaii.

4. Birds shall meet all Federal requirements.

5. Inspection of birds may be made at any time by representatives of the Division of Animal Industry, Hawaii Department of Agriculture. Birds shall be dusted with an approved pesticide on entry into the State to prevent the introduction of ectoparasites, or certified by a veterinarian as being ectoparasite-free.

6. Must be enclosed in fenced area.

7. Post entry inspection by Plant Quarantine staff.

8. All progenies must be pinioned and certified by a veterinarian.

Attachment 2

PQ-8b
5/87

Permit No. 12-93-M-7446

Date December 1, 1992

State of Hawaii
DEPARTMENT OF AGRICULTURE
Plant Quarantine Branch
701 Ilalo Street
Honolulu, Hawaii 96813-5524

IMPORT PERMIT

(Valid for One shipment(s) within One year(s) from date)

Permission is hereby granted to introduce the following, in accordance with Chapter 71, Rules of the Division of Plant Industry, Department of Agriculture, and the conditions listed below. *(Each lot must be inspected by a Plant Quarantine Inspector upon arrival before release.)*

Quantity	Commodity	Scientific Name
6	Magellanic penguins	Spheniscus magellanicus

Chap. 19

Conditions: Must be certified in accordance with ~~Reg. 11~~ attached and must be certified as the progeny of captive populations or have been held in captivity for a period of one year immediately prior to importation or have been specifically approved for importation by the board.

Condition: Items on this permit subject to cancellation due to changes in rules and policy at any time.

(NO SUBSTITUTIONS ALLOWED)

INSTRUCTION To Shipper: One copy of permit to accompany shipment to Hawaii.

Conditions or Object of Importation:

- [x] To be kept in captivity at all times.
- [ ] For propagation
- [ ] Other

Conditions: It is the responsibility of the named importer to personally contact the Federal Government as to their requirements which are contingent to this permit.

Name and Address of Shipper: International Animal Exchange., 130 East Nine Mile., Ferndale, MI 48220

Name and Address of Importer: Hyatt Regency Maui/Patricia Lonick., 200 Nohea Kai Drive., Lahaina, HI 96761 Phone: 661-1234

Gary R. Kokubun
CHIEF PLANT INSPECTOR

Yukio Kitagawa
CHAIRPERSON, BOARD OF AGRICULTURE

FOR OFFICIAL USE ONLY

PORT _____ ARRIVAL DATE _____ FLIGHT/SHIP _____

WAYBILL NO. _____ INSPECTION DATE/TIME _____ INSPECTOR _____

REMARKS _____

ASSOCIATION OF ZOOS & AQUARIUMS

AZA Species Survival Plan® Program Handbook

Created by the
AZA Animal Population Management Committee
in association with the
AZA Conservation, Management, and Welfare Sciences Department

Species Survival Plan® Program Handbook
Published by the Association of Zoos and Aquariums

Formal Citation: Association of Zoos and Aquariums (2021). Species Survival Plan® (SSP) Program Handbook. Association of Zoos and Aquariums, Silver Spring, MD.

Original Completion Date:
March 2011

Authors and Advisors:
AZA Animal Population Management Committee
AZA Conservation, Management, & Welfare Sciences Department

Table of Contents

Chapter 1. Introduction

Mission Statement

The mission of an ***Association of Zoos and Aquariums (AZA)*** cooperatively managed ***Species Survival Plan® (SSP) Program*** is to manage an *ex situ* species population with the interest and cooperation of AZA-accredited zoos and aquariums, ***Sustainability Partners***, and ***Certified Related Facilities*** (CRFs). An AZA SSP Program is identified through documented demand and potential sustainability within the AZA community; is selected by Taxon Advisory Groups (TAGs) through the RCP process; and develops a Breeding and Transfer Plan that identifies population goals and recommendations to manage a genetically diverse, demographically varied, and biologically sound population. Success is achieved when SSP animals are available to meet program goals and come from biologically sound populations as a result of a shared commitment to cooperative populations and program management.

Description

SSP Programs are led by expert ***advisors*** who cooperatively work together to maximize genetic diversity, appropriately manage the demographic distribution and long-term sustainability of TAG recommended Animal Programs within AZA member facilities. Each SSP Program manages the breeding of a select species or sub-species through a ***Breeding and Transfer Plan*** (previously referred to as a Master Plan). Breeding and Transfer Plans summarize the current demographic and genetic status of the population, describe the Animal Program's management designation, and recommend breeding pairs and transfers. Breeding and Transfer Plans are designed to maintain a healthy, genetically diverse and demographically stable population for the long-term future.

The AZA and its member facilities recognize that cooperative management is critical to the long-term survival of professionally managed Animal Programs and are fully committed to the goals and cooperative spirit of the SSP Program partnerships. Therefore, all AZA member facilities are required to fully participate in ***Green SSP Programs*** and their associated processes (see the AZA Policy for Full Participation in the SSP Program, Appendix A, and the AZA Animal Management Reconciliation Policy, Appendix B). Full participation in ***Yellow*** and ***Red SSP Programs*** is voluntary; however, cooperation among AZA facilities is strongly encouraged.

The AZA Animal Programs, along with the ***Animal Population Management Committee (APM Committee)***, must assure that the appropriate ***AZA Board approved policies*** are followed in all aspects of Animal Program management. All AZA member facilities and Animal Programs, regardless of management designation, must adhere to the AZA Policy on Responsible Population Management [formerly the Acquisition and Disposition (A&D)] Policy, the AZA Code of Professional Ethics, and the Sustainability Partner policies. All Board approved policies are found on the AZA website (https://www.aza.org/board-approved-policies-and-position-statements).

AZA Animal Programs

All AZA Animal Programs that have a ***published*** AZA Studbook, at least three defined goals, a minimum population size of 20 individuals, and are managed among three or more AZA member facilities are designated, in their TAG's ***Regional Collection Plan (RCP)***, and on the AZA website, as an SSP Program.

Animal Programs that have a published AZA Studbook, at least 3 defined goals and are designated as Extinct in the Wild, Critically Endangered, or Endangered (IUCN or other government agency) are not required to meet the minimum population size or number of participating facilities criteria in order to be designated by the TAG as an SSP Program. Whether the SSP Program is designated as Green, Yellow, or Red is dependent on the Animal Program's Sustainability Criteria (e.g., current population size, number of participating facilities, and projected gene diversity). The TAG may designate Animal Programs that do not qualify to be SSP Programs as ***Candidate Programs*** as long as the TAG has the goal of growing the Candidate Program to SSP status.

SSP Programs

Animal Programs designated as Green SSP Programs manage populations that are the most sustainable over time. Green SSP Program designations are made if Animal Programs:

- have a published AZA Regional Studbook,
- have at least 3 defined goals,
- are managed among at least three AZA member facilities,
- have a population that is able to retain >90.0% GD for 100+ years or 10+ generations, and
- have a population that is presently sustainable demographically with a sufficiently large population size and a positive growth rate to reach 100 years or 10 generations.

Animal Programs designated as Yellow SSP Programs manage populations that are potentially sustainable but require additional attention and effort to increase their sustainability. Factors such as reduced husbandry and breeding expertise/predictability, limited number of individuals, space, or founders, and/or poor demographics may prevent the Animal Program from achieving the Green SSP Program designation. Yellow SSP Program designations are made if Animal Programs:

- have a published AZA Regional Studbook,
- have at least 3 defined goals,
- have a population size equal to or greater than 50 individuals,
- are managed among at least three AZA member facilities, and
- have a population that is not able to retain at least 90.0% GD over for 100+ years or 10+ generations, or have a population that has never been formally planned, or was planned more than 5 years ago, so that the population's projected gene diversity cannot be properly assessed.

Animal Programs designated as Red SSP Programs manage populations that are currently unsustainable and in critical need of start-up efforts (e.g., importations) to help them increase their sustainability. This designation may change to a Yellow or Green SSP Program as sustainability increases. Red SSP designations are made if Animal Programs:

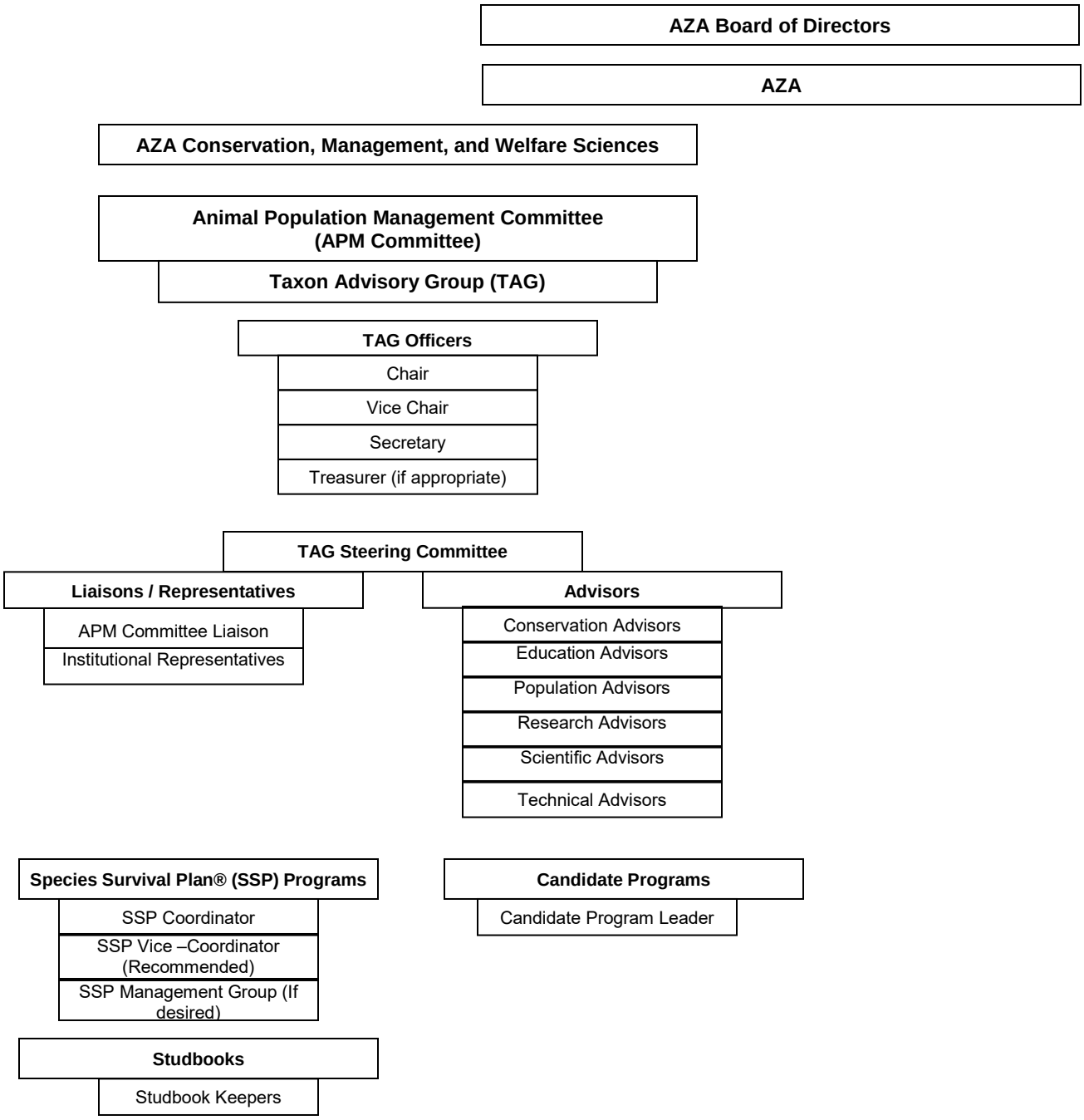
- have a published AZA Regional Studbook,
- have at least 3 defined goals,
- have a population size between 20 and 49 individuals, and
- are managed among at least three AZA member facilities.

Animal Programs that manage species designated as Extinct in the Wild, Critically Endangered, or Endangered (IUCN) do not need to meet minimum population size and number of participating facility criteria to be designated as an SSP Program. These SSP Programs only need to have a published AZA Regional Studbook and three defined goals.

More information on the designation and management of SSP Programs is in Chapter 3: SSP Program Management.

Chapter 2. Organization

Animal Program Overview



SSP Program Structure

All SSP Programs are composed of an ***SSP Coordinator***, Vice Coordinator (recommended), a ***Management Group*** (if preferred), and Advisors (if preferred) to assure that a significant amount of experience and diversity are represented. Required ***Officer*** positions include the SSP Coordinator and an AZA Regional ***Studbook Keeper***. Ideally the SSP Coordinator or SSP Vice Coordinator is also the AZA Regional Studbook Keeper; however this is not a requirement. In some cases the AZA Regional Studbook Keeper may also act as the International Studbook Keeper. The SSP Program may also include a Secretary and, if any financial components are incorporated into the SSP Program, a Treasurer. The SSP Coordinator or Vice Coordinator may fulfill the role of Secretary or Treasure, if necessary. It is not permissible for an SSP Program to have more than one Coordinator (Co-Coordinators) or Vice Coordinator (Co-Vice Coordinators). An SSP Coordinator is not required to be the ***Institutional Representative (IR)*** for their facility.

An SSP Program should include a Management Group if the SSP Program would benefit from the additional structure and administrative support. The Management Group must be composed of, and elected from, the SSP Program's Institutional Representatives (IRs). In addition, each SSP Program may be complimented by Advisors, who are not required to be affiliated with an AZA facility but are able to serve as experts in various fields such as nutrition, behavior, education, and field conservation, and may be members of an associated AZA ***Scientific Advisory Group (SAG)***. SSP Programs are encouraged to be creative in composing their Management Groups depending on the specific needs of their species.

Officer Positions

SSP Coordinator

Position Overview

The SSP Coordinator is elected by their TAG's ***Steering Committee*** (if the TAG has a current, approved RCP), or by the APM Committee (if the TAG does not have a current, approved RCP). An SSP Coordinator performs various duties to lead and support the AZA SSP Program. The SSP Coordinator works with IRs, the AZA Regional Studbook Keeper, the TAG, the APM Committee, and the AZA Conservation, Management, and Welfare Sciences Department, as well any associated governmental agencies, to develop, oversee, promote, and support the cooperative animal management, research, husbandry, and educational initiatives of the SSP Program. The primary responsibility of the SSP Coordinator is to regularly complete and distribute an SSP Breeding and Transfer Plan for the managed population.

Additional responsibilities include leadership and organization of the SSP Program in building and appropriately managing a sustainable population, and communication of recommendations and guidelines to the appropriate stakeholders**.** The SSP Coordinator serves as the primary contact and AZA expert for their species and abides by the duties and responsibilities set forth by the AZA, APM Committee, and the TAG.

Eligibility Requirements

The SSP Coordinator *must*:

- As of January 1, 2011, be a paid employee at an AZA member facility (AZA-accredited facility, Certified Related Facility, Society Partner, and Conservation Partner.) SSP Coordinators appointed prior to this date who were not employed at an AZA member facility are granted a personal variance as long as their existing circumstance remains in effect. When such individuals change circumstances they must resign as SSP Coordinator or gain employment at an AZA member facility within 6 months.
    - The term "paid" refers to hourly or salaried. The intent of this requirement is to assure that each Program Leader is fully integrated with his/her facility, serves a vital and consistent role within the facility that is outside of their role as a Program Leader, and has the facility's full support to serve as a Program Leader. Payment of a minimal amount to a Program Leader who is no longer integrated with their facility outside of their role as Program Leader does not fulfill this eligibility requirement.

- Have an individual AZA membership.
- Be well versed in the biology and behavior of the species covered by the SSP Program.
- Indicate any prior population management experience (i.e., completing AZA's Population Management 1 and/or 2 professional development courses, participation in a formal population planning meeting, prior ***Program Leader*** experience), as this is preferred.
- Uphold SSP business confidentiality.
- Be proficient in utilizing AZA web resources and the internet, and have email access.
- Have strong skills in organization, communication, facilitation, conflict resolution, and in establishing and maintaining effective working relationships with diverse groups of individuals.
- Provide a Statement of Commitment included in the application (Appendix C).

Essential Position Functions

Population Planning

- If the SSP Coordinator is the AZA Regional Studbook Keeper (or International Studbook Keeper in cases where a separate AZA Regional ***Studbook*** does not exist), s/he must:
  - Publish the AZA Regional component of the Studbook within 1 year of approval as SSP Coordinator/Studbook Keeper.
  - Coordinate development and publication of an SSP Breeding and Transfer Plan with the ***Population Management Center (PMC)***, a ***PMC Adjunct***, or an approved ***Small Population Management Advisory Group (SPMAG)*** Advisor.
  - If an SSP Coordinator is elected during the 3 year period of a current Breeding and Transfer Plan, the next Breeding and Transfer Plan will not be required until its scheduled due date, or as soon as possible after completing a planning session with the PMC, a PMC Adjunct, or SPMAG Advisor.
  - If an SSP Coordinator is elected past the 3 year period of the previous Breeding and Transfer Plan, a planning meeting date must be scheduled within 1 year of becoming SSP Coordinator. Publication of the Breeding and Transfer Plan will be due within 18 months of becoming SSP Coordinator.
  - Publish a complete Breeding and Transfer Plan with the PMC, a PMC Adjunct, or an Approved SPMAG member at least every 3 years after initial Breeding and Transfer Plan publication.
- If the SSP Coordinator is not the AZA Regional Studbook Keeper, s/he must:
  - If the AZA Regional Studbook is current, work with the AZA Regional Studbook Keeper and the PMC, a PMC Adjunct, or an approved SPMAG Advisor to publish an SSP Breeding and Transfer Plan as soon as possible after completing a planning session with the PMC, a PMC Adjunct, or SPMAG Advisor.
  - If the AZA Regional Studbook is not current, work with the AZA Regional Studbook Keeper to submit an up-to-date AZA Regional Studbook to the PMC within 1 year of approval as SSP Coordinator.
    - If it is a new AZA Regional Studbook and the AZA Regional Studbook Keeper does not meet the deadline within the 1 year period, then the AZA Regional Studbook Keeper can request an extension from the TAG, or if the TAG does not have a current, approved RCP, from the APM Committee Vice Chair of SSPs & Studbooks.
  - Coordinate development and publication of an SSP Breeding and Transfer Plan with the PMC, a PMC Adjunct, or an approved SPMAG Advisor as soon as possible after completing a planning session with the PMC, a PMC Adjunct, or SPMAG Advisor after the publication of an initial AZA Regional Studbook.
  - If an SSP Coordinator is elected during the 3 year period of a current Breeding and Transfer Plan, the next Breeding and Transfer Plan will not be required until its scheduled due date, or as soon as possible after completing a planning session with the PMC, a PMC Adjunct, or SPMAG Advisor.
  - If an SSP Coordinator is elected past the 3 year period of the previous Breeding and Transfer Plan, a planning meeting date must be scheduled within 1 year of becoming SSP

Coordinator. Publication of the Breeding and Transfer Plan will be due within 18 months of becoming SSP Coordinator.
    - Publish a complete Breeding and Transfer Plan with the PMC, a PMC Adjunct, or an Approved SPMAG member at least every 3 years after initial Breeding and Transfer Plan publication.
- Communicate and collaborate with species managers from other zoological regions on this SSP Program as needed for ***population sustainability***.
- Assure that the roles and goals of SSP Programs that are a part of a GSMP, or other formal international program, are well defined, and that participating facilities are aware of these roles.
- If the SSP Program is part of a GSMP or other formal inter-regional program, the SSP Program must coordinate with the TAG to determine whether the GSMP breeding and transfer information is sufficient for population management use among AZA facilities and therefore the publication of a separate SSP Breeding and Transfer Plan is not necessary.
    - If the GSMP breeding and transfer information is published in place of an SSP Breeding and Transfer Plan it must be published at least every 3 years, unless it is approved for a different time frame due to the species' natural history and/or WAZA accountability.
    - If the GSMP breeding and transfer information is published independently from the SSP Breeding and Transfer Plan, then the BTP must be published at least every 3 years and the GSMP will be required upon publishing at WAZA's accountability.
- Communicate any SSP Program data updates to the TAG Chair for inclusion in the TAG's ***Animal Program Summary Table*** (See TAG Handbook).
- Contribute to the ***SSP Sustainability Report*** and assure that all information in the report is current and complete.
- Work with the TAG Chair to assure that all goals in the SSP Sustainability Report are aligned with the TAG's RCP and TAG Annual Report.
- Copy the TAG Chair on all proposed changes to the SSP Sustainability Report.
- Communicate any SSP Program changes to the AZA Conservation, Management, and Welfare Sciences Department.

<u>Program Oversight</u>

- Consult with the PMC, a PMC Adjunct, or an approved SPMAG Advisor for genetic or demographic management questions, and to produce a Breeding and Transfer Plan (see Appendix F).
- Review the SSP's IR list on a regular basis. Contact ILs for those facilities that do not have a designated IR or when there is a discrepancy between the SSP's IR designation list and the IR designation list on the AZA website. IR lists can be downloaded on the SSP's Program page.
    - Communicate to the AZA Conservation, Management, and Welfare Sciences Department any IRs that should be assigned to SSP Programs for Sustainability Partners.
- Assure that all SSP Program participants have an opportunity to communicate their wants and needs in the planning process.
- Assess and address the wants and needs of AZA member facilities.
- Assure that all ***Sustainability Partners*** in all SSP Programs have been reviewed and approved by the TAG Chair and the APM Committee (Appendix G).
- Work with animal owners to assure that all SSP Program animals are relocated to an AZA member facility, or designated as nonessential to the population's demographic and/or genetic goals, within 2 years if a participating facility loses AZA accreditation and is not eligible to apply to be a Sustainability Partner, or chooses not to apply to be a Sustainability Partner.
- For the following qualifying event, the SSP Coordinator extends an invitation to a potential Sustainability Partner if the SSP wishes to include the facility in the Program and the facility is likely to pass the specific criteria in the Sustainability Partner application:
    - A facility participating in an SSP was formerly AZA-accredited, but is no longer accredited.
    - The SSP Coordinator should consult Appendix G: Sustainability Partner Policy & Application. The deadline to address Sustainability Partners will be 2 years from the change in facility accreditation OR approximately six months prior to the next SSP

Breeding and Transfer Plan publication, whichever comes first. If the non-AZA facility is not approved as a Sustainability Partner during that time, the facility will be excluded from the SSP.

- Respond to surveys and requests for information from the ***AZA Reproductive Management Center (RMC)***, as well as facilitate communication between the RMC and IRs when needed.
- Document issues related to IR or institutional ***accountability*** with respect to commenting on Draft and Final Breeding and Transfer Plans, and completing wants and needs surveys. A chart noting the institutional accountability should be included in the Breeding and Transfer Plan. In addition, the SSP Coordinator should make the AZA Conservation, Management, and Welfare Sciences Department and the APM Committee Chair aware of repeated events of poor IR or institutional accountability.
- Maintain detailed records, including institutional name, contact information, and Species360 mnemonics for each non-AZA member participating in SSP Programs.
- Immediately communicate any violations in AZA's SSP Sustainability Partner Policy to the TAG Chair, the APM Committee Vice Chair of Partnerships, the APM Committee Vice Chair of SSPs & Studbooks, and the AZA Conservation, Management, and Welfare Sciences Department.
- Attempt to reconcile any disagreements surrounding SSP recommendations through effective communication. Program Leaders should utilize their TAG Chairs, other Program Leaders, and the APM Committee to assist if needed. For Green SSP Programs, if disagreements cannot be reconciled, the SSP Coordinator must document the issue, communicate with the TAG Chair, and follow the AZA Animal Management Reconciliation Policy (Appendix B).
- Apply for an AZA SSP Sustainability Award if there has been a significant increase in the SSP population's sustainability.

<u>Administration</u>

- Develop an appropriate Management Group if necessary, and oversee the fulfillment of Management Group responsibilities.
  - If the SSP Program no longer requires an existing Management Group, the SSP Coordinator must explain the reasoning with the TAG Chair, and the TAG Steering Committee must approve the removal of the Management Group in its entirety.
- Assure that all Officers and Management Group members update their personal information to the TAG Chair and on the AZA website,
- Send copies of all significant SSP Program documents to the AZA Conservation, Management, and Welfare Sciences Department and TAG Chair.
- Respond to inquiries from the AZA office in a timely manner.
- Maintain regular contact with and respond in a timely fashion to inquiries/questions/ concerns from SSP Program members.
- Assure that the SSP Program adheres to the AZA Communications Policy (Appendix M).
- Support the AZA Animal Welfare Committee with the development and updating of ***Animal Care Manuals (ACMs).***
- Support the AZA Ambassador Animal Scientific Advisory Group with the development and updating of ***Ambassador Animal Guidelines (AAGs)***.
- Work closely with the AZA Conservation, Management, and Welfare Sciences Department if the SSP Program species is selected as an AZA SAFE species.
- Understand that Program Leaders are not responsible for providing engineering advice or letters of endorsement to facilities designing new exhibits for your Animal Program species. Rather, it is the responsibility of those who are developing new exhibit designs to approach a range of AZA member facilities to learn about various specifications or sources regarding the species to be exhibited.

The SSP Coordinator *is encouraged to*:

- Elect a Vice Coordinator. If no one within the Management Group or the SSP Program's IRs applies for this position then the SSP Coordinator may appoint an interested party from their

facility. As there may only be one vote per facility, the Vice Coordinator would not have an official vote in SSP Program elections and issues.
- Actively advocate and develop sustained interest on the part of member facilities to participate in the SSP Program and build a sustainable population.
- Arrange at least one working SSP Program meeting each year, either in person or electronically through tele- or web-conferencing. If this meeting is in person, the SSP Program is encouraged to hold the meeting in conjunction with the AZA Annual Conference and/or Mid-Year Meeting. Provide minutes from these meetings to the TAG.
- Provide routine SSP Program updates to IRs.
- Serve on or as an Advisor to the appropriate TAG and attend relevant meetings.
- Maintain contact with counterparts in other regional associations to facilitate inter-regional cooperation, if applicable.
- Engage with the appropriate International Union for the Conservation of Nature (IUCN) Species Survival Commission (SSC) Specialist Group and other relevant organizations, if applicable.
- Delegate responsibilities to the SSP Vice Coordinator and Management Group, as appropriate.
- Complete the AZA Professional Development Courses "Population Management 1 (PM1): Data Acquisition and Processing" and "Population Management 2 (PM2): Data Analysis and Breeding Recommendations."
- Review relevant AZA Online Training Modules (https://www.aza.org/online-training-modules/).

Vice Coordinator

Position Overview

The Vice Coordinator is a recommended role for SSP Programs and is elected from the Management Group or IRs through a publicly disclosed, democratic process. If no one within the Management Group or the SSP Program's IRs applies for this position then the SSP Coordinator may appoint an interested party from their facility. The Vice Coordinator's specific duties will be outlined by each SSP Program, but the primary role of the SSP Vice Coordinator is to assume leadership of the SSP Program should the SSP Coordinator be unavailable. It is presumed that if, for any reason, the SSP Coordinator must vacate the position, the SSP Vice Coordinator will assume all SSP Coordinator duties until a new SSP Coordinator is elected. Vice Coordinators are not automatically appointed as the SSP Coordinator upon an SSP Coordinator vacancy. Only one official Vice Coordinator may be appointed to the SSP; however, the SSP Program may design its operating structure as best it sees fit.

Eligibility Requirements

The SSP Vice Coordinator *must*:
- As of January 1, 2011, be a paid employee at an AZA member facility (AZA-accredited facility, Certified Related Facility, Society Partner, and Conservation Partner.) SSP Vice Coordinators appointed prior to this date who were not employed at an AZA member facility are granted a personal variance as long as their existing circumstance remains in effect. When such individuals change circumstances they must resign as Vice Coordinator **o**r gain employment at an AZA member facility within 6 months.
- Have an individual AZA membership.
- Be well versed in the biology and behavior of the species covered by the SSP Program.
- Uphold SSP business confidentiality.
- Have proficiency in utilizing AZA web resources and the internet, and have email access.
- Have strong skills in organization, communication, facilitation, conflict resolution, and in establishing and maintaining effective working relationships with diverse groups of individuals.
- Provide a ***Statement of Individual Commitment*** (Appendix E).
- Provide a ***Statement of Institutional Support*** from their employer (Appendix E).

Essential Position Functions

- Assume all Coordinator duties if Coordinator is unavailable, or the Coordinator position is vacant.

- Attend all TAG and SSP Program meetings, whenever possible.
- Respond to inquiries from the IRs, TAG, and AZA office in a timely manner.
- Assist the SSP Coordinator in supporting the SSP Program and building a sustainable population.
- Assist the Coordinator with filling the Secretary and Treasurer (if applicable) positions if vacant.

Secretary

Position Overview

If the SSP Program determines that a Secretary is needed to record and manage the SSP Program's details, the Secretary is elected from the IRs through a publicly disclosed, democratic process. The SSP Coordinator or Vice Coordinator may fulfill the role of Secretary, if necessary. In the event that a Secretary cannot be identified from within the Management Group membership, an IR may be appointed Secretary, but will not be allowed to vote as a Management Group member. The Secretary keeps a written record of the SSP Program's elections, votes and formal meetings, and communicates these records, and any programmatic changes to the TAG, the APM Committee, and the AZA Conservation, Management, and Welfare Sciences Department.

Eligibility Requirements

The Secretary *must*:

- As of January 1, 2011, be a paid employee at an AZA member facility (AZA-accredited facility, Certified Related Facility, Society Partner, and Conservation Partner.) Secretaries appointed prior to this date who were not employed at an AZA member facility are granted a personal variance as long as their existing circumstance remains in effect. When such individuals change circumstances they must resign as Secretary or gain employment at an AZA member facility within 6 months.
- Have an individual AZA membership.
- Uphold SSP business confidentiality.
- Have proficiency in word processing and spreadsheet programs, utilizing AZA web resources, and have email access.
- Have strong skills in organization, communication, and in establishing and maintaining effective working relationships with diverse groups of individuals.
- Provide a statement of individual commitment (Appendix E).
- Provide a statement of institutional support from their employer (Appendix E).

Essential Position Functions

- Attend all TAG and SSP Program meetings, when possible.
- Respond to inquiries from the IRs, TAG, and AZA office in a timely manner.
- Post all issues held to a vote within the SSP Program on the SSP's listserv, SSP Network Group, AZA website, etc.
- Distribute all Officer nominee applications to the Management Group, or if there is no Management Group, to the IRs.
- Oversee and mediate all components of issues and elections held to a vote within the SSP Program, including the issue and/or election, the voting record, and the outcome.
- Record and archive the results of all issues held to a vote within the SSP Program and submit them to the SSP Coordinator.
- Record, report, and archive IR responses to SSP related requests.
- Record, document, and ***AZA brand*** all SSP Program business (e.g., Action Plans, etc.) and submit these to the SSP Coordinator.
- Record, archive, AZA brand, and submit minutes from significant SSP Program meetings to the SSP Coordinator.
- Keep all application materials, statements of individual support, etc. on file.
- Communicate all programmatic changes (e.g., Program Leader, Officer, Management Committee member, Advisor, SSP Program designation, etc.) for the SSP Program to the TAG Chair and AZA Conservation, Management, and Welfare Sciences Department.

Treasurer

Position Overview

If any financial components are incorporated into the SSP Program, a Treasurer should be elected from the Management Group through a publicly disclosed, democratic process. The SSP Coordinator or Vice Coordinator may fulfill the role of Treasurer, if necessary. In the event that a Treasurer cannot be identified from within the Management Group membership, an IR may be elected Treasurer but will not be allowed to vote as a Management Group member. Treasurers collect, disperse, and archive written records of all financial transactions. Treasurers also coordinate and manage AZA designated fund accounts.

Eligibility Requirements

The Treasurer *must*:

- As of January 1, 2011, be a paid employee at an AZA member facility (AZA-accredited facility, Certified Related Facility, Society Partner, and Conservation Partner.) Treasurers appointed prior to this date who were not employed at an AZA member facility are granted a personal variance as long as their existing circumstance remains in effect. When such individuals change circumstances they must resign as Treasurer **o**r gain employment at an AZA member facility within 6 months.
- Have an individual AZA membership.
- Uphold SSP business confidentiality.
- Have proficiency in word processing and spreadsheet programs, and have email access.
- Have strong skills in organization, communication, and in establishing and maintaining effective working relationships with diverse groups of individuals.
- Provide a Statement of Individual Commitment (Appendix E).
- Provide a Statement of Institutional Support from their employer (Appendix E).

Essential Position Functions

- Apply for and manage the SSP Program's ***Dedicated Fund*** in compliance with "AZA's Management Guidelines for AZA Conservation Program Dedicated Funds" (www.aza.org/dedicated-funds), if appropriate.
- Respond to inquiries from the TAG and AZA office in a timely manner.
- Attend all TAG and SSP Program meetings, when possible.

Management Group

Overview

At a minimum, an SSP Management Group is composed of the Coordinator, Vice Coordinator (recommended), and AZA Regional Studbook Keeper. The SSP Program may find it useful to create a larger Management Group to assist in sharing the SSP Program's workload and to allow the AZA member facilities greater input into the SSP Program's management. The Management Group serves as the voting body for SSP Program business and all members are integrally involved in the SSP Program appointments, publications, and meetings. Each SSP Program should determine the Management Group's size (suggested ideal is 7 individuals with a maximum of 15 individuals), structure, and administrative responsibilities (e.g., election terms, term limits, duties, etc.). Management Group members must be elected from the SSP Program's IRs.

Eligibility Requirements

Members of the Management Group *must*:

- be a paid employee of their facility
- be their facility's IR
- Uphold TAG business confidentiality.
- Have proficiency in utilizing AZA web resources and the internet, and have email access.
- Have strong skills in organization, communication, facilitation, conflict resolution, and in establishing and maintaining effective working relationships with diverse groups of individuals.

Essential Position Functions

- Contribute to the development of the SSP Breeding and Transfer Plan
- Review and approve the species' GSMP, if applicable.
- Vote in all elections and issues brought to a vote.
- Attend TAG and SSP Program meetings, whenever possible.
- Inform the SSP Coordinator of any problems or issues within the Management Group.
- Contribute to and review the final draft of the ACM for the species represented by the SSP Program. This includes garnering information regarding ecology, nutrition, reproduction, behavior, etc., and conducting a complete literature review to incorporate the most recent scientific information, and working with the TAG, if applicable, to identify the required external reviewers.
- Provide expert review of ***Conservation Grants Fund (CGF)*** proposals directed to the SSP Program.
- Provide and update general SSP information for the public pages of the AZA website upon request.
- Respond to inquiries from the IRs, TAG, and AZA office in a timely manner.

Members of the Management Group are *encouraged* to:

- Solicit additional Management Group members to reach the Group's ideal capacity (5-15 individuals).
- Solicit new Management Group members to replace retired Management Group members.
- Implement a Program Leader Training and Mentoring Plan to help new incoming SSP Officers, Studbook Keepers, and Management Group members become familiar and comfortable with their responsibilities, especially with respect to building sustainable populations.
- Identify, assist, and provide, if appropriate, financial support for ***in situ*** and ***ex situ*** research related to the SSP Program.

Studbook Keeper

Position Overview

The AZA Regional Studbook Keeper is responsible for maintaining an accurate record of the histories of all individual animals in an *ex situ* population for the purpose of population management. This is an important responsibility because the global zoo and aquarium community depends on the maintenance of accurate Studbook records in order to manage populations and fulfill the goal of long-term sustainability. Ideally, the AZA Regional Studbook Keeper also serves as that Program's SSP Coordinator or the SSP Vice Coordinator and the Regional Studbook data will be used to create the SSP Program's Breeding and Transfer Plan. The AZA Regional Studbook Keeper works directly with the associated TAG and SSP Program, all participating AZA member facilities, the APM Committee, the PMC, a PMC Adjunct, or an approved SPMAG Advisor, and the AZA Conservation, Management, and Welfare Sciences Department to complete and distribute a timely and accurate AZA Regional Studbook to be used for demographic and genetic analyses relevant to the SSP Program's population management.

The AZA Regional Studbook Keeper serves as a contact and AZA expert for the species and abides by the duties and responsibilities set forth by the AZA, APM Committee, SSP Program, and the TAG. If the Studbook is an ***International Studbook***, and the International Studbook Keeper is employed in an AZA member facility, the Studbook Keeper is held to the same ***accountability*** requirements as an AZA Regional Studbook Keeper with regards to the AZA Regional Studbook data.

Eligibility Requirements

The AZA Regional Studbook Keeper *must*:

- As of January 1, 2011, be a paid employee at an AZA member facility (AZA-accredited facility, Certified Related Facility, Society Partner, and Conservation Partner.) AZA Regional Studbook Keepers appointed prior to this date who were not employed at an AZA member facility are granted a personal variance as long as their existing circumstance remains in effect. When such

individuals change circumstances they must resign as AZA Regional Studbook Keeper or gain employment at an AZA member facility within 6 months.
- Have an individual AZA membership.
- Uphold SSP business confidentiality.
- Be well versed in the biology and behavior of the Studbook species.
- Complete the AZA Professional Development Course "Population Management 1 (PM1): "Data Acquisition and Processing" within 2 years of becoming the AZA Regional Studbook Keeper. AZA Regional Studbook Keepers are also encouraged to take "Population Management 2 (PM2): Data Analysis and Breeding Recommendations" (https://www.aza.org/professional-development).
- Have proficiency in word processing and spreadsheet programs, population management software, utilizing AZA web resources, and have email access.
- Have strong skills in organization, communication, and in establishing and maintaining effective working relationships with diverse groups of individuals.
- Provide a Statement of Individual Commitment (see AZA Regional Studbook Keeper Handbook).
- Provide a Statement of Institutional Support from their employer (see AZA Regional Studbook Keeper Handbook).

Essential Position Functions

- Create, update and submit a current AZA Regional Studbook report to the AZA Conservation, Management, and Welfare Sciences Department for publication on the AZA website, in accordance with the requirements outlined in Appendix D of the AZA Regional Studbook Keeper Handbook.
  - within 12 months of completing PM1.
  - within 12 months of becoming AZA Regional Studbook Keeper if the Studbook Keeper has already completed PM1 prior to becoming an AZA Regional Studbook Keeper.
- Submit a complete, current AZA Regional Studbook report to the AZA Conservation, Management, and Welfare Sciences Department and the PMC at least once every 3 years in accordance with the current to date listed on the front cover of the previous Studbook publication; however, annual updates are preferred.
- Submit a complete, current AZA Regional Studbook database (PopLink, SPARKS, or Excel), if the studbook is not maintained in ZIMS for Studbooks.
  - to the AZA Conservation, Management, and Welfare Sciences Department and the PMC at least once every 3 years in accordance with the current to date listed on the front cover of the previous Studbook publication; however, annual updates are preferred.
  - to the SSP Program's Population Advisor (PMC, PMC Adjunct, SPMAG Advisor) prior to each formal population planning meeting, or as needed for population management purposes.
  - to the TAG Chair and SSP Coordinator after each publication.
  - If the Studbook is maintained in ZIMS for Studbooks, the Studbook cannot be exported. Instead of submitting a database, the PMC, PMC Adjunct, or SPMAG Advisor must have access to the database in ZIMS for Studbooks.
- Adhere to the "Guidelines for Data Entry and Maintenance of North American Regional Studbooks" (https://www.aza.org/assets/2332/standardsdataentry2.pdf).
- Send copies of all significant AZA Regional Studbook documents and correspondence to the AZA Conservation, Management, and Welfare Sciences Department, the relevant TAG Chair, and the SSP Coordinator (if the AZA Regional Studbook is for an SSP Program and if the SSP Coordinator is not the AZA Regional Studbook Keeper).
- Work closely with the appropriate TAG and SSP Coordinators.
- Attend relevant meetings, when possible.
- Update new contact information, including facility, phone, fax, and email via the AZA website by logging into their account on "My AZA."
- Serve as a contact and AZA expert for the Studbook species. Understand that Studbook Keepers are not responsible for providing engineering advice or letters of endorsement to facilities designing new exhibits for the Animal Program species. Rather, it is the responsibility of those

who are developing new exhibit designs to approach a range of AZA member facilities to learn about various specifications or sources regarding the species to be exhibited.
- Abide by the duties and responsibilities set forth by the AZA, the APM Committee, and the TAG.
- Maintain contact with counterparts in other regional associations to facilitate inter-regional cooperation, if applicable.
- If there is a separate International Studbook Keeper for the species or if data are combined in a single International Studbook, the AZA Regional Studbook Keeper is still responsible for current and accurate AZA regional data needed for AZA population analyses.
- Review relevant AZA Online Training Modules (https://www.aza.org/online-training-modules/).

Liaisons & Representatives

APM Committee TAG Liaison

Position Overview

The ***APM Committee Liaison*** is a member of the APM Committee who serves as the primary contact between the APM Committee and the Chair of the TAG(s) to which s/he has been assigned. The TAG should maintain consistent and open communication with their APM Committee Liaison. This will facilitate the Liaison in assisting the TAG during all RCP and accountability processes, and acting as a resource for TAG Program Leaders regarding APM Committee guidelines. The APM Committee Liaison will assist the TAG, and all SSP Programs within its purview, as needed.

Essential Position Functions

- Act as a Liaison between the TAG and APM Committee.
- Attend (or participate via conference call) as many of the TAG's Animal Program meetings as possible.
- Maintain consistent communication with the TAG Chair.
- Uphold TAG business confidentiality.
- Provide general assistance to the TAG's Animal Program oversight and operation, and assure the TAG maintains consistent communication with their Program Leaders, especially with respect to building sustainable populations.
- Serve as a conduit between the Animal Programs within the TAG's purview and the APM Committee.
- Provide a verbal summary of the TAG and the Animal Programs within the TAG's purview, including any accomplishments and/or concerns at both the Annual and Mid-Year APM Committee meetings.
- Assure that the TAG prioritizes, manages, and publishes the ACMs within their purview.
- Review the TAG's Annual Report and communicate any issues identified to the APM Committee during the AZA Annual Conference.
- Respond to any inquiries from TAG Chairs during the development of the RCP, review the Draft RCP as outlined in the RCP Handbook, and provide feedback to the TAG Chairs.
- Review the TAG's RCP, with the APM Committee Vice Chair of TAGs, and an additional appointed APM Committee member, and present this review to the APM Committee for final RCP approval consideration.
- Review SSP Sustainability Partner applications for completion.

Institutional Liaison

Position Overview

The default facility's single ***Institutional Liaison (IL)*** is the institutional CEO/Director, however s/he may appoint an alternate IL for the facility if desired. The IL assures that there is effective communication and participation between the facility and AZA's TAG and SSP Programs. It is assumed that all decisions/votes made by the IL are approved by the Institutional Director. The IL designates IRs and keeps the facility's IR list current. The IL serves as the default IR for any TAG or SSP Program which does not have an IR designated and is required to respond accordingly. The IL

works with Program Leaders and IRs to assure that their facility fully participates in all associated TAG and SSP Programs, and if necessary, will meet in conflict resolution processes.

Eligibility Requirements

The Institutional Liaison *must*:

- Be a paid employee of the facility s/he represents.
- Be designated by the CEO/Director of his/her facility.
- Have access to their facility's IR list through the AZA website.
- Be an individual member of AZA: Professional Affiliate or Professional Fellow.
- Uphold TAG business confidentiality.
- Have the capability to monitor and communicate with all IRs at his/her facility. Depending on the number of Animal Programs in which the facility participates, this can be a potentially large group of individuals.
- Have the ability to make decisions about his/her facility's animal populations, or be able to communicate with those who make decisions about these populations.
- Have proficiency in word processing and spreadsheet programs, utilizing AZA web resources, and have email access. In an effort to be as green as possible, most documents will be sent electronically or be available for download from the AZA website, and the IL must be able to view and download documents in Microsoft Word and PDF formats.
- Have the capability to disperse documents to the appropriate institutional personnel.

Essential Position Functions

- Designate IRs to appropriate TAG and SSP Programs with consideration as to who would be the most appropriate staff member to represent the needs of the species and the wishes of the facility when communicating with Program Leaders, and work with the Program Leaders on developing plans for building sustainable populations.
- Review and update their IR list via the AZA website on a regular schedule to assure currentness.
- Review the AZA Online Training Modules for instructions on how to manage your IR list.
- Fulfill the IR responsibilities for any TAG or SSP Program that does not have a designated IR.
- Review the list of upcoming SSP planning meetings at the PMC that is posted in the IL Network Group's announcement section each month. ILs should review this announcement as it serves as a reminder to update their IR list through the AZA website so that the Program Leaders obtain the most current IR list for their Animal Program.
- Assure that deadlines, including those for the completion of space surveys, are met by each IR.
- Assure that all Draft RCPs and Breeding and Transfer Plans are read and that all recommendations included within them are approved by each IR during the comment period.
- Provide Animal Program documents to IRs upon request if the IR is not an individual AZA member and does not have access to documents through the AZA website.
- Assure that studbook databases maintained by AZA Regional Studbook Keepers at their facility are archived, either at the facility or the AZA PMC.
- Assure that any RCP and Breeding and Transfer Plan recommendation disagreements are addressed by the IR with the SSP Coordinator during the comment period.
- Respond to initial inquiries of Program Leaders and IRs in a timely manner.
- Communicate with TAG and SSP Programs regarding problems that may arise with IR participation and work within the Reconciliation Process to resolve them if necessary.
- Follow up with Program Leaders who are approaching their accountability deadline for their Animal Program documents (i.e., RCPs, Breeding and Transfer Plans, and Studbooks). ILs are copied into automated accountability reminder emails at the 1 month, deadline reached, and 2 weeks past reminder emails.
- Follow up with AZA Regional Studbook Keepers who are approaching their Population Management 1 accountability deadlines; ILs are copied on reminder emails sent from the AZA Conservation, Management, and Welfare Sciences Department.

- If the institutional Director does not assume this responsibility, the IL must issue and communicate Program Leader extension approvals and denials to the AZA Conservation, Management, and Welfare Sciences Department if the Program Leader misses their accountability deadline.

Institutional Representative

Position Overview

The IR is the primary contact between his/her facility and the Program Leader of the TAG and SSP Programs to which s/he has been designated. The IR is responsible for maintaining open communication between the TAG and SSP Program and the facility, communicating to the Program Leader on behalf of the facility, and participating in TAG and SSP Program communications and activities.

Each facility is represented by one IR for each TAG and SSP Program in which the facility participates. If the TAG Chair moves to a facility that already has an IR represented in that TAG, or if a new Chair is appointed from a facility that already has an IR represented in that TAG, the TAG Chair will automatically be appointed as that facility's IR. The previous IR must relinquish his/her position because there can only be one IR, and one vote, per facility for each Animal Program. If the former IR served on the Steering Committee, the TAG will hold an immediate election to replace the Steering Committee member. SSP Coordinators and Studbook Keepers are not automatically approved as IRs; they must be designated as their facility's IR by the IL. Program Leaders who are not Steering Committee members may still participate in the TAG as non-voting advisory members.

One individual may serve as the IR for more than one Animal Program at a facility; however the duties for each Animal Program are independent of each other. IRs should be aware that being a representative to multiple Animal Programs involves a greater commitment. The IR is appointed by the IL unless the facility's Director assumes this responsibility.

Eligibility Requirements

The Institutional Representative *must*:

- Be a paid employee of the facility s/he represents.
- Be designated by the IL of the facility.
- Uphold TAG business confidentiality.
- Serve as the facility's IR for the TAG if s/he serves as TAG Chair.
- Be familiar with the species/taxa s/he represents. It is understood that there will not always be a staff member that specializes in a particular taxon or species. In these situations, the position should fall to the person on staff who is the most logical point of contact for the Animal Program.
- Have the ability to make decisions about the facility's animal collections, or be able to communicate with those who have the ability to make decisions about the collections.
- Have proficiency in word processing and spreadsheet programs, utilizing AZA web resources, and have email access. Most documents will be sent electronically or be available for download from the AZA website, and the IR must be able to view and download in documents in Microsoft Word and PDF formats.
- Have the capability to disperse documents to the appropriate facility personnel.

Essential Position Functions

- Communicate with and disseminate information among Animal Programs, Program Leaders, the IL, the institutional Director, Ambassador Animal staff, and the animal care staff, and work with and encourage Program Leaders to build sustainable populations.
- Respond to and fulfill inquiries by TAG and SSP Programs in a timely manner.
- Vote in all Steering Committee/Management Group elections.
- Review and complete "Institutional Wants and Needs" surveys within the requested time frame.
- Communicate Animal Program participation with the IL.
- Review and communicate comments for Draft Breeding and Transfer Plans and RCPs to the IL and Program Leaders during the 30-day comment period.

- Request Animal Program documents from the IL if the IR is not an individual AZA member and does not have access to documents through the AZA website.
- Assure that any RCP and Breeding and Transfer Plan recommendation disagreements are addressed with the IL and Program Leaders during the comment period.
- Complete and return space surveys for TAG RCPs within the requested time frame.
- Consider volunteering for Animal Program activities and standing for election to Animal Program committees.
- Communicate any contact information amendments or change of status to the IL.

Advisors

Position Overview

Advisors, often members of corresponding SAGs, play a critical role in advising, designing, and executing management decisions within AZA Animal Programs. If a member of the Management Group has the appropriate expertise in an advisory area, then s/he may serve as that Advisor. SSP Programs are encouraged to fill as many Advisor positions as appropriate for their SSP Program in order to implement superlative management initiatives. Advisors do not need to be employed by an AZA member facility.

Advisors do not vote in elections or on TAG issues unless they also serve as an IR or a member of the TAG Steering Committee.

Suggested Advisors

Ambassador Animal
Animal Welfare
Biomaterials Banking
Behavior
Contraception
Education
Endocrinology
Epidemiology
Field Conservation
Genetics
Government Affairs
Green Practices
Horticulture
Life Support Systems
Nutrition
Pathology
Public Relations
Registrar
Reintroduction
Research
Reproduction
Water Quality
Veterinary

Position Functions

- Advise the SSP in their efforts to identify, develop and implement Animal Program goals, as applicable.
- Work with the SSP Programs and provide input on the SSP Sustainability Reports.
- Provide content for AZA taxa-related stories of interest related to the Advisor's area of expertise.
- Provide expert advice regarding any topics, research proposals and inquiries related to the Advisor's area of expertise.
- Provide input on relevant Animal Care Manuals and Ambassador Animal Guidelines as requested.
- Uphold SSP Program business confidentiality.
- Assist in the development of education materials related to the Advisor's area of expertise.
- Assist with the development of research projects related to the Advisor's area of expertise.
- Assist the SSP Program and TAG in reviewing taxa-related CGF grant proposals as requested.

The AZA Population Management Center

The AZA PMC, hosted by the Lincoln Park Zoo in Chicago, Illinois, and San Diego Zoo Global in San Diego, California, was established in June, 2000 to provide assistance to zoo professionals across the country by conducting demographic and genetic analyses and preparing Breeding and Transfer Plan

for SSP Programs. For more information on the PMC and its role in AZA Animal Programs see the SSP and AZA Regional Studbook Keeper Handbooks.

PMC Functions

PMC Population Biologists provide many services for AZA Animal Programs including:

- Producing Breeding and Transfer Plans (BTPs) with SSP Programs
- Assisting AZA Regional Studbook Keepers with AZA Regional Studbook publication
- Researching unknown or partially-known pedigrees
- Creating analytical AZA Regional Studbooks
- Conducting research and helping develop software to improve methods of population management
- Advising on data conventions and entering abnormal data, and
- Troubleshooting problems with population management software (e.g., SPARKS, PopLink, PMx, ZIMS for Studbooks, ***PMCTrack***).

See Chapter 4 for more details on the PMC.

The AZA Reproductive Management Center

The mission of the AZA Reproductive Management Center (RMC) is to provide information and recommendations to the AZA community about contraceptive products that are safe, effective, and reversible. These recommendations are used by zoo professionals to make informed decisions on how to sustainably manage their animal collections. Contraception is an essential, proven, and humane tool for reproductive management while still allowing individuals to live in natural social and family groups. It allows managers to maximize available space by preventing births from animals that are not high priorities for breeding or animals that are not currently recommended for breeding, but will be in the future.

The RMC includes scientists, veterinarians, and animal managers with research and management expertise in wildlife contraception. The RMC houses a Contraception Database which contains over 30,000 records for animals treated with contraception. Using these data, the RMC is able to make taxon- and species-specific recommendations about products that are safe, effective, and reversible.

The RMC assures that contraceptives are safe and effective by:

- Maintaining databases that monitor all contraceptives used in all mammalian species.
- Analyzing data on the efficacy and safety of contraceptives.
- Conducting comprehensive pathologic examinations on reproductive tracts to detect if deleterious effects are associated with contraceptives through the Reproductive Health Surveillance Program.

RMC Functions

The RMC assists SSP Coordinators, mammal curators, wildlife managers, and veterinarians in choosing and administering appropriate contraceptives by:

- Annually producing and distributing up-to-date contraceptive recommendations for all mammals,
- Providing AZA SSP Coordinators and TAG Chairs with species-specific contraception guidelines for Animal Care Manuals,
- Providing a “Help Line” to assist animal managers with specific contraceptive questions or concerns,
- Maintaining a website with the latest wildlife contraceptive information,
- Attending SSP or TAG planning meetings if relevant to the population, and
- Providing written recommendations to be included in SSP Breeding and Transfer Plans as an Appendix, if needed.

The RMC relies on feedback from the zoo community to update and improve contraception recommendations. While safety and efficacy are vital components of a contraceptive suitable for zoo animals, reversibility is the third integral element that has far-reaching consequences for sustainable population management. The RMC’s goal is to produce reversibility data for different contraceptives so that managers are well-informed and know what to expect from a particular product. This is often the most challenging data to collect because pregnancies and births can occur years after treatment

or at a different facility than the one at which the contraceptive was administered. It is essential details be reported not only during treatment to obtain efficacy parameters, but also after treatment is stopped for breeding. The RMC requests reversal data in the annual Contraception Survey, but asks that program managers keep the RMC in mind when births occur in their respective populations throughout the year.

Sustainability Criteria

SSP Programs operate within three distinct management levels: Green SSP Programs, Yellow SSP Programs, and Red SSP Programs. The TAG may also designate populations that do not currently meet the minimum criteria to be an SSP as Candidate Programs. An Animal Program's Sustainability Criteria (i.e., population size, number of participating AZA member facilities, and projected gene diversity) directly affect its management designation. These criteria and how to define them are explained below and summarized in Table 1.

If there is no published AZA Regional Studbook, an Animal Program will be designated as a Candidate Program until a current, up-to-date AZA Regional Studbook has been submitted to the AZA Conservation, Management, and Welfare Sciences Department for publication.

Population Size

- To determine the current population size, refer to the published AZA Studbook, or the most recently published ***Population Viability Analysis (PVA)***, Breeding and Transfer Plan, and/or ***MateRx*** (whichever is most current).
- In order to be designated as an SSP Program, the population size (total N in the initial published Studbook, or the most recent Population Viability Analysis (PVA), Breeding and Transfer Plan, or MateRx) must be equal to or greater than 20 individuals. These SSP Programs are further designated as Green, Yellow, or Red SSP Programs through their population size and/or projected gene diversity.
- If the Animal Program manages a species which is classified as Extinct in the Wild, Critically Endangered, or Endangered (e.g., IUCN or other government agency), the minimum population size criterion does not apply and the Animal Program will qualify as an SSP Program upon publishing an AZA Regional Studbook.

Participating AZA Member Facilities

- To determine the official number of participating AZA member facilities in the managed population, refer to the AZA Studbook, or the most recently published Population Viability Analysis (PVA), Breeding and Transfer Plan, or MateRx (whichever is most current).
- In order to be designated as an SSP Program, the managed population must include at least three participating AZA member facilities. These SSP Programs are further designated as Green, Yellow, or Red SSP Programs through their population size and/or projected gene diversity.
- If the Animal Program is for a species which is classified as Extinct in the Wild, Critically Endangered, or Endangered then the minimum number of participating AZA member facility criterion does not apply and the Animal Program will qualify as an SSP Program upon publishing an AZA Regional Studbook.

Projected Gene Diversity

- For most populations, a projected gene diversity will be used to differentiate between Green and Yellow SSP Program designations, and this projected gene diversity is defined as the projected % gene diversity (%GD) at 100 years or 10 generations, although colonial populations such as herds, flocks and schools may require alternate modeling programs (to be developed) to determine their projected gene diversity.
- An Animal Program's projected gene diversity is measured during population analysis with the PMC, a PMC Adjunct, or an approved SPMAG Advisor.
- The projected gene diversity (%GD) at 100 years or 10 generations may be determined by a PVA or Breeding and Transfer Plan from the last 5 years.
- MateRx reports cannot be used to change projected gene diversity.
- If the population has never undergone formal population planning by the PMC, a PMC Adjunct or approved SPMAG Advisor, or was planned more than 5 years ago, the population's projected gene diversity cannot be properly assessed.

- If the Animal Program has at least 50 individuals in the population and three AZA facilities (determined as discussed above), it will be designated as a Yellow SSP Program until formal population planning occurs.
- If the Animal Program has between 20 and 49 individuals in the population and three participating AZA facilities, it will be designated as a Red SSP Program until formal population planning occurs.

- The designation of each SSP Program may change in accordance with the population becoming more or less sustainable over the course of time. The TAG should assist in making these updated designations available to AZA members on the AZA website.

Table 1. Applying Sustainability Criteria to Designate Animal Program Management Levels

Criterion	Green SSP Program	Yellow SSP Program	Red SSP Program	Candidate Program
Population size	50 and above	50 and above	20-49	19 and fewer
# AZA member facilities	3 and above	3 and above	3 and above	2 or fewer
Projected gene diversity	90.0% or above	Less than 90.0%	Less than 90.0%	NA

SSP Management Designations

SSP Programs fall into one of three designations: Green SSP Programs, Yellow SSP Programs, and Red SSP Programs. The differences in SSP Program management are described below, summarized in Table 2, and are outlined in a flow chart in Appendix S. The SSP Handbook provides complete SSP Program management details.

All AZA Animal Programs are held to the same established minimum criteria to be designated an SSP Program, unless the species is classified as Extinct in the Wild, Critically Endangered, or Endangered (IUCN or other government agency).

Green SSP Programs

- Green SSP Programs are overseen by the TAG, the AZA Conservation, Management, and Welfare Sciences Department, and the APM Committee.
- Green SSP Programs are cooperatively managed Animal Programs for selected populations that achieve the highest level of formal management due to their future sustainability.
- Green SSP Programs are managed by an SSP Coordinator, a Vice Coordinator (recommended), and a Management Group (if desired).
- Green SSP Programs are those populations that retain a minimum of 90% gene diversity at 100 years or 10 generations, and include at least 50 individual animals held among at least three AZA member facilities.
- Green SSP Programs must work with their TAG to identify their role in zoos and aquariums, at least three goals, and essential actions to work towards each goal.
- Green SSP Programs must record their population in a current, published AZA Regional Studbook.
- Each Green SSP Program Breeding and Transfer Plan manages breeding in order to maintain a healthy and self-sustaining population that is both genetically diverse and demographically stable.
- The PMC, PMC Adjuncts, and approved SPMAG Advisors are available to officially advise Green SSP Programs in the preparation of breeding and transfer recommendations.
- Green SSP Program participants must abide by the AZA Full Participation Policy in SSPs (Appendix A) and, if needed, the AZA Animal Management Reconciliation Policy (Appendix B).
- SSP Programs may partner only with Sustainability Partners that are approved by the APM Committee (See Appendix G for Sustainability Partner Policy and application).

- For all SSP Programs that have non-AZA partners, the facility will be approved as a Sustainability Partner or they will be excluded from the SSP within 2 years or prior to the next Breeding and Transfer Plan (whichever comes first).
- Adherence to the AZA Code of Professional Ethics and the AZA Policy on Responsible Population Management is still required.

Yellow SSP Programs

- Yellow SSP Programs are overseen by the TAG, the AZA Conservation, Management, and Welfare Sciences Department and the APM Committee.
- Yellow SSP Programs are cooperative population management Animal Programs for selected populations that receive formal management.
- Yellow SSP Programs are managed by an SSP Coordinator, a Vice Coordinator (recommended), and a Management Group (if desired).
- Yellow SSP Programs are those populations that retain less than 90% gene diversity at 100 years or ten generations, but include at least 50 individual animals (within AZA facilities and Sustainability Partner facilities) held among at least three AZA member facilities.
- Yellow SSP Programs must work with their TAG to identify their role in zoos and aquariums, at least three goals and essential actions to work towards each goal.
- Yellow SSPs must record their population in a current, published AZA Regional Studbook.
- Each Yellow SSP Program Breeding and Transfer Plan manages breeding in order to maintain as healthy and self-sustaining of a population as possible that is both genetically diverse and demographically stable.
- The PMC, PMC Adjuncts, and approved SPMAG Advisors are available to officially advise Yellow SSP Programs in the preparation of breeding and transfer recommendations.
- Although cooperation among AZA member facilities is strongly encouraged for the long-term benefit of the *ex situ* population, participation in Yellow SSP Programs is voluntary.
- SSP Programs may partner only with Sustainability Partners that are approved by the APM Committee (See Appendix G for Sustainability Partner Policy and application).
  - For all SSP Programs that have non-AZA partners, the facility will be approved as a Sustainability Partner or they will be excluded from the SSP within 2 years or prior to the next Breeding and Transfer Plan (whichever comes first).
  - Adherence to the AZA Code of Professional Ethics and the AZA Policy on Responsible Population Management is still required.
- When a Yellow SSP Program can retain 90% gene diversity it will be designated as a Green SSP Program.
  - Should a Yellow SSP Program change to a Green SSP Program, the draft SSP Breeding and Transfer Plan will be evaluated by the PMC Director, the APM Committee Chair, the APM Committee Vice Chair for SSPs and Studbooks, the APM Committee TAG Liaison, and the AZA Conservation, Management, and Welfare Sciences Senior VP. The evaluation will be based on criteria of Green SSP Programs, and the group will vote on the designation status. The SPMAG Chair will be consulted if additional assessment is needed.

Red SSP Programs

- Red SSP Programs are overseen by the TAG, the AZA Conservation, Management, and Welfare Sciences Department, and the APM Committee.
- Red SSP Programs are cooperative population management Animal Programs for selected populations that receive formal management.
- Red SSP Programs are managed by an SSP Coordinator, a Vice Coordinator (recommended), and a Management Group (if desired).
- Red SSP Programs are those populations that retain less than 90% gene diversity at 100 years or 10 generations, and include between 20 and 49 individual animals held among at least three AZA member facilities. Animal Programs managing species that are classified as Extinct in the Wild, Critically Endangered, or Endangered do not need to meet these criteria (e.g., a population

of 19 individuals, managed at only two facilities, but classified as Critically Endangered, would qualify as a Red SSP despite failing to meet SSP criteria).

- Red SSP Programs must work with their TAG to identify their role in zoos and aquariums, at least three goals, and essential actions to work towards each goal.
- Red SSP Programs must record their population in a current, published AZA Studbook.
- Each Red SSP Program Breeding and Transfer Plan manages breeding in order to maintain as healthy and self-sustaining of a population as possible that is both genetically diverse and demographically stable.
- The PMC, PMC Adjuncts, and approved SPMAG Advisors are available to assist Red SSP Programs.
- Although cooperation among AZA member facilities is strongly encouraged, participation in Red SSP Programs is voluntary.
- SSP Programs may partner only with Sustainability Partners that are approved by the APM Committee (See Appendix G for Sustainability Partner Policy and application).
    - For all SSP Programs that have non-AZA partners, the facility will be approved as a Sustainability Partner or they will be excluded from the SSP within 2 years or prior to the next Breeding and Transfer Plan (whichever comes first).
    - Adherence to the AZA Code of Professional Ethics and the AZA Policy on Responsible Population Management is still required.
- If a Red SSP Program population grows to 50 or more individual animals, then it will be designated as a Yellow SSP Program
- If a Red SSP Program can retain 90% gene diversity it will be designated as a Green SSP Program.

Candidate Programs

- Candidate Programs are overseen by the TAG, with no additional accountability requirements by the AZA Conservation, Management, and Welfare Sciences Department or the APM Committee.
- Candidate Programs are managed by a Candidate Program Leader.
- Candidate Programs are Animal Programs that the TAG hopes to grow to an SSP Program, and they are not considered AZA cooperatively managed Animal Programs at this time.
- Candidate Programs are those populations that have 19 or fewer individual animals and/or are held only at one or two AZA member facilities.
- Candidate Programs may also be populations which do not currently have a published AZA Regional Studbook.
- Candidate Programs must work with their TAG to identify their role in zoos and aquariums, at least three goals, and essential actions to work towards each goal.
- Once a Candidate Program Leader publishes an AZA Regional Studbook demonstrating that the population meets minimum SSP criteria it will be designated as an SSP. Candidate Program Leaders must take PM1 in order to publish an AZA Regional Studbook.
    - If the Candidate Program Leader has not taken PM1 prior to submitting an initial AZA Regional Studbook, the Candidate Program Leader must identify to the AZA Conservation, Management, and Welfare Sciences Department that they have a skilled mentor (one that has taken PM1) that guided them through the process, and present their Studbook for review by a PM1 instructor, a PMC staff member, a PMC Adjunct Population Biologist, or an AZA Conservation, Management, and Welfare Sciences staff member.
- Candidate Programs may work with private participants (organizations that are not AZA member facilities) without completing the APM Committee Sustainability Partner approval process. Adherence to the AZA Code of Professional Ethics and the AZA Policy on Responsible Population Management is still required.
- Candidate Programs should consider partners with the understanding that when they grow to an SSP Program, they must follow the Sustainability Partner Policy and application (see Appendix G).

Table 2. Animal Program Overview: Green SSP, Yellow SSP, Red SSP, and Candidate Program Management.

	Green SSP Program	Yellow SSP Program	Red SSP Program	Candidate Program
AZA Policies				
AZA Policy on Responsible Population Management	Required	Required	Required	Required
AZA Code of Professional Ethics	Required	Required	Required	Required
AZA Full Participation in SSP Program Policy	Required	Voluntary	Voluntary	NA
AZA Animal Management Reconciliation Policy	Required	Not Required	Not Required	NA
APM Committee Approval of Sustainability Partners	Required	Required	Required	Not Required
Sustainability Criteria				
Minimum population size (N)*	50	50	20	NA
Minimum number of participating AZA member facilities*	3	3	3	NA
Projected gene diversity (%GD) at 100 years or 10 generations	90.0% or above	Less than 90.0%	Less than 90.0%	NA
Cooperative Management				
TAG recommended Animal Program in RCP	Required	Required	Required	Required
AZA Regional Studbook	Required	Required	Required	Not Required^
Formal population planning by PMC, PMC Adjunct or SPMAG Advisor	Required	Required	Required	Not Required
Management Group	If Needed	If Needed	If Needed	If Needed
Accountability				
Develop three Program goals	Required	Required	Required	Required
AZA and APM Committee oversight	Yes	Yes	Yes	No
Breeding and Transfer Plan published at least every 3 years	Required	Required	Required	Not Required
AZA Regional Studbook published at least every 3 years	Required	Required	Required	Not Required
AZA Regional Studbook Keeper must take Population Management 1	Required	Required	Required	Recommended ^
Program Leader must take Population Management 2	Recommended	Recommended	Recommended	Recommended

*If a managed species is listed by IUCN or ESA as Extinct in the Wild, Critically Endangered, or Endangered, there will be no minimum number of participating facilities, nor minimum population size requirements, in order to qualify for management at the SSP level. In these cases, SSP status (Green, Yellow, Red) will be determined based upon population size and projected gene diversity at 100 years or 10 generations.

^For a Candidate Program to upgrade to management at the SSP level, an AZA Regional Studbook must be published. The Candidate Program Leader, therefore, must complete AZA's Population Management I in order to publish an official AZA Regional Studbook.

Animals Declared Out of the Managed Population

Some animals in the managed SSP population, due to their age, reproductive status, or other demographic or genetic characteristics, may be deemed out of the managed population.

- The SSP Program must document all decisions to designate an individual animal as out of the managed SSP population. SSP Coordinators should communicate with facilities housing any animals that are designated to assure mutual understanding.

- In some cases animals may be sent out of the SSP population to another region in order to facilitate global population goals. These animals may not necessarily be out of the SSP, but individuals should be selected so that any negative impact on the SSP is minimized.
- Please refer to the AZA Policy on Responsible Population Management before transferring any individuals that are designated as out of the SSP population to any non-AZA facilities.

Government Owned Species

The AZA Institutional Data Management Scientific Advisory Group (IDMAG) Government Ownership Working Group (IDMAG/GOWG) maintains a list of government owned species and develops record keeping protocols for many of these species. When managing government owned species, AZA Studbook Keepers and SSP Coordinators should be aware of record keeping protocols and loan agreements that may affect studbook record keeping or breeding and transfer recommendations in order to maintain data quality and assure legal compliance. It is suggested that documentation such as loan agreements or Memorandums of Understanding be obtained for all appropriate specimens. The GOWG is available to work with Animal Programs lacking record keeping protocols to develop them.

Any questions with regard to existing government-owned species, record keeping protocols, or general data management issues may be directed to the IDMAG/GOWG or IDMAG Chairs. Contact information and relevant documents are found on the AZA website (https://www.aza.org/institutional-data-management-scientific-advisory-group).

Assessment

SSP Programs should regularly assess the status and performance of its Officers, Management Group members, IRs, ILs, and other SSP Program participants within its purview,

Management Group Assessment

- SSP Coordinators should regularly communicate with their Officers, Management Group, Advisors, IRs, ILs, and the TAG regarding accountability deadlines, SSP Program management changes, policy guidelines, publications, population planning, etc. The Management Group may remove Management Group members if they do not adequately and/or appropriately perform their duties. The AZA Conservation, Management, and Welfare Sciences Department, the APM Committee Vice Chair of TAGs, and the TAG Chair are available to provide assistance with SSP Program participant performance issues.
- TAG Chairs with current, approved RCPs have the authority to remove SSP Coordinators and AZA Regional Studbook Keepers who do not fulfill their Animal Program responsibilities. Any such action should be reported to the APM Committee Vice Chair of TAGs and the AZA Conservation, Management, and Welfare Sciences Department.

Institutional Assessment

- The SSP Program should assess the status and performance of the facilities participating in its SSP Program.
- The SSP Program should track IR and IL responses or lack of responses to all information requests, and include this information in an appendix in their Breeding and Transfer Plan
- The AZA Policy for Full Participation in the SSP Program is required of all AZA member facilities caring for species designated as Green SSP Programs (Appendix A). Full participation is strongly encouraged for all AZA member facilities caring for species designated as Yellow and Red SSP Programs.
- TAG Chairs are responsible for arbitrating any full participation issues brought to their attention by their Green SSP Programs in effort to reach a mutually agreeable resolution.
- The SSP Coordinator should discuss any potential issues with the TAG Chair, who will then discuss with the APM Committee Vice Chair of TAGs, their APM Committee Liaison, and/or a representative from the AZA Conservation, Management, and Welfare Sciences Department.
- If a resolution cannot be obtained through this method, either party or the APM Committee may initiate AZA's Animal Management Reconciliation Process Policy (Appendix B).

Cooperating with Other Zoo and Aquarium Regional Associations

- It is important to cooperate with WAZA and other regional zoo and aquarium associations (i.e., the European Association of Zoos and Aquaria (EAZA), the Canadian Association of Zoos and Aquariums (CAZA), the Zoo and Aquarium Association (ZAA in Australasia), etc.) as Animal Programs strive toward sustainability.
- SSP Programs must work closely with their TAG as they pursue international relationships with these other regional zoo and aquarium associations.
- For some Animal Program populations, management at the regional level (solely within one regional association, e.g., AZA) may be sufficient to achieve the Animal Program's goals.
- The desired population size for maintaining optimal %GD for other Animal Programs may be greater than the current carrying capacity (maximum available space) within one regional association and cooperation with multiple regional associations may be necessary.
- Program Leaders are encouraged to consistently communicate with their regional counterparts as needed, and assure that the TAG's members, APM Committee Liaison, and the AZA Conservation, Management, and Welfare Sciences Department are kept informed about such discussions.
- The PMC is available to assist with questions relating to multi-regional population management (e.g., assessing genetic and demographic status regional populations, discussing potential value of global management, combining databases, selecting animals for transfer between regions, etc.).
- The AZA Conservation, Management, and Welfare Sciences Department and the TAG's APM Committee Liaison are available to assist Program Leaders in developing these relationships, if necessary.

Global Species Management Plans

The AZA and other WAZA member regional zoological associations have collectively identified addressing the sustainability of animal populations as a top priority. Thus, we seek to maximize the collective impact of our efforts in building the long-term sustainability of wildlife populations by working together in a manner that builds upon, respects, and optimizes existing regional processes and furthers science-based, inter-regional collaboration. For some Animal Programs forming an official WAZA ***Global Species Management Plan (GSMP)*** may be appropriate. The WAZA Committee for Population Management (CPM) is established to advance these relationships and collaborations in professionally managing species in zoos and aquariums globally. One way in which the CPM does this is through coordinating, administering, and overseeing GSMPs.

- When population goals cannot be met within a single regional association, global management may be an ideal method for increasing sustainability.
- Establishing a GSMP provides an opportunity to combine several regional populations, thus improving the genetic and demographic management potential by increasing the population's size, carrying capacity, and other resources. For certain populations, these additional resources may markedly increase their long-term management success and sustainability.
- Once approved by the CPM, a GSMP formalizes a series of clear goals and agreements to which the GSMP partners agree or aspire to achieve, with an underlying goal of increasing the long-term sustainability of zoo and aquarium populations.
- Cooperation in a GSMP may range from a series of aspirations to a formal Memorandum of Understanding on specific goals and commitments. The partners (e.g., the regional associations) determine the appropriate level (e.g., individual, regional) at which to manage the population, as well as define the scope and flexibility of the GSMP.

The WAZA regional associations continue to advance best practices aimed at increasing effective communications for building population sustainability. Over the past few years a small number of pilot GSMPs have served as guides for developing a common framework for defining the mechanisms and management of GSMPs. AZA has made a commitment to take a strong leadership role for pilot and future GSMPs, and will be deeply involved in the development of the GSMP Program and working integrally with all partners.

Establishing a GSMP

- Please review the WAZA GSMP Handbook, which may be obtained from the AZA Conservation, Management, and Welfare Sciences Department.
- An international studbook database is generally required for a GSMP. If an ISB for the species does not already exist, regional databases may be used to assist in completing the GSMP application but the PMC should be consulted to determine how to combine regional databases if global population management is the desired goal.
- Working with all partnering zoological regions, the zoological region that will be leading the GSMP will develop the GSMP application.
- Once the application is complete, it should be submitted the regional association office (e.g., the AZA Conservation, Management, and Welfare Sciences department) for review.
- The regional association office will send the completed application to the proposed partner regional associations for review and approval.
- Once all proposed partner regional associations have endorsed the application, the leading regional association will submit the application to WAZA's CPM for review and approval.

Sustainable Populations through Responsible Partnerships

AZA Animal Programs focus on select species through cooperative management of small populations at AZA-accredited zoos and aquariums and Certified Related Facilities (CRFs). These facilities undergo a thorough accreditation review process that includes the submission of an extensive application as well as an intensive, on-site inspection by a team of experts to assure the highest standards of animal care and management are met. Additionally, the facilities have access to members-only resources through the AZA Population Management Center, AZA Reproductive Management Center and the AZA office.

AZA Animal Programs can benefit from responsible partnerships with individuals, facilities, or organizations outside of AZA in the form of expertise, space, and other various resources. With a goal of creating genetically and demographically sustainable populations of animals that experience excellent welfare, AZA Animal Programs may explore such partnerships when they:

- Benefit individual animal(s) and/or the population as a whole through the goals of an AZA Animal Program
- Support AZA's mission of high quality animal care and welfare
- Recognize the contributions of like-minded entities in assuring a future for animals in expert care

See Appendix G for the Sustainability Partner Policy and application.

Sustainability Partners

The definition of a Sustainability Partner in an AZA Animal Program is an organization that regularly exchanges animals with AZA-accredited facilities and Certified Related Facilities, typically as part of the SSP Breeding and Transfer Plan or other SSP Program management process.

- A Sustainability Partner's species/animal(s) is regularly included in the SSP Breeding and Transfer Plan.
- Recommendations are made for individuals of that species in the Sustainability Partner's collection through the SSP Breeding and Transfer Plan process. This would include documented interim SSP Program recommendations.

If an AZA Animal Program (e.g., TAG, SSP) determines that an animal population may benefit from collaboration with a Sustainability Partner that can provide high quality genetic, demographic, conservation, husbandry, population management, and/or animal welfare benefits to an SSP Program, they must consider the information provided below. Sustainability Partners in any AZA Animal Program must adhere to AZA's Policy on Responsible Population Management, SSP Full Participation Policy, the AZA Code of Professional Ethics, and Accreditation Standards related to animal care and welfare regardless of Animal Program designation. SSP Programs may partner only with Sustainability Partners that are approved by the APM Committee (See Appendix G for the Sustainability Partner Policy and application). For SSP Programs that have non-AZA partners, the individual/organization

must be approved as a Sustainability Partner or they will be excluded from the SSP population. All SSP Programs that include non-AZA partners in their SSP Program population must assess these partners and should aim to submit any Sustainability Partner applications to the APM Committee six months prior to their next SSP Breeding and Transfer Planning meeting.

As stated in the *Guidelines for Data Entry and Maintenance of North American Regional Studbooks*: "Any and all facilities that can be verified as holding or having held specimens should be included in the studbook. Inclusion of data from a facility should not be contingent on whether it is a member or affiliate of AZA; if a facility provides data to the studbook keeper, it should be included in the studbook, provided it can be verified."

However, the inclusion of animals in a studbook does not indicate or imply that the facility is a Sustainability Partner in the Animal Program. Only AZA facilities and approved Sustainability Partners may be included in a Breeding and Transfer Plan. The AZA Population Management Center (PMC) may assist with evaluation of animals in the population regardless of their location, however discussion within the SSP and its associated TAG must occur to determine whether a facility/person needs to apply to be a Sustainability Partner, and thus continue participating in the SSP Program.

Sustainability Partners *are not considered accredited or certified*. Like AZA accreditations and certifications, approvals for a Sustainability Partner's continued participation in an SSP Program must be renewed every five years based on a review of the benefits to the Animal Program. Animal Program Leaders must evaluate existing partnerships (with the help of their associated TAG) when beginning a new SSP Program or taking over an established SSP Program, especially before the Breeding and Transfer Plan process. See Appendix H for the *Guidelines for Assessing Sustainability Partners in Species Survival Plan® Programs*.

Application Review and Approval

- The SSP Coordinator is responsible for compiling all Sustainability Partner application materials and sending the application as a complete package to the AZA Conservation, Management, and Welfare Sciences Department, with a copy to their TAG Chair.
- The AZA Conservation, Management, and Welfare Sciences Department will work with the APM Committee Vice Chair for Partnerships to review all application materials for completeness.
- Should any items be missing from the application or should the letter of justification from the SSP need adjustments, the APM Committee Vice Chair for Partnerships will return the incomplete application to the SSP Coordinator and specify the missing required information.
- The SSP Coordinator must submit requested materials or communicate with the APM Committee Vice Chair for Partnerships within 2 weeks of receiving the returned application. If the APM Committee Vice Chair for Partnerships does not hear from the SSP Coordinator within 2 weeks, the Sustainability Partner application will be considered inactive until the SSP Coordinator resubmits it.
- The APM Committee will hold monthly conference calls to review any complete Sustainability Partner applications that were submitted in the previous month.
- The AZA Conservation, Management, and Welfare Sciences Department will notify the SSP Coordinator, TAG Chair, and the applicant of the outcome as soon as possible.

Approved Partnerships

Once identified/approved, the Sustainability Partner must:

- Agree to adhere to AZA's Code of Professional Ethics, SSP Full Participation Policy, AZA Policy on Responsible Population Management, and Accreditation Standards related to animal care and welfare.
- Appoint an Institutional Liaison (IL) and Institutional Representative (IR) to serve as the primary point of contact(s) for SSP communications. Contact the AZA Conservation, Management, and Welfare Sciences Department with the IL and IR names and contact info for the Sustainability Partner.
- Not display the AZA logo or SSP logo.
- Upon request, agree to allow the sponsoring AZA-accredited facility, staff, Board, APM Committee, TAG, and/or SSP representatives to visit the applicant and view their facility to assure

adherence to AZA policies and animal care and welfare practices. Such visitors will provide feedback to the SSP, TAG, and the APM Committee.
- Submit a new, complete application for Sustainability Partner before the end of the five-year approval period in order to continue participation in the SSP Program.

Loss of Approval Status

A Sustainability Partner may have its approved status revoked by APM Committee if it fails to meet any of the Sustainability Partner responsibilities identified above and in the application.

If APM Committee deems it appropriate, the SSP Program may work with a Sustainability Partner that loses its approved status for up to two years to help manage the population, facilitate transfer of animals owned by AZA zoos and aquariums and, when possible, mentor re-approval of Sustainability Partner status. In such instances, the Sustainability Partner will not be an active participant in the SSP Program, however still may be considered during the planning processes. During this time, the SSP Program will not move SSP Program animals to the former Sustainability Partner facility.

For reinstatement as a Sustainability Partner, the potential Sustainability Partner's benefit to the SSP population's sustainability and adherence to AZA's Code of Professional Ethics, Policy on Responsible Population Management, and Animal Care and Welfare Standards must be reassessed.

Animal Program Roles, Goals, and Essential Actions

Animal Program Role

Although many species will qualify for more than one of the defined purposes below, the SSP Coordinator should work with the TAG to identify the primary role in zoos and aquariums for their managed population. The primary role for each SSP Program must be included in the Animal Program Roles, Goals, and Actions Table in the TAG's RCP and Annual Report (See TAG Handbook for more information). SSP Program roles may be selected from the following list, or develop alternate descriptors.

- **Conservation Action** – the taxon is under immediate threat and action, or reintroduction is underway.
- **Assurance Population** – the taxon is threatened or declining in some fashion and the managed population is serving as a genetic and demographic reservoir for the future, if required.
- **Education/Exhibit Needs** – the taxon is used for educational purposes and inspires guests to care for wildlife.
- **Research** – the taxon is in need of greater understanding and the managed population serves as a research population or a population that is just being founded within zoos and aquariums.

Setting Goals for your SSP Program

The SSP Coordinator should work with the TAG to set at least three goals, with corresponding essential actions, for their SSP Program. Working closely with the TAG, the SSP should prioritize the top three goals and outline the essential actions to meet these goals. The top three goals and essential actions must be included in the Animal Program Roles, Goals, and Actions Table in the TAG's RCP and Annual Report (See TAG Handbook for more information).

The first goal for each SSP Program should relate to the primary purpose of cooperatively managing the species within the AZA community. The second and third goals may be focused on items unique to the taxon and/or the managed population. Examples of additional goals might include, but are not limited to, increasing an SSP Program's current projected gene diversity to X% GD, increasing the population size to 50 individuals, or increasing the number of spaces for the population.

The essential actions for each goal must be specific actions or tasks that need to be achieved to accomplish the goals. Examples may include increasing the number of breeding and offspring spaces available by a certain number, increasing the number of breeding pairs, advancing artificial insemination techniques, obtaining new importation permits, compiling or researching effective husbandry protocols, or working with non-AZA partners to increase the number of founders in the population. Essential Actions should be articulated according to SMAART criteria as being: specific, measurable, achievable, relevant, and time-bound.

A SMAART goal explains a behavior using the following components:

Specific	A SMAART goal identifies a specific action or event that will take place.
Measurable	The description of a SMAART goal will allow you to determine your progress towards completion, and let you know when you are finished.
Achievable	A SMAART goal should be achievable given available resources.
Agreed-upon	A SMAART goal should encourage collaboration and cooperative ownership of plans.
Realistic	A SMAART goal should require you to stretch some beyond your normal routine and regular abilities, but allow for likely success based on your skills and the time available.
Time	A SMAART goal should state the specific time period in which it will be accomplished.

Chapter 4. Breeding and Transfer Plans

Overview

The goal of a Breeding and Transfer Plan is to maintain a healthy, genetically diverse, and demographically stable *ex situ* population of a particular species through cooperative management strategies among AZA member facilities, In order to assure the production of an effective Breeding and Transfer Plan, the SSP Coordinator must work with the PMC, a PMC Adjunct, or an approved SPMAG Advisor to summarize the current demographic and genetic status of the population, describe the SSP management designation, and recommend breeding pairs and transfers.

SSP Program Population Advisors

SSP Program advisors fall into three APM Committee approved categories:

- PMC Advisors are employed by and working at the AZA Population Management Center at Lincoln Park Zoo.
- PMC Adjuncts are trained by the PMC, and are employed by and working at an AZA member facility. An SSP Program is only approved or assigned to have their population planned by a PMC Adjunct if the SSP Coordinator or the AZA Regional Studbook Keeper is employed by the same AZA member facility that houses the PMC Adjunct.
- PMC Regional Adjuncts are trained by the PMC, and are employed by and working for a consortium of AZA members. An SSP Program is only approved or assigned to have their population planned by a PMC Adjunct if the SSP Coordinator or the AZA Regional Studbook Keeper is employed within the consortium of zoos and aquariums (e.g., the California Association of Zoos and Aquariums).
- Approved SPMAG Advisors are current member of the SPMAG who have historically planned particular populations. An SSP Program must receive APM Committee approval in order to have their population planned by a SPMAG Advisor.

The AZA PMC Director is responsible for assigning and reviewing AZA Animal Program advising assignments for all Population Biologists. These assignments always consider:

a. SSP Coordinator's hosting facility and if this is an Adjunct's supporting facility

b. Studbook Keeper's hosting facility and if this is an Adjunct's supporting facility

c. Adjunct existing agreements with their supporting facilities

d. Population Biologist's experience and history with the SSP and TAG

e. Population Biologist's experience and history with the taxonomic group

f. Population Biologist's experience with any other specific factors for the SSP (e.g., reintroduction species, conducting molecular genetics research)

g. Population Biologist's time availability within their schedule as well as during the timeframe in which the SSP needs advising

If a Program Leader wants to change Population Biologists, the Program Leader is encouraged to first have a one-on-one conversation with the Population Biologist and if needed, reach out to the AZA PMC Director for additional discussion. These requests will be carefully considered, mediation used if necessary, and additionally include all the same variables listed above.

Preparing for the PMC Planning Meeting

Scheduling the PMC Meeting

- All Green, Yellow, and Red SSPs will be assigned a planning date. Candidate species are not prioritized at this time but should contact the PMC if their status changes (e.g., if an initial AZA Regional Studbook is published, or if an updated published AZA Regional Studbook indicates that the population size or number of participating facilities have increased above minimum SSP criteria).

- The PMC may be able to provide informal assistance for Animal Programs in need of advice outside of scheduled formal planning dates. See "interim planning" below for more information.
- Animal Programs that have not scheduled planning meetings but need population management assistance should contact the PMC Planning Coordinator (PMC@lpzoo.org) to schedule a future planning date. Populations that have never been planned should contact the PMC as soon as possible.
- The SSP Coordinator must notify all people involved (i.e., SSP Vice Coordinator, AZA Regional Studbook Keeper, Management Group members, and IRs) of the planning meeting date and their responsibilities prior to meeting with the PMC.
- As many IRs as possible should participate in the planning process to increase institutional support and the effectiveness of the Breeding and Transfer Plan. SSP Coordinators are encouraged to reach out to their IRs and invite them to the planning meetings.
- PMC planning meetings may be conducted electronically (conference calls, internet conferencing) or in person (typically at Lincoln Park Zoo, the AZA Annual Conference or the AZA Mid-Year Meeting). It is preferred that first-time planning meetings be held in person.

Consulting with the AZA Reproductive Management Center (RMC)

The AZA RMC, hosted by the Saint Louis Zoo, provides information on safety, efficacy and reversibility of contraceptive products to the AZA community to help zoo professionals make informed decisions on how to manage their animal collections. Contraception is an essential, proven, and humane tool for reproductive management while still allowing individuals to live in natural social and family groups.

- The RMC is an integral part of AZA Animal Program management practices and is fundamental to managed breeding and population sustainability for individuals that are, or have ever been, contracepted.
- To assist AZA's Animal Programs the RMC maintains a database which monitors contraceptive records in one centralized location in order to facilitate meta-analyses and disseminate up-to-date recommendations.
- The SSP Coordinator should communicate with the RMC regarding the animals in their population prior to each formal planning meeting to review and update their status, as necessary.
- The RMC may provide written recommendations to be included in the Breeding and Transfer Plan as an appendix, if needed.
- An Advisor from the RMC may attend or conference into the SSP Program's planning meeting if relevant for the population.
- Communication between the SSP Coordinator and the RMC need not be limited to planning meetings, but can occur throughout the year as questions arise or new data become available.

The PMC Planning Meeting

Materials Required Prior to the PMC Planning Meeting

There are five types of materials that the SSP Program must compile and submit to the PMC in preparation for a scheduled planning meeting (See Appendix F). The PMC will work with the SSP Program to set deadlines by which each of these materials must be received by the PMC so that they can prepare for the planning meeting (for more guidance, visit: http://www.lpzoo.org/population-management-center).

These materials include:

1. An **AZA Regional Studbook database** for the SSP Program species containing all the currently living animals. The PMC Planning Coordinator will run data validation software or will validate the submitted data to assist AZA Regional Studbook Keepers in preparing for the meeting. Studbook Keepers should assess the validation provided by the Planning Coordinator and make updates to the Studbook accordingly.
2. A **list of institutional wants and needs** from all current or future holders of that SSP Program's species, including information on exhibits, holding facilities, breeding capabilities, or social groups, if applicable. Information regarding specific requests for breeding, holding, placing, or receiving animals should be included. IDs of animals should be included when relevant. The AZA and the APM Committee recommend utilizing PMCTrack to send and collect standardized,

customizable wants/needs surveys to IRs; see section regarding PMCTrack below for more information.

3. A **list of animals to be excluded** from the breeding population and the reason (e.g., medical, behavioral, age/post-reproductive, etc.).
4. An **up-to date contact list for IRs** for distribution of the Draft and Final Breeding and Transfer Plans.
5. A **list of potential pedigree assumptions** for those animals with unknown or MULT parentage (if applicable).

If the SSP Coordinator fails to meet the deadlines mutually agreed upon with the PMC, the PMC may cancel the meeting and reschedule for a time when the SSP Program is better prepared. If deadlines are not met, and the meeting cancelled, the PMC cannot guarantee a new planning date. The PMC will contact the TAG Chair and inform them of the situation. Repeated lack of preparedness may result in an SSP Coordinator's removal from their position by the TAG Steering Committee.

PMCTrack

PMCTrack (https://www.PMCTrack.org) is a web-based resource for SSP Coordinators who are preparing to plan with the PMC. SSP Coordinators should use PMCTrack to:

- See where the SSP Program is in the PMC planning process, as well as viewing information about their program's management history
- View recommendation outcomes, which are data on whether previous recommendations to hold, transfer, breed, or not breed occurred as requested in past breeding and transfer plans; these data can be used to better understand SSP management challenges and evaluate current breeding situations before planning
- Use standardized surveys to collect important institutional information before a planning meeting. Wants/needs surveys are sent to all IRs to gather information each holding facility's needs for the species; outcomes surveys are sent to IRs if a recommendation from the last breeding and transfer plan is not fulfilled according to the studbook, and solicit reasons that that recommendation was not completed as requested in the plan. PMCTrack includes automated reminder emails to encourage survey participation by IRs.

PMCTrack will help SSP Coordinators prepare for planning with the PMC, communicate with IRs, respond to problems completing plan recommendations, and will help to improve the planning and management processes over time for AZA Animal Programs. SSP Coordinators can log-in to PMCTrack at any time, but will most frequently utilize it when preparing for planning with the PMC.

For more information, contact PMCTrack@lpzoo.org.

Key Elements Produced in the Breeding and Transfer Plan

The PMC will work with the SSP Coordinator, other Animal Program participants, and their RMC Advisor (if necessary), throughout the course of the planning meeting to produce a Draft Breeding and Transfer Plan. This Draft will be AZA branded, structured to meet a standardized format and will include:

- A cover page with essential information such as the species common and scientific names, SSP Coordinator name and contact information, Studbook Keeper name and contact information, picture of the SSP species, date through which the data are current, and name of the PMC Advisor.
- A Table of Contents.
- A list of participating facilities with their corresponding IRs and Species360 mnemonics. This section must identify if the participant is an AZA-accredited facility, CRF, or approved Sustainability Partner.
- A genetic and demographic status summary of the population. Specific items to be included will be determined by the PMC Advisor but should include any assumptions made for the analyses.
- Animal-By-Animal Recommendations. The Breeding and Transfer Plan must include a list of the recommended actions for each individual animal or groups of animals in the population. These recommendations will consider genetic and demographic factors, social, nutritional, behavioral,

and medical concerns, practical day-to-day animal management considerations, and the wants and needs of the facilities.
- A general description of the SSP Program, identification of the SSP Program Officers, Management Group members, and Advisors, and a summary of the SSP Program's priorities and activities.

Reviewing the Draft Breeding and Transfer Plan

Upon completion of the planning meeting, the PMC will post the Draft Breeding and Transfer Plan on the AZA Animal Program webpage for 30 days and email the draft to IRs. This process is specifically designed to assure that all facility IRs view, fully comprehend, and provide feedback on the Draft Breeding and Transfer Plan recommendations before they are finalized. The IR is expected to communicate any recommendations effecting their facility's population to their IL and Director, and is required to provide feedback and address questions or concerns about these recommendations to the SSP Coordinator during the comment period. Lack of feedback from an IR will be interpreted as the facility's full acceptance and agreement to the recommendations presented in the Draft Breeding and Transfer Plan.

The following steps are vital to the review process:
- An automated email will be sent to all SSP Program designated IRs to inform them that the Draft Breeding and Transfer Plan is available for review.
- The IRs will have 30 days to provide feedback and address questions or concerns about the recommendations made in the Breeding and Transfer Plan with the SSP Coordinator.
- The SSP Coordinator must respond to institutional comments and address them promptly.
- The SSP Coordinator may wish to notify the IL if an IR does not respond to the Draft Breeding and Transfer Plan within three weeks. If the IR or IL does not respond within the next seven days, the SSP Coordinator may wish to notify the institutional Director.

IR and IL Responsibilities

- SSP Coordinators are responsible for tracking and reporting the response (or lack of response) and feedback provided by the IRs for their Breeding and Transfer Plans.
- If a non-responsive pattern becomes apparent with an IR, the SSP Coordinator should inform the IL of the potential problem. If it is deemed that the IR is not fulfilling his/her obligations, it is the IL's responsibility to contact the IR and inquire about the status of the delinquent duty. The IL will work with the IR and the SSP Coordinator until responsibilities are met.
- If the IL fails to properly oversee the completion of the SSP Program responsibilities of the IRs at his/her facility, the SSP Coordinator will likely contact the TAG, APM Committee, and the AZA Conservation, Management, and Welfare Sciences Department to formally register a complaint.
- Failure to meet these obligations will likely result in the recommendation of removal of the IR by the APM Committee.

Publication of the Final Breeding and Transfer Plan

Upon completion of the Draft Breeding and Transfer Plan 30 day comment period, the AZA Conservation, Management, and Welfare Sciences Department will publish the Final Breeding and Transfer Plan electronically on the AZA Animal Program webpage. A formal announcement of this publication will be distributed via an automated email that will be sent to all SSP Program designated IRs and in the publication month's Animal Programs Update. SSP Coordinators must assure that the following individuals/entities are notified of Final Breeding and Transfer Plan publication:
- All facilities holding the SSP Program species and participating in the SSP Program
- The SSP Vice Chair
- The AZA Regional Studbook Keeper
- The AZA TAG Chair
- The AZA Conservation, Management, and Welfare Sciences Department
- The United States Fish and Wildlife Service, if applicable.
- The IUCN Specialist Group Chair, if applicable.

- Invested individuals who do not have access to the member's only section of the AZA website (i.e., IUCN specialist group chairs, Program Leaders from other regional zoological associations) of the publication.

Interim Population Recommendations

Although Breeding and Transfer Plans are the official method of recommending and documenting population management actions, many populations will need assistance between plans due to changes in the population or institutional needs. The PMC, a PMC Adjunct, or an approved SPMAG Advisor can usually provide informal unscheduled assistance to Program Leaders for such interim planning needs.

A MateRx is one tool provided by the PMC, a PMC Adjunct, or an approved SPMAG Advisor that Program Leaders can use to assist them when making recommendations for their population between planning meetings.

- A MateRx is a matrix of all potential breeding pairs in a population which integrates four genetic factors to produce a single numeric Mate Suitability Index (MSI) for each male/female pair.
  - The MSI is calculated from considering the potential breeding pairs' mean kinship values, the difference in male and female mean kinship, the inbreeding coefficient of the potential offspring produced, and the amount of unknown pedigree in the potential pair. A MateRx allows users to simplify the decisions about which pairs should be bred by condensing all that we know about the genetics of a pair into a single number.
- Requests for a MateRx can be made to the PMC, a PMC Adjunct, or an approved SPMAG Advisor.
- Materials required for a MateRx include an updated studbook, a list of animals to be excluded from the breeding pool, and new information on pedigree assumptions.
- In some cases, a MateRx cannot be produced due to species biology or data quality (e.g., pedigree unknownness, population size, etc.). However, even in these cases the PMC highly encourages Programs to contact them for alternative assistance (PMC@lpzoo.org).
- Program Leaders are encouraged to record all recommendations made between their formal Breeding and Transfer Plans.

Chapter 5. SSP Sustainability Reports and Search Portal

A grant awarded to AZA by the Institute for Museum and Library Services (IMLS) allowed AZA to customize their database and merge existing data with new data from Animal Program documents and Program Leaders. The AZA community is now able to identify patterns in population challenges and to strategically address population needs. The SSP Sustainability Reports and Search Portal were launched to the AZA membership in May 2016 and are becoming incorporated into the daily management of AZA SSP Programs. This collection planning tool has profound impacts on TAG recommendations and management decisions, and facilitating action towards increasing SSP population sustainability. The primary sustainability challenges identified by SSP Coordinators and population biologists will help facilitate AZA members in aligning their resources (e.g., space, experience with partnership and imports, multi-species exhibit opportunities, husbandry/research expertise) with the essential actions of SSP Programs. The information gleaned from these reports allows zoo and aquarium staff to take direct action in addressing population sustainability. See Chapter 8 for more details.

SSP Sustainability Reports

The SSP Sustainability Reports are automatically generated, 5-page reports that summarize husbandry practices, exhibit management, species appeal, educational opportunities, multi-species exhibit considerations, species biology, SSP population dynamics, management priorities, challenges to sustainability, and research needs. They also include the major challenges impeding each SSP's population sustainability and the goals and essential actions needed to address them. The report is a compilation of the SSP Coordinator's expertise and the current and projected population summaries from the SSP Breeding and Transfer Plan or PVA. The main areas of the reports are:

Page 1:

- Photos of the species
- Marketing phrase
- Species conservation status, SSP designation, geographic information, and biome
- Exhibit design and management
- Species appeal
- Messaging opportunities

Page 2:

- Multi-species exhibit opportunities
- Non-SSP species that could be substituted by the SSP species
- Species biology
- Offspring housing and reproduction

Page 3:

- Sustainability profile that includes population size, demographics, genetics, and images such as census graphs, age pyramids, and population projections

Page 4:

- Challenges to SSP population sustainability, with identified goals, actions, and needs
- Reproductive technologies available
- Additional research opportunities
- Additional notes on SSP management

Page 5:

- Acquisitions and transfers with information about imports, exports, and reintroductions
- Challenges to acquisitions and transfers
- Disclaimer that includes the date that the report was last updated

SSP Sustainability Reports Search Portal

The SSP Sustainability Reports Search Portal is an online tool for collection planners, Program Leaders, ILs, IRs, research scientists, and other zoo and aquarium staff. The searchable format allows collection planning users to perform searches that identify appropriate species for their collection planning criteria, while also directing resources and attention to managed species. This portal contains 25 search fields, including IUCN status, species appeal, special exhibit considerations, opportunities in multi-species exhibits, messaging opportunities, and research opportunities. The user can select any number of criteria that will return links to the individual SSP Sustainability Reports. The SSP Search Portal can help collection planners at AZA facilities to incorporate SSP species into their institutional collection plan, while facilitating alignment of their specific resources and expertise with SSP needs. SSP Coordinators may use their SSP Sustainability Reports to communicate the challenges impeding population sustainability and encourage the zoos and aquariums participating in the SSP to take an active role in overcoming these challenges. Research scientists can use the "Research Opportunities" field in the Online Portal to align their interests and expertise with critical SSP research needs.

Updating the SSP Sustainability Reports

Quantitative Data

Each month, the quantitative data (e.g., population numbers, gene diversity, participating facilities) from recently finalized SSP Breeding and Transfer Plans is downloaded from PMCTrack and the AZA Conservation, Management, and Welfare Sciences staff add that information to the SSP Sustainability Reports.

Qualitative Information

To assure that the SSP Sustainability Reports are as accurate as possible, SSP Coordinators are encouraged, at any time, to submit updates to their qualitative information (e.g., major challenges to their SSP population sustainability, progress in importations) to the AZA Conservation, Management, and Welfare Sciences Department.

At the very least, SSP Coordinators will be asked to review their reports during each of their SSP planning sessions and send any updates to their information at that time.

TAG Chairs are requested to review all of the SSP Sustainability Reports within their purview at least once per year to make sure that the information is current, accurate, and in line with the TAG's goals. The TAG Chair will be asked if they have conducted their reviews in their TAG Annual Report.

A disclaimer is located on the last page of the SSP Sustainability Reports. A date is included in the disclaimer to show when the report was last updated.

Chapter 6. SSP Program Administration

SSP Program Accountability

SSP Coordinators are accountable for submitting a Breeding and Transfer Plan at least every 3 years in accordance with the submission date listed on the front cover of the previous publication, in order to meet their SSP Program accountability requirements. If a GSMP breeding and transfer information is also produced, it must be submitted at the time of publication in accordance with WAZA accountability. In addition, SSP Programs should also track IR responses for required SSP Program objectives, including wants and needs data.

There are a few SSP Programs that, due to the species' natural history, may not require or benefit from a traditional Breeding and Transfer Plan every 3 years. These SSP Programs will be considered on a case by case basis by the AZA Conservation, Management, and Welfare Sciences Department, the TAG, and the SSP Program's Population Advisor.

Automated Accountability Emails

Automated deadline reminders are emailed as a courtesy to remind the SSP Coordinator, and other associated parties, of an upcoming deadline. Each email includes the appropriate instructions, relevant contact information, and links to the Deadline Information pages on the AZA website (http://www.aza.org/animal-program-deadlines/). These automated emails are administered as follows:

- One year prior and 6 months prior to the deadline – Sent to the SSP Coordinator, and copied to the associated TAG Chair and the AZA Conservation, Management, and Welfare Sciences Department.
- One month prior – Sent to the SSP Coordinator, and copied to the associated TAG Chair, Institutional Liaison, APM Committee Chair, APM Committee VC of SSPs and Studbooks, APM Committee Liaison, and the AZA Conservation, Management, and Welfare Sciences Department.
- Deadline reached - Sent to the SSP Coordinator, and copied to the associated TAG Chair, Institutional Liaison, APM Committee Chair, APM Committee VC of SSPs and Studbooks, APM Committee Liaison, and the AZA Conservation, Management, and Welfare Sciences Department.
- Two weeks past- Sent to the SSP Coordinator, and copied to the associated TAG Chair, Institutional Liaison, APM Committee Chair, APM Committee VC of SSPs and Studbooks, APM Committee Liaison, and the AZA Conservation, Management, and Welfare Sciences Department.

Extension Requests

Prior to the accountability deadline date:

- The SSP Coordinator may request an extension to complete their Breeding and Transfer Plan prior to the due date by contacting their TAG Chair, if the TAG has a current, approved RCP.
    - If deemed appropriate, the TAG Chair must contact the AZA Conservation, Management, and Welfare Sciences Department with the approved new deadline.
- If the TAG does not have a current, approved RCP, the SSP Coordinator must also contact the APM Committee Vice Chair for SSPs & Studbooks to request an extension.
    - If deemed appropriate, the TAG Chair must contact the APM Committee Vice Chair and the AZA Conservation, Management, and Welfare Sciences Department with the proposed new deadline.
- The APM Committee Vice Chair will work with the APM Committee to approve/not approve the extension request and communicate the decision to the TAG Chair and SSP Coordinator.

After the accountability deadline has passed:

- If the Breeding and Transfer Plan extension request was not made prior to the Breeding and Transfer Plan deadline but the SSP Coordinator wishes to maintain their position, the IL or Director of the SSP Coordinator's facility must contact the APM Committee Vice Chair for SSPs

& Studbooks within 2 weeks of the missed deadline to discuss the reason for the missed deadline, and request a new deadline.
- The APM Committee will vote to determine if the reason for the missed deadline for the completion of the Breeding and Transfer Plan is valid, and if so, a new deadline will be set.
- If the APM Committee determines that the reason for the missed deadline is non-valid, the SSP Coordinator will likely be removed from their position.
- If the SSP Coordinator is removed, the position vacancy will be advertised on the AZA website and in the ***monthly Animal Programs Update***.

Voting

- All members of the Management Group, if one exists, are required to vote on issues and in elections; votes are determined by majority
- The Secretary will record the votes and submit the voting record to the SSP Coordinator.
- The SSP Coordinator will alert the members of the SSP, the candidates (if applicable), and the TAG of the outcome of all votes.
- Failure to meet these obligations may result in the removal of the Management Group member by the SSP Coordinator or the APM Committee.

Election Processes

SSP Coordinators are elected by their TAG's Steering Committee (if the TAG has a current, approved RCP) or by the APM Committee (if the TAG does not have a current, approved RCP or lacks a TAG Chair). SSP Officers and Management Group members are elected from the SSP Program's IRs. There are no SSP mandated term limits for SSP Officers or Management Group members. SSP Programs may determine whether to impose term limits on their Management Group members. All facilities are able to participate in the SSP Program through their IRs.

SSP Coordinator vacancies must be announced in the monthly Animal Programs Update: (https://www.aza.org/animal-programs-monthly-update), and are available on the Current Program Leader Vacancy page if they are not filled after the required 30-day posting: (http://www.aza.org/Program-Leader-Vacancies/).

SSP Coordinator

- Individuals interested in becoming an SSP Coordinator should consult the appropriate TAG Chair. If no TAG Chair exists, or the TAG does not have a current, approved RCP, interested individuals may consult the AZA Conservation, Management, and Welfare Sciences Department for advice on becoming an SSP Coordinator.
- Applicants for the position of SSP Coordinator must submit an SSP Coordinator Application (Appendix C), a Statement of Individual Commitment and a Statement of Institutional Support (Appendix C).
- All SSP Coordinator applications should be submitted directly to the TAG Chair, if the TAG has a current, approved RCP. If the TAG's RCP has not been approved or is not current, or there is no TAG Chair, SSP Coordinator applications should be submitted to the AZA Conservation, Management, and Welfare Sciences Department.
- TAG Chair contact information can be found on the TAG's Animal Program page, or on the AZA website: https://www.aza.org/contact-information.

Officers

- The SSP Coordinator will distribute a call for interest to the SSP Management Group, or to the IRs if there is no Management Group, to obtain a list of nominees for vacant Officer positions (except that of SSP Coordinator).
- Nominees for Officer positions must submit a Statement of Individual Commitment (Appendix E) and a Statement of Institutional Support (Appendix E) to the SSP Secretary (or the SSP Coordinator if the SSP Program does not have a Secretary) who will distribute the application to the Management Group, or if there is no Management Group, to the IRs.
- Elections, using an open democratic process, will be held if more than one Management Group member, or IR, is interested in the same Officer position.

- The SSP Coordinator will communicate the new appointment decision to the applicant, the rest of the applicant pool, and the AZA Conservation, Management, and Welfare Sciences Department.

Management Group Members

- The SSP Coordinator will send a request for Management Group nominees to all of the SSP Program's IRs if the Officers determine that a Management Group is necessary.
- Elections, using an open democratic process, will be held if the number of interested IRs exceeds the number of Management Group positions available.
- The Management Group may fill a vacated position by either holding a new election or appointing the IR who received the highest number of votes among the nominees not selected in the previous election.
- An IL may be involved with the Management Group as a non-voting member if an IR at the same facility is in the Management Group. There may only be one vote per facility.

AZA Regional Studbook Keeper

- Ideally, the SSP Coordinator or SSP Vice Coordinator is also the AZA Regional Studbook Keeper for the SSP Program.
- Individuals interested in becoming an AZA Regional Studbook Keeper should communicate with the TAG Chair, or review published RCPs to determine priority species.
- If the TAG does not have a current, approved RCP, or there is no TAG Chair, interested individuals should consult the AZA Conservation, Management, and Welfare Sciences Department for advice on acquiring an AZA Regional Studbook for a taxon of interest.
- All AZA Regional Studbook Keeper applications (See AZA Regional Studbook Keeper Handbook) should be submitted directly to the TAG Chair if the TAG has a current, approved RCP. If the TAG does not have an approved RCP, or there is no TAG Chair, applications should be submitted to the AZA Conservation, Management, and Welfare Sciences Department.

Change in Employment or Institutional Status

Change in Facility

Officers

- If the SSP Coordinator is leaving a facility and wishes to maintain the SSP Coordinator role and the facility does not wish to relinquish the SSP Program, the Director (or IL) must contact the TAG Chair (or the APM Committee Vice Chair for TAGs if the TAG does not have a current, approved RCP or the TAG Chair position is vacant) within 30 days of the departure of the SSP Coordinator. The position must be advertised as a vacancy in the monthly Animal Programs Update for a minimum of 30 days and a new SSP Coordinator candidate from the facility must submit an application (Appendix C).
    - The TAG Steering Committee (or the APM Committee Vice Chair for SSPs & Studbooks if the TAG does not have a current, approved RCP or the TAG Chair position is vacant) will request an updated application from the current SSP Coordinator if s/he wishes to retain their position.
    - Additional applications will also be received from any interested candidates.
    - The TAG Steering Committee will review and vote on the candidates to select the one most qualified.
    - For purposes of continuity of SSP Program management, applicants from the current supporting facility will be given serious consideration.
    - Upon selection of an SSP Coordinator, the TAG Chair (or the APM Committee Vice Chair for SSPs & Studbooks if the TAG does not have a current, approved RCP or the TAG Chair position is vacant) will inform the applicants, the supporting facilities, and the AZA Conservation, Management, and Welfare Sciences Department of the final decision.
- Officers moving to a new facility do not automatically become that facility's IR; they must be designated by the new facility's IL.

- If the new facility's current IR is involved in the SSP Program in a voting capacity, the facility must determine which of the two will serve as the IR for, and which will no longer act as a voting member of, the SSP Program to assure each facility has only one voting member.
- The IR required to step down may be appointed as a non-voting Advisor at the SSP Program's discretion.
- Officers who move to a new AZA member facility must, within 90 days of departure from their original facility, submit a new Statement of Individual Commitment (Appendix E) and Statement of Institutional Support (Appendix E) to the TAG Chair.
- Officers must update their new contact information, including facility, phone, fax, and email to the TAG Chair and via the AZA website by logging into their account on "My AZA."

Management Group Members

- If a Management Group member transfers to a new facility with an existing IR for the same SSP Program, the facility must determine which of the two will serve as the IR for, and which will no longer act as a voting member of, the SSP Program.
- The Management Group member required to step down may be appointed as a non-voting advisor at the SSP Program's discretion.

IL and IR

- ILs or IRs who transfer to a new facility will no longer serve as the previous facility's representative to the SSP Program. The IL position will revert to the Director, and the IR position will revert to the IL.

Loss of Employment

Officers

- If an Officer loses their position from an AZA member facility, they have 6 months to re-gain employment with another AZA member facility before they have to surrender their position within the SSP Program.
- If an Officer is no longer employed at an AZA member facility and fails to communicate with the TAG or the AZA Conservation, Management, and Welfare Sciences Department within one month, it will be assumed that the Officer has abandoned the role in the Animal Program and the TAG may proceed with filling the vacancy before the 6 month grace period is over.
- Officers who do not resign under these conditions will be removed by the TAG Chair or, if the TAG does not have a current, approved RCP, the APM Committee.

IL and IR

- If an IL or IR loses their position from an AZA member facility, they will immediately be removed from the SSP Program. The IL position will revert to the Director, and the IR position will revert to the IL.

Member Facility Loss of Accreditation or Certification

Officers

- If an Officer's facility loses accreditation or certification, they must communicate this to the TAG Chair.
- If an SSP Officer's facility loses accreditation or certification, the Officer has 6 months to resign from the SSP Program or find employment with another AZA member facility.
- Officers who do not resign from the SSP Program under these conditions will be removed by the TAG Chair or, if the TAG does not have a current, approved RCP, the APM Committee.

IL and IR

- The IL and all IRs of a facility that loses accreditation or certification will be removed from Green SSP Programs if accreditation is not regained within the two year grace period.

Member Resignation

Officers

- SSP Coordinator must provide a written notice of resignation to the TAG Chair.
- The SSP Vice Coordinator will act as interim SSP Coordinator until a replacement is elected.

- Officers, excluding the SSP Coordinator, must provide a written notice of resignation to the SSP Coordinator.
- Departing Officers should uphold SSP business confidentiality and, when possible, orient and provide all relevant SSP Program documents to their replacement.

Management Group

- SSP Management Group members must provide a written notice of resignation to the SSP Coordinator.
- Departing Management Group members should uphold SSP business confidentiality and, when possible, orient and provide all relevant SSP Program documents to their replacement.

IL and IR

- If an IR resigns, the IL will serve as the default IR for the SSP Program until a new IR is designated.
- If an IL resigns, the Director will serve as the default IL until a new IL is appointed.

Member Removal

Officers

- If an SSP Coordinator is removed by the TAG or the APM Committee, the position must be advertised and the TAG will select a new Coordinator from the pool of applicants if the TAG has an approved RCP. If the TAG does not have a current, approved RCP, the APM Committee will select the new SSP Coordinator.
- The SSP Management Group must vote to remove an Officer (excluding the SSP Coordinator) from the SSP Program.
- The SSP Coordinator will notify the TAG and the APM Committee in writing if an Officer (excluding the SSP Coordinator) is removed from the SSP Program and will include all reasons for, and documentation pertaining to the removal.
- The SSP Management Group will hold a new election to fill the vacant Officer position as soon as possible.

Management Group Members

- The Management Group may choose to remove a Management Group member.
- The SSP Coordinator will notify the TAG and the APM Committee in writing if a Management Group member is removed from the SSP Program and will include all reasons for, and documentation pertaining to, the removal.
- The Management Group may fill the position by either holding a new election or appointing the IR who received the highest number of votes among the nominees who were not selected in the previous election.

IL and IR

- If an IL removes an IR, the IL will serve as the default IR for the SSP Program until a new IR is designated.
- If a Director removes an IL, the Director will serve as the default IL until a new IL is appointed.

AZA Animal Program participants (e.g., Program Leaders, Officers, Steering Committee members,) may be removed at the discretion of the AZA Executive Director. In the rare case that this should occur, the Executive Director and the AZA Conservation, Management, and Welfare Sciences Department will work closely with the TAG or SSP Program to document this process.

Chapter 7. SSP Program Functions

Conservation Activities

While not a requirement, SSP Programs may want to support and/or engage in conservation activities on behalf of their species. Engagement is particularly encouraged if an SSP Program manages a species designated by the IUCN or other government agency as Extinct in the Wild, Critically Endangered, or Endangered.

- The AZA Wildlife Conservation Committee is available to assist SSP Programs with developing conservation programs with clear goals and objectives. The Wildlife Conservation Committee recommends that all conservation activities are part of an adaptive management plan that links activities to current threats and ultimate conservation goals. One recommended framework is the Open Standards for the Practice of Conservation (http://cmp-openstandards.org/).
- Additional recognition of specific projects may develop within the context of AZA SAFE: Saving Animals from Extinction.

SSP Officer and Management Group Training

- SSP Coordinators are encouraged to mentor incoming SSP Coordinators, Officers, AZA Regional Studbook Keepers, and Management Group members to help them become familiar and comfortable with their responsibilities as established by the APM Committee in the associated Animal Program Handbooks, especially with respect to building sustainable populations.
- Mentoring and training procedures should include identifying the protocols used to assure data are current and transferred from the outgoing Program Leader to the new incoming Program Leader.
- Effort should be taken to coordinate training for SSP Programs within a TAG so that training can address similar issues across the taxa.

Animal Program Meetings

The APM Committee holds an open meeting for AZA Program Leaders, Officers, ILs, IRs, and other interested parties at each AZA Annual Conference and Mid-Year Meeting. These meetings may include reporting and updates from the APM Committee, the PMC, the RMC, and/or the AZA Conservation, Management, and Welfare Sciences Department, as well as an open question and answer session. Minutes from these meetings are disseminated over the consci listserv after the meeting.

Program Leader Workshops

- Program Leader workshops may be held at AZA Annual and/or Mid-Year Meetings. These may be organized by the AZA, the APM Committee, or individual Animal Programs.
- These workshops should be advertised in the Animal Programs Update and other appropriate Network Groups and listservs.
- Minutes and reports from these meetings should be AZA branded and disseminated, as appropriate. The AZA Conservation, Management, and Welfare Sciences Department is available to assist with document branding, if needed.

AZA Online Training Modules

Online Training Modules are web-based video tutorials found on the AZA website that were created for AZA's Animal Program Leaders, Institutional Representatives (IRs), Institutional Liaisons (ILs), and other individuals interested in becoming involved in AZA's Animal Programs. These modules provide helpful hints on navigating the AZA website and the ***Animal Programs Database***, as well as downloading certain documents and contact information. PMCTrack and population management modules are available to help Animal Program participants navigate PMCTrack and understand the technical aspects of creating an AZA Regional Studbook or Breeding and Transfer Plan. Any new Online Training Modules will be announced in the monthly Animal Programs Update.

Conservation Grants Fund Reviews

Participation in the AZA Conservation Grants Fund (CGF) review process provides all Animal Programs with a direct tool for steering the research directives of the AZA. SSP Programs may be asked to provide first-tier reviews for relevant proposals.

- CGF application materials become available in January, with funds available the following October.
- SSP Programs are encouraged to provide input to parties interested in submitting CGF proposals in order to strengthen links between the project and the SSP Program priorities.
- Only one review per proposal may be submitted on behalf of the SSP Program. Requests for reviews will be forwarded to Program Leaders in April and are due at the end of May.
- Reviews should critically examine the project's justification for goals and anticipated outcomes, the conservation and/or management significance and importance, project team ability, and budget.
- Reviews are considered confidential, should identify those aspects of the proposal most important to the SSP Program, and describe whether and how the proposal reflects SSP Program priorities. If the SSP Program is given multiple proposals to review, it is helpful to provide a hierarchy which proposals best reflect these priorities.

Outputs

Each SSP Program is responsible for publishing and maintaining specific outputs including a Breeding and Transfer Plan, an SSP Sustainability Report, an Animal Care Manual (ACM), and an Ambassador Animal Guideline (AAG) (if applicable). Breeding and Transfer Plans must be developed with, and require approval from, the PMC, a PMC Adjunct, or an approved SPMAG Advisor. The SSP Sustainability Report may be updated at any time by emailing the AZA Conservation, Management, and Welfare Sciences Department. ACMs require AZA Conservation, Management, and Welfare Sciences Department approval prior to publication. All outputs must be AZA branded and published on the AZA website upon approval.

Breeding and Transfer Plans

Each SSP Program is required to develop a Breeding and Transfer Plan which summarizes the current demographic and genetic status of the population, describes the SSP Program management designation, and recommends breeding pairs and transfers. Breeding and Transfer Plans are designed to maintain a healthy, genetically diverse, and demographically stable population. In order to assure the production of an effective Breeding and Transfer Plans, the SSP Coordinator must work with the PMC, a PMC Adjunct, or an approved SPMAG Advisor. See Chapter 7 for more information on Breeding and Transfer Plans.

SSP Sustainability Reports

Each SSP Coordinator should work with the AZA Conservation, Management, and Welfare Sciences Department to complete their automatically generated 5-page report that summarizes husbandry practices, exhibit management, species appeal, educational opportunities, multi-species exhibit considerations, species biology, SSP population dynamics, management priorities, challenges to sustainability, and research needs. The report is a compilation of the SSP Coordinator's expertise and the current and projected population summaries from the SSP Breeding and Transfer Plan or PVA. This information can be updated at any time and is automatically generated from the SSP Search Portal located on the AZA website.

Animal Care Manuals

SSP Programs are required to assist their TAG in creating ACMs for their species. ACMs present a compilation of knowledge provided by recognized animal experts based on the current science, practice, and technology of animal management. The manual assembles basic requirements, best practices, and animal care recommendations to maximize capacity for excellence in animal care and welfare. The manual should be considered a work in progress, since practices continue to evolve through advances in scientific knowledge. The use of information within this manual should be in accordance with all local, state, and federal laws and regulations concerning the care of animals. The recommendations are not exclusive management approaches, diets, medical treatments, or procedures, and may require adaptation to the specific needs of individual animals and particular

circumstances in each facility. Commercial entities and media identified within the ACM are not necessarily endorsed by AZA. The statements presented throughout the body of the manual do not represent standards of care unless specifically identified as such in clearly marked sidebar boxes. See Chapter 9 for more information on ACMs.

Ambassador Animal Guidelines

SSP Programs are encouraged to assist the AASAG or their TAG in creating AAGs for their species, if applicable. AAGs provide a compilation of knowledge provided by recognized animal and education experts based on the current science, practice, and technology of ambassador animal management and presentation. Each AAG assembles basic requirements, best practices, and animal care recommendations to maximize capacity for excellence in animal care and welfare of ambassador species. The guidelines should be considered a work in progress, since practices continue to evolve through advances in scientific knowledge. The use of information within this document should be in accordance with all local, state, and federal laws and regulations concerning the care of animals. While some government laws and regulations may be referenced, these are not all-inclusive nor is this document intended to serve as an evaluation tool for those agencies. The recommendations included are not meant to be exclusive management approaches, diets, medical treatments, or procedures, and may require adaptation to meet the specific needs of individual animals and particular circumstances in each institution. See Chapter 10 for more information on AAGs.

Communication

Each SSP Program must develop a means to facilitate communication among its members, as well as distribute appropriate information about the SSP Program and its functions to the general public. The SSP Program may choose to distribute information via reporting sessions at AZA conferences and meetings, through AZA Annual Reports, ***monthly Animal Programs Update***, Listservs, and AZA Stories and/or through TAG websites, e-mail, and newsletters. All public communications must be AZA branded and it is recommended that the TAG utilize electronic resources as much as possible in order to engage in green practices.

Meetings

- SSP Coordinators should hold (electronically or in person) at least one working Animal Program meeting each year, and are encouraged to hold in person meetings in conjunction with the AZA Annual Conference and/or Mid-Year Meeting.
- If the SSP Program holds additional meetings in a venue outside of these conferences, the SSP Program must communicate the dates and locations of these to its TAG Chair, the APM Committee Liaison, and the AZA Conservation, Management, and Welfare Sciences Department.
- The SSP Coordinator or SSP Vice Coordinator is encouraged to schedule and moderate reporting sessions at AZA Mid-Year and/or Annual Conferences.
- All Officers are encouraged to attend all official SSP Program meetings.
- Minutes must be recorded, AZA branded, archived, disseminated among the SSP Program's IRs, and submitted to the TAG Chair and the AZA Conservation, Management, and Welfare Sciences Department.

Position Statements, White Papers and Guidelines

Position Statements

An AZA Position Statement must be approved by the AZA Board of Directors and defines an AZA Committee, SAG, or Animal Program (and therefore the Association's) position on a specific issue. AZA Position Statements most frequently supplement an AZA Board approved policy and are supported by an informational and science-based AZA White Paper. AZA Board approved Policies, Position Statements and White Papers are found here: https://www.aza.org/board-approved-policies-and-position-statements.

If an Animal Program, Committee, or Scientific Advisory Group is interested in developing Position Statement and White Papers relevant to taxa within their purview they should adhere to the following process:

- Draft the Position Statement using the AZA branded template (Appendix O).
- Draft a White Paper using the AZA branded template (Appendix P).
- Submit both Drafts to the AZA Conservation, Management, and Welfare Sciences Department (animalprograms@aza.org).
- The AZA Conservation, Management, and Welfare Sciences Department will review the drafts and, if deemed necessary, send them to the appropriate AZA Committees for review.
- All review comments will be returned to and discussed with you by the AZA Conservation, Management, and Welfare Sciences Department to develop final drafts.
- If deemed necessary, the AZA Conservation, Management, and Welfare Sciences Department will submit the Final Drafts to the AZA Board for review.

White Paper

An AZA White Paper may either support an AZA Position Statement and therefore require approval by the AZA Board of Directors, or it may be a stand-alone document that does not support an official AZA Position Statement. AZA White Papers may be informational articles that discuss a philosophy or initiative, or a description of recommended guidelines that are of relevance to the Association. If an Animal Program, Committee, or Scientific Advisory Group has a White Paper that they wish to submit for review, they should adhere to the following process:

- Draft a White Paper using the AZA branded template (Appendix P).
- Submit the Draft to the AZA Conservation, Management, and Welfare Sciences Department (animalprograms@aza.org).
- The AZA Conservation, Management, and Welfare Sciences Department will review the draft and, if deemed necessary, send it to the appropriate AZA Committees for review.
- All review comments will be returned to and discussed with you by the AZA Conservation, Management, and Welfare Sciences Department to develop final draft.
- If deemed necessary, the AZA Conservation, Management, and Welfare Sciences Department will submit the Final Draft to the AZA Board for review.

Guidelines

While the majority of Animal Program guidelines (i.e., hand-rearing protocols, mixed species exhibit suggestions, etc.) may not require AZA Board approval it is still important that they are required and approved by the AZA Conservation, Management, and Welfare Sciences Department before they are published and distributed to assure that they are appropriate and reflect the philosophy If an Animal Program, Committee, or Scientific Advisory Group has developed guidelines that they wish to submit for review, they should adhere to the following process:

- Draft Animal Program guidelines using the AZA branded template (Appendix Q).
- Submit the Draft guidelines to the AZA Conservation, Management, and Welfare Sciences Department (animalprograms@aza.org).
- The AZA Conservation, Management, and Welfare Sciences Department will review the draft and, if deemed necessary, send it to the appropriate AZA Committees for review.
- All review comments will be returned to and discussed with you by the AZA Conservation, Management, and Welfare Sciences Department to develop final draft.
- If deemed necessary, the AZA Conservation, Management, and Welfare Sciences Department will submit the Final Draft to the AZA Board for review.

Animal Programs Update

The Animal Programs Update is published monthly on the AZA website at (https://www.aza.org/animal-programs-monthly-update), and includes Animal Program announcements, vacancy advertisements and new publications. The TAG is responsible for submitting programmatic changes for Animal Programs within its purview, including SSP Program appointments and contact information, Animal Program upgrades and downgrades, and taxonomic changes for managed species to the AZA Conservation, Management, and Welfare Sciences Department. The SSP Program may also provide TAG-approved announcements and notices to be published in the monthly Animal Programs Update.

The TAG Chair must approve all Program Leader vacancy advertisements and assure that the TAG submits them to the AZA Conservation, Management, and Welfare Sciences Department. All Program Leader vacancies must be advertised for 30 days in the Animal Programs Update and on the Current Program Leader Vacancies page (www.aza.org/Program-Leader-Vacancies) before a new Program Leader may be appointed.

AZA Network

The ***AZA Network*** brings together great ideas, best practices and lessons learned from within the zoo and aquarium community. The diverse community allows for open professional interest groups or closed working groups. Your profile in the Network is where you will manage notifications of activity, allowing you options to receive emails as activity occurs, or in a daily, or weekly digest email. There are many open professional interest groups available, as well as closed Animal Program groups that can be maintained by the Animal Program Leaders themselves. These groups allow members to start discussions, add resources, and share documents.

SSP Programs are encouraged to establish an AZA Network Group for their SSP Program. Membership within Animal Program Network workspaces may be compartmentalized such that some portions may be restricted to the Management Group, while another section may be open to all IRs. All IR updates made in the AZA Animal Program Database will automatically be applied to the workspace membership. The workspace must have a Moderator who to will manage subscriptions to closed Management Group workspaces and establish rules for postings. To create a group within the AZA Network, the Program Leader should contact the AZA Conservation, Management, and Welfare Sciences Department.

SSP Highlights

SSP Highlights is a "member view" feature in AZA's *CONNECT* magazine that provides an opportunity to highlight and share efforts SSP Programs have made to increase population sustainability in zoos and aquariums and share their successes with the general AZA membership. This feature provides a way for SSP Programs to share their creative approaches to address population sustainability challenges. Examples include engaging in innovative research, advancing management practices, and developing partnerships to enhance SSP sustainability. SSP Highlights made its debut in the August 2016 issue of *CONNECT*. SSPs are encouraged to submit their draft SSP Highlights to the AZA Conservation, Management, and Welfare Sciences Department using the template found in Appendix N.

SSP Sustainability Award

The SSP Sustainability Award recognizes initiatives of AZA SSP Programs that have a quantifiable impact on the long-term sustainability of an SSP's managed population. Animal Program Leaders (i.e., TAG Chairs, SSP Coordinators, Studbook Keepers, and Scientific Advisory Group Chairs) may submit an application for this award that demonstrates how their significant and innovative efforts have resulted in a significant increase in an SSP population's sustainability. Award decisions will be based on the level of effort described that has resulted in significantly improving the SSP population's sustainability, the quantifiable impact that has occurred which demonstrates an increase in the SSP population's sustainability, how any resulting forward actions were made accessible to all appropriate facilities in an effective and timely manner, and how this initiative advances AZA TAG recommendations for that SSP population's sustainability. For more information on deadlines and application materials, visit the AZA website: https://www.aza.org/ssp-sustainability-award.

Social Media and *CONNECT* articles

AZA manages an AZA Facebook page and a Twitter account that have thousands of followers. To maximize exposure of the TAG's work, snippets of publicly appropriate information (including photos) should be provided for inclusion on the social media resources. To publish information on AZA's Facebook page and Twitter account, the TAG Chair should contact the AZA Digital Media Director. In addition, TAGs may wish to distribute information about their work in an article in *CONNECT* each year. To publish an article in *CONNECT* the TAG Chair should contact the AZA Publications and Brand Director.

Newsletter

SSP Programs may find it helpful and engaging to distribute annual or more frequent updates on their activities by publishing a newsletter. Newsletters may include updates and progress reports on all aspects of the SSP Program's work, such as membership, elections, vacancies, fundraising, research, statements, photos (rights must be obtained for all photos), and conservation projects. Newsletters must be AZA branded and may be distributed online via the AZA Animal Programs Database, or specifically to IRs and the AZA Conservation, Management, and Welfare Sciences Department.

AZA Animal Programs

In the late 1970's, the recognition that wildlife populations were declining in the wild and access to collection animals was becoming increasingly more difficult, inspired a group of visionary zoologists to create the Species Survival Plan® (SSP) concept as a cooperative animal management program administered by the AZA. AZA's first SSPs were created in 1981.

These SSPs functioned by managing each animal of a species held by all AZA member zoos and aquariums as a member of a single population for breeding purposes. The breeding plan for each species made breeding (or "do-not-breed") recommendations to maintain demographically stable populations with the greatest possible genetic diversity for the long-term future of a healthy and sustainable population. Sustainability of the population is related to many factors including its gene diversity, demographic stability, husbandry expertise, etc.

In 1994, AZA published *Species Survival Plans – Strategies for Wildlife Conservation*, which stated: "The SSP program was originally conceived to provide a blueprint for cooperative captive breeding programs in North America, but more recently the concept has also evolved to include field conservation efforts."

The AZA Conservation, Management, and Welfare Sciences Staff and the Animal Population Management Committee (APM Committee), which oversee 43 Taxon Advisory Groups (TAGs) and more than 500 Animal Programs, initiated a variety of processes to sustain zoo and aquarium collections and wild species. The TAGs became responsible for creating and maintaining RCPs which recommend species to be managed within AZA-accredited facilities given available space and resources. The AZA Population Management Center (PMC), created in 2000, became responsible for incorporating the data derived from Studbooks and RCPs to identify science-based breeding and transfer recommendations along with each SSP Program. The AZA ***Reproductive Management Center (RMC)*** at the St. Louis Zoo was also created in 2000 to assess contraception efficacy, reversibility, and safety for animals not recommended for breeding.

The 2009 publication titled *Sustaining the Ark: the challenges faced by zoos in maintaining viable populations* (International Zoo Yearbook. 43:6-18) highlighted a fact that many have increasingly recognized over the past several years: "Over the last decade . . . Ark-related activity" (i.e., maintaining sustainable populations) "has declined as zoos have diversified their conservation activities, re-directing efforts into other areas, such as conservation education, fund-raising and other support for *in situ* projects….Zoo populations are not achieving the conditions for sustainability."

The declining sustainability of zoo and aquarium populations likely results from a variety of factors including insufficient animal holding and breeding space, low breeding success, need for more advanced husbandry techniques, or, occasionally, lack of success in completing breeding recommendations. In 2008, Lincoln Park Zoo developed PMCTrack to evaluate the outcomes of breeding and transfer recommendations issued by AZA Program Leaders with the assistance of the PMC. AZA Program Leaders will be able to view their program's historical outcomes, monitor outcomes going forward, and use simple survey tools to solicit reasons why recommendations didn't occur from Institutional Representatives, so we can begin to understand how to improve recommendation outcomes. Institutional Liaisons will also have access to the system, so directors and their ILs can evaluate the participation of their staff in the cooperative management system and how their facility is doing at completing recommendations in comparison to the AZA average. Ultimately, the AZA community will be able to use the tools and data in PMCTrack to understand, monitor, and improve AZA's cooperative management system and the long-term viability of animal populations. The AZA Conservation, Management, and Welfare Sciences Department conducted an intensive qualitative and quantitative assessment of the Animal Programs to understand where simplification of processes may assist Program Leaders, how the involvement of non-AZA entities could make crucial founders available, and other aspects that could facilitate Animal Program success, in building sustainable populations.

In 2009, the AZA Board approved a simplified procedure to approve non-member participants in the SSP Programs, a new ***Full Participation Policy***, and a new Animal Management Reconciliation Policy to articulate the roles and responsibilities needed to enhance program success. The Board also formed a Task Force on the Sustainability of Zoo-based Populations and a Task Force on the Sustainability of Aquatic Populations.

The *Task Forces on the Sustainability of Zoo-Based Populations and Aquatic Populations*, comprised of AZA Board members, the APM Committee Chair, and AZA staff, obtained input from a diversity of individuals from the conservation community including: AZA Conservation, Management, and Welfare Sciences and Government Affairs staff, the PMC and RMC, the APM Committee and Small Population Management Advisory Group (SPMAG), U.S. Fish & Wildlife Service, Program Leaders, researchers, and other selected conservation professionals as needed to accomplish the following tasks:

- Review the mission, goals, and limits of the cooperative management of AZA's Animal Programs.
- Set minimum achievable goals for long-term sustainability of AZA's Animal Programs.
- Determine which factors have the greatest impact on the sustainability of zoo populations.
- Assess resources needed to sustain the cooperative management of AZA's Animal Programs.
- Plan for Program Leader succession.
- Assess the ability of the current program administration system to allow programs to meet sustainability requirements.
- Assess current relationships with U.S. government agencies and assess permitting regulations and practices that impede zoos and aquariums in maintaining sustainable populations. Provide recommendations to increase respect for the cooperative management of AZA's Animal Programs and facilitate legislative and regulatory changes that will maximize collection sustainability.
- Recommend modifications to the cooperative management system structure and the administration of AZA's Animal Programs to assure programs are positioned to achieve sustainability goals.

Variables Affecting Sustainability

The 2010 AZA Sustainability Task Force's assessment identified a combination of variables that have contributed to the reduced long-term sustainability of many of AZA's managed Animal Program populations. As there are a variety of causes, there is no single answer, direction, or solution. These variables include insufficient:

- Knowledge of current Animal Program population sustainability duration and genetic diversity.
- Number of holding and breeding spaces needed to increase the sustainability of the Animal Programs.
- Animal Program planning capacity.
- Institutional awareness surrounding the topic of sustainability.
- Institutional commitment to provide additional holding or breeding spaces.
- Permitting and/or regulatory availability to move animals.
- Advanced breeding expertise.

Enhancing Population Sustainability

In 2010, the Task Force identified, and the AZA Board approved, a variety of new Animal Program management strategies to address these variables and improve the sustainability of AZA's Animal Programs. These included:

- Assessing and providing each Animal Program population's projected gene diversity (% GD) at 100 years or 10 generations.
- Designating each Animal Program as a Green SSP Program, a Yellow SSP Program, or a Red Program.
- Increasing educational opportunities for Institutional Directors and staff to gain a detailed understanding of the new Animal Program management strategies including:
  - The critical need for an increased number of holding and breeding spaces.
  - The need for strong institutional support for all Program Leaders and their training.
  - The importance of following Breeding and Transfer Plan recommendations.
- Increasing training opportunities for zoo and aquarium staff to become more skilled at understanding permit application processes and permit writing techniques.

- Enhancing legislative and regulatory efforts to increase recognition of the vital roles of zoos and aquariums serve and better facilitate importation processes to help them build self-sustaining Animal Program populations.

In 2014, the APM Committee assessed and evaluated the effects that the new Animal Program designations had on AZA's cooperatively managed Animal Programs. After a thorough review, the APM Committee made a recommendation the AZA Board that all AZA cooperatively managed Animal Programs (i.e., Green, Yellow, and Red Programs) be designated as SSP Programs, that minimum SSP criteria be established for those Animal Programs that were not managing species classified as Extinct in the Wild, Critically Endangered, or Endangered, and that all Animal Programs would identify at least three goals. The Board approved establishing criteria that all other SSP Program populations include at least three AZA member facilities and be comprised of at least 20 individuals. At this time, the APM Committee established a new category of TAG managed Candidate Animal Programs that may include those populations that did not meet the minimum SSP Criteria, but where the TAG wishes to grow the Program to become an SSP in time.

Over the years, the AZA community has engaged in several initiatives including working with the Alexander Center for Applied Population Biology and the AZA PMC at Lincoln Park Zoo to develop new tools for sustainability. Funded by grants from the Institute for Museum and Library Services (IMLS), Lincoln Park Zoo has worked with Program Leaders at AZA facilities to conduct Population Viability Analyses (PVAs) for AZA Animal Programs. A PVA is a computer model that projects a population's likely future status and helps identify key factors that may be impacting the sustainability of the population. From 2011-2016, PVA reports were completed for 135 programs on a TAG-by-TAG basis. TAG summary reports, comparing and contrasting PVA results among different populations, have been completed for 16 AZA TAGs. As of the conclusion of IMLS funding in late 2016, PVAs are continuing to be conducted for additional SSPs to answer specific questions about long-term population viability. PVA reports are made available on the individual SSP or TAG pages within the Animal Programs Database.

An IMLS grant has also used IMLS funding to create a database that compiles extensive quantitative and qualitative information. Informed by this wealth of data, the AZA Conservation, Management, and Welfare Sciences Department can work with TAGs, SAGs, the PMC, and other working groups to identify patterns in population challenges and to strategically address population needs.

The online tools emerging from the SSP Sustainability Database include the SSP Sustainability Reports and Search Portal. The Database automatically generates SSP Sustainability Reports which summarize SSP species' basic care, exhibit design, and population management considerations and priorities. This information, which was originally provided by SSP Coordinators, TAG Chairs, and other Animal Program participants, is compiled in a searchable format, allowing collection planning users to perform searches that identify appropriate species for their collection planning criteria, while also directing resources and attention to managed species.

The SSP Sustainability Reports and Search Portal were designed as a member service for collection planners, Program Leaders, research scientists, and other zoo and aquarium staff. Access is available for staff at AZA-accredited facilities and Certified Related Facilities.

AZA SAFE: Saving Animals From Extinction

The mission of AZA's SAFE: Saving Animals From Extinction is to combine the power of zoo and aquarium visitors with the resources and collective expertise of AZA members and partners to save animals from extinction. The vision of SAFE is that together, we are saving the most vulnerable wildlife species from extinction and protecting them for future generations.

SAFE Species programs protect threatened animals; build on established recovery plans and track records of commitment; prioritize collaboration among AZA member facilities; implement both strategic conservation and public engagement activities; and measure and report conservation progress.

In 2015, ten inaugural SAFE Species were identified including the African penguin, Asian elephant, black rhinoceros, cheetah, gorillas, sea turtles, sharks and rays, vaquita, western pond turtle, and

whooping crane, and SAFE continues to grow. SAFE is a framework that encourages teams to use a collaborative process, incorporate a wide-range of species-specific expertise from AZA members and non-government and government partners, and identify the conservation actions needed to protect those species based on published recovery plans. Three-year SAFE Program Plans, include objectives and actions for conservation, stakeholder and public engagement, public awareness and communications, and fundraising that will make a positive impact on species' populations in the wild. Employees at AZA-accredited aquariums and zoos lead and implement these projects.

The Wildlife Conservation Committee administers SAFE, with support from AZA staff. Explore current SAFE Species programs or consider whether a species of interest to you may be eligible to become a SAFE Species at: https://www.aza.org/aza-safe.

Chapter 9. Animal Care Manuals

Overview

Animal Care Manuals (ACMs) provide a compilation of animal care and management knowledge that has been gained from recognized species experts based on the current science, practice, and technology of animal management. These manuals compile and organize our understanding of basic requirements, best practices, and animal care recommendations to advance the capacity for excellence in animal care and welfare. These dynamic manuals are considered works in progress, since practices continue to evolve through scientific learning. Once completed, the use of information within each manual should always be in accordance with all local, state, and federal laws and regulations concerning the care of the species specified.

Recommendations included in the manuals are not exclusive management approaches, diets, medical treatments, or procedures, and may require adaptation to the specific needs of individual animals and particular circumstances in each facility. The statements presented throughout the body of the manuals do not represent specific AZA accreditation standards of care unless specifically identified as such in clearly marked as such in sidebar boxes.

ACMs are composed by TAG and Animal Program representatives, managed by the AZA Animal Welfare Committee, and approved by the AZA Conservation, Management, and Welfare Sciences Department. The developmental procedures used to compose each manual follow a specific sequence that includes several review procedures (internal and external) before AZA reviews, and ultimately approves their publication. Because one of the most important outputs of a TAG is to develop and maintain a current ACM, a summary of the primary developmental procedures are described below, however, in addition, a specific ACM template (http://www.aza.org/animal-care-manuals/) has been composed to ease the process. The ACM template should be adjusted (e.g., edit headers and sub-headers, etc.) to match the needs of your species/taxa.

The key processes needed to compose an ACM are listed below; however the complete set of developmental processes (http://www.aza.org/animal-care-manuals/) should be used as a guide to produce the final publication.

Developmental Processes

Draft ACM Development

- TAGs and/or SSP Programs identify a contact person (Champion) who serves as the main communications conduit between the TAG/SSP and the AZA Conservation, Management, and Welfare Sciences Staff. The Champion is responsible for
  - seeking input from the TAG/SSP,
  - collecting TAG/SSP-based information,
  - compiling all scientific data and professional information about the natural history and management strategies of the taxa(on),
  - incorporating this information into the pre-existing sections of the ACM template, or editing the headers and sub-headers to match the needs of your species/taxa,
  - proof-reading and updating the TAG Chair on the ACM progress, and
  - communicating ACM issues with the AZA Conservation, Management, and Welfare Sciences Staff.
- The Champion will submit the first Draft of the ACM to the AZA Conservation, Management, and Welfare Sciences Department and the AZA Conservation, Management, and Welfare Sciences Department will complete a review of the draft and assure that all relevant bullets were addressed.
- The Champion will review the AZA Conservation, Management, and Welfare Sciences Department edits, develop a 2nd draft ACM, and submit this 2nd draft ACM to the AZA Conservation, Management, and Welfare Sciences Department.
- The Champion will review the AZA Conservation, Management, and Welfare Sciences Department edits, develop a 3rd draft ACM (if necessary), and submit this 3rd draft ACM to the AZA Conservation, Management, and Welfare Sciences Department.

- The AZA Conservation, Management, and Welfare Sciences Staff will return the edited final Draft ACM to the Champion to assure that mutually agreeable solutions are achieved for any editorial changes that may be necessary.

Final Draft ACM Review

- Prior to completion of the final Draft ACM, the Champion works with the TAG/SSP to identify two or more external review experts and submits the Final Draft ACM to the AZA Conservation, Management, and Welfare Sciences Department for editing.
- The AZA Conservation, Management, and Welfare Sciences Department will provide a digital copy of the final Draft ACM to the TAG Steering Committee, SSP Management Group, relevant AZA Committees and Scientific Advisory Groups, and external review experts. Additionally, the final Draft will be posted on the AZA website for the 30-day AZA member comment period.
- The AZA Conservation, Management, and Welfare Sciences Department will collect all comments from the members and external review experts, organize them according to their corresponding ACM sections, and distribute them via email to the Champion at the close of the 30 day review period.
- The Champion will work with the TAG/SSP to review all comments, incorporate suggestions as deemed necessary and then submit the Pending-Approval ACM to the AZA Conservation, Management, and Welfare Sciences Department.

ACM Approval

- If the ACM is approved, the AZA Conservation, Management, and Welfare Sciences Department will post the ACM on the AZA website.
- An updated and revised ACM should be published within 5 years of the last ACM publication. A TAG may update an ACM sooner if significant new information regarding animal care and welfare practices becomes available.

Required Elements of an ACM

The published ACM should include a variety of components that are clear enough for colleagues not familiar with the taxonomic group to understand how and why these recommendations were made. ACMs should provide up-to-date information gained from a large body of expertise including biologists, veterinarians, nutritionists, reproduction physiologists from the contraception center, behaviorists and researchers. TAGs/SSPs must address each of the following elements in the ACM template (www.aza.org/animal-care-manuals) if deemed relevant to the taxa (and may add additional elements if warranted). If data do not exist for particular areas listed below the ACM should state that fact as a clear identification of needed research and study. Each relevant area should be as comprehensive as existing knowledge allows.

Taxonomic Information

- Taxonomic classification
- Genus/species/status
- General Information

Ambient Environment

- Temperature/humidity
- Light
- Water/air quality
- Sound/vibration

Habitat Design and Containment

- Space and complexity
- Safety and containment

Records

- Definitions
- Types
- Permit considerations
- Government ownership
- Identification

Transport

- Preparations
- Protocols

Social Environment

- Group Structure and size
- Influence of others and conspecifics
- Introductions and reintroductions

Nutrition

- Nutritional requirements
- Diets
- Nutritional evaluations

Veterinary Care

- Veterinary services
- Transfer examination and diagnostic testing recommendations
- Quarantine
- Preventative medicine
- Capture, restraint, and immobilization
- Management of disease, disorders, injuries, and/or isolation

Reproduction

- Reproductive physiology and behavior
- Assisted reproductive technology
- Pregnancy, egg-laying/parturition
- Birthing/hatching facilities
- Assisted rearing
- Contraception

Behavior Management

- Animal training
- Environmental enrichment
- Staff and animal interactions
- Staff skills and training

Ambassador Animals

- Ambassador animal husbandry
- Institutional ambassador animal programs
- Handling and staff training
- Program evaluation

Research

- Known methodologies
- Future research needs

Other Considerations

- Additional information

Chapter 10. Ambassador Animal Guidelines

Overview

Ambassador Animal Guidelines (AAGs) provide a compilation of knowledge provided by recognized animal and education experts based on the current science, practice, and technology of ambassador animal management and presentation. Each AAG assembles basic requirements, best practices, and animal care recommendations to maximize capacity for excellence in animal care and welfare of ambassador species. These guidelines are considered works in progress, since practices continue to evolve through advances in scientific knowledge. Once completed, the use of information within each guideline should always be in accordance with all local, state, and federal laws and regulations concerning the care of the species specified.

While some government laws and regulations may be referenced, these are not all-inclusive nor is this document intended to serve as an evaluation tool for those agencies. The recommendations included are not meant to be exclusive management approaches, diets, medical treatments, or procedures, and may require adaptation to meet the specific needs of individual animals and particular circumstances in each institution.

AAGs are composed by TAG and Animal Program representatives, managed by the AZA Ambassador Animal Scientific Advisory Group, and approved by the AZA Conservation, Management, and Welfare Sciences Department. The developmental procedures used to compose each guideline follow a specific sequence that includes several review procedures (internal and external) before AZA reviews and ultimately approves their publication. The key processes needed to compose an AAG are listed below; however the complete set of developmental processes (https://www.aza.org/ambassador-animal-guidelines) should be used as a guide to produce the final publication.

Developmental Processes

Draft AAG Development

- In the event an Animal Care Manual (ACM) exists for a species, or is in the development process, the AAG will be incorporated into the ACM development process or integrated as appropriate at the next scheduled revision.
    - In the absence of an ACM, the AAG would continue through the development and revision process outlined below.
- The AASAG, TAG/SSP, or other relevant committee will identify a contact person (AAG Coordinator) who serves as the main communications conduit between the AASAG, TAG/SSP, and the AZA Conservation, Management, and Welfare Sciences Staff. The AAG Coordinator is responsible for
    - seeking input from the TAG/SSP,
    - collecting TAG/SSP-based information,
    - compiling all scientific data and professional information about the natural history and management strategies of the taxa(on),
    - incorporating this information into the pre-existing sections of the AAG template, or editing the headers and sub-headers to match the needs of your species/taxa,
    - proof-reading and updating the TAG Chair on the AAG progress, and
    - communicating AAG issues with the AZA Conservation, Management, and Welfare Sciences Staff.
- The AAG Coordinator will submit the first Draft of the AAG to the AASAG Steering Committee for review.
- Upon completion of the draft AAG, the AAG Coordinator will communicate with the TAG/SSP and AASAG to identify to or more external review experts and submit this information and the draft AAG to the AZA Conservation, Management, and Welfare Sciences Department for proofreading and editing.
- When all edits are agreed upon, the AZA Conservation, Management, and Welfare Sciences Department will provide a digital copy of the final Draft AAG to the external reviewers, TAG

Steering Committee, SSP Management Group, AASAG Steering Committee, relevant AZA Committees and Program Leaders, Chair of the Conservation Education Committee, and the Chair of the Animal Welfare Committee. Additionally, the final Draft will be posted on the AZA website for the 30-day AZA member comment period.

- The AAG Coordinator will work with the TAG/SSP and AASAG to review all comments, incorporate suggestions as deemed necessary and provide a written justification report for omitting suggestions deemed unnecessary.

AAG Approval

- Once completed, the AZA Conservation, Management, and Welfare Sciences Department will provide final approval and post the AAG on the AZA website.
- An updated and revised AAG should be published within 5 years of the last AAG publication. An AAG Coordinator may update an AAG sooner if significant new information regarding animal care and welfare practices becomes available.

Required Elements of an AAG

The published AAG should include a variety of components that are clear enough for colleagues not familiar with the taxonomic group to understand how and why these recommendations were made. AAGs should provide up-to-date information and must address each of the following elements in the AAG template (https://www.aza.org/ambassador-animal-guidelines) if deemed relevant to the taxa (and may add additional elements if warranted). Each relevant area should be as comprehensive as existing knowledge allows.

Husbandry

- Housing
- Diet
- Enrichment
- Animal Training
- Social Grouping
- Signs of Stress

Programs

- Program Types
- Temperature Guidelines
- Transport
- Display Options
- Messaging

Handling and Staff Training

- Handling Limits
- Handlers and Handler Training
- Handler Certification

Contacts

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AZA Web Resources

AZA Board Approved Policies
https://www.aza.org/board-approved-policies-and-position-statements

Animal Exchange
To access ***Animal Exchange***, the user must be logged in to the AZA website and have Animal Exchange privileges assigned to your individual record in order to use this feature. Never share your log-in information with anyone as you will directly be held responsible for any changes or edits made to secured areas. Once logged in, the Animal Exchange link will be found on the Animals & Conservation > Animal Care & Management dropdown.

Animal Programs Database
The Animal Programs Database contains all Animal Program Data, and is separated out into Animal Program pages. There are separate pages for TAGs, SSP Programs, Studbooks and SAGs. Each Animal Program page can be accessed by going through the:

Animal Program Page Search Portal
https://ams.aza.org/eweb/DynamicPage.aspx?Site=AZA&WebKey=8f652949-31be-4387-876f-f49a2d7263b2

Each Animal Program page contains the following (*information only available if logged in):
- Program Leaders, Officers, Advisors
- Program Leader, Officers, Advisors contact information*
- Animal Program details (start dates, websites, etc.)
- Animal Program Species
- Related Animal Programs
- Animal Program Documents*
- Animal Program IR list*

SSP Sustainability Reports and Search Portal
https://ams.aza.org/eweb/DynamicPage.aspx?WebCode=LoginRequired&expires=yes&Site=AZA

The SSP Sustainability Reports summarize SSP species' basic care, exhibit design, and population management considerations and priorities. The Search Portal automatically generates these reports which allow collection planners to perform searches that identify appropriate species for their collection planning criteria, while also directing resources and attention to managed species. The SSP Sustainability Reports and Search Portal were designed as a member service for collection planners, Program Leaders, ILs, IRs, research scientists, and other zoo and aquarium staff. Access is available for staff at AZA-accredited facilities and Certified Related Facilities.

Animal Programs Resources
https://www.aza.org/animal-programs-resources
The Animal Programs Resources page contains numerous links, documents and templates aimed to assist Program Leaders. These include:
- Program Leader Handbooks
- Animal Program Applications
- Resource Documents (including templates, guides, and resources related to being a new Program Leader, assessing Sustainability Partners, TAG strategic planning, PVA FAQs, and maintaining Studbooks)
- Contact information for TAGs Chairs, Institutional Liaisons, APM Committee TAG Liaisons, SPMAG TAG Liaisons
- Animal Program ***Sustainability Designations*** (updated quarterly)
- Animal Programs Monthly Update
- Current Program Leader Vacancies
- Illustrative protocols to help Program Leaders navigate the Animal Programs Database

Accountability Information and Instructions
http://www.aza.org/animal-program-deadlines/

PMCTrack
Website: www.pmctrack.org; Email: pmctrack@lpzoo.org

PMCTrack evaluates breeding and transfer recommendations to:
- Determine whether each recommendation occurred based on studbook data

- Collect reasons from Institutional Representatives for recommendations not occurring as planned
- Improve management of AZA's Animal Programs and increase long-term viability of these populations

Population Management Center
Website: http://www.lpzoo.org/population-management-center; Email: pmc@lpzoo.org

Reproductive Management Center
Website: https://www.stlzoo.org/animals/scienceresearch/reproductivemanagementcenter/; Email: contraception@stlzoo.org

ZIMS for Studbooks
Website: https://zims.species360.org/
Email: support@species360.org
ZIMS for Studbooks is an online database where Studbook Keepers maintain and track their studbook databases.
Resources:

- A Reference Guide to ZIMS for Studbooks for Animal Program Leaders
- Starting a New AZA Studbook in ZIMS for Studbooks
- AZA Guidelines for I. Roles and Access to ZIMS for Studbooks, and II. Sharing Studbook Data
- Working Together in a Shared Studbook Database

Glossary

Accountability- Accountability refers to the processes by which Animal Program participants including Program Leaders, Institutional Representatives (IRs), and Institutional Liaisons (ILs) are responsible for producing and reviewing documents, and communicating among appropriate individuals. Accountability of Animal Programs includes meeting deadlines, requesting extensions if needed, maintaining communication with all individuals, and adhering to the AZA's Full Participation Policy and the Species Survival Plan® Animal Management Reconciliation Policy.

Advisor- An advisor is a non-voting participant of an AZA Animal Program (AP) that provides advice to the AP in their efforts to identify, develop and implement goals related to their species. An advisor may also provide input on Animal Care Manuals and assist with the development of education materials and research projects related to the Advisor's area of expertise.

Animal Care Manuals (ACMs)- Animal Care Manuals (ACMs) are a compilation of animal care and management knowledge that has been gained from recognized species experts, including AZA Taxon Advisory Groups (TAGs), Species Survival Plan® Programs (SSPs), biologists, veterinarians, nutritionists, reproduction physiologists, behaviorists and researchers. Content is based on the current science, practice, and technology of animal management. The manual assembles best practices, animal care recommendations and AZA accreditation standards to maximize capacity for excellence in animal care and welfare and is updated every 5 years. All ACMs are peer reviewed, widely valued, and acclaimed by other regional associations. All TAGs are required to coordinate the publication of ACMs for the taxa within their purview.

Animal Exchange- The Animal Exchange allows representatives from AZA-Accredited Facilities, Certified Related Facilities and Approved Non-Member Participants to list and search for individuals of a species that can be exchanged to meet the goals of their Institutional Collection Plan (ICP) or the Regional Collection Plan (RCP).

Animal Population Management Committee (APM Committee)- The Animal Population Management Committee (APM Committee) works collaboratively with other Committees and is responsible for facilitating the professional and scientific management of the animals cared for in AZA-Accredited zoos and aquariums, Certified Related Facilities, and Approved Non-Member Participants. Committee members serve up to two three-year terms and consist of Directors, Vice Presidents (VPs), curators, and registrars. APM Committee develops, oversees, promotes, evaluates, and supports the cooperative animal management, conservation, sustainability, and scientific initiatives of the AZA.

Animal Population Management Committee (APM Committee) Liaison- Each Taxon Advisory Group (TAG) is assigned one member of the Animal Population Management Committee (APM Committee) who serves as a liaison for that TAG. APM Committee members typically serve as a liaison for 3 TAGs. They communicate with the TAG regularly and serve a crucial advisory role for any policy, procedure, or processes questions the TAG may have, and act as the primary contact and mentor during the TAG's Regional Collection Plan (RCP) developmental and review process.

Animal Programs Database- The AZA Animal Programs Database allows anyone to access general information about AZA's Taxon Advisory Groups (TAGs), Species Survival Plan® (SSP) Programs, Studbooks, the individual species included in these AZA Animal Programs (APs), and view Program Leader, Officer and Advisor contact information. AZA members can log in to the AZA Animal Programs Database to gain access to more detailed AP information and have the ability to download Institutional Representative (IR) lists and associated final and draft documents.

Animal Program Summary Table- Animal Program Summary Table identifies each AZA Animal Program (AP) (Species Survival Plan® (SSP) Programs and Studbooks) recommended by the TAG for cooperative management. The following information is included for each AP: the date of the last Breeding and Transfer Plan; the current population size, current gene diversity, designation, and target population size; the number of additional spaces needed to achieve the target population size; and the 5-year population trend, conservation status, and top three goals. This table must be updated as APs are analyzed by the Population Management Center (PMC), a PMC Adjunct or an approved Small Population Management Scientific Advisory Group (SPMAG) Advisor, and is a required component of the TAG Annual Report and the TAG's Regional Collection Plan (RCP).

Association of Zoos and Aquariums (AZA)- Founded in 1924, the Association of Zoos and Aquariums (AZA) is a nonprofit organization dedicated to the advancement of accredited zoos and aquariums in the areas of animal care, wildlife conservation, education and science. AZA is America's leading accrediting organization for zoos and aquariums and accredits only those facilities that have achieved rigorous standards for animal care, education, wildlife conservation and science.

AZA Animal Program- AZA Animal Programs (APs) include Taxon Advisory Groups (TAGs), Species Survival Plan® (SSP) Programs and Studbook Programs. APs are responsible for the extraordinary leadership, development,

oversight, promotion, evaluation and support of AZA's cooperative animal management, conservation, and scientific initiatives. Management tools, databases, reference materials, policies, and management plans have been developed to facilitate exceptional AP collaboration within and amongst AZA-accredited facilities.

AZA Brand/Branded The signature for the Association of Zoos & Aquariums is a unique piece of artwork that has been designed specifically for our brand. Consisting of the AZA wordmark and the AZA ampersand symbol, the signature is an extremely valuable asset and the most concise visual representation of our brand.

AZA Board Approved Policies- AZA policies may be drafted by AZA Committees, Scientific Advisory Groups (SAGs), and Animal Programs (APs) in collaboration with their AZA Staff and Board Liaisons but all AZA-related policies must be approved by the AZA Board of Directors before being finalized, published, or distributed. AZA policies may cover topics such as animal management, animal programs, conservation, ethics, health, husbandry and welfare, research and technology, and safety.

AZA Dedicated Funds Account- AZA Committees, Scientific Advisory Groups (SAGs), Taxon Advisory Groups (TAGs), Species Survival Plan® Programs, and SAFE Species Programs who hold and distribute money raised specifically to support projects initiated or coordinated by their group must use an AZA Dedicated Funds to manage all transactions.

AZA Mission- The Association of Zoos & Aquariums (AZA) provides its members the services, high standards and best practices needed to be leaders and innovators in animal care, wildlife conservation and science, conservation education, the guest experience, and community engagement.

AZA Network- The Association of Zoos & Aquariums' online private social networking tool.

AZA Policy for Full Participation- AZA policy stating that all AZA-accredited facilities and Certified Related Facilities having a Green SSP animal in their collection are required to participate in the collaborative SSP planning process (e.g., provide relevant animal data to the AZA Studbook Keeper, assign an Institutional Representative (IR) who will communicate institutional wants and needs to the SSP Coordinator, comment on the draft plan during the 30-day review period, and abide by the recommendations agreed upon in the final plan). All AZA member facilities and Animal Programs (APs), regardless of management designation, must adhere to the AZA Acquisition, Transfer and Transition Policy, as well as the AZA Code of Professional Ethics.

AZA Strategic Plan- AZA accredited zoos and aquariums will be recognized for leading a compelling wildlife conservation movement. We will achieve this by caring for wildlife and wild places; educating and engaging public, professional and government audiences; serving and increasing membership; and developing a robust and sustainable economic model which empowers AZA to provide superlative member services.

Breeding and Transfer Plans- Breeding and Transfer Plans (BTPs) summarize the current demographic and genetic status of a Species Survival Plan® (SSP) Program, describe the SSP Program management designation, and recommend breeding pairs and transfers. Breeding and Transfer Plans are designed to maintain a healthy, genetically diverse and demographically stable population.

Candidate Programs- TAG managed Animal Programs are not considered official AZA cooperatively managed Animal Programs; however the TAG has the goal to grow these populations to meet minimum criteria to be an SSP Program. Candidate Programs manage smaller populations (19 or fewer individual animals), and/or manage populations among only one or two participating AZA member facilities. New Animal Programs that do not have a published AZA Regional Studbook will also be classified as Candidate Programs until an AZA Regional Studbook is published.

Certified Related Facilities- Organizations holding wildlife that are not commercial entities, and are not open to the public on a regularly scheduled, predictable basis. The facility shall be under the direction of a professional staff trained in animal husbandry, and shall be further defined as having conservation and preservation as part of its mission—a mission that shall have a beneficial, tangible, supportive impact on the zoological and aquarium professions. This includes wildlife ranches, wildlife refuges or rehab centers, research facilities, survival centers, breeding farms, and/or similar organizations.

Conservation Grants Fund (CGF) - Established in 1984, CGF supports the cooperative conservation-related scientific and educational initiatives of AZA and AZA-accredited zoos and aquariums and their collaborators. CGF grants are awarded in six categories: Animal Health, Animal Welfare, Conservation Education, Field Conservation and/or Reintroduction, Management and/or Breeding, Research.

Conservation Partner- Organizations that support the vision, mission and goals of zoos and aquariums. Conservation Partners represent AZA-Accredited Facility member societies and associated organizations, professional societies, conservation organizations, universities, some government entities and other non-profits.

***Ex situ* Conservation**- Preservation of species outside of their native habitat.

Global Species Management Plan (GSMP)- GSMPs are formal, international population management plans among a minimum of two regional zoological associations, and are overseen by WAZA. GSMPs are a valuable partnership when population goals for increasing sustainability cannot be met within a single region. A GSMP provides an opportunity to combine several regional populations, thus improving the genetic and demographic management potential by increasing the population's size, carrying capacity, and other resources.

Green Species Survival Plan® (Green SSP) Program- A Green SSP Program has a population size of 50 or more animals and is projected to retain 90% gene diversity for a minimum of 100 years or 10 generations. Green SSP Programs are subject to AZA's Full Participation and Sustainability Partner Policies.

International Studbook- The World Association of Zoos and Aquariums' (WAZA) Committee of Population Management (CPM) administers and provides oversight to International Studbooks. International Studbooks provide a valuable service to the zoological community by offering the most complete and accurate global data on the *ex situ* population's pedigree and demography, if possible including husbandry and veterinary guidance, and enhancing management of the *ex situ* population through analysis of the International Studbook data.

***In situ* Conservation**- Preservation of natural communities and populations of species in the wild.

Institutional Liaison (IL)- The Institutional Liaison (IL) assures that there is effective communication and participation between the facility and AZA's Animal Programs (APs). The IL designates Institutional Representatives (IRs), keeps the facility's IR list current, and is responsible for updating IR contact information on the AZA website. The IL serves as the default IR for any AP which does not have an IR assigned and is required to respond accordingly. The IL works with Program Leaders and IRs to assure that their facility fully participates in all associated Taxon Advisory Groups (TAGs) and Species Survival Plan® (SSP) Programs, and if necessary, will meet in conflict resolution processes.

Institutional Representative (IR)- The Institutional Representative (IR) is the primary contact between his/her facility and the Program Leader of the Animal Programs (APs) to which s/he has been designated. The IR is responsible for maintaining open communication between the AP and the facility, communicating to the Program Leader on behalf of the facility, and participating in the AP communications and activities.

Management Group- At a minimum, the Management Group is composed of the Coordinator, Vice Coordinator, and AZA Regional Studbook Keeper. The Management Group serves as the voting body for Species Survival Plan® (SSP) Program business and all members are integrally involved in the SSP Program appointments, publications, and meetings. Management Group members must be elected from the SSP Program's Institutional Representative (IRs).

MateRx- The primary output is a matrix of genetic ratings for every possible breeding pair in a population which allow Program Leaders to quickly discover how the genetic status of animals in their collections compare to the rest of a managed population. Note that this does not include any demographic, logistic, or other variables that should be considered when recommending breeding.

Monthly Animal Programs Update- AZA's Monthly Animal Programs Update contains information about the most recent news pertaining to Animal Programs (APs), Professional Development Courses, workshops, conferences, meetings, funding and award opportunities, new Program Leaders, Program Leader vacancies, new publications, and information regarding Breeding and Transfer Plans.

Officer- Officer positions for an Animal Program (AP) include the Program Leader Taxon Advisory Group (TAG) Vice Chair or Species Survival Plan® (SSP) Program Vice Coordinator, TAG or SSP Secretary, and if any financial components are incorporated into the Animal Program, a TAG or SSP Treasurer. Officers, with the exception of the TAG Chair or SSP Coordinator, are elected from the TAG Steering Committee or SSP Management Group and the Steering Committee/Management Group forms the electorate for that vote.

PMC Adjunct- PMC Adjunct Population Biologists are advisors that are approved by AZA and advise AZA Animal Programs from their home facilities. PMC Adjuncts provide many services for AZA Animal Programs including producing Breeding and Transfer Plans, providing informal genetic or demographic advice between plans, investigating unknown or partially-known pedigrees, developing pedigree assumptions and creating analytical studbooks, conducting research and helping to develop software to improve methods of population management, and troubleshooting software problems.

PMCTrack- PMCTrack is a web-based database and monitoring system designed to evaluate the outcomes of breeding and transfer recommendations made through the AZA Animal Programs (APs) such as Species Survival Plan® (SSP) Programs. PMCTrack provides the necessary tools and data to understand, monitor, and improve AZA's cooperative population management system. PMCTrack includes survey functionality to request additional information from facilities on the information needed for preparing for SSP Breeding and Transfer Plans (wants/needs, reasons for unfulfilled outcomes).

Population Management Center (PMC)- The AZA Population Management Center (PMC) hosted by the Lincoln Park Zoo in Chicago, Illinois, as well as San Diego Zoo Global in San Diego, California, is responsible for conducting demographic and genetic analyses needed to develop and distribute population management recommendations for all SSP Programs. PMC staff, including Population Biologists, Planning Coordinator, and Research Assistant, assist each SSP in the development of their population management plans by making sure the data are accurate, determining the current population status, predicting the future population status, identifying specific breeding and transfer recommendations, and distributing the plan to all participating AZA-accredited facilities. In addition, the PMC contributes valuable information for AZA Sustainability Reports and Regional Collection Plans (RCPs).

Population Sustainability- AZA's cooperatively managed Animal Programs reach population sustainability when the projected gene diversity (% GD) at 100 years or 10 generations is greater than or equal to 90%. The SSP Breeding and Transfer Plan for each species makes recommendations to maintain demographically stable populations with the greatest possible genetic diversity for the long-term future of a healthy and sustainable population. Sustainability of the population is related to many factors including its gene diversity, demographic stability, husbandry expertise, etc.

Population Viability Analysis (PVA)- A PVA is a computer model that projects the likely future status of a population. PVAs are used for evaluating long-term sustainability, setting population goals, and comparing alternative management strategies. Several quantitative parameters are used in a PVA to calculate the extinction risk of a population, forecast the population's future trajectory, and identify key factors impacting the population's future.

Program Leader- Program Leaders include Taxon Advisory Group (TAG) Chairs, Species Survival Plan® (SSP) Program Coordinators, AZA Regional Studbook Keepers, and Candidate Program Leaders.

Publish- An SSP Breeding and Transfer Plan, AZA Regional Studbook, Population Viability Analysis, MateRx, or a TAG Regional Collection Plan is considered published once the document is posted on that Animal Program's page in the AZA Animal Programs Database.

Red Species Survival Plan® (Red SSP) Program- A Red Species Survival Plan® (SSP) Program has a population size of twenty or more animals managed among three or more participating AZA facilities. If a population does not meet these minimum criteria, but has an IUCN designation of Critically Endangered, Endangered, or Extinct in the Wild, and the TAG has developed three goals to sustain this population, then the population will be considered a Red SSP Program. Red SSPs cannot retain 90% gene diversity for 100 years or 10 generations and participation by AZA facilities is voluntary. Red SSP Programs are subject to AZA's Sustainability Partner Policy.

Regional Collection Plan (RCP)- Taxon Advisory Groups (TAGs) develop Regional Collection Plans (RCPs) to recommend species for cooperative management among the Association of Zoos and Aquariums (AZA) member facilities, determine the sustainability goals for each recommended Animal Program (AP) within its purview, identify objectives relevant to their long-term collection plans, and assure adherence to AZA's animal management and conservation goals.

Reproductive Management Center (RMC)- The AZA Reproductive Management Center (RMC), hosted by the Saint Louis Zoo, is responsible for assessing factors such as contraception type efficacy, reversibility, and safety; an animal's age, reproductive status, behavioral and social needs, and delivery system practicality when recommending appropriate contraception methods for the animals cared for in AZA-accredited facilities.

Scientific Advisory Group (SAG)- Established in 1991, Scientific Advisory Groups (SAGs) help facilitate, support, network and coordinate the relevant research activities of its member facilities. SAGs are made up of experts in a particular field of wildlife science. Members include veterinarians, researchers and zoo- and aquarium-based curators with appropriate scientific training, as well as university, government and other outside scientists with a commitment to sharing their particular expertise.

Small Population Management Scientific Advisory Group (SPMAG)- A Scientific Advisory Group (SAG) that provides technical advice pertaining to population management for AZA Animal Programs. SPMAG helps advance the science of applied small population biology and develops tools for use by small population managers.

Species Survival Plan® (SSP) Program- An AZA SSP Program is an AZA cooperatively managed program that strives to manage an *ex situ* species population with the interest and cooperation of AZA-accredited facilities; is identified through documented demand and potential sustainability within the AZA community; is selected by TAGs through the RCP process; and develops a Breeding and Transfer Plan that identifies population goals and recommendations to manage a genetically diverse, demographically varied, and biologically sound population. Success is achieved when SSP animals are available to meet program goals and come from biologically sound populations as a result of a shared commitment to cooperative populations and program management.

Species Survival Plan® (SSP) Coordinator- An Species Survival Plan® (SSP) Program Coordinator performs various duties to lead and support the AZA SSP program. The SSP Coordinator works with Institutional Representative (IRs), the AZA Regional Studbook Keeper (if different from the Coordinator), the Taxon Advisory Group (TAG), the Animal Population Management Committee (APM Committee), and the AZA Conservation, Management, and Welfare Sciences Department, as well as any associated governmental agencies, to develop, oversee, promote, and support the cooperative animal management, conservation, and research initiatives of the SSP Program. The primary responsibility of the SSP Coordinator is to regularly complete and distribute an SSP Breeding and Transfer Plan for the managed population.

SSP Sustainability Report- An automatically generated 5-page report that summarizes husbandry practices, exhibit management, species appeal, educational opportunities, multi-species exhibit considerations, species biology, SSP population dynamics, management priorities, challenges to sustainability, and research needs. The report is a compilation of the SSP Coordinator's expertise and the current and projected population summaries from the SSP Breeding and Transfer Plan or PVA.

SSP Sustainability Search Portal- An online tool for collection planners, Program Leaders, ILs, IRs, research scientists, and other zoo and aquarium staff. The searchable format allows collection planning users to perform searches that identify appropriate species for their collection planning criteria, while also directing resources and attention to managed species.

Statement of Individual Commitment- A signed statement by the potential new Animal Program (AP) officer to show that the individual is willing and able to meet the commitments and responsibilities of the AP and leading the group in its mission.

Statement of Institutional Support- A signed statement by the potential new Animal Program (AP) officer's facility to show that the facility is willing and able to support this individual in meeting the commitments and responsibilities of the AP and leading the group in its mission.

Steering Committee- The Steering Committee serves as the voting body for Taxon Advisory Group (TAG) business, and all members are integrally involved in TAG decision making, appointments, publications and meetings. The Steering Committee is composed of 5-15 members, including Officers. Each TAG may determine the optimal size and management of its Steering Committee.

Studbooks- An AZA Regional Studbook dynamically documents the pedigree and entire demographic history of each individual in a population of species. These collective histories are known as the population's genetic and demographic identity and are invaluable tools that track and manage each individual cared for in AZA-Accredited Zoos and Aquariums, Certified Related Facilities and by Approved Sustainability Partners as part of a single *ex situ* population.

Studbook Keeper- The AZA Regional Studbook Keeper is responsible *for maintaining an accurate record of the histories of all individual animals in an ex situ population.* The AZA Regional Studbook Keeper works directly with the associated Taxon Advisory Group (TAG) and Species Survival Plan® (SSP) Program, all participating AZA member facilities, the Animal Population Management Committee (APM Committee), Population Management Center (PMC), a PMC Adjunct, or an approved Small Population Management Advisory Group (SPMAG) advisor, and the AZA Conservation, Management, and Welfare Sciences Department to complete and distribute a timely and accurate AZA Regional Studbook to be used for demographic and genetic analyses relevant to the SSP Program's population management.

Sustainability Designations- An initial Studbook, or a Population Viability Analysis (PVA), Breeding and Transfer Plan, or MateRx determines an Animal Program's (AP's) designation. Sustainability Designations include Green Species Survival Plan® (SSP) Programs, Yellow SSP Programs, and Red SSP Programs. This list is updated quarterly on the Association of Zoos and Aquariums (AZA) website.

Sustainability Partners- AZA Animal Population Management Committee (APM Committee) approved wildlife facilities that regularly exchange animals with AZA-accredited facilities and certified related facilities, typically as part of the Species Survival Plan® (SSP) Program Breeding and Transfer Plan or other SSP Program management process.

Target Population Size (TPS)- The desired number of SSP animals to be held across AZA and approved partner facilities over a specific, stated timeframe. This number is determined with consideration for program roles and goals (genetic, demographic, and others), logistical constraints, spatial competition with other TAG-managed species, and other population-specific concerns. Target Population Size is determined by the Taxon Advisory Group (TAG) and published in their Regional Collection Plan (RCP).

Taxon Advisory Group (TAG) Annual Report- Taxon Advisory Group (TAG) Annual Reports update the Animal Population Management Committee (APM Committee) and the Association of Zoos and Aquariums (AZA) Conservation, Management, and Welfare Sciences Department on the conservation work of the TAG, and the Animal Programs (APs) within the TAG's purview. TAG Annual Reports provide the Chair an opportunity to document and communicate any potential issues within the TAG's programs, and allow an opportunity for the TAG to submit AP meeting minutes and other materials to AZA on an annual basis. Reports are due to the AZA Conservation, Management, and Welfare Sciences Department July 15 of each year.

Taxon Advisory Group (TAG)- Established in 1990, Taxon Advisory Groups (TAGs) examine the conservation and management needs of entire taxa, or groups of related species. TAGs establish priorities for management, research, and conservation. TAGs select appropriate species for AZA conservation and management programs and provide a forum for discussing husbandry, veterinary, ethical, and other issues that apply to entire taxa.

Taxon Advisory Group (TAG) Chair- The primary responsibility of the Taxon Advisory Group (TAG) Chair is to assure the completion and distribution of a Regional Collection Plan (RCP). Additional responsibilities include leadership of the TAG, organization of its members, oversight and consistent communication with all Animal Programs within the TAG's purview (Species Survival Plan® (SSP) Program, AZA Regional Studbooks, and Candidate Programs), the Institutional Liaisons (ILs), Institutional Representatives (IRs), and reporting to the Animal Population Management Committee (APM Committee). The TAG Chair serves as the primary contact and AZA expert for the taxon and abides by the duties and responsibilities defined for the position.

Yellow Species Survival Plan® (Yellow SSP) Program- A Yellow Species Survival Plan® (SSP) Program has a population size of 50 or more animals but cannot retain 90% gene diversity for 100 years or 10 generations. Yellow SSP participation by AZA facilities is voluntary. Yellow SSP Programs are subject to AZA's Sustainability Partner Policy.

Appendix A: Policy on Full Participation in SSPs

Policy for Full Participation in the Species Survival Plan®

Cooperative animal management and conservation are among the primary goals of the Association of Zoos & Aquariums (AZA). These goals are best exemplified by the Association's shared commitment to its cornerstone animal management and conservation program: the Species Survival Plan® (SSP). The AZA Board of Directors recognizes that: 1. Cooperative animal management is vital to the long-term survival of professionally managed zoological parks and aquariums and their valuable and often irreplaceable live animal collections; and 2. All AZA-accredited institutions and Certified Related Facilities should be fully committed to the animal management, conservation, and public education goals as well as the collaborative spirit of the SSP partnership. Therefore, in 2000, the Board adopted the first policy of Full Participation in the SSP program by all AZA member institutions.

An SSP Breeding and Transfer Plan articulates long- and short-term goals for a population. It plans the "family tree" of each managed population to minimize the rate of loss of genetic diversity and maintain the long-term demographic stability of the population. Breeding and other population management recommendations are made for each animal with consideration of logistical feasibility, animal welfare, and other factors that can improve SSP outcomes. In addition to breeding recommendations, Breeding and Transfers also include a recommendation not to breed certain animals for sound husbandry reasons and the betterment of the population. The Board recognizes that, in the collaborative process of managing the SSPs, the responsibility of each SSP Management Group is to make sound Breeding and Transfer Plan recommendations, and also recognizes that, at times, these may conflict with a member institution's plans.

The Board emphasizes the responsibility of all institutions to cooperate in SSP Master Planning. If differences occur between an SSP's recommendations and a participating institution, the SSP Coordinator and the IR have a joint responsibility to work collaboratively to resolve it. When an SSP recommendation is fundamental to the collaborative management of the ex situ population, then the SSP recommendation should take precedence. In this process, all institutions' clearly stated and reasonable needs will be considered. If an SSP recommendation is not fundamental to the collaborative management of the ex situ population, then the SSP Management Group may elect to change it before the Breeding and Transfer Plan is finalized. Thus, when an SSP Breeding and Transfer Plan is approved its animal management recommendations will accurately reflect the vital needs of both the SSP and the participating institutions.

The Policy for Full Participation in the SSP Program assures that AZA Accredited Institutions and Certified Related Facilities have input into the SSP Master Planning process and that they fully comprehend, agree to, and follow the final SSP recommendations. The Board now further defines Full Participation in the SSP program, and the processes used to achieve Full Participation, as follows:

- The Institutional Liaison (IL) at AZA Accredited Institutions or Certified Related Facilities will assure that an Institutional Representative (IR) is appointed for each SSP species the institution/facility owns or holds, or for which the institution selects to support as defined by the SSP Management Group.
- Each IR must serve as the primary point of contact for all matters relating to their assigned SSP and will assure that their institution responds to SSP needs for information during Master Planning.
- Periodically and regularly, the SSP Coordinator will ask each participating institution's IR how their institution will participate in the SSP: breeding, non-breeding (where an institution cannot breed due to space, or other factors), or support.
- Prior to the Breeding and Transfer Plan development, at the request of the SSP Coordinator, each IR will provide all relevant data regarding individual SSP animals to the corresponding SSP Coordinator and Studbook Keeper in a timely manner. Further, IRs must assure that all proposed acquisitions or dispositions of the SSP species are included in the SSP Breeding and Transfer Plan or, if the Breeding and Transfer Plan is already published, are approved in advance by the SSP Coordinator or, preferably the SSP Management Group. SSP Coordinators and IRs must work collaboratively to develop an SSP Breeding and Transfer Plan that strives to meet the needs of the SSP program and the needs of participating institutions.
- A draft of the SSP Breeding and Transfer Plan, which must include a written record of all animal management recommendations, will be published on the AZA web site for a 30-day comment period and the SSP Coordinator will notify all IRs as soon as the Plan is available for comment. IRs at all participating institutions must inform the SSP Coordinator during the comment period that they will adhere to the Breeding and Transfer Plan recommendations, or why they cannot, which will initiate the resolution discussions described below. If all participants agree with the recommendations, the final Breeding and Transfer Plan will be published and implemented.

- Each IR must assure that their institution's Director and IL are aware of the Breeding and Transfer Plan and its recommendations and must initiate a collaborative discussion with the SSP Coordinator to resolve differences regarding Breeding and Transfer Plan recommendations during the comment period. All involved should maintain accurate records of all related communications and discussions.
- If a resolution with no change to the SSP recommendations is found, then the final Breeding and Transfer Plan will be published and implemented.
- If a resolution that causes changes in the SSP recommendations is reached, the edited Breeding and Transfer Plan will be re-posted for a final 30-day comment period. IRs at institutions affected by the edited recommendation(s) must respond to the SSP Coordinator during the final comment period regarding their agreement to adhere to the recommendations; institutions not affected by the changes will not need to respond again. At this stage, the finalized Breeding and Transfer Plan will be published and all institutions agreeing to adhere to the Breeding and Transfer Plan's recommendations will commence implementing the Breeding and Transfer Plan.
- If no resolution is found through direct discussion between the SSP Coordinator and the IR(s), they must work cooperatively with the IL, institutional Director, and corresponding TAG Chair to find one. If necessary, the discussion can extend for an additional 30 days, during which time the institution disputing a recommendation must not engage in any breeding or acquisitions and / or dispositions of species that run counter to the SSP recommendations.

If differences are not resolved by the steps outlined above, then the SSP Coordinator and / or any other involved parties must request that AZA's Animal Population Management Committee (APM Committee) mediate the situation as defined in the AZA Animal Management Reconciliation Policy and, again, the institution disputing the recommendation must not engage in any breeding, acquisitions and / or dispositions that run counter to the SSP recommendations until the mediation and, if necessary, the reconciliation process is complete. Emergencies or other extraordinary circumstances will be considered for the health and welfare of the animals. Institutions not affected by the disagreement will continue carrying out their recommendations.

Approved by the AZA Board of Directors 26 Mar 09

Appendix B: AZA Animal Management Reconciliation Policy

Species Survival Plan® – Animal Management Reconciliation Policy

The success of cooperative breeding programs depends on all institutions supporting Species Survival Plan® (SSP) recommendations. Therefore, the Board emphasizes the crucial nature of the cooperative process in the development of SSP Breeding and Transfer Plans to assure that animal management recommendations accurately reflect the vital needs of both the SSPs and participating Accredited Institutions and Certified Related Facilities.

If differences regarding SSP recommendations occur between the SSP Management Group and a member Institution, AZA's Full Participation Policy clearly articulates the process that both parties must utilize to resolve them prior to engaging in the Animal Management Reconciliation process. However, if such differences cannot be resolved, then the parties involved must request that AZA's Animal Population Management Committee (APM Committee) mediate the situation.

- APM Committee will (1) determine if all efforts to resolve differences have been exhausted and, (2) determine if the recommendations in question are fundamental to the cooperative management of the *ex situ* population. If both situations are true, then APM Committee will notify all parties and appoint a Mediation Task Force which includes the APM Committee Chair / designee, one member of APM Committee selected by each party to represent them, the SSP Coordinator, the institution's Director and two other institutional representatives, and AZA's VP of Animal Conservation, or designee.
- The Mediation Task Force will conduct a confidential review of the situation in less than 30 days. Within 2 weeks of the completed review, the APM Committee Chair / designee will draft a mediation report describing a consensus decision, which will be reviewed by the participating parties. Comments on the draft report must be returned within a week of distribution. The APM Committee Chair / designee will consider all comments and produce a final mediation report. Assuming a resolution is reached, the report will be submitted to all participants involved in the process and the matter will be closed.
- If the mediation process yields no resolution, APM Committee must notify all parties and initiate the reconciliation process, during which the institution in question must not engage in any breeding, acquisitions and / or dispositions that run counter to the SSP until a resolution is found. The Reconciliation Committee, over which the APM Committee Chair / designee presides, will include the institution's Director or designee, the APM Committee Board Liaison, and AZA's Sr. VP of Conservation, VP of Animal Conservation, or designee, and Executive Director, or designee. The Reconciliation Committee will consider the Mediation Task Force report and determine if additional information is required.
- In its call for greater accountability, the AZA Board holds that action by the Accreditation Commission and / or the Ethics Board can be taken against a member institution that: (1) demonstrates a pattern of a failure to participate and / or (2) demonstrates an action contrary to an SSP program recommendation which threatens the short- or long-term management of the *ex situ* population. Therefore, the Reconciliation Committee will specifically consider if either of these instances is found to be valid.
- If it is determined that the member institution's action is not detrimental to the cooperative management of the *ex situ* population, then the Breeding and Transfer Plan will be changed accordingly and the results of these findings will be incorporated into a reconciliation final report submitted to the AZA Conservation Office.
- If it is determined that the member institution's action is detrimental to the cooperative management of the *ex situ* population, and / or is part of a pattern of a failure to participate, then the Breeding and Transfer Plan will stand as is and the Reconciliation Committee will notify the institution that they must comply with it. If the institution refuses this directive, the Reconciliation Committee will note this in the reconciliation final report filed with AZA's Conservation Office and provide the report to the Accreditation Commission and the Ethics Board for consideration.

Approved by the AZA Board of Directors 26 Mar 09

Appendix C: SSP Coordinator Application

Individuals interested in becoming an SSP Coordinator must complete the following application and submit it to The TAG Chair, or if the TAG does not have a current RCP or there is no TAG Chair, the AZA Conservation, Management, and Welfare Sciences Department.

*Please note that this application is available in a digitized Word form at https://www.aza.org/animal-program-applications

1. Applicant Name: _____

   AZA supporting facility: _____

   Are you an AZA Individual Member? _____

   Phone: _____

   Email: _____

   Date Application Submitted: _____

2. Common and Scientific name(s) of the species: _____

3. Which TAG oversees this SSP? _____

4. Name of the current AZA Regional Studbook Keeper, if other than you: _____

5. Date of program's last Breeding & Transfer Plan: _____

6. List all other AZA Program Leader positions (e.g., Studbook Keeper, SSP Coordinator, etc) you hold or have held, and the most recent publication date of relevant Animal Program documents (e.g., Studbook, Breeding and Transfer Plan, RCP).

Program Leader Position	Term dates	Publication	Date Last published
E.g.: XX Studbook Keeper	*2010 – present*	*Studbook*	*12 May 2014*

7. Attach a current *curriculum vitae*.

SSP Coordinator Statements of Commitment and Support

AZA SSP Coordinators and their supporting facilities must be willing and able to devote the necessary resources to oversee and manage an AZA Species Survival Plan®. As outlined in the AZA Species Survival Plan® Program Handbook these duties and responsibilities include:

- Publishing a complete Breeding and Transfer Plan with the PMC, a PMC Adjunct, or an Approved SPMAG member at least every three years after initial Breeding and Transfer Plan publication.
- Communicating any SSP Program data updates to the TAG Chair.
- Ensuring that SSP Program participants fully understand and abide by the AZA Policy on Responsible Population Management, the AZA Code of Professional Ethics, and the Sustainability Partner Policy.
- Ensuring that Green SSP Program participants fully understand and abide by the AZA Policy for Full Participation in the SSP Program and the AZA Animal Management Reconciliation Policy.
- Ensuring that all holding facilities have a designated IR, and that this designation has updated on the AZA website. Contact the Institutional Liaison (IL) when a discrepancy between IR designations is found between the SSP Coordinator's IR list and the IR list on the AZA website.
- Ensuring that all Officers and Management Group members update their personal information to the TAG Chair and on the AZA website.
- Sending copies of all significant SSP Program documents to the AZA Conservation, Management, and Welfare Sciences Department and TAG Chair.
- Maintaining regular contact with and respond in a timely fashion to inquiries/questions/concerns from SSP Program members, the TAG Chair, and the AZA office.
- Understanding that failure to meet these obligations and those outlined within the AZA Species Survival Plan® Program Handbook could result in removal from the Animal Program.

The _____ (Name of facility) is committed to providing the necessary resources to oversee and manage the AZA _____ SSP program as outlined above. This may include:

- Access to computers and software necessary for database management, assembling a complete Breeding and Transfer Plan for distribution and communication via email.
- Funding for travel to professional meetings, workshops or to meet with Population Advisors.
- Scheduled time within routine work schedules to accomplish Animal Program related tasks.

The above-named facility further acknowledges that information gathered for SSP Programs supported by the facility is not the exclusive property of the facility and enters public domain upon publication on the AZA website. The facility also understands that part of any SSP Program Officers' responsibility is to promote the development of sustainable populations.

Name of Applicant: _____

Date: _____

The following will serve as your digital signature:
I, _____ (Name of Applicant) have read and agree to the terms and conditions stated above.

Name of the Director/Governing Official: _____

Date: _____

The following will serve as your digital signature:
I, _____ (Name of Director/Governing Official) have read and agree to the terms and conditions stated above

Appendix D: SSP Coordinator and Regional Studbook Keeper Application

Individuals interested in becoming an SSP Coordinator and Studbook Keeper must complete the following application and submit it to the TAG Chair, or if the TAG does not have a current RCP or there is no TAG Chair, the AZA Conservation, Management, and Welfare Sciences Department.

*Please note that this application is available in a digitized Word form at https://www.aza.org/animal-program-applications

1. Applicant Name: _____

   AZA supporting facility: _____

   Are you an AZA Individual Member? _____

   Phone: _____

   Email: _____

   Date Application Submitted: _____

2. Common and Scientific name(s) of the species: _____
3. Which TAG oversees this SSP? _____
4. Date of program's last Breeding & Transfer Plan: _____
5. Date of program's last Studbook: _____
6. List all other AZA Program Leader positions (e.g., Studbook Keeper, SSP Coordinator, etc) you hold or have held, and the most recent publication date of relevant Animal Program documents (e.g., Studbook, Breeding and Transfer Plan, RCP).

Program Leader Position	Term dates	Publication	Date Last published
E.g.: XX Studbook Keeper	*2010 – present*	*Studbook*	*12 May 2014*

7. Attach a current *curriculum vitae*.

SSP Coordinator and Studbook Keeper Statements of Commitment and Support

AZA SSP Coordinators and Studbook Keepers and their supporting facilities must be willing and able to devote the necessary resources to oversee and manage an AZA Species Survival Plan®. As outlined in the AZA Species Survival Plan® Program and AZA Regional Studbook Keeper Handbooks these duties and responsibilities include:

- Completing the Population Management 1 (PM1) Professional Development course.
- Creating, updating and submitting a current AZA Regional Studbook report to the AZA Conservation, Management, and Welfare Sciences Department for publication on the AZA website.
- Submitting a complete, current AZA Regional Studbook database to the AZA Conservation, Management, and Welfare Sciences Department and the PMC at least once every three years. In the event of loss of employment/resignation, assure that the Studbook Keeper and/or supporting facility provide relevant AZA Regional Studbook documents to the TAG Chair and to the replacement AZA Regional Studbook Keeper
- Providing an up to date AZA Regional Studbook database to the SSP Program's Population Advisor (PMC, PMC Adjunct, SPMAG Advisor) prior to each formal population planning meeting, or as needed for population management purposes.
- Publishing a complete Breeding and Transfer Plan with the PMC, a PMC Adjunct, or an Approved SPMAG member at least every three years after initial Breeding and Transfer Plan publication.
- Ensuring that SSP Program participants fully understand and abide by the AZA Acquisition, Transfer, and Transition Policy and the AZA Code of Professional Ethics, both of which apply to all AZA Animal Programs.
- Ensuring that Green SSP Program participants fully understand and abide by the AZA Policy for Full Participation in the SSP Program, the AZA Animal Management Reconciliation Policy and the AZA SSP Sustainability Partner Policy.
- Maintaining regular contact with and respond in a timely fashion to inquiries/questions/ concerns from SSP Program members, the TAG Chair, and the AZA office.
- Understanding that failure to meet these obligations and those outlined within the AZA Species Survival Plan® Program and AZA Regional Studbook Keeper Handbooks could result in removal from the Animal Program.

The _____ (Name of facility) is committed to providing the necessary resources to oversee and manage the AZA _____ SSP and Studbook as outlined above. This may include:

- Funding to attend Population Management 1 and 2.
- Access to computers and software necessary for database management, assembling a complete Breeding and Transfer Plan and AZA Regional Studbook for distribution and communication via email.
- Funding for travel to professional meetings, workshops or to meet with Population Advisors.
- Scheduled time within routine work schedules to accomplish Animal Program related tasks.

The above-named facility further acknowledges that information gathered for SSP Programs supported by the facility is not the exclusive property of the facility and enters public domain upon publication on the AZA website. The facility also understands that part of any AZA Animal Program Leader's responsibility is to promote the development of sustainable populations.

Name of Applicant: _____

Date: _____

The following will serve as your digital signature:
I, _____ (Name of Applicant) have read and agree to the terms and conditions stated above.

Name of the Director/Governing Official:
_____

Date: _____

The following will serve as your digital signature:
I, _____ (Name of Director/Governing Official) have read and agree to the terms and conditions stated above.

Appendix E: Statements of Commitment and Support for Officers

*Please note that these statements are available in a digitized Word form in the Program Leader Applications at https://www.aza.org/animal-program-applications

Statement of Individual Commitment for SSP Vice Coordinator, Secretary, or Treasurer

As the _____ position of the _____ SSP Program, I am willing and able to devote the necessary time to fulfill the deadlines, commitments and responsibilities as outlined in the SSP Program Handbook. I understand that failure to meet these obligations could result in my removal from the SSP Program.

Name of Applicant: _____

Signature of Applicant: _____

Date: _____

Statement of Institutional Support for Vice Coordinator, Secretary, or Treasurer

The _____ (Name of Facility) is committed to providing adequate resources and support for the _____ position of the _____ SSP Program as outlined in the SSP Program Handbook. I understand that failure to meet these obligations could result in his/her removal from the SSP Program.

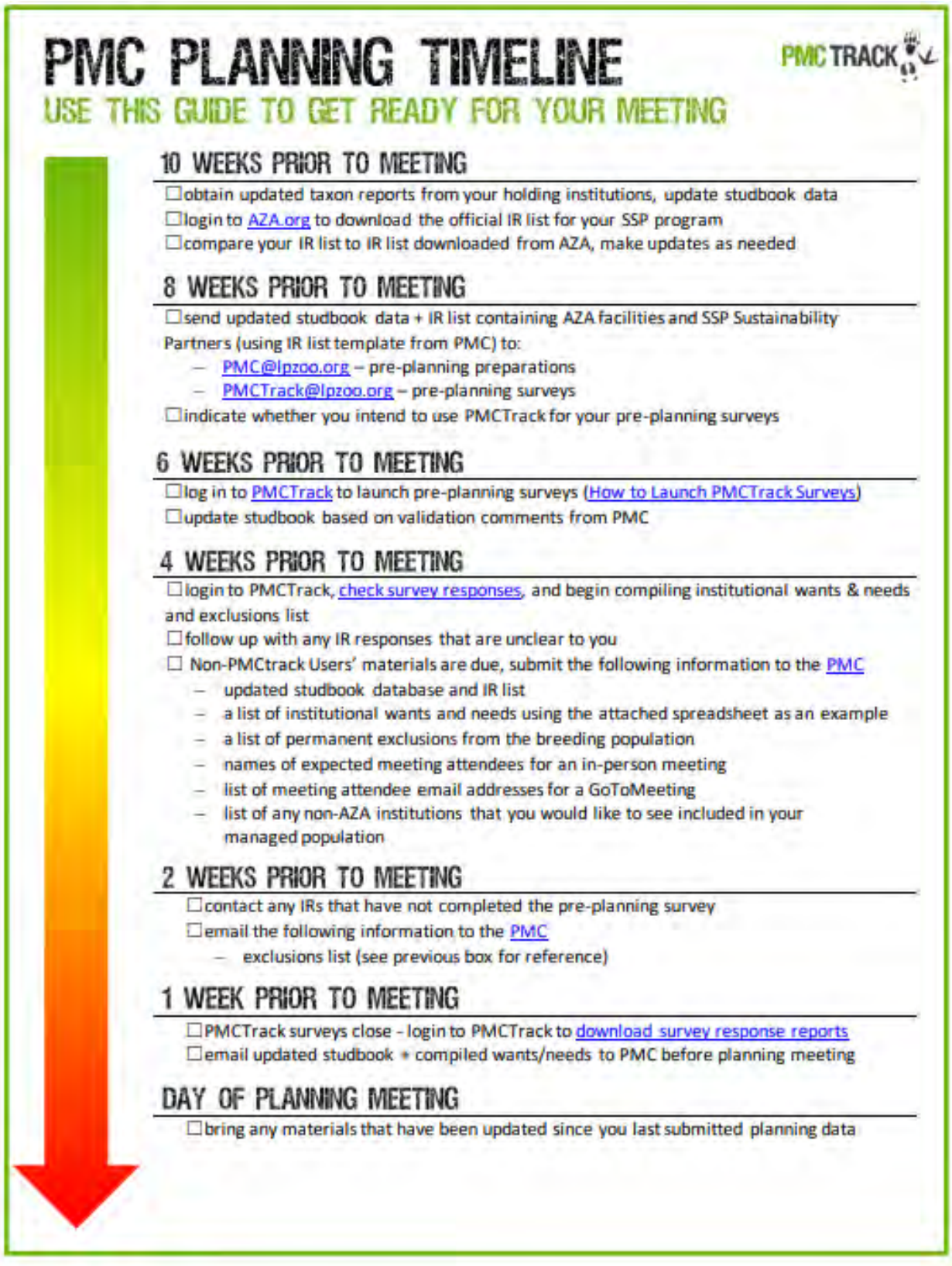
The above-named facility further acknowledges that information gathered for SSP Programs supported by the facility is not the exclusive property of the facility and enters public domain upon publication on the AZA website. The facility also understands that part of any SSP Program Officers' responsibility is to promote the development of sustainable populations.

Name of the Director/Governing Official: _____

Signature of Director/Governing Official: _____

Date: _____

Appendix F: Preparing for a Planning Meeting with the PMC



PMC PLANNING TIMELINE

PMC TRACK

USE THIS GUIDE TO GET READY FOR YOUR MEETING

10 WEEKS PRIOR TO MEETING

- ☐ obtain updated taxon reports from your holding institutions, update studbook data
- ☐ login to AZA.org to download the official IR list for your SSP program
- ☐ compare your IR list to IR list downloaded from AZA, make updates as needed

8 WEEKS PRIOR TO MEETING

- ☐ send updated studbook data + IR list containing AZA facilities and SSP Sustainability Partners (using IR list template from PMC) to:
  - PMC@lpzoo.org – pre-planning preparations
  - PMCTrack@lpzoo.org – pre-planning surveys
- ☐ indicate whether you intend to use PMCTrack for your pre-planning surveys

6 WEEKS PRIOR TO MEETING

- ☐ log in to PMCTrack to launch pre-planning surveys (How to Launch PMCTrack Surveys)
- ☐ update studbook based on validation comments from PMC

4 WEEKS PRIOR TO MEETING

- ☐ login to PMCTrack, check survey responses, and begin compiling institutional wants & needs and exclusions list
- ☐ follow up with any IR responses that are unclear to you
- ☐ Non-PMCtrack Users' materials are due, submit the following information to the PMC
  - updated studbook database and IR list
  - a list of institutional wants and needs using the attached spreadsheet as an example
  - a list of permanent exclusions from the breeding population
  - names of expected meeting attendees for an in-person meeting
  - list of meeting attendee email addresses for a GoToMeeting
  - list of any non-AZA institutions that you would like to see included in your managed population

2 WEEKS PRIOR TO MEETING

- ☐ contact any IRs that have not completed the pre-planning survey
- ☐ email the following information to the PMC
  - exclusions list (see previous box for reference)

1 WEEK PRIOR TO MEETING

- ☐ PMCTrack surveys close - login to PMCTrack to download survey response reports
- ☐ email updated studbook + compiled wants/needs to PMC before planning meeting

DAY OF PLANNING MEETING

- ☐ bring any materials that have been updated since you last submitted planning data

Appendix G: Sustainability Partner Policy & Application

Information & Application to Become an Approved Sustainability Partner in an AZA Species Survival Plan® Program

Approved by the AZA Board of Directors 1 December 2009, Revised March 2014, Revised July 2018

The digitized Full Application to become an Approved Sustainability Partner can be obtained on the AZA website at https://www.aza.org/resource-documents.

The Association of Zoos and Aquariums (AZA) is a professional organization representing accredited zoological parks and aquariums and certified related facilities (CRFs). Among its objectives, AZA strives to raise professional standards that foster the continued development of superior zoos and aquariums and best practices in animal population management that uphold high standards of animal welfare.

AZA-accredited zoos and aquariums serve as centers of excellence in animal welfare, wildlife conservation, and public education and create animal exhibits that provide society the opportunity to develop personal connections with the animals and nature. As such, AZA-accredited zoos and aquariums are concerned about ecosystem health, take responsibility for species survival, contribute to research, and promote the highest standards of animal care and welfare in the management of small populations of earth's precious wildlife species.

Expectations for Approved Sustainability Partners in an AZA Species Survival Plan® (SSP) Program

AZA SSP Programs focus on the conservation of select and typically threatened or endangered species through the cooperative management of small populations at AZA-accredited zoos and aquariums and Certified Related Facilities. These facilities undergo a thorough accreditation review process that includes the submission of an extensive application as well as an intensive, on-site inspection by a team of experts to assure the highest standards of animal care and management are met.

A **Sustainability Partner** is defined as an organization that has **regularly exchanged** animals of the SSP with AZA-accredited facilities and CRFs, typically as part of the SSP Breeding and Transfer Plan or other SSP Program management process.

- A Sustainability Partner's species/animal(s) is **regularly included** in the SSP Breeding and Transfer Plan.
- Recommendations are made for individuals of that species in the Sustainability Partner's collection through the SSP Breeding and Transfer Plan process. This would include documented interim SSP Program recommendations.

It is not necessary to apply for a facility to be a Sustainability Partner if exchanges occur as infrequent, "one-way," or "one-off" transfers and the facility will not receive SSP Breeding and Transfer Plan recommendations. A facility may be sent animals excluded from the SSP population without becoming a Sustainability Partner. If the facility does not currently have the species in question then they do not qualify as a Sustainability Partner and the SSP should not apply. They may apply to include the facility as a Sustainability Partner in the future once the facility acquires the animals.

AZA believes that the highest standards of animal care, welfare, and population management are of paramount importance, and Sustainability Partners are expected to agree and abide by AZA's Code of Professional Ethics, SSP Full Participation Policy, Policy on Responsible Population Management, and Accreditation Standards related to animal care and welfare for all animals in the facility's collection.

Sustainability Partners in SSP Programs are ***not considered accredited or certified*** and may not display the AZA logo or the AZA SSP logo. Like AZA accreditations and certifications, approvals for a Sustainability Partner's continued participation in an SSP Program must be renewed every five years.

Who is Eligible to Apply for Approval as a Sustainability Partner?

If an AZA SSP Program determines that a potential Sustainability Partner cares for animals that can provide genetic, demographic, conservation, husbandry, population management, and/or animal welfare benefits to the SSP Program, the following are eligible to apply for approval as Sustainability Partners:

Category 1. Zoos/aquariums located outside the U.S. that are accredited members of a World Association of Zoos and Aquariums (WAZA) Recognized Super-Regional Zoological Association

Note: Recognized Super-Regional Zoological Associations that have a formal accreditation program include: Asociación Latinoamericana de Parques Zoológicos y Acuarios (ALPZA), Zoo and Aquarium Association - Australasia (ZAA), Asociación de Zoológicos Criaderos y Acuarios de México (AZCARM), Canadian Association of Zoos and Aquariums (CAZA), European Association of Zoos and Aquaria (EAZA), and Pan African Association of Zoos and Aquaria (PAAZA)

Note: Members of the Asociación Mesoamericana y del Caribe de Zoológicos y Acuarios (AMACZOOA), the Eurasian Regional Association of Zoological Parks and Aquariums (EARAZA), the Japanese Association of Zoos and Aquariums (JAZA), South East Asian Zoos Association (SEAZA), South Asian Zoo Association for Regional Cooperation (SAZARC), and unaccredited members of a qualifying recognized super-regional association must apply under Category 2.

Submission Requirements

A. A Letter of Justification from an AZA SSP Program for the applicant to participate in their SSP Program;
B. A completed and signed Sustainability Partner Application; and
C. A Letter affirming their accreditation from a WAZA recognized Super-Regional Zoological Association.

Category 2. Wildlife facilities that are not accredited by a WAZA recognized Regional Zoological Association

Wildlife facilities include, but are not limited to zoos and aquariums not accredited by a WAZA recognized regional zoological association (including zoos and aquariums that are *members* of such associations but not *accredited* by them), ranches, refuges, rehabilitation centers, research facilities, sanctuaries, survival centers, breeding facilities, private individuals, and educational outreach organizations. These facilities can be within or outside the U.S., and do not need to be open to the public.

Submission Requirements

A. A Letter of Justification from an AZA SSP Program for the applicant to participate in their SSP Program;

B. A completed and signed Sustainability Partner Application;
C. Letters of Sponsorship from the Directors of two different AZA-accredited facilities; and
D. If within the United States, submission of the most recent USDA licenses and inspection report(s), if applicable.

Application Processes

Submission Process

The SSP Coordinator must work with the applicant to assure all application materials are complete. The SSP Coordinator may fill out the application through a phone call to the applicant facility. The SSP Coordinator must submit the completed application, required letters, and attachments to the AZA Conservation, Management, & Welfare Sciences Department (animalprograms@aza.org), and assure that the applicant does not participate, or continue to participate, in the SSP Program until they are formally approved as a Sustainability Partner.

The AZA Conservation, Management, & Welfare Sciences Department will review the application materials, and forward applications deemed to be complete and appropriate for review to the APM Committee Vice Chair for Partnerships who will act as the coordinator of the Sustainability Partner's application. The Vice Chair of Partnerships will review application materials to identify potential needs for clarification. The Vice Chair of Partnerships will notify the AZA Conservation, Management, & Welfare Sciences Department when the application is ready for APM Committee review, and distribute the application to the committee for review.

Review Process

Applications are reviewed by AZA's Animal Population Management Committee (APM Committee) throughout the year via committee conference calls, as well as twice each year – during the AZA Annual Conference and the AZA Mid-Year Meeting. No fees are charged for the Sustainability Partner application.

APM Committee will evaluate the benefits of the applicant being approved as an SSP Program Participant in the context of the entire AZA *ex-situ* population which includes all SSP Program animals at AZA- Accredited zoos and aquariums, Certified Related Facilities, and Sustainability Partners.

Reviews are held either by conference call or in closed sessions, and are attended by APM Committee members and advisors and AZA staff; in addition, members of the AZA Board of Directors, the SSP Coordinator, and/or the corresponding Taxon Advisory Group (TAG) Chair may attend as well. Approval decisions are based on the information that exists at the time of the application review, not on future plans. Crucial elements in APM Committee's consideration include:

- Completion of application
- Application support letters, documents, and photographs
- Whether there is sufficient evidence that the applicant follows the tenets of AZA's Code of Professional Ethics, SSP Full Participation Policy, Policy on Responsible Population Management, and Accreditation Standards related to animal care and welfare for all animals in the facility's collection.
- Whether the participation of the proposed partner in the SSP will significantly enhance the SSP program population's sustainability, while supporting high standards of animal welfare.

APM Committee may take one of the following actions:

- **Approval:** APM Committee will grant approval when it determines that the applicant facility meets the requirements of an approved Sustainability Partner.
- **Table Approval:** APM Committee may table a facility's application if it determines that certain conditions must be met or additional information submitted before the facility can be considered as a Sustainability Partner. If the facility is able to meet those requirements within one year, and if the SSP Program still wishes, the APM Committee will re-review the application.
- **Deny Approval:** APM Committee will deny approval when a facility does not meet the minimum requirements (see "expectations") to be recognized as an approved Sustainability Partner at the present time and, in its opinion, would require in excess of one year to successfully do so. Applicants may work with the SSP Program and reapply to be a

Sustainability Partner after the APM Committee's concerns have been addressed.

Once a decision is reached, the APM Committee Vice Chair for Partnerships will inform the SSP Coordinator and the facility's Owner/CEO/Director of the outcome. An official letter noting the decision and points of discussion will be sent from the AZA Conservation, Management, & Welfare Sciences Department to the SSP Coordinator and the facility Director within 30 days of the application review.

Sustainability Partner Responsibilities

Once approval has been granted the Sustainability Partner will:

- Agree to adhere to AZA's Code of Professional Ethics, SSP Full Participation Policy, Policy on Responsible Population Management, and relevant Accreditation Standards, especially those related to animal care and welfare for all animals in the facility's collection.
- Appoint an Institutional Liaison (IL) to serve as the primary point of contact for SSP communications.
- Not display the SSP logo.
- Agree to allow the sponsoring AZA-accredited zoo or aquarium, staff, Board, APM Committee, TAG, and/or SSP representatives to visit and view the applicant facility, upon request, to assure adherence to AZA policies and animal care and welfare practices. Such visitors will provide feedback to the SSP, TAG, and the APM Committee.
- Submit a new, complete application for Sustainability Partner before the end of the five-year approval period in order to continue participation in the SSP Program.

Loss of Approval Status

A Sustainability Partner may have its approved status revoked by the APM Committee if it fails to meet any of the Sustainability Partner responsibilities identified above and in the application.

If AZA/APM Committee deems it appropriate, the SSP Program may work with a Sustainability Partner that loses its approved status for up to two years to help manage the population, facilitate transfer of animals owned by AZA zoos and aquariums and, when possible, mentor re-approval of Sustainability Partner status. In such instances, the Sustainability Partner will not be an active participant in the SSP but still may be considered during the planning processes. During this time the SSP will not move SSP animals to the former Sustainability Partner facility.

For reinstatement as a Sustainability Partner, the potential Sustainability Partner's benefit to the SSP population's sustainability and adherence to AZA's Code of Professional Ethics, Policy on Responsible Population Management, and Animal Care and Welfare Standards must be reassessed.

Sustainability Partner Application

Part A: To be completed by facilities in Categories 1 & 2

Facility Information

1. Facility Name:
2. Mailing Address (street, city, state, zip code, country):
3. Physical Address (if different than mailing address):
4. Telephone Number:
5. Website:
6. Is this organization open to the public on a regularly scheduled and predictable basis?
   Yes, Hours: No ☐
7. If located outside of the United States, is the organization **accredited by** a WAZA recognized Super-Regional Zoological Association: Yes ☐ No ☐

   Name of Accrediting Zoological Association:

   ☐ALPZA

   ☐AZCARM

   ☐CAZA

   ☐EAZA

   ☐PAAZA

   ☐ZAA - Australasia

8. If located outside of the United States, is the organization a **member of** a WAZA recognized Super-Regional Zoological Association: Yes ☐ No ☐

   Name of Zoological Association:

   ☐AMACZOOA

   ☐EARAZA

   ☐JAZA

   ☐SAZARC

   ☐SEAZA

9. Who has ultimate responsibility for decisions relating to animal care, welfare, and management at the facility?

   ☐Owner

   ☐Director

   ☐Board of Directors

   ☐Animal Care Manager/Curator

   ☐Veterinarian

   ☐Other (name):

10. The organization is (check all that apply):

    ☐owned/operated by municipality, city, county, state, or federal government

☐not for profit

☐part of a larger corporation

☐privately funded

☐privately owned

☐other/more information:

11. Type of organization (check all that apply):

☐Zoo

☐Aquarium

☐Rescue facility

☐Research facility

☐Sanctuary/refuge[1]

☐Open to the general public

☐Guided tours only

☐Drive-thru park

☐Ranching operation that manages non-domestic/domesticated wildlife species

☐Private breeder (not open to the public)

☐Other/more information:

Facility's Representative Information

1. Name of the Facility's Director/CEO:
    a. Director/CEO Telephone Number:
    b. Director/CEO E-mail:
2. Name of the Facility's Institutional Representative (IR):
    a. IR's Telephone Number:
    b. IR's E-mail Address:

SSP Program Information

1. Name of SSP Program:
2. Number of SSP Program animals owned by/at your facility that are to be included in the SSP Program:
3. Does the facility currently house this species?
4. Describe any breeding of this species at the organization (e.g., have you bred this species previously? How many times? When?)
5. List all other AZA SSP Programs in which the organization participates.

[1] Sanctuaries being distinct from zoos by housing only non-breeding animals and only receiving animals (e.g., does not send animals to facilities or individuals)

Part B: To be completed by facilities in Category 2

Animal Housing, Animal Care, Safety, and Records

1. If an AZA Animal Care Manual (ACM) exists for the SSP Program species, has your facility reviewed the ACM and does your facility meet the guidelines identified in the ACM?
2. Describe the spaces available for this SSP Program species (indoor and outdoor facilities; holding areas; shifting doors; locks and pins for all doors; public barriers and containment). Attach photos of exhibit and holding areas. Approximate the size of the animals' space.
3. Describe the staff experience in care and management of the SSP Program species.
4. Describe the facility's veterinarian's experience with the SSP Program species (or similar or closely related species). If the veterinarian does not have experience with the SSP Program species, is there a consulting veterinarian that does have experience with the species?
5. Describe the typical diet of the SSP Program species at the organization and how this diet was or will be created to meet the nutritional needs of the animal(s).
6. Describe the organization's internal procedures for reviewing animal welfare of this SSP Program species.
7. Describe the standard operating procedure (SOP) for daily husbandry and/or exhibition of the SSP Program species. The SOP typically includes a list of daily staff duties required for safe care and exhibition of the species with assigned or rough estimates of sequence and time allocation.
8. What type of contact will staff have with the SSP Program species?
   - ☐ Unprotected contact – staff will intentionally make physical contact with the species and will share space with the animal(s) regularly.
   - ☐ Incidental – staff will regularly share space with the animal(s) and may occasionally make physical contact with the animals.
   - ☐ Protected – Physical contact with the animal(s) by staff will always be across or through a protective barrier; staff will share space with the animals only in emergency situations.
   - ☐ None – no physical contact with this species is ever permitted at this facility regardless of whether staff shares space with the animal(s) or interacts with them across a barrier. Exceptions regarding physical contact are only made in the event of emergencies.
9. What type of contact will visitors have with the SSP Program species?
   - ☐ Unprotected contact – visitors will share space with the animal(s) and they will be granted the ability to make physical contact with the animal(s).
   - ☐ Incidental – visitors will share space with the animals, the ability to touch the animals will be incidental and contingent on animal(s) seeking contact with visitors.
   - ☐ Protected – visitors will not share space with the animals; physical contact with the animal(s) will always be across or through a protective barrier
   - ☐ None – visitors will not be permitted to have physical contact with the animal(s) under any circumstances.
10. If the facility houses dangerous animals (including, but not limited to large felids, large canids, bears, any great ape species, large crocodilians, large snakes, large ratites,

venomous or toxic species), attach the risk management plan describing escape protocols, contingency plans for disasters/emergencies, and immobilizations/lethal weapons protocols. Please also describe any additional safety measures taken at the facility when working with these species (e.g., animal shifting protocols, lock checking protocols, communication protocols, and delivery of feed).

   a. For venomous species, does your facility maintain a stock of the appropriate anti-venin, if available? Are health practitioners in your area aware that you are housing venomous species?

11. Has the facility had any permits and/or licenses related to wildlife suspended or revoked? If yes, please explain the reasons for suspension or revocation. If permits or licenses were revoked or suspended, when were they re-instated?
12. Has the facility received any fines from local, state or federal wildlife regulatory agencies? If yes, please explain by providing dates of fining and circumstances that lead to the fine.
13. Is the facility in compliance with all local, state, and federal ordinances, laws, regulations, permits, etc. related to wildlife? If no, please explain how the organization is working towards becoming compliant.
14. Submit the facility's USDA license and the last 5 years of the USDA inspection reports, if applicable.
15. Submit any applicable (local, state, federal) wildlife permits.
16. What animal recordkeeping system does the facility use?
17. What method(s) will be used to permanently identify animals born/hatched at this facility in accordance with SSP Breeding and Transfer recommendations?
18. Submit a current inventory list and census of the animal collection at the facility.

Part C: To be completed by facilities in Categories 1 & 2

Applicant Agreement to Accept All Sustainability Partner Responsibilities

By signing below, I, (Name), (this must be the Director or CEO) of the (Organization Name), fully agree to meet the responsibilities listed below if approved as a Sustainability Partner in the (species name) SSP Program and further acknowledge that failure to do so may result in a revocation of this approved status:

- ☐I and my staff will adhere to AZA's Code of Professional Ethics, SSP Full Participation Policy, Policy on Responsible Population Management, and Animal Care and Welfare Standards for all animals in our collection.
- ☐I will provide the AZA SSP Program with complete studbook histories for all animals proposed for inclusion in the SSP managed population within 30 days of approval.
- ☐I will not display the SSP logo.
- ☐I will complete the full application process before the end of the five-year approval period ends in order to continue my Organization's participation in the SSP Program.
- ☐I will assure that the sponsoring AZA-accredited facility, staff, Board, APM Committee, TAG, and/or SSP representatives can schedule a visit to the applicant institution/zoo upon request to view the facilities and assure adherence to AZA policies and animal care and welfare practices.

Applicant Director/CEO Signature: Date:

Checklist of Supporting Materials and Attachments for each Sustainability Partner Category

1. *Zoos/aquariums located outside the U.S. that are accredited members of a World Association of Zoos and Aquariums (WAZA) Recognized Super-Regional Zoological Association*

   ☐Does this application include a completed Letter of Justification from the SSP Program Coordinator?

   ☐Does this application include a completed Letter Affirming the applicant's accreditation from a WAZA Super-Regional Zoological Association?

2. *Wildlife facilities that are not accredited by a WAZA recognized Regional Zoological Association*

   ☐Does this application include a completed Letter of Justification from the SSP Program Coordinator?

   ☐Does this application include completed letters of sponsorship from two Directors of two different AZA-accredited facilities? If the SSP Coordinator submitting the application is also a Director from an AZA-accredited facility or CRF, they must include a letter of sponsorship from a Director of a different AZA-accredited facility. The SSP Coordinator cannot write their letter of support.

   Photographs of SSP Program species':

   ☐Indoor facilities

   ☐Outdoor facilities

   ☐Holding areas

   ☐Animal shifting doors

   ☐Locks and pins for all doors, including keeper to exhibit/holding, animal to exhibit, animal to animal

   ☐Public barriers and containment

   Documents:

   ☐Standard operating procedure for daily husbandry of the species

   ☐Risk management plan

   ☐Copies of USDA License and applicable permits, if applicable

   ☐Last 5 years of USDA inspection reports, if applicable

   ☐Current inventory list and census of the animal collection

Guidelines for Letters of Justification, Accreditation, and Sponsorship

Required for facilities in Categories 1 & 2:

Letter of Justification from an AZA SSP Program for a Sustainability Partner to Participate in their Program

The letter from an SSP Coordinator must:

1. Clearly identify the SSP Program for which the potential Sustainability Partner participant's involvement is being requested, as well as the SSP Coordinator's identity, telephone number and e-mail address;
2. Indicate that the TAG Chair has reviewed the Sustainability Partner application and the TAG supports the approval of the potential Sustainability Partner;
3. Make a clear formal request for approval of the potential Sustainability Partner;
4. Describe the challenges that the population faces and how this partner could alleviate some of those challenges. Identify specifically why the potential Sustainability Partner's participation would benefit the SSP Program and describe how the Sustainability Partner applicant's animals and/or holding/breeding spaces are critical to the success of the SSP Program. How would the approval of the potential Sustainability Partner for participation in the SSP Program provide genetic, demographic, population management, husbandry, welfare, conservation or other benefits to the SSP Program? Identify any additional factors that substantiate how approval of the potential Sustainability Partner's participation will benefit the SSP Program and conservation of the species (e.g., reference to the SSP Breeding and Transfer Plan, Global Species Management Plans, Population Viability Analysis results suggesting a need for additional holding space, unique genetics, additional demographics, TAG Regional Collection Plan goals, etc.). Present facts and accurate data. Indicate if the facility is currently working successfully with the TAG in other ways or with other SSP Programs, and include examples of when/how the facility effectively communicated with the SSP, other AZA SSP Programs, and/or other facilities. Describe any animal transfers that have occurred between the applicant and the SSP Program;
5. Provide confirmation that the SSP Coordinator has discussed in detail with the potential Sustainability Partner the responsibilities that the organization will assume upon approval as an SSP participant including assurance that the Sustainability Partner applicant has fully read and understood:
    - AZA's Code of Professional Ethics,
    - the Full Participation in the SSP Policy,
    - the Policy on Responsible Population Management, and
    - the AZA Accreditation Standards related to animal care and welfare.
6. Provide a clearly stated description of how the Sustainability Partner applicant provides a level of animal care and welfare in keeping with AZA's standards and identify how the SSP Coordinator has attained this understanding. Describe the Sustainability Partner applicant's history of experience with the SSP Program species or similar species, and identify staff qualifications in caring for the species, related expertise, and resources at the facility. This section should also include any forms of information (such as photographs, veterinary statements, USDA inspection reports, etc.) that corroborate the applicant's adherence to AZA standards of animal care and welfare;
7. Indicate whether the SSP Coordinator or any current participating facility in the SSP Program has conducted a site visit of the proposed Sustainability Partner in the last 3 years. Provide copies of site visit reports, if available;
8. End with the signature of the SSP Coordinator and the date.

9. Submit the completed application, required letters, and attachments to the AZA Conservation, Management, & Welfare Sciences Department (animalprograms@aza.org) for APM Committee approval.

*The APM Committee Vice Chair for Partnerships is a valuable resource to use when the SSP is beginning to gather the application materials. The current APM Committee Vice Chair for Partnerships is listed on the AZA website (https://www.aza.org/animal-population-management-committee).

Required for Facilities in Category 1:

Letter Affirming Accreditation by a WAZA Recognized Zoological Association for Organizations which are Applying for Approval in an AZA SSP Program

The letter from the appropriate association should:

1. Clearly identify the name of the organization / entity applying for approval as a participant in an AZA SSP Program;
2. Clearly identify the name of the Association and the Association Director's name and contact information;
3. Affirm that the applicant organization/entity is a currently accredited member in good standing of the Association providing this letter;
4. Provide clearly stated assurance that the applicant organization/entity provides a level of animal care and welfare in keeping with the standards of the Association providing this letter and identify how this information is known;
5. End with the signature of the Association Director and the date.

Required for Facilities in Category 2:

Letters From the Directors of Two AZA-accredited Facilities Sponsoring the Approval of a Non- Member Wildlife Facility as an SSP Sustainability Partner

The letters from two, separate AZA-accredited Facility Directors must:

1. Identify the SSP for which the potential Sustainability Partner participant's involvement is being sponsored;
2. Provide a description articulating why it is believed that the potential Sustainability Partner applicant will provide a benefit to the SSP Program, and identify any additional sources that substantiate how approval of the potential Sustainability Partner applicant's participation will benefit the SSP Program and conservation of the species (e.g., reference to the SSP Breeding and Transfer Plan, Population Viability Analysis results suggesting a need for additional holding space, unique genetics, additional demographics, TAG Regional Collection Plan goals, etc.);
3. Provide a statement that it is understood that the signature on the letter of sponsorship serves as assurance that the applicant facility provides a level of animal care and welfare equivalent to or above that of the AZA Accreditation Standards. When referencing the site, please describe if the Director (or identify who on senior staff) visited the applicant's facility and outline the observations
   / experiences that substantiate why it is believed the applicant adheres, or will adhere, to AZA's Code of Professional Ethics, SSP Full Participation Policy, Policy on Responsible Population Management, and Accreditation Standards related to animal care and welfare for all animals in the facility's collection.

   For wildlife facilities in North America both sponsoring letters must be written by a Director (or designate) of two different AZA facilities. For wildlife facilities outside of North America,

one of the two required letters may be written by the Director of a zoo or aquarium accredited by another WAZA recognized regional zoological association.

4. End with the signature of the Director and the date.

Appendix H: Guidelines for Assessing Sustainability Partners in SSP Programs

Partnerships in animal management are unique relationships between facilities accredited by the Association of Zoos and Aquariums (AZA) and entities external to AZA that are designed to benefit the population viability of the species while upholding high standards of animal care and welfare. A **Sustainability Partner** is an AZA Animal Population Management (APM) Committee approved wildlife facility* (see definition below) that has regularly exchanged animals of the Species Survival Plan® (SSP) Program with AZA-accredited facilities and certified related facilities, typically as part of the SSP Breeding and Transfer Plan or other SSP Program management process.

- A Sustainability Partner's species/animal(s) is regularly included in the SSP Breeding and Transfer Plan.
- Recommendations are made for individuals of that species in the Sustainability Partner's collection through the SSP Breeding and Transfer Plan process. This would include documented interim SSP Program recommendations.

AZA Animal Programs can benefit from responsible partnerships with appropriate wildlife facilities in the form of expertise, space, and other various resources. For the purpose of this and other associated materials, **wildlife facilities** include, but are not limited to, zoos and aquariums, ranches, refuges, rehabilitation centers, research facilities, sanctuaries, survival centers, breeding facilities, private individuals, and educational outreach organizations. These facilities can be within or outside the U.S., and do not need to be open to the public.

The SSP Program and Taxon Advisory Group (TAG) must first decide if a partnership is warranted before a facility is invited to apply and considered for formal inclusion in an SSP Program as a Sustainability Partner. It is understood that there is no "one size fits all" model regarding how to assess partners, and each SSP Program should consider their own specific needs as they work through these guidelines. SSP Coordinators should communicate with relevant parties (SSP Program Officers, Steering Committee members, the TAG Chair, population advisors, etc.) and follow these guidelines to help them make an informed and responsible partnership decision.

This assessment process will be two-fold. APM Committee Liaisons will work with TAGs and the SSP Programs within their purview, and use these Guidelines to evaluate the current and potential SSP Program partners in order to make an initial assessment of their value to the SSP Program's population sustainability, as well as their appropriateness as an AZA partner. During this process some existing and potential partners may be removed from the SSP Program, for a variety of reasons.

After this initial assessment, SSP Programs should work closely with their AZA Population Management Center (PMC) Population Biologist as they initiate their next Breeding and Transfer Plan (BTP) process. The analyses needed to assess the remaining partners may be time-consuming, depending on the species, population dynamics, the number of remaining potential partners, and other factors. APM Committee will allow for extensions to SSP Program accountability deadlines as needed. If the SSP Program determines that it wants to include the facility in the SSP Program then it must move forward with submitting the Sustainability Partner application to APM Committee.

Step 1: Please discuss the questions (a-e) below within the SSP Program management group to determine if the partnership meets basic requirements to be forwarded for APM Committee consideration

The first and foremost question to consider when determining whether or not to include a specific Sustainability Partner to the SSP Program should be:

Does the potential Sustainability Partner provide the AZA Animal Program (TAG or SSP) population with any of the following benefits?

a. Genetic considerations
    i. Could the partner provide genetically unique or valuable animals to the SSP population if they joined the SSP Program? Could the partner provide a unique individual or multiple unique individuals?
b. Demographic considerations
    i. Could the partner provide individual animals of a desired age, life stage, or gender to the SSP population?
    ii. Could the partner provide additional animals that would make the population more demographically stable?
c. Field conservation considerations
    i. Does this partner's involvement affect current field conservation initiatives for the SSP Program?
    ii. Does this partner play a significant role in a release or recovery plan for the species?
    iii. Will the loss of the partnership negatively affect conservation strategies for the SSP or Recovery Program?
d. Husbandry, well-being, and welfare considerations
    i. Does the partner provide some unique opportunity that would benefit the species' husbandry that may be difficult to replicate or is only replicable on a small scale in AZA facilities (e.g., hundreds of acres for herds of animals, direct access to saltwater for marine species)?
    ii. Will the partner be able to provide appropriate husbandry for the species at all life- stages?
    iii. Are there concerns regarding the potential commercialization of the animals or their offspring? Would the partner be willing to only accept non-breeding animals in this situation?

iv. Will the partner accept animals recommended by the SSP Program as directed?
v. Is there a specific husbandry role the partnership may provide (housing males, housing groups instead of individuals, etc.)?
vi. Will the partnership provide a husbandry benefits to individual animals as well as to the species as a whole?
vii. Will the partnership provide additional expertise to the SSP Program, such as veterinary, behavior, education, research, etc.?

e. International partners, if applicable
i. Is it logistically feasible for the partner to regularly import or export this species across its international boundary?
ii. Is the partner willing and able to meet any pre-export quarantine requirements relevant for this species as dictated by the USDA?
iii. Does the partner have a member of staff experienced with international animal transfers and/or permits?
iv. Is the facility experienced with international imports/exports among major zoological regions?

If you answer "no" to many of the above questions, then it may be most appropriate not to partner with the facility in question in future SSP BTPs. This does not imply that the SSP Program or an AZA facility can never send or receive animals from this facility. However, if exchanges occur, they must be infrequent or "one-way," and the facility will not receive BTP recommendations.

If the answer is "yes" to many of the above questions, the partner may warrant additional deliberation to include them in the SSP Program's next BTP. Potential partners will require additional evaluation to assure that they are vital to the SSP Program and an appropriate partner for AZA. Consider the potential partner carefully. Consult with fellow members of the TAG and other SSP Programs that may have partnered with them in the past. Proceed to step 2

Step 2: Please ask your potential partner - and yourself - the following questions:

How does this new or continued partnership benefit the sustainability of the animal population?

The AZA PMC population biologists are available to help with your decision. They may be able to assess whether a potential partner's animals are valuable to the SSP population or how valuable additional space(s) is/are and help think critically about the impact that the partner could have on the population. For example, while increasing numbers may seem automatically positive, simply adding one more space or one more animal does not necessarily mean the partner will significantly enhance the population's sustainability. Answering this type of question may require consultation with the SSP Program's Population Biologist, and may need to wait until the BTP process.

Will the facility actively support and participate in AZA Animal Programs?

Supporting and participating includes providing requested information regarding its animals and their husbandry and welfare upon request to AZA Program Leaders, including Studbook

Keepers, SSP Coordinators, and TAG Chairs, assigning Institutional Representatives (IRs) to the AZA Animal Program, and following agreed upon recommendations (e.g., acquisitions, breeding, transfers, etc.). An existing partner who has regularly failed to provide requested information in the past should not be moved forward through the current application process.

If you are assessing an existing partner, has the facility been actively involved in previous SSP Breeding and Transfer Plans with the SSP Program in question?

Has the facility communicated with the SSP Coordinator during the planning process such as answering wants and needs surveys, reviewing draft plans and providing feedback? Do they have a history of following SSP BTP recommendations? Are they equally likely to follow recommendations for hold as they are for breed with, or transfer? Do they regularly communicate and share their animal data with the Studbook Keeper?

If the answer to the above questions is yes, do you think they will continue to do so in the future?

Will the movement of SSP animals with the partner be considered infrequent "one-way" or "one-off" transfers?

The best way to make this decision is with an AZA Population Biologist and using PMx software; however, SSP Program Leaders can also do some investigation on their own to help make these decisions. When potentially bringing an animal into the SSP, it is best to first determine if this animal will add value to the SSP population. This value can come in many forms (e.g., genetically, demographically, husbandry, ambassador needs). When potentially sending an animal out of the SSP, there is always a cost and many variables must be carefully considered, including what will the welfare of the animal be outside of the SSP, will losing the animal hurt the demographic or genetic stability of the SSP, etc.

It is not necessary to apply for a facility to be a Sustainability Partner if exchanges occur as infrequent, "one-way," or "one-off" transfers and the facility will not receive SSP Breeding and Transfer Plan recommendations. A facility may be sent animals excluded from the SSP population without becoming a Sustainability Partner. If the facility does not currently have the species in question then they do not qualify as a Sustainability Partner and the SSP should not apply. They may apply to include the facility as a Sustainability Partner in the future once the facility acquires the animals.

What can SSP Program Leaders do to help determine which individual animals to infrequently send out of the SSP (export) and/or receive into the SSP (import) (i.e., "one- way," or "one-off" transfers)?

The best way to make these decisions is with an AZA Population Biologist and using PMx software; however, SSP Program Leaders can also do some investigation on their own to help make these decisions. When potentially bringing an animal into the SSP, it is best to first determine if this animal will add value to the SSP population. This value can come in many forms (e.g., genetically, demographically, husbandry, ambassador needs). When potentially sending an animal out of the SSP, there is always a cost and many variables must be carefully considered, including what will the welfare of the animal be outside of the SSP, will losing the animal hurt the demographic or genetic stability of the SSP, etc.

As each SSP is unique, there is no way to create an extensive list, but below are some examples of ways to further investigate these potential values and considerations.

- What do you know about the potential animal(s) that are proposed to join the SSP? If you are adding them for genetic reasons, do you know their pedigree and are they linked to the SSP

population? If they are intended for breeding, are they of an appropriate age, reproductively viable, experienced?

- Look at the studbook and previous Breeding and Transfer Plan for the SSP.
    - Demography
        - Are more animals demographically needed for this SSP (i.e., it has a very small population size or lacks young, breeding-aged animals)?
        - Is the SSP population demographically robust enough to send out and potentially lose animals (i.e., is the animal(s) you plan to export in pre-reproductive or reproductive age classes and will you have enough reproductive-aged animals remaining in the SSP to meet future breeding goals)?
    - Genetics
        - Would the SSP population benefit from adding more unique genes (i.e., is gene diversity low and projected to decline quickly? Do you have a small number of founders represented in the SSP?)?
        - Are the proposed non-SSP animals related to the SSP population? If so, how closely related? Has the SSP previously imported animals from this source?
        - Would the SSP population be able to withstand sending out and potentially losing some genes? Are the animals you plan to export over-represented, having high mean kinship and many living relatives in the SSP?
    - Husbandry
        - Is the husbandry known and consistent for this SSP population? If not, could this non-SSP facility share knowledge, expertise, or experienced breeding animals to help the SSP?
        - Was this non-AZA facility included in the last BTP? If so, what were their breeding and transfer recommendations?
- Are these potential animals included in the SSP studbook database? If so, look in the database to identify closely related individuals to minimize inbreeding when making new breeding recommendations. Use the Antecedent and Descendant Pedigree Reports in PopLink, the Sibling Tables and Descendant Lists in SPARKS, or the Pedigree tools in ZIMS for Studbooks.

Think about the logistics, resources, abilities, and acquisition/disposition policies of the facilities potentially involved in these transfers. Are they conducive to making the proposed transfers occur?

Does the Animal Program believe the partner will adhere to relevant AZA policies and accreditation standards?

The partner must agree to adhere to the AZA's Code of Professional Ethics, SSP Full Participation Policy, Policy on Responsible Population Management, and Accreditation Standards related to animal care and welfare. Do you know if the partner is familiar with these documents - have you provided them? Did the partner express any concerns or reservations?

The agreement to follow these policies signals the partner's intent to provide high quality animal care and operate in ways consistent with AZA principles and ethics.

Is the facility involved with other AZA Animal Programs?

Reach out to those Animal Program Leaders and ask about the potential partner. What is the nature of their relationship? Are they communicative? Do they provide information? Do they actively participate in the program?

The AZA Conservation, Management, & Welfare Sciences Department and the AZA PMC may be able to help SSP Programs identify what other SSP Programs are working with a potential partner.

Has anyone from an AZA-accredited facility (e.g., staff, an AZA Board member, APM Committee member, TAG, and/or SSP Program representative) visited the potential partner and viewed their facility to assure adherence to AZA policies and animal care and welfare practices?

Communicate with your TAG Chair and Steering Committee, as well as other SSP Programs within your TAG (and others). Utilize the AZA Network groups for your taxa or at the Curator level. Reach out to the person who conducted a site visit to gain insight into the facility. Make sure that it is an appropriate fit for the SSP Program animals. Request documents or written opinions.

Has the partner's participation in another SSP Program been denied?

Talk to the SSP Programs for which the partnership application was denied to identify the exact reasons why the application did not go through. It could be that a facility that is appropriate or capable of working with one species is less appropriate for another.

Step 3: Decide if this partnership will be formally submitted for APM Committee consideration

Given the answers to all of the questions above:

A. The SSP Program feels that the facility is essential to the SSP Program
   - The SSP Coordinator should review the Sustainability Partner and other related AZA policies and Accreditation Standards with the partner.
   - The SSP Coordinator should work with the Sustainability Partner and begin completing the Sustainability Partner application.

- The AZA Conservation, Management, & Welfare Sciences Department and APM Committee Vice Chair for Partnerships are available to assist with any questions you may have during this process.

B. The SSP Program believes the facility would be an appropriate partner, but is unsure if the facility is essential to the SSP Program from a sustainability standpoint
   - The SSP Coordinator should review the Sustainability Partner and other related AZA policies and Accreditation Standards with the partner.
   - The SSP Coordinator should let their AZA PMC Population Advisor know that they will be requesting additional analyses during their next SSP BTP so that schedules and materials can be planned accordingly.
   - If, once these analyses are complete, the partner is deemed essential to the SSP Program then the SSP Coordinator should work with the Sustainability Partner and begin completing the Sustainability Partner application.
   - The AZA Conservation, Management, & Welfare Sciences Department and APM Committee Vice Chair for Partnerships are available to assist with any questions you may have during this process.

C. The SSP Program does not believe the partner is essential to the SSP Program
   - The partner must not be listed in subsequent SSP BTPs. They are not an SSP Program partner.
     - This does not prohibit occasional animal moves that are permitted by AZA's Policy on Responsible Management.
     - This does prohibit the facility's holdings from appearing in the BTP and from them receiving written recommendations.

Appendix I: Sustainability Partner Policy FAQ

The APM Committee recommends that Program Leaders contact Michael Ogle, APM Committee Vice Chair of Partnerships (mogle@zooknoxville.org), with any questions prior to submitting applications for review.

Expectations for Approved Sustainability Partners in an AZA Species Survival Plan® (SSP) Program

AZA SSP Programs focus on the conservation of select and typically threatened or endangered species through the cooperative management of small populations at AZA-accredited zoos and aquariums and Certified Related Facilities. These facilities undergo a thorough accreditation review process that includes the submission of an extensive application as well as an intensive, on-site inspection by a team of experts to assure the highest standards of animal care and management are met.

A **Sustainability Partner** is defined as an organization that has **regularly exchanged** animals of the SSP with AZA-accredited facilities and CRFs, typically as part of the SSP Breeding and Transfer Plan or other SSP Program management process.

- A Sustainability Partner's species/animal(s) is **regularly included** in the SSP Breeding and Transfer Plan.
- Recommendations are made for individuals of that species in the Sustainability Partner's collection through the SSP Breeding and Transfer Plan process. This would include documented interim SSP Program recommendations.

It is not necessary to apply for a facility to be a Sustainability Partner if exchanges occur as infrequent, "one-way," or "one-off" transfers and the facility will not receive SSP Breeding and Transfer Plan recommendations. A facility may be sent animals excluded from the SSP population without becoming a Sustainability Partner. If the facility does not currently have the species in question then they do not qualify as a Sustainability Partner and the SSP should not apply. They may apply to include the facility as a Sustainability Partner in the future once the facility acquires the animals.

AZA believes that the highest standards of animal care, welfare, and population management are of paramount importance, and Sustainability Partners are expected to agree and abide by AZA's Code of Professional Ethics, SSP Full Participation Policy, Policy on Responsible Population Management, and Accreditation Standards related to animal care and welfare for all animals in the facility's collection.

Sustainability Partners in SSP Programs are **not considered accredited or certified** and may not display the AZA logo or the AZA SSP logo. Like AZA accreditations and certifications, approvals for a Sustainability Partner's continued participation in an SSP Program must be renewed every five years.

Accountability and Planning

My SSP Program is scheduled to be planned with the AZA Population Management Center (PMC) or Adjunct Advisor in the next few months. Do all Sustainability Partner applications need to be submitted and approved before my planning date?

SSP Program populations that had a scheduled planning meeting between October 1, 2018 and March 31, 2019 were included in a six-month grace period and could include any not yet approved non-AZA facilities in the Breeding and Transfer Plan (BTP), if the Program Leaders chose to do so. Program Leaders should think about potential Sustainability Partners and start working with them to fill out the application(s) in order to be ready for the next Breeding and Transfer Plan planning process. SSP Programs will need to get their non-AZA facilities approved before the planning meeting in order to include them in the BTP. Non-AZA facilities that are not yet approved Sustainability Partners are not allowed to be included in the BTPs, even if their applications are in process.

Who enforces the implementation of the new policy, and how is it assured that only approved Sustainability Partners are included in BTPs?

The AZA Animal Population Management Committee (APM Committee) ultimately enforces all Animal Program policies. The SSP Coordinator and TAG Chair should conduct the first review of their Sustainability Partner application(s) and remove any non-AZA facilities that clearly do not meet the definition of a Sustainability Partner. During the development of the Draft BTP, the SSP Program's Population Biologist will be engaged in conversations with the SSP Program and discuss all remaining non-AZA participants, and will be aware of which participants have been approved to be Sustainability Partners.

The AZA Conservation, Management, & Welfare Sciences Department will review the most recent published SSP BTPs every six months or so to track Sustainability Partners that are listed in the Final Plans. The Conservation, Management, & Welfare Sciences Department will share these data with the APM Committee and the TAGs on a regular basis.

What is the turnaround time for application review and approval by APM Committee once the SSP Coordinator submits the application?

APM Committee holds monthly conference calls to review and approve Sustainability Partner applications on a rolling schedule as they are submitted. The SSP Program and the applicant will be notified via email as soon as possible whether or not the Sustainability Partner application was approved. SSP Coordinators must assure that application materials are provided and all questions answered completely. TAG Chairs may assist in assuring applications are ready for submission. If the APM Committee has questions about the application(s) during review, a representative will contact the SSP Coordinator and provide an opportunity for further clarification. While this may delay the approval process, it will be valuable in helping the APM Committee understand why the SSP Coordinator feels the partner should be considered for Sustainability Partner status.

If a Green SSP Program includes previously approved Sustainability Partners, do these Sustainability Partners have to go through the new approval process to be included in the next BTP?

Previously approved Sustainability Partners are approved for five years, and these approvals will remain in place until their original expiration date. Once the five years have passed, the Sustainability Partner must submit the new application to remain as a Sustainability Partner in the SSP Program.

Do facilities participating in SSP Programs that include government-owned/managed animals follow the same policy and processes?

The AZA Board approved a waiver to the Sustainability Partner Policy when certain conditions are met. SSP Programs that include animals owned by a US state or federal agency, or equivalent foreign government partner, may not need to complete Sustainability Partner applications for each facility housing individuals of that species in order for them to participate in the SSP. These agencies and designated facilities are considered Government Partners, which allows them to be part of the SSP and included in the SSP Breeding and Transfer Plan. Official documentation may be requested by the APM Committee.

When the animals at a specific facility meet all of the following conditions with regard to the SSP program, a waiver for that facility would apply:

- The agency owns the animals the SSP program is requesting for participation,
- The agency holds responsibility and authority for adding (or removing) the facility as a holder of the agency owned individuals,
- The government agency must approve of the housing and management of the species at the facility, and
- The agency must approve animal breeding and transfer activity at this facility.

If specific facilities within your SSP Program meet the criteria above, please notify animalprograms@aza.org at AZA as soon as possible. If you have documentation from the agency that empowers the SSP Coordinator to manage the population for the agency please submit this to animalprograms@aza.org. Doing so will help the AZA staff and the APM Committee help facilitate the planning process for your program with the PMC.

The APM Committee strongly encourages Program Leaders for these programs to have up-to- date facility profiles on participating non-AZA facilities as these may be helpful in facilitating transfers of animals between AZA and non-AZA facilities. The Sustainability Partner application could be used as a template for these profiles as the application covers most, if not all, of the common questions AZA facilities may have when working with non-accredited facilities on animal transfers and may reduce the paperwork burden on non-AZA facilities participating in transfers with AZA facilities in your SSP Program.

It is important to note that requirements to acquire permits for possession, transport or import/export for your species are NOT sufficient government oversight to waive the Sustainability Partner Policy.

Application and Process

Who submits the Sustainability Partner application to APM Committee?

The SSP Coordinator and the non-AZA facility should work together to assure that the application and materials are complete. The SSP Coordinator is the point of contact and the person who should collate the application and submit all materials to the AZA Conservation, Management, & Welfare Sciences Department (animalprograms@aza.org) and copy their TAG Chair.

How should SSP Programs notify their current non-AZA partners of the new policy?

We hope that SSP Coordinators have developed relationships with their current partners and feel comfortable introducing them to the application and walking them through the process, if they meet the definition of a Sustainability Partner. APM Committee has developed template letters that SSP Coordinators may wish to use to assist in their communications. Email the AZA Conservation, Management, & Welfare Sciences Department (animalprograms@aza.org) for these templates.

Some TAGs where multiple SSP Programs are working with the same partners may wish to coordinate their outreach efforts, while others may prefer for SSP Coordinators to reach out to their non-AZA partners directly to explain the new policy and application process. APM Committee TAG Liaisons are available for additional assistance.

How should an SSP Program select Directors to write their Letters of Sponsorship? Can someone other than Directors (such as a Curator) write the Letters of Sponsorship?

SSP Coordinators may ask Directors who have had recent animal transfers with the non-AZA facility to write the letter as they may have the most current knowledge of and communication with the facility. Alternatively, it may be useful to ask a Director of an accredited facility nearby to provide one of the letters since they may be aware of, have relationships with, or have visited these neighboring non-accredited facilities.

A Curator, or another designee, at an AZA-accredited facility may write the letter, but the Director of the facility must assume responsibility for the contents of the letter by signing it.

If the SSP Coordinator is also a Director from an AZA-accredited facility or CRF, they must include a letter of sponsorship from a Director of a different AZA-accredited facility.

Who should conduct the site visit for a potential Sustainability Partner facility?

Site visits are NOT a required component of a Sustainability Partner application. However, if a Director references a site visit in the letter of support then it must have occurred within the last five years. Visits should have an inspection report associated with them, and may be conducted by someone delegated by the signing Director, such as a member from the SSP Program or TAG Steering Committee or a representative (curator, director, or veterinarian) from a neighboring AZA-accredited facility.

It should be noted, however, that in the application, applicants agree to site visits after they are approved.

Do Candidate Programs need to follow the Sustainability Partner policy process with their non-AZA participants?

No, this policy and application process applies only to current SSP Programs. Candidate Programs will need to assess their non-AZA participants if they become SSP Programs, and facilities that meet the definition of a Sustainability Partner must be approved by APM Committee before they can be included in a BTP.

If a non-AZA facility participates in multiple SSP Programs, can they submit one Sustainability Partner application that encompasses all of these SSP Programs?

APM Committee requires a separate application for each SSP Program in which a non-AZA facility wishes to continue participating. Regulations, standards of care and welfare, facilities, and experience at the proposed partner facility may vary among species, so each must be assessed separately.

Several sections of the application form (e.g., Part A: Facility Information and Facility's Representative Information; and most of Part B) may be easily cut and pasted from application to application, easing the workload of those facilities that may be applying to participate in multiple SSP Programs.

If a facility loses AZA accreditation during the SSP Breeding and Transfer Planning process, does the facility need to become an approved Sustainability Partner to be included in the current, final Plan?

In the event that a facility loses its AZA accreditation during the SSP Breeding and Transfer Plan planning process (i.e., after the planning meeting), if the SSP Program chooses, the newly non-accredited facility may still be included in the Final BTP. The SSP Program should work with the facility to submit a Sustainability Partner application to the APM Committee within six months from the Final BTP publication and must be an approved Sustainability Partner or rejoin AZA before the next BTP. The Final BTP must include a note next to the facility's recommendations table indicating that in order to remain an SSP participant; this facility must work with the SSP to submit a Sustainability Partner application.

Partnerships

What are the benefits of being a Sustainability Partner to a non-AZA facility?

Sustainability Partners benefit in many ways. A Sustainability Partner is part of collective population management and information exchange. They can participate with the SSP Program, be affiliated with professionally-managed AZA programs, receive scientifically-based breeding and transfer recommendations to assist with managing animals at their facility, or acquire new animals.

Being a Sustainability Partner offers a designation of engagement in the preservation of the species in human care that goes beyond simply occasionally providing or receiving animals to/from zoos and aquariums. Sustainability Partners may also have enhanced access to

information about ways to support in situ conservation of the species through more regular and formal interaction with AZA colleagues, or they may be better placed to seek support from the AZA community for their own in situ initiatives.

We also hope that having a centralized, comprehensive, and reasonably up-to-date (five years old or less) profile maintained by AZA and the SSP Coordinator will help reduce the burden of paperwork down the road when the Sustainability Partner is sending or receiving animals from AZA-accredited facilities, as these AZA facilities will have access to the Sustainability Partner applications, which will likely answer many of the questions they typically have when they work with non-AZA facilities. We hope that current non-AZA partners recognize these benefits and value continued participation in AZA SSP Programs.

This application may be a lot of work for applicants; what if a facility doesn't want to fill it out?

The types of questions found within the Sustainability Partner application are very similar to those found in recipient profile forms that many zoos and aquariums already use when they work with non-AZA facilities. Therefore, many potential Sustainability Partners may be familiar with these types of questions and have much of the information already available. SSP Coordinators and TAG Chairs may assist partners that are finding the application process difficult. If the facility does not want to apply to be a Sustainability Partner then they cannot be considered part of the SSP nor included in the BTP.

If an SSP Program no longer wants to include a Sustainability Partner, can they remove them from the SSP Program? Do they need APM Committee approval to do this?

An SSP Program may choose to no longer partner with a Sustainability Partner at any time without APM Committee approval. SSP Programs should let the AZA Conservation, Management, & Welfare Sciences Department know of any changes in participation, and the reasons why the partnership has ended.

Must all Sustainability Partners adhere to relevant AZA policies and accreditation standards, regardless of SSP designation?

The Sustainability Partner must agree to adhere to the AZA's Code of Professional Ethics, SSP Full Participation Policy, and Policy on Responsible Population Management for all animals in their collection. The agreement to follow these policies signals the partner's intent to provide high quality animal care and operate in ways consistent with AZA principles and ethics. As well, it acknowledges the Program Leader's responsibility and authority to maintain best practice animal care and scientific population management standards.

The Sustainability Partner must agree to AZA's SSP Full Participation Policy regardless of the SSP Program designation (Red, Yellow, or Green). This also signals the partner's intent to cooperate fully with the SSP Program and abide by BTP recommendations.

Must all Sustainability Partners adhere to relevant AZA animal care and welfare accreditation standards?

The Sustainability Partner must agree to adhere to Accreditation Standards related to animal care and welfare. The agreement to follow these policies signals the partner's intent to provide high quality animal care and operate in ways consistent with AZA principles and ethics.

What if a non-AZA facility is not "regularly exchanging animals" with the SSP Program population (e.g., movements of animals between the facility and AZA are infrequently recommended by the SSP Program)? Does that mean that they do not qualify to be a Sustainability Partner?

A facility must be "regularly exchanging animals" of the SSP to meet the definition of a Sustainability Partner. The period of time considered "regular" is dependent upon the characteristics of the SSP Program's species, such as lifespan and frequency of breeding events. SSP Coordinators should assess the likelihood of moving animals between the partner and AZA facilities in the next five years and use that as a guideline in determining who to invite to go through the Sustainability Partner application process at this time.

Facilities that are likely to only be receiving OR sending animals to AZA facilities, but not likely to be involved in both types of transactions in the next five years, may not be priorities for applying to become Sustainability Partners at this time. However, they may apply at a later date if deemed appropriate by the SSP Coordinator. SSP Coordinators may ask their Population Biologists for assistance in making some of these decisions. All facilities and all animals are encouraged to be tracked within the Studbooks, regardless of their Sustainability Partner status, so that SSP Coordinators and Population Biologists may potentially assess whether animals at non-Sustainability Partner facilities should be brought into the SSP population.

If a non-AZA facility is expected to ONLY RECEIVE individuals deemed non-essential to the SSP and there is NO REASONABLE EXCEPTION that any individuals from that facility will be transferred into an SSP facility in the future, that facility does not need to be an SSP Sustainability Partner. If an individual is declared non-essential to an SSP and is available for export to a non-SSP facility, no demographic and/or genetic analyses will be conducted to either inform which non-SSP facility the individual should be transferred to or to provide breeding recommendations for that individual at a receiving non-SSP facility.

If a non-AZA facility has expressed a willingness to follow future SSP recommendations and holds one or more individuals that ARE EXPECTED to be transferred into an SSP facility in the future, requiring the demographic and/or genetic value of the individual(s) to be REPEATEDLY ASSESSED during Breeding and Transfer Plan or interim recommendation development to identify when transfer into an SSP facility is beneficial, then that facility should become an SSP Sustainability Partner. A non-AZA facility should also become a Sustainability Partner if it would like to receive breeding and/or transfer recommendations for its animals.

Demographic and/or genetic analyses to evaluate the transfer of individual(s) from a non-SSP facility to an SSP facility will only be completed when a specific need has been defined and transfer(s) have a REASONABLE EXPECTATION of occurring. The demographic and/or genetic value of individual(s) at non-SSPs facilities will not be repeatedly assessed during

Breeding and Transfer Plan or interim recommendation development to identify when the transfer to an SSP facility might be beneficial.

How should the SSP Program document in the BTP when an animal is transferred out of the SSP population to a non-AZA facility that is not a Sustainability Partner for that SSP Program?

In the BTP, the animal should be given a "SEND TO" transfer recommendation, labeled as "excluded", and have a note indicating that the animal will be transferring "Out of the SSP." The receiving non-AZA facility will not be listed in the BTP. The SSP Coordinator will be responsible for discussing the potential transfer options with the current holding facility, separate from the BTP. The holding facility will adhere to its own policies when transferring the animals to the non-AZA facility. The Studbook Keeper should record this transfer, and the destination, in the Studbook.

When an AZA member-owned animal is currently held at a non-AZA facility, does that facility need to be a Sustainability Partner to remain in the SSP Program?

When an AZA member-owned animal is held at a non-AZA facility, the best course of action will vary depending on the needs of the SSP Program. There are several possibilities: 1) The non- AZA facility may apply to become a Sustainability Partner to the SSP Program so that the animal remains part of the SSP population; 2) The non-AZA facility does not become a Sustainability Partner, and the animal is not included in the SSP nor BTP and remains at the non-AZA facility; or 3) The owning AZA facility moves the animal to an AZA facility and it remains in the SSP population. Animals should be moved as soon as possible; however, it is understood that this may sometimes take additional time.

What are the options if a non-AZA facility does not want to complete the Sustainability Partner application but still wants to be involved in the SSP Program?

There are several categories of AZA membership that are available including AZA-accredited members and Certified Related Facilities (https://www.aza.org/organization-membership). AZA membership provides many benefits and many opportunities. See more information at https://www.aza.org/benefits-of-accreditation, or contact membership@aza.org.

If a non-accredited facility does not become a Sustainability Partner, does this mean I cannot work with them in any way?

No, AZA facilities may still send animals and/or receive animals to/from non-AZA facilities at the recommendation of the SSP Coordinator according to their own animal transfer policies. When these transactions are mostly one-way (send to OR receive from) and/or rare, those non-AZA facilities are not engaging with the SSP at the level of Sustainability Partner. It does not mean that these facilities are less valuable or not necessary. AZA-accredited facilities may continue to work with non-AZA facilities in accordance with AZA Accreditation guidelines, Code of Professional Ethics, and Policy on Responsible Population Management.

In some cases, a non-AZA facility may hold individual animals for a long time before those animals become genetically and/or demographically beneficial to the SSP population. AZA recognizes that these facilities are still performing a valuable contribution and hopes the facility will provide data on the animals to the Studbook Keeper so that the animals can be tracked. The level of engagement a potential partner has with an SSP Program can change over time and thus there may be periods when it does and does not make sense for the SSP Program to try and move the facility through the Sustainability Partner process.

Are AZA Certified Related Facilities required to apply as Sustainability Partners?

No. Certified Related Facilities are full institutional AZA members, subject to the same standards, policies and processes as accredited members, with one exception, because they are not regularly open to the public, they are not required to maintain education programs.

What can SSP Program Leaders do to help determine which individual animals to infrequently send out of the SSP (export) and/or receive into the SSP (import) (i.e., "one- way," or "one-off" transfers)?

The best way to make these decisions is with an AZA Population Biologist and using PMx software; however, SSP Program Leaders can also do some investigation on their own to help make these decisions. When potentially bringing an animal into the SSP, it is best to first determine if this animal will add value to the SSP population. This value can come in many forms (e.g., genetically, demographically, husbandry, ambassador needs). When potentially sending an animal out of the SSP, there is always a cost and many variables must be carefully considered, including what will the welfare of the animal be outside of the SSP, will losing the animal hurt the demographic or genetic stability of the SSP, etc.

As each SSP is unique, there is no way to create an extensive list, but below are some examples of ways to further investigate these potential values and considerations.

- What do you know about the potential animal(s) that are proposed to join the SSP? If you are adding them for genetic reasons, do you know their pedigree and are they linked to the SSP population? If they are intended for breeding, are they of an appropriate age, reproductively viable, experienced?
- Look at the studbook and previous Breeding and Transfer Plan for the SSP.
    - Demography
        - Are more animals demographically needed for this SSP (i.e., it has a very small population size or lacks young, breeding-aged animals)?
        - Is the SSP population demographically robust enough to send out and potentially lose animals (i.e., is the animal(s) you plan to export in pre-reproductive or reproductive age classes and will you have enough reproductive-aged animals remaining in the SSP to meet future breeding goals)?
    - Genetics
        - Would the SSP population benefit from adding more unique genes (i.e., is gene diversity low and projected to decline quickly? Do you have a small number of founders represented in the SSP?)?
        - Are the proposed non-SSP animals related to the SSP population? If so, how closely related? Has the SSP previously imported animals from this source?
        - Would the SSP population be able to withstand sending out and potentially losing some genes? Are the animals you plan to export over-

represented, having high mean kinship and many living relatives in the SSP?

- Husbandry
  - Is the husbandry known and consistent for this SSP population? If not, could this non-SSP facility share knowledge, expertise, or experienced breeding animals to help the SSP?
  - Was this non-AZA facility included in the last BTP? If so, what were their breeding and transfer recommendations?

- Are these potential animals included in the SSP studbook database? If so, look in the database to identify closely related individuals to minimize inbreeding when making new breeding recommendations. Use the Antecedent and Descendant Pedigree Reports in PopLink, the Sibling Tables and Descendant Lists in SPARKS, or the Pedigree tools in ZIMS for Studbooks.
- Think about the logistics, resources, abilities, and acquisition/disposition policies of the facilities potentially involved in these transfers. Are they conducive to making the proposed transfers occur?

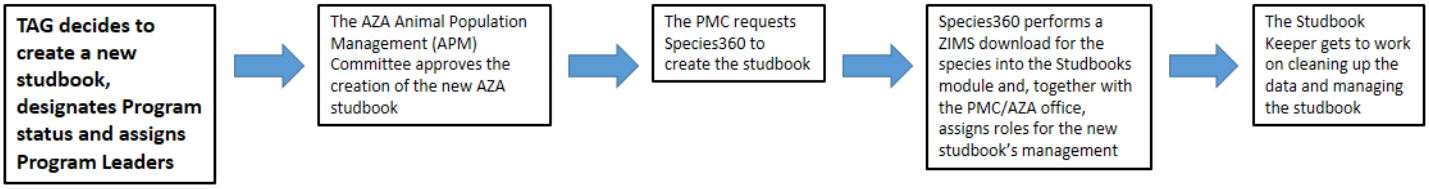
Appendix J: Starting a New AZA Studbook in ZIMS for Studbooks

This document outlines how a new AZA studbook can be started in ZIMS for Studbooks. There are several ways this is possible:

1. A new studbook in ZIMS for Studbooks can be created from ZIMS for Husbandry data.
2. An existing Candidate or TAG-monitored studbook already in ZIMS for Studbooks can be reclassified to become a new SSP studbook.
3. A studbook that exists in another software can be migrated over to ZIMS for Studbooks.
4. A new blank studbook can be created for data to be entered manually by the Studbook Keeper.
5. An existing studbook within ZIMS for Studbooks but managed by a different region or institution can be transferred to AZA.

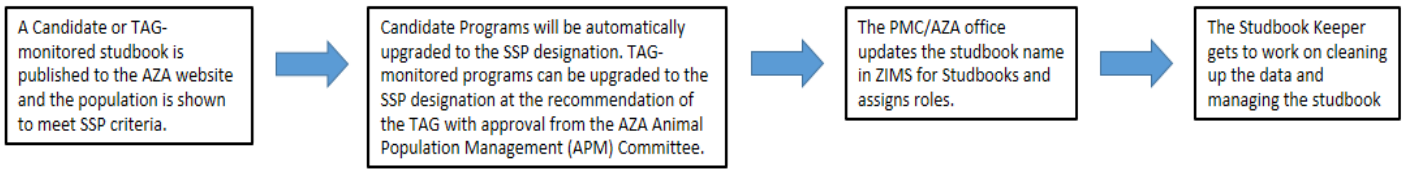
1. Starting a new AZA studbook from ZIMS for Husbandry data

A new studbook can be created using data from the ZIMS for Husbandry module, which contains data entered by Species360 member institutions. This can be done for a new AZA Animal Program or to restart a studbook that was lost or corrupted in legacy software. Creating this studbook would be an AZA TAG level decision, and dependent on approval from the Animal Population Management (APM) Committee. Species360 will download data for the species from ZIMS for Husbandry into the ZIMS for Studbooks module, and the PMC/AZA office will assign roles to the Studbook Keeper and other program participants.



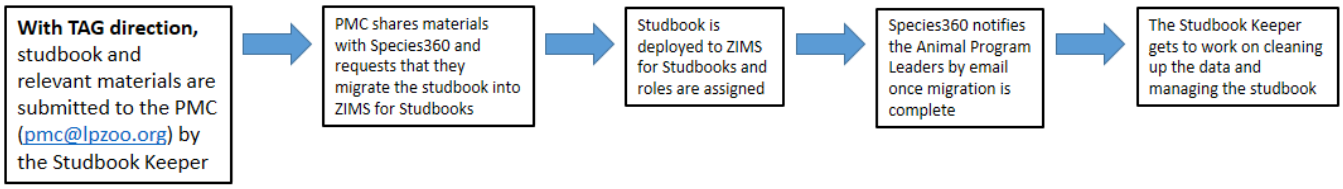
2. Starting a new AZA studbook by upgrading to SSP

A studbook may exist in ZIMS for Studbooks but is not a designated SSP Program; for example, it may be a Candidate or TAG-monitored program. In the event that it is eligible to become an SSP (i.e. a published studbook indicates the population meets SSP criteria), a Candidate Program will automatically be upgraded to the SSP designation and a TAG-monitored program can be upgraded at the recommendation of the TAG with approval from the Animal Population Management (APM) Committee. The PMC/AZA office will change the name on the studbook and assign roles to the Studbook Keeper and other program participants.



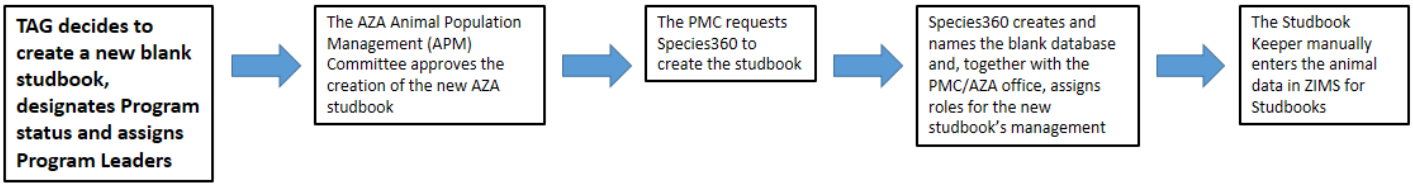
3. Starting a new AZA studbook by migrating from a legacy software

There may be a studbook that is maintained in PopLink, SPARKS, Excel, Access, etc. There are various reasons studbooks may not yet have migrated. Some may not have been able to migrate to ZIMS for Studbooks previously due to special functionality required for their management not yet developed by ZIMS. When the necessary functionality becomes available, the Studbook Keeper should send the studbook and other relevant materials to the PMC (pmc@lpzoo.org), who then will share them with Species360 for migration.



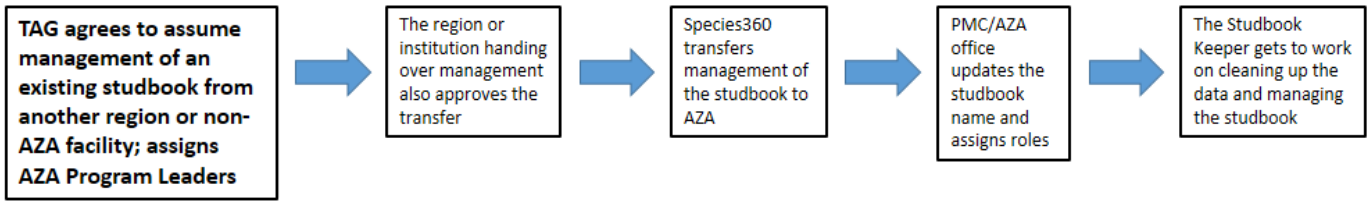
4. Starting a new AZA studbook by manually inputting data into a blank database in ZIMS for Studbooks

While uncommon, there may be reason to start a new studbook by manually entering data only. Species360 can create a blank studbook in these cases and the designated Studbook Keeper enters the data by hand.



5. Starting a new AZA studbook by transferring an existing studbook from a different region or institution over to AZA

Species360 can transfer over an existing ZIMS for Studbooks database from a different region or institution if all parties/regions approve of the transfer.



Resource documents:

- Studbooks from ZIMS Data
- A Reference Guide to ZIMS for Studbooks for Animal Program Leaders
- Working Together in a Shared Studbook Database
- Tidying Your Studbook in 5 Steps

Contacts:

Topic	Person	Role	Email
General questions; giving others access to your studbook database	Rebecca Greenberg	AZA	animalprograms@aza.org
	Kendra Strohmayer	PMC	pmc@lpzoo.org
Population questions	Your Population Biology Advisor	PMC, Adjunct, SPMAG	Contact pmc@lpzoo.org for referral if you don't yet have an advisor
ZIMS for Studbooks software technical questions	Species360 Support	Species360	support@species360.org

Appendix K: AZA Guidelines for Roles and Access to ZIMS for Studbooks

ZIMS for Studbooks allows for different roles to be created to give access to specific features in each studbook database, such as the ability to view or edit data, run reports, and use available tools. Multiple people can have access at the same time to a single studbook database. The intention of this document is to outline AZA's currently existing roles and give guidance on whom they could be assigned.

At the request of approved AZA Studbook Keepers, access roles are currently assigned by the Regional Association Administrators (i.e., AZA Conservation, Management, & Welfare Sciences Program Assistant and PMC Planning Coordinator). To simplify the customizability of ZIMS for Studbooks and protect data quality, the features available to roles are preset as described below.

Not all SSPs are managed the same. For this reason, SSP Coordinators and Studbook Keepers may request custom roles. Depending on the nature of the request and parties involved, a request must be submitted to animalprograms@aza.org and may need additional approval by the APM Committee and corresponding TAG. As ZIMS for Studbooks is updated with new features, populated with more studbook databases, and we learn from the requests coming from SSPs, these roles and access options may change to accommodate the perceived needs.

If any AZA member facilities wants access to the AZA studbook database, but are not involved in the SSP in any way, they must request access from both the Studbook Keeper and APM Committee Vice Chair of SSPs and Studbooks (animalprograms@aza.org).

We encourage collaboration within AZA and WAZA-recognized regional associations, but sharing data outside of the AZA/WAZA community is more sensitive. For guidance on when it is appropriate to share studbook data, please see the AZA's 'Guidance for SSP Coordinators and Studbook Keepers on Sharing Studbook Data' (Section II, page 3).

AZA Studbook Data Ownership:

Access and use of studbook data is vital to the AZA mission to maintain and perpetuate healthy populations of animals. To this end, AZA owns the compilation of studbook data contributed at any time by AZA members (current and archived), Sustainability Partners, SSP Programs, and other participants in its animal programs (hereafter referred to as "Contributors"). By its participation as a Contributor, a Contributor gives AZA the non-exclusive right to use its contributed data for studbook purposes including unrestricted use by AZA members and the AZA right to authorize access and use by third parties (including researchers and members of other WAZA-recognized regional associations) without further notice or additional permission. (Updated approval by the AZA Board, July 2019).

AZA Roles and Their Access for ZIMS for Studbooks

1. Studbook Keeper
   - Full view and edit access (except for the overlay tool)
   - Given to: TAG-appointed Studbook Keepers recognized by AZA will receive this access as soon as the studbook database is in ZIMS for Studbooks. For Candidate Programs and TAG monitored populations, the TAG studbook maintainer will be treated the same as an SSP Studbook Keeper and receive access as soon as the studbook database is in ZIMS for Studbooks.
   - Access: Can view and edit all data, and run any report or tool, except for overlays.
   - This role needs approval by the AZA office, to verify that they are an approved AZA Studbook Keeper, TAG-approved Candidate Program or TAG monitored population, or AZA member facility sponsored studbook database.

2. All View and Edit Access
   - Full view and edit access (customizable, as needed)
   - Given to: TAG-appointed Studbook Keepers and SSP Coordinators recognized by AZA or others, as approved by the Studbook Keeper and SSP Coordinator
   - Access: Can view and edit all data, and run any report or tool, including overlays, with the tool access being customizable, as needed.
   - If it is a Studbook Keeper or SSP Coordinator requesting this access, they need to have completed the PM2 Course.
   - This role needs approval by the Studbook Keeper, SSP Coordinator, and Advising Population Biologist.

3. View and Export Only Access
   - Given to: the Studbook Keeper and SSP Coordinator approve who to give this access to and may include the former Program Leaders, International Studbook Keeper that maintains a different studbook database, another WAZA-recognized regional association's equivalent Program Leader, Apprentice Studbook Keeper, TAG Chair, APM Committee member, SSP Advisors, TAG Advisors, ILs, PM1/PM2 instructors, researchers, etc.
   - Access: Can view all studbook data, run any report or tool, and export data to Excel, but cannot edit data or use overlays.
   - Duration: Some 'View and Export Only' roles may include a specific timeframe that must be re-approved after each period.

4. Population Biology Advisor
   - Full view and edit access (all features)
   - Full access to data, editing, reporting, and tools
   - Given to: AZA Population Biology Advisors will receive this access as soon as the studbook database is in ZIMS for Studbooks.
   - Population Biology Advisors from other WAZA-recognized regional associations can also request this type of access in order to advise and manage programs in their association. The regional association must verify this request before access is granted

5. Regional Administrator
   - Full administrative access
   - Given to: AZA Administrators (AZA Conservation, Management, & Welfare Sciences Program Assistant, PMC Planning Coordinator, AZA Director Animal Programs, PMC Director)
   - Assign individuals to access studbook databases using one of the roles listed above

A description of all features and how to create, delete, or edit roles can be found in the ZIMS for Studbooks Roles document.
(http://training.species360.org/Documents/ZIMShelp/ZIMSHelp-Studbooks-Update%20Roles.pdf).

Conflict Resolution

There may be cases of conflict between users over their roles and access to a studbook and their handling of data. This conflict may be within AZA, the larger WAZA community, or when sharing with outside users. If needed, the 'AZA Animal Management Reconciliation Policy' (found as an Appendix in the AZA SSP Program Handbook; https://www.aza.org/animal-program-handbooks) should be used for any conflict resolution needs that arise.

Appendix L: Guidance for SSP Coordinators and Studbook Keepers on Sharing AZA Studbook Data

The intention of this document is to provide guidance on when it is appropriate to share studbook data or allow studbook access. This information is specific for AZA studbooks; international studbooks or studbooks from other regions may be subject to other processes.

Sharing studbook data

There are many ways for SSP Coordinators and Studbook Keepers to share studbook data. Summarized data can be shared via exported population figures, tables, and reports (using SPARKS, PopLink, ZIMS for Studbooks, PMx, Excel, R), Population Viability Analyses (PVAs), and Breeding and Transfer Plans (BTPs). Historic and current studbook data are viewable via AZA Studbook Publication documents. An entire PopLink, SPARKS, or Excel studbook database may also be shared, and ZIMS for Studbooks allows for roles to be created to give individuals customized access to a studbook database.

Sharing access to your studbook database within ZIMS for Studbooks

ZIMS for Studbooks allows for different roles to be created to give access to specific features in each studbook database, such as the ability to view or edit data, run reports, and use available tools. Multiple people can have access at the same time to a single studbook database. If any SSP participants would like access to the studbook data in ZIMs for Studbooks, 'View and Export Only' access may be the best choice and can be requested from the Studbook Keeper and SSP Coordinator, who then request it from the AZA Administrators (animalprograms@aza.org). For more information on sharing data from studbook databases within ZIMS for Studbooks see 'AZA Guidelines for Roles and Access to ZIMS for Studbooks' (Section I, page 1).

While a distinction can be made between sharing studbook data and sharing studbook access, from here on for the purposes of this document, "sharing data" will be used to include both descriptions.

Sharing studbook data with colleagues within the AZA Community

We encourage collaboration within the AZA community. Potential SSP collaborators may include an SSP Advisor, TAG Chair, TAG Advisor, participating SSP facility, researcher affiliated with an AZA facility, or other colleagues involved in the SSP. Even when sharing studbook data within the AZA community, SSP Coordinators and Studbook Keepers should consider the following:

- The AZA Studbook Keeper will always remain ultimately responsible for their studbook database and all data within it.
- Be aware that information in a studbook database has been contributed by numerous facilities, with the understanding that these data are only to be used for collaborative population management within AZA.
- Be aware that the studbook database may contain sensitive information or information that may be considered controversial (e.g., transfers, management euthanasia, individual animal's notes).
- Never share the log in information to your personal ZIMS account. People with whom you would like to share information must have their own log in access to ZIMS. If they do not already have an account, contact AZA Administrators (animalprograms@aza.org) for guidance.
- Collaborators planning to publish research or analysis based on studbook data that could be distributed outside of the AZA community are required to fill out and submit the consent form (Appendix L) to both the Studbook Keeper and APM Committee Vice Chair of SSPs and Studbooks (animalprograms@aza.org) before data are shared.

Sharing studbook data with collaborators from WAZA-recognized regional associations

We encourage collaboration within the WAZA community. AZA Studbook Keepers and SSP Coordinators may need to share studbook data with colleagues working at institutions within WAZA-recognized regional associations (e.g., EAZA, Australasia's ZAA, SEAZA), particularly population biologists or Studbook Keepers for the same species in other regions. Studbook Keepers and SSP Coordinators wishing to grant access should contact AZA Administrators (animalprograms@aza.org) to have an access role assigned within ZIMS

for Studbooks. Reciprocal access can also be requested to view studbook data maintained within other WAZA-recognized regional associations.

Sharing studbook data with collaborators outside the AZA/WAZA Community
Sharing studbook data with collaborators outside AZA or WAZA-recognized regional associations can lead to more effective *ex situ* population management and species conservation. Potential outside collaborators include advisors, researchers at academic institutions, or partner NGOs. However, data sharing is not always mutually beneficial, and comes with risk including the misuse or misinterpretation of data, and sharing of information beyond the intended audience.

It is important that you initially create an agreement with any outside collaborator(s) concerning sharing data from a studbook, as well as publication of the data or any research results based on these data. Creating an agreement before sharing access to the studbook database will allow you to feel more comfortable with sharing these data. Researchers, or anyone planning to publish or present results based on studbook data, are required to fill out and submit the consent form (Appendix L) to both the Studbook Keeper and APM Committee Vice Chair of SSPs and Studbooks (animalprograms@aza.org) before data are shared. It is recommended that the following disclaimer be added to any data shared outside of the AZA/WAZA community or any published research or results based on the shared studbook data.

AZA Studbook Data Sharing Disclaimer
The data shared here are Copyright of AZA (date). All rights reserved. None of these data may be used in any future research or publication, or reproduced in hard copy, machine-readable or other forms without consent from the Studbook keeper and the APM Committee Vice Chair of SSPs and Studbooks and a written agreement in place. Members of the Association of Zoos and Aquariums (AZA) may copy this information for their own use as needed. AZA strongly recommends that users of this information consult with the Studbook Keeper in all matters related to data analysis and interpretation.

When considering sharing studbook data, be aware of the following:

- Before sharing studbook data, request a research proposal from the researcher to better understand why the studbook database is being requested, what specific data will be needed from the studbook database, and how the researcher intends to use the data. For an example of such a research proposal form, see the AZA Research and Technology Committee's 'AZA Standardized Research Application Form' here: https://www.aza.org/research_and_technology_committee.
- Check in with your TAG before sharing data. Several AZA TAGs have existing processes for evaluating potential research involvement.
- Verify who the researcher is that you are communicating with and about to potentially collaborate. You can do this by looking at their academic websites, LinkedIn, previously published articles, etc.
- Even those that have 'View and Export Only' access in ZIMS for Studbooks can export the entire studbook database to Excel, which allows them to analyze and share these data with others.
- It is important that you initially create an agreement with the collaborator concerning sharing the studbook data with third parties as well as publication of the data or any research results based on these data (see Appendix L). Creating an agreement before sharing access to the studbook database will allow you to feel more comfortable with sharing these data.
- We encourage SSP Coordinators and Studbook Keepers to at least be listed in the acknowledgements section and should be considered as co-authors on any publications using the studbook database (e.g., published journal articles, talks, posters), depending on their involvement and how prominently the studbook data are used.
- Studbook data are best interpreted by those trained in small population management and studbook data conventions and software. An AZA Population Biologist must be involved with data requests from external researchers to guide data analyses and interpretation.
- Identify the end date for the collaborator's access to the studbook database. Depending on the research, this may be several weeks to several months or longer. If an end date is not identified, collaborators will be assigned the default access of six months. It is good practice to annually review who has access to your studbook database and update, as needed.

- Researchers are required to include both the studbook Currentness date and date of access in any publications and presentations.
- The consent form in Appendix L must be filled out and submitted to both the Studbook Keeper and APM Committee Vice Chair of SSPs and Studbooks (animalprograms@aza.org).

As a reminder, all AZA Program Leaders (e.g., Studbook Keepers, SSP Coordinators, TAG Chairs) can get access to ZIMS for Studbooks, even if your AZA facility is not a Species360 member. Contact the AZA Administrators (animalprograms@aza.org) for assistance if your AZA member facility is not a member of Species360.

For colleagues that are neither an AZA member nor a Species360 member and want access to a studbook database, they first need to get a ZIMS login from Species360. In addition to permission from the Studbook Keeper, permission will also be required from the APM Committee Vice Chair of SSPs and Studbooks (animalprograms@aza.org).

*Please note that these guidelines are available in a digitized Word form at https://www.aza.org/resource-documents

Required Consent Form for Access to an AZA Studbook Database for Analyses

*****For researchers and anyone outside of the AZA/WAZA community who are planning to publish or present results based on Studbook data

Attach a research proposal to briefly explain why the studbook data are being requested, what specific data will be needed from the studbook database, and how data will be analyzed and used. The SSP must also attach a letter of support for this specific researcher and their intended research.

I have read both the "AZA Guidelines for Roles and Access to ZIMS for Studbooks" and "Guidance for SSP Coordinators and Studbook Keepers on sharing studbook data" and I agree to the following terms (initial on each line):

____ I will only use the studbook data for analyses relevant to population management and species conservation.

____ Analyses of data from the _____ (insert individual or multiple species' name(s)) studbook database will never be presented or published without consent of the SSP Coordinator and Studbook Keeper.

____ Individual facility information contained in these records will not be shared in any way, without specific written permission from the respective submitting facilities.

____ Any publications and presentations resulting from analyses of data from the studbook listed above will have shared authorship with the SSP Coordinator and Studbook Keeper as well as any AZA staff or Population Biologist involved, as appropriate to the regional scope of the analysis.

____ All authors on any reports resulting from analyses of data from this studbook database will fully review the material to be submitted, will be willing to support the conclusions of the study, and can defend it.

____ The AZA Animal Population Management Committee reserves the right to block publication and presentation of results if agreement cannot be reached on the content of the reports.

____ When submitting manuscripts using studbook data to journals requiring deposit of data for public access and later use, the author must include the italicized statement below in the document to be deposited. All individual animal and institutional identifying information must be stripped from the deposited document and replaced with dummy codes.

> *The authors gratefully acknowledge the use of aggregate studbook data by permission of the Association of Zoos & Aquariums (AZA), the owner of this compilation of studbook data contributed by AZA members and other participants in its animal programs. That permission prohibits identification of particular facilities or identifiable details of particular animals.*

Signature of Applicant: _____ Date: _____

Printed Name of Applicant: _____

Approved by the AZA APM Committee Vice Chair of SSPs and Studbooks

Signature: _____ Date: _____

Printed Name: _____

Date that studbook database and/or access to studbook database was given to applicant

Date: _____

Appendix M: Communications Guidelines

Guidelines on communications that represent the Association of Zoos and Aquariums and its Members

All public statements* that may be construed to represent a communication from the Association of Zoos & Aquariums (AZA) or are made by or on behalf of any AZA Program** must be reviewed and approved by the appropriate AZA Department prior to public release or publication. In addition, plans to create such documents should involve input from AZA and other appropriate AZA entities** during their conceptualization and development.

* For example, but not limited to, position or advocacy statements, letters of support / endorsement or censure, policies, petition signatures, proposals, and comments on legislative / regulatory actions

** For example, but not limited to, Committees, Scientific Advisory Groups, Taxon Advisory Group, Species Survival Plan® Programs, Conservation Action Partnerships, Task Forces, the Population Management Center, and the Reproductive Management Center.

Appendix N: SSP Highlights Template

*Please note that this template is available on the AZA website (https://www.aza.org/resource-documents)

The brief parameters for SSP Highlights:

- 300-500 words
- "Catchy" title
- Author/SSP Coordinator (with job title)
- Photo (5*7 300dpi)
- Appropriate for the broad audience that receive *CONNECT* magazine

Introduction should include one or more:

- General species information
- Conservation status
- What is their role in AZA zoos and aquariums?
- Why are they cool/interesting/important animals?
- General SSP information (population size, number of organizations in the SSP)

Body should include one or more:

- Sample challenges to SSP population sustainability that will be discussed in the highlight (e.g., low gene diversity, small population size, need for husbandry/management enhancements, etc.).
- New, innovative, and/or creative ways that the SSP is engaging in to address the challenge identified (e.g., international collaborations, research projects, artificial reproductive technologies, new husbandry and management practices, etc.).
- While there may be more than one sustainability challenge and more than one initiative in place, given the limited space it is recommended to focus on only one initiative in the SSP Highlight.

Conclusion should include:

- The results and impacts of the initiative on the SSP population in lay terms
- Lessons learned. Sharing the SSP's story is important as it may inspire other SSPs with similar challenges to try something new or think about their situation in a new light.
- Future plans to further enhance sustainability, if necessary

Please submit draft SSP Highlights to animalprograms@aza.org.

Appendix O: AZA Position Statement Template

*Please note that this template is available on the AZA website (https://www.aza.org/resource-documents)

ASSOCIATION OF ZOOS & AQUARIUMS

Title

Approved by the AZA Board of Directors on ????

A Position Statement defines the Association's position on a specific issue and most frequently supplements an AZA Board approved policy (http://www.aza.org/board-policies/). Please concisely describe AZA's position on the identified subject matter. If this Position Statement is associated with an AZA Board approved Policy, be sure to indicate to which policy [e.g., Acquisition and Disposition Policy (2008), Policy on the Presentation of Animals (2008), Program Animal Policy (2011)] the statement is related.

Please use Arial 10pt font for all text and separate each paragraph within a section by a 5pt. space.

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Appendix P: AZA White Paper Template

*Please note that this template is available on the AZA website (https://www.aza.org/resource-documents)

AZA White Paper

ASSOCIATION OF ZOOS & AQUARIUMS

Title

Approved by the AZA Board of Directors on ????

AZA Position

Include this section in the White Paper only if there is an AZA Board approved Position Statement on this topic. If there is not an approved Position Statement then please remove this section. If this White Paper is being submitted alongside a Draft Position Statement, then you may include the Draft Position Statement here.

Please use Arial 10pt font for all text and separate each paragraph within a section by a 5pt. space.

Rationale

A White Paper may be an article that provides additional information to supplement and support a specific AZA Position Statement, or an informational article that discusses a philosophy or an initiative that is of relevance to the Association.

Please compose a concise, well-cited article that provides the evidence to supplement and support the AZA Position Statement above, or the identified philosophy or initiative. If this White Paper is informational and does supplement a Position Statement, then this "Rationale" section will be the first section of the White Paper.

Please number each citation in the References section below and include citation numbers as superscripts at the end of the appropriate sentence [1].

AZA Action

Detail the recommended actions that AZA institutions and/or members should engage in to adhere to the AZA Position Statement supplemented by this White Paper. If this white paper is informational, then this section is optional and may be removed.

References

References should be numbered and in APA format

1. Last name, First Initial and Last Name, First Initial. (year). Title of article. *Title of Journal, Issue*, page – page.

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Appendix Q: AZA Guidelines Template

*Please note that this template is available on the AZA website (https://www.aza.org/resource-documents)

ASSOCIATION OF ZOOS & AQUARIUMS

Title

Approved by the AZA Conservation & Science Department (or other relevant party) on ????

While the majority of Guidelines (e.g., hand-rearing protocols, mixed species exhibit suggestions, etc.) may not require AZA Board approval it is still important that they are reviewed and approved by the AZA Conservation & Science Department before they are published and distributed to ensure that they are appropriate and reflect the philosophy of the Association.

Guidelines may provide potential strategies, suggest procedures, and /or provide additional information regarding a specific topic. If these Guidelines are associated with an AZA Board approved Policy, be sure to indicate to which policy [e.g., Acquisition, Transfer, and Transition Policy (updated 2014), Policy on the Presentation of Animals (2008), Program Animal Policy (2011)] the Guidelines are related.

Please use Arial 10pt font for all text and separate each paragraph within a section by a 5pt. space.

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Appendix R: Sample IR Statement of Commitment

AZA Animal Program Institutional Representative (IR) Guidelines

Overview

The Institutional Representative (IR) is the primary contact between their institution and the Program Leader of the Animal Program(s) to which they have been designated. The IR is responsible for maintaining open communication between the Animal Program and the institution, communicating to the Program Leader on behalf of the institution, and participating in Animal Program communications and activities.

Each institution is represented by one IR for each Animal Program the institution participates in. If the TAG Chair moves to an institution that already has an IR represented in that TAG, or if a new Chair is appointed from an institution that already has an IR represented in that TAG, the TAG Chair will automatically be appointed as that institution's IR. The previous IR must relinquish his/her position because there can only be one IR, and one vote, per institution for each Animal Program. If the former IR served on the Steering Committee, the TAG will hold an immediate election to replace the Steering Committee member. SSP Coordinators and Studbook Keepers are not automatically approved as IRs; they must be designated as their institution's IR by the Institutional Liaison (IL). Program Leaders who are not Steering Committee members may still participate in the TAG as non-voting advisory members.

One individual may serve as the IR for more than one Animal Program at an institution; however, the duties for each Animal Program are independent of each other. IRs should be aware that being a representative to multiple Animal Programs involves a greater commitment. The IR is appointed by the Institutional Liaison (IL) unless the institution's Director assumes this responsibility.

Eligibility Requirements

The Institutional Representative must:

- be an employee of the institution they represent.
- be designated by the IL of the institution.
- uphold TAG business confidentiality.
- serve as the institution's IR for the TAG if they serve as TAG Chair.
- be familiar with the species/taxa they represent. It is understood that there will not always be a staff member that specializes in a particular taxon or species. In these situations, the position should fall to the person on staff who is the most logical point of contact for the Animal Program.
- have the ability to make decisions about the institution's animal collections or be able to communicate with those who have the ability to make decisions about the collections.
- have proficiency in word processing and spreadsheet programs, utilizing AZA web resources, and have email access. Most documents will be sent electronically or be available for download from the AZA website, and the IR must be able to view and download in documents in Microsoft Word and PDF formats.
- have the capability to disperse documents to the appropriate institution personnel.

Essential Position Functions

- Communicate with and disseminate information among Animal Programs, Program Leaders, the IL, the Institutional Director and the animal care staff, and work with and encourage Program Leaders to build sustainable populations.
- Respond to and fulfill inquiries by TAG and SSP Programs in a timely manner.
- Vote in all Steering Committee/Management Group elections.
- Review and complete “Institutional Wants and Needs” surveys within the requested time frame.
- Communicate Animal Program participation with the IL.
- Review and communicate comments for Draft Breeding and Transfer Plans and RCPs to the IL and Program Leaders during the 30-day comment period.
- Request Animal Program documents from the IL if the IR is not an individual AZA member and does not have access to documents through the AZA website.
- Ensure that any Regional Collection Plans (RCPs) and Breeding & Transfer Plan recommendation disagreements are addressed with the IL and Program Leaders during the comment period.
- Complete and return space surveys for TAG RCPs within the requested time frame.
- Consider volunteering for Animal Program activities and standing for election to Animal Program committees.
- Communicate any contact information amendments or change of status to the IL.

When requests are made for Taxon or Specimen reports from AZA Animal Program Leaders, please contact the Curator-Zoological Records to assist in providing the correct information as necessary.

Many AZA Animal Programs use the PMC Track software (www.pmctrack.org) prior to assembling Breeding & Transfer Plans. While not a requirement for Animal Programs, this software is a valuable tool in tracking recommendations in such plans. IRs are strongly encouraged to use this system when requested by a Program Leader for their Animal Program.

Surveys and Breeding & Transfer Plans (drafts and final) must be discussed with the appropriate Zoological Curators before being submitted or confirmed. IRs must be familiar with their roles and responsibilities, and are expected to communicate questions or concerns in a timely manner.

By signing, I understand the above information and accept the responsibilities and duties as explained.

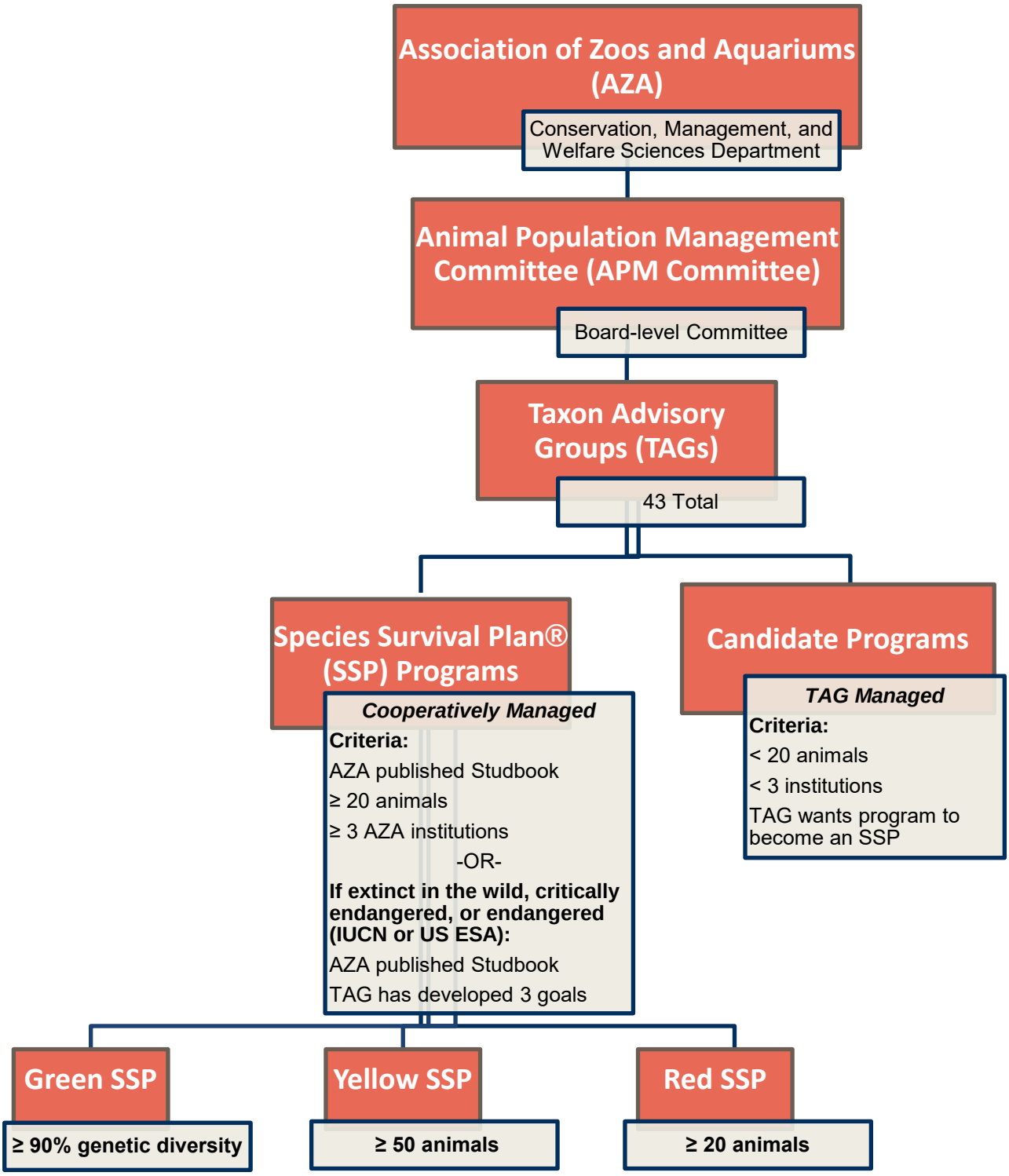
Print Name _____ Signature _____

Date _____

A copy of this signed form will be kept on file in the office of the Institutional Liaison.

Date created/Last edited: 23 April 2013 / 13 Jan 2021

Appendix S: AZA Animal Programs Chart



Appendix T: Citation Formats

Citation of an SSP Breeding and Transfer Plan:

*SSP Coordinator should be the first author, then Studbook Keeper, then the Population Biologist.

SSP Coordinator last name, first initial., Studbook Keeper last name, first initial., and Population Biologist last name, first initial. Year published. Species common name (*Scientific name*). AZA Species Survival Plan® Designation color Program Population Analysis & Breeding and Transfer Plan. AZA Population Management Center: Chicago, IL.

McAuliffe, J., Ross, S., and Andrews, J. 2017. Chimpanzee (*Pan troglodytes*). AZA Species Survival Plan® Green Program Population Analysis & Breeding and Transfer Plan. AZA Population Management Center: Chicago, IL.

Citation of a Global Species Management Plan

GSMP Coordinator last name, first initial. and Population Biologist last name, first initial. Year published. Species common name (*Scientific name*) WAZA Global Species Management Plan. Institution name: City, State.

Myers, M., Gardner, L., and Lynch, C. 2018. Blue-crowned Laughingthrush (*Dryonastes courtoisi*). WAZA Global Species Management Plan. Riverbanks Zoo: Columbia, SC.

Citation of an AZA Regional Studbook:

Studbook Keeper last name, first initial. Year published. Species common name (*Scientific name*) AZA Regional Studbook. Institution name: City, State.

Ross, S. 2015. Chimpanzee (*Pan troglodytes*) AZA Regional Studbook. Lincoln Park Zoo: Chicago, IL.

Citation of a Regional Collection Plan:

TAG Chair last name, first initial. Year published. TAG name Regional Collection Plan. Institution name: City, State.

Holmes, C. 2018. Galliformes TAG Regional Collection Plan. Houston Zoo: Houston, TX.

Citation of a Population Viability Analysis:

(all Last name, First initial) Population Biologist., SSP Coordinator., Studbook Keeper., TAG Chair., and TAG Vice-Chair. Year. Species common name (*Scientific name*) AZA Animal Program Population Viability Analysis Report. Lincoln Park Zoo: Chicago, IL.

Johnson, B., Ray, J., Reinartz, G., Meinelt, A., Stoinski, T., and Fenn, T. 2016. Bonobo (*Pan paniscus*) AZA Animal Program Population Viability Analysis Report. Lincoln Park Zoo: Chicago, IL.

Citation of an SSP Sustainability Report:

SSP Coordinator last name, first initial. Year published. Species common name (*Scientific name*) Species Survival Plan® Sustainability Report. Association of Zoos and Aquariums: Silver Spring, MD.

McAuliffe, J. 2017. Chimpanzee (*Pan troglodytes*) AZA Species Survival Plan® Sustainability Report. Association of Zoos and Aquariums: Silver Spring, MD.

Citation of a Survival Statistic Report:

(all Last name, First initial) SSP Coordinator., SSP Vice Coordinator., Studbook Keeper., Population Biologist. Year. Descriptive Survival Statistics Report for Species common name (*Scientific name*). Chicago (IL): Lincoln Park Zoo.

Fischer, M., Gray, C., Keele, M., Ray, J., Long, S. 2014. Descriptive Survival Statistics Report for Asian Elephant (*Elephas maximus*). Chicago (IL): Lincoln Park Zoo.

Citation of PMCTrack:

Faust, L., Theis, M., Long, S., and Shell, S. 2011b. PMCTrack: A Website for Monitoring Breeding and Transfer Recommendations for Zoo Programs. Lincoln Park Zoo, Chicago, IL. <https://www.pmctrack.org>.

Citation of an Animal Care Manual:

AZA (X) Species Survival Plan® (or Taxon Advisory Group). (YEAR). XXX Care Manual. Silver Spring, MD: Association of Zoos and Aquariums.

Citation of an Ambassador Animal Guideline:

AZA Ambassador Animal Scientific Advisory Group, Species Common Name Species Survival Plan® (or Taxon Advisory Group). (YEAR). Species common name Ambassador Animal Guidelines. Silver Spring, MD: Association of Zoos and Aquariums.

ASSOCIATION OF ZOOS & AQUARIUMS

PENGUIN (Spheniscidae) CARE MANUAL

CREATED BY THE
PENGUIN TAXON ADVISORY GROUP
IN ASSOCIATION WITH THE
AZA ANIMAL WELFARE COMMITTEE

Penguin (Spheniscidae) Care Manual

Published by the Association of Zoos and Aquariums in association with the AZA Animal Welfare Committee

Formal Citation:

AZA Penguin Taxon Advisory Group. (2014). Penguin (Spheniscidae) Care Manual. Silver Spring, MD: Association of Zoos and Aquariums.

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Table of Contents

Introduction

Preamble

AZA accreditation standards, relevant to the topics discussed in this manual, are highlighted in boxes such as this throughout the document (Appendix A).

AZA accreditation standards are continuously being raised or added. Staff from AZA-accredited institutions are required to know and comply with all AZA accreditation standards, including those most recently listed on the AZA website (http://www.aza.org), which might not be included in this manual.

Taxonomic Classification

Table 1. Taxonomic classification for penguins

Classification	Taxonomy	Additional information
Kingdom	Animalia	
Phylum	Chordata	
Class	Aves	
Order	Neognathae	
Suborder	Sphenisciformes	
Family	Spheniscidae	

Genus, Species, and Status

Table 2. Genus, species, and status information for penguins

Genus	Species	Common Name	USA Status	IUCN Status	AZA Status
Aptenodytes	*patagonicus*	King penguin	Not listed	Least Concern	Green SSP
Aptenodytes	*forsteri*	Emperor penguin	Not listed	Least Concern	Red SSP
Eudyptes	*pachyrynchus*	Fiordland penguin	Threatened	Vulnerable	
Eudyptes	*robustus*	Snares penguin		Vulnerable	
Eudyptes	*sclateri*	Erect-crested penguin	Threatened	Endangered	
Eudyptes	*chrysocome*	Southern rockhopper penguin	Threatened	Vulnerable	Green SSP
Eudyptes	*moseleyi*	Northern rockhopper penguin	Not listed	Endangered	Red SSP
Eudyptes	*chrysolophus*	Macaroni penguin	Not listed	Vulnerable	
Eudptyes	*schelegeli*	Royal Penguin	Not listed	Vulnerable	
Eudyptula	*minor*	Little blue penguin	Not listed	Least Concern	Yellow SSP
Pygoscelis	*adeliae*	Adélie penguin	Not listed	Least Concern	Green SSP
Pygoscelis	*antarctica*	Chinstrap penguin	Not listed	Least Concern	Yellow SSP
Pygoscelis	*papua*	Gentoo penguin	Not listed	Least Concern	Green SSP
Megadyptes	*antipodes*	Yellow-eyed penguin	Threatened	Endangered	
Spheniscus	*magellanicus*	Magellanic penguin	Not listed	Near Threatened	Green SSP
Spheniscus	*humboldti*	Humboldt penguin	Threatened	Vulnerable	Green SSP
Spheniscus	*mendiculus*	Galapagos penguin	Endangered	Endangered	
Spheniscus	*demersus*	African penguin*	Endangered	Endangered	Green SSP

*Also known as the black-footed, Cape, and jackass penguin.

General Information

The information contained within this Animal Care Manual (ACM) provides a compilation of animal care and management knowledge that has been gained from recognized species experts, including AZA Taxon Advisory Groups (TAGs), Species Survival Plan® Programs (SSPs), Studbook Programs, biologists, veterinarians, nutritionists, reproduction physiologists, behaviorists and researchers. They are based on the most current science, practices, and technologies used in animal care and management and are valuable resources that enhance animal welfare by providing information about the basic requirements needed and best practices known for caring for *ex situ* penguin populations. This ACM is considered a living document that is updated as new information becomes available and at a minimum of every five years.

Information presented is intended solely for the education and training of zoo and aquarium personnel at AZA-accredited institutions. Recommendations included in the ACM are not exclusive management approaches, diets, medical treatments, or procedures, and may require adaptation to meet the specific needs of individual animals and particular circumstances in each institution. Statements presented

throughout the body of the manuals do not represent specific AZA accreditation standards of care unless specifically identified as such in clearly marked sidebar boxes. AZA-accredited institutions which care for penguins must comply with all relevant local, state, and federal wildlife laws and regulations; AZA accreditation standards that are more stringent than these laws and regulations must be met (AZA Accreditation Standard 1.1.1).

AZA Accreditation Standard

(1.1.1) The institution must comply with all relevant local, state, and federal laws and regulations, including those specific to wildlife. It is understood that, in some cases, AZA accreditation standards are more stringent than existing laws and regulations. In these cases the AZA standard must be met.

The ultimate goal of this ACM is to facilitate excellent penguin management and care, which will ensure superior penguin welfare at AZA-accredited institutions. Ultimately, success in our penguin management and care will allow AZA-accredited institutions to contribute to penguin conservation, and ensure that penguins are in our future for generations to come.

Penguins are flightless, highly specialized marine birds which spend the majority of the year at sea, coming ashore to nest and molt. On land, they are highly social animals, often occurring in large flocks that can number into the tens of thousands. They are dependent on prey items such as fish, crustaceans, and squid. This dependence creates a great vulnerability to pressures from fisheries as well as global climate change, oil spills, marine pollution, human disturbance, hunting, degradation of nesting habitats, and disease. All of these factors have led to the decline of most of the 18 species of penguins.

All species of penguin are found in a wide range of habitats throughout the Southern Hemisphere, from the snow and ice in Antarctica, to temperate rain forests in New Zealand. Breeding, egg-laying, and nest building vary across the species. The largest species of penguins—the Emperor and King penguins—will lay one egg, and instead of building a nest structure, will hold the egg in place on top of their feet. Other species build rock nests or burrows and lay two eggs. Penguins are normally monogamous and will often nest with the same partner for a number of years.

Penguins are long-lived; some individuals will breed at 20 years of age in the wild, and at over 30 years of age in zoos and aquariums. Some species will start nesting at 2 years of age, but others may not breed until they are 5 years old. Most species nest once a year during times of favorable environmental conditions, but for some species the nesting season is variable. A few species will nest twice during the same year.

Due to their adaptation to a marine environment, all penguin species are similar in morphology and physiology. The body is streamlined and the wings are adapted for swimming. Feathers are specialized, improving swimming performance while providing insulation and waterproofing. During molt, penguins lose waterproofing and insulation and should remain on land until molt is complete. This requires penguins to gain weight prior to molt while fasting during molt. (This physiological process has significant implications in an *ex situ* environment, and is addressed in this manual.) Plumage is similar in all species: the dorsal side is darkly colored and the ventral side is white. This coloration provides visual protection from both above and below.

Because of their aquatic adaptations penguins spend significant time in the water. Cold, clean water is essential to their well-being. Penguins will utilize deep pools and pathways that allow for circular swimming. In the wild, penguins will “porpoise,” a natural movement behavior that also occurs in zoo and aquarium environments if the aquatic habitat provides adequate space. Despite their aquatic nature, land space is also important for penguins; if provided in a zoological setting, penguins will spend significant time on land. Land areas should to be designed for roosting, nesting, and walking.

Their beaks are specialized and vary in size and shape depending on their prey. In the wild, penguins eat a variety of marine species including fish, squid, and krill. During nesting season, they will forage within a limited area near their nesting location, but they spend the majority of the year at sea. Recent advances in data trackers have allowed researchers to determine important foraging locations. This information has been used to protect important marine systems.

Penguins are not regulated by the US government other than those species listed as endangered or threatened by the Endangered Species Act. Regulations under this act can create challenges in importing or exporting birds to other countries, but do not affect movements within the United States.

Chapter 1. Ambient Environment

1.1 Temperature and Humidity

> **AZA Accreditation Standard**
>
> **(1.5.7)** The animals must be protected from weather, and any adverse environmental conditions.

The animals must be protected from weather, and any adverse environmental conditions. (AZA Accreditation Standard 1.5.7). Animals not normally exposed to cold weather/water temperatures should be provided heated enclosures/pool water. Likewise, protection from excessive cold weather/water temperatures should be provided to those animals normally living in warmer climates/water temperatures.

Temperature: Penguins are warm-blooded, with average body temperatures ranging from 37.8–38.9 °C (100–102 °F). Penguin species range from the equator to the Antarctic Circle, but are generally found in waters that are relatively cool for the latitude. Temperature regulation is accommodated by both behavioral and physiological adaptations. Apart from behavior and weight, overlapping feathers with downy shafts and a thick layer of blubber provide very effective insulation against the cold. Penguins found in warmer latitudes may face problems with excess heat. These birds generally have thinner layers of blubber than polar species, and also have less dense feathers on the head and flippers. Heat can be lost by ruffling feathers to expose the skin, shading the feet, holding the flippers away from the body, panting, or by remaining in sheltered burrows. Feathers are replaced yearly in a “catastrophic” molt, which generally follows the breeding season.

Air temperature: The following optimum air temperature ranges are recommended for indoor exhibits, and can be used as a guide by northern facilities that seasonally exhibit these species outside.

Table 3. Recommended temperature ranges for penguins

Species	Air temperature range
Emperor	-6 to 0 °C (20 to 32 °F)
Adelie	-6 to 1 °C (20 to 34 °F)
Chinstrap, gentoo	-4.5 to 7 °C (24 to 45 °F)
King, macaroni, rockhopper	0 to 11.5 °C (32 to 52 °F)
Little blue	12 to 22 °C (54 to 72 °F)
African, Magellanic, Humboldt	4.5 to 26.5 °C (40 to 80 °F)

Antarctic and sub-Antarctic penguin species (emperor, Adelie, chinstrap, gentoo, king, macaroni, rockhopper) need to be kept in climate controlled indoor facilities that can maintain the appropriate temperatures. Temperate species (African, Humboldt, Magellanic, little blue) can be successfully housed indoors or outdoors, or in exhibits using a combination of both. The success of an outside exhibit depends chiefly on the ambient temperature and the relative humidity of the area. When housing temperate penguins outdoors in areas where the temperature rises above 26.5 °C (80 °F), provisions should be made to allow the birds a means of heat relief. Sprinklers, misters, shaded areas, and forced-air movements are recommended methods. Chilled water and access to climate controlled areas should be provided. Heat stress problems are not confined to warm southern areas; hot, humid days in the upper mid-east of the United States are warm enough to cause problems. Signs of heat stress include panting, lethargy, and decreased appetite. The penguins may not automatically go into their pool or climate controlled holding areas and may need to be forced into these areas if heat stress becomes apparent. Fans, sprinklers, and misters should also be placed in or around the exhibit and indoor holding areas

Outside exhibits should be constructed so that the birds have shelter from freezing winds in the winter months. When the temperature falls below freezing, all birds should have access to shelter. Open water should be available all winter, and pools should not be allowed to freeze. Penguin species that naturally inhabit temperate climates (e.g., Spheniscid species) may suffer frostbite to the flippers if housed outdoors in cold climates with inadequately heated or accessed shelters.

Water temperature: Acceptable water temperature ranges for penguins housed in zoos and aquariums can be found below.

Table 4. Recommended temperature ranges for penguin pools

Species	Water temperature range
Adélie and emperor	1–7 °C (33–45 °F)
King, gentoo, chinstrap, macaroni, rockhopper	2–13 °C (35–55 °F)
Little blue	12-22 °C (54-72 °F)
African, Magellanic, Humboldt	4–18 °C (40–65 °F)

Some outside exhibits may have ambient temperatures that could rise above 29 °C (84 °F) during the summer months without causing adverse effects to the birds. Chilled water in these situations can assist birds in thermoregulation during these environmental conditions.

Humidity: Penguins do not thrive in humid climates. Warm, humid climates may be conducive to aspergillus infection. In addition, warm wet environments are breeding grounds for mosquitoes and penguins are highly susceptible to malarial infection. Outside exhibits in humid areas with heavy mosquito populations should not be considered for penguin enclosures. A mosquito abatement program should be in place in areas where mosquitoes are present.

In situ populations of penguins may experience a variety of humidity ranges depending on the season and their location (e.g., on the Antarctic continent, the coast of Chile, or the beaches of Australia), however an optimal humidity range has not been scientifically demonstrated. In zoos and aquariums, great care should be taken to ensure that penguins are provided the ability to regulate their own temperatures at all times through their behavior. Systems employed to raise or lower humidity within indoor and outdoor exhibits include air conditioning, dehumidifiers, misters, sprinklers, and fans.

AZA institutions with exhibits which rely on climate control must have critical life-support systems for the animal collection and emergency backup systems available, while all mechanical equipment should be included in a documented preventative maintenance program. Special equipment should be maintained under a maintenance agreement or records should indicate that staff members are trained to conduct specified maintenance (AZA Accreditation Standard 10.2.1).

AZA Accreditation Standard

(10.2.1) Critical life-support systems for the animals, including but not limited to plumbing, heating, cooling, aeration, and filtration, must be equipped with a warning mechanism, and emergency backup systems must be available. All mechanical equipment must be kept in working order and should be under a preventative maintenance program as evidenced through a record-keeping system. Special equipment should be maintained under a maintenance agreement, or a training record should show that staff members are trained for specified maintenance of special equipment.

Climate control: The AZA Penguin TAG recommends that each institution identify the most appropriate climate control systems suitable for their penguin exhibits in order to meet the temperature and humidity recommendations provided above.

Climate control systems can include but are not limited to the following items: HVAC system, heat exchanger, air handling unit, chiller, furnace or boiler system, and the computronics to run the system. All employees should have a general knowledge of the mechanical system to identify any unusual signs that the system may need repair. Daily mechanical/equipment checks should be conducted and information recorded. Any anomalies (e.g., high temperatures, mechanical failures, oil leaks) should be addressed. Critical repairs should be completed as soon as possible. Routine and preventative maintenance on equipment is recommended and all repairs documented.

Backup generators are recommended in the event of a power failure. The type of generator required will be dependent on the needs of the exhibit (e.g., small or portable generator for incubators, or large diesel backup generators for the exhibit). Facilities should have a contingency plan for moving animals in the event of a catastrophic event (e.g., natural disaster, motor failure, wide spread power failure, complete system breakdown). These contingency plans may include moving penguins to alternate housing.

1.2 Light

AZA-accredited zoos and aquariums should give careful consideration to the provision of proper lighting for penguins. For indoor exhibits, special attention should be given to the spectral quality of the light, the light intensity, and the photoperiod. Where feasible, the provision of natural light should be considered. It is recommended that designers plan ahead for the likely potential that more light will be required than what is projected to be needed. The configuration of the exhibit, along with the variation in

exhibit elements and number of birds housed, will influence light absorption and reflectivity within the enclosure and has ultimate impact on the amount of light needed to be delivered inside the exhibit.

Types of lighting that have been used with penguins include skylights, HID lamps (mercury vapor and metal halide), quartz halogen, fluorescent (normal and full-spectrum), incandescent and, most recently, LED. Each type of light installation has unique characteristics and photometrics. For example, HID lamps produce heat and this should be considered when assessing overall exhibit heat load. However, metal halides are a relatively energy-efficient means of providing good quality, high intensity light. Fluorescent lamps are frequently used providing good energy efficiency and spectral output but may not provide sufficient intensity. When evaluating lighting needs, it is recommended to use a variety of bulbs to assure a balanced appearance and appropriate spectral environment. Bulb manufacturers can provide information on color temperature, color rendering index (CRI), and spectral power distribution (the distinct spectrum of light produced by the bulb). It is recommended to consult with other penguin exhibitors before making final decisions about light installations.

Proper maintenance of light fixtures is essential to good quality light. Institutions should make provision for annual replacement of light bulbs because many types of lamps experience a change in their spectral output with use. Skylights or windows through which light passes should be kept clean to maximize light transmittance.

Exposure to a consistent photoperiod is essential to promoting proper breeding and molting cycles. Although penguins have reproduced on a simple turn on/turn off lighting system, some zoos and aquariums report enhanced reproductive success by varying annual day length and light intensity. Lighting schedules should reflect definitive photoperiods to encourage natural molting and breeding cycles. Several zoos and aquariums use lighting schedules that approximate that of the latitudes in which the species exhibited are found. Variations in molt patterns have been correlated with lighting schedules. Penguins are maintained successfully in both northern and southern photoperiod. Birds that are transferred from one cycle to another will usually adapt biologically within three years.

1.3 Water and Air Quality

AZA-accredited institutions must have a regular program of monitoring water quality for aquatic animals and a written record must document long-term water quality results and chemical additions (AZA Accreditation Standard 1.5.9). Monitoring selected water quality parameters provides confirmation of the correct operation of filtration and disinfection of the water supply available for the collection. Additionally, high quality water enhances animal health programs instituted for aquatic collections.

AZA Accreditation Standard

(1.5.9) The institution must have a regular program of monitoring water quality for fish, pinnipeds, cetaceans, and other aquatic animals. A written record must be maintained to document long-term water quality results and chemical additions.

Water quality: Both fresh water and salt water can be used in penguin exhibits. The water in a penguin exhibit pool should be clear and of good color with a low bacterial count. (Coliform bacteria levels should not exceed 1,000 MPN (most probable number) per 100 mL of water (Animal Welfare Regulations, 2013). A coliform bacteria count over 1,000 MPN is an indicator of potentially harmful conditions. There are several ways of controlling coliform levels. Water treatment filtration systems include sand, diatomaceous earth, ozone, biological, and ultraviolet light (UV). The addition of a chlorine or bromine system in conjunction with the filtering system also aids in controlling coliform levels. Older exhibits without filtration should maintain a clean supply of constantly running water, with adequate surface water skimming. Skimming capacity is essential for the health of the birds. Oils that build up on the water should be removed in order to maintain healthy feather condition. The number of skimmers should correspond to pool size and configuration. Noxious odors such as ammonia and chlorine that can cause health problems at high concentrations should be carefully monitored.

Performing routine water chemistries assures proper maintenance of water quality for pools. Chemistries should be taken at least once a month but a more frequent schedule is recommended. A record of results should be maintained and reviewed. When collecting water for testing, the sample should be taken from 61–91 cm (2–3 ft.) below the surface in about the same location at each collection. Tests can be performed by various methods such as with a refractometer, spectrophotometer or water quality test stripes such as HACH® AquaChek strip. The tests to be run may include but are not limited to ammonia, nitrite, nitrate, pH, temperature, and specific gravity.

Ammonia (NH_3) should be kept at a level below 0.1 ppm and nitrite (NO_2) levels below 0.5 ppm, although Spotte (1992) lists concentrations 3 ppm as being safe for adult marine fish. Nitrate (NO_3) is the final product in the nitrogen cycle and is safer than nitrite or ammonia. Nitrate readings below 50 ppm are safe for adult marine fish. Nitrate will not react out of the system and is removed only through water changes. The pH for saltwater should range from 8.0 to 8.3 and for fresh water 5.5 to 7.5. Specific gravity for saltwater pools should range from 1.020 to 1.030. Ozone can be utilized for disinfection of penguin water sources. When ozone is used, institutions should develop specific water filtration and disinfection protocols. The following information on the use of ozone has been adapted from approaches used at one institution (see www.zoolex.org). Ozone disinfection can be achieved by using a 10% by-pass flow supplied by a 40 g (1.41 oz.) ozonator through dry air (2 mg/L) that is mixed with filtered water in a vortex mixing chamber with a contact time of two minutes. The oxidation reduction potential (ORP) taken from the mixing chamber can be used to measure and monitor the automation of the ozonator, along with oxidation-reduction probes in the return to pool line. In all cases, a back-up oxidization treatment system should be available (e.g., 1.0 mg/L sodium hypochlorite), and should become operative if the ozonator experiences any mechanical difficulties. If any of the water quality results are above the target levels appropriate, water changes should be performed. Penguin pools require a turnover rate of three to five times the system volume per hour.

Table 5. Recommended water quality parameters

	Temp (°C (°F))	pH	Oxidant (mg/L)	ORP (mVolts)	Turbidity (NTU)	Salinity (0/00)	Coli (/1000mL)	NH3 (mg/L)
Antarctic	42–45 (6–7)	7.2–8.2	0	400–600	<0.20	30–34	<1000	<0.10
Spheniscus	54–57 (12–14)	7.2–8.2	0	275–325	<0.20	30–34	<1000	<0.10

Drainage: Drainage systems for land areas and pool areas should be separate to avoid pool contamination from run-off or exhibit maintenance. Drains, intake valves, and skimmers should be covered so that direct contact by birds is not possible. In filtered systems, care should be taken to provide a large enough bottom drain cover to prevent the possibility of a bird being sucked onto the drain.

Surface drainage should be adequate to allow for quick drying, and all floors should slope to the drain. One of the major reasons to have large exhibits is so penguins can come in and out of the water and dry quickly. Low spots that puddle should be avoided because a constantly wet substrate will eventually cause foot problems in penguins, as well as added staff hours needed for servicing the facility.

Air quality: Penguins as a group are highly susceptible to air-borne fungal infections. For this reason, the air quality in an indoor penguin exhibit should be optimal. Airflow, fresh air exchange, and filter capacity should be researched to provide the cleanest air possible. *Aspergillus fumigatus* spores range in size from 2.5–3 microns with other aspergillus species spores as large as 10 microns. In order to remove these spores from the air, a filter should remove particles in that size range or smaller. If possible sources of aspergillus are external to the exhibit then consideration should be given to reducing fresh air intake and providing a high-quality filter on the incoming air line as well as in the recirculation line. If the possible sources of aspergillus are internal to the exhibit, then a high-quality filter in the recirculating system, a high volume air change per hour, and increased fresh air exchange—as well as identifying and removing the aspergillus source within the exhibit—should be considered. Collection of regular air cultures in the exhibit as well as the air-handling system is a good practice in preventative maintenance. To aid in control of malaria in outdoor exhibits, consideration should be given to installing fans, since mosquitoes avoid persistent air movement.

Air turnover rates in the range of 15 air changes per hour have been recommended for laboratory animals (Lane-Petter, 1976). These parameters may be acceptable for penguins; however, the specific design of an air system needs to balance the tradeoffs between: (1) filter efficiency and airflow or ventilation; and (2) fresh air exchange and temperature regulation capacity. The exhibits of some 1993 AZA Penguin TAG Survey respondent institutions are under positive pressure, which allows air to be forced out instead of into the exhibit when a door is open (Henry, 1993). Doors should be well sealed to prevent air exchanges with outside areas. These rates are acceptable for closed indoor systems.

Daily records of air/water parameters should be recorded to monitor for any changes. If a significant variation in air/water parameters occurs, the penguins' behavior should be carefully monitored for correlations. Immediate steps should be taken to correct problems. Appropriate air monitoring is important for maintaining proper air quality. Air filters, at least 3 microns, are recommended. Filters should be

changed on a regular basis; as often as once a month or more as air quality dictates. Air handlers can be disinfected monthly to reduce the risk of fungal growth. Air testing using agar plates can be conducted every few months to ensure that fungal growth is not occurring. Prior to adding penguins to a new or refurbished exhibit, the air should be monitored for any signs of fungal growth. If spores are grown the area should be cleaned and disinfected, filters changed and another set of air testing should be completed.

1.4 Sound and Vibration

Consideration should be given to controlling sounds and vibrations that can be heard by animals in the care of AZA-accredited zoos and aquariums.

In general, penguins appear adaptable to auditory stimuli within their environments, and can acclimate to new noises and vibrations that are slowly introduced and associated with positive stimuli. However, new sounds and/or sources of vibrations (e.g., generators, water filters, construction noise, concerts, etc.), and activities that may create chronic or acute auditory stressors, should be eliminated or minimized during sensitive animal management periods such as animal introductions, nesting, chick rearing, the arrival of animals in quarantine, and when animals are sick.

Results from formal and informal research into the responses of penguins to sounds and vibrations within zoo and aquarium environments, the welfare issues that may result from this exposure, and methods of minimizing the effect of these stimuli, should be reported to the AZA Penguin TAG and individual species SSP Programs. The AZA Penguin TAG and its SSP programs support research that advances the development of management recommendations and exhibit designs to best meet the needs of penguins in AZA-accredited zoos and aquariums.

Penguin colonies in general can be quite noisy environments (i.e., 90–100 dBA), and penguins seem to adapt to frequent high noise levels (A. Bowles, personal communication). Pending further research, it is recommended that sound levels suitable for humans without hearing protection (i.e., OSHA standards for an 8-hour day) are adequate for penguins.

Chapter 2. Habitat Design and Containment

2.1 Space and Complexity

Careful consideration should be given to exhibit design so that all areas meet the physical, social, behavioral and psychological needs of the species. Penguins should be presented in a manner reflecting modern zoological practices in exhibit design (AZA Accreditation Standard 1.5.1). Penguins must be housed in enclosures and in appropriate groupings which meet their physical, psychological, and social (AZA Accreditation Standard 1.5.2).

AZA Accreditation Standard

(1.5.1) Animals should be presented in a manner reflecting modern zoological practices in exhibit design, balancing animals' functional welfare requirements with aesthetic and educational considerations.

AZA Accreditation Standard

(1.5.2) Animals should be displayed, whenever possible, in exhibits replicating their wild habitat and in numbers sufficient to meet their social and behavioral needs. Display of single specimens should be avoided unless biologically correct for the species involved.

Enclosure space and complexity: Throughout most of the year, the behavior of penguins in zoos and aquariums is fairly predictable, consisting primarily of eating, swimming, and generalized social interaction. Penguins require a multi-faceted exhibit that encompasses enough space for species-appropriate behaviors such as breeding, nesting, and swimming, as well as areas for holding, isolating, and quarantining birds.

Isolation area: Isolation areas should be separate areas for housing birds that need to be isolated for forced pairing, behavioral challenges, parent and hand-rearing of chicks, and non-contagious health problems.

Quarantine area: The quarantine facility for penguins should be a separate facility for accommodating newly acquired birds, or birds that should be separated from the group for health-related reasons. This area should provide separate air and water systems from the main exhibit. A quarantine area can serve as an isolation area if not in use for its intended purpose, or if the isolated birds are treated as quarantine birds whenever quarantine is active. An isolation area without separate air and water systems should not be considered as an appropriate quarantine area.

At the present time, the AZA Penguin TAG adopts minimum guidelines for housing penguins (see Table 6). Additional space should be provided so that penguins are able to perform their full range of species-appropriate behaviors. The same criteria apply to the pool surface area in order to allow sufficient space for the swimming habits of the colony. Penguins within the facility should be able to lie down and turn in a complete circle. The following guidelines are recommended as minimum and only minimum criteria for exhibit and holding standards. These minimum areas do not include land required for nesting for all penguins other than *Aptenodytes*.

Table 6: Minimum space requirements

Species	Land Area	Pool Area	Pool Depth	Pool Volume
King/Emperor				
Exhibit - (per bird for 1st 6 birds)	1.7 m^2 (18 ft^2)	0.8 m^2 (9 ft^2)	1.2 m (4 ft.)	6156 liters (1620 gallons)
Each additional bird	0.8 m^2 (9 ft^2)	0.5 m^2 (5 ft^2)	---	593 liters (156 gallons)
Short-term holding area <6 mo/per bird	0.8 m^2 (9 ft^2)	0.5 m^2 (5 ft^2)	0.9 m (3 ft.)	
All other species (includes program animals)				
Exhibit - (per bird for 1st 6 birds)	0.7 m^2 (8 ft^2)	0.4 m^2 (4 ft^2)	0.9 m (3 ft.)	2052 liters (540 gallons)
Each additional bird	0.4 m^2 (4 ft^2)	0.2 m^2 (2 ft^2)	---	171 liters (45 gallons)
Short-term holding area (per bird)	0.4 m^2 (4 ft^2)	0.3 m^2 (3 ft^2)	0.6 m (2 ft.)	

Enough land mass is needed to accommodate the number of birds housed in the exhibit allowing for territorial disputes, and providing areas for nesting during the breeding season. Penguins also use vertical space and all land space accessible to the birds should be considered usable space. Adequate space will be determined by the particular species and the particular birds and best determined by the animal staff that works with and knows the birds. The amount of land space provided to birds within a breeding colony

of penguins needs to be the size that it takes for individuals to build a nest far enough away from conspecifics that they are out of reach from a neighboring nesting bird's beak. This ensures that neighboring birds do not peck chicks. Larger penguin colonies may benefit from an open area to facilitate individual feeding of supplemented fish.

The AZA Penguin TAG understands that there may be circumstances for short term holding during maintenance of an existing facility or construction of a new exhibit where an institution may find it necessary to house birds in a facility that maintains a healthy and appropriate life support system but may fall outside the square footage recommendations of land or water. The TAG encourages those institutions designing or renovating penguin exhibits to provide enriching and generous land space and as deep of a pool as financially possible to offer the birds an opportunity to perform their natural diving behaviors.

Enclosure design: Penguins are colonial, and the need for visual barriers within enclosures is usually not necessary. Barriers like whalebones, rocks, etc. may be used during breeding seasons between nest sites, and nest boxes or burrows should be 2 m (6.6 ft.) apart. This distance helps to prevent injury of a chick, and does not necessarily keep the birds out of sight from one another. In general, penguins do not seem to be disturbed by visitors, but they should be given an area within their enclosure where they can get away from the public view if they choose.

Hiding places for penguins can include nest boxes, caves, or rock areas that they can duck behind. There should be sufficient hiding places to allow as many opportunities for individual animals (or all individuals) to get out of sight as possible. Penguins should be allowed to move a comfortable flight distance, a minimum of four feet, from the public

Penguins appear to be very adaptable to changes in their physical environment. Changes in the exhibit are enriching to the animals and should be encouraged. Design flexibility can This can include moving rocks around the exhibit, using waves and ice blocks in the pool, and utilizing misting systems. The following list identifies facility design considerations recommended for appropriate and effective care of penguins in AZA-accredited zoos and aquariums:

Observations: Video cameras are an excellent tool to assist in recording events such as breeding, nesting, and chick rearing behavior. Underwater viewing areas for staff and visitor observations are also useful.

Exhibit maintenance: Various land areas where birds can safely get in and out of the water should be provided. Safe entryways and exits should also be provided for keepers and maintenance workers going down into the pool area, and for divers entering and exiting the pool. Walkways and land areas should be safe for keepers to walk on with no trip hazards. Barriers to block birds from the exhibit pool during draining or maintenance should be included within the design of the exhibit.

Enclosure landscaping: The land area should be large enough for various feeding stations to be provided. All areas should be landscaped to minimize bumblefoot by including different levels and different substrates and to encourage natural behaviors. It should also be possible to clean exhibit areas, and good drainage is essential to prevent puddles from accumulating. Care should be taken to ensure that nesting areas are located where the birds feel comfortable and where the public can have at least a partial view.

Miscellaneous: Adding general storage areas for nesting material and behavioral enrichment items near or on exhibit, as well as mixing chambers for adding chemicals to pool water, is helpful for the daily management of the animals.

Enclosure substrates: At this time, there is no single product that meets all of the requirements necessary for optimum penguin substrate. Many institutions use a combination of the following products to provide effective substrate for their birds: Astroturf, concrete, dirt, Dri-Dek®*, fiberglass, grass, Gunite, ice, cat litter, Nomad™* matting, peanut shells, polyurethane*, rocks (river, pea gravel), sand, and sport track surfacing (See Appendix K for product information). Some zoos add soil and vegetation in outdoor exhibits.

Cat litter: Because of its desiccating nature, cat litter has been reported to decrease foot problems and respiratory issues caused by molds. However, caution should be used as cat litter labels now include an OSHA warning relating to the percentage of silica dust contained in the product. Cat litter will also find its

way into the pool drains, as well as the water and filtering systems, where it will clog mechanical equipment, creating additional keeper and maintenance work.

Ground peanut shells: Care should be taken when using ground peanut shell litter products. Although peanut shells do not fall under OSHA regulations, they can serve as a natural media for aspergillus growth. If this product is used, it is recommended that a fungal retardant be added at the manufacturers' level. As a precaution, it is recommended that the product be cultured for fungi before use.

Concrete: Historically, concrete has been used as a substrate for penguin enclosures. It is easy to clean and readily available. Over a period of time, however, the abrasive nature of concrete takes its toll on a penguin's foot, and the result can be pododermatitis or bumblefoot (see Chapter 6, section 6.6). For this reason, concrete or any substrate that remains wet for long periods of time should be avoided altogether. Many accredited zoos and aquariums have found it advantageous to use matting over concrete in selected areas of the exhibit. Some facilities place a protective coating of lacquer over concrete surfaces to reduce abrasiveness and to fill in the small pores where bacterial colonies can become established. Fiberglass and polyurethane have been reported to cause fewer foot problems than plain concrete.

Ice: Ice machines are used in some facilities to create a constant supply of ice, which can be used effectively as substrate. Ice has been used successfully over concrete floors to provide a less abrasive surface for the penguins to stand and walk on. Ice substrate should be used only in exhibits where the temperature is near freezing, as wet ice can contribute to foot problems.

Pebbles: Pebbles and small rocks of various sizes (e.g., 6–15 cm/2.4–5.9 in.) have been used in some exhibits with good success. Adequate drainage is important to ensure that the rocks can be hosed and disinfected regularly.

The AZA Penguin TAG recommends that a variety of materials and textures be provided on which the birds may stand. Plain concrete surfaces should be kept to a minimum, and some type of covering such as ice, matting, or cat litter should be provided. To reduce foot problems, it is recommended to encourage penguins to spend several hours each day swimming, as standing for long periods of time may contribute to foot health problems.

Holding areas: The same careful consideration regarding exhibit size and complexity and its relationship to the penguin's overall well-being should be given to the design and size of all enclosures, including those used in exhibits, holding areas, hospital, and quarantine/isolation (AZA Accreditation Standard 10.3.3). Sufficient shade must be provided by natural or artificial means when sunlight is likely to cause overheating or discomfort to the animals (AZA Accreditation Standard10.3.4).

All penguin exhibits should include an isolation area. There should also be a separate incubation room and/or nursery area away from other bird areas. Holding areas may contain a pool and barriers to separate birds. Adequate lighting, electrical, and temperature monitoring should be included within all indoor and holding areas. Transfer passages between exhibit areas and holding areas so birds do not need to be handled are important for the effective management of the birds.

AZA Accreditation Standard

(10.3.3) All animal enclosures (exhibits, holding areas, hospital, and quarantine/isolation) must be of a size and complexity sufficient to provide for the animal's physical, social, and psychological well-being; and exhibit enclosures must include provisions for the behavioral enrichment of the animals. AZA housing guidelines outlined in the Animal Care Manuals should be followed.

AZA Accreditation Standard

(10.3.4) When sunlight is likely to cause overheating of or discomfort to the animals, sufficient shade (in addition to shelter structures) must be provided by natural or artificial means to allow all animals kept outdoors to protect themselves from direct sunlight.

Enclosure cleaning: Many facilities use wash-downs to clean areas on a periodic basis. These are sprinkler systems that come on for a short duration to prevent accumulation of fecal material. A broad-spectrum disinfectant and fungicide should be used to clean penguin exhibits on a daily basis. Some veterinarians recommend periodic rotation of these products. Care should be taken not to use products that produce strong or toxic fumes.

Enrichment through design:
Penguins are curious animals and appreciate a complex exhibit with multiple layers and textures. Care should be taken in design to create enriching features in the water (jets, vortex, and bubbles) and the dry area. Caves, rock ledges, alcoves, canyons and rock steps are some ways to create an interesting multi-faceted exhibit for the birds. Large rocks can be used for penguins to stand on. Many species also exit the water in one rocket throttle and various large rock perches 0.9–1.8 meters (3–6 feet) above the water are very popular. Ice machines can also be left on during the day and many of the birds enjoy laying and standing in the snow piles. In addition, sprinklers that spray at randomly have also been used successfully and may also contribute to easy cleaning in the exhibit as well. Wave machines provide variation in the water's surface.

2.2 Safety and Containment

Penguins should not be housed in free-ranging environments. Animal exhibits and holding areas in all AZA-accredited institutions must be secured to prevent unintentional animal egress (AZA Accreditation Standard 11.3.1). Exhibit design must be considered carefully to ensure that all areas are secure and particular attention must be given to shift doors, gates, keeper access doors, locking mechanisms and exhibit barrier dimensions and construction.

AZA Accreditation Standard

(11.3.1) All animal exhibits and holding areas must be secured to prevent unintentional animal egress.

Containment: For burrowing penguins, containment barriers should be buried at least 0.6 m (2 ft.) into the ground, and they should be angled inwards in an 'L' shape a total of 0.9 m (3 ft.) down.

Predator and pest control: If pests or predators are a problem at an institution, then efforts should be made to protect the colony using appropriate containment barriers and management practices. These pest control methods must be administered so there is no threat to the animals, staff, and public (AZA Accreditation Standard 2.8.1). These methods can include trapping or making the exhibit area predator-proof by using predator-proof barriers such as fences or electrical barriers. Trapping should be used to remove potential predators from the area. Local laws concerning trapping or depredation of native wildlife should be checked prior to predator removal in this manner.

AZA Accreditation Standard

(2.8.1) Pest control management programs must be administered in such a manner that the animals, staff, and public are not threatened by the pests, contamination from pests, or the control methods used.

Native gulls (*Larus* spp.) will often raid penguin exhibits for fish, sometimes even taking fish from the beaks of the penguins. Several methods can be employed to discourage gulls, including placing fake predators in the area, playing recorded gull distress calls, placement of gull taxidermy specimens, and placing monofilament line over the exhibit. It is important that these methods be varied as gulls are likely to habituate quickly to a single method. Modifying the penguins' feeding times and method of feeding may reduce the competition from the gulls. Providing fish underwater has been successfully used in some exhibits. It is important to remember that gulls are protected by the U.S. Migratory Bird Treaty Act, and federal permits are required for culling or capture.

On land, depending on geographical location, penguin eggs and chicks may be lost to gulls, dogs, foxes, cats, rats, or small mustelids. Fish should not be left outside overnight to avoid attracting rats. Additionally, if there are other exhibits nearby that attract rats, efforts should be made to keep these areas rodent-free as well. It is critical not to place any poison or traps in areas to which the birds have access.

Public barriers: Exhibits in which the visiting public may have contact with penguins must have a guardrail/barrier that separates the two (AZA Accreditation Standard 11.3.6). Most penguin exhibits are designed so the birds are maintained inside the boundary of the exhibit by acrylic, glass or a moated area with walls. If the exhibit is designed to allow penguins to come into close proximity with visitors, where they could possibly touch the birds, the area should also be constantly monitored by appropriate staff. If the exhibit is an open-air design where the public has potential access to the pool, it is recommended that there be a system in place to monitor for the presence of foreign objects (e.g., regular policing of the area, regularly radiographing the birds, etc.).

AZA Accreditation Standard

(11.3.6) Guardrails/barriers must be constructed in all areas where the visiting public could have contact with other than handleable animals.

Exhibits without a solid barrier between penguins and guests: Several penguin species will "pop" out of the water on to land gaining height of as much as six feet. Consideration should be given to this fact, especially for gentoo penguins. It would be appropriate for exhibits with a low barrier between the guest pathway and the penguin pool to add a staff oversight during the day and a night time barrier to prevent birds from jumping out of the exhibit during the night.

Exhibits should be designed so that the birds, and especially the chicks, can easily move in and out of the water from the land mass. This will usually involve some type of ramp system. Sharp materials that birds could hit as they exit the water (walking or porpoising) should be avoided. Acrylic, glass, concrete and rockwork have all proven safe materials within a penguin exhibit.

Selecting the species of penguin for a new exhibit: Prior to committing to and designing a new penguin facility, institutions should consult the AZA Penguin TAG to identify which penguin Species Survival Plan® (SSP) populations have the greatest need for the additional spaces you will be providing. This will ensure that your facility is contributing to increasing the SSP's long-term sustainability. The polar birds will require a much more sophisticated life support system and a climate controlled facility and there should be a considerable cost differential between displaying the sub-Antarctic and the more temperate species who can be housed outside in many climates

Monitoring: Most zoos and aquariums use some type of identification band around each penguin's flippers to maintain records on each bird and on the collection. A color coded system that includes colored cable ties is used by many. Implanted transponder chips are also used by many institutions. Some institutions use a combination of bands and implants to protect against a lost band. Care should be taken to constantly monitor the ID bands to make sure they are sitting properly on the flipper and that the bird's flipper has not swollen prior to molt. Bands are changed regularly as needed and it is good practice in a large colony to use a band on each flipper in case one of the bands falls off.

Education and conservation: Education and Conservation outreach programs are very popular and many zoos allow guests the opportunity to have an up close and personal penguin experience. Penguins may also have a presence in the local and national community. The penguins should be conditioned to be around strangers and trained staff should always accompany the birds and be present when the penguins are in close contact with the guests. Penguins should travel in a kennel and portable display cases can be used at the remote site to safely house the penguins and allow guests a good view of the birds.

Emergency protocols: All emergency safety procedures must be clearly written, provided to appropriate staff and volunteers, and readily available for reference in the event of an actual emergency (AZA Accreditation Standard 11.2.3).

There should be enough crates and nets on site to be able to quickly transport all your birds in case of emergency evacuations. There should be a written evacuation plan that includes alternate locations to hold the animal should your facility have to be evacuated.

Staff training for emergencies must be undertaken and records of such training maintained. Security personnel must be trained to handle all emergencies in full accordance with the policies and procedures of the institution and in some cases, may be in charge of the respective emergency (AZA Accreditation Standard 11.6.2). AZA accredited institutions must also ensure that written protocols define how and when local police or other emergency agencies are contacted and specify response times to emergencies (AZA Accreditation Standard 11.2.7)

In the event of a fire or emergency weather event, a secondary holding area should be available for the penguins. The area should have adequate space and life support and be available quickly in the event of an emergency. It may be advantageous to prepare a contingency plan ahead of time in the event the main and secondary facilities are damaged. Arrangements can be made with other nearby zoological facilities in the case of emergency and a current phone tree of other AZA institutions in your

AZA Accreditation Standard

(11.2.4) All emergency procedures must be written and provided to staff and, where appropriate, to volunteers. Appropriate emergency procedures must be readily available for reference in the event of an actual emergency.

AZA Accreditation Standard

(11.6.2) Security personnel, whether staff of the institution, or a provided and/or contracted service, must be trained to handle all emergencies in full accordance with the policies and procedures of the institution. In some cases, it is recognized that Security personnel may be in charge of the respective emergency (i.e. shooting teams).

AZA Accreditation Standard

(11.2.7) A written protocol should be developed involving local police or other emergency agencies and include response times to emergencies.

area would be helpful to have. Due to the special natural history of the penguins, life support systems should be hooked up to a generator capable of running critical life support for several days in the event of emergency.

Training for emergency holding for penguins should consist of an SOP noting the plan, where the birds can be moved to, agreement with a refrigerated truck rental business and potential arrangements for ice and fish. Emergency drills should be conducted at least once annually for each basic type of emergency to ensure all staff is aware of emergency procedures and to identify potential problematic areas that may require adjustment. These drills should be recorded and evaluated to ensure that procedures are being followed, that staff training is effective and that what is learned is used to correct and/or improve the emergency procedures. Records of these drills should be maintained and improvements in the procedures duly noted whenever such are identified (AZA Accreditation Standard 11.2.5). AZA-accredited institutions must have a communication system that can be quickly accessed in case of an emergency (AZA Accreditation Standard 11.2.6).

Due to the nature of the animal, there is no need to develop an animal attack or escape plan for penguins. In the event of a penguin escape, appropriate zoological staff should be notified to recapture the bird. In the event of a bird bite, the institution should be notified and their health care protocol followed.

AZA Accreditation Standard

(11.2.5) Live-action emergency drills must be conducted at least once annually for each of the four basic types of emergency (fire; weather/environment appropriate to the region; injury to staff or a visitor; animal escape). Four separate drills are required. These drills must be recorded and evaluated to determine that procedures are being followed, that staff training is effective, and that what is learned is used to correct and/or improve the emergency procedures. Records of these drills must be maintained and improvements in the procedures documented whenever such are identified.

AZA Accreditation Standard

(11.2.6) The institution must have a communication system that can be quickly accessed in case of an emergency.

Chapter 3. Transport

3.1 Preparations

Animal transportation must be conducted in a manner that adheres to all laws, is safe, and minimizes risk to the animal(s), employees, and general public (AZA Accreditation Standard 1.5.11). All temporary, seasonal, and traveling live animal exhibits must meet the same accreditation standards as the institution's permanent resident animals (AZA Accreditation Standard 1.5.10). Safe animal transport requires the use of appropriate conveyance and equipment that is in good working order. Animals should be caught up and placed in kennels and transport vehicles with the least amount of stress just prior to transport.

AZA Accreditation Standard

(1.5.11) Animal transportation must be conducted in a manner that is safe, well-planned and coordinated, and minimizes risk to the animal(s), employees, and general public. All applicable local, state, and federal laws must be adhered to.

AZA Accreditation Standard

(1.5.10) Temporary, seasonal and traveling live animal exhibits (regardless of ownership or contractual arrangements) must meet the same accreditation standards as the institution's permanent resident animals.

Transport container/crate: IATA regulations require that the transport container allow a penguin being transported to stand fully erect without touching the roof and sides of the container. IATA regulations can be found at www.iata.org. Penguins can be transported in pet kennels or rigid plastic containers/crates. The proper substrate (e.g., cat litter, rubber matting, Astroturf), depending on the species, should be provided for all crates. It is recommended that the containers be divided so that animals have their own compartment to reduce the threat of injury or over-heating. Bonded pairs, however, can be kept together. A #300 size kennel can hold up to six birds (depending on species).

Transportation containers are typically made of hard white plastic or another safe, water proof non-toxic material, to reduce heat absorption as black crates may get too warm. These containers or crates can be modified for taller species by adding a screened area around the top which will also allow for increased air circulation. Lids should fit the dimensions of the crate and can be bolted on to the crate in all four corners. Figure 1 illustrates these features. Transportation crates should also have slots for the forklift to easily move them from one location to another and lift them up and down off of a vehicle (Figure 2).

Figure 1. Example of a crate made of hard white plastic with a modified screened area added around the top to increase height and air flow.
Photo courtesy of Lauren DuBois

Figure 2. Crates should have slots built into them so that a forklift can easily move them. Photo courtesy of Lauren DuBois

Large crates: Large crates are used on charter flights primarily due to the size and weight. Dimensions of the most commonly used boxes are 1.1 m x 1.2 m x 1.0 m (44 in. x 48 in. x 40 in.). Large crates will hold four medium sized penguins (Gentoos and Macaronis) or five to six small sized penguins (Chinstraps, Adelies, Rockhoppers, etc.). For birds that are aggressive, no more than four small penguins should be placed in a crate. One to three King penguins can be shipped in these large containers.

Small crates: Small crates are typically 1.1 m x 0.7 m x 0.7 m (42 in. x 29 in. x 28 in.) and have been used on commercial flights. They are appropriate for small penguins (Adelie, chinstrap, macaroni, rockhopper), two to three medium sized penguins (gentoo) and one to two King penguins.

Individual pet crates (Sky Kennels): Dimensions of a standard pet crate is 0.7 m x 0.5 m x 0.5 m (27 in. x 21.5 in. x 20 in.). All “windows” and doors of the pet crate should be covered with a breathable and flexible material like bar mat/shelf liner or burlap. Pieces can be cut to fit the exposed areas and attached with cables ties. Doors should be secured with cable ties. Gentoos, Magellanics, Humboldts, Africans, Chinstraps, and Macaronis have been transported in pet crates.

Climate control: Polar species of penguins are susceptible to overheating and special considerations should be taken when these species are placed into transport containers/crates. To ensure that crates (of any size) remain adequately controlled for temperature, they should contain a bottom layer of pre-filled frozen large 0.2 m x 0.04 m x 0.2 m (7 in. x 1.63 in. x 6.75 in.) BlueIce® containers or a layer of frozen water (ice). If using BlueIce®, the best way to prevent slippage is to layer the BlueIce® containers between two industrial rubber floor mats (Figure 3). If using a layer of frozen water, fill crates with 7.6 cm (3 in) of water and freeze them overnight. To prevent slippage, place an industrial rubber floor mat on the ice and add a thin layer of water to the container and re-freeze to allow the mat to freeze to the ice (Figure 4).

Figure 3.

Figure 4.

Figure 3 illustrates how a crate can be temperature controlled by lining the bottom with frozen (size) BlueIce®
Figure 4 illustrates how a crate can be temperature controlled by lining the bottom with a 7.6.cm (3 in.) layer of water that was frozen. Both methods add an industrial rubber floor mat for the birds to stand on to prevent slippage.
Photos courtesy of Lauren DuBois

Transportation plan: A transportation plan should be developed prior to any transports. The plan should identify the point persons and their contact information for both the shipping and receiving institutions and the emergency numbers for the trucking companies, airline contacts, etc. The point person should be responsible for updating their institution regularly on their progress and also notifying the receiving institution of progress and any possible problems and making sure adequate animal checks are being done if applicable. The mode of transportation selected will determine the number of staff needed but there should be at least one experienced penguin person aboard the transport.

Trucking companies generally have contingency plans for truck breakdowns, refrigeration issues or other problems that may occur and these should be detailed in the transportation plan. For a ground transport over 4 hours, also identify institutions having penguin accommodations along the route in case of emergency. The point person should contact these institutions prior to the transport to let them know that they will be in the area and to make sure that they would be able to assist if needed. Transport protocols and contingency plans should be well defined in the transportation plan and discussed with all animal care staff on the transport prior to the trip.

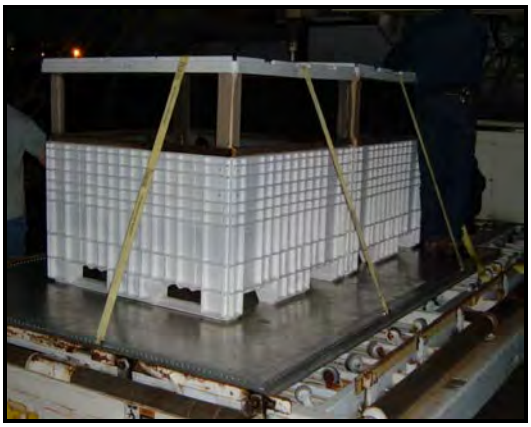
Modes of transportation: Climate control considerations should be taken in all modes of transportation moving polar species of penguins that are susceptible to overheating. If the transportation distance is not too great (e.g., not more than a 10-hour drive), penguins can be transported by being secured into a truck or van (Figure 5). If the ambient temperature is above 4 °C (39.2 °F), it is recommended that a refrigerated truck be used. If the ambient temperature is below 4 °C (39.2 °F), the animals can be transported in an unrefrigerated truck or passenger van. It is recommended that shipping occur during cooler weather 0–21 °C (32–70 °F) and/or during the cooler parts of the day. Ensure that the interior of the truck (van) is cleared of sharp edges and organic debris and that the inside is cleaned, aired out, and disinfected several times over several days prior to transporting penguins.

When the birds are being transported by truck, there should be enough drivers so that they reach their destination in the shortest amount of travel time. Longer truck transports will require several staff members. Contact the Department of Transportation with any questions and keep in mind that when crossing state lines, regulations can differ. However, if the transport is more than a one-day drive, it is recommended that the drivers stop and rest during the evening. This not only gives the drivers needed rest, but allows the penguins time to recover from the continual motion of the transport. Contact the Department of Transportation with questions and for the most updated regulations on driving time vs resting time. Keep in mind that when crossing state lines, regulations can differ

Commercial air transportation can be used for penguins but it is easier for *Spheniscus* (and other non-polar) species because they are more heat-tolerant. Adequate communication with the airlines is essential and it is important to contact the airlines prior to shipping animals to understand their policy for transporting live animals. Staff should communicate the need to move the birds in a timely fashion so that

the time interval to and from the air freight office to the plane can be minimized. If possible, the animals should be transported through the VIP or DASH systems of freight transportation that many airlines have available. The most direct flights should always be used. Accompanying staff should ask the airline if the birds can be loaded onto the plane last, so that they can be the first off-loaded. Prior to loading the birds on to the aircraft, airline personnel will strap the penguin crates down to the" cookie sheets" which then slide into and are fastened onto the bottom of the plane for a secure ride. Airlines will often accommodate special needs of penguins so it is important that these are discussed in advance.

Figure 5. Penguins being transported in crates secured in a refrigerated truck.
Photo courtesy of Lauren DuBois



Figures 6. Figure 7.
Figures 6 & 7. Figure 6 illustrates how a crate is secured to "cookie sheet" being loaded on to an aircraft. Figure 7 illustrates how the "cookie sheet" is secured to the aircraft.
Photos courtesy of Lauren DuBois

3.2 Protocols

The equipment should provide for the adequate containment, life support, comfort, temperature control, food/water, and safety of the animal(s).

Equipment: Batteries, extra light for ambient lighting, flashlight, thermometers, tools to repair barriers/kennels, extra cable ties, extra matting, extra ice, water and tubs are important to have on hand during transport. For possible medical conditions towels, plastic bags, spray bottle, paper towels, vet wrap, Quick Stop, silver nitrate sticks, sodium chloride solution, Povidine solution, triple antibiotic ointment, gauze, superglue are also important.

Physical condition: There are certain physical conditions experienced by penguins that can influence the timing of animal shipments. Penguins that are gravid or in any phase of the molt cycle should not be shipped. The timing of molt varies by species. As there is considerable physiological stress associated with molting, the AZA Penguin TAG recommends that birds should not be transported at least six weeks prior to their anticipated molt. Birds may be shipped one to two months after completing molt as long as they have sustained their pre-molt weight for two to four weeks. It is also best to avoid shipping animals just prior to or during the breeding season. The safest time to move penguins is during the cold months of the year as penguins can easily overheat (Boersma, 1991).

Food and water: In the wild, penguins commonly fast for several weeks at a time and drink only every few days. As penguins regularly go through these periods of feast and famine, it is recommended that they be fed well before transport. Once penguins have gained some weight for their trip they can they be fasted for at least eight hours before transport. If the trip lasts more than 48 hours, it is recommended that the birds be fed during transport. It is important that the birds have access to fresh water or clean ice at all times.

Bedding and substrate: For polar penguin species, a suitable substrate is necessary to provide adequate footing for the animals. Smaller rocks (5–10 cm/2–4 in. in diameter) covered with ice provide good footing while allowing drainage of melting ice and fecal materials. It is important to ensure that drains are clear to avoid backup. For non-polar species, the transport container should be bedded with cat litter or rubber matting. Blue ice can be placed below the rubber matting to cool the container.

Temperature, light, and sound: It is necessary that light be provided at all times in the animal transport area. The light source can be the truck light in the refrigerated compartment, or a low wattage bulb that is powered with a 12-volt to 110-volt converter. If accompanying staff will be spending the night in transit, it will be necessary to run an extension cord with a light so that there is lighting throughout the night for the birds.

For truck transportation, a temperature monitor should be installed in the animal area that has a readout in the truck's cabin. This allows the staff traveling with the penguins to constantly monitor the temperature. A backup thermometer should also be placed in the animal area, secured away from the birds in case there is a question of the temperature monitor working properly.

Sub-Antarctic and Adelie penguins: The recommended temperature for truck or plane transport is -5–11.1 °C (22–52 °F). These penguins should be shipped with ice or blue ice in their crates. Air temperature in the plane or truck should not exceed 12.8 °C (55 °F). For short durations (e.g., transport between exhibit and transport vehicle), 23.9 °C (75 °F) is acceptable. If Adelie penguins are housed in exhibits with temperatures below freezing, they should be acclimated to higher temperatures before transport.

Emperor penguins: The recommended transport temperature for emperor penguins is below freezing in the range of -7.2– -1.1 °C (19–30 °F). Emperor penguins overheat easily and should only be exposed to a maximum temperature of 4.4 °C (40 °F) for short durations when the animals are moved between the exhibit and transport vehicle.

Sphensicus: The temperature should be kept between 4.4–15.6 °C (40–60 °F) for air and truck travel involving *Sphensicus* penguins. During short periods of time when the animals are transported between the exhibit and transport vehicles, temperatures should not exceed 23.9 °C (75 °F). *Sphensicus* penguins should be acclimated to cooler or warmer temperatures prior to transport if the receiving institution maintains a different temperature than the sending.

Animal monitoring: The animal area in the back of the refrigerated truck should be separated from the door with a barrier. This will ensure that the animals will not be able to exit when the door is opened. A video camera should be installed in the animal area with a monitor in the cabin, so that the animals can be observed during transport. If a video camera is not available or breaks during transport, staff should check on animals every two to four hours.

Post-transport release: It is important that the environmental conditions in quarantine be similar between the sending and receiving institutions. It is also important, where possible, to have two or more birds quarantined together because of the social needs of the animals. If this is not possible, efforts should be made for quarantined birds to have visual or auditory contact with other penguins. For more information on quarantine, see Chapter 6, section 6.3.

Egg transport: An alternative to transporting live adult birds is to transport eggs and then complete incubation and hand-rearing of the animals at the final destination. One institution has developed techniques for transporting eggs from the wild to their incubation and rearing facilities (Todd, 1987). Eggs have also recently been transported between facilities. A portable incubator that maintains a constant temperature may be used however, for shorter intra-continental flights, a well-insulated cooler with a hot water bottle or hand/feet warmers with a mounted temperature probe can successfully maintain the temperature of the eggs.

The timing of egg transport is important. Eggs should be transported either during the last one-third of their incubation period or before incubation begins (C. Kuehler, personal communication). For species that lay two eggs, it is best to transport the eggs after the second egg is laid, because egg incubation does not begin until the clutch is complete and they can withstand changes in temperatures. Eggs are quite tolerant to periods of neglect throughout the incubation period. The temperature in the cooler or incubator should be maintained at approximately 35.6 °C (96 °F). When the temperature drops below this, additional water should be added to the hot water bottle from a thermos carried for this purpose. If necessary, the airline can usually supply hot water. Upon arrival at the destination, eggs should be placed in an incubator and the procedures and protocols for artificial incubation followed. Safe transport requires the assignment of an adequate number of appropriately trained personnel (by institution or contractor) who are equipped and prepared to handle contingencies and/or emergencies that may occur in the course of transport. Planning and coordination for animal transport requires good communication and planning.

Chapter 4. Social Environment

4.1 Group Structure and Size

Careful consideration should be given to ensure that animal group structures and sizes meet the social, physical, and psychological well-being of those animals and facilitate species-appropriate behaviors. Penguins are highly social, colonially-nesting birds. There is good evidence that reproduction in penguins, as in other colonial waterbirds, is socially facilitated, and that adequate stimulation by conspecifics is essential to successful reproduction in zoo and aquarium conditions (Berger, 1981). Boersma (1991) suggested that small colony sizes in zoo and aquarium populations of penguins might show decreased productivity. A minimal social grouping of three pairs for a single species of penguins was suggested by Gailey-Phipps (1978). The TAG has since revisited this recommendation and as stated in the 2010–2013 Regional Collection Plan TAG Guidelines now recommends that institutions maintain a minimum of 10 penguins in an exhibit. This recommendation supports the importance of the social structure in a penguin colony and allows the birds to select mates and establish a social hierarchy.

Penguins are generally considered to be perennially monogamous, except king and emperor penguins, which are serially monogamous. Mate fidelity in one colony of Adélie penguins housed in a zoological institution has been reported to be 75% over a 13-year period, which is markedly higher than the 51% reported for wild Adélie penguins (Ellis-Joseph, 1992; Ainley et al., 1983). In another case, one pair of wild Magellanic penguins was faithful for 16 years until one of the individuals died (Boersma, 2008). Mate fidelity may be affected by transfer, separation caused by management of illness, or mortality in a zoo or aquarium setting.

In emperor and king penguins, pair bond formation and egg fertility are often positively correlated with competition for new mates (A. Bowles, personal communication). Breeding pairs of Magellanic penguins are more likely to break up after a reproductive failure, compared to situations where breeding pairs have successfully reared a chick (D. Boersma, personal communication). Facilities should be strongly encouraged to build or renovate exhibits to allow any offspring to be housed for up to two years.

Same-sex groups and pairings: Single-sexed groups of penguins can be maintained for management purposes. Having single-sex groups can be an effective management tool for exhibiting birds without any breeding occurring. Same-sex pair bonding does not appear to pose any problems for the health and management of penguins. This phenomenon has been seen in Magellanic, gentoo, little blue, king, northern and southern rockhopper, and African penguins. Pairs of this nature have even been successfully used to raise fostered chicks. Bonds between same-sex individuals have also been successfully split, and the birds have successfully re-paired with individuals from the opposite gender.

Sex ratios: Managers of penguins should strive for fairly balanced sex ratios within their breeding colony. However, a perfect 1:1 ratio is not necessary for harmony within the group. Not all individuals seek out a mate and seem content in the company of conspecifics. Penguin caretakers should be cognizant of each individual's behavior and social interactions. For ideal breeding situations, an even sex ratio and varied age structure among all social groups is best. Over representation of one sex may lead to same sex pairings, while over representation of age classes, especially among older penguins may lead to decreased breeding success.

Multigenerational groups: Individual interactions will be seen among multigenerational groups. Care should be taken to insure that related birds do not breed together. Penguins in general are long-lived, prolific birds. In most colonies where breeding is occurring or younger animals are occasionally brought in, the age structure of the group is suitable for long-term sustainability (i.e., geriatric individuals are replaced by younger birds). Managers who are faced with static collections should consider making changes in order to balance the age structure to avoid loss of the collection through attrition.

Fledging: The age of fledging, or independence from parents, varies among penguin species (see Table 7). Penguins usually achieve their peak weight just prior to fledging.

Table 7. Average age and peak weight at fledging for penguins*

Species	Age at fledging	Approximate peak weight
Emperor	4–6 months	Varies
King	4–8 months	Varies
Adélie	40–60 days	2.5–3 kg (5.5–6.6 lb.)
Chinstrap	55–60 days	3.1–4.2 kg (6.8–9.3 lb.)
Gentoo	70–75 days	6.5–7.5 kg (14.3–16.5 lb.)
Little blue	50–55 days	0.8–0.9 kg (1.8–2 lb.)
Macaroni	60–65 days	3.0–4.1 kg (6.6–9 lb.)
Rockhopper	50–60 days	1.4–1.8 kg (3.1–4 lb.)
Humboldt	70–90 days	3.0–3.6 kg (6.6–7.9 lb.)
African	70–84 days	3.0–3.3 kg (6.6–7.3 lb.)
Magellanic	65–120 days	3.2–4.2 kg (7.1–9.3 lb.)

*Information derived from one zoological institution's unpublished data

4.2 Influence of Others and Conspecifics

Animals cared for by AZA-accredited institutions are often found residing with conspecifics, but may also be found residing with animals of other species.

Mixed-penguin species: Many facilities successfully house and breed several species of penguins in one enclosure, and in some cases mix penguins with other species such as Inca terns (*Larosterna inca*) or blue-eyed cormorants (*Phalacrocorax atriceps*). Concerns for mixed species exhibits include inter-specific compatibility and aggression, differential life support and temperature requirements, differential habitat use and habitat requirements, and avoidance of hybridization. Hybridization among several penguin genera, in particular *Spheniscus* spp. and *Eudyptes* spp., has been documented. It is strongly recommended that *Spheniscus* spp. be housed as single-species populations. One facility has housed northern and southern rockhoppers together for over 25 years without hybridization. Managers contemplating mixed-species exhibits should carefully select desired species.

Aside from a few cases where multi-penguin species exhibition may be problematic, housing several species together can work well if seasonality is maintained. At one zoological institution, king and Gentoo penguins are housed together and utilize the same nesting area. Gentoos nest first, and as their chicks are fledging, the king penguins begin to occupy the rookery and breed. In mixed-species exhibits, sufficient space is needed for each species so that conflict can be avoided. Plenty of nesting areas and feeding stations are needed, with consideration for the natural behaviors of each species. For example, feeding stations for flighted birds housed with penguins can be located off the ground and away from the penguins. Another consideration is the size of the nesting burrow entrances. If little blue penguins are to be held with a larger species of burrow nesting penguins, the nest openings should be smaller to keep the larger birds from exploiting these burrows.

Any time a new species is introduced into an exhibit, it's advisable to section them off to get them accustomed to their "territory" for at least a week before opening them up to the rest of the exhibit. This allows the birds to know where their area is and cuts down on the desire to nest or feed elsewhere once full exhibit access is allowed. Most species of penguins are territorial by nature and having established areas will reduce the need to aggressively defend their "home turf."

Keepers with good observational skills are needed to watch for signs of stress, aggression, and competition in mixed-species exhibits. A plan should be in place to be able to remove problem individuals or make changes to the exhibit, such as changing feeding station areas, adding nesting areas, or adding barriers and dividers between nests if problems arise.

Mixed-species: Appropriate non-penguin species may include waterfowl and shorebird species found also occurring in the penguins' home ranges. Competition for food and nesting resources can be an issue. One species may have to defer to another before gaining access to desired resources. Being alert and responding properly to this will help decrease stress in the colony. Some species within the same exhibit may show preferences for different areas of the exhibit for nesting. For example, one species may prefer flat beach areas versus higher cliff ledges. Make sure to provide ample nesting site possibilities for all exhibited species.

Social groups of penguins used for education: The Penguin TAG recognizes that penguins are valuable additions to education, outreach, and visitor experiences. For institutions that use their birds solely for the purposes of education and outreach, ten is still recommended as the minimum colony size. Acclimating penguins for educational programs can be accomplished by slowly conditioning the birds to being handled in a non-threatening way. Positive reinforcement of calm behavior seems to be most effective. Not all individuals have the demeanor to be involved in education programs. Managers should recognize the signs of intolerance to handling and be prepared to allow these individuals to rejoin their social group.

Imprinting in penguins: During the hand rearing process, penguin chicks can become imprinted on their caregivers. In some cases, this bond is encouraged especially if these individuals are to be used in educational programs. Humans can provide some social stimulation but should not be the only source of social activity for these penguins. All penguins require time with conspecifics in order to develop appropriate behaviors. In some juveniles, aggression towards humans develops. Heavily imprinted African penguins have gone on to select mates and successfully reproduce.

4.3 Introductions and Reintroductions

Managed care for and reproduction of animals housed in AZA-accredited institutions are dynamic processes. Animals born in or moved between and within institutions require introduction and sometimes reintroductions to other animals. It is important that all introductions are conducted in a manner that is safe for all animals and humans involved.

In general, introduction of novel stimuli, including new birds, to a social group of penguins is met with curiosity and investigation. As with all animal introductions, staff should closely monitor both the introduced bird as well as the social group for signs of stress and aggression. The introduction of a new bird or introduction of a group of birds to an exhibit has been approached in several ways:

- Gradual introduction: Use of this technique will depend on exhibit design as well as the temperament of the birds. In gradual introductions, birds are introduced to an exhibit for a few hours at a time, with close monitoring over a several-day period. The time the birds are left in the exhibit is gradually increased until the birds appear to be acclimated. This technique is the most conservative, and most likely to result in successful integration of new birds into an existing social group.
- Group introduction: Most penguin managers feel that it is inadvisable to introduce a single bird into a colony. New birds can be isolated with one or more conspecifics removed from the social group for a period of time. Birds can then be introduced into an exhibit together and monitored by staff.
- “Howdy” cage introduction: Birds are placed in a small enclosure within the exhibit for several hours daily and slowly acclimated to the exhibit and other penguins. Generally, a gradual introduction procedure, as described above, can then be followed.
- Immersion introduction: Birds are placed in the exhibit and regularly monitored by staff.

Hand-reared *Spheniscus* chicks can be introduced into the colony when they are nearly fledged (approximately 80 days). It is best to introduce all species of chicks in a group or in pairs if possible. It is advisable to supervise the interactions of the newly introduced birds during the initial visit to the colony to ensure the chicks’ safe movement through exhibit and that aggression from older birds is not an issue.

Chicks can be left unattended after a few days, provided they are able to emerge from the water without trouble, and are not being harassed by other birds. Juveniles tend to congregate together and will fight to establish a hierarchy of their own (Gailey-Phipps, 1978). Chicks should be encouraged to join the other birds at the feeding station rather than be provided with special treatment. It may be a few weeks before they are regularly feeding with the others. Some institutions find it advantageous to use an off-site area to introduce the chicks to members of the colony. A Plexiglas® barrier or screen can also be used for the first introduction within the exhibit. Introduction of hand-reared chicks into exhibits requires close monitoring and is likely to be most successful if a gradual introduction procedure is followed.

Animal separations: In large colonies, removal of individual birds does not seem to have a well-defined effect on social dynamics, except for individuals whose mates have been removed. In these cases, birds may show some signs of lethargy or may repeatedly visit the nest site during breeding season, as if

searching for the bird that has been removed. For example, when moving one bird off exhibit for medical reasons, also move its mate if possible. This seems to decrease stress while off exhibit, helps to maintain the pair bond, and makes for an easier reintroduction to the exhibit. In smaller colonies, removal of a dominant individual may cause a shift in the dominance hierarchy and as equilibrium in the social group is re-established, may lead to a short-term increase in aggressive behavior.

Chapter 5. Nutrition

5.1 Nutritional Requirements

A formal nutrition program is recommended to meet the nutritional and behavioral needs of all penguins (AZA Accreditation Standard 2.6.2). Diets should be developed using the recommendations of nutritionists, the Nutrition Scientific Advisory Group (NAG) feeding guidelines: (http://www.nagonline.net/Feeding%20Guidelines/feeding_guidelines.htm), and veterinarians as well as AZA Taxon Advisory Groups (TAGs), and Species Survival Plan® (SSP) Programs.

AZA Accreditation Standard

(2.6.2) The institution should have a written nutrition program that meets the behavioral and nutritional needs of all species, individuals, and colonies/groups in the institution. Animal diets must be of a quality and quantity suitable for each animal's nutritional and psychological needs.

Diet formulation criteria should address the animal's nutritional needs, feeding ecology, as well as individual and natural histories to ensure that species-specific feeding patterns and behaviors are stimulated. Penguins feed almost exclusively on aquatic prey, predominately pelagic schooling fish, crustaceans (often Euphausiidae species) and cephalopods (squid). All species consume more than one type of food, although some smaller-sized, higher-latitude species (e.g., gentoo and chinstrap) rely almost exclusively on Euphausiidae crustaceans (Croxall & Lishman, 1987). Macaroni and Adélie penguins rely heavily on krill, but fish consumption has been reported in some locations (Lishman, 1985). Penguins that live at lower latitudes, such as little blue penguins and the *Spheniscus* spp., tend to rely more heavily on fish than do the high-latitude species (Croxall & Lishman, 1987). The prey fish taken most often are small-bodied, surface-schooling species.

Although qualitative information on feeding habits is available for most penguin species, information on consumed quantities of specific foods is exceedingly rare. Some food intake data are available for little blue, Humboldt, and African penguins, for both non-breeding and breeding seasons (Rand, 1960; Hobday, 1992; Herling et al., 2005). More recent ecological research has focused on the dietary effects on reproductive success (Fonseca et al., 2001; Putz et al., 2001; Clausen & Putz, 2002; Tremblay & Cherel, 2003); the foraging strategies and trophic levels of feeding (Raclot et al., 1998; Forero et al., 2002; Lenanton et al., 2003); and the effect of environmental change on penguin populations (Putz et al., 2001; Gauthier-Clerc et al., 2002; Chiaradia et al., 2003; Boersma, 2008).

Digestive system morphology and physiology: The digestive system of the penguin is relatively simple; it is anatomically and functionally similar to other carnivorous birds. The esophagus is large, expandable, and muscular, allowing for the consumption of large prey items; however, the crop is completely absent, similar to owls (Paster, 1992; Duke, 1997; Olsen et al., 2002). The stomach contains two distinct chambers: the proventriculus and the ventriculus. The proventriculus has two major functions: the secretion of gastric juices for chemical digestion, and the storage of food to feed chicks. The ability to store food for long periods of time is achieved through mechanisms that raise the pH of the gastric juices and regulates stomach temperatures, which disrupts digestive enzymatic activity (Gauthier-Clerc et al., 2002; Olsen et al., 2002; Thouzeau et al., 2004).

The proventriculus empties into the ventriculus (gizzard), which is characterized by a massive muscular wall, often containing grit or small stones (reviewed by Beaune et al., 2009). These stones are believed to aid in digestion and/or be used to regulate buoyancy during foraging; however, the absence of stones in zoo or aquarium penguin exhibits do not appear to affect digestibility (Splettstoesser & Todd, 1999). Small intestines are relatively long, compared with other birds, and correlates positively with body mass (Jackson, 1992). Although limited data exist on the functional features of the ceca in penguins, they are present, but small and vestigial (Clench & Mathias, 1995).

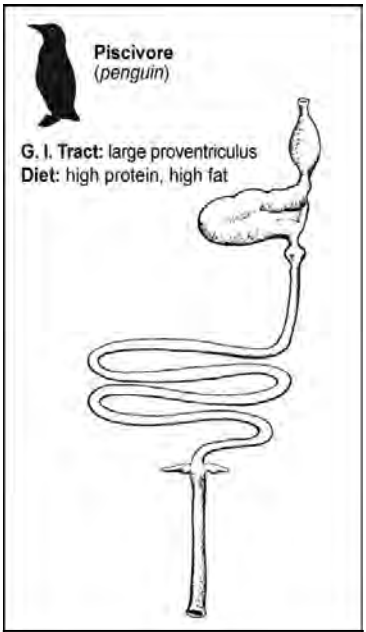


Figure 11. Digestive system of the penguin (adapted from Stevens & Hume (1995) by McClements (2007)).

Nutrient requirements: While many items consumed by various species

of free-living penguins are known, the nutrient content of these items have not been completely characterized. The National Research Council (NRC) has published estimated nutrient requirements of domestic birds and the carnivorous domestic cat (National Resources Council, 1994; 2006). Using these NRC estimates as guidelines, plus data on nutrient composition of free-ranging penguin foods and foods available in zoos and aquariums, target nutrient ranges for penguin diets are proposed in Table 8. Target nutrient ranges encompass needs for growing, reproducing, and maintenance animals.

Vitamin A: Dietary vitamin A requirements for studied avian species are between 1,100–5,600 IU/kg of diet on a DM basis (National Resources Council, 1994). Based on limited data, the vitamin A requirement for cats is between 3,333–7,500 IU/kg of dietary DM (National Resources Council, 2006). It is possible that penguins, as fish-eating birds, have a high tolerance for vitamin A because comparatively high levels occur in their natural diet (Crissey et al., 1998). Whether this infers a high dietary vitamin A requirement has not been established.

Most diets that contain a variety of fish species should contain adequate levels of vitamin A without supplementation. Studies of free-ranging macaroni penguins showed that vitamin A was mobilized from body stores during molt and reproduction (Ghebremeskel et al., 1991; 1992). In zoos and aquariums, serum levels of vitamin A in Humboldt penguins and plasma levels of vitamin A in gentoo and rockhopper penguins vary with diet fed and physiologic conditions, such as molt (Crissey et al., 1998; Monroe, 1993). Dietary levels of 12,000–100,000 IU/kg DM were offered to African and Humboldt penguins in the U.S. with no signs of vitamin A deficiencies or toxicities. Eggs produced by these birds contained vitamin A concentrations of 4.0–7.5 μg/g wet weight (McClements, 2007).

Vitamin E: Vitamin E is destroyed over time in stored marine foods (Bernard & Allen, 1997). It has been proposed that foods for marine animals should be supplemented with 100 IU of vitamin E per kg of diet on a wet basis, or approximately 400 IU/kg DM (Geraci, 1986). In zoos and aquariums, serum levels of vitamin E in Humboldt penguins and plasma levels of vitamin E in Gentoo and rockhopper penguins vary with diet and physiologic conditions, in the same way as serum and plasma vitamin A levels (Crissey et al., 1998; Monroe, 1993). Vitamin E can be purchased in capsules, paste, injectable form, or as a multivitamin designed specifically for piscivorous species, which can be hidden inside the fish and hand-fed to individual penguins.

Although limited data exists on the effect of dietary concentrations of vitamin E on egg composition and hatchability, McClements (2007) showed concentrations between 39–250 IU/kg of natural and commercially available vitamin E resulted in egg yolk concentrations between 180–356 μg/g. Although these data could not be used to determine a minimum requirement for reproductive success, it did appear that these dietary levels resulted in eggs containing sufficient levels of vitamin E for embryonic development. These sufficiency estimates were based on levels observed in eggs collected from reproductively successful free-ranging penguin and piscivorous birds (Surai et al., 2001a; Surai et al., 2001b).

Thiamin: Thiaminases have been identified in mackerel, herring, smelt, and clams with activity sufficient to destroy much of the tissue thiamin during frozen storage (Bernard & Allen, 1997; National Resources Council 1982). It has been proposed that thiamin supplements should be added to marine animal diets, providing 25–30 mg/kg diet on a wet weight basis or approximately 100–120 mg/kg DM (Geraci, 1986). Thiamin can be purchased in tablet, paste, injectable form, or as a multivitamin designed specifically for piscivorous species which can be hidden inside the fish and hand-fed to individual penguins.

Vitamin D, calcium, and phosphorus: Calcium concentrations in whole fish and krill (0.9–6.4% of DM) appear adequate, even for breeding and laying penguins, and calcium supplements should not be required (Bernard & Allen, 1997). Squid, however, are relatively low in calcium (0.1–0.2% of DM) and have an inverse calcium:phosphorus ratio. Some institutions have reported problems (without dietary details) in penguins housed in zoos and aquariums that were ascribed to calcium deficiency during production of multiple clutches, and calcium supplements were used with no apparent ill effect (Ellis & Branch, 1994). However, consideration should be given to the concentrations of calcium, phosphorus, and vitamin D in dietary items (using analyses, if necessary), and to the calcium:phosphorus ratio, as a disproportionate supply of one of these nutrients can adversely influence metabolism of the others, Appropriate UV lighting should be provided as a source of vitamin D if birds are housed indoors.

Sodium: Sodium is an essential nutrient for all animals. It was generally considered by some that the requirement for sodium is a special consideration for the functional development of the nasal glands of marine birds with access only to fresh water (Ellis & Branch, 1994). Some institutions, with both fresh and saltwater environments, supplement penguin diets with salt at approximately 250 mg of NaCl per bird per day, without apparent harm (Ellis & Branch, 1994). However, recent studies with *ex situ* African penguins, housed in a fresh water environment and offered a diet of herring, capelin, and squid, were found to maintain electrolyte balance without additional salt supplementation (Mazzaro et al., 2004). These electrolyte balances have been maintained in the six years since the end of the experimental period (L. M. Mazzaro, personal communication). Gentoo and rockhopper penguins have been maintained in freshwater with no sodium supplementation for eleven years, and king penguins for eight years with no reported ill effects (E. Diebold, personal communication). It is noteworthy that the fish and invertebrates that have been analyzed, whether of marine or freshwater origin, contain sodium concentrations (0.2–5.5% of DM) that are higher than the minimum need of any species for which a requirement has been established (Bernard & Allen, 1997).

Fatty Acids: Fish lipids contain high concentrations of both saturated and unsaturated long chain fatty acids. Henderson & Tocher, (1987) reviewed the major fatty acid fractions of a number of fish species and showed that generally freshwater fish contain considerably higher concentrations of omega-6 (ω-6) fatty acids than fish caught in the marine environment. Generally freshwater fish contain higher concentrations of linoleic (C18:2ω-6) and arachidonic (C20:4ω-6) acids compared to all other marine fish resulting in a 4–14 times reduction in the ω-6 to ω-3 fatty acid ratios (Henderson & Tocher, 1987; Ackman, 1989). Salmonids, such as rainbow trout, are the exception to this generalization, as they contain high concentrations of both ω-6 and ω-3 fatty acids. In contrast, all fish species contain high concentrations of ω-3 fatty acids, including docosahexaenoic acid (DHA; C22:6 ω-3) and eicosapentaenoic acid (EPA) (C20:5 ω-3). Krill and squid are also very good sources of highly unsaturated fatty acids, with squid containing very high levels of DHA (Passi et al., 2002; Ackman & Kean-Howie, 1994).

Based on analytical values for other nutrients in fish and marine invertebrates, it seems unlikely that other deficiencies would appear unless unwise food choices have been made or storage and handling of these foods has been below standards (Crissey, 1998). If a variety of high quality fish are offered, and if they are stored and thawed properly, it is unlikely that supplements, other than of vitamin E and thiamin, will be needed. Adjustments in the amounts of supplement provided should be made in proportion to the mass of food offered.

Chicks: Nutrient requirements for growing chicks have not been defined. Diets that meet the target nutrient ranges should be adequate. During periods of rapid growth, the higher ranges of values for calcium and vitamin D are recommended. Metabolic bone disease has been reported in juvenile Humboldt penguins (Adkesson & Langan, 2007). Long chain polyunsaturated fatty acids are known to impart very important roles in birds, and they are especially apparent in the high concentrations of arachidonic acid and docosahexaenoic acid in the hearts and brains of developing chicks of many species (Noble & Cocchi, 1990; Speake et al., 1998).

Table 8. Target nutrient ranges for adult penguin diets[a] based on requirements of domestic poultry (NRC, 1994); cats (NRC, 2006); and inferences from composition of wild foods (Bernard & Allen, 1997; McClements, 2007) (on a dry matter basis)

Nutrient	Concentration
Gross energy, kcal/g	4.5–6.5
Crude protein, %	45–75
Fat, %	10–40
Vitamin A, IU/g	1.1–7.5
Vitamin D, IU/g	0.2–0.5
Vitamin E, IU/kg	400[b]
Thiamin, mg/kg	100[c]
Calcium, %	0.78–2.5
Phosphorus, %	0.26–0.76
Magnesium, %	0.04–0.07
Potassium, %	0.33–0.5
Sodium, %	0.14–0.17[d]
Iron, mg/kg	60–80
Copper, mg/kg	4–9
Manganese, mg/kg	5–67
Zinc, mg/kg	35–75
Selenium, mg/kg	0.1–0.4

[a] Other nutrients, such as essential fatty acids, essential amino acids, vitamin K, and the other B-complex vitamins are probably required. Nevertheless, there is no evidence that inadequate concentrations are provided by fish and marine invertebrates. Whether or not vitamin C can be synthesized by penguin tissues has not been established. Freshly caught fish contain significant concentrations of this vitamin, and some destruction undoubtedly occurs during storage. However, signs of vitamin C deficiency in the penguin have not been described.

[b] Although this concentration of vitamin E may exceed the minimum requirement, about 400 IU/kg of DM provided by the supplement of 100 IU of vitamin E/kg of fresh fish is recommended to compensate for losses during peroxidation of unsaturated fatty acids.

[c] This concentration of thiamin undoubtedly exceeds the minimum requirement, but about 100–120mg/kg of DM are provided by the supplement of 25–30mg of thiamin/kg of fresh fish to compensate for destruction by thiaminases.

[d] Recent studies with African penguins fed a diet of herring, capelin, and squid, indicate that salt supplementation is not necessary to maintain electrolyte balance (Mazzaro et al., 2004).

Energy requirements: On a yearly cycle, penguin behavior consists of periods of inactivity, such as molting and egg incubation, and periods of increased activity, such as nest building and raising chicks. Some institutions have seen migratory swimming behavior when the birds think that they are “out to sea.” The birds’ caloric requirements will vary as activity levels fluctuate. Most penguins in zoos and aquariums are given the opportunity to eat until the point of satiation. When the proper environmental conditions are in place, a penguin’s food consumption will oscillate with the normal cycles of activity. Nutrient and energy requirements should continue to be met.

Breeding: The appetite of penguins often increases in conjunction with breeding and egg-laying, and distinctive food preferences may be exhibited. Females may increase their weight by as much as 20–25%. It is currently recommended that a variety of whole fish be fed to nesting penguins, in quantities adequate to supply energy and protein needs. It does not appear necessary to provide supplemental fat in the diet. Adélie penguins have been found to feed exclusively on krill when nesting (Nagy & Obst, 1992).

Chick rearing: Energy requirements are considerable for the growth of chicks. King penguin (*Aptenodytes patagonicus*) chicks were estimated, by mass and energy density of stomach contents, to consume an average of 3,646 kJ (871 kcal) of gross energy (GE) per chick per day during a 3-month growth period (Cherel & Ridoux, 1992). The fish consumed contained 22–26 kJ (5.26–6.21 kcal) GE/g, DMB. Free-ranging emperor penguins fed their single chick the equivalent of about 7.5% of adult emperor penguin body mass in a 24-hour period (Robertson et al., 1993). The most important dietary adjustment to make when the parents are rearing chicks in zoos and aquariums is to offer enough fish to the parents so they may adequately feed themselves and their offspring. During chick rearing, parents should be fed *ad libitum* and frequently.

Molting: There are notable alterations in energy intake that are associated with molt (Ghebremeskel et al., 1992). The cues that induce the molting process include changes in ambient temperature, day length, food resource availability (possibly including food nutrient content), and associated hormonal changes (Ghebremeskel et al., 1992). It appears that if fed an adequate diet *ad libitum*, and the environment

accurately mimics seasonal light and temperature changes, most penguins in zoos and aquariums will exhibit a normal annual cycle of food intake, and will molt and reproduce normally (Wilson, 1985; Monroe, 1993). Appetite usually increases during the pre-molt period and decreases during molt. In a study with *ex situ* rockhopper penguins, all birds gained about 23–38% in body mass just prior to molting (Monroe, 1993). Among the penguin species that have been studied, most will fast during incubation and molting. In the wild, mean loss of body mass during molt is as much as 40% in macaroni penguins and 47% in king penguins (Ghebremeskel et al., 1991; Cherel et al., 1994). During molt in zoos and aquariums, losses can be as much as 50% of body mass. After these periods, penguins consume vast quantities of food and deposit considerable body fat and protein (Ghebremeskel et al., 1991).

5.2 Diets

The formulation, preparation, and delivery of all diets must be of a quality and quantity suitable to meet the animal's psychological and behavioral needs (AZA Accreditation Standard 2.6.2). Food should be purchased from reliable, sustainable and well-managed sources. The nutritional analysis of the food should be regularly tested and recorded.

The nutrient composition of fish and marine invertebrates fed to piscivorous animals in zoos and aquariums has been discussed by Bernard & Allen (1997) in the AZA Nutrition Advisory Group Handbook Fact Sheet 005. More recently, McClements (2007) analyzed fish fed to Humboldt and African penguins at ten U.S. zoos. This data encompasses most species of fish utilized for all species of penguin maintained in a zoo or aquarium (see Appendix F). It should be noted that fish nutrient values will vary with species, age, gender, physiologic state, season, and locale of harvest.

The quantity of food provided to penguins in zoos and aquariums to consume per day can be estimated based on their body mass. An average but active adult penguin's daily food consumption on an as-fed basis is approximately 2–3% of body mass for the larger species, such as kings and emperors, and 10–14% for smaller species, such as Humboldts and rockhoppers (Ellis & Branch, 1994). However, the specific quantities consumed depend on the activity level and physiologic state of each individual. In one study, free-ranging king penguins consumed (wet basis) an average of 1.84 kg (4.06 lb.) of food daily (Cherel & Ridoux, 1992). Estimated daily consumption (wet basis) in another study with free-ranging king penguins was an average of 2.32 kg (5.1 lb.). Mean body mass of the king penguins was 11.8 kg (26 lb.), resulting in a calculated daily intake equivalent to as much as 20% of their body mass (Putz & Bost, 1994).

When formulating diets for *ex situ* penguins, flexibility is required to account for variations in food preferences, body mass, activity, physical condition, environment, and behavior, as well as food availability and nutrient content. Vitamin mineral supplementation should be included in the diet where appropriate according to label indications and or recommendations from a qualified nutritionist or veterinarian. Ideally, the items chosen (e.g., high-fat and low-fat fish) and supplements fed should complement each other so that nutrient and energy requirements are met. It should be noted that when examining nutrient data for whole fish and marine invertebrates, the nutrient concentrations can vary among species, among individual lots within a species, among individual fish within a lot, as well as over a period of storage. Thus, published values may or may not reflect the nutrients actually fed to penguins at a specific time. Fish should be routinely sampled and analyzed according to industry standards via commercial laboratory for the determination of macro and micro nutrient concentrations.

Sample diets: Sample diets from institutions housing penguins can be found in Appendix G. The nutrient composition of these diets is presented in Appendix H. Refer to section 7.5 Assisted Rearing or the Penguin Husbandry Manual (Henry & Sirpenski, 2005) for specific diet information regarding hand-rearing of any species.

Food provision: The recommended method of feeding is to hand-feed individual penguins, particularly when offering fish that have been injected with nutrient supplements or in which supplement tablets or capsules have been placed. This ensures that each bird will receive intended nutrients and allows caretakers to monitor food and energy consumption. However, birds conditioned to hand-feeding may develop poor swimming habits, and may spend most of their time standing around on the exhibit surface. To encourage swimming, institutions may opt to pool feed. Individual appetites should still be closely monitored during the feeding. Adult penguins are commonly fed to appetite twice daily, although the number of feedings may be increased during pre-molt and breeding.

Methods of penguin self-feeding can sometimes be used, but keepers should ensure that food items remain cool, clean, and are consumed within a short time after being thawed. In exhibits held at or below 4 °C (39.2 °F), fish may be offered in feeding trays for several hours, as long as birds are neither defecating nor walking in the trays. However, fish should not be left in standing water because of the potential for nutrient loss. Supplemented fish should not be fed in trays because of the potential for under- or over-dosing if individual penguins consume either no or several fish containing supplements. If penguins are fed outdoors in hot, humid, or sunny weather, it is important to feed only the amount that will be consumed immediately or while still iced to avoid microbial proliferation, nutrient loss, and contact by disease-spreading pests.

The size of food items offered to penguins should be appropriate for easy manipulation and swallowing. Purchasing specifications for fish and squid should include size designations so that they can be fed whole. Whole food is accepted most readily, but if it has to be cut because it is too large, all portions should be fed to ensure that the entire supply of nutrients contained in the whole food is consumed. Lengths of fish consumed by free-ranging adult emperor penguins range between 6–12 cm (2.4–4.7 in.), and lengths of squid consumed range from 1.9–28 cm (0.7–11 in.). The largest squid consumed weighed 460 g (1 lb.) (Robertson et al., 1993). Free-ranging adult king penguins consumed prey estimated to be. 7–9 cm (2.8–3.5 in.) long, substantially smaller than the fish commonly fed in zoos and aquariums (Cherel & Ridoux, 1992). The larger average body size and bill dimensions of male penguins may result in consumption of somewhat larger prey than consumed by females. This sex-related difference has been documented in Gentoo penguins, but such differences have not been seen in macaroni, chinstrap, and Adélie penguins (Williams et al., 1992).

Food variability: Among penguin species that have been studied at more than one site or during more than one season, there are suggestions of within species diet variations (Croxall & Lishman, 1987; Cullen et al., 1992). Much of the variation may relate to differences in prey availability, but not all feeding patterns are clear (Croxall & Lishman, 1987; Cullen et al., 1992; Adams & Klages, 1987; Croxall et al., 1988; Clausen & Putz, 2002). Both seasonal and site-based differences in quantities of specific prey items have been reported for most species, including little blue, African, king, and others (Adams & Klages, 1987; Rand, 1960; Montague, 1982; Moore & Wakelin, 1997; Coria et al., 2000; Ainley et al., 2003; Lynnes et al., 2004). African penguins appear to exhibit seasonal variations in food selection that appear unrelated to prey supply. Nevertheless, prey supply appears to be the single largest contributor to seasonal variations and is often associated with reduced reproductive success in free-living species (Clausen & Putz, 2002; Rombola et al., 2003; Lynnes et al., 2004).

Supplies of prey items may shift with major oceanographic events, such as El Niño (Radl & Culik, 1999; Bakun & Broad, 2003; Hays, 1984; 1986). The increased risk of prey disappearance may result from climate change, major disease outbreaks in prey items, and increased competition of human fisheries on prey species (Tonn, 1990; Walther et al., 2002; Perry et al., 2005; Chiaradia et al., 2001; Chiaradia et al., 2003). The impact of fisheries on prey species should not simply be considered a free-living animal issue, especially given that prey items available to zoos and aquariums are a direct result of commercial fisheries. Considerable data exist on both the direct and indirect effects of fisheries on free-living avian species, including penguins (Furness & Tasker, 2000; Tasker et al., 2000; Furness, 2003; Crawford & Shelton, 1978; Shelton et al., 1984; Croll & Tershy, 1998). Therefore, it is recommended that all institutions understand where and how their prey items are being harvested and whether these practices are ecologically sustainable. Data can be found regarding many of the commonly offered species at a number of non-profit and government websites, including the National Oceanic and Atmospheric Administration's FishWatch® initiative and Seafood Watch®. Not all of the fish that are commonly offered to penguins are listed in these two websites, but other countries have similar websites listing many of these other species and their ecological status.

In zoos and aquariums, it is generally accepted that penguins have food preferences. The types and species of prey available for feeding are limited and may be quite different from the variety with which penguins evolved. Even data from free-ranging penguins suggest that the food items most consumed may not be those most preferred, but may be foods that are most available (Hays, 1986; Hobday, 1992; Boersma, 2008). Differences in food choice also may be influenced by physiologic circumstances, such as stage of the reproductive cycle (Boersma, 2008).

A penguin's selection of particular food items may be an expression of food preference, but since penguins in zoos and aquariums lack a historical and long-term association with the dietary items they

are provided, they do not appear to make choices on the basis of nutritional wisdom. Food refusal, on the other hand, may be an indication of spoilage, and if fish are refused, their quality should be checked in addition to normal quality inspections. To avoid dependence on a particular food item, it is prudent to offer a variety of prey species. If a penguin becomes "imprinted" on a specific food item, and if that item becomes unavailable, it may be difficult to coax acceptance of an alternative. In addition, offering a variety of foods will help ensure that the diet provides a complementary and complete nutrient profile.

Food preparation must be performed in accordance with all relevant federal, state, or local laws and/or regulations (AZA Accreditation Standard 2.6.1). Meat processed on site must be processed following all USDA standards. The appropriate hazard analysis and critical control points (HACCP) food safety protocols for the diet ingredients, diet preparation, and diet administration should be established for the taxa or species specified. Diet preparation staff should remain current on food recalls, updates, and regulations per USDA/FDA. Remove food within a maximum of 24 hours of being offered unless state or federal regulations specify otherwise and dispose of per USDA guidelines. Refer to Crissey (1998) for proper assessment, handling and storage of fish.

AZA Accreditation Standard

(2.6.1) Animal food preparation and storage must meet all applicable laws and/or regulations.

Typically browse is not offered to penguins. However, any plant species used in the exhibit or for enrichment should be identified with regards to safety by the veterinarians or horticulturalists. If browse plants are used within the animal's diet or for enrichment, all plants must be identified and assessed for safety. The responsibility for approval of plants and oversight of the program should be assigned to at least one qualified individual (AZA Accreditation Standard 2.6.3). The program should identify if the plants have been treated with any chemicals or near any point sources of pollution and if the plants are safe for the penguins. If animals have access to plants in and around their exhibits, there should be a staff member responsible for ensuring that toxic plants are not available.

AZA Accreditation Standard

(2.6.3) The institution should assign at least one person to oversee appropriate browse material for the animals.

5.3 Nutritional Evaluations

Taking regular weights is important for monitoring the health of individual animals. The weighing of individuals should be carried out opportunistically. This can be done on a routine basis if exhibit design and bird behavior allows it. The birds should always be weighed when they are handled for other reasons. Individual weight records should be maintained over time and utilized for comparison when a bird appears sick. The use of operant conditioning to train birds to stand on a scale (e.g., scale training) can assist in the daily management of the birds. In most cases, there is no need to limit food intake below *ad libitum* levels unless the penguin is extremely overweight.

Vitamin excesses: Fat-soluble vitamins A, D, E and K accumulate in the body when intakes exceed need, and excessive amounts over extended periods will produce signs of toxicity (Machlin, 1984). It should be noted, however, that there are seasonal differences in the availability of these vitamins for some animal species in the wild, and the accumulation of body stores during comparatively short natural periods of plenty may be critical for health during periods of short supply.

Vitamin A: Chronic vitamin A toxicity typically results from long-term intakes that are 100–1,000 times dietary requirements, although toxic signs have been reported from dietary levels as low as 10 times the requirement in domestic animals (National Resources Council, 1987). Elevated serum levels of vitamin A have been observed in Humboldt penguins fed diets containing 59,800 IU of vitamin A/kg (DMB) for 12 months, but no toxicity signs were seen (Crissey et al., 1998).

Vitamin E: Maximum tolerable levels of dietary vitamin E are quite high, but interference with blood clotting has been reported in pelicans with supplements of vitamin E adding 1,000–2,000 IU/kg of dietary DM (Nichols et al., 1989). Elevated serum levels of vitamin E have been observed in Humboldt penguins fed diets containing 58.6 IU of vitamin E/kg (DMB) for 12 months, but there were no signs of toxicity (Crissey et al., 1998).

Chapter 6. Veterinary Care

6.1 Veterinary Services

Veterinary services are a vital component of excellent animal care practices. A full-time staff veterinarian is recommended. In cases where this is not practical, a consulting/part-time veterinarian must be under contract to make at least twice monthly inspections of the animal collection to respond to emergencies (AZA Accreditation Standard 2.1.1). In some instances, because an institution's size or nature, exceptions may be made to the twice-monthly inspection requirement Veterinary coverage must also be available at all times so that medical needs can be responded to in a timely fashion (AZA Accreditation Standard 2.1.2). The AZA Accreditation Standards recommend that AZA-accredited institutions adopt the guidelines for medical programs developed by the American Association of Zoo Veterinarians (AAZV): http://www.aazv.org/displaycommon.cfm?an=1&subarticlenbr=839.

AZA Accreditation Standard

(2.1.1) A full-time staff veterinarian is recommended. In cases where such is not practical, a consulting/part-time veterinarian must be under written contract to make at least twice monthly inspections of the animals and to respond as soon as possible to any emergencies

AZA Accreditation Standard

(2.1.2) So that indications of disease, injury, or stress may be dealt with promptly, veterinary coverage must be available to the animal collection 24 hours a day, 7 days a week

The current Penguin TAG veterinary advisors can be found at: https://ams.aza.org/eweb/DynamicPage.aspx?Site=AZA&WebKey=8f652949-31be-4387-876f-f49a2d7263b2. Basic information on penguin husbandry, behavior and medicine is available in the current scientific literature, including *Zoo and Wildlife Medicine 3rd edition* (Fowler, 1993), and the 5th editions (Fowler & Miller, 1999). Additional veterinary references can be found in the reference section of this document. There are no penguin-specific training programs in veterinary medicine currently available, although several institutions that house penguins may offer general veterinary medicine internships which include on the job training with penguins.

AZA-accredited institutions must have a clear process for identifying and addressing penguin animal welfare concerns within the institution (AZA Accreditation Standard 1.5.8) and should have an established Institutional Animal Welfare Committee. This process should identify the protocols needed for animal care staff members to communicate animal welfare questions or concerns to their supervisors, their Institutional Animal Welfare Committee or if necessary, the AZA Animal Welfare Committee. Protocols should be in place to document the training of staff about animal welfare issues, identification of any animal welfare issues, coordination and implementation of appropriate responses to these issues, evaluation (and adjustment of these responses if necessary) of the outcome of these responses, and the dissemination of the knowledge gained from these issues.

AZA Accreditation Standard

(2.3.2) Hospital facilities should have radiographic equipment or have access to radiographic services.

AZA Accreditation Standard

(2.5.1) Deceased animals should be necropsied to determine the cause of death. Cadavers must be stored in a dedicated storage area. Disposal after necropsy must be done in accordance with local/federal laws.

Given the wide variety of zoos and aquariums that house penguins, the AZA Penguin TAG cannot provide specific recommendations for the best approaches to take to communicate animal welfare issues effectively within every institution. Some institutions have an animal welfare committee to whom concerns can be relayed. Committee members include both frontline care staff, animal managers, curators as well as staff from other institution departments. Some additionally recruit one or two outside consultants to be members that can voice non-institutional opinions. All animal caretakers that work with penguins should be aware of institutional protocols in place for them to identify, communicate, and hopefully address potential animal welfare issues that are associated with the care and management of these animals.

Protocols for the use and security of drugs used for veterinary purposes must be formally written and available to animal care staff (AZA Accreditation Standard 2.2.1). Protocols should include a list of persons authorized to administer animal drugs, situations in which they are to be utilized, location of animal drugs and those persons with access to them, and emergency procedures in the event of accidental human exposure.

AZA Accreditation Standard

(2.2.1) Written, formal procedures must be available to the animal care staff for the use of animal drugs for veterinary purposes, and appropriate security of the drugs must be provided.

Animal recordkeeping is an important element of animal care and ensures that information about individual animals and their treatment is always available. A designated staff member should be responsible for maintaining animal records and for conveying relevant laws and regulations to the animal care staff (AZA Accreditation Standard 1.4.6). Recordkeeping must be accurate and documented on a daily basis (AZA Accreditation Standard 1.4.7). Complete and up-to-date animal records must be retained in a fireproof container within the institution (AZA Accreditation Standard 1.4.5) as well as be duplicated and stored at a separate location (AZA Accreditation Standard 1.4.4).

AZA Accreditation Standard

(1.4.6) A staff member must be designated as being responsible for the institution's animal record-keeping system. That person must be charged with establishing and maintaining the institution's animal records, as well as with keeping all animal care staff members apprised of relevant laws and regulations regarding the institution's animals.

A specific individual should be assigned to handle endangered species permits. For transport across state lines or out of country, contact the receiving state for its requirements regarding health certificates, preshipment tests, and permit numbers.

AZA Accreditation Standard

(1.4.7) Animal records must be kept current, and data must be logged daily.

Detailed medical records should be kept regarding an individual's complete medical history. This includes information on all preventive medical care, diagnostic exams, illnesses, injuries, associated treatments, vaccinations, lab reports, abnormal physiology and abnormal behavior. Water quality results should be documented and readily available. Key information for veterinary care should be recorded on a daily basis and include changes in behavior, appetite, diet offered, fecal consistency, reproductive activity, and any overt signs of illness or abnormal health, such as regurgitation/vomiting, bleeding, abnormal swelling, lameness, and respiratory problems, including coughing. It is a critical to follow up with information on response to treatment and procedures, or changes in condition. If medications are being administered, record this information and whether or not delivery of medication was successful. Weights should be documented regularly. Necropsies should be done if at all possible and results maintained as part of the permanent record as a way to monitor the health of the overall collection.

AZA Accreditation Standard

(1.4.5) At least one set of the institution's historical animal records must be stored and protected. Those records should include permits, titles, declaration forms, and other pertinent information.

AZA Accreditation Standard

(1.4.4) Animal records, whether in electronic or paper form, including health records, must be duplicated and stored in a separate location.

Reproductive recordkeeping: Recordkeeping related to reproductive management should begin at the time of egg laying. Marking the first egg laid is important when calculating expected hatch dates. Egg logs should contain data such as lay date, number of days incubated, sire and dam, sibling identification, and method of rearing. Fertility results should be noted for each egg as well as survivability of chicks. By tracking a pair's reproductive history, trends in success or failure can be identified. One simple method for recording reproductive data for penguins, using large rookery maps, is described by Ellis-Joseph (1990).

Hatch weights and subsequent daily or weekly weights are important to monitor overall growth rate. For hand-reared penguins, many institutions develop records which include first morning weight, weight before and after each feeding, amount of food consumed at each feeding, types of food consumed, vitamins and medications given, and comments on behavior, and. It is useful to record ambient temperature and brooder temperature (if applicable). Chick records should be maintained through fledging. For more information on assisted rearing practices for penguins, see Chapter 7.5.

6.2 Identification Methods

Ensuring that penguins are individually identifiable allows for more better care of each individual. And individual animals should have corresponding ID numbers whenever practical. A system for accurately maintaining animal records must be created if individual identifications are not practical (AZA Accreditation Standard 1.4.3).

AZA Accreditation Standard

(1.4.3) Animals must be identifiable, whenever practical, and have corresponding ID numbers. For animals maintained in colonies/groups or other animals not considered readily identifiable, the institution must provide a statement explaining how record keeping is maintained.

To maintain individual records, animals should be banded or marked so individuals can be identified at a distance. In birds, an additional system of permanent identification is recommended in

case the band is lost, and to track birds from one institution to another if banding techniques should change. Cheney (1989) reported that most institutions use flipper bands with good success. In lieu of actual flipper bands, colored cable ties can be placed around the flipper. When using this method, the band should be tightened to the point where a finger can be slipped between the band and the bird's flipper. As bands can continue to tighten after applied, either the fastener should be glued to the band to prevent slippage when in place or monitored to ensure that they do not tighten further and impede circulation to the flipper.

The band should be placed in such a manner that the fastener does not rub against the penguin's flipper or get hooked on protruding objects. Flipper bands should be monitored closely during molt, as the penguins' flippers often swell during this time, potentially restricting circulation. During molt many institutions replace the flipper band with a looser band to accommodate swelling or leave it off during molt if there are other methods if identifying the bird. Regardless of the method of visible individual identification used, the AZA Penguin TAG recommends that transponders also be used with penguins. The AZA Penguin TAG recommends subcutaneous placement of the transponder in the loose skin of the back of the neck, or on top of the head, but Boersma recommends the fleshy part of the foot in the front of the tarsus (D. Boersma, personal communication). Chicks weighing as little as 500 g (1.1 lb.) can be micro chipped if needed. For smaller collections, identification of adults can be made based on spot patterns of the breast feathers based on photographs taken after the molt into adult plumage.

Sexing: DNA sexing from feather, blood, or egg membranes can be done by commercial laboratories and is very reliable. This is the recommended method for sexing penguins (see Appendix I for laboratories). When pulling feathers, be sure to remove them so the root is intact. If commercial labs are not available, penguins can be sexed by cloacal examination. The most reliable use of this technique is constrained to a two-week period following egg laying (Boersma & Davies, 1987). Sladen (1958) indicated that a cloacoscope method for sexing Adélie, Humboldt, and African penguins has been used with some success. The differences between male and female physical characteristics are slight, and extensive training is needed for this method to be used accurately. Although sexing based on morphometrics has been published for some species, this has been shown to be unreliable in managed populations of Humboldt penguins (Wallace et al., 2008) and thus might be expected to be unreliable for other spheniscid species.

AZA member institutions must inventory their penguin population at least annually and document all penguin acquisitions and dispositions (AZA Accreditation Standard 1.4.1). Transaction forms help document that potential recipients or providers of the animals adhere to the AZA Code of Professional Ethics, the AZA Policy on Responsible Population Management: Acquisitions, Transfers and Transitions by Zoos & Aquariums (see Appendix B), and all relevant AZA and member policies, procedures and guidelines. In addition, transaction forms must insist on compliance with the applicable laws and regulations of local, state, federal and international authorities. All animals owned by an AZA institution must be listed on the inventory, including those animals on loan to and from the institution (AZA Accreditation Standard 1.4.2).

AZA Accreditation Standard

(1.4.1) An animal inventory must be compiled at least once a year and include data regarding acquisitions and dispositions at the institution.

AZA Accreditation Standard

(1.4.2) All species owned by the institution must be listed on the inventory, including those animals on loan to and from the institution. In both cases, notations should be made on the inventory.

6.3 Transfer Examination and Diagnostic Testing Recommendations

The transfer of animals between AZA-accredited institutions or certified related facilities as a result of AZA Animal Program recommendations often occurs as part of a concerted effort to preserve these species. These transfers should be done as altruistically as possible and the costs associated with preshipment examination and diagnostic testing should be considered.

Complete preshipment examinations are recommended to ensure that individuals are healthy enough to withstand the stress of shipment, and to screen for disease to prevent spread to another institution. A full physical exam should be conducted, including but not limited to weight, inspection of the feet, oral cavity and eyes, general body and feather condition, and review of medical history, appetite, and behavior.

Minimally, most institutions request blood for a routine CBC and chemistry profile, fecal exam for parasites, and fecal culture for pathogens. Radiographs can be requested provided that the sending institution has access to anesthesia and a radiograph machine, but not all institutions can provide this. Other diagnostic tests might be required by the receiving state/country, and the state/country should be contacted prior to shipment to find out what additional rests and what permits are required. Local, state, or federal regulations that are more stringent than AZA Standards and recommendations have precedence.

6.4 Quarantine

AZA institutions must have holding facilities or procedures for the quarantine of newly arrived animals or for the treatment of sick/injured animals (AZA Accreditation Standard 2.7.1). All quarantine, hospital, and isolation areas should be in compliance with AZA standards/guidelines (AZA Accreditation Standard 2.7.3; Appendix C). Local, state or federal regulations that are more stringent take precedence. All quarantine procedures should be formally written, available to staff working with quarantined animals, and supervised by a veterinarian (AZA Accreditation Standard 2.7.2). If no specific quarantine facility exists, newly acquired animals should be kept separate from the established collection to prohibit physical contact, prevent disease transmission, and avoid aerosol and drainage contamination. If the receiving institution lacks appropriate facilities for quarantine, pre-shipment quarantine at an AZA or American Association for Laboratory Animal Science (AALAS) accredited institution may be applicable.

AZA Accreditation Standard

(2.7.1) The institution must have holding facilities or procedures for the quarantine of newly arrived animals and isolation facilities or procedures for the treatment of sick/injured animals.

AZA Accreditation Standard

(2.7.3) Quarantine, hospital, and isolation areas should be in compliance with standards/guidelines contained within the *Guidelines for Zoo and Aquarium Veterinary Medical Programs and Veterinary Hospitals* developed by the American Association of Zoo Veterinarians (AAZV), which can be obtained at: http://www.aazv.org/associations/6442/files/veterinary_standards_2009_final.docx.

AZA Accreditation Standard

(2.7.2) Written, formal procedures for quarantine must be available and familiar to all staff working with quarantined animals.

Quarantine protocols: Penguins should be quarantined for a minimum of 30 days unless otherwise directed by the staff veterinarian. It may be extended if problems are diagnosed. It can be shortened if examination has shown no problems and it is behaviorally necessary for the well-being of the animals. If additional birds are introduced during the quarantine period, the quarantine should begin again. However, the addition of animals besides birds may not require the re-initiation of the quarantine period. If the new additions do not show signs of infectious disease, the first set of animals may clear quarantine without re-examination.

Separate facilities are recommended to accommodate newly acquired birds, or birds that should be separated from the group for health-related reasons. This area should have air and water systems separate from the main exhibit. It can serve as an isolation area if not in use for quarantine. An area without separate air and water systems should not be considered an appropriate quarantine or isolation area. If possible, two or more birds should be quarantined together because of their social needs. If this is not possible, efforts should be made for quarantined birds to have visual or auditory contact with other penguins. Designated keepers should care only for quarantined animals if possible. If keepers must care for both quarantined and resident animals of the same taxa, they should care for the quarantined animals only after caring for the resident animals. Any equipment or enrichment items used for quarantined animals should be used only with these animals. If this is not possible, then all items should be appropriately disinfected, as designated by the veterinarian supervising quarantine, before being used elsewhere. Standard disinfection with quaternary ammonium or bleach is adequate unless a mycobacterial disease is suspected, in which case ammonium-based products are not suitable. Phenolics can be used but can be corrosive. Enrichment items that are not easily cleaned can be thrown out and replaced if needed (infectious disease diagnosed or suspected).

AZA institutions must have zoonotic disease prevention procedures and training protocols established to minimize the risk of transferable diseases (AZA Accreditation Standard 11.1.2) with all animals, including those newly acquired in quarantine. Although transmission of tuberculosis from penguins to humans

AZA Accreditation Standard

(11.1.2) Training and procedures must be in place regarding zoonotic diseases.

is not of concern, penguins can potentially carry gastrointestinal bacteria that cause disease in people. A separate set of Personal Protective Equipment (PPE) should be worn when handling or cleaning quarantined animals. This includes outerwear such as washable or disposable smocks, aprons, overalls or gowns, surgical masks, gloves and a separate set of boots or shoe covers. Recommended minimum quarantine space, pool, and temperature recommendations are listed in space recommendations (Chapter 2). Non-abrasive flooring or matting should be used, if at all possible.

Quarantine veterinary procedures: During the quarantine period, a complete physical examination and specific diagnostic tests should be conducted for each animal (see Appendix C). Animals should be permanently identified during quarantine if not already, Animals should be evaluated for ectoparasites and gastrointestinal parasites, and treated accordingly. Blood should be collected, analyzed and the sera banked long-term in either a -70 °C (-94 °F) freezer or short-term in -20 °C (-4 °C) freezer (frost-free or self-defrosting freezer should not be used because of the freeze-thaw cycles) for retrospective evaluation. Vaccinations should be updated as appropriate, and if the vaccination history is not known, the animal should be treated as immunologically naive and given the appropriate series of vaccinations. Detailed medical records for each animal should be maintained and kept easily available.

Release from quarantine should be contingent upon normal results from diagnostic testing, and three negative fecal parasite exams and fecal/cloacal cultures that are spaced a minimum of 1 week apart. If at all possible, radiographs should be taken to establish a baseline reference for each individual and to check for evidence of disease, gastrointestinal foreign bodies, or evidence of previous trauma (fractures).

Aspergillus prevention: Aspergillosis is a severe fungal disease and often affects penguins under stress. In addition to receiving anti-fungals prior to shipment (AZA standard 6.3), animals should also receive it for at least two weeks after arrival into quarantine until they are acclimated to their new surroundings.

6.5 Preventive Medicine

AZA-accredited institutions should have an extensive veterinary program that must emphasize disease prevention (AZA Accreditation Standard 2.4.1). The American Association of Zoo Veterinarians (AAZV) has developed an outline of an effective preventative veterinary medicine program that should be implemented to ensure proactive veterinary care for all animals: (www.aazv.org/associations/6442/files/zoo_aquarium_vet_med_guidelines.pdf).

AZA Accreditation Standard

(2.4.1) The veterinary care program must emphasize disease prevention.

Depending on the disease and history of the animals, testing protocols for animals may vary from an initial quarantine test to yearly repetitions of diagnostic tests as determined by the veterinarian. Animals that are taken off zoo/aquarium grounds for any purpose have the potential to be exposed to infectious agents that could spread to the rest of the institution's healthy population. AZA-accredited institutions must have adequate protocols in place to avoid this (AZA Accreditation Standard 1.5.5). To minimize risk, some institutions have separate program animals that used solely for that purpose and that are housed separately from the main collection. If this is not possible, then penguins taken off grounds for any reason, whether for educational programs or diagnostic testing, should not come into contact with other birds or areas where other birds have been, if not adequately disinfected.

AZA Accreditation Standard

(1.5.5) For animals used in offsite programs and for educational purposes, the institution must have adequate protocols in place to protect the rest of the animals at the institution from exposure to infectious agents.

Routine physical exams: Physical exam frequency for penguins can depend on the situation of the institution. Some institutions will perform medical assessments of the birds more frequently, especially if they are screening regularly for diseases or parasites, or specifically after treatments to assess effectiveness. For smaller flocks, monthly weights are recommended—penguins can be trained to step onto a platform scale to facilitate weighing. Blood samples may be collected from penguins weekly or biweekly in a flock of birds with malaria problems. It is recommended that a physical exam should be performed at least annually, and include blood sampling, weighing, and general health assessments, if staffing and resources permit. If possible, radiographs should be performed on birds where the possibility of ingestion of foreign objects exists. During annual exams, and whenever birds are caught up for other reasons, the opportunity should be taken to weigh the animal, as well as to check the eyes, feet, and mucous membranes for indicators of any health issues. Routine vaccinations are rarely given to

penguins, but in those collections housed outdoors and exposed to mosquitos, vaccination against West Nile Virus and against Eastern or Western encephalitis if the diseases are endemic to a location, may be warranted.

Blood parameters: Each institution should establish its own set of normal blood parameters for every species maintained, preferably on MedARKS or ZIMS software. Outside laboratories or other institutions will often have different normal values. (See Appendix N for normal blood values for various managed species) Data from free-ranging individuals has been published for several species (Wallace et al., 1995; Wallace et al., 1996; Travis et al., 2006; Karesh et al., 1999.). Blood may be collected from the inter-digital, medial tarsometatarsal, flipper, and jugular veins. It appears that more institutions are utilizing the jugular because of the speed and ease of acquisition of large quantities of blood. One institution collects blood from a venous sinus located on the dorsal aspect of the vertebral column at the base of the tail. The amount of blood that may be removed depends on the size of the individual, but generally follows normal avian standards (no more than 1% body weight). Complete blood counts (CBCs) are usually done by hand (using either the eosinophil method or Natt and Herricks method); estimates from a smear are considered less accurate. The Celldyne shows promise in accurately counting white blood cells. Chemistry profiles should include assays for glucose, alanine aminotransferase (ALT), asparginine aminotransferase (AST), calcium, urea, uric acid and bile acids. Increases in cholesterol, calcium, phosphorus, and occasionally alkaline phosphatase are often seen in reproductively active females beginning about a month prior to egg laying and persisting until shortly after the egg(s) is laid (Wallace, unpublished data).

Medical management of molt: Molt is physiologically stressful for penguins. Regeneration of new feathers requires a large amount of energy. Penguins usually molt once a year after the breeding season, but some species (e.g., Galapagos penguins) molt before breeding (Boersma, 1977; 1978). The onset of molt occurs as the days begin to shorten, and is thought to be initiated by a decrease in daylight, especially in the polar species. Some species, such as the African penguins, molt over a longer period of time. African penguins at one zoological institution have molted in every month of the year, but the majority of molts occur between March and August (Bennett, 1991). At another zoological institution, Humboldt penguins have typically molted during August, September, and October, while the rockhoppers and gentoos housed indoors on a Southern hemisphere light cycle typically molt in January to March, and March to April respectively. In Europe, most *Speniscus* species molt in July and August. It is important that institutions are familiar with their normal birds molting times and plan management appropriately.

Prior to molt there is a significant increase in appetite that corresponds with a visible gain in weight. Once penguins begin to molt their appetites decrease dramatically. Some birds refuse food altogether. This corresponds to behavior in the wild, where molting occurs on land and birds do not have access to food, resulting in a fasting period lasting as long as three weeks. For wild African penguins, Cooper (1978) reported a 31% weight gain in pre-molt birds, with a subsequent loss of 41% of their peak body mass during molt. For Humboldt penguins housed at one zoological institution, it is not unusual for them to gain and lose 25% of their body weight.

During molt, the birds lose all their feathers in a short period of time. Bennett (1991) reported that the average molt length is 16.75 days in African penguins. Other penguins have similar molting periods. In zoo and aquarium environments, this large loss of feathers can cause problems for some filtration systems, and it may be necessary to remove birds from the exhibit during this time. If birds are to be moved off-exhibit, it is recommended that they are moved before they drop their feathers. Shed tail shafts have been reported to be ingested by some penguins in the wild, and the ingestion of some feathers by penguins should be considered normal (D. Boersma, personal communication). Another consideration during molt is the potential need to change flipper bands. The swelling that occurs during molt can cause the bands to constrict around the flippers. Bands may need to be removed and replaced with looser bands during molt; birds can then be re-banded after molt is completed. If the band is not removed, it is important that the birds are closely observed to ensure that the bands do not impede circulation.

Sometimes birds will either not go into or not complete their molt. In zoos and aquariums, this condition appears to occur most frequently in chinstrap penguins. Abnormal, inconsistent, or incomplete molts have been noted in various species under different circumstances. Birds from the wild, or those recently acquired from another institution, may skip a molt for the first season at a new location. Molt may also be affected by illness in an individual. Factors that may be linked to molt problems include improper light cycle, improper light intensity (i.e., coverage throughout exhibit), improper light spectrum (UV, type,

spectrum of artificial light), nutrition (i.e., body condition, weight gain, vitamins, and protein components), levels of fatty acids, and humidity.

One zoological park has tried several different methods to stimulate molt including hormonal treatments, increased day length, and natural sunlight, with varying success. The potential role of circulating thyroid and hormone levels in molt problems has also been investigated. Treatment with medroxyprogesterone compounds has been shown to induce or speed up molting, though there is some concern that this is symptomatic relief rather than a true cure. Timing of its use should coincide with the peak portion of the light cycle used in the exhibit (Reidarson et al., 1999). Fatal complications with this treatment have occurred, as has obesity with associated fatty liver syndrome. This treatment, or other types of hormonal therapy, should be used only when environmental factors (e.g., light) have been thoroughly investigated, and when all other changes in husbandry techniques and remedies have failed. There have been cases of arrested molt at varying zoos that have not responded to any treatment, resulting in penguins that are almost devoid of feathers. For these individuals, hypothermia is a concern and management adjustments should be made.

6.6 Capture, Restraint, and Immobilization

The need for capturing, restraining and/or immobilizing penguins for normal or emergency husbandry procedures may be required. All capture equipment must be in good working order and available to authorized and trained animal care staff at all times (AZA Accreditation Standard 2.3.1).

AZA Accreditation Standard

(2.3.1) Capture equipment must be in good working order and available to authorized, trained personnel at all times.

Manual restraint: Penguins are hardy animals and can normally tolerate routine handling for nail and beak trimming, banding, and weighing. The individual to be captured should be separated from the colony. There are several different methods for capturing the animal; initial restraint is done by grabbing the back of the head or very high on the neck and lifted from behind. Penguins should not be grabbed by the flippers; several institutions have reported broken flippers during handling. Two people should work together when capturing and restraining king and emperor penguins. The people capturing the birds should wear eye protection to avoid injury from a bird's beak, especially when restraining king penguins. Once the bird has been secured, a black bag can be placed over its head with the beak and nares exposed so the birds can breathe easily. Covering the eyes will immediately calm the bird (D. Boersma, personal communication).

Once captured, there are a variety of restraint techniques for penguins. Non-invasive procedures may necessitate only minimal restraint. However, medical procedures, such as blood collection, which require the bird to be immobile, dictate stronger restraint. One method used successfully involves placing the penguin between the handler's legs so that the flippers are held secure. In this way, the handler's hands are free to restrain and position the head and neck to facilitate procedures such as blood collection and re-banding. With king and emperor penguins, a second person may be needed to avoid injury to the bird and/or handler. Other methods of restraint include using large diameter PVC pipe or traffic cones to hold the bird secure. If a penguin needs to be moved a short distance, it is recommended that the handler carry the bird close to his/her body with the head at their side facing their back. If the bird needs to be moved to a different location, such as the hospital or a different holding area, it can be placed in an appropriate container such as an air kennel or large tub.

Immobilization: Animals should be fasted 18–24 hours prior to anesthesia to prevent regurgitation and aspiration of gastric content. Isoflurane is still the most commonly used gas anesthetic, although many institutions are now successfully using sevoflurane. Induction may be accomplished by use of a facemask or cone with subsequent intubation.

It should be noted that the trachea bifurcates at different levels in some species. Therefore, use of a standard length endotracheal tube may result in unilateral intubation if the clinician is not careful. Because of the extensive pulmonary/air sac system, unilateral intubation does not lead to the severe problems of hypoventilation/hypooxygenation seen in mammals. If the tracheal size diminishes distal to the bifurcation, however, tracheal trauma may occur if an inappropriately sized tube is used. If a clinician is unsure where the trachea bifurcates, radiographs may be helpful as a double trachea may frequently be seen.

Maintenance of anesthesia may be complicated by shallow breathing in the patient, resulting in a chronic excitement phase indicated by swimming like behavior. A smoother plane of anesthesia may be

achieved by assisting ventilation two to three times per minute. Ketamine has also been used, although recovery can be prolonged when compared to isoflurane. One institution recommends ketamine/valium or just ketamine given IM for induction over isoflurane for Little Blue penguins because of the fragile nature of this species and its tendency to traumatize itself during anesthetic induction with isoflurane. Once the ketamine takes effect, anesthesia may be maintained with isoflurane. If cold climate penguin species are immobilized for extended periods, some institutions use ice, ice packs, or other methods to prevent hyperthermia during the immobilization procedure. For minor procedures that just require sedation, or to reduce the stress of handling, birds may be given midazolam intranasally or intramuscularly. Sedation may then be reversed with flumazenil if needed once the procedure is finished.

6.7 Management of Diseases, Disorders, Injuries and/or Isolation

AZA-accredited institutions should have an extensive veterinary program that manages animal diseases, disorders, or injuries and has the ability to isolate these animals in a hospital setting for treatment if necessary. Penguin keepers should be trained for meeting the animal's dietary, husbandry, and enrichment needs, as well as in restraint techniques, and recognizing behavioral indicators animals may display if their health becomes compromised (AZA Accreditation Standard 2.4.2). Protocols should be established for reporting these observations to the veterinary department. Penguin hospital facilities should have radiographic equipment or access to radiographic services (AZA Accreditation Standard 2.3.2), contain appropriate equipment and supplies on hand for treatment of diseases, disorders or injuries, and have staff available that are trained to address health issues, manage short and long term medical treatments and control for zoonotic disease transmission.

AZA Accreditation Standard

(2.4.2) Keepers should be trained to recognize abnormal behavior and clinical signs of illness and have knowledge of the diets, husbandry (including enrichment items and strategies), and restraint procedures required for the animals under their care. However, keepers should not diagnose illnesses nor prescribe treatment.

AZA Accreditation Standard

(2.3.2) Hospital facilities should have radiographic equipment or have access to radiographic services.

Aspergillosis: Aspergillosis is one of the most commonly reported illnesses in penguins. It is a fungal infection caused by aspergillus organisms. The organism is ubiquitous in the outdoor environment and is often found in various areas of indoor exhibits. It can exist in low numbers without causing problems if the birds are healthy and well adapted to their exhibit and social group. Disease may occur in stressed or debilitated animals. Stressors that have been associated with the occurrence of aspergillosis include: substandard air quality, poor ventilation, elevated ammonia levels; social incompatibility; introduction to a new social group; inappropriate, prolonged or stressful relocation; introduction of new aspergillus species via new substrate or nesting material; change in location, which may expose birds to new fungal species; and excessive environmental heat or cold. High standards in exhibit air quality are an important consideration in prevention of the disease.

Early clinical signs of Aspergillosis can be subtle, and missed by keepers and veterinarians unfamiliar with the course of this infection in penguins. Signs may include open-mouth breathing, coughing, an inability to vocalize, and mucus may be evident at the glottis (opening to the trachea). Other common signs that are frequently but not always exhibited include inappetance, lethargy, weight loss, isolation, and lying down. These signs are often nonspecific and early diagnosis is difficult. Auscultation of the lungs and air sacs are commonly unremarkable. A complete blood count (CBC) may show an increase in the white blood cell count with a monocytosis, but early in the course of the disease may not show changes. Fungal cultures may be taken of the throat, trachea, or air sacs. Radiographs are helpful in looking for pulmonary or air sac granulomas or general cloudiness to air sac or lung fields. Fluoroscopy, if available, is also useful to detect granulomas. Serologic titers to aspergillus may be helpful, but it is often difficult to differentiate an acute infection from previous exposure. Changes in the plasma (heparinized) protein electrophoretic pattern compatible with chronic inflammation may be present. While there is some variation in the electrophoretic pattern among different penguin species, the inflammatory response elicited by aspergillosis typically results in elevated beta and gamma levels, and a notably depressed albumin : globulin ratio. However, these findings are nonspecific indicators of inflammation, and so can be found with other inflammatory conditions such as malaria, intestinal obstruction, and non-fungal coelomitis. Standard serum or plasma analysis for albumin and globulin values, and hence the ratio between the two, are not accurate in penguins and cannot be used in lieu of electrophoresis as a diagnostic aid.

The method and success of treatment depends on the stage and severity of disease when diagnosed. The veterinarian may often tailor the type of drug used and other therapy modalities. It is important to consult with veterinarians experienced in the treatment of this disease in penguins. Antifungal drugs may be given systemically (oral or intravenous), by nebulization, or intratracheally. Fluids may also be given orally by tube, subcutaneously or intravenously. Force-feeding fish gruel by tube can be used for short-term nutritional support, and any weight loss should be closely monitored. Drugs utilized with some measure of success include (see Appendix J):

- Voriconazole
- Terbenafine
- Itraconazole
- Clotrimazole: (nebulized)
- Amphotericin: (nebulized, intra-tracheal, intravenous)
- Enilconazole: nebulized (very thick, needs dilution)
- Antibacterials (for concurrent bacterial infections)

Commercial formulations of itraconazole should be used. Compounded formulations have been shown to have poorer absorption and may not reach therapeutic levels (Smith et al., 2010). Itraconazole appears to be losing its efficacy in some collections. In those cases where itraconazole is not effective, treatment with voriconazole is recommended, although this drug currently is very expensive and might be cost prohibitive for some institutions.

Treatment is typically long-term, frustrating, and often unsuccessful if begun in the latter stages of disease. Early intervention may yield a better survival rate in aspergillosis cases**.**
It has been observed that during serious outbreaks, mortality of acutely affected birds follow a “bell-shaped curve”, with sporadic deaths initially, a central period of increased deaths followed by another period of sporadic deaths. Loss of acutely affected birds is often followed by another rise in mortalities in birds that have been chronically affected. Prevention of the disease is best. Historically, many major outbreaks of aspergillosis have occurred after major environmental changes. Environmental stressors should be kept to a minimum, especially those involved with social factors (e.g., overcrowding). Prophylactic antifungal drugs, typically oral itraconazole) should be administered when shipping, relocating, or introducing new birds to an exhibit, and it is important not to ship or relocate birds during molt period (including pre- and post-molt periods). Although a fungal vaccination exists, it is not commercially available, and its efficacy is not proven. Maintaining high standards in exhibit air quality is crucial to prevention for species housed indoors. Regular fungal air cultures should be taken from the exhibit area to monitor levels of aspergillus. If it is necessary to shut down the air filtration system in a penguin exhibit, it is recommended to run the system for at least a week after it is restarted to clear the system before putting penguins back into the exhibit. Air cultures and disinfection for aspergillus spp. should be taken at this time. Construction in the surrounding areas may affect the air quality inside the exhibit, and should be carefully monitored. Precautions should be taken prior to the start of any construction.

Malaria: Malaria is a blood parasite carried by mosquitoes and/or biting flies. The causative agent is a *Plasmodium* organism, usually *Plasmodium relictum* or occasionally *P. elongatum*. Most cases of penguin malaria occur in animals that are currently or have historically been housed outside. Although penguins of all ages can be clinically affected, those particularly susceptible include chicks and juvenile birds, naïve adults previously housed indoors, or those that have been transported from areas with low mosquito/malaria problems. Clinical signs for malaria may vary, and range from acute death with no signs, sudden onset of respiratory difficulty with death rapidly following, to lethargy, inappetance, pale mucous membranes (from anemia), and behavioral separation from the group (Graczyk et al., 1995). Signs in more chronic courses are similar to heavy metal toxicity. Diagnostic tests for malaria include a CBC with blood smears (although this test to detect malarial organisms is not very sensitive), postmortem smear of blood, or splenic impression. A serologic test has been validated for black-footed penguins (*Spheniscus demersus*), and may be useful for other sphenscid species, but is not commercially available (Graczyk et al., 1995a; Hoogestyn & Cunningham, 1996). Research is currently underway to try to detect

malarial organisms in blood using PCR techniques, but accurate tests have yet to be developed. In penguins, the mortality rate from malaria infection is high, therefore, regular screening of birds housed outside can be attempted. All birds considered high risk can have blood collected every two weeks, and stained smears of the blood checked for the presence of malaria organisms. Even though it is not a very sensitive test, it may be helpful. Death can often be acute, with malarial protozoa visible only after the onset of severe clinical signs or during necropsy.

Treatment of malaria involves the use of Primaquine with Chloroquine, or if primiquine is not available, mefloquine (Tavernier et al 2005, Willette et al., 2009) can be used. Prophylaxis can be attained using mefloquine, primiquine or using the following drug regimen: A compounded capsule containing 125 mg sulfadiazine, 4 mg Daraprim (pyrimethamine) and 0.4 mg folic acid can be formulated. One capsule should be given orally for 3–5 kg (6.6–11 lb.) penguins every other day throughout the mosquito season. However, as Daraprim is a folic acid inhibitor and is teratogenic (i.e., causes birth defects), it should not be used in laying females. Administration of either prophylactic treatment is risky in parent birds that are feeding chicks, as the parent may regurgitate the medication to a small chick. Institutions may want to discontinue treatment for a week or two while the chick is small, and then restart treatment first in the parent that is less involved in feeding the chick. If using the every other day therapy, treat the parents on alternate days so that the chick does not receive two doses in a day. Doxycycline is used in humans for both malaria treatment and prevention, and should hold promise for treatment in birds, but to date no studies have been published indicating dose or efficacy.

Mosquito control is paramount to reducing exposure to malaria if penguins are housed outdoors. This includes minimizing standing water or removing standing water on a weekly basis, larvicide application to standing water that cannot be routinely removed (including in any drains in the penguins indoor and outdoor enclosures), and minimizing foliage near animal exhibits. Exposure to adult mosquitoes can be reduced by bringing the penguins in during peak mosquito hours (e.g., dusk to dawn), ensuring door sweeps and screens are in good condition, placing screens over intake fans, and providing fans wherever possible to keep the air moving, which may discourage mosquitoes.

Viral encephalitides: There are a number of viruses that can cause encephalitis in birds. Disease spread is typically by the bite of an infected mosquito, and wild birds can act as a reservoir for, and amplify, the virus. There has been some evidence that bird-to-bird transmission may also occur via semen and other infected bodily fluids. Diseases relevant to penguins include eastern equine encephalitis (EEE), western equine encephalitis (WEE), and West Nile fever, caused by the West Nile virus (WNV). Both EEE and WNV have been reported in *Spheniscid* penguins, and these penguins can have high rates of morbidity and mortality in response to these diseases.

West Nile virus: This disease is caused by a flavivirus. West Nile virus was first reported in the United States of America in 1999 after being discovered in a dead crow found on the grounds of the Wildlife Conservation Center (formerly the Bronx Zoo) in New York City. The virus spread rapidly across the US over the course of the next few years, and now has been reported in all 48 contiguous states. Species susceptibility to severe morbidity and mortality varies widely, with Sphensicid penguins being one of the more highly susceptible avian groups. Birds that survive infections with this disease have some latent immunity to reinfection, but it is not known how long this immunity lasts.

Acute death can occur with few premonitory signs, or death may occur within 3–4 days. With supportive care, the course of the disease may be protracted, with death occurring after a couple weeks. Recovery can be prolonged in those animals that do not die, with weakness and decreased appetite lasting for several weeks. When clinical signs are seen, they usually include anorexia, weakness (lying down frequently), and vomiting, with the inability to retain even small amounts of water or oral electrolyte solutions. Bile-stained diarrhea may occur. Dyspnea from excessive mucoid tracheal/pulmonary secretion may also occur, secondary to myocardial involvement. In Humboldt penguins, neurologic abnormalities are not a common sign and tend to occur only in those animals that survive longer before succumbing (R. Wallace, personal communication, 2007).

There is no specific treatment for this disease, and therapy is limited to supportive care. Supplemental fluids given subcutaneously, intravenously, and orally may be necessary for adequate hydration. Antifungal or antibacterial therapy can be given as needed for secondary infections. Oral supplementation of fluids or gruel is not recommended until a penguin's condition has stabilized, or signs begin to resolve, as there is a tendency for these birds to vomit (R. Wallace, personal communication). The oral cavity and glottis should be carefully suctioned if excess mucus is obstructing the airway, and

supplemental oxygen may also be necessary. The zoonotic potential of infected penguins for the keeper staff is unknown. However, virus can be shed in the respiratory secretions, and possibly urates/feces. In addition, horizontal transmission of the virus to humans from other avian species has been documented. Therefore, appropriate protective clothing should be worn when handling or working around infected birds. This should include N-95 masks if there is a chance for inhalation of aerosolized matter (cleaning).

As with malaria, adequate mosquito control is paramount in the prevention of this disease, especially if penguins are housed outdoors. Vaccination is recommended for susceptible species. Currently, there are no commercially available vaccines produced specifically for birds. Two vaccines developed for horses are commercially available (Innovator™ and Recombitek™). Innovator™ is a killed, inactivated vaccine produced by Fort-Dodge. Recommended doses are 1 mL IM given 3–4 weeks apart for three doses, and given to naïve animals prior to mosquito season, followed by annual boosters prior to mosquito season. The efficacy of this vaccine, as measured by serologic titers, differs in different avian species. Recombitek™ is a recombinant canary pox vaccine produced by Merial. There are anecdotal reports of this being used, but efficacy and safety in birds is unknown at this point. Birds known to have had and recovered from the disease are most likely immune, and may not need to be vaccinated, but more information is required to determine the extent of this immunity.

Eastern equine encephalitis (EEE): Eastern equine encephalitis is caused by an alphavirus. The virus was first reported in a group of African penguins (*S. demersus*) housed outdoors at an aquarium (Tuttle et al., 2005). Approximately 60% of the colony had noticeable clinical signs. Common clinical signs include acute anorexia, lethargy, and intermittent vomiting, along with penguins showing antisocial (isolation) behavior. Bile-stained diarrhea may occur. Ataxia can develop after 3–4 days, and with signs progressing to recumbency and seizures in about 25% of affected penguins. Signs in less severely affected penguins began to resolve in 6–9 days, but only after 14 days in more severely affected penguins. Stress-induced secondary infections such as aspergillosis may occur.

Standard complete blood cell count and serum chemistry diagnostic tests show non-specific changes such as an increased white blood cell count with a heterophilia, mild anemia, and a mild increase in glucose and sodium. Serologic testing using a hemagglutin-inhibition test for titers to the EEE virus is performed by the USDA National Veterinary Services Laboratory, and can confirm exposure to the disease. Reference limits for penguins have not been established, although a high titer suggests either exposure or disease, and a rising titer taken 2–4 weeks apart suggests active disease.

As with West Nile infections, there is no specific treatment, and any therapy is limited to supportive care. Supplemental fluids given subcutaneously, intravenously, and orally may be necessary for adequate hydration. Anticonvulsants (diazepam) may be needed to control seizures. Antifungal or antibacterial therapy should be provided as needed for secondary infections. As with WNV and malaria, adequate mosquito control is key for effective prevention of this disease, particularly if penguins are housed outdoors. A killed vaccine against EEE is available for horses and has been used, but the dose required and efficacy for penguins has not been determined.

Chlamydia psittaci: *C. psittaci* is thought to be a pathogen primarily in psittacines and columbiformes. However, *C. psittaci* has caused outbreaks of disease in penguins (F. Dunker, personal communication). Signs include poor appetite, lethargy, and lime-green stools/urates. Bloodwork typically shows an elevated WBC with a heterophilia/lymphopenia with toxic changes. The total protein is elevated with increases in the beta- and gamma-globulins.

Post-mortem lesions seen include splenic and hepatic enlargement, with pulmonary congestion. Necrotizing splenitis, hepatitis, interstitial pneumonia and nephritis may be seen histologically. Gimenez stain shows elementary bodies in affected tissues. The organism can be confirmed using a *C. psittaci* PCR (DNA) probe and tissues, or by culture (Jencek et al., 2006)

Diagnostic tests: The general confusion surrounding the testing methods for *C. psittaci*, and interpretation of test results to determine if a bird's illness is due to an active infection complicates the diagnosis in live birds. Tests are offered by many labs and veterinary diagnostic laboratories. The veterinary clinician is urged to thoroughly investigate the latest diagnostic techniques, and to have a good understanding of what each test result signifies. Some available tests are listed below.

- PCR (DNA) probe of *C. psittaci* (feces, choanal/cloacal swabs, fresh tissue): This test is useful in the diagnosis of infected birds and helps determine shedding, as well as if therapy is working.

- PCR (DNA) probe for *C. psittaci* (blood): False negatives can be seen in birds begin treated with enrofloxacin. This test is of questionable value as known infected birds in one outbreak tested negative.
- Complement fixation (CF) (blood): This test measures IgG antibodies. It is useful to ascertain exposure to chlamydia. However, its value as a diagnostic aid for current infections or as an indicator for cleared infections is still uncertain. It is unknown how long titers remain elevated in affected or recovered penguins.
- Elementary body agglutination (EBA) (blood): This test measures IgM antibodies, indicating current infection. The value of this test in penguins in still unknown.

Treatment:

- Doxycycline is the drug of choice. Either oral doxycycline (Vibramycin) 25–50 mg/kg orally once a day for 45 days (if possible) or parenteral doxycycline (Vibrovenos) 50–75 mg/kg IM once weekly for 6–7 weeks (preferably). Both of these drugs can cause inappetance and possible photophobia.
- Enrofloxacin 15 mg/kg orally once or twice a day. In one outbreak, the Baytril treatment resolved clinical signs but the blood picture did not change. Therefore, it may not be an effective treatment to resolve infection.
- Other supportive care measures such as fluids should be given to ill birds.

C. psittaci is a zoonotic disease, and risk of transmission to the public or animal care staff is real. Public Health officials should be notified if chlamydia infection is confirmed. Affected birds or flocks should be quarantined to protect other collection birds as well as animal keepers. Protective clothing, including N-95 masks, should be worn by persons working with the birds. If birds are kept on display, the area should be hosed with a disinfectant prior to public hours.

Avian pox: Avian pox infection has been observed in both managed and wild penguin populations (Kane et al., 2012). Based on phylogenetic structure of the virus, it was determined that infection was transmitted from wild birds. Transmission is via arthropod vectors or contact of mucosal membranes, broken or abraded skin with infected individuals or their secretions. Pox virus can live a long time in the scabs shed by infected individuals. Infection can be manifested by both the wet and dry forms. There currently is no treatment, and supportive care should be provided while the disease runs its course, usually in 2–3 weeks. Because the virus can survive in the scabs or other dried infected lesions, meticulous disinfection should be performed in any areas where ill animals were housed to prevent infection of other individuals.

Toxoplasmosis: Deaths from toxoplasmosis have occurred in black-footed penguin chicks exposed to cat feces. Signs were primarily neurologic, with death occurring within 24 hours. (Ploeg et al., 2011) At necropsy, peritonitis, pneumonia, hepatomegaly, splenomegaly, and renomegaly were evident. Aside from the direct threat of predation that cats can pose to penguins, toxoplasma oocysts transmitted from infected cat feces can pose a risk; therefore penguin exhibits should be secured to prevent entry by domestic cats.

Pododermatitis (bumblefoot): Penguins, like other birds, may be predisposed to pododermatitis by the following factors: change in normal activity patterns (e.g., decreased swimming, increase in sedentary behaviors), and prolonged standing on hard, abrasive surfaces or surfaces with excessive moisture or fecal contamination. Prevention can be attempted by encouraging penguins to swim on a daily basis. The original lesion may be the result of a bacterial infection from a puncture wound or soft tissue damage caused by pressure necrosis. Once the epithelium is compromised, secondary bacterial invasions may occur, resulting in deep soft tissue infections. If left untreated, severe complications can occur, including mineralized soft tissue, deep granulomas, and osteomyelitis. Examination for pododermatitis should involve an evaluation of the behavior and posture of the penguins. Indicators include:

- Abnormal stance
- Increased lying down
- Abnormal gait (limp)
- Footpad ulceration; scab formation, epithelial thinning, laceration or puncture; drainage; swelling; increased redness; and discomfort on palpation
- Soft tissue mineralization or osteomyelitis seen radiographically

Thermography may be useful both as a diagnostic technique and for monitoring response to therapy. Therapy should be aimed at protecting the foot from further damage, instituting local and systematic treatment of the current lesion, and changing conditions to prevent future occurrences (e.g., improving hygiene and changing to an appropriate substrate or flooring). Treatments that have been used include systemic antibiotics; local antibiotics with or without dimethyl sulfoxide (DMSO); surgical debridement; cryotherapy; and chronic bandaging in conjunction with various salves and ointments (chronic exposure to DMSO within a bandage can cause severe skin irritation) accompanied by intermittent debridement of devitalized tissue.

While there is often initial improvement with many of the techniques listed above, there is a tendency for reoccurrence once therapy is discontinued. Since most treatments involve wrapping the affected feet, it is helpful to provide padding to minimize pressure on the wound site. If the wound site is not surgically closed, the area should be kept moist to encourage granulation. Gauze, GORE-TEX® cast padding, ointment, Vetrap bandaging tape, and waterproof tape or booties made from soft material have all been used (Reidarson et al., 1999a). Booties can be made from old wet suits and Velcro or are commercially available in various sizes. Healing efficiency can also be improved with proper debridement and the use of hydroactive dressings, which may retain moisture better than gauze and ointment. Environmental temperature may affect healing rates. There is some evidence that allowing birds with bandages to swim in salt water during therapy may promote healing, as the saltwater may help in drying out the tissue. Prevention of bumblefoot is a priority, as treatment is typically long-term and frustrating. Prevention should be geared toward encouraging swimming and avoiding hard, rough, wet surfaces that retain contaminated water.

Preen gland infections: Diagnosis is based on the presence of an enlarged, swollen gland containing purulent or caseous material. Early diagnosis and treatment may prevent impaction. The specific etiology of preen gland infections is unknown, but there may be many potential factors, including sedentary birds with decreased swimming patterns, poor plumage, non-preening birds who do not molt regularly, and nutritional deficiencies. Encouraging swimming and making birds stay in the water for longer periods may also reduce this problem, as penguins are more likely to preen when they come out of the water. Once a bird has preen glans problems, they are more susceptible to future episodes. Preen gland infections have not been seen in penguins in the wild (D. Boersma, personal communication).

Cultures of preen gland fluid have contained numerous bacteria. *Candida* is commonly cultured, even following antifungal therapy. Histologic examination of the gland suggests the possibility of vitamin A deficiency, although supplementation of vitamin A has not resolved the condition. While a limited number of birds may respond to symptomatic therapy, such as flushing the gland or infusing it with a proteolytic enzyme ointment, surgical removal may be needed to avoid eventual rupture and secondary septicemia (MacCoy & Campbell, 1991). It is important to encourage birds, particularly those that are nesting, to swim regularly as a preventative measure. For birds that are nesting, if one of the pair voluntarily leaves the nest to feed, it should be encouraged to swim before returning to the nest. If, for medical reasons, birds are housed without a pool, daily showers can be given to stimulate preening activity.

Pulmonary disease: While aspergillosis is usually the most common disease involving the respiratory system, there are other respiratory problems that are primarily related to bacterial pathogens. In some cases, it is difficult to distinguish between primary or secondary aspergillosis involvement. Upper respiratory diseases also include disease of the sinuses, and dyspnea can occur from plugged nares. Antibiotic therapy should be based on culture and sensitivity results whenever possible.

General bacterial disease; Penguins as with other animals can acquire bacterial infections. Trauma, stress, egg-yolk retention, age, and poor food quality can all predispose an animal to infection with a variety of bacteria, including mycobacteria (Boerner et al., 1994; Fisher et al., 2008). Good husbandry and management help reduce the incidence of bacterial disease.

Renal disease: The diagnosis of severe renal disease by serum chemistries is difficult in penguins. In some cases, the uric acid levels are elevated. However, normal increases in uric acid concentrations that occur after a meal should be differentiated from increases reflecting renal disease. A blood urea nitrogen (BUN) greater than 5 mg/dl may indicate dehydration. Fluid supplementation given orally, subcutaneously, or intravenously may be helpful, although systemic or visceral gout may result in rapid death with very few prior symptoms. On postmortem, there may be bright white flecks of uric acid

deposits in the muscle, air sac, or serosa of organs. Uric acid crystals can be visualized under polarized light. For histologic verification, tissues should be placed in alcohol, since formalin will dissolve the deposits. Articular gout (gout in the joints) occasionally occurs in penguins. Lameness is the primary clinical sign. Nephritis (renal infection) or amyloidosis may be present without clinical signs of gout.

Foreign object ingestion: Penguins are curious animals, and young penguins in particular will investigate small and novel items within their enclosures. When manipulating these objects they may ingest them. Ingestion of foreign objects can cause medical problems and even death (Perpiñan & Curro, 2009). Some of the items that have been reported being ingested include nesting material (e.g., sticks and stones), bristles from brushes used for cleaning (the use of nylon scrub brushes that easily lose their bristles should be avoided), coins, fence clips, lead pellets from dive belt weights, and even molted tail feather shafts. Zinc, lead, and other heavy metal toxicities are always possible when metal objects are ingested. Initial symptoms may mimic malaria. Therefore, radiographs should be performed to detect metallic foreign objects. Some institutions regularly radiograph their penguins to ensure that they are not retaining such items. Some zoos and aquariums use commercial metal scanners on their birds. Although penguins regurgitate easily, foreign objects are not always present in the regurgitated material. These objects frequently remain in the stomach, and do not moved further down the gastrointestinal tract. If attempts to get the penguin to regurgitate are unsuccessful, treatment is usually by endoscopic removal. Penguins have large stomachs. When foreign objects settle in the distal aspect of the stomach, radiographically they often appear to be in the distal intestine near the cloaca. This frequently leads clinicians to believe that the object is about to pass through on its own. But most likely it is still in the stomach. When performing endoscopy for foreign body retrieval, it is necessary to examine all the way to the most distal aspect of the stomach to locate the object.

Nervous system disorders: Incoordination and “stargazing” are occasionally reported as clinical symptoms. Thiamine deficiency has been implicated as a cause when fish quality is compromised (Griner, 1983). Differential diagnoses for non-specific signs of central nervous system involvement should include disease problems seen in other species, including viral or bacterial encephalitis, fungal granuloma, sepsis, nutritional deficiencies, and tumors. Domoic acid poisoning was reported to cause the total loss of a rockhopper penguin collection (Broadbent, 2009). Exposure to the toxin came from eating fish contaminated by the algal toxin. Consideration should be given regarding the source of fish fed to penguins (caught in shallow vs. deep water).

Neoplasia: A variety of neoplasias have been reported in penguin species including adenocarcinomas, melanoma, and lymphoma (Cho et al., 1998; Yonemaru et al., 2004; Rambaud et al., 2003; Ferrell et al., 2006)**.**

Egg-related health issues: Pathology of the reproductive system is uncommon in penguins, although salpingitis, egg binding, and cloacal prolapse have been reported. Treatment for egg binding is similar to that of other avian species. Manual extraction of the egg is preferable. If that is not possible, surgical removal of the egg may be required. Removal of the entire oviduct may be necessary if egg retention leads to oviductal rupture or necrosis. Problem birds should have their calcium level checked periodically. Like other avian species, these birds may benefit from calcium supplementation.

Fluid administration: Fluid may be given to penguins by stomach tube, subcutaneously, intraperitoneally, or intravenously. Intravenous catheters for administration of fluids and therapeutic agents have been successfully placed and maintained in the flipper vein (brachial or medial) of several species of penguin (if the penguin is kept out of water). Penguin bones are not pneumatic and are much denser than those of other species of birds, therefore, intraosseous administration of fluids is quite difficult.

Surgery: Surgery to assess air sacs, reproductive, and gastrointestinal tracts has been successfully performed in a variety of penguin species. It is important to remember to keep Antarctic and sub-Antarctic species cool during surgery. Standard surgical technique may be employed. Intubation, standard patient monitoring (i.e., ECG, oxygen saturation), and fluid administration are generally easy to perform. Birds should be kept out of the water until the skin incision has healed.

Most institutions find that it is easy and less damaging to the patient’s skin if the feathers are shaved in preparation for surgery, not plucked. The feather shafts will fall out and normal feathers will grow in

during the next molt. Surgical scrubbing may be gentler and avoid skin trauma. Where feathers are plucked, alcohol may cause excessive damage and impede skin healing.

Blood transfusions: Transfusions may be performed when birds are severely anemic from malaria (blood phase), blood loss, or clotting disorders, and they can stabilize a bird until a diagnosis can be made and treatment initiated. It is indicated when the hematocrit (HCT) or packed cell volume (PCV) drops rapidly into the teens or less and does not stabilize. If the HCT is stable and the cause of the anemia is removed, penguins generally have a good bone marrow response (if not old or debilitated by concurrent disease), and generally respond well to supportive care alone (i.e., fluids, oral or injectable iron supplementation, oxygen and B-vitamins). In birds with malaria with a stable hematocrit in the teens, it has been reported that a transfusion appears to shorten the convalescent time while the treatment with chloroquine/primaquine takes effect.

Blood transfusion procedure: Approximately 1–1.5% of the donor's weight in blood volume can be safely collected (60 mL from a 4–5 kg/8.8–11 lb. bird). Acid citrate dextrose (ACD) solution (available from Metrix Co. Dubuque IA) is used as the anticoagulant at 0.15 mL ACD/ml blood collected. The blood is then collected slowly over 10–15 minutes using a butterfly catheter from the jugular or metatarsal vein while the bird is under anesthesia. IV fluids up to, or equal to the blood volume collected can be given using the same butterfly catheter used to collect blood. The donor bird is given supportive care post-blood collection in the form of subcutaneous fluids (50 mL/kg), B-vitamins (0.5 mL in fluids or IM), and iron dextran (10 mg/kg IM).

Prior to the administration of blood, a partial cross match should be performed on the recipient using the donor blood and recipient bird serum. Absence of hemolysis or agglutination will suggest compatibility. The recipient bird is given dexamethasone sodium phosphate (0.25–1.0 mg/kg IM/IV). The blood is administered through intravenous or intraosseous routes (difficult) using either an IV with either a disposable blood filter or an inline filter, both of which can be attached directly to a 60 mL syringe. It is advisable to administer 60 mL of blood over 45–60 minutes, while constantly rocking the blood in the syringe while monitor the recipient's heart and respiratory rates closely. If either increases, slow or stop the transfusion until parameters have returned to normal, then resume at a slower rate.

With 60 mL of blood (for one 4–5 kg/8.8–11 lb. penguin), one should expect an increase in pre-transfusion HCT by 25–50%. Homologous (same species) transfusions are preferred since the blood cells probably remain in the recipient's circulation longer.

AZA-accredited zoos and aquariums provide superior daily care and husbandry routines, high quality diets, and regular veterinary care to support penguin longevity. In the occurrence of death however, information obtained from necropsies is added to a database of information that assists researchers and veterinarians in zoos and aquariums to enhance the lives of penguin both in their care and in the wild. As stated in Chapter 6.4, necropsies should be conducted on deceased penguin to determine their cause of death, and the subsequent disposal of the body must be done in accordance with local, state, or federal laws (AZA Accreditation Standard 2.5.1). Necropsies should include a detailed external and internal gross morphological examination and representative tissue samples form the body organs should be submitted for histopathologic examination. Many institutions utilize private labs, partner with Universities or have their own in-house pathology department to analyze these samples. The AZA and American Association of Zoo Veterinarians (AAZV) websites should be checked for any AZA Penguin SSP Program approved active research requests that could be filled from a necropsy.

AZA Accreditation Standard

(2.5.1) Deceased animals should be necropsied to determine the cause of death. Cadavers must be stored in a dedicated storage area. Disposal after necropsy must be done in accordance with local/federal laws.

Euthanasia: The AZA Penguin TAG does not have specific recommended protocols for penguin euthanasia within zoos and aquariums. Veterinarians at each institution are encouraged to contact the AZA Penguin TAG veterinary advisors for more specific information or advice on the most effective, safe, and humane approaches to utilize. Each institution housing penguins should have a euthanasia protocol in place, developed by the veterinary team, in case euthanasia becomes necessary in a particular situation. The AZA Animal Welfare Committee also encourages each institution to develop a process to determine when elective euthanasia might be appropriate from a quality of life perspective, taking into account behavioral, health, social, nutritional, and animal caretaker perspectives. Examples of approaches used by institutions are available from the AZA Animal Welfare Committee. If a penguin's

quality of life has diminished to the point where euthanasia is the humane option, anesthesia followed by injection of an approved euthanasia solution (chemical euthanasia) should be performed.

Egg euthanasia: The American Association of Zoo Veterinarians (AAZV) states that the neural tube of avian embryos has developed sufficiently for pain perception by 50% gestation, and so any bird embryos that have reached this stage or beyond should be euthanized using methods appropriate for hatched birds (i.e., chemical euthanasia).

Necropsy: Post-mortem examination is an important component of any comprehensive veterinary medical program. Thorough necropsies include detailed external and internal gross morphological examinations and findings should be documented. Eggs that did not hatch should be opened and checked for fertility and age of embryonic death. Bacterial cultures should be taken of the yolk/albumin or embryo to identify bacterial infection as a cause of embryonic death. Representative tissue samples form the body organs should be submitted for histopathologic examination. Thorough necropsy examination and records will aid assessment of the overall health, and causes of morbidity and mortality in penguin collections. In turn this should lead to better husbandry, management and treatment of the collection. The full Humboldt penguin and egg necropsy protocols can be found in Appendix O. These may be used as a guideline for other penguin species. Further copies and updates may be found at either the AAZV or AZA website under necropsy protocols that can be used as a guideline for other penguin species. Copies of final reports should be sent to the Penguin TAG veterinary pathology advisor and then to the SSP veterinary advisors.

Chapter 7. Reproduction

7.1 Reproductive Physiology and Behavior

It is important to have a comprehensive understanding of the reproductive physiology and behaviors of the animals in our care. This knowledge facilitates all aspects of reproduction, artificial insemination, birthing, rearing, and even contraception efforts that AZA-accredited zoos and aquariums strive to achieve.

The exact age of sexual maturity is difficult to determine for some zoo-housed species. The sex ratio and age distribution of the colony will have an impact on the sexual behavior of the younger penguins. Young males generally will not compete with older males for mates. They will, however, engage in courtship behavior at an early age (1–2 years). The approximate ages of sexual maturity are shown for wild penguins in Table 9.

Table 9. Average age of sexual maturity (*in situ*)

Species	Age at sexual maturity (male / female if available)
Emperor	5 yrs. / 6 yrs.
King	5–7 yrs.
Adélie	3–8 yrs.
Gentoo	2–3 yrs.
Chinstrap	3 yrs.
Macaroni	6 yrs.
Rockhopper	4 yrs. (likely)
African	4 yrs.
Humboldt	3–4 yrs.
Magellanic	4–5 yrs. / 5–6 yrs.
Little Blue	2–3 yrs.

(Williams, 1995; Garcia & Boersma, 2012).

On a yearly cycle, penguins show some predictable changes in sociality related to breeding. Penguins can be seen in large social groups on land during molting and breeding season. They are generally antisocial during molting, although they remain in close proximity. Courtship behaviors can be seen at the beginning of breeding season. The breeding season can be defined in terms of four major phases: courtship, incubation, chick-rearing and fledging. In zoo and aquarium conditions, some behaviors, such as mutual displays, observed during the early phases of the breeding season may be seen year-round, albeit less intensely. In one study, Adélie penguin pairs were observed to occupy their nest sites year-round, even during periods when nesting materials were not available (Ellis-Joseph, 1988). Adélie penguins that pair and lay their eggs earlier in the season were also reported to be significantly more likely to fledge chicks (Ellis-Joseph, 1988; 1992).

The onset of the breeding season, which varies between species, may create a flurry of activity similar to what is reported for wild penguins (Sladen, 1958; Penney, 1968; Ainley et al., 1983). In the wild, the onset of the breeding season takes place when birds return to the colony (*Pygoscelis* spp., *Eudyptes* spp., and *Aptenodytes* spp.) or to the nesting territory (*Spheniscus* spp.). In general, behaviors associated with pairing are observed more intensively 3–4 weeks prior to egg-laying. Depending on the species and exhibit, initiation of courtship can be enhanced by manipulation of artificial lighting (photoperiod, refer also to Chapter 1.2) or introduction of nesting materials.

Aggressive behavior in penguins is most pronounced during courtship and pairing and again once chicks are hatched. Although it is a natural part of the reproductive cycle, staff should monitor aggression closely during the breeding season to ensure that reproduction is not deterred because of excess aggression or competition. Some institutions report mate “stealing” in exhibits with skewed sex ratios. Emperor and king penguins, for example, may require the construction of removable barriers to allow isolation of pairs or individuals, as unpaired birds may attempt to “steal” eggs or chicks from conspecifics that may be incubating or brooding. Some institutions report that penguins attack, and may kill birds that are weak or ill. There is also a need to closely monitor birds that have been isolated and subsequently

returned to the group. Harassment by groups is not common in penguins. Most aggressive exchanges take place between individual birds or pairs (Williams, 1995).

Agonistic displays increase during the breeding season as birds begin to reclaim and defend nest territory, or compete for prime nest locations (Renison et al., 2002; 2003). Overall rates of vocalization and display may increase throughout the exhibit during breeding. It is important to note that injuries from disputes (such as jab wounds in king penguins and corneal abrasions in *Spheniscus, Eudyptes,* and *Pygoscelis* species) may occur more frequently, particularly in multi-species exhibits with a high density of penguins. For Adélie penguins, aggression is lowest during incubation and at highest levels once chicks are hatched (Ellis-Joseph, 1988).

Mating and mate selection: Penguins are usually housed in colonies large enough that birds can select their own mates. Atypical pairing behaviors have been noted in zoos and aquariums. For example, same-sex pairing has been reported for emperor, king, gentoo, Humboldt, Magellanic, and African penguins. One zoological institution reported a male/male pair to which eggs were successfully cross-fostered for two breeding seasons. Other unusual behaviors include: copulations in which the traditionally effective male on top/female on the bottom position is switched; extra-pair copulations; or polyandrous or polygynous trios. In wild Adélie penguins, Muller-Schwarze (1984) described two types of pairing: trial pairing, which is temporary, and true pairing, which results in a clutch and a season-long bond. Such pairings have not been observed in Adélie penguins in zoos and aquariums, possibly because there is no seasonal emigration from the colony and subsequently no advantage to trial pairing. Occasionally, it may be necessary to selectively pair adults when undesirable pair bonding takes place (e.g., sibling, polygynous, polyandrous, same-sex bonds, or non-recommended program pairs). In *Spheniscus* spp., a successful pair bond may be encouraged by isolating the desired pair through egg-laying and incubation. It is desirable to use the male's territory for this isolation.

Approximately 3–4 weeks from onset, courtship and nest building are complete. Copulations, which usually occur at the nest site, may be observed within one week of the onset of the breeding cycle. In *Spheniscus* spp., copulations may be noted frequently during courtship and nest building. Copulations for *Eudyptes* and *Pygoscelis* are generally observed within days of occupation of the rookery. In *Aptenodytes*, particularly emperor penguins, copulation is rarely observed. It is important to note that emperor penguins in zoos and aquariums appear to be much heavier than their wild counterparts, which may hamper copulation and thus adversely affect reproduction.

Hormone tracking: Currently, no hormonal tracking methods are used to assess reproductive condition in penguins. Normal hormonal values have not been established for these taxa. This is an area that may be better understood with future investigation into reproductive technology. All reproductive physiological information can be found in Chapter 7.3.

7.2 Assisted Reproductive Technology

The practical use of artificial insemination (AI) with animals was developed during the early 1900s to replicate desirable livestock characteristics to more progeny. Over the last decade or so, AZA-accredited zoos and aquariums have begun using AI processes more often with many of the animals residing in their care. AZA Studbooks are designed to help manage animal populations by providing detailed genetic and demographic analyses to promote genetic diversity with breeding pair decisions within and between our institutions. While these decisions are based upon sound biological reasoning, the efforts needed to ensure that transports and introductions are done properly to facilitate breeding between the animals are often quite complex, exhaustive, and expensive, and conception is not guaranteed.

AI has become an increasingly popular technology that is being used to meet the needs identified in the AZA Studbooks without having to re-locate animals. Males are trained to voluntarily produce semen samples and females are being trained for voluntary insemination and pregnancy monitoring procedures such as blood and urine hormone measurements and ultrasound evaluations. Techniques used to preserve and freeze semen have been achieved with a variety, but not all, taxa and should be investigated further.

Semen preservation and AI have the potential to enhance natural breeding programs of penguins by reducing or eliminating reproductive problems associated with inbreeding, behavioral compatibility, bird transport, human-imprinting of hand-raised birds and disease transmission. Costs of establishing an assisted reproductive program may initially be greater than relative costs of animal transport, and substantial research on basic reproductive biology is still needed for each penguin species to successfully

apply AI, but such costs would be outweighed in the long-term through benefits resulting from improved genetic and reproductive management.

Artificial insemination has been developed in the Magellanic penguin using fresh, chilled semen. Table 10 outlines the methodologies that have been used in some species in the area of semen collection, characterization, and preservation. Females can be conditioned for insemination using similar training methods described for semen collection, except that females are conditioned to accept manipulation of the cloaca and insertion of a 1 mL syringe and catheter. Alternatively, females can be anesthetized for the artificial insemination procedure (O'Brien, 2013). Candling observations are used to monitor egg fertility status and embryonic development.

Table 10. Assisted reproductive techniques

Assisted reproductive technology	Penguin species	Methodologies	Reference
Semen collection	*Spheniscus magellanicus*	Voluntary semen collection method (n=1 male)	O'Brien et al., 1999
	Eudyptes chrysocome	Voluntary semen collection method (n=6-14 males)	Waldoch et al., 2007, 2012
	Aptenodytes patagonicus	Voluntary semen collection method (n=1 male)	O'Brien & Robeck, 2013
	Spheniscus demersus	Voluntary semen collection Method (number of males not specified)	Unknown
Semen characterization & preservation	*Spheniscus magellanicus*	Short-term chilled storage, long-term cryostorage (n=1 male)	(O'Brien et al., 1999)
	Spheniscus magellanicus	Short-term chilled storage, long-term cryostorage (n=7 males)	2012–2013 unpublished
	Eudyptes chrysocome chrysocome	Semen characterization only (n=14 males)	Waldoch et al., 2007, 2012
	Aptenodytes patagonicus	Short-term chilled storage, long-term cryostorage (directional freezing method)	O'Brien & Robeck, 2013
Artificial insemination	*Spheniscus magellanicus*	Artificial insemination using fresh, chilled semen (n=4 chicks derived from AI, as confirmed by genetic analysis)	O'Brien, 2013

Research into all areas associated with the development of AI using chilled and cryopreserved semen is still required in penguins, in particular, the characterization of female reproductive hormones and temporal relationships of such hormones with physiological events such as ovulation.

7.3 Pregnancy & Egg-laying

It is extremely important to understand the physiological and behavioral changes that occur throughout an animal's pregnancy.

Egg-laying and incubation: Table 11 shows the most commonly reported timing of laying of first clutches for various penguin species in North American facilities. In conjunction with breeding and egg-laying, appetite often increases and distinctive food preferences may be exhibited. Females may increase their weight by as much as 20–25%, and in some cases females may become inappetent 1–2 days before laying. In *Aptenodytes* species, incubation of rocks or ice may indicate that egg-laying is imminent. Gentoos and *Eudypteds* will lie in the nest and dig with their feet. After a frenzied period of nest construction, *Spheniscids* will stop digging and gathering nesting material. Within a month of egg lay females will show changes to blood parameters as outlined in Chapter 6.7: Egg Related Health Issues. Estrogen, progesterone, and prolactin all interact to facilitate brood patch formation in both sexes (Hutchison et al., 1967).

Table 11. Timing of first clutch egg-laying (Henry & Sirpenski, 2005)

Species	Month											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Emperor						A	A	A				
King					N A	N A	N	N		A	A	A
Adélie										A	A	
Gentoo				N						A	A	
Chinstrap				N	N					A		
Macaroni			N	N					A	A		
Rockhopper				N	N				A	A	A	
African	N	N	N	N	N	N	N	N	N	N	N	N
Humboldt	N	N A	N	N	N	N	N	N	N	N	N	N
Magellanic			N	N								
Little Blue								N	N	N		

A = Austral lighting schedule (30° S Latitude–77° S Latitude)
N = Northern Hemisphere or natural lighting conditions

Following a period of ritualized courtship, penguins normally lay 1–2 eggs, depending on the species (refer to Table 12 for clutch size and other egg-laying data). With the exception of emperor and king penguins, both parents take part in nest construction, incubation and chick rearing. For *Pygoscelis* spp., courtship behaviors such as rock presentation and nest building continue throughout egg lay and incubation. On rare occasions, king penguins have laid a replacement clutch when their only egg has been lost early in the term (J. Jozwiak, personal communication). In *Eudyptes* spp., the first laid eggs are much smaller than the second eggs and hatch much later. Table 13 shows expected egg measurements for various species.

Table 12. Egg-laying intervals and incubation data (Henry & Sirpenski, 2005)

Species	Clutch size	Egg lay interval	Mean incubation period	Incubation period range	Pip-to-hatch	Multiple clutches
Emperor	1	-	67 days	64–73 days	48–72 hrs.	No
King	1	-	56 days	53–62 days	48–72 hrs.	No
Adélie	2	3–4 days	36 days	34–42 days	24–48 hrs.	No
Gentoo	2–4	3–5 days	38 days	36–44 days	36–48 hrs.	Yes
Chinstrap	2	3–4 days	37 days	35–39 days	36–48 hrs.	No
Macaroni	2	4–5 days	36 days	36–42 days	24–48 hrs.	No
Rockhopper	2	3–5 days	35 days	32–36 days	24–48 hrs.	No
African	2	3–4 days	38 days	36–42 days	24–48 hrs.	Yes
Humboldt	2	2–4 days	42 days	40–46 days	24–48 hrs.	Yes
Magellanic	2	3–4 days	42 days	38–48 days	24–48 hrs.	Yes
Little Blue	2	1–4 days	36 days	33–37 days	48–56 hrs.	No

Table 13. Egg measurements (includes *ex situ* and *in situ* * laid eggs)

Species	Sample Size	Mean length x width (mm)	Range length (mm)	Range width (mm)	Range weight (g)
Emperor *	10	121 x 82	100–130	78–86	350–502
King	301	106 x 76	90–122	65–82	100–391
Adélie *	72	69 x 53	60–79	42–60	64–119
Chinstrap	52	66 x 52	61–71	48–56	72–113
Gentoo	111	70 x 58	61–78	53–61	93–145
Macaroni “A” egg	25	77 x 52	71–85	46–54	93–136
Macaroni “B” egg	26	78 x 60	75–90	52–61	138–184
Rockhopper “A” egg	50	63 x 49	56–67	41–52	47–88
Rockhopper “B” egg	84	70 x 54	64–80	50–57	83–123
Humboldt *	30	73 x 52	62–85	46–56	-
Magellanic	101	73 x 55	68–82	50–60	94–134
African *	7	65 x 49	62–72	44–60	72–98
	196	65 x 49	59–84	40–70	50–117
Little blue	10		37–40	53–58	36–42

Nest management: It is important to be familiar with the breeding history of a pair during nest establishment and before an egg is produced. Nest sites should be evaluated for prior success or failure, neighboring aggression and level of rookery traffic. Natural barriers such as large rocks or logs can be placed between neighboring nests to discourage intrusion and decrease aggression. An ample supply of nesting materials will decrease resources competition and may help contribute to nesting success. Familiarity with each species natural history can help determine normal versus irregular behavior. Macaroni penguins frequently practice obligate brood reduction and eject their alpha egg from the nest in preparation for the arrival of the beta egg (St. Clair et al., 1995). One zoological institution routinely collects alpha eggs from all pairs of this species at lay for artificial incubation. Any pair with a history of poor incubation, crushing eggs, or ejecting eggs should be evaluated for assisted rearing options.

The health of each parent should be taken into consideration before the onset of egg-laying. Some common antibiotics and malaria preventatives may pose threats to embryonic development, and should be discontinued in advance of egg development. For example, Daraprim (pyrimethamine) is a folic acid inhibitor and is teratogenic (i.e., causes birth defects); Daraprim should not be used in laying females (see Chapter 6.7).

When a female is preparing to lay, she will occupy the nest continuously for a period of 1–2 days while the male stands nearby. It is sensible during this time period to minimize disturbance around the nest and avoid handling of the female. Behavioral changes associated with impending egg lay include, lethargy, dehydration, and a sleepy-eyed appearance. Females may frequently show fluffed contour feathers and sometimes labored respirations. Soon after the egg is produced, the male should provide nest relief, which allows the female to leave the rookery to bathe and feed. The pair should be observed for several days after the clutch is completed to ensure they are sharing incubation duties and performing them adequately. If one parent is left with the sole responsibility of egg care supplemental feedings at the nest can be provided to ensure parental health, or the egg(s) can be removed for assisted rearing or fostering. A decision to supplement any parent at the nest during incubation should include an assessment of the likelihood for adverse impacts on incubation and/or disruption of adjacent pairs on nests.

Gravid females should be monitored for proper egg delivery. Hens who have never laid before are more likely to experience cloacal tearing and associated cloacal bleeding post egg-lay. Depending on rookery cleanliness and individual bird behavior the hen can be placed in the pool for a swim to help clean the vent. Veterinary exam will indicate whether a course of antibiotics is necessary. Females with histories of thin shelled eggs, egg binding, or cloacal prolapse should be watched closely so any difficulties can be addressed early in the process. A bird that has had difficulty with egg lay in the past may be at increased risk to continue the pattern in successive seasons. A female who is showing visible discomfort, seen straining, tail pumping, or has a noticeably distended cloaca for more than 12–18 hours without production of an egg may be experiencing complications and should be examined by a veterinarian.

Complications may include egg binding or an inability to pass an egg that has been broken before or during delivery. If a gravid female has experienced external trauma (e.g., during competition for a mate or a nest site) this could cause an unlaid egg to break in the canal. Thin-shelled eggs may also be broken during delivery due to their fragility. Shell fragments left behind could result in lacerations and fecal matter introduced into the bloodstream might lead to septicemia. Again, veterinary treatment is recommended and may include manually flushing the cloaca to remove the egg fragments. Cloacal prolapse is a serious and life threatening condition that requires immediate veterinary attention. The female should be removed quickly from the rookery to an isolated area with a heat source to await veterinary care (See Chapter 6.7).

Eggs in the nest should be checked visually for damage. Any egg found to have cracks or holes can be repaired. Many zoos have had success repairing eggs with Tegaderm®, or paper towels and white glue. After repair, the egg may be returned to the nest (depending on the extent of the repair) or can be placed in the incubator for careful monitoring for appropriate weight loss and development through incubation. See Chapter 7.5 for details on incubation.

It is recommended that emperor pairs with eggs be separated from the main colony as soon as an egg is detected. One zoological institution utilizes a removable Plexiglas® barrier that physically separates incubating birds within the exhibit without adversely affecting visual and vocal stimuli from the group. Emperors with eggs are slowly walked to the barrier entrance. Initially, pairs are moved inside the barrier together. The female can be released back into the colony as soon as she transfers the egg to the male and begins to pace the enclosed area. Like wild emperors, females do not incubate. In the wild, food

requirements of chicks greater than 40 days of age require both parents to forage simultaneously, leaving chicks alone on the ice to congregate with other chicks in crèches. Crèching is not observed in zoos and aquariums, presumably because of constant food sources, lack of predators, and environmental conditions. In these conditions, emperor penguins continuously brood chicks for approximately 4–6 weeks, and parent-reared chicks fledge at approximately 4–6 months. Huddling for thermoregulation is not generally observed in zoos and aquariums because of constant environmental conditions.

Like emperor penguins, king penguins build no nest, but defend a small nest "territory." For facilities housing king penguins, it is advisable to provide one area suitable for a nesting territory. Although gentoo penguins housed in the same exhibit may attempt to utilize this area for off-season nest-building, king penguins dominate the site during their breeding cycle (S. Branch, personal communication).

Emperor penguins generally eat from keepers' hands without difficulty during incubation. *Pygoscelis* spp., *Eudyptes* spp., and *Spheniscus* spp. penguins can be offered food on the nest as long as it does not cause unnecessary stress for the birds. These species may be aggressive and reluctant to accept food. King penguins may show inappetence at the time of incubation; more commonly, they are too aggressive to eat while on the egg. But keepers familiar with incubation exchanges can locate the bird for feeding when it is off the egg. Some facilities continue to feed the chick-rearing parent the normal morning vitamin fish, and some others prefer to wait until the chick is old enough to take vitamins in their own diet. It is extremely important to remove all dropped fish if parents of smaller species are fed on the nest. The ease with which fish can be removed should be considered when the decision is made to offer food at the nest. If feeding or removal of fish elicits excessive aggression from the parents, an alternative to feeding on the nest should be considered.

If nesting birds are in an off-exhibit area or do not have access to water, it is prudent to give the non-incubating partner the opportunity to swim sometime during the day. Most penguins quickly catch on to this routine and are willing to leave the nesting area for short periods.

7.4 Birthing/Hatching Facilities

As parturition approaches, animal care staff should ensure that the mother is comfortable in the area where the birth will take place, and that this area is "baby-proofed."

Penguins are highly social, colonially-nesting birds. Evidence supports that reproduction in penguins is socially facilitated, and that adequate stimulation by conspecifics is essential for successful reproduction in managed populations (Berger, 1981; Setiawan et al., 2007). Depending on the species, penguins incubate and hatch their eggs either on the nest or in a nest area, and then rear their chicks on or proximal to the nest or nest area. Many institutions provide a nesting area within the main exhibit or provide a designated rookery or nursery in close proximity to, but separate from, the main exhibit space. In either case, provisions for nesting area should be in addition to the recommended land space parameters described in Chapter 2. Some species or individuals may benefit from either a partial or complete separation from the colony due to intra-specific or inter-specific competition and aggression. It may be necessary to partition select nests to prevent aggression or wandering chicks. A partial separation can be achieved by utilizing a barrier such as a Plexiglas® bin, gated and fenced area or a log.

It is important to provide more nesting sites than needed to alleviate competition. The safety and well-being of parents and chicks should always be of the utmost consideration when choosing a nesting area. If the nesting area is located within the exhibit space, areas of high activity, such as proximal to a feeding station, should be avoided. The nesting area should also be located far enough away from pool access to avoid accidental drowning of chicks. Nesting areas should be ventilated well, have good drainage, and be easy to clean, disinfect, and monitor.

It is not recommended to move penguins between facilities during the breeding season. Moving females during egg-formation, laying and post-laying intervals should be avoided due to possible internal egg breakage from handling, and an increased risk of secondary infection from aspergillosis (refer to Transport Protocols, Chapter 3.2). Seasonally, penguins exhibit a great degree of nest site fidelity. With the exception of *Aptenodytes* spp., it is not advisable to relocate a breeding pair once the nest has been established.

Nests and nesting materials: Timing of the addition of nest materials should correlate with other reproductive stimuli that should be approximated to the natural cycle (e.g., artificial lighting and photoperiod), and are generally offered at the onset of breeding season. While nest materials are not necessary for the comfort of the chicks, the collection of nesting material seems to be a strong

component in pair bonding. It is important to provide adequate amounts of suitable nesting material to avoid competition.

Burrows: Nests for penguin species that burrow (*Spheniscus* spp. and *Eudyptula* spp.) can be permanent or seasonal structures, either indoors or outdoors depending on time of egg-laying. Typical burrows for wild *Spheniscus* penguins are fairly wide at the entrance (approximately 40–58 cm [16–23 in.]), narrow slightly, and then widen again in the egg-laying chamber. The dimensions may range from 14–39.9 cm (5.5–5.7 in.) in height by 59.9 cm (23.6 in.) in length (Boersma, 1991). *Ex situ*, burrows can be in the form of natural-excavated scrapes or holes, human-made caves, covers or boxes, or airline kennels (Macha & Sirpenski, 2011; Martir, 2012; Sarro & Kottyan, 2012). When using airline kennels (Vari-Kennel® brand, large, or Sky-Kennel® brand # 300), many facilities recommend removing the door. Some institutions find that using only the top portion of the airline kennel facilitates better monitoring and management due to ease of lifting and moving. Small nest boxes with only enough room so the pair is touching each other when lying down with less than 5.1 cm (2 in.) behind them encourages the penguins to defecate outside the nest which keeps the nest site cleaner than kennels with more room. Burrow nest sites should be at least 1.9 m (6.6 ft.) apart for temperate penguins (Henry & Sirpenski, 2005). The key components to consider are: burrow opening size; adequate air circulation and drainage; ease of cleaning and disinfecting; and adequate number of burrows. *Spheniscus* spp. penguins will utilize almost any nesting option provided. Conservation programs designed to improve *in situ* nesting options have even used 120 L refuse bins, divided in half longitudinally, as artificial burrows (Simeone, 2011).

Artificial burrows may be constructed from wood, providing they are painted or sealed in order to seal out moisture. A burrow of this material should be refurbished or replaced at the conclusion of the breeding season. It may be advisable to consider synthetic wood options such as Trex®, due to the difficulty in adequately sealing and disinfecting wood and for improved durability. All nest boxes should allow the keepers access without unnecessary disruption of the nest. One type of artificial burrow uses 91.4 cm (36 in.) sections of cement pipe, open at both ends. One aquarium uses a similar design made from expanded PVC pipe (45.7 cm [18 in.] long with a 45.7 cm [18 in.] opening) (Macha & Sirpenski, 2011). At another zoological institution, Humboldt penguins are housed in an outdoor exhibit where birds excavate burrows into the natural substrate. Excavation is augmented with a painted plywood tent, box, or fiberglass cover. Most recently a vinyl clad-wire-and-shade-cloth constructed cover has been used successfully (Martir, 2012). In exhibits where birds are allowed to burrow, the soil mixture should be at least 20% clay to prevent nest cave-ins (Beall & Branch, 2005).

Adequate air circulation and drainage are important from the standpoint of humidity and disease control. Proper air circulation is essential in a humid environment; this is especially true if birds are coming from the water and going directly into the nest. Holes or vents can be placed along the sides of the nest box. In exhibits where burrow flooding may occur (due to rain or an overflowing pool) a small drain inside the nest can expedite recovery of the burrow.

The degree of daily maintenance of nest boxes or burrows seems to vary among facilities. Some institutions clean nests daily while others do not clean the nest until the parents abandon it following chick removal. Daily cleaning of nest boxes does not appear to be necessary and may be disruptive. Many institutions remove nest boxes from the exhibit entirely at the close of the breeding cycle. It is recommended that nest boxes be removed for annual disinfection and maintenance (L. Henry, personal communication).

Burrow substrate and nesting material: The substrate used beneath the nesting material should be absorbent, and provide good drainage and ventilation. Nest box substrates that have been safely used include dust-free, non-clumping clay litter, sand or rounded stones (that are too large to swallow) and artificial grass. Materials that have been reported to produce fungal spores (e.g., crushed corncob, peanut shell, potting soil, and shredded newspaper—or bedding made from it) should be avoided. Nesting material might include rounded stones (indoors or outdoors), grasses (e.g., pampas grass), dried heater, and thick leaves like mangrove, evergreens, or dried kelp. Although used successfully by some institutions, managers should be aware of the danger of introducing fungal spores through the use of fresh vegetation as nesting material. When used, it is recommended that vegetation be used outdoors only. Vegetation should be changed frequently if possible. Pencil-sized, dry sticks are an example of a nesting material that should be avoided, as mortality has been reported in adults from eating sticks. Additionally, sticks could be dangerous for young chicks that may be impaled or become trapped under

them. One aquarium uses semi-flexible tubing that is easy to clean and disinfect. The tubing is heated at each end to seal the end closed and to prevent bacteria from getting inside (Macha & Sirpenski, 2011).

Above ground nests: Nests for species that nest above ground (*Pygoscelis* spp. and *Eudyptes* spp.*)* are built to varying degrees. Wild *Pygoscelis* spp. and *Eudyptes* spp. penguins nest in the open or among vegetation. They commonly make a shallow scrape and utilize small rocks as the primary nest material. Feathers or even vegetation may also be incorporated depending on locale. *Ex situ* nests can consist of depressions built into artificial rockwork, forms made from large rocks or pavers, or rubber tubs. Nests should have good drainage and can be cleaned by carefully using a hose to "flush-out" any debris. This procedure should be discontinued prior to egg-laying, and throughout egg incubation and chick rearing on the nest.

One zoological institution reports that they add beach pebble and river rock to a depth of 10.2–12.7 cm (4–5 in.) on the rookery area to provide an adequate base and a good rock source. Care should be taken to provide rocks large enough to preclude ingestion by chicks. It is unknown whether rock eating is dangerous, since wild penguins are known to eat rocks as well. However, given the need to optimize success in the *ex situ* environment, managers would be well advised to avoid smaller sized rocks.

Aptenodytes spp. do not build nests, but defend a small nest "territory," and therefore, do not require the addition of nesting material. The nesting area should be relatively flat and have good drainage. Substrate used in the nesting area can include Dri-dek® mats or a layer of river rock. For both king and emperor penguins, it may be advantageous to separate incubating and chick-rearing pairs from the colony to avoid aggression and egg or chick stealing by conspecifics. Emperor penguins do not generally occupy a single area for nesting; after an egg is produced it is recommended to move the pair to a separate area to avoid disturbance by conspecifics (L. Henry, personal communication). See Chapter 7.3 for more on nest management.

Assisted hatching: Penguin chicks normally hatch without assistance from the parents. Depending on the species, it takes approximately 12–72 hours for penguin chicks to emerge from the shell (refer to Table 12 in Chapter 7.3). Occasionally, hatching chicks become lethargic or malpositioned within the egg, and may need assistance with hatching. Managers should be familiar with the parental breeding history, the pip-to hatch interval for the species, and the normal appearance of a newly hatched chick. Hatching eggs should be monitored frequently throughout the day. Some general indicators of hatching difficulty (either artificial or parent-incubated eggs) include: an internal or external pip that has failed to progress for 12–15 hours or is well beyond the expected incubation period; the chick has rotated away from the pip site such that the bill is no longer visible at the pip hole; a change in parental behavior (e.g., *Aptenodytes* spp. will lift the brood pouch and bow more frequently); a change in chick sounds coming from the egg (high pitched and frequent suggest stress; too few sounds may indicate lethargy).

Good observations and recordkeeping are essential to determine if intervention is needed. It is recommended that institutions with a penguin breeding program invest in the equipment and training necessary to complete egg-incubation, hatching, hand-rearing and supportive care in the event that intervention is required. Equipment and protocols should be in place prior to the start of the breeding season. For more information on incubator and hatcher recommendations, and incubation and hatching parameters, see Artificial Incubation Protocol in Chapter 7.5.

Once it has been determined that a chick is having hatching difficulty, the egg should be removed from under the parent. When performing an assisted hatch, care should be taken not to introduce bacteria to the chick. Hands should be washed and gloved, and all instruments should be sterilized. The egg should be carefully examined and the problem evaluated before attempting an assisted hatch. A candler can be used to assess the pip site (externally and internally), vascularization, and the position and respiration rate of the chick. A penlight can then be used to look inside of the pip hole for unabsorbed yolk, residual albumen, and for further vessel assessment. In some cases, radiographs may be useful for determining the position of the chick (e.g., when an internal pip has not occurred). If the chick has failed to internally and/or externally pip, a manual internal or external pip may be required.

If an external pip has occurred, forceps can be used to remove small pieces of shell from the pip site. As the pip area becomes further exposed, the membrane should be moistened with warm, sterile water (using a sterile swab) to check for active vessels. If the vessels have receded, the membrane can be peeled back to expose the chick. It is important that the temperature of the egg/chick be monitored during assisted hatching to avoid chilling. Be sure to keep the nares clear of membrane during assistance. Depending on chick vitality and the availability of a hatcher, hatching assistance may be accomplished

over a few hours in a step-wise process. The first goal should be to open the pip hole and create more space for the chick. Assistance over time allows chicks to better absorb their yolk. Some chicks will be able to complete hatching on their own with only minor help. In the case of "sticky chicks" (chicks with a lot of residual albumen) it is best to fully assist the chick to hatch. The chick should then be carefully extracted from the shell, preferably head first. For more information on common problems associated with pipping and hatching eggs, and their solutions, please refer to the Penguin Husbandry Manual (Henry & Sirpenski, 2005).

If a chick is to be parent-reared, it should be carefully assessed for any other problems, and then returned to the nest as soon as possible. Chicks that are "sticky" or have protruding yolk sacs should be considered for hand-rearing. Chicks that are returned for parent-rearing should be closely monitored. In general, a healthy chick will vocalize as it solicits food from the parents. If a problem is suspected or to ensure that the chick is being fed sufficiently, chicks can be carefully removed from the nest, examined, and weighed. Weight gain within the first 5–7 days should be substantial. To check for adequate hydration, pinch the skin (usually on the back of the neck) and assess resilience. The chick is dehydrated if the skin "tents" (stays in the pinched position). The eyes should be moist and the feet plump; the lungs should sound clear. Other ways to determine whether a chick is being adequately fed include checking for the presence of regurgitated fish in the nest, establishing keeper observation times and the use of video cameras.

Staff should be prepared to provide assistance to chicks that are small or malnourished. If a chick appears dehydrated, a supplemental feeding of 2–4 cc of Pedialyte® can be beneficial for sustaining very young chicks until the parents are adequately providing food. Small or dehydrated chicks should be monitored closely for complications.

Weaning/fledging procedure: Penguins raised *ex situ* do not crèche like those raised in the wild. The age of fledging, or independence from parents, varies among penguin species. Penguins usually achieve their peak weight just prior to fledging (refer to Chapter 4 Table 7 for the age of fledging and peak fledging weight for each species). Keeper staff should begin transitioning chicks to hand-feeding at this time. At this stage, parents tend to leave chicks unattended for longer periods. It is good practice to hand-feed chicks when parents are away from the nest to avoid aggression by parents. Once chicks are readily hand-feeding, it may be beneficial to separate chicks from the parents and the main exhibit for controlled introductions. Monitored visits to the social group and colony should then occur. It is best to introduce chicks in pairs or groups if possible. Food consumption, weight and acclimation should be closely monitored during this time and for several weeks post-fledging.

Smaller species of penguins can be given access to water when their abdomen and back are completely molted of down. Larger species may not venture near the water until near completion of the molt. Efforts should be made to ensure that chicks gain experience with entering and exiting the pool prior be being left in the exhibit unattended. Inexperienced chicks should be monitored at all times while swimming and entering or exiting the pool to avoid accidental drowning. For more information on chick removal, weaning and habituation, and introductions see also Partial Rearing in Chapter 7.5 and Chapter 4.3.

7.5 Assisted Rearing

Although mothers may successfully give birth, there are times when they are not able to properly care for their offspring, both in the wild and in *ex situ* populations. Fortunately, animal care staff in AZA-accredited institutions are able to assist with the rearing of these offspring if necessary.

Intervention may be warranted in cases where one or both parents has a health concern, perhaps due to irregular exchange of incubation or brooding bouts; for dropped or abandoned eggs; or where parents are not observed to regularly feed a chick or a chick fails to thrive.

Artificial incubation: Institutions should be familiar with expected incubation behavior for a given species, in order to properly manage eggs on the nest. Many times eggs are removed from a pair based on an assumption of inadequate incubation when, in fact, incubation had not yet begun. Eggs removed from the parents because of improper incubation may be returned to the nest at or before pip for parent rearing if the pair has continued to incubate a dummy egg during the period the egg was in the incubator.

Artificial incubation protocol: It is recommended that institutions undertaking to artificially incubate penguin eggs be familiar with proper hatchery setup, sanitation and maintenance.

Good record keeping is important to egg management. It is recommended that all eggs be documented with a unique egg log identification number and that all eggs laid are recorded along with their outcomes. As eggs come into the incubator, the egg log identification number should be written on the small end of the egg for continuity of identification (see Chapter 6.1: Reproductive Recordkeeping).

A variety of incubators may be suitable for penguin egg incubation. Factors to consider when choosing an incubator include: an automatic-turning mechanism sufficient to accept the larger size and weight of penguin eggs; the ability to maintain a stable temperature and humidity, especially if manual turning is required; and a size sufficient to hold the likely number of eggs to be brought into care. Some types that have been used successfully but are no longer manufactured (though still available) include Petersime Models 1 and 4 and Humidaire models 20 and 50. Other types include Grumbach, Roll-X, Brinsea® and R-Com (Standard Model). Most institutions have reported using Grumbach incubators.

Artificial incubation temperatures reported by 23 institutions vary from 35.2–37.5 °C (95.5–99.5 °F) on the dry bulb and 26.6–30 °C (80–86 °F) on the wet bulb. The most commonly used dry bulb temperature is 35.8 °C (96.5 °F). The wet bulb temperature should range from 27.7–28.8 °C (81–84 °F). Depending on geographic locale and rainfall, this may necessitate more or less frequent additions of water to the incubator reservoir. Type of incubator and the number of eggs being held at one time will affect overall humidity. Monitoring eggs through egg weight loss measurements is well described in the literature, and can assist managers in establishing humidity requirements for their egg incubation (Lomholt, 1976; Anderson-Brown, 1979; Johnson, 1984; and Hoffman, 1987).

Eggs should be set flat, not on end, in the incubator. The majority of institutions that have attempted artificial incubation have reported mechanical turning of the eggs every 1–2 hours. In addition to mechanical turning, some institutions also perform a 180° manual turn of the egg. This practice facilitates a more even development of the vascularization in the egg (Jordan, 1989). For incubators without automatic turning capability, manual turning can be done five or seven times (an uneven number of turns) within a 12-hour day. Eggs should be turned slowly to avoid rupture of developing blood vessels in the egg.

A penguin egg is ready to move to the hatcher following external pip. Turning of the egg is no longer necessary at this time but hatching eggs should be checked 4–5 times per day. Problems have been reported when moving *Pygoscelis* spp. eggs to the hatcher prior to the chipping of the shell by the chick. Some institutions use playback recordings of the colony to stimulate the chick during hatching.

At the time of pip, humidity should be increased by 1–2 °C (2–3 °F) on the wet bulb. This can best be accomplished in a hatcher separate from the incubator. Shell membranes may become dry during hatching. This can be alleviated by adding a small reservoir of warm-water (35.8 °C [95.5 °F]) to the hatcher (away from chick access) to temporarily increase humidity, by rolling a moistened, sterile cotton-tipped applicator over the membrane or by lightly misting the egg. Water for misting should be kept in the hatcher so that the temperature is the same as the hatcher. For more information on common problems associated with pipping/hatching eggs, and their solutions, please refer to the Penguin Husbandry Manual (Henry & Sirpenski, 2005).

Once chicks hatch, they should remain in the hatcher for 12–24 hours to allow for their down to dry before transfer to a chick brooder. Check for yolk sac absorption and closure of the umbilicus (seal). Be extremely careful handling the chick if the yolk is not properly absorbed and/or the umbilical opening is not properly sealed. Swab the umbilicus with a dilute, iodine-based disinfectant (such as Betadine®) or a sterile PDI® Iodine Duo-Swab® Prep and Scrub SwabStick can be rolled gently over the area. For more information on the medical management of neonates, see Chapter 6.5. If two or more chicks are hatching simultaneously in the same hatcher, measures should be taken to separate eggs/chicks in order to maintain individual identification (hatching egg to hatched chick).

Hand-rearing: It is advisable for all institutions managing penguins to gain experience in hand-rearing. A separate hand rearing area is recommended with provision for good air movement, temperature commensurate with the species and reduced humidity.

Important factors to consider before deciding to remove eggs or chicks from the nest should include the age of the pair, their reproductive experience, environmental and social conditions, and the goals of the reproductive program. Prior to undertaking hand-rearing of penguin chicks, managers should consider the time and cost involved in hand-rearing penguins, because this is a labor-intensive undertaking. Staff hours required to tend to the chicks along with the cost of the necessary equipment (brooder, formula, etc.) may have an impact on the decision whether or not to hand-rear chicks.

As with most species, parental rearing is always preferred to hand-rearing. It may be necessary to remove an egg or chick for hand-rearing in the event of the death of a parent or the failure of a chick to thrive in the nest. Sticky chicks (those with residual albumen) or chicks with protruding yolk sacs should be considered for hand-rearing. Success with hand-rearing chicks can be as high as 90% once a well-defined protocol has been established (Cheney, 1990). Hand-rearing may be used to maximize founder representation within a colony, particularly if underrepresented birds do not exhibit successful parental behavior. Hand-rearing can also be used to increase productivity, as some species will often breed again within one season if chicks or eggs are removed. Hand-reared chicks seem to be more tolerant of handling than parent-reared chicks. Depending on the routine husbandry practices of the facility, this may or may not be important. It should also be stressed that penguins are social animals and need to be in the company of conspecifics or congeners, even at a very young age, if they are to develop socially and not imprint. Therefore, if possible, chicks of similar age should be hand-reared together.

Occasionally, when birds are hand-reared, they develop a preference for human companionship over that of conspecifics. Depending on the species, highly imprinted birds may or may not eventually reproduce. Imprinted hand-reared *Pygoscelid* penguins, for example, may not breed. Highly imprinted *Spheniscus* spp. penguins, however, have been reported to breed and may make very good parents. Imprinted birds can be disruptive in penguin colonies, wandering over other birds' nesting territories. Social dysfunction sometimes can be overcome in imprinted birds, especially if they pair with a non-imprinted bird. In general, it is advisable to discourage staff from reinforcing attention from imprinted birds. As with most species, the best strategy is the avoidance of imprinting during rearing.

Introduction of hand-reared chicks into exhibits requires close monitoring and is likely to be most successful if a gradual introduction procedure is followed (see Chapter 4.3). Hand-reared *Spheniscus* chicks can be introduced into the colony when they are nearly fledged (approximately 80 days). It is best to introduce chicks in a group or in pairs if possible. It is advisable to supervise the interactions of the newly introduced birds during the initial visit to the colony. Chicks can be left unattended after a few days provided they are able to emerge from the water without trouble and are not being harassed by other birds. Juveniles tend to congregate together and will fight to establish a hierarchy of their own (Gailey-Phipps, 1978).

Chicks should be encouraged to join the other birds at the feeding station rather than be provided with special treatment. It may be a few weeks before they are regularly feeding with the others. Some institutions find it advantageous to use an off-site area to introduce the chicks to members of the colony. A Plexiglas® barrier can also be used at first introduction in the exhibit. If chicks have not yet lost their entire down, adult birds may attempt to brood fledglings. Emperor penguins in zoos and aquariums, for example, have been observed to compete aggressively to brood newly introduced hand-reared *Pygoscelid* chicks. Once chicks are hatched and have been allowed to dry in the hatcher for 12–24 hours they can be moved to a brooder.

Brooder: Penguin chicks require low humidity and good air circulation, which is best achieved in an open-topped brooder style. Some institutions have successfully used closed baby incubators, or AICU, but managers should be vigilant in order to avoid high humidity and the resultant increased risk for aspergillosis. Brooders should be chosen based on adequate air circulation, ease of cleaning and disinfection, and size and temperature gradient. Brooders can be constructed of a wood alternative (such as Trex®), an ice-chest type plastic cooler or a plastic storage container without a lid; one institution uses a Plexiglas® Acrylic Sheet frame with an open top. Some facilities have successfully used a cooler-type brooder (such as The Original Cooler Brooder); it is important to keep the top open for sufficient air circulation. Typical early brooder dimensions might be 40 cm x 83 cm x 38 cm (12 in. x 33 in. x 15 in.) to accommodate one to four chicks of smaller species (e.g., *Spheniscus* spp., *Pygoscelis* spp., *Eudyptes* spp.) or one to two chicks of larger species (e.g., *Aptenodytes* spp.). Chicks should not be overcrowded. The brooder surfaces may be cleaned and disinfected at least twice per day or more frequently depending on the number of chicks, their age and fecal load. As chicks grow and their needs change, older birds can be housed in a larger area such as in a contained floor area or in an elevated bin.

Substrate: The substrate used my most institutions in the brooder is clean toweling without holes or frays (that might catch a chick's toenails). Some facilities include a non-adhesive and non-slip type of shelf liner (such as Cont-Tact® Grip Ultra Shelf Liner) to provide traction for the chick (on top of the base toweling). Dri-Dek® can also be placed under the toweling to provide a better grip for the chicks' developing legs. Other facilities put a few rocks under the towel to improve the gripping surface. The toweling can be

changed as fecal load dictates. Chicks under 7 days old may tend to wander away from the heat source so a rolled towel can be fashioned to contain the chick(s) in the early brooding period. Older chicks can be moved to an area that provides a substrate for proper foot health such as rocks (similar to that described in 7.1 for nesting), matting (e.g., AstroTurf® roll mat) or Dri-dek®. As chicks approach fledging it may be advantageous to consider providing housing in the exhibit. Chicks can be separated from the colony but still in visual and vocal contact, at a similar temperature and on a similar substrate as their conspecifics, which may facilitate later introductions to the group.

Temperature: The brooder should have a heat source (such as a 250-watt infrared heat lamp). Temperature gradients within the brooder will be increasingly important as the chick grows in its second down toward the end of the guard stage. Gradients allow chicks to find a comfortable temperature within the brooder. Generally, chicks at 1–7 days should be maintained at about 26.7–32.2 °C (80–90 °F); 8–14 day old chicks are usually ready for a slightly reduced temperature of about 21.1–26.7 °C (70–80 °F). These temperatures are dependent on the species and individual chicks' needs. Temperature requirements will change for chicks greater than 14–21 days. Sub-Antarctic and high latitude species will require less or no heat, and may even need reduced temperatures closer to exhibit temperatures. Downy *Spheniscus* chicks may do well at 18.3–21.1 °C (65–70 °F) but should still be monitored for overheating.

A common problem in penguin chick rearing is over- or under-heating chicks. Under-heating is most often seen in chicks less than 14 days old. Under-heated chicks may shiver, huddle against the side of the brooder, have feet and flippers drawn in and/or be cold to the touch. Under-heated chicks are often slow to respond to a feeding stimulus. As chicks get older overheating is a more common concern. Overheating can lead to illness in penguin chicks. Overheating may be indicated by any one or a combination of the following signs and symptoms: chick's posture is spread out, feet and flippers are extended and/or are very warm to the touch, panting, lethargy, dehydration, and disinterest in food. Many of these symptoms are also indicators of illness in a chick. Measures should be taken to discern if under- or overheating is indicated and veterinary intervention should be sought for a chick that does not respond to adjustments in temperature.

Record keeping: Complete records for each chick are extremely important. Records should include the daily morning weight of the chick, the type and volume or weight of the food fed, assessments of the chick's health and vitality including fecal output, temperature adjustments, and any notable milestones such as when eyes open, downing stages, etc. Such records will help monitor proper health and determine if chicks are developing consistent with documented growth rates. Fecal output is an important measure of a chick's response to hand feeding regimes. Feces should be slightly runny and squirt out a good distance during defecation. Color may vary but in general an orange/brown fecal is often reported as normal. Older chicks receiving fish pieces will have a slightly thicker fecal but it will still be quite soft. Feces should not be pasty, dry or pellet-like, excessively green (green is normal in 1–2 day old chicks), black or yellow, or contain blood (orange or red oily spots in the fecal will be normal if krill is part of the diet).

Feeding: Detailed feeding guidelines for penguin chicks (*Spheniscus* spp., *Pygoscelis* spp., *Eudyptes* spp., and *Aptenodytes* spp.) are well described in the Penguin Husbandry Manual (Henry & Sirpenski, 2005). Safe food practices should be followed for fish handling and in the preparation of all diets. Feeding apparatus will include syringes (3 cc, 6 cc, 12 cc, and later 35 cc) sometimes with a short (2.3 cm [1 in.]) portion of a 14-fr catheter tube (such as Kendall Sovereign® Feeding Tube and Urethral Catheter) securely glued to the hub end. A small extension on the syringe can help facilitate the delivery of formula to the chick.

It is important to continue to monitor the absorption of the yolk after the chick is moved to the brooder and feeding begins; slow absorption or a tight distention of the abdomen might be an indicator of a yolk sac infection. Yolk sac infections commonly occur through 14–17 days of age and require a veterinary exam and treatment. The seal should continue to be swabbed once daily (as described above for newly hatched chicks) until the seal is fully closed, usually within a few days following hatching.

In general, young penguin chicks of all species are started on a mixture of fish, krill (if available), water, and vitamins (Penguin Chick Hand Rearing Diet see Appendix L), ground in a blender and fed by syringe five times per day at 3-hour intervals. The very first feeding might be water only in order to determine the vitality of the chick and to introduce it to syringe feeding. Chicks are fed by eliciting a feeding response by extending the first and second fingers in an inverted "V"-shape over the chick's bill,

then wiggling the fingers. The chick should respond by opening its bill and pushing up into the fingers. At this time, the syringe should be placed in the mouth and the formula fed. The amount of food to feed penguin chicks is based on their morning weight. After a few days of initial introduction to feeding, where volumes might be less, chicks can be given a food amount equivalent to 10% of their morning weight at each feeding. It is important not to over-feed penguin chicks.

As the chick grows, fish pieces (usually without skin and bone), and later whole fishes can be introduced. The timing of when to introduce fish, reduce temperature in the brooder, and then later reduce the relative ratio of fish and formula in the diet is all based on weight milestones rather than age. An exception is made for the *Aptenodytes* where fish might be introduced starting at 7–10 days of age. Weight (or age) milestones can serve as a guideline for when to introduce various changes to diet and brooding temperature but hand rearing should always be based on the individual bird's responses. As the smaller species of chicks grow toward about 500 g (18 oz.) the feeding interval should be evaluated and lengthened to 4 hours with feedings reduced to four times per day; the weight milestone here will be different for *Aptenodytes*, This change in feeding interval is in response to the increased amount of food fed per feeding as well as the change in the relative ratio of formula to fish (which is usually 50:50 by this time). Once a maximum of about 30 mL of formula (40 mL for *Aptenodytes*) is being given per feeding, this amount can remain stable with the balance of food making up the feeding coming from fish fillets, fish pieces or whole fishes. In this way, as chicks grow, they are gradually weaned off formula to a whole fish diet. By about 1000 g (35 oz.), most of the smaller species of penguin chicks may start to refuse syringe feeding in favor of fish, need a reduced temperature environment and larger brooder area, and reduce to three feedings per day. As before, timing for this change will be at a different weight target for *Aptenodytes*. As chicks begin to fledge they can be fed consistent with the feeding times they will encounter once they are introduced to the social group.

It is important to note that as chicks (species *Spheniscus* spp.*, Pygoscelis* spp.*, Eudyptes* spp.*)* approach 1000–1500 g (35–53 oz.) and beyond they may not eat all the food offered per feeding (i.e., the 10% threshold). At this time, it may be difficult to discern whether the chick is exhibiting normal behavior or whether the behaviors are suggestive of a subclinical illness. Over feeding and overheating are common problems encountered at all stages in penguin rearing, but particularly at this age and stage. *Spheniscus* spp. may also become "head shy" at about 1000 grams (35 ounces) or about 30 days of age, which may be accompanied by a reluctance to give a feeding response. This behavior is normal and roughly correlates to when these chicks would be starting to investigate outside the burrow. However, all chicks exhibiting a reluctance to eat should be assessed for overheating, whether they have been overfed (and/or need a reduction in feeding interval or amount) and monitored for early signs of illness. Dehydration is one good indicator of both overfeeding and overheating. Foul smelling fecal matter should be addressed immediately with a veterinary exam.

Vitamin supplementation: Refer to Chapter 5.1 and the Penguin Husbandry Manual (Henry & Sirpenski, 2005). The preceding is a summary of feeding and rearing procedures. More details are available in the Penguin Husbandry Manual (Henry & Sirpenski, 2005). Penguin managers rearing penguins should consider consulting other institutions with penguin hand-rearing experience before or during the hand rearing process. The preceding is a summary of feeding and rearing procedures. More detailed guidelines for hand-rearing penguins can be found in Appendix M.

Partial rearing: Eggs removed for fostering to another pair can be taken at any point during incubation. Options at this time include placing the egg in an incubator until the target (foster) pair is ready to receive the egg, or transferring the egg immediately to the target pair. The target or surrogate pair should always be incubating an egg or dummy egg prior to replacement with a viable fostered egg.

The fostering of eggs to a surrogate pair for chick rearing is an option used by many facilities to maximize chick survivability and reduce the need for hand rearing. In managing eggs, once viable eggs are identified, one egg from a fertile clutch can be fostered to pairs with infertile eggs. In cases where two chicks could be produced from a pair, this arrangement allows the parents to rear only one chick while a pair that is known to be successful at rearing cares for the second chick. The timing of egg-laying for both pairs should be within two weeks of each other. The eggs of the surrogate pair should be replaced with dummy eggs immediately. The egg(s) to be fostered can be placed under the surrogate pair a few days prior to the expected hatch date or at the time of pipping. Some facilities allow the first egg to hatch successfully before fostering the other egg. Fostering eggs can also be used to give younger or less experienced pairs, or even same sex pairs, an opportunity to rear a chick.

Chicks should be monitored at the nest to assure proper growth and vitality by recording feeding observations. Pairs rearing chicks should be fed frequently and *ad libitum*. It may be advisable to feed smaller, more digestible fishes (such as capelin or silversides) for the first parental feeding of the day so that chicks can be fed quickly. Parents with soliciting chicks have been reported trying to feed chicks too soon after eating larger fishes (such as herring) resulting in large chunks that young chicks cannot accept. Feeding smaller fishes or smaller meals allows for better digestion before it is fed to the chick. Chicks can also be removed from the nest for periodic weights and physical assessments. Chick weights can then be compared with published data for the same age and species to assure adequate growth. It is worth noting that parent-reared chicks should demonstrate a steeper growth rate than that for hand-reared chicks; most available growth rate data will be for hand-reared birds. If a chick requires medical treatment unrelated to parental care, treatments may be accomplished without removing the chick for hand-rearing but instead removing the chick only for needed procedures then returning it to the nest for continued parental care. Some institutions have reported supplementing parents or chicks with vitamins at the nest (see Chapter 5.1 for chicks' nutritional requirements). When chicks are older and able to accept whole fish, they may take fish from hand offered at the nest.

Many facilities remove chicks from parents prior to fledging to habituate the birds to hand feeding. Age at removal varies from 21–50 days depending on the facility and the species. Removing chicks allows for improved monitoring of chicks' growth and development, especially if there are two chicks in a nest, as the second chick may be out-competed by the first chick. Other institutions remove chicks at the end of the guard stage if a pool is nearby and there is concern for chicks' access and welfare. Additionally, chicks weaned in this way are reported to accept routine handling better, are much more relaxed in the colony, and accept hand-feeding better than parent-reared and fledged birds. Chicks removed from parental care can be housed with hand-raised birds of similar age and size. Introduction into the colony follows a similar course as outlined for hand-reared birds' introductions. In rare cases, juveniles may return to the parents or nest area and continue to be fed by one or both of the parents. This does not usually result in adverse outcomes. However, if a parent continues to feed a chick for a prolonged post-fledging time period, a second separation of the chick from the parent should be considered.

7.6 Contraception

Many animals cared for in AZA-accredited institutions breed so successfully that contraception techniques are implemented to ensure that the population remains at a healthy size. The use of invasive contraceptive methods with penguins has not been described. Penguins, as with other birds, provide easy contraception management via the removal of eggs immediately at lay. Dummy eggs may be needed to prevent double-clutching. Should the need arise to cull an egg that has undergone some development, the egg should be refrigerated at 4.4 °C (40 °F) for at least 3 days. This will humanely stop development (Leary, 2013).

Chapter 8. Behavior Management

8.1 Animal Training

Classical and operant conditioning techniques have been used to train animals for over a century. Classical conditioning is a form of associative learning demonstrated by Ivan Pavlov. Classical conditioning involves the presentation of a neutral stimulus that will be conditioned (CS) along with an unconditioned stimulus that evokes an innate, often reflexive, response (US). If the CS and the US are repeatedly paired, eventually the two stimuli become associated and the animal will begin to produce a conditioned behavioral response to the CS.

Operant conditioning uses the consequences of a behavior to modify the occurrence and form of that behavior. Reinforcement and punishment are the core tools of operant conditioning. Positive reinforcement occurs when a behavior is followed by a favorable stimulus to increase the frequency of that behavior. Negative reinforcement occurs when a behavior is followed by the removal of an aversive stimulus to also increase the frequency of that behavior. Positive punishment occurs when a behavior is followed by an aversive stimulus to decrease the frequency of that behavior. Negative punishment occurs when a behavior is followed by the removal of a favorable stimulus also to decrease the frequency of that behavior.

AZA-accredited institutions are expected to utilize reinforcing conditioning techniques to facilitate husbandry procedures and behavioral research investigations. A structured training program that utilizes operant conditioning of natural behaviors, a structured desensitization program to reduce aversive stimuli within the zoo and aquarium environment, and classical conditioning have been effective with penguins. Penguins are relatively easy to condition as they respond well to consistent routines. As a tool for operant conditioning purposes, bridges or markers such as clickers, whistles, and verbal stimuli have all been successfully trained. Food reinforcement is most commonly used, but tactile stimulation, novel objects, and social interaction have also been utilized. Penguins have successfully been scale trained, trained for restraint during physical exams, voluntary blood collection, semen collection, foot exams, shifting and recall. Common recall signals are verbal or mechanical such as a whistle. These behaviors have also been utilized for research purposes.

8.2 Environmental Enrichment

Environmental enrichment, also called behavioral enrichment, refers to the practice of providing a variety of stimuli to the animal's environment, or changing the environment itself to increase physical activity, stimulate cognition, and promote natural behaviors. Stimuli, including natural and artificial objects, scents, and sounds are presented in a safe way for the penguins to interact with. Some suggestions include providing food in a variety of ways (i.e., frozen in ice or in a manner that requires an animal to solve simple puzzles to obtain it), using the presence or scent/sounds of other animals of the same or different species, and incorporating an animal training (husbandry or behavioral research) regime in the daily schedule.

Enrichment programs for penguins should take into account the natural history of the species, individual needs of the animals, and facility constraints. The penguin enrichment plan should include the following elements: goal setting, planning and approval process, implementation, documentation/record-keeping, evaluation, and subsequent program refinement. The penguin enrichment program should ensure that all environmental enrichment devices (EEDs) are "penguin" safe and are presented on a variable schedule to prevent habituation AZA-accredited institutions must have a formal written enrichment program that promotes penguin-appropriate behavioral opportunities (AZA Accreditation Standard 1.6.1).

AZA Accreditation Standard

(1.6.1) The institution must have a formal written enrichment and training program that promotes species-appropriate behavioral opportunities.

Penguin enrichment programs should be integrated with veterinary care, nutrition, and animal training programs to maximize the effectiveness and quality of animal care provided. AZA-accredited institutions must have specific staff members assigned to oversee, implement, train, and coordinate interdepartmental enrichment programs (AZA Accreditation Standard 1.6.2).

AZA Accreditation Standard

(1.6.2) The institution must have specific staff member(s) or committee assigned for enrichment program oversight, implementation, training, and interdepartmental coordination of enrichment efforts.

Utilizing the natural, individual, and facility information, goals should be set to address either specific behaviors or to provide a stimulating environment. Due to the colonial nature of penguins, enrichment will most often be presented to the entire flock, but can be utilized for individuals as needed. A specific staff person and/or a committee should determine appropriate procedures for setting goals, documentation, and how to determine whether the enrichment is meeting the goals both before and after use. Routine screening of devices for wear as well as determining their "enrichment value" should be conducted on a regular basis. Safety should always be a primary concern and should be in the forefront of any program.

Behavioral enrichment for penguins can easily be achieved by creating a complex water habitat where small fish can hide and survive. Foraging is an important natural behavior and penguins will spend time hunting and capturing these fish, which keeps them swimming and on display. Beyond normal stimuli in a zoo and aquarium environment, such as snow, water, and conspecifics, penguins generally tend to respond with curiosity to novel objects and increase their exploratory behavior. Enrichment does not require elaborate or costly apparatus. One zoological institution reports good success with brightly-colored rubber balls, sprinklers, and also with blocks of frozen fish placed into pools. Having variety in the water by manipulating water currents or using wave machines can stimulate penguins. Sawhorses with securely affixed strips of fabric under which the birds can run is an example of a novel device. Underwater visual barriers may also provide enrichment. Some facilities report good success with the use of different feeding strategies, such as multiple feedings, extended feedings, and scatter feedings.

Enrichment areas should always be built into exhibit rockwork to provide slides, covered areas, burrows, and different sized pathways and land areas. The ability to alter the "furniture" is a benefit. There should be places where it is easy to retrieve devices from the water. By incorporating these types of elements into exhibits natural behaviors such as locomotion, foraging, courtship and breeding are facilitated. Enrichment devices should be provided on a variable schedule. This can be accomplished by varying time of day and duration of presentation. Catalogs and calendars for enrichment initiatives can also be created to allow a variable schedule of enrichment delivery to be developed. It is important to consider sub-aquatic landscape or furbishing in order to promote the surface and underwater activity. This will allow for an increase in natural behaviors that include foraging and exploration. Enrichment devices can be utilized to mitigate stereotypic or aggressive/fearful behaviors as well as facilitate introductions.

Participation in training programs and in behavioral research programs can be enriching as they allow the bird to have differing cognitive stimulations from the normal zoo or aquarium experience. Interaction and mental stimulation are important aspects of training and are essentially enriching. Training reinforcers can include items that the birds find enriching such as novel foods or favorite devices. Training and enrichment can also be utilized to address issues such as veterinary or nutritional needs. Lack of activity can be addressed by enrichment and offering different food choices and presentations can be used to deal with nutritional requirements. Training can make necessary interactions more cooperative and create an environment of choice and control.

As with all taxa, safety is of utmost concern with environmental enrichment devices. Carefully examine all devices for small, ingestible pieces, parts that could easily be broken off, entanglement issues and so on. New devices should always be monitored after presentation to assure that they are safe. Food enrichment should be appropriate for the species and follow the institutional approval process prior to offering. It is also important to be sure that the devices do not cause undue stress on the animals. All devices should be examined on a regular basis to assure that there has been no degradation and if there has been they should be disposed of. An example schedule of penguin enrichment can be found in Appendix P.

Browse: If browse plants are used for enrichment or nesting materials, all plants need to be identified and assessed for safety. The responsibility for approval of plants and oversight of the program should be assigned to at least one qualified individual. The program should identify if the plants have been treated with any chemicals or near any point sources of pollution and if the plants are safe for the species. If animals have access to plants in and around their exhibits, there should be a staff member responsible for ensuring that toxic plants are not available.

8.3 Staff and Animal Interactions

Animal training and environmental enrichment protocols and techniques should be based on interactions that promote safety for all involved. Penguins adapt to humans quickly (Walker et al., 2005; 2006). When

animal caretakers are present within an exhibit with the birds during visitor hours, it is recommended that some interpretation be provided so that the public can learn more about the role of the caretakers, and that their actions are acceptable. Common keeper-penguin activities include feeding, training, handling, herding the birds into the water, and tactile interactions. Interpretation can be achieved through graphics, keeper explanations, volunteers, pool attendants, etc. At a minimum, interpretation efforts should explain what the keeper is doing, and why it is important.

Facilities should be designed to take advantage of training opportunities. Off exhibit holding should be designed to accommodate scales and have sufficient room to allow for training of individuals. This space should have a flat, non-slip surface that is large enough for more than one staff person. Shifts should be large enough to accommodate more than one bird at a time, but easily opened/shut to be able to separate birds. Penguins do not require protected contact, but care should always be used when working in close proximity. They have extremely strong flippers and beaks, and they are capable of causing serious injury. Eye protection may be necessary, depending upon the bird and circumstance.

Program animals: In contact and behind the scenes programs, the keeper has an opportunity to explain more thoroughly the contact that keepers have with the birds. The keeper should explain about the benefits of training, how there are proper ways to handle and desensitize a bird, and that a lot of time is taken to get the birds used to the keepers so they can feel comfortable being handled. Natural history and conservation topics should also be discussed; and it should be made clear that wild birds would not react this way. Finally, the visitors should be told what to expect from their visit, whether they can touch the bird, proper techniques to use, and how the bird might react. See Chapter 9 for additional information on conservation/education program animals.

8.4 Staff Skills and Training

Penguin staff members should be trained in all areas of penguin behavior management. Funding should be provided for AZA continuing education courses, related meetings, conference participation, and other professional opportunities. A reference library appropriate to the size and complexity of the institution should be available to all staff and volunteers to provide them with accurate information on the behavioral needs of the animals with which they work. The following skills are important for all animal caretakers involved in the management of penguins:

- Knowledge of basic husbandry.
- Knowledge of natural history, and the ability to apply this knowledge in the design of effective exhibits.
- Knowledge of exhibit history and collection history.
- General knowledge of life support systems involved with the exhibit.
- Knowledge of incubation and rearing practices.
- General knowledge of morbidities, avian triage, and diseases associated with penguins in zoos and aquariums.
- SCUBA certification, if applicable.
- Ability to lift, shovel, and scrub.
- Ability to safely restrain a penguin.
- Knowledge of operant conditioning techniques prior to training animals.
- General enrichment knowledge that includes an understanding of enrichment that promotes natural behavior, safe enrichment, the importance of varied schedules of enrichment delivery, as well as the ability to recognize that certain types of enrichment can be used for reinforcement.
- Knowledge of in-house policies and procedures, approval processes and safety issues.

Chapter 9. Program Animals

9.1 Program Animal Policy

AZA recognizes many public education and, ultimately, conservation benefits from program animal presentations. AZA's Conservation Education Committee's Program Animal Position Statement (Appendix D) summarizes the value of program animal presentations.

For the purpose of this policy, a program animal is described as an animal presented either within or outside of its normal exhibit or holding area that is intended to have regular proximity to or physical contact with trainers, handlers, the public, or will be part of an ongoing conservation education/outreach program.

Program animal presentations bring a host of responsibilities, including the welfare of the animals involved, the safety of the animal handler and public, and accountability for the take-home, educational messages received by the audience. Therefore, AZA requires all accredited institutions that give program animal presentations to develop an institutional program animal policy that clearly identifies and justifies those species and individuals approved as program animals and details their long-term management plan and educational program objectives.

AZA's accreditation standards require that the conditions and treatment of animals in education programs must meet standards set for the remainder of the animal collection, including species-appropriate shelter, exercise, sound and environmental enrichment, access to veterinary care, nutrition, and other related standards (AZA Accreditation Standard 1.5.4). In addition, providing program animals with options to choose among a variety of conditions within their environment is essential to ensuring effective care, welfare, and management. Some of these requirements can be met outside of the primary exhibit enclosure while the animal is involved in a program or is being transported. For example, housing may be reduced in size compared to a primary enclosure as long as the animal's physical and psychological needs are being met during the program; upon return to the facility the animal should be returned to its species-appropriate housing as described above.

AZA Accreditation Standard

(1.5.4) A written policy on the use of live animals in programs must be on file. Animals in education programs must be maintained and cared for by trained staff, and housing conditions must meet standards set for the remainder of the animals in the institution, including species-appropriate shelter, exercise, social and environmental enrichment, access to veterinary care, nutrition, etc. Since some of these requirements can be met outside of the primary enclosure, for example, enclosures may be reduced in size provided that the animal's physical and psychological needs are being met.

Penguins, in general, can be used as program animals. Program penguins can be held in a colony situation or in separate dedicated housing. Penguins are not a significant zoonotic risk and specific housing or shelter options do not lessen this risk. An animal care program with dedicated clothing and latex gloves will limit disease transfer from penguins to human and other animals in the facility.

The physical needs of penguins as program animals are virtually the same as penguins as exhibit animals. The TAG does suggest colony management of program penguins but that does not mean that off-exhibit holding pens are inadequate. The floor and water requirements are exactly the same and allow for adequate swimming and ambulatory exercise. The TAG recommends that penguins be housed with a minimum of six individuals, which is the same for colonies. Penguins can be trained to enter a "transport crate" to go to educational programming events, although they are also easily placed into these crates manually. Generally, penguins are easy to monitor for medical concerns through animal care staff observations and records keeping, and program animals may more easily allow tactile medical inspection due to their familiarity with people.

Penguin psychological needs are not very extensive. Penguins thrive with other penguins for social interactions but often also engage in social behaviors with their caretakers and visitors. Providing unique or novel enrichment, such as floating balls and/or sinking balls with flag ends, may momentarily enrich a penguin's daily routine but that interest is short-lived. Utilization of laser pointers on a wall or floor has been used with some success as well. Adding live fish to an exhibit may provide interest but there are other considerations with this form of enrichment.

In contact and behind the scenes programs, the keeper has an opportunity to explain more thoroughly the contact that keepers have with the birds. The keeper should explain about the benefits of training, how there are proper ways to handle and desensitize a bird, and that a lot of time is taken to get the birds used to the keepers so they can feel comfortable being handled. Natural history and

conservation topics should also be discussed; and it should be made clear that wild birds would not react this way. Finally, the visitors should be told what to expect from their visit, whether they can touch the bird, proper techniques to use, and how the bird might react.

9.2 Institutional Program Animal Plans

AZA's policy on the presentation of animals is as follows: AZA is dedicated to excellence in animal care and welfare, conservation, education, research, and the presentation of animals in ways that inspire respect for wildlife and nature. AZA's position is that animals should always be presented in adherence to the following core principles:

- Animal and human health, safety, and welfare are never compromised.
- Education and a meaningful conservation message are integral components of the presentation.
- The individual animals involved are consistently maintained in a manner that meets their social, physical, behavioral, and nutritional needs.

AZA Accreditation Standard

(1.5.3) If animal demonstrations are a part of the institution's programs, an educational/conservation message must be an integral component.

AZA-accredited institutions that have designated program animals are required to develop their own Institutional Program Animal Policy that articulates and evaluates the program benefits (see Appendix E for recommendations). Program animals should be consistently maintained in a manner that meets their social, physical, behavioral, and nutritional needs. Education and conservation messaging must be an integral component of any program animal demonstration (AZA Accreditation Standard 1.5.3).

Penguins are flagships for numerous conservation messages. The list includes human overpopulation impacts, over-fishing concerns, oil-spills, global warming, pollution, invasive species impacts, and predator-prey dynamics, to name a few. Certain species of penguins lend themselves to different types of educational programming. The *Sphensicid* species (African, Humboldt, and Magellanic) and rockhoppers are commonly used for off-site outreach programs, as they are tolerant of a wide range of temperatures. This does not exclude cold-weather species from outreaches but adds an additional layer to the logistics. For programs that are held on-site, either close to the exhibit/holding pen or in the exhibit, many more of the species may be utilized within the confines of the facility's policies.

Penguins, by nature, are social animals and thrive with interaction with others. Program penguins, and even exhibit animals, often will court and socially interact with their caretakers. The TAG recommends that program penguins be kept in a colony situation although separate accommodations for program birds are acceptable as long as spatial considerations and population numbers are appropriate. Penguin nutrition, daily consumption, and vitamin supplements should be monitored and records kept.

Animal care and education staff should be trained in program animal-specific handling protocols, conservation and education messaging techniques, and public interaction procedures. These staff members should be competent in recognizing stress or discomfort behaviors exhibited by the program animals and be able to address any safety issues that arise. Both exhibit animal and program animal locations require the land and water space formula delineated in this document. Penguins do not pose a large zoonotic risk to the handlers other than occasional bites from beaks and/or impacts from flippers.

The TAG recommends that each institution create their program animal handling policy that conforms to AZA guidelines as well as any local legislation. In general, penguins make good program animals and are usually displayed on a stage, floor or table, with constant monitoring of the handlers. Penguins may try to bite/poke guests, or even handlers, at any time during a program and it are suggested that handlers know the personality of the program birds before utilizing them. Handlers should always be aware of the bird's demeanor and the location of visitors. It is imperative that the penguins be kept away from human faces.

The TAG recommends that the handler of program penguins be aware of visitor interaction at all times. Food and beverage consumption for the handlers should be limited to non-animal areas always. Monitoring of the visitors requires ever-present vigilance. Penguins often poke at people that are within beak-range. Monitoring close approaches of visitors and knowing the personality of the penguin will help ensure a positive interaction for the guests.

Penguin stress, including heat stress and over stimulation, may manifest its presence in a number of ways. Some of the signs of stress are: reduced appetite, abnormally aggressive behavior, agitated attitude, lying down, attempts to get away from the presentation area, and heavy/open mouthed

breathing. If the animal is showing heat stress, check feet for warmth and isolate the bird in a cool dark area or return it as soon as possible to its exhibit or pen. For stress that appears to be from over stimulation, remove the bird from the presentation and kennel it in a quiet area. Later, gauge if the animal will be able to continue with its performance by judging its attitude. Do not continue if the penguin shows continued stress. The animal should be returned it to its exhibit or pen as soon as possible and supervisory staff should be alerted of the situation. Medical staff can also be contacted, if warranted.

The Penguin TAG recommends that when injuries occur to animals, they receive medical attention as soon as possible. The injury may not seem significant but to ensure continued health, seek medical counsel. Before an injury to a visitor or handler occurs, consult your Human Resource Department to determine the proper protocol if an injury should occur. Follow the protocol and contact HR as soon as possible.

Penguins are used in presentations often. The entire program including birds, programs and handlers should be reviewed annually. At this time, handler competency may be evaluated as well as during periodic institutional performance reviews. Any concerns with training performance may be addressed at this time and re-training or additional lessons may be instituted.

Program animals that are taken off zoo or aquarium grounds for any purpose have the potential to be exposed to infectious agents that could spread to the rest of the institution's healthy population. AZA-accredited institutions must have adequate protocols in place to avoid this (AZA Accreditation Standard 1.5.5).

Disease risk is inherent in all environments and it is impossible to eradicate this risk totally. It is best to review each program event and look at potential risks and try to minimize them. The TAG suggests that all outreach events with penguins ensure that only their facility has birds at the event. Additionally, at all events, indoor or outdoor, it is recommended that the program birds have dedicated kennels which will hold the birds any time they are not needed for a presentation and these kennels are kept away from visitors, other animals, and disturbance.

The TAG recommends using hand-washing stations, wipes and/or gels to limit disease transfer and contamination for all staff involved with program animals. All transport kennels should be cleaned thoroughly with facility-approved cleansers and disinfectants to help prevent disease after each use.

The use of chemical sanitation is important for all transport kennels, presentation surfaces and maintenance tools. There are a variety of sanitation chemicals available for proper hygiene. Consult with your animal management team and/or medical staff to identify the best chemical compounds for your situation.

Careful consideration must be given to the design and size of all program animal enclosures, including exhibit, off-exhibit holding, hospital, quarantine, and isolation areas, such that the physical, social, behavioral, and psychological needs of the species are met and species-appropriate behaviors are facilitated (AZA Accreditation Standard 10.3.3; AZA Accreditation Standard 1.5.2).

Similar consideration needs to be given to the means in which an animal will be transported both within the Institution's grounds, and to/from an off-grounds program. Animal transportation must be conducted in a manner that is lawful, safe, well planned, and coordinated, and minimizes risk to the animal(s), employees, and general public (AZA Accreditation Standard 1.5.11).

AZA Accreditation Standard

(1.5.5) For animals used in offsite programs and for educational purposes, the institution must have adequate protocols in place to protect the rest of the animals at the institution from exposure to infectious agents.

AZA Accreditation Standard

(10.3.3) All animal enclosures (exhibits, holding areas, hospital, and quarantine/isolation) must be of a size and complexity sufficient to provide for the animal's physical, social, and psychological well-being; and exhibit enclosures must include provisions for the behavioral enrichment of the animals. AZA housing guidelines outlined in the Animal Care Manuals should be followed.

AZA Accreditation Standard

(1.5.2) All animals must be housed in enclosures and in appropriate groupings which meet their physical, psychological, and social needs. Wherever possible and appropriate, animals should be provided the opportunity to choose among a variety of conditions within their environment. Display of single specimens should be avoided unless biologically correct for the species involved.

AZA Accreditation Standard

(1.5.11) Animal transportation must be conducted in a manner that is safe, well-planned and coordinated, and minimizes risk to the animal(s), employees, and general public. All applicable local, state, and federal laws must be adhered to. Planning and coordination for animal transport requires good communication among all involved parties, plans for a variety of emergencies and contingencies that may arise, and timely execution of the transport. At no time should the animal(s) or people be subjected to unnecessary risk or danger.

There are two basic methods to removing a penguin from an exhibit: train the bird to enter a kennel and then remove the kennel, or manually pick up/restrain the bird where upon it can be placed into an open kennel for transport or walked being hand-held to the desired location. It is not recommended to allow a penguin free-run of a van or other transport vehicle. The penguin will usually walk out of the open kennel once it has arrived at the desired location. When transporting a program bird from one location to the next, it is suggested that the penguin remain in the kennel for the duration of the transport. Crating suggestions are delineated in the above text.

The temperature restrictions for penguins depend upon the species that are being used in programs, the destination of the program and the policy of the institution's animal management team. With weather tolerant species such as African, Humboldt, or Magellanic penguins, extremes in temperature should be avoided. Be cautious having the penguin exposed to temperatures above 26 °C (80 °F) and below 4.4 °C (40 °F). Monitor behavior closely if rising temperatures or direct sunlight exposure is present. If a cold-weather species is to do a program in a situation where it is not climate controlled, please discuss the logistics with your animal management team to discuss the risks. There may be times when the physical environment can be modified to accommodate these species to maintain them is a safe and healthy manner.

As with all program animals, penguins will need breaks from being "on-stage." The TAG suggests a 30-minute on, 10-minute rest schedule for a penguin that is working in a program. The TAG does acknowledge that some programs may run somewhat longer and certain individual penguins can handle a longer "stage performance." Handlers that know their program animals well, how they react to stress, and are able to watch for signs is the key. Many penguins handle travel very well and overnight outreaches are acceptable as long as the animal's basic husbandry needs are addressed and a medical protocol is in place in case of concerns.

9.3 Program Evaluation

AZA-accredited institutions that have Institutional Program Animal Plan are required to evaluate the efficacy of the plan routinely (see Appendix E for recommendations). Education and conservation messaging content retention, animal health and well-being, guest responses, policy effectiveness, and accountability and ramifications of policy violations should be assessed and revised as needed.

The TAG suggests an annual review of all program animal plans. The supervisory staff of the program animals should monitor accountability. Biting issues with visitors, behavioral changes and/or reproductive concerns should be reported to the management in a timely manner. These concerns should be written on accident reporting forms, in daily reports or some other appropriate formal documentation.

The TAG does not mandate any specific disciplinary action in the event of mistakes or violations of policy in a program animal protocol. The TAG will suggest that violations be viewed as serious in nature with re-training, close review of handling privileges, additional supervisory monitoring and probationary implementation as possible actions items. Expectation surveys and other measurement techniques are on the market that may provide insight into the program's effectiveness. There are many facilities that have proven, in-house development/marketing department plans that address measurement and success of programs.

The TAG recommends an annual review of all animal programs as well as the formation and utilization of an Animal Welfare Policy that may address any and all staff concerns in a written and formal method. The TAG suggest that some type of program evaluation form be associated with penguin outreaches. A simple check-off form will often provide valuable information on the effectiveness of the success of a program and give additional insight into how to modify it to include conservation messages, natural history details, and other educational messaging in an engaging, highly palatable form.

Chapter 10. Research

10.1 Research Methods

AZA believes that contemporary animal management, husbandry, veterinary care and conservation practices should be based in science, and that a commitment to both basic and applied, scientific research, is a trademark of the modern zoological park and aquarium. An AZA accredited institution must demonstrate a commitment to scientific research that is in proportion to the size and scope of its facilities, staff and animal collections. AZA accredited institutions have the invaluable opportunity, to conduct or facilitate research both in *in situ* and *ex situ* settings with the goal of maximizing the scientific knowledge of the animals in our care and enhancing the conservation of wild populations. This might be achieved by participating in AZA Penguin TAG sponsored research, conducting original research projects, affiliating with local universities or conservation organizations, and/or employing staff with scientific credentials (AZA Accreditation Standard 5.3).

AZA Accreditation Standard

(5.3) The institution should maximize the generation of scientific knowledge gained from the animals. This might be achieved by participating in AZA TAG/SSP sponsored research when applicable, conducting original research projects, affiliating with local universities, and/or employing staff with scientific credentials.

Research, whether observational, behavioral, physiological, or genetically based, should have a clear scientific purpose with the reasonable expectation that it will increase our understanding of the species being investigated and may provide results which benefit animals in wild populations. Many AZA accredited institutions incorporate superior positive reinforcement training programs into their routine schedules to facilitate sensory, cognitive, and physiological research and these efforts are strongly encouraged by the AZA.

As with all taxa, thorough understanding of natural history, behavior, physiology, and other aspects of organismal biology are critical to providing the highest possible quality of husbandry. Penguins are among the taxa most closely managed on the individual level in AZA bird collections, with a large proportion of animals interacting directly with animal care staff on a daily basis. This makes penguins, as a group, easily accessible for many types of research. Many wild penguin populations have been intensively studied over the past 40 years, and therefore data exists for wild populations. Few avian taxa have such a superb interface of zoo and wild animal population research. As populations decline in the wild, and *ex situ* populations experience concerns surrounding sustainability, research in both managed and wild settings are of increasing and complimentary importance.

AZA-accredited institutions are required to have a clearly written research policy that identifies the types of research being conducted, methods used, staff involved, evaluations of the projects, the animals included, and guidelines for the reporting or publication of any findings (AZA Accreditation Standard 5.2). Institutions must designate a qualified individual to oversee and direct its research program (AZA Accreditation Standard 5.1). If institutions are not able to conduct in-house research investigations, they are strongly encouraged to provide financial, personnel, logistical, and other support for priority research and conservation initiatives identified by Taxon Advisory Groups (TAGs) or Species Survival Plans® (SSP) Programs.

AZA Accreditation Standard

(5.2) The institution must have a written policy that outlines the type of research that it conducts, methods, staff involvement, evaluations, animals to be involved, and guidelines for publication of findings.

AZA Accreditation Standard

(5.1) Research activities must be under the direction of a person qualified to make informed decisions regarding research.

10.2 Future Research

This Animal Care Manual is a dynamic document that will need to be updated as new information is acquired. Knowledge gaps have been identified throughout the manual and are included in this section to promote future research investigations. Any knowledge gained will help maximize AZA accredited institutions' capacity for excellence in animal care and welfare as well as advance conservation initiatives for the species.

Lighting: Artificial lighting in relation to the management of penguins in zoos and aquariums is an area that merits further research. Seasonal variation in light cycle, intensity and spectrum are essential for proper breeding and molting cycles. Some zoos and aquariums have reported enhanced reproductive

success with appropriate changes in day length and light intensity. Variations in molt have also been correlated with lighting schedules.

Diet: The mineral requirements of penguins have not been determined. Research may be helpful to determine if vitamin C can be synthesized by penguin tissues, and whether vitamin C deficiencies are relevant to penguin health. Definitive studies on the water requirements of penguins in zoo and aquariums have also not yet been conducted, and may be beneficial.

Mosquito control: The use of high velocity fans that are strategically placed within outdoor penguin enclosures to generate air currents in the hopes of creating an environment undesirable to mosquitoes warrants further consideration and testing. Further research on the success of this approach and other mosquito abatement research is needed.

West Nile virus: Penguins known to have had and recovered from this disease are believed to have some immunity against the virus, and may not need further vaccination. However, more information is required to determine the extent and duration of this immunity.

Irregular and incomplete molting patterns: Abnormal molting in some penguin species is a fairly common occurrence. Research is needed to determine the extent of the problem and to find ways to prevent and treatment this condition. Several pharmacological agents have been documented to induce molt in penguins with abnormal or arrested molts but further testing is needed.

Pharmacokinetic studies: Antibiotic and antifungal drugs are frequently administered to penguins empirically without actually knowing whether the amount or frequency of administration is adequate to reach and sustain effective levels. Pharmacokinetic studies of commonly used antimicrobial drugs are needed. Studies, even on an opportunistic basis, should be considered in managed penguins, or penguins in rehabilitation centers. Drug metabolism frequently varies among species, therefore these studies should occur across penguin species.

Field research: There are numerous opportunities to conduct or support field studies on species population size, dispersal patterns, migration, fishery use, artificial nest use, changing climate, and other factors that are affecting penguin populations and distribution. The use of geolocators and other technologies have created opportunities for additional areas of research. The Penguin TAG encourages institutions to support field programs and researchers.

The Global Penguin Society (www.globalpenguinsociety.org) is a non-profit conservation and research organization that “is dedicated to the survival and protection of the world´s penguin species, fostering integrated ocean conservation through science, management and community education.” The Penguin TAG supports the initiatives of GPS and supports its goals.

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Appendix A: Accreditation Standards by Chapter

The following specific standards of care relevant to penguins are taken from the AZA Accreditation Standards and Related Policies (AZA, 2011) and are referenced fully within the chapters of this animal care manual:

General Information

(1.1.1) The institution must comply with all relevant local, state, and federal laws and regulations, including those specific to wildlife. It is understood that, in some cases, AZA accreditation standards are more stringent than existing laws and regulations. In these cases the AZA standard must be met.

Chapter 1

(1.5.7) The animals must be protected from weather, and any adverse environmental conditions.

(10.2.1) Critical life-support systems for the animals, including but not limited to plumbing, heating, cooling, aeration, and filtration, must be equipped with a warning mechanism, and emergency backup systems must be available. All mechanical equipment must be kept in working order and should be under a preventative maintenance program as evidenced through a recordkeeping system. Special equipment should be maintained under a maintenance agreement, or a training record should show that staff members are trained for specified maintenance of special equipment.

(1.5.9) The institution must have a regular program of monitoring water quality for fish, pinnipeds, cetaceans, and other aquatic animals. A written record must be maintained to document long-term water quality results and chemical additions.

Chapter 2

(1.5.1) Animals should be presented in a manner reflecting modern zoological practices in exhibit design, balancing animals' functional welfare requirements with aesthetic and educational considerations.

(1.5.2) All animals must be housed in enclosures and in appropriate groupings which meet their physical, psychological, and social needs. Wherever possible and appropriate, animals should be provided the opportunity to choose among a variety of conditions within their environment. Display of single animals should be avoided unless biologically correct for the species.

(10.3.3) All animal enclosures (exhibits, holding areas, hospital, and quarantine/isolation) must be of a size and complexity sufficient to provide for the animal's physical, social, and psychological well-being; and exhibit enclosures must include provisions for the behavioral enrichment of the animals. AZA housing guidelines outlined in the Animal Care Manuals should be followed.

(10.3.4) When sunlight is likely to cause overheating of or discomfort to the animals, sufficient shade (in addition to shelter structures) must be provided by natural or artificial means to allow all animals kept outdoors to protect themselves from direct sunlight.

(11.3.3) Special attention must be given to free-ranging animals so that no undue threat is posed to either the institution's animals, the free-ranging animals, or the visiting public. Animals maintained where they will be in contact with the visiting public must be carefully monitored, and treated humanely at all times.

(11.3.1) All animal exhibits and holding areas must be secured to prevent unintentional animal egress.

(2.8.1) Pest control management programs must be administered in such a manner that the animals, staff, and public are not threatened by the pests, contamination from pests, or the control methods used.

(11.3.6) In areas where the public is not intended to have contact with animals, some means of deterring public contact with animals (e.g., guardrails/barriers) must be in place.

(11.2.4) All emergency procedures must be written and provided to staff and, where appropriate, to volunteers. Appropriate emergency procedures must be readily available for reference in the event of an actual emergency.

(11.2.5) Live-action emergency drills must be conducted at least once annually for each of the four basic types of emergency (fire; weather/environment appropriate to the region; injury to staff or a visitor;

animal escape). Four separate drills are required. These drills must be recorded and evaluated to determine that procedures are being followed, that staff training is effective, and that what is learned is used to correct and/or improve the emergency procedures. Records of these drills must be maintained and improvements in the procedures documented whenever such are identified.

(11.6.2) Security personnel, whether staff of the institution, or a provided and/or contracted service, must be trained to handle all emergencies in full accordance with the policies and procedures of the institution. In some cases, it is recognized that Security personnel may be in charge of the respective emergency (i.e. shooting teams).

(11.2.6) The institution must have a communication system that can be quickly accessed in case of an emergency.

(11.2.7) A written protocol should be developed involving local police or other emergency agencies and include response times to emergencies.

(11.5.3) Institutions maintaining potentially dangerous animals (e.g. large carnivores, large reptiles, medium to large primates, large hoofstock, killer whales, sharks, venomous animals, and others, etc.) must have appropriate safety procedures in place to prevent attacks and injuries by these animals. Appropriate response procedures must also be in place to deal with an attack resulting in an injury. These procedures must be practiced routinely per the emergency drill requirements contained in these standards. Whenever injuries result from these incidents, a written account outlining the cause of the incident, how the injury was handled, and a description of any resulting changes to either the safety procedures or the physical facility must be prepared and maintained for five years from the date of the incident.

(11.5.2) All areas housing venomous animals, or animals which pose a serious threat of catastrophic injury and/or death (e.g. large carnivores, large reptiles, medium to large primates, large hoofstock, killer whales, sharks, venomous animals, and others, etc.) must be equipped with appropriate alarm systems, and/or have protocols and procedures in place which will notify staff in the event of a bite injury, attack, or escape from the enclosure. These systems and/or protocols and procedures must be routinely checked to insure proper functionality, and periodic drills must be conducted to insure that appropriate staff members are notified.

(11.5.1) Institutions maintaining venomous animals must have appropriate antivenin readily available, and its location must be known by all staff members working in those areas. An individual must be responsible for inventory, disposal/replacement, and storage of antivenin.

Chapter 3

(1.5.11) Animal transportation must be conducted in a manner that is safe, well-planned and coordinated, and minimizes risk to the animal(s), employees, and general public. All applicable laws and/or regulations must be adhered to. Planning and coordination for animal transport requires good communication among all involved parties, plans for a variety of emergencies and contingencies that may arise, and timely execution of the transport. At no time should the animal(s) or people be subjected to unnecessary risk or danger.

(1.5.10) Temporary, seasonal and traveling live animal exhibits (regardless of ownership or contractual arrangements) must meet the same accreditation standards as the institution's permanent resident animals.

Chapter 5

(2.6.2) The institution should have a written nutrition program that meets the behavioral and nutritional needs of all species, individuals, and colonies/groups in the institution. Animal diets must be of a quality and quantity suitable for each animal's nutritional and psychological needs.

(2.6.1) Animal food preparations must meet all applicable laws and regulations.

(2.6.3) The institution should assign at least one person to oversee appropriate browse material for the collection.

Chapter 6

(2.1.1) A full-time staff veterinarian is recommended. In cases where such is not practical, a consulting/part-time veterinarian must be under written contract to make at least twice monthly inspections of the animals and to respond as soon as possible to any emergencies.

(2.1.2) So that indications of disease, injury, or stress may be dealt with promptly, veterinary coverage must be available to the animal collection 24 hours a day, 7 days a week.

(2.2.1) Written, formal procedures must be available to the animal care staff for the use of animal drugs for veterinary purposes, and appropriate security of the drugs must be provided.

(1.4.6) A staff member must be designated as being responsible for the institution's animal record-keeping system. That person must be charged with establishing and maintaining the institution's animal records, as well as with keeping all animal care staff members apprised of relevant laws and regulations regarding the institution's animals.

(1.4.7) Animal records must be kept current, and data must be logged daily.

(1.4.5) At least one set of the institution’s historical animal records must be stored and protected. Those records should include permits, titles, declaration forms, and other pertinent information.

(1.4.4) Animal records, whether in electronic or paper form, including health records, must be duplicated and stored in a separate location.

(1.4.3) Animals must be identifiable, whenever practical, and have corresponding ID numbers. For animals maintained in colonies/groups or other animals not considered readily identifiable, the institution must provide a statement explaining how record keeping is maintained.

(1.4.1) An animal inventory must be compiled at least once a year and include data regarding acquisitions and dispositions at the institution.

(1.4.2) All species owned by the institution must be listed on the inventory, including those animals on loan to and from the institution. In both cases, notations should be made on the inventory.

(2.7.1) The institution must have holding facilities or procedures for the quarantine of newly arrived animals and isolation facilities or procedures for the treatment of sick/injured animals.

(2.7.3) Quarantine, hospital, and isolation areas should be in compliance with standards/guidelines contained within the *Guidelines for Zoo and Aquarium Veterinary Medical Programs and Veterinary Hospitals* developed by the American Association of Zoo Veterinarians (AAZV), which can be obtained at: http://www.aazv.org/associations/6442/files/veterinary_standards_2009_final.docx.

(2.7.2) Written, formal procedures for quarantine must be available and familiar to all staff working with quarantined animals.

(11.1.2) Training and procedures must be in place regarding zoonotic diseases.

(11.1.3) A tuberculin (TB) testing/surveillance program must be established for appropriate staff in order to ensure the health of both the employees and the animals. Each institution must have an employee occupational health and safety program.

(2.5.1) Deceased animals should be necropsied to determine the cause of death. Cadavers must be stored in a dedicated storage area. Disposal after necropsy must be done in accordance with local/federal laws.

(2.4.1) The veterinary care program must emphasize disease prevention.

(1.5.5) For animals used in offsite programs and for educational purposes, the institution must have adequate protocols in place to protect the rest of the animals at the institution from exposure to infectious agents.

(2.3.1) Capture equipment must be in good working order and available to authorized, trained personnel at all times.

(2.4.2) Keepers should be trained to recognize abnormal behavior and clinical signs of illness and have knowledge of the diets, husbandry (including enrichment items and strategies), and restraint procedures required for the animals under their care. However, keepers should not diagnose illnesses nor prescribe treatment.

(2.3.2) Institution facilities should have radiographic equipment or have access to radiographic services.

(1.5.8) The institution must develop a clear process for identifying, communicating, and addressing animal welfare concerns within the institution in a timely manner, and without retribution.

Chapter 8

(1.6.1) The institution must have a formal written enrichment and training program that promotes species-appropriate behavioral opportunities.

(1.6.2) The institution must have specific staff member(s) or committee assigned for enrichment program oversight, implementation, training, and interdepartmental coordination of enrichment efforts.

Chapter 9

(1.5.4) A written policy on the use of live animals in programs must be on file. Animals in education programs must be maintained and cared for by trained staff, and housing conditions must meet standards set for the remainder of the animals in the institution, including species-appropriate shelter, exercise, social and environmental enrichment, access to veterinary care, nutrition, etc. Since some of these requirements can be met outside of the primary enclosure, for example, enclosures may be reduced in size provided that the animal's physical and psychological needs are being met.

(1.5.3) If animal demonstrations are part of the institution's programs, an educational/conservation message must be an integral component.

(1.5.5) For animals used in offsite programs and for educational purposes, the institution must have adequate protocols in place to protect the rest of the animals at the institution from exposure to infectious agents.

(10.3.3) All animal enclosures (exhibits, holding areas, hospital, and quarantine/isolation) must be of a size and complexity sufficient to provide for the animal's physical, social, and psychological well-being; and exhibit enclosures must include provisions for the behavioral enrichment of the animals. AZA housing guidelines outlined in the Animal Care Manuals should b e followed.

(1.5.2) All animals must be housed in enclosures and in appropriate groupings which meet their physical, psychological, and social needs. Wherever possible and appropriate, animals should be provided the opportunity to choose among a variety of conditions within their environment. Display of single animals should be avoided unless biologically correct for the species.

(1.5.11) Animal transportation must be conducted in a manner that is safe, well-planned and coordinated, and minimizes risk to the animal(s), employees, and general public. All applicable laws and/or regulations must be adhered to. Planning and coordination for animal transport requires good communication among all involved parties, plans for a variety of emergencies and contingencies that may arise, and timely execution of the transport. At no time should the animal(s) or people be subjected to unnecessary risk or danger.

Chapter 10

(5.3) The institution should maximize the generation of scientific knowledge gained from the animals. This might be achieved by participating in AZA TAG/SSP sponsored research when applicable, conducting original research projects, affiliating with local universities, and/or employing staff with scientific credentials.

(5.2) Institutions must have a written policy that outlines the type of research that it conducts, methods, staff involvement, evaluations, animals to be involved, and guidelines for publication of findings.

(5.1) Research activities must be under the direction of a person qualified to make informed decisions regarding research.

Appendix B: AZA Policy on Responsible Population Management: Acquisitions, Transfers and Transitions by Zoos & Aquariums

PREAMBLE

The Association of Zoos & Aquariums (AZA) was established, among other reasons, "…to foster continued improvement of the zoological park and aquarium profession through the development and regulation of high standards of ethics, conduct, education and scholarly attainments." The stringent requirements for AZA accreditation and high standards of professional conduct are unmatched by similar organizations and also far surpass the United States Department of Agriculture's Animal and Plant Health Inspection Service's requirements for licensed animal exhibitors. Every AZA member must abide by a Code of Professional Ethics (https://www.aza.org/Ethics/). In order to continue these high standards, AZA-accredited institutions and certified related facilities should make it a priority, when possible, to acquire animals from and transfer them to other AZA member institutions or other regional zoo associations and their members.

AZA-accredited institutions and certified related facilities cannot fulfill their important missions of conservation, education, and science without living animals. Responsible management and the long-term sustainability of living animal populations necessitates that some individuals be acquired and that others be transferred or transitioned at certain times. Furthermore, priority for acquisition and transfer activities should be the long-term sustainability of living animal populations among AZA-accredited and certified related facilities, and between AZA member institutions and non-AZA entities with animal care and welfare standards aligned with AZA. AZA member institutions that acquire animals from the wild, directly or through commercial vendors, should perform due diligence to ensure that zoos/aquariums are not creating a commercial market that promotes the taking of those animals from nature and/or that is detrimental to the survival of species in the wild. Animals should only be solicited and acquired from non-AZA entities that are known to operate legally and conduct their business in a manner that reflects and/or supports the spirit and intent of the AZA Code of Professional Ethics as well as this Policy.

In this Policy "AZA member institutions" refers to AZA-accredited institutions and certified related facilities (zoological parks and aquariums). "AZA members" may refer to either institutions or individuals.

Non – AZA entities includes facilities not accredited or certified by the AZA, facilities in other zoological regions, academic institutions, museums, research facilities, private individuals, etc.

I. INTRODUCTION

The AZA Acquisition, Transfer and Transition Policy was created to help (1) guide and support AZA-accredited and certified related facilities in their animal acquisition and transfer/transition decisions, and (2) make certain that all acquisitions and transfers/transitions are compatible with the Association's stated commitment to save and protect the wonders of the living natural world. This AZA Acquisition, Transfer and Transition Policy applies to individual animals, groups/colonies, and specimens (animal parts, materials, and products). More specifically, the AZA Acquisition, Transfer and Transition Policy provides guidance to AZA members to:

1. assure that the health and welfare of individual animals is considered during acquisition and transfer/transition activities,

Acquisition of animals can occur through breeding (births, hatchings, cloning, and division of marine invertebrates = "fragging"), trade, donation, lease, loan, transfer (inter- and intra-institution), purchase, collection, confiscation, appearing on zoo property, or rescue and/or rehabilitation for release.

Transfer/transition occurs when an animal leaves the institution for any reason. Reasons for transfer or transition may include cooperative population management (genetic, demographic or behavioral management), animal welfare or behavior management reasons (including sexual maturation and individual management needs). Types of transfer include withdrawal through donation, trade, lease, loan, inter- and intra-institution transfers, sale, escape, theft. Types of transition include reintroduction to the wild, humane euthanasia or natural death.

"Dispose/Disposing of" in this document is limited to complete and permanent removal of an individual via incineration, burying or other means of permanent destruction.

2. assure that the health and conservation of populations, species, and ecosystems are carefully considered during acquisition and transfer/transition activities,

3. maintain a proper standard of conduct for AZA members during acquisition and transfer/transition activities, including adherence to all applicable laws and regulations,

4. assure that animals from AZA member institutions and certified related facilities are not transferred to individuals or organizations that lack the appropriate expertise or facilities to care for them [*see taxa specific appendices (in development)*], and

5. support the goals of AZA's cooperatively managed populations and associated Animal Programs [Species Survival Plans® (SSPs), Studbooks, and Taxon Advisory Groups (TAGs)].

Attempts by members to circumvent AZA Animal Programs in the acquisition of animals can be detrimental to the Association and its Animal Programs. Such action may also be detrimental to the species involved and may be a violation of the Association's Code of Professional Ethics.

AZA's scientifically-managed Animal Programs, including SSPs, have successfully bred and reintroduced critically endangered species for the benefit of humankind. To accomplish these critical conservation goals, populations must be managed within "carrying capacity" limits. At times, the number of individual animals in a population exceeds carrying capacity, and while meaning no disrespect for these individual animals, we refer to these individual animals as "extra" within the managed population.

This AZA Acquisition, Transfer and Transition Policy will serve as the default policy for AZA member institutions. Institutions may develop their own Acquisition, Transfer and Transition Policy in order to address specific local concerns. Any institutional policy must incorporate and not conflict with the AZA acquisition and transfer/transition standards.

II. LAWS, AUTHORITY, RECORD-KEEPING, IDENTIFICATION AND DOCUMENTATION

The following must be considered with regard to the acquisition or transfer/transition of all living animals and specimens (their living and non-living parts, materials, and/or products):

Examples of specimens include animal parts, materials and products including bodily fluids, cell lines, clones, digestive content, DNA, feces, marine invertebrate (coral) fragments ("frags"), germplasm, and tissues

1. Any acquisitions, transfers, and transitions must meet the requirements of all applicable local, state, federal and international laws and regulations. Ownership and any applicable chain-of-custody must be documented. If such information does not exist, an explanation must be provided regarding such animals and specimens. Any acquisition of free-ranging animals must be done in accordance with all local, state, federal, and international laws and regulations and must not be detrimental to the long-term viability of the species in the wild.

2. The Director/Chief Executive Officer of the institution must have final authority for all acquisitions and transfers/transitions.

3. Acquisitions or transfers/transitions must be documented through institutional record keeping systems. The ability to identify which animal is being transferred is very important and the method of identifying the animal should be documented. Any existing documentation must accompany all transfers. To standardize institutional animal records data, records guidelines have been developed for certain species (https://www.aza.org/AnimalCare/detail.aspx?id=3150).

Examples of colonial, group-living, or prolific species include and are not limited to certain terrestrial and aquatic invertebrates, fish, sharks/rays, amphibians, reptiles, birds, rodents, bats, big herds, and other mammals,

4. For some colonial, group-living, or prolific species, it may be impossible or highly impractical to identify individual animals when these individuals are maintained in a group. When considered as a group, these species are therefore maintained, acquisitioned, transferred, and transitioned as a group or colony, or as part of a group or colony.

5. If the intended use of specimens is to create live animal(s), their acquisition and transfer should follow the same guidelines. If germplasm is acquired or transferred with the intention of creating live animal(s), ownership of the offspring must be clearly defined in transaction documents (e.g., breeding loan agreements).

   Institutions acquiring, transferring, transitioning or disposing of specimens should consider current and possible future uses as new technologies become available. All specimens from which nuclear DNA could be recovered should be carefully considered as these basic DNA extraction technologies already exist.

6. AZA member institutions must maintain transaction documents (e.g., confirmation forms, breeding agreements) which provide the terms and conditions of animal acquisitions, transfers and loans, including documentation for animal parts, products and materials. These documents should require the potential recipient or provider to adhere to the AZA Acquisition, Transfer and Transition Policy, all relevant AZA and member policies, procedures and guidelines, and the AZA Code of Professional Ethics, and must require compliance with the applicable laws and regulations of local, state, federal, and international authorities.

7. In the case of animals (living or non-living) and their parts, materials, or products (living or non-living) held on loan, the owner's written permission should be obtained prior to any transfer and should be documented in the institutional records.

8. AZA SSP and TAG necropsy and sampling protocols should be accommodated.

9. Some governments maintain ownership of the species found within their borders. It is therefore incumbent on institutions to determine whether animals they are acquiring or transferring are owned by a government entity, foreign or domestic, and act accordingly by reviewing the government ownership policies available on the AZA website. In the case of government owned animals, proposals for and/or notifications of transfers must be sent to the species manager for the government owned species.

Transaction documents must be signed by the authorized representatives of both parties, and copies must be retained by both parties*. In the case of loans, the owner's permission for appropriate activities should be documented in the institutional records. This document(s) should be completed prior to any transfer. In the case of rescue, confiscation, and evacuation due to natural disasters, it is understood that documents may not be available until after acceptance or shipping. In this case documentation (e.g., a log) must be kept to reconcile the inventory and chain of custody after the event occurs.

*In the case of government owned animals, notification of transfers must be sent to species manager for the government owned species.

III. ACQUISITION REQUIREMENTS

A. General Acquisitions

1. Acquisitions must be consistent with the mission of the institution, as reflected in its Institutional Collection Plan, by addressing its exhibition/education, conservation, and/or scientific goals.

2. Animals (wild, feral, and domestic) may be held temporarily for reasons such as assisting governmental agencies or other institutions, rescue and/or rehabilitation, research, propagation or headstarting for reintroduction, or special exhibits.

Feral animals are animals that have escaped from domestication or have been abandoned to the wild and have become wild, and the offspring of such animals. Feral animals may be acquired for temporary or permanent reasons.

3. Any receiving institution must have the necessary expertise and resources to support and provide for the professional care and management of the species, so that the physical, psychological, and social needs of individual animals and species are met.

4. If the acquisition involves a species managed by an AZA Animal Program, the institution should communicate with the Animal Program Leader and, in the case of Green SSP Programs, must adhere to the AZA Full Participation Policy (http://www.aza.org/full-participation-in-ssp-program-policy/).

5. AZA member institutions should consult AZA Wildlife Conservation and Management Committee (WCMC)-approved TAG Regional Collection Plans (RCPs), Animal Program Leaders, and AZA Animal Care Manuals (ACMs) when making acquisition decisions.

6. AZA member institutions that work with commercial vendors that acquire animals from the wild, must perform due diligence to assure the vendors' collection of animals is legal. Commercial vendors should have conservation and animal welfare goals similar to those of AZA institutions.

7. AZA member institutions may acquire animals through public donations and other non-AZA entities when it is in the best interest of the animal and/or species.

B. Acquisitions from the Wild

Saving species and wild animal populations for education and wildlife conservation purposes is a unique responsibility of AZA member zoos and aquariums. The AZA recognizes that there are circumstances where acquisitions from the wild are needed in order to maintain healthy, diverse animal populations and to support the objectives of managed species programs, in which case acquisitions from the wild may be a preferable choice to breeding in human care.

Acquiring animals from the wild can result in socioeconomic benefit and environmental protection and therefore the AZA encourages environmentally sustainable/beneficial acquisition from the wild when conservation is a positive outcome.

1. Before acquiring animals from the wild, institutions are encouraged to examine alternative sources including other AZA institutions and other regional zoological associations or other non-AZA entities.

2. When acquiring animals from the wild, both the long-term health and welfare impacts on the wild population as well as on individual animals must be considered. In crisis situations, when the survival of a population is at risk, rescue decisions will be made on a case-by-case basis by the appropriate agency and institution.

3. Institutions should only accept animals from the wild after a risk assessment determines the zoo/aquarium can mitigate any potential adverse impacts on the health, care and maintenance of the permanently housed animals, and the animals being acquired.

The Lacey Act prohibits the importation, exportation, transportation, sale, receipt, acquisition or purchase of wildlife taken or possessed in violation of any law, treaty or regulation of the United States or any Indian tribal law of wildlife law.

In cases when there is no documentation accompanying an acquisition, the animal(s) may not be transferred across state lines. If the animal was illegally acquired at any time then any movement across state or international borders would be a violation of the Lacey Act.

IV. TRANSFER AND TRANSITION REQUIREMENTS

A. Living Animals

Successful conservation and animal management relies on the cooperation of many entities, both AZA and non-AZA. While preference is given to placing animals with AZA-accredited institutions or certified related facilities, it is important to foster a cooperative culture among those who share AZA's mission of saving species.

Attempts by members to circumvent AZA Animal Programs in the transfer or transition of animals may be detrimental to the Association and its Animal Programs (unless the animal or animals are deemed extra in the Animal Program population by the Animal Program Coordinator). Such action may be detrimental to the species involved and may be a violation of the Association's Code of Professional Ethics.

1. Any transfer must abide by the Mandatory Standards and General Advisories of the AZA Code of Professional Ethics which indicates that AZA members should assure that all animals in their care are transferred and transitioned in a manner that meets the standards of the Association, and that animals are not transferred or transitioned to those not qualified to care for them properly.

2. If the transfer of animals or their specimens (parts, materials, and products) involves a species managed by an AZA Animal Program, the institution should communicate with that Animal Program Leader and, in the case of Green SSP Programs must adhere to the AZA Full Participation Policy (http://www.aza.org/full-participation-in-ssp-program-policy/).

3. AZA member institutions should consult WCMC-approved TAG Regional Collection Plans, Animal Program Leaders, and Animal Care Manuals when making transfer decisions.

4. Animals acquired as animal feed are not typically accessioned into the collection. There may be occasions, however, when it is appropriate to use accessioned animals that exceed population carrying capacity as feeder animals to support other animals. In some cases, accessioned animals may be transitioned to "feeder animal" status by the local institution as part of their program for long-term sustained population management of the species.

5. In transfers to non-AZA entities, AZA members must perform due diligence and should have documented validation, such as a letter of reference, that the recipient has the expertise and resources required to properly care for and maintain the animals. Supporting documentation must be kept at the AZA member institution.

Examples of documentation include ZIMS records, "Breeding Loan" agreements, chain-of-custody logs, letters of reference, transfer agreements, and transaction documents

6. Domestic animals should be transferred in accordance with locally acceptable farm practices, including auctions, and subject to all relevant laws and regulations.

Examples of domestic animals may include certain camelids, cattle, cats, dogs, ferrets, goats, pigs, reindeer, rodents, sheep, budgerigars, chickens, doves, ducks, geese, pheasants, turkeys, and goldfish or koi.

7. AZA members must not send any non-domestic animal to auction or to any organization or individual that may display or sell the animal at an animal auction. *See certain taxa-specific appendices to this Policy (in development) for information regarding exceptions*.

8. Animals must not be sent to organizations or individuals that allow the hunting of these individual animals; that is, no animal from an AZA institution may be hunted. For purposes of maintaining sustainable zoo and aquarium populations, AZA-accredited institutions and certified related facilities may send animals to non-AZA organizations or individuals. These non-AZA entities (for instance, ranching operations) should follow appropriate ranch management practices and other conservation minded practices to support population sustainability.

9. Every loaning institution must annually monitor and document the conditions of any loaned specimen(s) and the ability of the recipient(s) to provide proper care. If the conditions and care of animals are in violation of the loan agreement, the loaning institution must recall the animal or assure prompt correction of the situation. Furthermore, an institution's loaning policy must not be in conflict with this AZA Acquisition, Transfer and Transition Policy.

10. If living animals are sent to a non-AZA entity for research purposes, it must be a registered research facility by the U.S. Department of Agriculture and accredited by the Association for the Assessment & Accreditation of Laboratory Animal Care, International (AAALAC), if eligible. For international transactions, the receiving facility must be registered by that country's equivalent body having enforcement over animal welfare. In cases where research is conducted, but governmental oversight is not required, institutions should do due diligence to assure the welfare of the animals during the research.

11. Transition: reintroductions and release to the wild. The reintroduction of animals must meet all applicable local, state, and international laws and regulations. Reintroductions may be a part of a recovery program and must be compatible with the IUCN Reintroduction Specialist Group's Reintroduction Guidelines (http://www.iucnsscrsg.org/index.php).

Examples of "Transition" include movements of animals from zoo/aquarium populations to the wild through reintroductions or other legal means, or the transition of an animal from living to dead.

12. Transition: humane euthanasia. Humane euthanasia may be employed for medical reasons to address quality of life issues for animals or to prevent the transmission of disease. AZA also recognizes that humane euthanasia may be employed for managing the demographics, genetics, and diversity of animal populations. Humane euthanasia must be performed in accordance with the established euthanasia policy of the institution and follow the recommendations of current AVMA Guidelines for the Euthanasia of Animals (2013 Edition https://www.avma.org/KB/Policies/Documents/euthanasia.pdf) or the AAZV's Guidelines on the Euthanasia of Non-Domestic Animals.

B. Non-Living Animals and Specimens

AZA members should optimize the use and recovery of animal remains. All transfers must meet the requirements of all applicable laws and regulations.

1. Optimal recovery may include performing a complete necropsy including, if possible, histologic evaluation of tissues which should be a key component of optimal recovery before specimens' use in education/exhibits. AZA SSP and TAG necropsy and sampling protocols should be accommodated. This information should be available to SSP Programs for population management.

It is best practice for modern zoos and aquariums to establish relationships with nearby museums or other biorepositories, so that they can maximize the value of animals when they die (e.g., knowing who to call when they have an animal in necropsy, or specimens for cryopreservation).

Natural history museums that are members of the Natural Science Collections Alliance (NSCA) and frozen biorepositories that are members of the International Society of Biological and Environmental Repositories (ISBER) are potential collaborators that could help zoos find appropriate repositories for biological specimens.

2. The educational use of non-living animals, parts, materials, and products should be maximized, and their use in Animal Program sponsored projects and other scientific projects that provide data for species management and/or conservation must be considered.

3. Non-living animals, if handled properly to protect the health of the recipient animals, may be utilized as feeder animals to support other animals as deemed appropriate by the institution.

4. AZA members should consult with AZA Animal Program Leaders prior to transferring or disposing of remains/samples to determine if existing projects or protocols are in place to optimize use.

5. AZA member institutions should develop agreements for the transfer or donation of non-living animals, parts, materials, products, and specimens and associated documentation, to non-AZA entities such as universities and museums. These agreements should be made with entities that have appropriate long term curation/collections capacity and research protocols, or needs for educational programs and/or exhibits.

When specimens are transferred, the transferring and receiving institutions should agree on data that must be transferred with the specimen(s). Examples of associated documentation include provenance of the animal, original permits, tags and other metadata, life history data for the animal, how and when specimens were collected and conserved, etc.

Appendix C: Recommended Quarantine Procedures

Quarantine facility: A separate quarantine facility, with the ability to accommodate mammals, birds, reptiles, amphibians, and fish should exist. If a specific quarantine facility is not present, then newly acquired animals should be isolated from the established collection in such a manner as to prohibit physical contact, to prevent disease transmission, and to avoid aerosol and drainage contamination.

Such separation should be obligatory for primates, small mammals, birds, and reptiles, and attempted wherever possible with larger mammals such as large ungulates and carnivores, marine mammals, and cetaceans. If the receiving institution lacks appropriate facilities for isolation of large primates, pre-shipment quarantine at an AZA or American Association for Laboratory Animal Science (AALAS) accredited institution may be applied to the receiving institutions protocol. In such a case, shipment must take place in isolation from other primates. More stringent local, state, or federal regulations take precedence over these recommendations.

Quarantine length: Quarantine for all species should be under the supervision of a veterinarian and consist of a minimum of 30 days (unless otherwise directed by the staff veterinarian). Mammals: If during the 30-day quarantine period, additional mammals of the same order are introduced into a designated quarantine area, the 30-day period must begin over again. However, the addition of mammals of a different order to those already in quarantine will not have an adverse impact on the originally quarantined mammals. Birds, Reptiles, Amphibians, or Fish: The 30-day quarantine period must be closed for each of the above Classes. Therefore, the addition of any new birds into a bird quarantine area requires that the 30-day quarantine period begin again on the date of the addition of the new birds. The same applies for reptiles, amphibians, or fish.

Quarantine personnel: A keeper should be designated to care only for quarantined animals or a keeper should attend quarantined animals only after fulfilling responsibilities for resident species. Equipment used to feed and clean animals in quarantine should be used only with these animals. If this is not possible, then equipment must be cleaned with an appropriate disinfectant (as designated by the veterinarian supervising quarantine) before use with post-quarantine animals.

Institutions must take precautions to minimize the risk of exposure of animal care personnel to zoonotic diseases that may be present in newly acquired animals. These precautions should include the use of disinfectant foot baths, wearing of appropriate protective clothing and masks in some cases, and minimizing physical exposure in some species; e.g., primates, by the use of chemical rather than physical restraint. A tuberculin testing/surveillance program must be established for zoo/aquarium employees in order to ensure the health of both the employees and the animal collection.

Quarantine protocol: During this period, certain prophylactic measures should be instituted. Individual fecal samples or representative samples from large numbers of individuals housed in a limited area (e.g., birds of the same species in an aviary or frogs in a terrarium) should be collected at least twice and examined for gastrointestinal parasites. Treatment should be prescribed by the attending veterinarian. Ideally, release from quarantine should be dependent on obtaining two negative fecal results spaced a minimum of two weeks apart either initially or after parasiticide treatment. In addition, all animals should be evaluated for ectoparasites and treated accordingly.

Vaccinations should be updated as appropriate for each species. If the animal arrives without a vaccination history, it should be treated as an immunologically naive animal and given an appropriate series of vaccinations. Whenever possible, blood should be collected and sera banked. Either a 70 °C (-94 °F) frost-free freezer or a 20 °C (-4 °F) freezer that is not frost-free should be available to save sera. Such sera could provide an important resource for retrospective disease evaluation.

The quarantine period also represents an opportunity to, where possible, permanently identify all unmarked animals when anesthetized or restrained (e.g., tattoo, ear notch, ear tag, etc.). Also, whenever animals are restrained or immobilized, a complete physical, including a dental examination, should be performed. Complete medical records should be maintained and available for all animals during the quarantine period. Animals that die during quarantine should have a necropsy performed under the supervision of a veterinarian and representative tissues submitted for histopathologic examination.

Quarantine procedures: Penguins should be quarantine for a minimum of 30 days unless otherwise directed by the staff veterinarian. It may be extended problems are diagnosed. It can be shortened if examination has shown no problems and it is behaviorally necessary for the well-being of the animals.

If additional birds are introduced during the quarantine period, the quarantine must begin again. However, the addition of animals besides birds may not require the re-initiation of the quarantine period. If the new additions do not show signs of infectious disease, the first set of animals may clear quarantine without re-examination

Separate facilities are recommended to accommodate newly acquired birds, or birds that must be separated from the group for health-related reasons. This area should have air and water systems separate from the main exhibit. It can serve as an isolation area if not in use for quarantine. An area without separate air and water systems should not be considered an appropriate quarantine or isolation area .. If possible, two or more birds should be quarantined together because of their social needs.. If this is not possible, efforts should be made for quarantined birds to have visual or auditory contact with other penguins. Designated keepers should care only for quarantined animals if possible. If keepers must care for both quarantined and resident animals of the same taxa, they should care for the quarantined animals only after caring for the resident animals. Any equipment or enrichment items used for quarantined animals should be used only with these animals. If this is not possible, then all items must be appropriately disinfected, as designated by the veterinarian supervising quarantine, before being use elsewhere. Standard disinfection with quaternary ammonium or bleach is adequate unless a mycobacterial disease is suspected, in which case ammonium-based products are not suitable. Phenolics can be used but can be corrosive. Enrichment items that are not easily cleaned can be thrown out and replaced if needed (infectious disease diagnosed or suspected).

AZA institutions must have zoonotic disease prevention procedures and training protocols established to minimize the risk of transferable diseases (AZA Accreditation Standard 11.1.2) with all animals, including those newly acquired in quarantine. Although transmission of tuberculosis from penguins to humans is not of concern, penguins an potentially carry gastrointestinal bacteria that cause disease in people. A separate set of Personal Protective Equipment (PPE) should be worn when handling or cleaning quarantined animals. This includes outerwear such as washable or disposable smocks, aprons, overalls or gowns, surgical masks, gloves and a separate set of boots or shoe covers.

Recommended minimum quarantine space, pool, and temperature recommendations are listed in space recommendations (Chapter 2). Use non-abrasive flooring or matting if at all possible.

Quarantine veterinary procedures: During the quarantine period, a complete physical examination and specific diagnostic tests should be conducted for each animal (see Appendix C). .. Animals should be permanently identified during quarantine if not already, Animals should be evaluated for ectoparasites and gastrointestinal parasites, and treated accordingly. Blood should be collected, analyzed and the sera banked long-term in either a -70 °C freezer or short-term in -20 °C freezer (frost-free or self-defrosting freezer should not be used because of the freeze-thaw cycles) for retrospective evaluation. Vaccinations should be updated as appropriate, and if the vaccination history is not known, the animal should be treated as immunologically naive and given the appropriate series of vaccinations. Detailed medical records for each animal should be maintained and easily available

Release from quarantine should be contingent upon normal results from diagnostic testing, and three negative fecal parasite exams and fecal/cloacal cultures that are spaced a minimum of 1 week apart. If at all possible, radiographs should be taken to establish a baseline reference for each individual and to check for evidence of disease, gastrointestinal foreign bodies, or evidence of previous trauma (fractures).

Aspergillus prevention: Aspergillosis is a severe fungal disease and often affects penguins under stress. In addition to receiving anti-fungals prior to shipment (AZA standard 6.3), animals should also receive it for at least 2 weeks after arrival into quarantine until they are acclimated to their new surroundings.

The following are recommendations and suggestions for appropriate quarantine procedures for penguins:

Penguin (Spheniscidae):

Required:

1. Direct and floatation fecals
2. Vaccinate as appropriate

Strongly recommended:

1. CBC/sera profile
2. Urinalysis
3. Appropriate serology (FIP, FeLV, FIV)
4. Heartworm testing in appropriate species

Appendix D: Program Animal Policy and Position Statement

Program Animal Policy

Originally approved by the AZA Board of Directors—2003
Updated and approved by the Board—July 2008 & June 2011

The Association of Zoos & Aquariums (AZA) recognizes many benefits for public education and, ultimately, for conservation in program animal presentations. AZA's Conservation Education Committee's *Program Animal Position Statement* summarizes the value of program animal presentations (see pages 42–44).

For the purpose of this policy, a Program Animal is defined as "an animal whose role includes handling and/or training by staff or volunteers for interaction with the public and in support of institutional education and conservation goals." Some animals are designated as Program Animals on a full-time basis, while others are designated as such only occasionally. Program Animal-related Accreditation Standards are applicable to all animals during the times that they are designated as Program Animals.

There are three main categories of Program Animal interactions:

1. On Grounds with the Program Animal Inside the Exhibit/Enclosure:
    a. Public access outside the exhibit/enclosure. Public may interact with animals from outside the exhibit/enclosure (e.g., giraffe feeding, touch tanks).
    b. Public access inside the exhibit/enclosure. Public may interact with animals from inside the exhibit/enclosure (e.g., lorikeet feedings, 'swim with' programs, camel/pony rides).
2. On Grounds with the Program Animal Outside the Exhibit/Enclosure:
    a. Minimal handling and training techniques are used to present Program Animals to the public. Public has minimal or no opportunity to directly interact with Program Animals when they are outside the exhibit/enclosure (e.g., raptors on the glove, reptiles held "presentation style").
    b. Moderate handling and training techniques are used to present Program Animals to the public. Public may be in close proximity to, or have direct contact with, Program Animals when they're outside the exhibit/enclosure (e.g., media, fund raising, photo, and/or touch opportunities).
    c. Significant handling and training techniques are used to present Program Animals to the public. Public may have direct contact with Program Animals or simply observe the in-depth presentations when they're outside the exhibit/enclosure (e.g., wildlife education shows).
3. Off Grounds:
    a. Handling and training techniques are used to present Program Animals to the public outside of the zoo/aquarium grounds. Public may have minimal contact or be in close proximity to and have direct contact with Program Animals (e.g., animals transported to schools, media, fund raising events).

These categories assist staff and accreditation inspectors in determining when animals are designated as Program Animals and the periods during which the Program Animal-related Accreditation Standards are applicable. In addition, these Program Animal categories establish a framework for understanding increasing degrees of an animal's involvement in Program Animal activities.

Program animal presentations bring a host of responsibilities, including the safety and welfare of the animals involved, the safety of the animal handler and public, and accountability for the take-home, educational messages received by the audience. Therefore, AZA requires all accredited institutions that make program animal presentations to develop an institutional program animal policy that clearly identifies and justifies those species and individuals approved as program animals and details their long-term management plan and educational program objectives.

AZA's accreditation standards require that education and conservation messages must be an integral component of all program animal presentations. In addition, the accreditation standards require that the

conditions and treatment of animals in education programs must meet standards set for the remainder of the animal collection, including species-appropriate shelter, exercise, appropriate environmental enrichment, access to veterinary care, nutrition, and other related standards. In addition, providing program animals with options to choose among a variety of conditions within their environment is essential to ensuring effective care, welfare, and management. Some of these requirements can be met outside of the primary exhibit enclosure while the animal is involved in a program or is being transported. For example, free-flight birds may receive appropriate exercise during regular programs, reducing the need for additional exercise. However, the institution must ensure that in such cases, the animals participate in programs on a basis sufficient to meet these needs or provide for their needs in their home enclosures; upon return to the facility the animal should be returned to its species-appropriate housing as described above.

Program Animal Position Statement

Last revision 1/28/03
Re-authorized by the Board June 2011

The Conservation Education Committee (CEC) of the Association of Zoos and Aquariums supports the appropriate use of program animals as an important and powerful educational tool that provides a variety of benefits to zoo and aquarium educators seeking to convey cognitive and affective (emotional) messages about conservation, wildlife and animal welfare.
Utilizing these animals allows educators to strongly engage audiences. As discussed below, the use of program animals has been demonstrated to result in lengthened learning periods, increased knowledge acquisition and retention, enhanced environmental attitudes, and the creation of positive perceptions concerning zoo and aquarium animals.

Audience Engagement

Zoos and aquariums are ideal venues for developing emotional ties to wildlife and fostering an appreciation for the natural world. However, developing and delivering effective educational messages in the free-choice learning environments of zoos and aquariums is a difficult task.
Zoo and aquarium educators are constantly challenged to develop methods for engaging and teaching visitors who often view a trip to the zoo as a social or recreational experience (Morgan & Hodgkinson, 1999). The use of program animals can provide the compelling experience necessary to attract and maintain personal connections with visitors of all motivations, thus preparing them for learning and reflection on their own relationships with nature.

Program animals are powerful catalysts for learning for a variety of reasons. They are generally active, easily viewed, and usually presented in close proximity to the public. These factors have proven to contribute to increasing the length of time that people spend watching animals in zoo exhibits (Bitgood, Patterson & Benefield, 1986, 1988; Wolf & Tymitz, 1981).

In addition, the provocative nature of a handled animal likely plays an important role in captivating a visitor. In two studies (Povey, 2002; Povey & Rios, 2001), visitors viewed animals three and four times longer while they were being presented in demonstrations outside of their enclosure with an educator than while they were on exhibit. Clearly, the use of program animals in shows or informal presentations can be effective in lengthening the potential time period for learning and overall impact.

Program animals also provide the opportunity to personalize the learning experience, tailoring the teaching session to what interests the visitors. Traditional graphics offer little opportunity for this level of personalization of information delivery and are frequently not read by visitors (Churchman, 1985; Johnston, 1998). For example, Povey (2001) found that only 25% of visitors to an animal exhibit read the accompanying graphic; whereas, 45% of visitors watching the same animal handled in an educational presentation asked at least one question and some asked as many as seven questions. Having an animal accompany the educator allowed the visitors to make specific inquiries about topics in which they were interested.

Knowledge Acquisition

Improving our visitors' knowledge and understanding regarding wildlife and wildlife conservation is a fundamental goal for many zoo educators using program animals. A growing body of evidence supports the validity of using program animals to enhance delivery of these cognitive messages as well.

- MacMillen (1994) found that the use of live animals in a zoomobile outreach program significantly enhanced cognitive learning in a vertebrate classification unit for sixth grade students.
- Sherwood and his colleagues (1989) compared the use of live horseshoe crabs and sea stars to the use of dried specimens in an aquarium education program and demonstrated that students made the greatest cognitive gains when exposed to programs utilizing the live animals.
- Povey and Rios (2002) noted that in response to an open-ended survey question ("Before I saw this animal, I never realized that . . . "), visitors watching a presentation utilizing a program animal provided 69% cognitive responses (i.e., something they learned) versus 9% made by visitors viewing the same animal in its exhibit (who primarily responded with observations).
- Povey (2002) recorded a marked difference in learning between visitors observing animals on exhibit versus being handled during informal presentations. Visitors to demonstrations utilizing a raven and radiated tortoises were able to answer questions correctly at a rate as much as eleven times higher than visitors to the exhibits.

Enhanced Environmental Attitudes

Program animals have been clearly demonstrated to increase affective learning and attitudinal change.

- Studies by Yerke and Burns (1991), and Davison and her colleagues (1993) evaluated the effect live animal shows had on visitor attitudes. Both found their shows successfully influenced attitudes about conservation and stewardship.
- Yerke and Burns (1993) also evaluated a live bird outreach program presented to Oregon fifth-graders and recorded a significant increase in students' environmental attitudes after the presentations.
- Sherwood and his colleagues (1989) found that students who handled live invertebrates in an education program demonstrated both short and long-term attitudinal changes as compared to those who only had exposure to dried specimens.
- Povey and Rios (2002) examined the role program animals play in helping visitors develop positive feelings about the care and well-being of zoo animals.
- As observed by Wolf and Tymitz (1981), zoo visitors are deeply concerned with the welfare of zoo animals and desire evidence that they receive personalized care.

Conclusion

Creating positive impressions of aquarium and zoo animals, and wildlife in general, is crucial to the fundamental mission of zoological institutions. Although additional research will help us delve further into this area, the existing research supports the conclusion that program animals are an important tool for conveying both cognitive and affective messages regarding animals and the need to conserve wildlife and wild places.

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Appendix E: Developing an Institutional Program Animal Policy

Last revision 2003
Re-authorized by the Board, June 2011

Rationale

Membership in AZA requires that an institution meet the AZA Accreditation Standards collectively developed by our professional colleagues. Standards guide all aspects of an institution's operations; however, the accreditation commission has asserted that ensuring that member institutions demonstrate the highest standards of animal care is a top priority. Another fundamental AZA criterion for membership is that education be affirmed as core to an institution's mission. All accredited public institutions are expected to develop a written education plan and to regularly evaluate program effectiveness.

The inclusion of animals (native, exotic, and domestic) in educational presentations, when done correctly, is a powerful tool. CEC's **Program Animal Position Statement** describes the research underpinning the appropriate use of program animals as an important and powerful educational tool that provides a variety of benefits to zoo and aquarium educators seeking to convey cognitive and affective messages about conservation and wildlife.

Ongoing research, such as AZA's Multi-Institutional Research Project (MIRP) and research conducted by individual AZA institutions will help zoo educators to determine whether the use of program animals conveys intended and/or conflicting messages and to modify and improve programs accordingly and to ensure that all program animals have the best possible welfare.

When utilizing program animals our responsibility is to meet both our high standards of animal care and our educational goals. Additionally, as animal management professionals, we must critically address both the species' conservation needs and the welfare of the individual animal. Because "wild creatures differ endlessly," in their forms, needs, behavior, limitations and abilities (Conway, 1995), AZA, through its Animal Welfare Committee, has recently given the responsibility to develop taxon- and species-specific animal welfare standards and guidelines to the Taxon Advisory Groups (TAG) and Species Survival Plan® Program (SSP). Experts within each TAG or SSP, along with their education advisors, are charged with assessing all aspects of the taxons' and/or species' biological and social needs and developing Animal Care Manuals (ACMs) that include specifications concerning their use as program animals.

However, even the most exacting standards cannot address the individual choices faced by each AZA institution. Therefore, each institution is required to develop a program animal policy that articulates and evaluates program benefits. The following recommendations are offered to assist each institution in formulating its own Institutional Program Animal Policy, which incorporates the AZA Program Animal Policy and addresses the following matters.

The Policy Development Process

Within each institution, key stakeholders should be included in the development of that institution's policy, including, but not limited to representatives from:

- The Education Department
- The Animal Husbandry Department
- The Veterinary and Animal Health Department
- The Conservation & Science Department
- The Behavioral Husbandry Department
- Any animal show staff (if in a separate department)
- Departments that frequently request special program animal situations (e.g., special events, development, marketing, zoo or aquarium society, administration)

Additionally, staff from all levels of the organization should be involved in this development (e.g., curators, keepers, education managers, interpreters, volunteer coordinators).

To develop a comprehensive Program Animal Policy, we recommend that the following components be included:

I. Philosophy

In general, the position of the AZA is that the use of animals in up close and personal settings, including animal contact, can be extremely positive and powerful, as long as:

1. The use and setting is appropriate.
2. Animal and human welfare is considered at all times.
3. The animal is used in a respectful, safe manner and in a manner that does not misrepresent or degrade the animal.
4. A meaningful conservation message is an integral component. Read the AZA Board-approved Conservation Messages.
5. Suitable species and individual specimens are used.

Institutional program animal policies should include a philosophical statement addressing the above, and should relate the use of program animals to the institution's overall mission statement.

II. Appropriate Settings

The Program Animal Policy should include a listing of all settings both on and off site, where program animal use is permitted. This will clearly vary among institutions. Each institution's policy should include a comprehensive list of settings specific to that institution. Some institutions may have separate policies for each setting; others may address the various settings within the same policy. Examples of settings include:

1. On-site programming
    a. Informal and non-registrants:
        i. On-grounds programming with animals being brought out (demonstrations, lectures, parties, special events, and media)
        ii. Children's zoos and contact yards
        iii. Behind-the-scenes open houses
        iv. Shows
        v. Touch pools
    b. Formal (registration involved) and controlled settings
        i. School group programs
        i. Summer camps
        ii. Overnights
        iii. Birthday parties
        iv. Animal rides
        v. Public animal feeding programs
    c. Offsite and outreach
        i. PR events (TV, radio)
        ii. Fundraising events
        iii. Field programs involving the public
        iv. School visits
        v. Library visits
        vi. Nursing home visits (therapy)
        vii. Hospital visits
        viii. Senior centers
        ix. Civic group events

In some cases, policies will differ from setting to setting (e.g., on-site and off-site use with media). These settings should be addressed separately, and should reflect specific animal health issues, assessment of distress in these situations, limitations, and restrictions.

III. Compliance with Regulations

All AZA institutions housing mammals are regulated by the USDA's Animal Welfare Act. Other federal regulations, such as the Marine Mammal Protection Act, may apply. Additionally, many states, and some cities, have regulations that apply to animal contact situations. Similarly, all accredited institutions are bound by the AZA Code of Professional Ethics. It is expected that the Institution Program Animal Policy address compliance with appropriate regulations and AZA Accreditation Standards.

IV. Collection Planning

AZA accredited institutions should have a collection planning process in place. Program animals are part of an institution's overall collection and must be included in the overall collection planning process. The AZA Guide to Accreditation contains specific requirements for the institution collection plan. For more information about collection planning in general, please see the Collection Management pages in the Members Only section.

The following recommendations apply to program animals:

1. Listing of approved program animals (to be periodically amended as collection changes). Justification of each species should be based upon criteria such as:
    a. Temperament and suitability for program use
    b. Husbandry requirements
    c. Husbandry expertise
    d. Veterinary issues and concerns
    e. Ease and means of acquisition / disposition according to the AZA code of ethics
    f. Educational value and intended conservation message
    g. Conservation Status
    h. Compliance with TAG and SSP guidelines and policies
2. General guidelines as to how each species (and, where necessary, for each individual) will be presented to the public, and in what settings
3. The collection planning section should reference the institution's acquisition and disposition policies.

V. Conservation Education Message

As noted in the AZA Accreditation Standards, if animal demonstrations are part of an institution's programs, an educational and conservation message must be an integral component. The Program Animal Policy should address the specific messages related to the use of program animals, as well as the need to be cautious about hidden or conflicting messages (e.g., "petting" an animal while stating verbally that it makes a poor pet). This section may include or reference the AZA Conservation Messages.

Although education value and messages should be part of the general collection planning process, this aspect is so critical to the use of program animals that it deserves additional attention. In addition, it is highly recommended to encourage the use of biofacts in addition to or in place of the live animals. Whenever possible, evaluation of the effectiveness of presenting program animals should be built into education programs.

VI. Human Health and Safety

The safety of our staff and the public is one of the greatest concerns in working with program animals. Although extremely valuable as educational and affective experiences, contact with animals poses certain risks to the handler and the public. Therefore, the human health and safety section of the policy should address:

1. Minimization of the possibility of disease transfer from non-human animals to humans, and vice-versa (e.g., hand washing stations, no touch policies, use of hand sanitizer).
2. Safety issues related to handlers' personal attire and behavior (e.g., discourage or prohibit use of long earrings, perfume and cologne, not eating or drinking around animals, smoking, etc.).

AZA's Animal Contact Policy provides guidelines in this area; these guidelines were incorporated into accreditation standards in 1998.

VII. Animal Health and Welfare

Animal health and welfare are the highest priority of AZA accredited institutions. As a result, the Institutional Program Animal Policy should make a strong statement on the importance of animal welfare. The policy should address:

1. General housing, husbandry, and animal health concerns (e.g. that the housing and husbandry for program animals meets or exceeds general AZA standards and that the physical, social and psychological needs of the individual animal, such as adequate rest periods, provision of enrichment, visual cover, contact with conspecifics as appropriate, etc., are accommodated).
2. Where ever possible provide a choice for animal program participation, e.g., retreat areas for touch tanks or contact yards, evaluation of willingness/readiness to participate by handler, etc.)

3. The empowerment of handlers to make decisions related to animal health and welfare; such as withdrawing animals from a situation if safety or health is in danger of being compromised.
4. Requirements for supervision of contact areas and touch tanks by trained staff and volunteers.
5. Frequent evaluation of human / animal interactions to assess safety, health, welfare, etc.
6. Ensure that the level of health care for the program animals is consistent with that of other animals in the collection.
7. Whenever possible have a “cradle to grave” plan for each program animal to ensure that the animal can be taken care of properly when not used as a program animal anymore.
8. If lengthy “down” times in program animal use occur, staff should ensure that animals accustomed to regular human interactions can still maintain such contact and receive the same level of care when not used in programs.

VIII. Taxon Specific Protocols

We encourage institutions to provide taxonomically specific protocols, either at the genus or species level, or the specimen, or individual, level. Some taxon-specific guidelines may affect the use of program animals. To develop these, institutions refer to the Conservation Programs Database.

Taxon and species -specific protocols should address:

1. How to remove the individual animal from and return it to its permanent enclosure, including suggestions for operant conditioning training.
2. How to crate and transport animals.
3. Signs of stress, stress factors, distress and discomfort behaviors.

Situation specific handling protocols (e.g., whether or not animal is allowed to be touched by the public, and how to handle in such situations):

1. Guidelines for disinfecting surfaces, transport carriers, enclosures, etc. using environmentally safe chemicals and cleaners where possible.
2. Animal facts and conservation information.
3. Limitations and restrictions regarding ambient temperatures and or weather conditions.
4. Time limitations (including animal rotation and rest periods, as appropriate, duration of time each animal can participate, and restrictions on travel distances).
5. The number of trained personnel required to ensure the health and welfare of the animals, handlers and public.
6. The level of training and experience required for handling this species
7. Taxon/species-specific guidelines on animal health.
8. The use of hand lotions by program participants that might touch the animals

IX. Logistics: Managing the Program

The Institutional Policy should address a number of logistical issues related to program animals, including:

1. Where and how the program animal collection will be housed, including any quarantine and separation for animals used off-site.
2. Procedures for requesting animals, including the approval process and decision-making process.
3. Accurate documentation and availability of records, including procedures for documenting animal usage, animal behavior, and any other concerns that arise.

X. Staff Training

Thorough training for all handling staff (keepers, educators, and volunteers, and docents) is clearly critical. Staff training is such a large issue that many institutions may have separate training protocols and procedures. Specific training protocols can be included in the Institutional Program Animal Policy or reference can be made that a separate training protocol exists.

It is recommended that the training section of the policy address:

1. Personnel authorized to handle and present animals.
2. Handling protocol during quarantine.

3. The process for training, qualifying and assessing handlers including who is authorized to train handlers.
4. The frequency of required re-training sessions for handlers.
5. Personnel authorized to train animals and training protocols.
6. The process for addressing substandard performance and noncompliance with established procedures.
7. Medical testing and vaccinations required for handlers (e.g., TB testing, tetanus shots, rabies vaccinations, routine fecal cultures, physical exams, etc.).
8. Training content (e.g., taxonomically specific protocols, natural history, relevant conservation education messages, presentation techniques, interpretive techniques, etc.).
9. Protocols to reduce disease transmission (e.g., zoonotic disease transmission, proper hygiene and hand washing requirements, as noted in AZA's Animal Contact Policy).
10. Procedures for reporting injuries to the animals, handling personnel or public.
11. Visitor management (e.g., ensuring visitors interact appropriately with animals, do not eat or drink around the animal, etc.).

XI. Review of Institutional Policies

All policies should be reviewed regularly. Accountability and ramifications of policy violations should be addressed as well (e.g., retraining, revocation of handling privileges, etc.). Institutional policies should address how frequently the Program Animal Policy will be reviewed and revised, and how accountability will be maintained.

XII. TAG and SSP Recommendations

Following development of taxon-specific recommendations from each TAG and SSP, the institution policy should include a statement regarding compliance with these recommendations. If the institution chooses not to follow these specific recommendations, a brief statement providing rationale is recommended.

Appendix F: Nutrient Composition of Fish

	Capelin	Herring	Marine smelt	Freshwater smelt	Rainbow trout	Krill	Squid
Dry Matter (%)	19.9 ± 1.02	27.8 ± 3.51	23.9 ± 4.40	19.3 ± 3.70	27.5 ± 1.80	14.0 ± 6.58	22.9 ± 2.01
Energy (kcal/g)	5.4 ± 0.29	6.0 ± 0.38	5.6 ± 0.73	5.3 ± 0.22	5.9 ± 0.25	4.7 ± 0.79	5.1 ± 0.25
Crude Protein (%)	65.7 ± 5.03	56.6 ± 5.00	62.7 ± 6.40	66.9 ± 5.00	55.2 ± 2.95	54.6 ± 12.18	66.8 ± 2.29
Crude Fat (%)	15.3 ± 4.01	30.6 ± 7.04	19.4 ± 10.3	15.2 ± 4.30	29.6 ± 6.60	25.1 ± 5.66	13.7 ± 7.00
Calcium (%)	1.5 ± 0.23	2.0 ± 0.42	2.9 ± 1.43	2.3 ± 0.96	2.0 ± 0.31	1.6 ± 0.22	0.2 ± 0.15
Phosphorus (%)	1.6 ± 0.20	1.7 ± 0.28	2.4 ± 0.98	1.8 ± 0.61	1.7 ± 0.25	1.5 ± 0.13	1.0 ± 0.38
Magnesium (%)	0.2 ±0.07	0.2 ± 0.04	0.2 ± 0.09	0.1 ± 0.06	0.1 ± 0.02	0.4 ± 0.07	0.2 ± 0.10
Potassium (%)	1.4 ±0.18	1.2 ± 0.16	1.5 ± 0.50	1.1 ± 0.28	1.1 ± 0.16	0.6 ± 0.37	1.3 ± 0.43
Sodium (%)	1.1 ± 0.53	0.8 ± 0.28	0.8 ± 0.46	0.5 ± 0.28	0.4 ± 0.16	1.7 ± 0.64	1.4 ± 0.56
Iron (ppm)	46.5 ±13.65	67.0 ±11.44	57.9 ± 29.97	29.8 ±11.14	50.5 ± 22.4	58.9 ± 22.50	77.7 ± 69.46
Copper (ppm)	2.8 ±1.13	4.3 ± 2.32	4.0 ± 3.55	6.1 ± 2.42	5.4 ±1.46	82.8 ± 28.23	133.5 ± 45.5
Zinc (ppm)	59.2 ±17.4	57.1 ±11.85	109.1 ± 50.94	83.8 ± 24.40	109.3 ± 45.3	63.1 ± 28.23	89.6 ± 22.93
Manganese (ppm)	1.6 ± 0.51	6.01 ± 2.63	6.4 ± 2.93	6.5 ± 1.58	4.2 ± 1.25	3.0 ± 0.06	2.2 ± 0.88
Molybdenum (ppm)	0.6 ± 0.36	0.8 ± 0.19	1.3 ± 0.55	0.7 ± 0.27	0.7 ± 0.13	N/A	1.0 ± 0.34
Vitamin A (IU/g)	29.3 ± 3.50	19.6 ± 4.56	68.3 ± 16.16	44.5 ± 15.12	62.1 ± 22.14	45.3 ± 35.6	45.7 ± 35.46
Vitamin E (IU/g)	17.5 ± 1.45	10.8 ± 1.46	21.5 ± 6.05	44.0 ± 8.08	32.1 ± 6.18	79.3 ± 36.4	79.2 ± 38.4
Total wt FA (g/kg)	14.6 ± 5.13	22.7 ± 8.46	17.8 ± 7.82	14.3 ± 5.49	20.9 ± 7.49	17.8 ± 8.79	12.8 ± 4.28
Saturated (% of FA)	16.9 ± 2.26	23.5 ± 4.17	24.4 ± 2.85	22.4 ± 0.87	24.6 ± 1.4	10.66	22.9 ± 3.21
MUFA (% of FA)	34.8 ± 3.33	37.9 ± 4.49	36.8 ± 6.92	25.8 ± 3.45	31.3 ± 4.2	8.92	19.8 ± 4.40
PUFA (% of FA)	19.8 ± 4.38	18.2 ± 5.94	23.6 ± 6.02	35.9 ± 3.38	29.8 ± 2.25	7.90	40.6 ± 4.81
Total ω-6 (% of FA)	1.4 ± 0.45	1.9 ± 0.47	2.2 ± 0.80	8.3 ± 2.05	7.4 ± 1.49	3.23	2.2 ± 0.19
Total ω-3 (% of FA)	18.4 ± 4.14	16.3 ± 5.55	21.3 ± 5.54	27.6 ± 1.74	22.7 ± 2.67	12.35	38.4 ± 4.78
18: 2 ω-6 (% of FA)	1.0 ± 0.42	1.2 ± 0.31	0.9 ± 0.16	4.5 ± 1.39	6.0 ± 1.13	1.29	0.7 ± 0.14
20: 4 ω-6 (% of FA)	0.4 ± 0.12	0.7 ± 0.37	1.1± 0.56	3.7 ± 0.93	1.2 ± 0.38	1.18	1.5 ± 0.18
18: 3 ω-3 (% of FA)	0.4 ± 0.14	0.9 ± 0.26	0.5 ± 0.32	4.2 ± 1.83	1.3 ± 0.07	0.53	0.5 ± 0.09
20: 5 ω-3 (% of FA)	8.5 ± 1.83	7.4 ± 2.65	7.5 ± 2.69	8.2 ± 1.44	7.2 ± 1.28	5.59	12.5 ± 2.86
22: 6 ω-3 (% of FA)	8.7 ± 2.42	7.2 ± 3.10	9.6± 3.35	11.2 ± 1.80	10.9 ± 0.97	3.62	24.5 ± 2.00

Data from McClements (2007) except krill

Appendix G: Sample Maintenance Diets for Various Penguin Species

Penguin species	King		Rockhopper		Gentoo	Humboldt		African		Magellanic	Little blue	
Institution	**A**	**B**	**C**	**B**	**D**	**E**	**F**	**G**	**H**	**A**	**D**	**C**
Est. Amt/day/bird (g)	800	800	550	600	430	650	650	600	600	625	120	150
Fish type by percentage:												
Capelin	15	50	45	40	32.5	17	77	25	100	100	70	50
Herring	85			15	32.5	11	15	33.5				
Trout		50	15	15		57						20
Krill					17.5						15	
Silversides				10	17.5						15	
Sardines			5	10				16.5				
Squid								25				
Marine Smelt			35	10		15	8					30
Total	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**	**100**
Supplements/bird/day:												
Mazuri Vita-Zu 5TLB1	1 tab									1 tab		
Mazuri Vita-Zu 5M23 (with Vit A)1					1 tab							
Mazuri Vita Zu 5TLC1												
Mazuri Vita Zu 5M25 (with Vit A)1						1 tab		1 tab	1 tab		1 tab	
Thiamin E Paste1		0.8 ml		0.6 ml								
Vitamin E			100 IU 3x/week									100 IU 1x/week
Thiamin			50 mg 3x/week									50 mg 3x/week
CVS Multivit2			0.5 tab 1x/week									0.25 tab 2x/week
CaCO3		1.4 g		1.1 g								
BZ Penguin vit 3							1 tab					

*The AZA Penguin TAG does not endorse any products mentioned.
1PMI Nutrition International. Brentwood, MO 63144
2CVS Corporation. Woonsocket, RI 02895
3Manufactured by Bomac Vets Plus, Inc. Knapp, WI 54749

Appendix H: Nutrient Composition of Sample Diets (Dry Matter Basis)

Species	King		Rockhopper		Gentoo	Humboldt		African		Magellanic	Little Blue	
Institution	A	B	C	B	D	E	F	G	H	A	D	C
Dry Matter (%)	26.70	23.70	22.94	23.85	22.17	25.71	21.42	25.55	19.90	19.90	19.62	22.62
Energy (kcal/g)	22.05	24.30	24.52	23.81	25.72	22.68	25.94	21.99	27.14	27.14	27.59	24.89
Crude Protein (%)	57.97	60.45	62.64	61.67	60.28	58.26	64.10	60.66	65.70	65.70	63.59	62.70
Crude Fat (%)	28.31	22.25	18.88	20.13	22.71	25.52	17.92	20.46	15.30	15.30	17.39	19.31
Calcium (%)	1.93	1.75	2.16	2.16	1.93	2.05	1.69	2.22	1.50	1.50	1.73	2.03
Phosphorus (%)	1.69	1.65	1.93	1.90	1.76	1.79	1.68	1.71	1.60	1.60	1.71	1.86
Magnesium (%)	0.20	0.15	0.19	0.19	0.24	0.14	0.20	0.20	0.20	0.20	0.23	0.18
Potassium (%)	1.23	1.25	1.39	1.36	1.21	1.22	1.38	1.29	1.40	1.40	1.30	1.37
Sodium (%)	0.85	0.75	0.88	0.88	1.06	0.62	1.03	0.97	1.10	1.10	1.15	0.87
Iron (ppm)	61.38	48.50	59.03	68.25	56.28	52.42	50.04	97.19	46.50	46.50	50.01	50.72
Copper (ppm)	4.08	4.10	4.02	3.69	17.50	4.63	3.12	25.12	2.80	2.80	14.98	4.63
Zinc (ppm)	57.42	84.25	85.08	78.16	67.93	95.01	62.88	68.00	59.20	59.20	67.27	84.20
Manganese (ppm)	5.35	2.90	4.04	4.31	4.12	4.29	2.65	4.66	1.60	1.60	2.53	3.75
Mo (ppm)	0.77	0.65	0.88	0.82	0.68	0.78	0.69	0.74	0.60	0.60	0.62	0.87
Vitamin A (IU/g)	20.81	48.59	51.90	40.39	80.10	68.67	37.24	31.44	46.76	30.00	77.47	57.31
Vitamin E (IU/g)	0.60	0.45	0.20	0.44	1.33	0.91	0.47	0.19	0.17	1.02	2.91	0.50
Thiamin (mg/g)	0.54	0.21	0.24	0.21	1.15	0.21	0.18	0.15	0.19	0.92	2.34	0.64
Saturated (g/kg)	50.20	40.25	37.14	39.03	39.37	47.04	32.05	39.38	24.80	24.80	27.58	37.17
MUFA (g/kg)	82.20	59.41	59.60	63.84	64.23	66.02	59.20	63.26	51.06	51.06	49.93	59.12
PUFA (g/kg)	39.98	48.35	41.12	40.93	34.87	52.60	32.61	43.09	29.05	29.05	29.81	41.23
Total ω-6 (g/kg)	4.13	9.81	5.18	4.57	3.77	10.72	2.70	3.29	2.10	2.10	2.81	5.88
Total ω-3 (g/kg)	35.92	38.87	34.98	34.49	32.61	42.19	29.93	35.42	27.00	27.00	28.45	35.42
18: 2 ω-6 (g/kg)	2.52	7.91	3.58	3.09	2.08	8.41	1.70	2.02	1.44	1.44	1.57	4.22
20: 4 ω-6 (g/kg)	1.48	1.70	1.59	1.61	1.43	2.06	0.92	1.78	0.60	0.60	1.02	1.50
18: 3 ω-3 (g/kg)	1.93	1.78	1.11	1.28	1.28	2.09	0.86	1.46	0.51	0.51	0.63	1.16
20: 5 ω-3 (g/kg)	16.45	14.20	14.07	15.13	14.11	14.78	13.77	17.51	12.97	12.97	12.52	13.59
22: 6 ω-3 (g/kg)	16.04	18.58	16.43	16.21	14.39	19.92	13.87	18.15	12.77	12.77	12.88	16.57

Appendix I: Institutions for *Aspergillus* Testing

University of Miami
Division of Comparative Pathology
1550 NW 10th Avenue, Room 105
Miami, Florida 33136
Phone: (305) 243-6927 or 800-596-7390
Fax: (305) 243-5662
Questions: Dr. Carolyn Cray

Elisa tests for both antibodies and galactomannan. Optional protein electrophoresis to aid diagnosis. Call for submission forms and shipping instructions.

Zoologix Inc.: www.zoologix.com
9811 Owensmouth Avenue
Suite 4
Chatsworth CA 91311-3800info@zoologix.com
Phone: (818) 717-8880
Fax: (818) 717-8881

Qualitative real-time PCR test for *Aspergillus fumigatus*. Recommended samples: throat or cloacal swab. Call to confirm specimen acceptability and shipping instructions.

Research Associates Laboratory
14556 Midway Rd.
Dallas, TX 75224
Phone: (972)-960-2221
Fax: (972)-960-1997

DNA-based real-time PCR for detection of *Aspergillus fumigatus* infection. Samples recommended: swab of trachea, air sac granuloma,

Sex Determination

Avian Biotech
1336 Timberlane Road ·
Tallahassee, FL 32312-1766
Phone: (850) 386-1145 or (800) 514-9672 (Office)
Fax: (850) 386-1146

Zoogen DNA Services
P.O. Box 1157
1046 Olive Drive, Ste. A
Davis, CA 95616
Phone: (530) 750-5757
Toll Free Tel: (800) 995-2473
Fax: (530) 750-5758
Email: zoogenservices@yahoo.com

Loyola Medical Center
2160 South First Avenue
Bldg. #101. RM #2718
Maywood, IL 60153
Phone: (708) 216-2341
Email: jeandubach@gmail.com

Sexing now can be done on feather shafts and eggshell membrane as well as whole blood.

Appendix J: Drugs Commonly Used in Penguin Species

Drug	Use
Terbinafine	Antifungal
Clotrimazole	Antifungal—nebulize
Voriconazole	Antifungal
Itraconazole **	Antifungal
Amoxicillin	Antibacterial
Cephalosporins	Antibacterial (may cause regurgitation in higher doses)
Ivermectin	Parasiticide
Pyrantel pamoate	Parasiticide
Fenbendazole	Parasiticide
Medroxyprogesterone	Molt Induction, suppression of egg-laying
Ibuprofen	Pain reliever (use with care because of renal toxicity)
Meloxicam	Pain relief (use with care because of renal toxicity)
Calcium EDTA	Chelation for heavy metal toxicity
Chloroquine	Malaria treatment
Primaquine	Malaria treatment or prevention
Mefloquine	Malaria treatment or prevention
Daraprim/sulfadiazine	Malaria prevention (compounded formulation)

Pharmocokenetic studies have not been done for most of these drugs in any of the penguin species. Therefore dosage and dosing interval for many of the drugs are empirical. Consult a formulary that includes avian species (Veterinary Drug Handbook by Dr. Donald Plumb, or the Exotic Animal Formulary by Dr. James Carpenter). Some dose and treatment regimens for certain species of penguins may be listed in the references.

** Commercial formulations of itraconazole should be used. Compounded formulations have been shown to have poorer absorption and may not reach therapeutic levels (Smith et al., 2010).

Appendix K: Product Information

1. Dri-Dek®, Kendall Products, 2706 South Horseshoe Drive, Maples, FL 33942 USA. http://www.dri-dek.com
2. Grumbach Incubators, Loher Straße 17, DE-35614 Asslar, Germany. http://www.grumbach-brutgeraete.de/english Lyon Technologies, Inc. is a dealer for supply and repair in North America. www.lyonusa.com
3. Trex® Trex Company, Inc., 160 Exeter Drive, Winchester, VA 22603-8605 USA. http://www.trex.com
4. Roll-X Incubators, Lyon Technologies, Inc., 1690 Brandywine Avenue, Chula Vista, CA 91911 USA. http://www.lyonusa.com
5. Brinsea® Incubators, Brinsea Products Inc., 704 N. Dixie Avenue, Titusville, FL 32796 USA. http://www.brinsea.com
6. R-com Incubators, Autoelex Co., Ltd., 612, Deokam-ri, Juchon-myeon, Grimhae city, Gyeongsangnam-do, Korea. www.autoelex.com (For USA distributor see also Lyon Technologies).
7. Betadine® Solution, Purdue Products L.P., One Stamford Forum, Stamford, CT 06901-3431 USA. http://www.betadine.com
8. PDI® Iodine Duo-Swab® Prep and Scrub SwabStick, PDI, Two Nice-Pak Park, Orangeburg, NY 10954 USA. http://www.pdipdi.com
9. Plexiglas® Acrylic Sheet, Altuglas International, Arkema Inc., 100 PA Route 413, Bristol, PA 190007, USA. www.plexiglas.com
10. The Original Cooler Brooder, Avey Incubator, PO Box 279, Hugo, CO 80821 USA. www.aveyincubator.com
11. AstroTurf roll mat, Grass Tech, S.P.R.L/B.V.B.A, 11, Rue Granbonpre, 1348 Louvain-la-Neuve, Belgium. http://www.astroturfmats.com
12. Con-Tact® Grip Ultra Shelf Liner, Kittrich Corporation, La Mirada, CA. Con-Tact shelf liner is widely available at kitchen and home stores.
13. Kendall Sovereign® Feeding Tube and Urethral Catheter, Tyco Healthcare Group LP, Mansfield, MA 02048 USA. Size 14 Fr (4.7 mm), length 16 in (41 cm). www.tycohealthcare.com
14. Hi-Intensity Egg Candler (Special Zoo Model), Lyon Technologies, Inc., 1690 Brandywine Avenue, Chula Vista, CA 91911 USA. www.lyonusa.com
15. Animal Intensive Care Unit, Lyon Technologies, Inc., 1690 Brandywine Avenue, Chula Vista, CA 91911 USA. www.lyonusa.com
16. Pedialyte®, Abbott Laboratories, 3300 Stelzer Road, Columbus, OH 43219-3034 USA. http://pedialyte.com
17. Mazuri® Vita-Zu Bird Tablet w/o Vitamin A, Land O' Lakes, PO Box 64101, Saint Paul, MN 55164-0101 USA. www.mazuri.com
18. Enfamil® Poly-vi-sol® Infant drops with iron, Mead Johnson Global Headquarters, 2701 Patriot Boulevard, Fourth Floor, Glenview, IL 60026 USA. www.enfamil.com
19. Tegaderm®, Tegaderm Brand Products, 3M Corporate Headquarters, 3M Center, St. Paul, MN 55144-1000 USA.

Appendix L: Penguin Chick Hand-rearing Diet (Formula)

Fish handling and preparation: Fish to be used for the making of Penguin Chick Hand Rearing Diet should be prepared in accordance with safe food handling procedures. Fish should be pulled in a semi-frozen condition straight from the air-thawed fish block. Similar preparation is recommended for krill. This assures the best fish quality for young chicks with naïve immune systems. The goal is to use the least thawed, more frozen fish, from the air-thawed blocks to avoid excessive warming of the food items during preparation. All fish items should be maintained at or below 4.4 °C (40 °F) during preparation.

Full Batch: Average volume pre-strain approximately 1.5 liters

440 g	5–7 in. long whole herring (with head, tail, fins & skin removed)
440 gm	Krill (squeeze water out after defrosting & before measuring)
600 ml	Filtered water
8 each	7.5 grain Brewer's yeast tablets
550 mg	B_1
1 each	5 lb Mazuri® Vita-Zu Bird tab w/o Vitamin A
4 each	10 grain calcium carbonate tablets
1200 IU	Vitamin E
2 cc	Poly-vi-sol® with iron

Blend ingredients thoroughly. Strain through a large colander. Keep refrigerated. Mark with date and time; use within 24 hours.

Prior to feeding, warm the diet using a reservoir of warm water to heat the formula to 35 °C (95 °F) just before feeding; if formula exceeds 37.8 °C (100 °F) during the heating process, discard and do not feed. It is recommended to stir in a pinch of ground B_1 (thiamine) powder to the diet prior to feeding. The powder can be made by grinding 100 mg B_1 tablets; mix one pinch per 30 cc warmed formula.

If a smaller volume of formula is needed within a 24-hour period a half portion can be prepared. Due to the vitamin formulation it is not recommended to make batches smaller than an half batch.

Half Batch: Average volume pre-strain approximately 850 cc

220 gm	5–7 in. whole herring (with head, tail, fins & skin removed)
220 gm	Krill (squeeze water out after defrosting & before measuring)
300 ml	Filtered tap water
4 each	7.5 gr. Brewer's yeast tablets
275 mg	B_1
1 each	2.5 lb Mazuri® Vita-Zu Bird tab w/o Vitamin A
2 each	10 grain calcium carbonate tablets
600 IU	Vitamin E
1 cc	Poly-vi-sol® with iron

Prepare as for Full Batch.

Appendix M: Penguin Chick Hand-rearing Protocols

The guidelines can be used for the Aptenodytes, but modifications must be made for the larger size of these chicks at each stage. The information contained is intended as a guideline only. It is recommended to review this entire document before undertaking to hand rear penguins. Depending on the physical plant, availability of products and materials, and the individual needs of chicks, modifications to these guidelines may be necessary.

Feeding: A note about fish preparation: Before preparing any other fish for the day, fish to be used in preparing the hand-rearing diet or to be used to feed chicks directly should be removed from the air-thawed blocks of fish in a semi-frozen state. Be vigilant for foreign objects often found in frozen fish. The fish should be placed in an appropriate container, topped with ice immediately and stored in the refrigerator. Krill should be prepared in the same way, so that it too is removed straight from the air-thawed block, placed in a separate container and topped with ice. When storing in the refrigerator, do not mix the krill with the fish. If, in the course of feeding during the day, additional food items are needed, it should be pulled from freshly thawing blocks of fish or krill. No fish should be used that has been prepared longer than 12 hours. Such preparation of the fish for use in formula or for feeding assures the best fish quality for young chicks whose guts are more sensitive. The goal is to use fish as freshly thawed as possible to avoid excessive warming of the constituent food items before use in formula or used for direct feeding. Proper fish handling is the foundation of good animal husbandry.

A note about formula storage and preparation: Prepared penguin formula should be stored in the refrigerator until use and will remain fresh for approximately 24 hours from the time it is made. The formula to be fed is heated prior to feeding. The recommended manner of heating formula is by setting the container of formula in hot (not boiling) water until the temperature reaches approximately 35 °C (95 °F). (For very young or finicky chicks, formula may need to be heated to 36.7 °C [98 °F]). Formula should be stirred continually during the heating process to prevent curdling. If curdling occurs, dispose of that formula. Do not boil. Do not reheat. Do not heat in microwave. The unused portion of heated formula should be discarded. When feeding several chicks, the formula container is placed in a warm water bath to maintain temperature for the duration of the feeding bout.

General intake guidelines: Feeding is based on a calculated percentage of the first daily or morning weight of the chick measured before the first feeding (e.g., if chick weighs 100 grams (3.5 oz.), the chick should be fed no more than 10 grams (0.35 oz.) per feeding. Chicks that are 3 days and under are generally fed much less than the calculated 10% because they are still using yolk and learning to eat). Treat chicks individually; the range in amounts listed for the first 3 days is due to the wide range in chicks' weights during this time, depending on species, from 60–120 g (2.1–4.2 oz.).

Figure 12. Syringes with both catheter tip and applied portion of short feeding tube along with baby food jar containing formula and dishes with pre-measured fish amounts.
Photo courtesy of Linda Henry.

Initial days of feeding

Day 1: 50:50 formula: water: 1–5 g (cc), but not to exceed the calculated 10% of the first daily weight total intake per feeding. (1 g formula=1 cc formula.) **Note:** Day 1 here is defined as the first day of feeding; this may differ from the chick's age where day 1 equals day of hatch. In these early days, the chick may still be absorbing yolk sac. This is an important factor in judging intake for young chicks—it is wise to be conservative.

Day 2: 75:25 ration of formula to water: 4–8 g (cc) total intake per feeding, not to exceed 10% of chick's first daily weight.

Day 3: Introduce straight formula: 4–10 g (cc) total intake per feeding, not to exceed 10% of chick's first daily weight. (If not well accepted, go back to a 75:25 ratio of formula: to water.)

Day 4 through Day 6: Try 10% of first daily weight total intake per feeding of straight formula - do not exceed. Use 10% of morning weight as a guide for each feeding's total intake. When the chick reaches 7 days of age, but not before reaching 100 g (3.5 oz.) first daily/morning weight, begin evaluating the chick for the ability to accept fish in the diet as described below.

7 days of age until chick achieves 500 grams first daily weight: At or about 7 days of age, but not before 100 g, first daily weight of the chick, evaluate adding fish to the diet. This evaluation should include the following: chick has been tolerating 100% (or full-strength) formula for three days; hydration is good; chick is bright, active and alert; fecal output is normal for chick's age; chick is thermoregulating appropriately for its age. Fish is most often introduced using herring filets cut into 2.5–3.8 cm (1–1.5 in.) x 0.6 cm (0.25 in.) pieces. Dip the fish or fish pieces in warm water just prior to feeding—this hydrates fish, warms it a little, and makes it easier for the chick to swallow. Gentoos usually begin fish at slightly greater than 100 g morning weight (approximately 110–115 g [3.9–4.1 oz.] morning weight) due to their larger hatch weights. Their first day on fish should not be any earlier than 7 days of age. Humboldts may also begin fish at greater than 100 g morning weight (between 100–200 g [3.5–7.1 oz.] first daily weight) because Humboldt penguins often have a longer readiness period to accept fish.

The guidelines for the amount of fish to be fed are as follows:

- **7 days of age:** Evaluate for fish introduction. If ready, give 3 g (0.1 oz.) fish once a day (SID) for the first day at the second feeding; fish is given in combination with formula to equal, but not exceed, 10% of the first daily weight.
- **2nd day on fish**: 3–5 g (0.1–0.2 oz.) maximum fish given twice a day (BID)—at second and fourth feedings—in combination with formula to equal, but not exceed, 10% of first daily weight
- **3rd day on fish**: 3–5 g (0.1–0.2 oz.) maximum fish given every other feeding in combination with formula to equal, but not exceed, 10% of first daily weight.
- **4th day on fish**: 3–5 g (0.1–0.2 oz.) maximum fish given every feeding in combination with formula to equal, but not exceed, 10% of first daily weight.
- **5th day on fish**: 5–7 g (0.2–0.25 oz.) maximum fish given every feeding in combination with formula to equal, but not exceed, 10% of first daily weight.
- **6th day on fish**: 7–10 g (0.25–0.35 oz.) maximum fish given every feeding in combination with formula to equal, but not exceed, 10% of first daily weight.

After 6 days of transitioning fish into the diet, fish amounts can be determined using the first daily weight as a guide:

- **300 g (10.5 oz.):** 10–15 g (0.35–0.5 oz.) fish every feeding maximum with formula to equal, but not exceed, 10% of first daily weight.
- **400 g (14 oz.):** Fish is 50% of total intake every feeding maximum in proportion with formula, not to exceed 10% of morning weight per feeding. Consider adding vitamin supplements at this time. **Note:** Heating formula to the full 35 °C (95 °F) becomes less critical as chick is consuming a higher percentage of cold fish. 32 °C (90 °F) is an acceptable formula temperature at this time.
- **500 g (18 oz.):** Decrease the number of feedings to 4 per day (QID), every 4 hours, at approximately 500 g (18 oz.) first morning weight. Let the chick's appetite guide you.

After the chick reaches approximately 600 g (21 oz.) or greater, and has been doing well on a 50:50 fish to formula diet ratio, then the feeding schedule may be altered to increase the percentage of fish in the diet.

Maintain the formula amount given at 30 cc, and then adding fish to make the feeding intake total equal to 10% of first daily weight. Water may be given as needed. The size of fish given can usually be increased at this time to include cut up herring and capelin chunks, including entrails. Fish size can progress gradually to whole capelin as chicks are able to accept it; herring is a dense-fleshed fish and may be difficult for younger birds to digest when given whole so use herring chunks a little longer before offering whole herring fish. Maintain formula at 30 cc of formula per feeding so that a natural transition occurs from formula to fish. As the chick grows the percentage of fish in the diet, relative to formula, will increase with increasing daily weights.

When chick is 1000 g (35 oz.) or greater at the first morning weight: Chicks may start to "wean" themselves from formula by refusing to feed from a syringe. Formula may be reduced to 15 cc four times per day. Formula is eventually reduced to 30 cc once a day and given at the first feeding when chick is most hungry. Formula will eventually be eliminated from the diet altogether. Fish fed to chicks that are not receiving formula should be dipped in water or hydrated by injecting water into the fish just prior to feeding. If this is not enough to hydrate chicks, an electrolyte replacement solution should be used.

Although chicks may be on four feedings per day, they may not eat the full amount of fish offered at each of those feedings, especially the fourth feeding of the day. Feeders should be thinking in terms of the total daily intake for each individual chick and whether chicks are maintaining proper weight gains. Be vigilant for early signs of illness or overheating at this time, which also will adversely affect a chick's appetite.

An additional reduction of numbers of feedings per day may also be indicated at around 1500 g (53 oz.). Chicks that are not hungry at the second feeding for several days are probably ready for three feedings per day, given about every 6 hours.

When chicks go to three times a day (TID) feedings monitor weight gains; birds may be reaching their asymptotic weight at this time. Chicks should still be eager to eat at each feeding. As chicks start to moult, they may not eat the full amount offered. Once chicks have completed moult and have reached a good, stable weight, fish may be fed on "demand" (or on the same schedule as the other birds in the primary penguin exhibit).

Note: As chicks progress through various feeding stages, they will respond differently. Sometimes chicks will not eat all food items offered at all feedings. Never force a chick to eat. Evaluate each chick individually and then determine the cause for inappetence. Information contained in the Chapters 6 Veterinary Care and 7 Reproduction have details on assessing chick health and vitality relative to hand rearing regimes.

There are typically two stages at which many chicks become finicky, at 500 g (18 oz.) for a day or two, and at 1,000 g (35 oz.) for several days (this often corresponds to head-shyness in *Spheniscus* at 30 days of age). Chicks may refuse food at one feeding or not eat full amounts at each feeding. Check for overheating. Evaluate the environment. If low appetite continues for more than one or two feedings, a veterinary exam should be scheduled. The chick may be ill. Once chicks molt into juvenile plumage and fledge they can be introduced to the primary colony. After birds are stable and well-integrated into the colony, vitamin supplementation can be consistent with adult maintenance vitamins.

Figure 13. Two Plexiglas® brooder boxes set on top of brooder bases with heat lamps secured. Towels are draped over one or both sides to control airflow. Note the fans in the upper left corner; these provide cooling and good air movement. It is important that the room be cooled to offset the production of heat by the heat lamps. Lighting is provided by dimmable full-spectrum 40W fluorescent light bulbs. Photoperiod during the neonatal period is set to match exhibit parameters.
Photo courtesy of Linda Henry.

Figure 14. A closer view of Plexiglas® brooder boxes on brooder stands. Note arrangement of toweling inside. Digital readouts are mounted on each vertical pole with temperature probes extending into brooders.
Photo courtesy of Linda Henry

Figure 15. An Adélie chick in the brooder with a towel to prevent the young chick from wandering away from the heat source. A temperature probe and an Onset HOBO® temperature data logger have been placed in the brooder to record temperature variations. Photo courtesy of Linda Henry.

Figure 16. Left: A brooder bin in the corner; note how it is elevated on legs above the floor. In this instance a heat lamp has been provided on a portable stand; such provision of heat may be needed for some chicks during the initial transition to the bin following the end of the guard stage. Right: Gentoo chicks in one side of the divided bin with toweling over the rock substrate.
Photos courtesy of Linda Henry.

Penguin hand-rearing vitamin regimen: Recommended for small species (*Spheniscus magellanicus, S. humboldti, Pygoscelis adeliae, P. papua, P. antarctica, Eudyptes chrysolophus*.)

Early Vitamins**:** Provided in three ways in the formula: Poly-vi-sol® infant multi-vitamin, oral B-Comp, and oral B-1 tablets. See as follows:

- Just prior to feeding formula, stir in one pinch of ground 100 mg. B-1 and one pinch of ground B-Complex (B-50) per 100 cc formula prepared. Do this starting with the introduction of full strength formula until chick is 400 gm. at the first daily weight.
- 25 mg B-1 BID and 1/8 of a B-comp –BID beginning at 400 g first daily weight (or when the amount of fish fed is equal to or greater than the amount of formula fed) until 1000 g first daily weight.
- Poly-vi-sol® infant multivitamin drops (without iron) starting at 4 days of age through 1000 g first daily weight as outlined:

4 days of age:

250 g/ 8.8 oz. (a.m. weight):	0.10 cc Poly-vi-sol® drops SID
251–500 g /8.8–18 oz. (a.m. weight):	0.15 cc Poly-vi-sol® drops SID
501–750 g/ 18–26 oz. (a.m. weight):	0.20 cc Poly-vi-sol® drops SID
751–1000 g/ 26–35 oz. (a.m. weight):	0.25 cc Poly-vi-sol® drops SID

First daily weight = 1000 g (or when chick receives BID formula)

AM	1/2 children's multi-vitamin 1/8 tablet 10 grain Calcium carbonate 50 mg. B-1
PM	100 I.U. Vitamin E EOD 25 mg. B-Complex (1/2 tablet B-50) 1/8 tablet 10 grain Calcium carbonate

First daily weight = 2000 g (or greater)

AM	1 children's multi-vitamin 1/8 tablet 10 grain Calcium carbonate 50 mg. B-1
PM	100 I.U. Vitamin E EOD 25 mg. B-Complex (1/2 tablet B-50) 1/8 tablet 10 grain Calcium carbonate

Vitamins may be inserted into the gills of the fish before feeding, or fed to the chicks with a feeding response followed by the fish fillets if no whole fish is being fed.

Children's poly-vitamin drops: One zoological institution has used Enfamil® Poly-vi-sol® Infant Drops

Vitamin	Amount per 1 mL
Vitamin A	1500 IU
Vitamin C	35 mg
Vitamin D	400 IU
Vitamin E	5 IU
Vitamin B_1	0.5 mg
Vitamin B_2	0.6
Niacin	8 mg
Vitamin B_6	0.4 mg
Vitamin B_{12}	2 mcg

Children's poly-vitamin drops with iron: One zoological institution has used Enfamil® Poly-vi-sol Infant Drops with Iron

Vitamin	Amount per 1 mL
Vitamin A	1500 IU
Vitamin C	35 mg
Vitamin D (cholicalciferol)	400 IU
Vitamin E (d-alpha-tocopheryl succinate)	5 IU
Thiamin (as thiamin HCl)	0.5 mg
Niacin (as niacinamide)	8 mg
Vitamin B_6 (as pyridoxine HCl)	0.4
Iron (as ferrous sulfate)	10 mg

Children's Multi-vitamin: One zoological institution uses My First Flintstones™

Vitamin	Amount per tablet
Vitamin A	1998 IU
Vitamin C	60 mg
Vitamin D (D_3)	400 IU
Vitamin E	15 IU

Thiamin (B_1)	1.05 mg
Riboflavin (B_2)	1.2 mg
Niacin	10 mg
B_6	1.05 mg
Folic Acid	300 mcg
Vitamin B_{12}	4.5 mcg
Sodium	10 mg

Contents of Mazuri® Vita-Zu Bird Tablet w/o Vitamin A

	Each ½ lb. tablet (5TLC) supplies:	**Each 5 lb. tablet (5TLB) supplies:**
Vitamin A, I.U.	0	0
Vitamin E, I.U.	26	130
Vitamin C, mg	28	140
Thiamin Mononitrate, mg	23	117
Riboflavin, mg	1.7	8.6
Pyridoxine	1.7	8.6
Pantothenic Acid, mg	1.71	8.54
Biotin, mcg	0.0	0.1
Folic Acid, mg	0.06	0.29
Magnesium, mg	0.1	0.3

Juvenile Penguin Vitamin Supplementation Schedule
Begin supplementation at completion of first molt until 4 months post fledge

Gentoo, Humboldt, Magellanic:
1 each 2.5 lb. Mazuri Tab without Vitamin A once daily
½ each 50 mg B-complex once daily
100 IU Vitamin E twice weekly

Macaroni, chinstrap, Adélie:
2 each ½ lb. Mazuri Tab without Vitamin A once daily
½ each 50 mg B-complex once daily
100 IU Vitamin E twice weekly

Mazuri® Vita-Zu Bird Tablet w/o Vitamin A www.mazuri.com
(See table above for contents)

My First Flintstones www.bayercare.com
(See table above for contents)

Enfamil® Poly-vi-sol® Infant drops www.enfamil.com
(See table above for contents)

Onset HOBO®
Pendant temp/light datalogger www.onsetcomp.com/products/data-loggers/ua-002-64

Appendix N: ISIS Physiological Blood Values

International Species Information System
12101 Johnny Cake Ridge Road
Apple Valley, MN 55124 USA.
www.isis.org

Blue Penguin (*Eudyptula minor*)

Samples contributed by 8 institutions.

Sample Selection Criteria:

- No selection by gender
- All ages combined
- Animal was classified as healthy at the time of sample collection
- Sample was not deteriorated

Physiological Reference Intervals for Eudyptula minor

Test	Units	Reference Interval	Mean	Median	Low Sample[a]	High Sample[b]	Sample Size[c]	Animals[d]
White Blood Cell Count	*10^3 cells/µL	2.93 - 34.60	13.39	12.00	1.98	39.40	220	138
Red Blood Cell Count	*10^6 cells/µL	1.19 - 3.05	2.06	2.05	1.00	3.80	125	102
Hemoglobin	g/dL	*	17.2	18.1	9.3	23.9	30	28
Hematocrit	%	29.4 - 57.8	44.3	44.5	24.0	64.0	209	130
MCV	fL	123.0 - 362.3	222.1	214.2	98.6	437.5	125	102
Heterophils	*10^3 cells/µL	0.55 - 19.83	6.74	5.59	0.03	24.80	219	137
Lymphocytes	*10^3 cells/µL	1.02 - 16.19	5.65	4.60	0.53	20.00	219	138
Monocytes	cells/µL	48 - 2095	579	385	30	2340	162	115
Eosinophils	cells/µL	0 - 460	210	181	30	700	80	60
Basophils	cells/µL	0 - 1014	407	339	20	1600	112	82
Glucose	mg/dL	51 - 328	205	209	1	405	212	120
Uric Acid	mg/dL	0.6 - 38.4	12.5	8.2	0.2	44.7	222	124
Calcium	mg/dL	8.4 - 13.2	10.3	10.3	6.9	14.3	145	70
Phosphorus	mg/dL	1.3 - 11.0	4.2	3.5	1.1	12.0	123	53
Ca/Phos ratio		0.0 - 6.0	3.2	2.9	0.8	8.6	117	50
Sodium	mEq/L	142 - 163	152	153	136	168	89	31
Potassium	mEq/L	1.6 - 6.2	4.0	3.9	1.8	7.1	98	39
Na/K ratio		13.1 - 66.7	42.0	39.9	22.3	87.8	88	31
Total Protein	g/dL	3.9 - 8.2	5.6	5.5	3.0	8.9	186	101
Albumin	g/dL	1.1 - 3.4	2.1	2.1	0.6	3.8	133	57
Globulin	g/dL	0.5 - 6.8	3.4	3.3	0.0	7.6	128	54

Alkaline Phosphatase	IU/L	0 - 500	255	229	47	584	40	18
Lactate Dehydrogenase	IU/L	0 - 1002	417	323	20	1553	67	36
Aspartate Aminotransferase	IU/L	110 - 587	262	228	50	690	233	132
Creatine Kinase	IU/L	28 - 874	255	189	0	1096	221	124
Amylase	IU/L	0 - 8466	2879	2850	1	6420	51	30
Cholesterol	mg/dL	102 - 384	242	243	66	470	89	43

[a] Lowest sample value used to calculate the reference interval.
[b] Highest sample value used to calculate the reference interval.
[c] Number of samples used to calculate the reference interval.
[d] Number of different individuals contributing to the reference interval.
[*] Sample size is insufficient to produce a valid reference interval.

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Suggested citation format:
Teare, J.A. (ed.): 2013, "Eudyptula_minor_No_selection_by_gender__All_ages_ combined_Conventional_American_units__2013_CD.html " *in* ISIS Physiological Reference Intervals for Captive Wildlife: A CD-ROM Resource., International Species Information System, Eagan, MN.

Chinstrap Penguin (*Pygoscelis antarcticus*)

Samples contributed by 2 institutions.

Sample Selection Criteria:

- No selection by gender
- All ages combined
- Animal was classified as healthy at the time of sample collection
- Sample was not deteriorated

Physiological Reference Intervals for Pygoscelis antarcticus

Test	Units	Reference Interval	Mean	Median	Low Sample[a]	High Sample[b]	Sample Size[c]	Animals[d]
White Blood Cell Count	*10^3 cells/µL	0.00 - 16.22	8.24	7.62	2.30	23.40	52	21
Hematocrit	%	36.3 - 54.0	44.6	45.1	32.0	51.0	52	20
Heterophils	*10^3 cells/µL	0.00 - 10.50	4.82	4.30	1.30	16.40	52	21
Lymphocytes	*10^3 cells/µL	0.00 - 5.80	2.76	2.27	0.52	7.26	51	21
Monocytes	cells/µL	*	514	522	23	1596	33	9
Glucose	mg/dL	*	255	261	168	346	33	19
Creatinine	mg/dL	*	0.2	0.2	0.0	0.3	32	19
Uric Acid	mg/dL	*	10.9	8.4	3.5	28.1	32	19
Calcium	mg/dL	*	10.4	10.3	9.0	11.8	32	18
Phosphorus	mg/dL	*	3.8	3.5	1.6	6.9	30	19
Sodium	mEq/L	*	154	156	136	165	30	19
Chloride	mEq/L	*	108	110	92	117	30	19
Total Protein	g/dL	*	4.7	4.8	3.4	5.7	33	19
Albumin	g/dL	*	1.8	1.8	1.2	2.3	31	19
Globulin	g/dL	*	2.9	2.9	2.0	3.6	32	19
Alkaline Phosphatase	IU/L	*	202	110	30	749	30	19
Aspartate Aminotransferase	IU/L	*	185	173	90	363	32	19
Alanine Aminotransferase	IU/L	*	118	96	18	369	30	19
Creatine Kinase	IU/L	*	337	272	4	934	32	19
Cholesterol	mg/dL	*	324	320	167	547	33	19

[a] Lowest sample value used to calculate the reference interval.
[b] Highest sample value used to calculate the reference interval.
[c] Number of samples used to calculate the reference interval.
[d] Number of different individuals contributing to the reference interval.

* Sample size is insufficient to produce a valid reference interval.

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al_Ameõêcan_uniIs__201’j CD.h&l"ÿ?n

Gentoo Penguin (*Pygoscelis papua*)

Samples contributed by 12 institutions.

Sample Selection Criteria:

- No selection by gender.
- All ages combined
- Animal was classified as healthy at the time of sample collection
- Sample was not deteriorated

Physiological Reference Intervals for Pygoscelis papua

Test	Units	Reference Interval	Mean	Median	Low Sample[a]	High Sample[b]	Sample Size[c]	Animals[d]
White Blood Cell Count	*10^3 cells/µL	3.63 - 22.38	11.36	10.68	1.60	28.00	372	131
Red Blood Cell Count	*10^6 cells/µL	1.34 - 3.21	2.30	2.28	1.50	3.25	54	30
Hemoglobin	g/dL	4.9 - 30.7	15.1	17.8	2.0	22.4	48	33
Hematocrit	%	36.3 - 57.7	48.4	48.7	31.0	63.0	400	134
MCV	fL	148.3 - 308.5	227.8	228.4	150.2	306.3	48	27
MCHC	g/dL	27.0 - 45.4	36.8	36.2	23.3	49.9	41	29
Heterophils	*10^3 cells/µL	2.41 - 16.31	7.49	6.98	1.15	20.00	370	130
Lymphocytes	*10^3 cells/µL	0.61 - 9.36	3.33	2.74	0.17	11.00	371	130
Monocytes	cells/µL	60 - 1378	413	299	47	1620	284	107
Eosinophils	cells/µL	0 - 528	235	193	41	740	95	67
Basophils	cells/µL	0 - 295	144	121	35	440	91	57
Glucose	mg/dL	147 - 298	234	237	108	344	361	125
Blood Urea Nitrogen	mg/dL	0 - 7	4	4	1	9	54	39
Creatinine	mg/dL	0.0 - 0.6	0.2	0.2	0.0	1.0	52	46
Uric Acid	mg/dL	2.3 - 20.4	7.8	6.1	1.4	24.7	351	123
Calcium	mg/dL	7.8 - 12.4	10.2	10.2	6.7	13.9	351	128
Phosphorus	mg/dL	1.3 - 8.0	3.9	3.8	0.4	9.7	298	106
Ca/Phos ratio		1.2 - 5.5	2.9	2.7	0.6	6.6	292	106
Sodium	mEq/L	145 - 164	155	155	138	169	291	99
Potassium	mEq/L	1.4 - 6.8	3.2	3.1	1.0	8.1	276	98
Na/K ratio		20.9 - 112.8	55.3	49.6	16.6	147.3	277	97
Chloride	mEq/L	101 - 123	111	112	98	120	58	43
Total Protein	g/dL	3.8 - 7.0	5.4	5.3	2.6	7.9	314	127
Albumin	g/dL	1.4 - 3.7	2.4	2.3	0.6	5.2	345	123

Globulin	g/dL	0.6 - 4.6	2.7	2.9	0.2	5.6	344	123
Alkaline Phosphatase	IU/L	0 - 378	119	102	0	454	192	58
Lactate Dehydrogenase	IU/L	153 - 963	453	420	23	1248	206	56
Aspartate Aminotransferase	IU/L	67 - 590	248	225	2	706	372	133
Alanine Aminotransferase	IU/L	*	94	92	5	210	36	32
Creatine Kinase	IU/L	81 - 742	279	232	4	861	266	99
Amylase	IU/L	148 - 1302	702	716	0	1529	158	34
Cholesterol	mg/dL	232 - 417	326	326	195	451	218	68

[a] Lowest sample value used to calculate the reference interval.
[b] Highest sample value used to calculate the reference interval.
[c] Number of samples used to calculate the reference interval.
[d] Number of different individuals contributing to the reference interval.

[*] Sample size is insufficient to produce a valid reference interval.

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Humboldt Penguin (*Spheniscus humboldti*)

Samples contributed by 21 institutions.

Sample Selection Criteria:

- No selection by gender
- All ages combined
- Animal was classified as healthy at the time of sample collection
- Sample was not deteriorated

Physiological Reference Intervals for Spheniscus humboldti

Test	Units	Reference Interval	Mean	Median	Low Sample[a]	High Sample[b]	Sample Size[c]	Animals[d]
White Blood Cell Count	*10^3 cells/µL	6.16 - 49.88	23.53	21.99	1.37	74.50	2191	468
Red Blood Cell Count	*10^6 cells/µL	1.27 - 3.16	2.16	2.12	0.67	4.20	448	167
Hemoglobin	g/dL	9.5 - 21.5	15.8	15.9	5.0	24.0	889	234
Hematocrit	%	28.9 - 60.0	47.8	49.0	18.0	79.0	2589	503
MCV	fL	135.3 - 342.5	226.1	225.1	57.8	389.3	444	167
MCH	pg	52.3 - 114.8	79.8	79.7	20.6	146.5	328	112
MCHC	g/dL	26.3 - 45.8	33.2	32.7	16.3	50.3	884	233
Heterophils	*10^3 cells/µL	3.33 - 30.22	14.03	13.26	1.05	42.40	2183	468
Lymphocytes	*10^3 cells/µL	1.17 - 21.47	7.56	6.09	0.14	28.10	2176	467
Monocytes	cells/µL	103 - 4200	1210	859	32	5550	1709	435
Eosinophils	cells/µL	84 - 1495	457	348	22	1785	964	335
Basophils	cells/µL	99 - 1786	602	468	22	2387	1330	371
Glucose	mg/dL	154 - 326	236	235	69	406	2276	424
Blood Urea Nitrogen	mg/dL	1 - 7	4	4	1	8	1078	240
Creatinine	mg/dL	0.1 - 0.9	0.4	0.4	0.0	1.8	1028	183
Uric Acid	mg/dL	2.3 - 22.0	8.0	6.4	0.8	24.6	2473	462
Calcium	mg/dL	8.7 - 12.8	10.4	10.3	6.9	14.3	2183	437
Phosphorus	mg/dL	1.2 - 8.0	3.4	3.1	0.0	9.9	2171	409
Ca/Phos ratio		1.3 - 7.8	3.7	3.4	0.4	10.1	2156	406
Sodium	mEq/L	140 - 164	152	152	128	176	2204	420
Potassium	mEq/L	2.1 - 6.1	3.8	3.7	0.5	8.2	2142	412
Na/K ratio		23.7 - 74.1	43.1	41.0	4.2	95.0	2149	413
Chloride	mEq/L	100 - 124	113	114	89	136	1781	349

Total Protein	g/dL	3.7 - 6.9	5.2	5.2	2.1	8.4	2274	425
Albumin	g/dL	1.0 - 2.7	1.8	1.7	0.0	3.5	2109	411
Globulin	g/dL	0.6 - 5.3	3.4	3.5	0.3	6.6	2080	405
Alkaline Phosphatase	IU/L	36 - 387	137	112	3	447	1580	271
Lactate Dehydrogenase	IU/L	79 - 654	248	204	40	786	1171	210
Aspartate Aminotransferase	IU/L	83 - 435	209	192	4	571	2454	466
Alanine Aminotransferase	IU/L	11 - 105	42	37	0	137	1431	250
Creatine Kinase	IU/L	56 - 849	272	206	0	1065	1617	427
Gamma-glutamyltransferase	IU/L	0 - 18	7	7	0	26	516	184
Amylase	IU/L	718 - 3288	1665	1545	2	4502	401	190
Lipase	IU/L	0 - 50	23	19	2	64	80	67
Total Bilirubin	mg/dL	0.0 - 1.6	0.4	0.3	0.0	1.8	1264	235
Direct Bilirubin	mg/dL	0.0 - 0.1	0.0	0.0	0.0	0.1	384	27
Indirect Bilirubin	mg/dL	0.0 - 2.2	0.6	0.4	0.0	2.5	387	27
Cholesterol	mg/dL	131 - 380	244	240	13	493	1679	337
Triglyceride	mg/dL	20 - 138	56	49	13	158	671	189
Bicarbonate	mEq/L	17.9 - 34.3	26.2	26.1	15.9	39.0	69	55
Magnesium	mg/dL	1.46 - 3.23	2.42	2.34	1.80	3.89	48	40
Iron	µg/dL	32 - 258	148	145	40	277	69	18
Carbon Dioxide	mEq/L	15.6 - 39.0	27.7	28.0	11.0	48.4	417	120

[a] Lowest sample value used to calculate the reference interval.
[b] Highest sample value used to calculate the reference interval.
[c] Number of samples used to calculate the reference interval.
[d] Number of different individuals contributing to the reference interval.

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Jackass Penguin (*Spheniscus demersus*)

Samples contributed by 37 institutions.

(Citation Format)

Sample Selection Criteria:

- No selection by gender.
- All ages combined
- Animal was classified as healthy at the time of sample collection
- Sample was not deteriorated

Physiological Reference Intervals for Spheniscus demersus

Test	Units	Reference Interval	Mean	Median	Low Sample[a]	High Sample[b]	Sample Size[c]	Animals[d]
White Blood Cell Count	*10^3 cells/µL	4.11 - 39.01	15.34	13.53	0.17	50.40	2105	626
Red Blood Cell Count	*10^6 cells/µL	0.97 - 3.30	1.83	1.77	0.16	3.68	1130	467
Hemoglobin	g/dL	4.7 - 19.9	12.5	12.7	1.3	27.5	1066	429
Hematocrit	%	27.6 - 57.2	45.1	46.0	14.0	70.0	2884	788
MCV	fL	97.8 - 356.4	238.0	245.4	26.2	457.1	1153	469
MCH	pg	17.7 - 125.9	67.7	64.0	5.4	195.2	985	396
MCHC	g/dL	15.1 - 43.2	29.1	29.9	3.5	63.3	1048	423
Heterophils	*10^3 cells/µL	1.77 - 21.50	8.48	7.51	0.02	28.70	2084	625
Lymphocytes	*10^3 cells/µL	0.64 - 16.78	5.32	4.04	0.07	22.20	2078	623
Monocytes	cells/µL	78 - 2099	599	435	23	2550	1593	548
Eosinophils	cells/µL	73 - 1508	428	289	25	1894	989	386
Basophils	cells/µL	59 - 1080	369	287	30	1428	894	401
Glucose	mg/dL	137 - 290	220	220	91	349	2320	736
Blood Urea Nitrogen	mg/dL	2 - 10	4	4	1	11	536	251
Creatinine	mg/dL	0.2 - 1.1	0.5	0.4	0.0	1.5	377	182
Uric Acid	mg/dL	2.3 - 23.0	8.7	7.2	0.0	27.4	2384	726
Calcium	mg/dL	8.5 - 13.4	10.5	10.4	6.4	15.0	2267	732
Phosphorus	mg/dL	1.1 - 8.2	3.6	3.3	0.0	11.1	2033	664
Ca/Phos ratio		1.3 - 7.7	3.5	3.2	0.0	10.2	1980	646
Sodium	mEq/L	142 - 168	155	155	129	180	1880	637
Potassium	mEq/L	2.7 - 7.5	4.5	4.3	1.2	8.9	1827	617
Na/K ratio		16.7 - 55.5	35.9	35.6	2.8	74.8	1850	627
Chloride	mEq/L	103 - 129	116	116	88	141	1304	461
Total Protein	g/dL	3.7 - 7.3	5.3	5.3	1.7	9.3	2378	736
Albumin	g/dL	1.0 - 3.2	1.8	1.8	0.0	3.9	2241	699
Globulin	g/dL	0.6 - 5.1	3.2	3.3	0.0	7.0	2118	685
Fibrinogen	mg/dL	*	1	1	0	1	36	14

Alkaline Phosphatase	IU/L	22 - 459	141	100	0	550	1315	461
Lactate Dehydrogenase	IU/L	80 - 1908	581	436	30	2581	995	364
Aspartate Aminotransferase	IU/L	58 - 378	164	146	2	489	2413	748
Alanine Aminotransferase	IU/L	21 - 268	101	88	2	353	646	300
Creatine Kinase	IU/L	77 - 1052	362	290	0	1296	2065	668
Gamma-glutamyltransferase	IU/L	0 - 10	3	2	0	13	358	168
Amylase	IU/L	1247 - 6866	3277	2793	3	7987	609	206
Total Bilirubin	mg/dL	0.1 - 0.8	0.2	0.2	0.0	1.0	340	189
Cholesterol	mg/dL	153 - 437	273	267	24	536	1722	560
Triglyceride	mg/dL	44 - 269	128	126	39	350	133	92
Bicarbonate	mEq/L	13.8 - 32.5	23.1	23.1	10.0	34.0	86	54
Carbon Dioxide	mEq/L	15.6 - 34.0	25.0	25.5	10.0	36.0	207	62
Body Temperature	F	*	100.4	101.3	94.3	104.0	35	32

[a] Lowest sample value used to calculate the reference interval.
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King Penguin (*Aptenodytes patagonicus*)

Samples contributed by 11 institutions.

Sample Selection Criteria:

- No selection by gender
- All ages combined
- Animal was classified as healthy at the time of sample collection
- Sample was not deteriorated

Physiological Reference Intervals for Aptenodytes patagonicus

Test	Units	Reference Interval	Mean	Median	Low Sample[a]	High Sample[b]	Sample Size[c]	Animals[d]
White Blood Cell Count	*10^3 cells/µL	2.89 - 22.49	9.40	8.50	0.80	29.80	167	65
Red Blood Cell Count	*10^6 cells/µL	*	2.04	1.88	0.76	3.25	38	14
Hemoglobin	g/dL	12.5 - 20.9	16.2	16.7	10.0	19.6	57	22
Hematocrit	%	33.0 - 58.5	47.2	48.1	23.0	62.0	193	68
MCV	fL	*	237.9	242.1	144.6	310.0	37	13
MCHC	g/dL	29.7 - 38.5	34.2	34.1	30.0	40.5	55	20
Heterophils	*10^3 cells/µL	0.95 - 9.74	4.19	3.90	0.48	11.10	165	64
Lymphocytes	*10^3 cells/µL	0.55 - 11.37	3.69	3.01	0.22	14.20	164	65
Monocytes	cells/µL	56 - 1527	473	354	38	1856	128	56
Eosinophils	cells/µL	0 - 453	202	155	27	670	71	38
Basophils	cells/µL	0 - 1672	699	552	60	2415	118	50
Glucose	mg/dL	147 - 321	233	230	101	369	191	76
Blood Urea Nitrogen	mg/dL	2 - 6	4	4	2	6	50	25
Creatinine	mg/dL	0.0 - 0.7	0.4	0.4	0.1	0.8	44	19
Uric Acid	mg/dL	2.6 - 23.2	10.0	9.0	1.6	28.0	191	77
Calcium	mg/dL	8.1 - 12.4	10.3	10.3	6.4	14.0	176	75
Phosphorus	mg/dL	1.6 - 8.5	3.9	3.6	0.1	9.7	178	73
Ca/Phos ratio		1.2 - 6.3	3.0	2.8	0.2	7.4	169	72
Sodium	mEq/L	141 - 170	155	155	131	172	133	59
Potassium	mEq/L	1.4 - 6.8	3.4	3.2	0.7	7.5	129	59
Na/K ratio		19.3 - 103.1	50.8	47.9	3.8	108.0	126	58
Chloride	mEq/L	99 - 127	113	113	88	131	102	49
Total Protein	g/dL	2.5 - 6.9	5.1	5.1	1.9	8.0	164	72
Albumin	g/dL	1.0 - 3.3	2.0	1.9	0.0	4.3	151	72
Globulin	g/dL	0.5 - 4.9	2.9	3.1	0.2	5.9	142	69
Alkaline Phosphatase	IU/L	0 - 224	119	106	35	304	90	40
Lactate Dehydrogenase	IU/L	0 - 550	235	166	54	789	85	51

Aspartate Aminotransferase	IU/L	91 - 366	202	191	54	419	190	77
Alanine Aminotransferase	IU/L	1 - 121	64	61	13	149	69	38
Creatine Kinase	IU/L	66 - 891	312	272	4	968	132	66
Total Bilirubin	mg/dL	0.0 - 0.7	0.2	0.1	0.0	1.2	42	19
Cholesterol	mg/dL	134 - 513	318	317	46	573	120	59

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Macaroni Penguin (*Eudyptes chrysolophus*)

Samples contributed by 3 institutions.

Sample Selection Criteria:

- No selection by gender
- All ages combined
- Animal was classified as healthy at the time of sample collection
- Sample was not deteriorated

Physiological Reference Intervals for Eudyptes chrysolophus

Test	Units	Reference Interval	Mean	Median	Low Sample[a]	High Sample[b]	Sample Size[c]	Animals[d]
White Blood Cell Count	*10^3 cells/µL	3.16 – 19.54	8.70	7.88	1.38	24.00	178	41
Red Blood Cell Count	*10^6 cells/µL	1.73 – 3.50	2.66	2.62	1.67	4.30	98	18
Hemoglobin	g/dL	13.3 – 20.9	17.0	17.1	10.5	20.0	69	14
Hematocrit	%	31.6 – 60.4	49.3	49.8	25.0	64.0	214	45
MCV	fL	123.7 – 275.2	196.0	199.4	63.0	290.0	99	18
MCH	pg	44.6 – 88.5	65.9	66.6	19.6	90.9	68	14
MCHC	g/dL	24.6 – 38.1	31.6	31.4	25.9	39.2	70	14
Heterophils	*10^3 cells/µL	1.30 – 9.70	4.09	3.61	1.04	12.20	177	41
Lymphocytes	*10^3 cells/µL	0.50 – 12.45	4.14	3.61	0.21	15.20	178	41
Monocytes	cells/µL	0 – 904	331	238	28	1511	79	32
Eosinophils	cells/µL	0 – 624	244	181	28	990	82	29
Basophils	cells/µL	0 – 543	253	218	32	815	103	26
Glucose	mg/dL	146 – 276	215	218	75	318	196	46
Uric Acid	mg/dL	2.2 – 27.2	10.2	8.1	1.7	30.7	185	44
Calcium	mg/dL	7.7 – 13.5	10.0	9.9	6.9	14.2	192	46
Phosphorus	mg/dL	0.0 – 5.9	3.1	2.8	0.7	7.8	114	45
Ca/Phos ratio		0.0 – 8.2	4.1	3.6	1.2	13.0	113	45
Sodium	mEq/L	142 – 165	154	154	133	168	109	39
Potassium	mEq/L	2.1 – 5.6	4.0	3.9	1.9	6.4	107	39
Na/K ratio		21.0 – 57.3	40.1	39.2	15.6	70.9	107	38
Chloride	mEq/L	101 – 128	115	114	94	134	84	37
Total Protein	g/dL	3.4 – 7.0	4.9	4.8	2.0	7.6	185	43
Albumin	g/dL	1.3 – 4.3	2.6	2.6	1.1	4.6	132	35

Globulin	g/dL	0.2 – 3.8	1.4	0.9	0.2	5.3	127	34
Alkaline Phosphatase	IU/L	4 – 201	107	103	24	205	42	30
Lactate Dehydrogenase	IU/L	0 – 391	204	188	62	548	49	29
Aspartate Aminotransferase	IU/L	126 – 401	247	243	52	471	192	45
Alanine Aminotransferase	IU/L	*	47	43	14	131	38	27
Creatine Kinase	IU/L	72 – 730	242	193	63	813	121	42
Cholesterol	mg/dL	176 – 438	309	307	142	476	98	39
Bicarbonate	mEq/L	*	25.8	26.0	17.0	35.0	31	27

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Magellanic Penguin (*Spheniscus magellanicus*)

Samples contributed by 12 institutions.

Sample Selection Criteria:

- No selection by gender
- All ages combined
- Animal was classified as healthy at the time of sample collection
- Sample was not deteriorated

Physiological Reference Intervals for Spheniscus magellanicus

Test	Units	Reference Interval	Mean	Median	Low Sample[a]	High Sample[b]	Sample Size[c]	Animals[d]
White Blood Cell Count	*10^3 cells/µL	4.79 - 37.51	15.07	13.20	2.30	44.60	908	238
Red Blood Cell Count	*10^6 cells/µL	0.87 - 3.41	1.97	1.98	0.51	4.67	412	142
Hemoglobin	g/dL	10.7 - 21.8	16.1	16.2	8.0	24.3	107	77
Hematocrit	%	27.7 - 58.9	45.6	46.8	15.0	75.0	955	243
MCV	fL	117.8 - 441.1	241.7	231.0	10.0	536.4	399	140
MCH	pg	36.2 - 106.5	75.5	71.3	40.7	114.0	86	63
MCHC	g/dL	25.1 - 41.3	33.5	33.2	19.4	48.3	106	77
Heterophils	*10^3 cells/µL	1.70 - 20.05	7.42	6.27	0.03	26.20	891	237
Lymphocytes	*10^3 cells/µL	1.04 - 18.12	6.31	5.07	0.06	23.70	897	238
Monocytes	cells/µL	66 - 1673	478	342	26	2045	454	185
Eosinophils	cells/µL	63 - 1306	384	277	40	1560	392	172
Basophils	cells/µL	64 - 696	261	208	30	915	391	172
Glucose	mg/dL	149 - 283	215	215	87	342	791	240
Blood Urea Nitrogen	mg/dL	0 - 9	5	3	1	12	61	44
Creatinine	mg/dL	0.0 - 0.7	0.3	0.2	0.0	0.9	77	50
Uric Acid	mg/dL	1.9 - 26.1	9.6	7.3	0.6	35.8	818	239
Calcium	mg/dL	8.6 - 12.0	10.2	10.2	7.1	13.6	753	237
Phosphorus	mg/dL	1.1 - 8.7	3.8	3.4	0.4	10.5	585	205
Ca/Phos ratio		1.1 - 8.2	3.3	2.9	0.0	9.4	555	200
Sodium	mEq/L	141 - 165	153	153	132	170	360	161
Potassium	mEq/L	2.1 - 8.5	4.4	4.2	1.7	10.4	366	163
Na/K ratio		18.9 - 75.3	39.0	36.5	10.3	92.2	359	163
Chloride	mEq/L	94 - 126	109	109	85	137	162	102
Total Protein	g/dL	3.7 - 8.4	5.8	5.7	2.3	10.6	916	239

Albumin	g/dL	0.3 - 3.7	1.9	1.8	0.0	4.2	619	209
Globulin	g/dL	0.1 - 5.9	3.1	3.4	0.0	7.6	626	208
Alkaline Phosphatase	IU/L	29 - 388	140	116	2	475	191	88
Lactate Dehydrogenase	IU/L	65 - 1033	375	288	1	1406	295	152
Aspartate Aminotransferase	IU/L	59 - 538	206	176	21	628	767	239
Alanine Aminotransferase	IU/L	0 - 191	78	63	0	312	71	48
Creatine Kinase	IU/L	56 - 1121	336	250	0	1315	658	222
Amylase	IU/L	558 - 7001	3838	4022	87	7426	187	71
Total Bilirubin	mg/dL	*	0.4	0.3	0.1	1.4	35	29
Cholesterol	mg/dL	165 - 463	300	299	66	580	458	198

[a] Lowest sample value used to calculate the reference interval.
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Southern Rockhopper Penguin (*Eudyptes chrysocome*)

Samples contributed by 14 institutions.

Sample Selection Criteria:

- No selection by gender
- All ages combined
- Animal was classified as healthy at the time of sample collection
- Sample was not deteriorated

Physiological Reference Intervals for Eudyptes chrysocome

Test	Units	Reference Interval	Mean	Median	Low Sample[a]	High Sample[b]	Sample Size[c]	Animals[d]
White Blood Cell Count	*10^3 cells/µL	2.45 - 19.51	8.30	7.44	1.40	24.20	513	150
Red Blood Cell Count	*10^6 cells/µL	*	2.11	2.10	1.43	3.13	33	22
Hemoglobin	g/dL	*	18.4	17.9	11.9	26.4	38	26
Hematocrit	%	33.1 - 59.9	48.7	49.6	25.0	68.0	553	157
MCV	fL	*	223.3	226.5	129.6	293.7	33	22
MCHC	g/dL	*	38.8	36.2	31.6	50.6	38	26
Heterophils	*10^3 cells/µL	1.07 - 9.65	4.14	3.60	0.02	14.20	512	149
Lymphocytes	*10^3 cells/µL	0.39 - 8.49	3.01	2.47	0.05	11.00	495	147
Monocytes	cells/µL	45 - 959	315	240	16	1275	431	137
Eosinophils	cells/µL	44 - 1378	378	272	20	1629	217	99
Basophils	cells/µL	35 - 952	295	229	24	1278	286	114
Glucose	mg/dL	167 - 319	239	238	126	361	500	152
Blood Urea Nitrogen	mg/dL	1 - 6	3	3	1	9	111	57
Creatinine	mg/dL	0.0 - 0.9	0.4	0.3	0.1	1.3	91	51
Uric Acid	mg/dL	2.5 - 24.0	9.3	7.5	1.5	33.0	472	149
Calcium	mg/dL	7.8 - 11.7	9.7	9.7	6.7	12.5	479	152
Phosphorus	mg/dL	0.4 - 6.8	2.5	2.2	0.0	8.5	443	148
Ca/Phos ratio		1.3 - 14.9	5.3	4.3	0.8	17.3	425	144
Sodium	mEq/L	141 - 163	153	153	135	171	419	136
Potassium	mEq/L	2.0 - 7.1	4.0	3.8	1.0	8.4	388	134
Na/K ratio		20.9 - 76.4	41.4	39.6	14.6	91.8	387	134
Chloride	mEq/L	106 - 122	115	115	102	124	149	85
Total Protein	g/dL	3.1 - 6.0	4.4	4.4	1.8	6.8	390	150
Albumin	g/dL	1.1 - 3.2	1.9	1.7	0.3	4.1	437	144
Globulin	g/dL	1.0 - 4.1	2.6	2.7	0.3	5.3	432	142
Alkaline Phosphatase	IU/L	1 - 289	94	76	0	337	298	88
Lactate Dehydrogenase	IU/L	48 - 368	164	149	24	410	279	81
Aspartate Aminotransferase	IU/L	123 - 445	255	245	32	533	456	149

Alanine Aminotransferase	IU/L	0 - 101	48	40	10	149	103	56
Creatine Kinase	IU/L	91 - 1145	385	302	54	1338	329	117
Gamma-glutamyltransferase	IU/L	0 - 9	3	3	0	12	48	27
Amylase	IU/L	1392 - 8877	5001	5135	1483	7962	108	28
Total Bilirubin	mg/dL	0.0 - 0.4	0.1	0.1	0.0	0.9	58	37
Cholesterol	mg/dL	194 - 497	325	321	133	621	305	96
Carbon Dioxide	mEq/L	15.7 - 41.0	29.1	28.3	13.0	52.5	110	46

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Clinical Pathology Records Report: ISIS/In-House Reference Values (2002)
Milwaukee County Zoo
Scientific name: *Eudyptes pachyrhynchus*
Common Name: Fiordland penguin

ISIS Values

		Mean	S.D.	Min.	Max.	(N)
WBC	*10^3/UL	9.440 +	3.319	5.720	12.10	(3)
RBC	*10^6/UL	2.13 +	0.48	1.70	2.65	(3)
HCT	%	47.0 +	5.6	41.0	52.0	(3)
MCV	fL	223.8 +	24.2	196.2	241.2	(3)
HETEROPHILS	*10^3/UL	3.607 +	3.540	0.970	7.630	(3)
LYMPHOCYTES	*10^3/UL	5.307 +	2.169	3.990	7.810	(3)
MONOCYTES	*10^3/UL	0.323 +	0.100	0.210	0.400	(3)
EOSINOPHILS	*10^3/UL	0.220 +	0.141	0.120	0.320	(2)
BASOPHILS	*10^3/UL	0.230 +	0.000	0.230	0.230	(1)
GLUCOSE	MG/DL	188 +	4	185	191	(2)
CREAT.	MG/DL	1.2 +	0.8	0.7	1.8	(2)
URIC ACID	MG/DL	26.0 +	10.1	11.1	32.9	(4)
AST (SGOT)	IU/L	715 +	212	476	980	(4)
CPK	IU/L	721 +	218	459	985	(4)

Clinical Pathology Records Report: ISIS/In-House Reference Values (2002)
Milwaukee County Zoo
Scientific name: *Pygoscelis adeliae*
Common Name: Adelie penguin

ISIS Values

		Mean	S.D.	Min.	Max.	(N)
WBC	*10^3/UL	9.186 +	2.265	5.400	11.80	(7)
RBC	*10^6/UL	3.00 +	0.97	2.31	3.68	(2)
HGB	GM/DL	16.4 +	1.5	14.3	17.4	(4)
HCT	%	49.4 +	4.4	42.0	54.0	(7)
MCHC	uug	35.1 +	1.2	34.0	36.9	(4)
MCV	fL	184.6 +	57.3	144.0	225.1	(2)
HETEROPHILS	*10^3/UL	5.301 +	1.972	2.970	8.900	(7)
LYMPHOCYTES	*10^3/UL	3.316 +	2.038	0.740	5.430	(7)
MONOCYTES	*10^3/UL	0.327 +	0.336	0.074	0.708	(3)
EOSINOPHILS	*10^3/UL	0.255 +	0.170	0.054	0.472	(6)
BASOPHILS	*10^3/UL	0.245 +	0.159	0.074	0.472	(6)
GLUCOSE	MG/DL	284 +	46	215	353	(7)
BUN	MG/DL	3 +	0	3	3	(5)
CREAT.	MG/DL	0.3 +	0.1	0.2	0.5	(6)
URIC ACID	MG/DL	8.6 +	6.3	2.5	18.7	(7)
CA	MG/DL	10.9 +	0.8	10.0	12.5	(7)
PHOS	MG/DL	2.6 +	0.6	1.7	3.1	(4)
NA	MEQ/L	153 +	5	146	161	(7)
K	MEQ/L	2.9 +	0.6	2.2	3.8	(7)
CL	MEQ/L	114 +	3	110	117	(7)
CHOL	MG/DL	304 +	65	256	415	(5)
T.PROT. (C)	GM/DL	4.9 +	0.5	4.0	5.6	(7)
ALBUMIN (C)	GM/DL	2.1 +	0.3	1.7	2.6	(7)
GLOBULIN (C)	GM/DL	2.7 +	0.3	2.2	3.0	(7)
AST (SGOT)	IU/L	155 +	56	95	234	(7)
ALT (SGPT)	IU/L	25 +	15	7	45	(5)
T. BILI.	MG/DL	0.3 +	0.1	0.2	0.4	(5)
ALK.PHOS.	IU/L	64 +	27	26	96	(5)
LDH	IU/L	415 +	277	139	940	(6)
CPK	IU/L	147 +	151	43	371	(4)
ALPHA-1 GLOB	GM/DL	0.3 +	0.0	0.3	0.3	(1)
ALPHA-2 GLOB	GM/DL	0.4 +	0.0	0.4	0.4	(1)
BETA GLOB.	GM/DL	0.5 +	0.0	0.5	0.5	(1)
CO2	MMOL/L	21.0 +	0.0	21.0	21.0	(1)

Appendix O: AZA Recommended Penguin Egg, Chick & Adult Bird Necropsy Protocols

Egg Necropsy:

1. Refrigerate the egg if there will be a delay before necropsy. Do not freeze eggs or embryos unless the primary goal is virus isolation or bacterial culture, rather than histologic evaluation.
2. Weigh and measure the egg as soon as possible after the embryo is confirmed dead.
    a. Record weight in grams.
    b. Measure length and greatest diameter of egg in centimeters.
3. Describe egg shell characteristics (abnormal shape, shell thickness, presence of cracks, degree of fecal staining, external calcium deposits, etc.).
4. Open the egg by carefully removing the shell overlying the aircell. This can be accomplished with a pair of sharp-blunt scissors, or by gently cracking the shell and removing fragments with forceps.
    a. Examine the aircell membrane for integrity, thickenings, hemorrhages, etc.
5. For small (early stage) embryos, obtain separate swabs of yolk and albumen for culture and cytology. Skip to step 7 for larger embryos.
    a. Peel back the aircell membrane and insert a swab to obtain the albumen culture. Note: if the fluid is watery, it is likely allantoic fluid rather than albumen.
    b. The egg contents may have to be dumped out in order to obtain the yolk cultures.
    c. A second swab of yolk (not a culture swab) may then be taken and rolled onto three microscope slides. The smears should be as thin as possible. NOTE: Avoid vigorous swabbing of the internal aspect of the yolk sac; hematopoietic cells which reside there may be dislodged and give a false impression that there is inflammation in the yolk sac. Recommended stains include Wright-Giemsa (or Diff-Quik) and gram. Save the third slide for additional stains, if needed.
6. For larger (late stage) embryos, remove enough egg shell to expose the embryo. Note the position of the head relative to other body parts, and in relation to the aircell. The normal position for embryos ready to pip is head under the right wing, with the tip of the beak pointing up toward the aircell.
    a. If the yolk sac is still external (has not retracted into the body cavity), and is accessible, puncture the wall with a sterile scalpel and obtain a culture. If the yolk sac is inaccessible, skip to step 8.
    b. Obtain a second swab of yolk for cytology as described above.
    c. Save the yolk sac (in formalin) for histopathology
    d. Record the color and consistency (relative thickness or viscosity) of the yolk.
7. Remove the embryo and membranes from the shell by gently dumping the contents into a clean shallow container.
    a. If swabs of yolk for culture and cytology have not yet been collected, obtain them now (as described under step 6). Record the color and consistency (relative thickness or viscosity) of the yolk.
    b. Weigh the embryo with and without the yolk sac (if external).
    c. Measure the length of the embryo and if possible estimate the stage of development using The Normal Stages of The Chick as a guideline.
    d. Note any external abnormalities, such as musculoskeletal deformities, abnormal skin color, skin hemorrhages, edema, dryness, residual albumen, etc. If possible photograph any abnormalities.
    e. Record the degree of internalization (retraction) of the yolk sac.
    f. Examine the pipping muscle at the back of the neck for edema or hemorrhages.
    g. Note the contents of the mouth, nares, and gizzard.
8. Small embryos along with yolk sac and fetal membranes may be immersed whole in formalin. The volume of formalin should be at least ten times the total volume of the tissues.
9. If the embryo is large enough, conduct a mini-necropsy, retaining representative samples of all organs and tissues for histopathology.

   a. Open the coelomic cavity by making a ventral midline incision with a scalpel or scissors, being careful to avoid tearing the yolk sac if it is internalized. Proceed with yolk sac cultures and cytology as described under steps 6 and 7 above.
   b. Save the yolk sac (in formalin) for histopathology along with the embryo and membranes. The volume of formalin should be at least ten times the total volume of the tissues.
10. Send a copy of the final pathology report and a recut set of H&E stained slides to Dr. Judy St. Leger, SeaWorld San Diego, 500 SeaWorld Drive, San Diego, CA 92109-7904. Ph: 619-222-6363.

Chick and Adult Necropsy:

1. Refrigerate the body if there will be a delay before necropsy. Do not freeze the body unless the primary goal is virus isolation or bacterial culture, rather than histologic evaluation.
2. Record all relevant historical information as indicated on the necropsy form.
3. Weigh the bird as soon as possible after death.

EXTERNAL EXAMINATION:

4. For chicks, note condition of the umbilicus or seal, particularly whether it dry and completely closed.
5. Note any musculoskeletal abnormalities, ectoparasites, evidence of trauma, proliferative skin lesions, etc.
6. Examine the feet carefully for evidence of pododermatitis (bumblefoot).
7. Examine body orifices for patency, exudates, fecal staining around cloaca, etc.
8. Make an evaluation of nutritional condition based on fat stores and relative muscle mass.

INTERNAL EXAMINATION:

9. Make a ventral midline skin incision from the mandible to the cloaca with a sharp scalpel or scissors, being careful to avoid rupturing the yolk sac in young birds.
   a. If the yolk sac ruptures, immediately obtain a yolk culture as the yolk spills out and prepare smears for cytology.
   b. Note the size of the yolk sac and, if sufficient yolk remains, obtain separate swabs for culture and cytology.
10. Remove the keel to expose the thoracic organs.
   a. Note any accumulations of fluid or exudate in the body cavity and obtain a swab for bacterial and/or fungal culture if appropriate.
11. Obtain blood for smears and bacterial culture by direct heart puncture using a 1 to 3 cc syringe with a 20 to 22 gauge needle.
   a. Prepare at least two blood smears for hemoparasite screening (only a few drops of blood are needed).
   b. If enough blood was obtained, bacterial cultures should be submitted on young birds to rule out septicemia.
   c. If no blood can be obtained from the heart by syringe, smears can be prepared by dabbing the cut surface of the lung or liver onto two or three microscope slides.
12. Collect the thyroids (with parathyroids), thymus, and spleen for histopathology.
   a. Determine gender by examining the gonads prior to removal.
13. Remove the internal organs and examine each systematically.
   a. Obtain samples for histopathology using the tissue list below as a guide. Save samples of all lesions.
   b. Note especially the quantity and nature of the ingesta throughout the GI tract.
   c. The bursa of Fabricius lies dorsal to the cloaca, close to the cloacal orifice (vent). Make sure the bursa does not remain attached to the body when the GI tract is removed.

<u>Tissue Checklist</u>

All of the following tissues may be placed together in a single container of 10% neutral buffered formalin. THE VOLUME OF FORMALIN SHOULD BE 10 TIMES THE VOLUME OF ALL TISSUES COLLECTED. The tissues should be no thicker than 0.5cm to ensure proper fixation.

- Skin Muscle (pectoral and thigh)

- Sciatic nerve (with thigh muscle)
- Tongue
- Esophagus
- Crop
- Proventriculus
- Gizzard
- Duodenum
- Jejunum
- Ileum
- Cecum
- Colon
- Cloaca with Bursa of Fabricius
- Liver with gallbladder
- Pancreas
- Spleen
- Kidney with Gonad
- Oviduct
- Adrenal (with kidney)
- Thyroid and Parathyroid Thymus
- Trachea
- Lung
- Heart
- Aorta
- Pituitary
- Eye
- Brain
- Femoral Bone Marrow

FREEZE PORTIONS OF THE FOLLOWING IF POSSIBLE FOR FURTHER TESTING:

- Liver
- Spleen
- Lung
- Brain
- Heart
- Skeletal Muscle

Freeze each tissue separately by wrapping in foil and placing in separate plastic bags (at least 10 grams of each tissue if large enough. These tissues can be valuable for ancillary diagnostics. They may be discarded after a definitive diagnosis is established, but if possible, should be saved for future research purposes.

Send a copy of the pathology report and a recut set of H&E slides to Dr. Judy St. Leger, Pathology Department SeaWorld San Diego, 500 SeaWorld Dr. San Diego CA 92109-7904. Ph:619-222-6363.

Appendix P: Sample Enrichment Schedules for Penguins

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
			1	2	3	4
			Sprinkler / mister	Keeper play in habitat	Relocate pm pans	Ice cubes throughout habitat
			W	K	FH	T
5	6	7	8	9	10	11
Puzzle ball w/fish & ice cubes	Guests in habitat	Relocate pm pans	Wading area on east side w/sunken fish	Keeper's choice of toy w/interaction	Boomer ball	Radio or penguin sounds CD
T, FD	G	FH	W, FD	TK	T	A
12	13	14	15	16	17	18
Keeper play in habitat	Bubbles	Ice cubes on west & north sides	Sprinkler on east side	Relocate pm pans	Keeper's choice of toy w/interaction	Ice cubes throughout habitat (iphone)
K	V	T	W	FH	TK	T
19	20	21	22	23	24	25
"Keeper play" outside habitat	Radio or penguin sounds CD	Relocate pm pans	Wading area on east side w/boomer ball	Ice cubes throughout habitat	Multiple boomer balls, interact with for 10 minutes	Keeper's choice of toy w/interaction
V	A	FH	W	T	TK	TK
26	27	28	29	30	31	
Puzzle ball w/fish & ice cubes	Bubbles	Keeper play in habitat	Sprinkler / mister	Relocate pm pans	Ice cubes throughout habitat	
T, FD	V	K	W	FH	T	

Week of:	Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday
	Traffic cones, car mats, painting	Fish from heaven, seasonings, kelp with fish	Hula hoop chain, jingle bells	Mandatory swim, pool float	Showers, pool noodles, small colored plates	Mirror-in exhibit or at underwater viewing, jumbo tennis balls	Balloon freezes, open house encounter room
	Mandatory swim, hanging ball from feed hook	Water sprinkler, dog toys, kazoos	Bubbles, cauldrons, puzzle mats	Turtle pool &/or top, kayak	Trash can lids, baby bath, xylophone	Hose pieces, ice sculpture, baby mobile	Kelp, flashlight in exhibit or at underwater viewing
	Ice treats, bells, frisbees	Boogie board, roll ball at underwater viewing	Smiley toy, open house HR and HP	Tv at UWV, fish inside octoballs	Water feed from heaven, mandatory swim, buoys	Snow cones, chalk drawings, painting	Tent, boogie board, extracts
	Color-changing ball, yoga mats	Mandatory swim, penguin soccer	Big red ball, wind chimes, large ice floe	Music, water feed from side door of exhibit	Kiddie pool, small balls, small colored mats	Water sprinkler, fire hose pieces	Ice alone or with fish/fish juice/extracts
	Bubbles, plastic box toys, plastic bowling pins	Pinwheels, window clings, piano mat	Wheelbarrow with ice and fish/juice/extract, beans in a can interactive	Yellow surf board, in water fountain/light show	Hula hoops, singing and dancing penguin	Ice alone or with fish/fish juice/extracts, mega blocks towers or loose	Towels & mandatory swim

Attachment 5

MAP KEY

- Elevators
- Restrooms
- Walking Path
- Accessible Path
- Accessible Facilities
- Stairs
- Safe Swimming
- Coral Reef
- Walking Bridge
- Suspension Bridge

*Map Not to Scale

DINING
1. Drums of the Pacific Lu'au/ Sunset Terrace
2. Ululani's Hawaiian Shave Ice
3. Son'z Steakhouse
4. Swan Court
5. 'Umalu
6. Honolulu Coffee
7. Japengo
8. Pau Huaka'i Tiki Bar

ACTIVITIES
9. Fitness Center
10. Spa
11. Beach Activity Center & Scuba
12. Recreation and Pool Desk
13. Lava Tube Waterslide
14. Keiki Lagoon (Children's Pool)
15. Residence Club Pools
16. Tennis Courts

TOWEL CADDY
TC. 24-hr Self-Serve Access to Towels

HOTEL SERVICES
17. Laundry Room
18. Business Center
19. Regency Club
20. Sunglass Hut
21. Events and Weddings Office
22. Grins2Go
23. Concierge
24. Weekends
25. Accents General Store
26. Maui Hands
27. Na Hoku
28. Hertz
29. SpeediShuttle
30. Front Desk, Valet & Bell Stand
31. Hyatt Vacation Ownership Sales Office
32. Trolley Stop

TOWEL CADDY RETURN
TR. 24-hr Self-Serve Towel Return Only

EVENT SPACES
33. Monarchy Ballroom (Promenade Level)
34. Maui Suites (Promenade Level)
35. Lahaina Suites (Retail Level)
36. Oriental Gardens
37. Halona Kai
38. Ka Hale Aloha Gazebo
39. Napili Pool Lawn
40. Makai Lawn
41. Napili Garden

WILDLIFE
42. Swan Lagoon
43. Koi Fish
44. Penguins

W S N E

LAHAINA POOL

NAPILI POOL

WAILELE LAGOON

MALUHIA POOL

LAHAINA TOWER
Rooms 350 - 982

ATRIUM TOWER
Rooms 1401 - 2234

NAPILI TOWER
Rooms 101 - 836

HYATT RESIDENCE CLUB

LAHAINA SELF PARKING

NAPILI SELF PARKING

VALET PARKING

KA'ANAPALI GOLF COURSE

GUEST ROOMS

- LAHAINA TOWER Rooms 350 - 982
- ATRIUM TOWER Rooms 1401 - 2234
- NAPILI TOWER Rooms 101 - 836

Photograph 1: Depicts one side of the exterior wall surrounding the penguin enclosure.

Photograph 2: Depicts another view of the exterior wall surrounding the penguin enclosure.

Photograph 3: Depicts another view of the exterior wall surrounding the penguin enclosure.

Photograph 4: Depicts another view of the exterior wall surrounding the penguin enclosure.

Photograph 5: Depicts locked gate to the entrance of the penguin enclosure.

Australian Government
Department of Agriculture, Fisheries and Forestry

Australian Wildlife Health Network

ZOO AQUARIUM ASSOCIATION AUSTRALASIA

National **Zoo Biosecurity** Manual

MARCH 2011

DEPARTMENT OF AGRICULTURE, FISHERIES AND FORESTRY

National Zoo Biosecurity Manual

A cooperative initiative between the Zoo and Aquarium Association, the Australian Wildlife Health Network, the Commonwealth Department of Agriculture, Fisheries and Forestry and the Australian Zoo Industry.

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Australian Government Department of Agriculture, Fisheries and Forestry

Zoo and Aquarium Association (the Association)

ZAA Veterinary Specialist Advisory Group

Australia Zoo

Zoos South Australia

Perth Zoo

Sea World Australia

Taronga Conservation Society Australia (Taronga Zoo)

Zoos Victoria (Melbourne Zoo)

The Zoo and Aquarium Association and the Australian Wildlife Health Network support the group and assisted in production.

COMMENT HAS ALSO BEEN PROVIDED BY THE FOLLOWING:

Auckland Zoo

Australasian section of the CBSG of the IUCN SSC

ZAA Veterinary Specialist Advisory Group

Australian Animal Welfare Standards working group

The Zoo and Aquarium Association

Victorian Department of Primary Industries

Members of the ZAA Legislation and Standards Review Working Group

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CONTENTS

DISCLAIMER

This National Zoo Biosecurity Manual is not intended to be prescriptive and is not a set of standards requiring compliance by zoo industry members. This Manual has been specifically designed as an industry resource to raise awareness of best practice in zoo biosecurity. The information and guidelines within the Manual should not be used for any other purpose, nor interpreted outside this context.

DEVELOPMENT AND REVIEW PROCESS

This Manual has been developed as a cooperative initiative between the Zoo and Aquarium Association (the Association), the Australian Wildlife Health Network (AWHN) and the Australian Government Department of Agriculture, Fisheries and Forestry (DAFF), on behalf of the Australian Zoo Industry. The Zoo Animal Health Reference Group, the Board of the Zoo and Aquarium Association and Australia's Chief Veterinary Officer have endorsed this Manual for use by the zoo industry. The Manual is published as a working draft for 12 months from May 2011 to May 2012. During this period the Association welcomes feedback on the Manual from Association members and other stakeholders (send to NZBMreview@zooaquarium.org.au). Significant updates or changes to this document will be indicated by a change in Edition number and date of publication.

CONTEXT

The National Zoo Biosecurity Manual (NZBM) has been developed by veterinary leaders and advisors within the Australian zoo industry to document best practice biosecurity measures currently being adopted by the zoo industry. The Manual can be tailored by zoos to suit their individual circumstances and can also be utilised as a training tool, to assist zoos in educating staff on biosecurity measures. The Manual acknowledges the wide range of circumstances under which zoos within Australia operate in terms of geographic location, species and numbers of animals held, work practices and available resources. All zoos are encouraged to use the information in this Manual to assess their own biosecurity risks, and to develop and maintain an appropriate level of biosecurity for their premises.

INTRODUCTION

Biosecurity is the set of precautions taken to minimise the risk of introducing an infectious disease into an animal (or human) population.

Each zoo's unique characteristics will influence its biosecurity requirements. This Manual identifies areas of common risk to all zoos and outlines appropriate measures to minimise these risks. Individual zoos are encouraged to develop their own site-specific Biosecurity Plan.

Biosecurity is important for all zoos. Good biosecurity practices help to:

- Keep zoo animals safe and healthy
- Keep zoo staff and visitors safe and healthy
- Minimise costs associated with treating disease in zoo animals
- Keep zoos open and running if an infectious disease outbreak occurs within, or near a zoo
- Promote the good reputation of the individual zoo and the zoo industry as a whole
- Assist zoos in acquiring and managing exotic species.

The National Zoo Biosecurity Manual (NZBM)is intended to be used by individual zoos, including fauna parks, sanctuaries, aquaria and marine parks, holding native and/or exotic species, as a tool to help them to gauge their own biosecurity requirements and to assist them to develop a biosecurity plan suitable for their particular circumstances. It is not expected that every zoo will have a need to, nor be in a position to, implement all of the guidelines in daily practice.

Zoo Biosecurity includes but is not limited to:

- Appropriately constructed and maintained facilities
- Management of stray and pest species
- Management of drainage and waste products
- Good hygiene and work practices
- A preventative medicine program
- Appropriate quarantine of newly arrived and sick animals and
- Veterinary diagnosis and treatment of sick animals.

These guidelines complement and support the Australian Animal Welfare Standards and Guidelines: Exhibited Animals (Zoos) (AAWS), developed as part of the Australian Animal Welfare Strategy and should be read in conjunction with that document. Relevant standards from the draft AAWS document (November 2010, Version 7.1) are referenced throughout this Manual. Association member zoos should also refer to the ZAA Accreditation Standards http://www.zooaquarium.org.au/Accreditation/default.aspx.

Zoos must also comply with the legislation of relevant agencies and jurisdictions (local, federal and state/territory).

The development of Zoo-specific practices and institutional-specific Biosecurity Plans is fundamental to the success of improved biosecurity for the entire zoo industry. It is acknowledged that each zoo will have differing biosecurity challenges and operating environments, which should be addressed based upon the objectives identified within this Manual.

Each institution is encouraged to develop its own Zoo specific Plan to guide their biosecurity activities. An institution which does not develop a Zoo specific Plan can achieve best practice by meeting the guidelines within the National Zoo Biosecurity Manual.

A biosecurity self audit checklist for ongoing assessment and improvement is available, in electronic format, as a supplement to this Manual. The checklist can be downloaded from www.zooaquarium.org.au and adapted as needed by each institution.

Guidelines (numbered sequentially for each section) outline the recommended practices to achieve best practice zoo biosecurity outcomes.

The Manual outlines both **basic guidelines** and **higher level guidelines** for all zoos. Most guidelines are considered to be basic guidelines which will be relevant to most zoos in most circumstances. Some guidelines, marked **higher level guidelines** may not be applicable in all situations or in all zoos, but may be implemented in individual zoos according to their needs or in all zoos during periods of higher biosecurity risk.

Definitions and **abbreviations** are found at the end of this document and are mostly drawn from the AAWS document. Some defined words in this document are capitalised. Some important definitions are included in the main body of the document for clarity.

PRINCIPLES OF ZOO BIOSECURITY

Good biosecurity is integral to the successful management of all zoos.

GOOD ZOO BIOSECURITY AIMS TO:

- prevent the introduction of infectious disease and contaminants to zoo animals
- prevent the spread of disease from an infected area to an uninfected area within the zoo
- prevent the spread of infectious disease from zoo animals to animals outside the zoo
- prevent the spread of infectious disease from animals to humans or humans to animals.

Biosecurity is important for all zoos, regardless of size. Historically, Australia's larger zoos have been expected to maintain strong biosecurity practices, due to the perceived higher risks associated with importing and holding exotic species. With today's growing focus on biosecurity management, it is important that zoo biosecurity focuses on all risks, not just those arising from exotic species. All zoos (including smaller zoos and fauna parks holding few or no exotic species) need to be aware of, and address the biosecurity risks relevant to their circumstances. All zoo staff need to be aware of the principles of biosecurity and how this applies to their work at the zoo.

Biosecurity is the responsibility of everyone at the zoo.

Biosecurity is concerned with minimising the negative consequences of infectious disease introduction and spread. Infectious disease within the zoo collection impacts on individual health and welfare, and can have long term impacts on reproduction, longevity, behaviours and population and species viability. Subclinical and chronic diseases can exert their effects for years and even decades. Ill health, death and reproductive failure in collection animals leads to greater costs (husbandry, veterinary, acquisition) and reduces the financial viability of the zoo as a business. Infectious disease spread to humans or domestic animals can have serious social, economic and ethical costs. A zoo's ability to protect itself from a disease outbreak will be greatly improved if it has appropriate biosecurity arrangements.

Biosecurity is an insurance policy against disease outbreak and its consequences. Biosecurity is a prudent and necessary investment.

Biosecurity is concerned with recognising and managing risk. This Manual identifies areas of risk common to most zoos and appropriate measures to minimise those risks. Individual zoos can

achieve best practice by conducting an institution-specific **biosecurity risk assessment** to establish the level of risk that exists in each area of its operations, and by using this Manual as a guide to identifying and implementing appropriate control measures for their circumstances. Zoos are encouraged to develop their own institution-specific Biosecurity Plans.

It is important to consider **all factors that may impact on zoo biosecurity**, including:

- species, origin and number of collection animals
- location and layout of the zoo
- source of water supply
- source of food supply
- method of waste management
- disease status of collection animals
- disease status and proximity to animals in the surrounding area
- presence and type of wildlife and pest species
- zoonotic disease potential
- animal movements and transactions
- movement of staff, visitors, contractors and deliveries.

TYPICAL ZOO BIOSECURITY MANAGEMENT PRACTICES INCLUDE:

- a preventative medicine program for all zoo animals
- inspection, testing and quarantine of incoming animals, including species bred for release as part of a sanctioned recovery program
- isolation and treatment of sick animals
- veterinary investigation of illness and death in collection animals
- control of wild, stray and pest animals
- hygiene procedures for staff and visitors
- appropriately constructed and maintained facilities
- controlling drainage and waste disposal and
- ensuring food, water, equipment or work practices do not introduce or spread pests or disease.

MAJOR ROUTES FOR DISEASE AND PATHOGEN TRANSMISSION

An understanding of the major routes for disease and pathogen movement from, or into, a zoo is essential for assessing and managing risk and creating effective work practices. Managing risk is the key to good biosecurity.

Diseases and pathogens may enter or exit the zoo via many routes. Any animal, human or product entering or leaving the zoo should be seen as a possible route or vehicle for disease transmission. The management of inputs and outputs is discussed in greater detail in the relevant sections of this Manual (see Routine Biosecurity Procedures).

Inputs

Inputs refer to any human, animal, biological or non-biological product which enters the zoo. Inputs into zoos vary depending on the type of facility. Each input into the zoo should be assessed for its biosecurity risk. This Biosecurity Manual deals primarily with the recognition and management of the risks associated with these inputs. Each section is covered in greater detail in the document, but the following general principles apply:

I. ANIMALS

Animal inputs include: animals introduced from other institutions either from within Australia or imported from overseas; animals imported from commercial properties, including animals used as food items; sick, injured or orphaned wildlife brought in by members of the public, wildlife care groups or wildlife officers; animals confiscated by customs/quarantine officers; native animals caught from the wild for captive breeding purposes; free-ranging animals, (either native, feral or stray, including birds, rodents, cats and dogs) from adjacent areas; pet animals brought into the zoo grounds and disability animals accompanying visitors. It also includes insects and other invertebrates which may carry, or mechanically transmit, infectious diseases. Any animal input may pose a biosecurity risk.

II. FEED

Feed inputs include dry feed (concentrates, hay, pellets, seed) and wet feed (fresh fruit, poultry, fish, meat, vegetables, browse and pasture silage). Feed may carry pathogens, and may be contaminated by the raw materials used, post-production, during transport and storage or by exposure to rodents, birds, other pests, insects and other free-ranging species on or off the property. Bacteria and mould in poor quality or damaged feed may be a biosecurity concern.

III. BIOLOGICAL SPECIMENS

Biological specimens may be brought to zoos by researchers, wildlife officers, customs and quarantine officers or others. Wildlife carcases may be brought to the zoo for post mortem investigation. Semen, embryos and other biological specimens may be brought to the zoo for reproductive or laboratory work. These inputs can pose a risk of disease and pathogen transmission.

IV. VEHICLES, MACHINERY, TOOLS AND OTHER EQUIPMENT

Vehicles moving into the zoo may transfer infectious agents, especially on contaminated tyres. Other equipment entering the facility includes tools, materials used for animal housing (straw, litter, mulch, sand and gravel), equipment used during the transportation of animals (hay, sawdust and crates), medicines and other veterinary products. Animal waste products may enter the facility with imported or transferred animals.

V. PEOPLE

Zoo staff, including volunteers and students, enter the premises for normal work purposes and may have contact with other animals (domestic pets/ rehabilitating or “pet” wildlife/ livestock or feral species) outside of work hours. Zoo personnel and family members may live on-site. Local and international visitors pass through the premises on a daily basis and may have close contact with zoo animals. Contractors, maintenance personnel and service people also visit the site regularly. Researchers, wildlife rehabilitators and wildlife officers may also visit the facility, often bringing animals with them. Disease agents can be transmitted from people to animals, for example, via hands, boots, clothing or equipment. Humans can transmit diseases from other animals they have been in contact with outside the zoo, or can transmit human diseases such as influenza, common colds and other zoonoses to zoo animals.

VI. AIR

Some disease agents can be transmitted on air-borne particles, including dust, aerosolised water and aerosolised faeces.

VII. WATER SUPPLY

Water supplies used for drinking, bathing and cleaning may carry pathogens to, or from, animals. Water may become contaminated with waste products or animal faeces, for example from feral or wild birds, rodents or native mammals which poses a risk to both animals and staff.

Outputs

Outputs refer to any human, animal, biological or non-biological product which leaves the zoo. Outputs will vary on the type of facility. Each output from the zoo should be assessed for its biosecurity risk. This Biosecurity Manual deals primarily with the recognition and management of the risks associated with both inputs and outputs. Each section is covered in greater detail in the document, but the following general principles apply:

I. ANIMALS

Animal outputs include: animals leaving the zoo for other institutions either within Australia or overseas; sick, injured or orphaned wildlife being moved to rehabilitation facilities or released into the wild; confiscated animals returned to owners or other authorities; captive-bred animals for release to the wild as part of a sanctioned recovery program; free-ranging animals (either native, feral or stray, including birds, rodents, cats and dogs) moving out from zoo properties. Any animal output may pose a biosecurity risk to humans, livestock and the environment.

II. WASTE PRODUCTS INCLUDING FAECES, URINE, WATER, BIOLOGICAL PRODUCTS, CARCASES

Waste outputs including waste food products, faeces, animal bedding and biological products such as zoo animal carcases often leave the zoo property for disposal at a remote site. Some of these waste products can transmit disease and pathogens. Waste management both on and off property is important for good zoo biosecurity.

III. BIOLOGICAL SPECIMENS

Biological specimens may leave the zoo for diagnostic or research purposes. Dead animals may go to independent facilities for post mortem investigation, research, taxidermy or skeletal preparation for study or display. These outputs can pose a risk of disease and pathogen transmission.

IV. VEHICLES, MACHINERY, TOOLS AND OTHER EQUIPMENT

Vehicles moving from the zoo may transfer infectious agents, especially on contaminated tyres. Other equipment leaving the facility includes tools, materials used for animal housing (straw, litter, mulch, sand and gravel), equipment used during the transportation of animals (hay, sawdust, crates), medicines and other veterinary products. Animal waste products may leave the facility with exported or transferred animals.

V. PEOPLE

Zoo staff, including volunteers and students, leave the zoo premises and return to the community each day, where they may have contact with other animals (domestic pets/ rehabilitating or "pet" wildlife/ livestock or feral species) outside of work hours. Researchers, wildlife rehabilitators and wildlife officers may have contact with non-zoo animals after visiting the facility. Disease agents can be transmitted from people to animals, for example, via hands, boots, clothing or equipment. Humans can transmit diseases to other animals they are in contact with outside the zoo.

LEVELS OF BIOSECURITY

ROUTINE BIOSECURITY PROCEDURES

The majority of biosecurity measures outlined in this document will be applied on a routine or daily basis by most zoos in most circumstances. Maintaining these levels of routine biosecurity will give a high assurance that disease agents are not carried into animal enclosures and will reduce the risk of disease transmission between enclosures.

HIGHER LEVEL BIOSECURITY PROCEDURES

Some biosecurity measures may not be a necessary part of routine practice in zoos, but may be implemented in situations or circumstances outside the normal. Higher level biosecurity procedures may be adopted by individual zoos, according to Zoo-specific circumstance and risk. Some higher level biosecurity guidelines are included in this document and may be adopted as needed, within the Zoo's individual Biosecurity Plan.

In the event of an increased disease risk (e.g. infectious disease event in one enclosure, changed health status of individuals), an **increased** level of biosecurity should be implemented as determined by the circumstances.

EMERGENCY BIOSECURITY RESPONSE PLANS

In the case of an emergency animal disease and where applicable, standard operating procedures (SOPs) will be implemented in line with the relevant AUSVETPLAN disease strategy (see www.animalhealthaustralia.com.au). Zoos should also develop a Zoo-specific Emergency Biosecurity Response Plan, to increase biosecurity protection in the event of a suspected outbreak of an emergency disease or serious endemic disease.

ROUTINE BIOSECURITY PROCEDURES

1. Record keeping, animal identification, staff training and documentation

RECORD KEEPING

Objective

To record all the necessary and appropriate information essential for good biosecurity practices.

Records include individual animal or group identification, date and place of birth, medical history of individual, including preventative medicine program, breeding history and movements of animals both externally and within the facility. This information will allow tracing of movements and events.

Tracing, either forward or back, allows the pathway of disease introduction and spread to be identified in the event of a disease outbreak or a breakdown in biosecurity. Tracing facilitates risk identification and management.

RELEVANT AAWS STANDARDS
Section 12: Animal identification and records
S12.1, S12.4, S12.5, S12.6, S12.7, S 12.8, S 12.9, S12.10, S12.11

Guidelines

G1.1 Records should be permanently maintained for veterinary and husbandry activities concerning individual collection animals, including acquisition and disposition of animals to and from the collection.

G1.2 When a Zoo is managed with different Biosecurity Zones, records should be maintained of movement of animals from one biosecurity zone to another (see **Section 2 Property management – Biosecurity zones and compartmentalisation**).

G1.3 Records should be kept of all significant animal illness and all collection animal deaths. (see **Section 7 Management of sick animals** and **Section 8 Animal deaths, post mortem examination and carcase disposal**).

G1.4 A **minimum** set of information should be recorded for each significant animal illness and all collection animal deaths:

a. date

b. location

c. species

d. clinical signs/ circumstance and/or syndrome

e. tests performed and results

f. diagnosis (definitive or suspected)

g. response - any associated actions put in place as a result including reporting.

ANIMAL IDENTIFICATION

RELEVANT AAWS STANDARD
Section 12: Animal identification and records
S12.1

Guidelines

G1.5 Whenever possible, individual animals should be permanently identified. Identification methods such as microchip or tattoo are recommended over other methods such as ear tags and leg bands (although these may be used in addition to microchips or tattoos).

G1.6 Permanent identification should be verified whenever possible (i.e. confirm tattoo or microchip present, functional, with both site and number verified).

STAFF TRAINING AND DOCUMENTATION

RELEVANT AAWS STANDARDS
Section 1: Responsibilities
S1.1, S1.2, S1.3, S1.4, S1.7

Objective

To ensure all zoo staff are aware of the significance of biosecurity issues.

To ensure zoo management and staff working with animals have a good understanding of the major routes for disease and pathogen movement both from, and into, the zoo.

To ensure zoo management and staff working with animals have a good understanding of work practices which minimise the risk of disease and pathogen movement.

All personnel involved with the operations of the zoo require a basic understanding of biosecurity and biosecurity risks. **Staff working with animals**, in animal enclosures, or with animal products are expected to have a high level of knowledge of biosecurity as relevant to their work practices. Every staff member, including volunteers and students assisting in work practices, has a responsibility for zoo biosecurity. The biosecurity environment in Australia is rapidly changing. National, linked information networks allow rapid access to biosecurity information and new developments.

Definition: **Staff - all persons who have been given a level of responsibility associated with the zoo and includes, but is not limited to, keepers, volunteers, researchers, students and contractors.**

Definition: **Staff working with animals – any staff member involved in work activities within the zoo, which involves direct or close contact with animals, animal enclosures or animal products.**

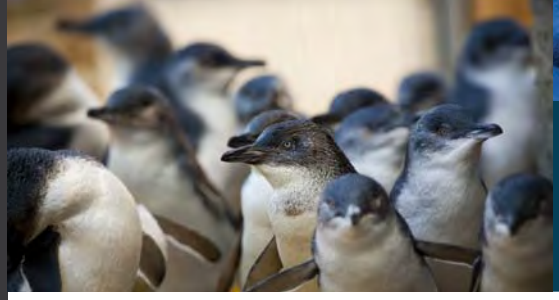
Guidelines

G1.7 Each zoo should keep a copy of the National Zoo Biosecurity Manual and a copy of a more detailed, site-specific document (the Institution's Biosecurity Plan) that encompasses the National Zoo Biosecurity Manual. These documents should be readily accessible to all staff.

G1.8 All zoo staff should have an appropriate awareness of biosecurity and its importance to the zoo and to Australia.

G1.9 Staff working with animals and other staff with biosecurity responsibilities should have an appropriate level of understanding of biosecurity risks and management procedures relevant to their work responsibilities.

G1.10 Staff should receive regular training in the relevant aspects of the National Zoo Biosecurity Manual and the Zoo's site-specific Biosecurity Plan. Records of training should be maintained for the duration of the employment of the staff member.



2. Property management

Objective

To minimise the risk of spread of disease or contaminants into, from or within the zoo collection through effective use of daily zoo management practices and protocols.

INPUTS AND OUTPUTS

Objective

Inputs and outputs are managed to reduce biosecurity risks.

An understanding of the major routes for disease and contaminant movement from, or into, a zoo is essential for assessing and minimising biosecurity risk (see **Major routes for disease and pathogen transmission**).

Guidelines

G2.1 Zoo management and staff working with animals should have a good understanding of the major routes for disease and contaminant movement into, from and within, the zoo.

G2.2 Zoo management and staff working with animals should have a good understanding of work practices which minimise the risk of disease and pathogen movement.

G2.3 Inputs and outputs should be assessed for potential biosecurity risks.

G2.4 If the zoo runs a domestic animal ("petting zoo"), these animals should be sourced from low biosecurity risk facilities; risk assessment should occur and they should be housed and managed to minimise the biosecurity risk to other zoo animals.

See also **Section 6 Quarantine.**

PERIMETER AND ANIMAL ENCLOSURE SECURITY

Objective

To limit and control unauthorised access by people to zoo grounds and animal enclosures.

To prevent or minimise access by wild, feral, stray pet and other animals to zoo grounds and animal enclosures.

To ensure all zoo animals are safely and appropriately secured within their enclosures.

To have emergency response plans established in the event of animal escape or other emergency.

RELEVANT AAWS STANDARDS
Section 2: Security and Section 3: Enclosures
S2.1, S2.2, S2.3, S2.4, S2.5, S2.6, S2.7, S2.8, S2.9, S2.10, S2.11, S2.12, S2.13, S3.3, S3.4, S3.5, S3.6, S3.8, S3.11

Guidelines

G2.5 The property should have a secure perimeter fence or otherwise well-defined boundary, establishing a clearly defined biosecurity zone.

G2.6 Entrances to the property should be able to be closed and locked to vehicle and foot traffic. Entrances should be locked during all non-visitor hours.

G2.7 All animal enclosures should be appropriately constructed and secured to prevent animal escape.

G2.8 Each enclosure should be individually and permanently identified with a unique name, number or alphanumeric code for identification purposes.

G2.9 Zoos should ensure that all animals are housed in appropriate enclosures with a suitable level of enclosure security for the species, including all species listed as either Extreme or Serious Threat species under the Vertebrate Pests Committee Guidelines (http://www.feral.org.au/guidelines-for-the-import-movement-and-keeping-of-exotic-vertebrates-in-australia/).

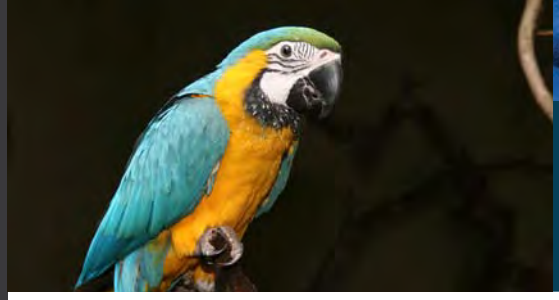
G2.10 Zoos should have a written management action plan in the event of an escape or theft of an animal from the institution, including for all species listed as either Extreme or Serious Threat species under the Vertebrate Pests Committee Guidelines.

AN INSTITUTION-SPECIFIC BIOSECURITY PLAN

Objective

To develop and maintain an institution-specific, documented, Biosecurity Plan

The development of Zoo-specific practices and Zoo-specific Biosecurity Plans is fundamental to the success of improved biosecurity for the entire zoo industry. Because each zoo will have differing biosecurity challenges and operating environments, a site-specific Plan is the most effective way to achieve excellent biosecurity for each zoo.



Guidelines

G2.11 Each zoo should develop and implement an effective, documented institution-specific Biosecurity Plan. Those zoos which chose not to develop their own Biosecurity Plan should implement the relevant guidelines within the National Zoo Biosecurity Manual.

G2.12 Each zoo should have an up-to-date map of the property, showing identified enclosures, service buildings, veterinary and quarantine facilities, food sheds, access roads and gates.

See also **Section 10 A Zoo-specific Biosecurity Plan.**

BIOSECURITY ZONES AND COMPARTMENTALISATION

Objective

To identify and document different areas of the zoo, based on biosecurity risk.

Dividing a property into distinct biosecurity zones, based on differing levels of biosecurity risk, allows for more effective risk management and planning. For example, areas to which the public have access may require a different level of biosecurity management compared to working areas which are not accessible to the public. Biosecurity zones may also vary depending on the species held, their origin and differences in zoonotic disease transmission potential (see also Section 4 Prevention of transmission of disease between animals and people). Some typical biosecurity zones include: quarantine area; main zoo collection; domestic animal enclosures; public-animal interaction areas; mobile zoo; hospitalised zoo animals; wildlife hospital; confiscated animals and endangered species bred for release.

Higher Level Guidelines

G2.13 Each zoo property should be divided into distinct biosecurity zones based on differing levels of biosecurity risk.

G2.14 There should be an up-to-date map of the property showing the different biosecurity zones, and a written plan, which documents the biosecurity requirements of each zone.

G2.15 If animals of lower biosecurity risk are housed with animals of higher biosecurity risk, they should be assumed to have a similarly high biosecurity risk profile.

ENCLOSURE AND GROUND MAINTENANCE

Objective

To minimise the introduction and spread of disease agents and contaminants in the zoo grounds and enclosures and reduce the attraction of pest species which may transmit disease.

Good hygiene and sanitation are vital components of a biosecurity plan.

RELEVANT AAWS STANDARDS
Section 3: Enclosures and Section 5: Heath and wellbeing
S3.19, S3.20, S3.25, S5.3

Guidelines

G2.16 Enclosures should be maintained at an appropriate level of cleanliness for the species, with the aim of minimising biosecurity risk.

G2.17 Zoo grounds (including maintenance and holding areas) should be maintained at a suitable level of cleanliness.

G2.18 Enclosure equipment and furnishings (including enrichment items) should be managed, using practices aimed at minimising disease and contaminant transmission, so as to minimise biosecurity risk.

G2.19 All enclosures and furnishings should be cleaned regularly to maintain a level of hygiene appropriate for the species involved.

G2.20 Enclosures should be adequately drained to prevent accumulation and stagnation of water.

G2.21 Enclosures and zoo grounds should be designed and maintained in a manner which actively reduces access and attractiveness to pest species (see also Management of Pest and Stray Animals).

G2.22 Water used for cleaning enclosures and waste products including faeces and urine should not drain into adjacent enclosures, other areas with animal access or waterways (see also Drainage and waste disposal and Water quality and supply).

G2.23 Equipment, furnishings and enrichment items should be dedicated to one enclosure or management area. If equipment, furnishings and enrichment have to be moved to different enclosures, they should be thoroughly cleaned and disinfected before use in the new area, or appropriate consideration and management of biosecurity risks should occur prior to movement (e.g. use of faeces of one species for behavioural enrichment of another species).

DRAINAGE AND WASTE DISPOSAL

Objective

To minimise the risk of spread of disease or contaminants, through drainage and waste disposal.

Disposal of waste water, waste food and biological products including faeces and urine presents potential biosecurity risks. Waste products may need to be disinfected prior to disposal. Waste products may also need to be transported off-site for disposal. Waste management practices should follow biosecurity guidelines to minimise risks. Containment, transport and disposal of waste products and water must also comply with local, state/ territory and federal requirements.

RELEVANT AAWS STANDARDS
Section 2: Enclosures and Section 5: Health and wellbeing
S3.19, S3.20, S5.3

Guidelines

G2.24 Zoo management should have a knowledge of drainage routes. Preferably, the zoo should maintain a map showing drainage routes.

G2.25 Water and waste draining from enclosures and holding areas should be assessed for biosecurity risks.

G2.26 Drainage from enclosures should not enter other enclosures or management areas or waterways.

G2.27 Enclosures should be adequately drained to prevent accumulation and stagnation of water likely to attract wild birds, especially in the areas around collection waterfowl.

G2.28 Substrate should be removed and replaced as needed to maintain good enclosure hygiene.

G2.29 Waste products including substrate, food matter, faeces and other biological products should be assessed for biosecurity risks before disposal or subsequent use (e.g. zoo animal faeces composted and used within zoo or made available outside the zoo as a commercial product such as “Zoo Poo”).

G2.30 Waste products should be disposed in a manner appropriate to the biosecurity risks of the product, species, enclosure and individual.

G2.31 Containment, transport and disposal of waste products and water leaving the property should minimise disease transmission risks.

G2.32 If necessary, waste products should be disinfected or destroyed, using methods such as:

a) composting

b) autoclaving

c) chemical sterilisation

d) radiant sterilisation (UV, gamma irradiation)

e) incineration.

See also **Section 8 Animal deaths and carcase disposal.**

FOOD QUALITY AND SUPPLY

Objective

To ensure that animal food is procured, stored, prepared and presented to minimise biosecurity risk.

All food products entering the zoo have the potential to bring in disease and contamination and may pose a biosecurity risk. Food brought into the zoo should be assessed for biosecurity risks.

RELEVANT AAWS STANDARD
Section 4: Dietary and water requirements
S4.4

Guidelines

G2.33 Food offered to zoo animals should be free from known disease risks and should at a minimum meet health and hygiene levels applicable to livestock or equivalent domestic animals.

G2.34 Food storage, preparation and presentation practices, particularly those concerning food of animal origin, should consider and minimise the risks of introduction and spread of infectious disease and contaminants. Food should be stored under conditions (correct temperature and humidity) that minimise spoilage and contamination.

G2.35 Feed offered to zoo animals should be procured, stored, prepared and presented in a manner to minimise or prevent accessibility by pest species.

G2.36 Food that has been damaged by pest species or has obvious contamination from pests (e.g. rodent faeces) should not be fed out.

G2.37 Staff should be trained in appropriate hygiene, including personal hygiene procedures, to ensure that hygiene in food preparation areas is maintained at an appropriate level.

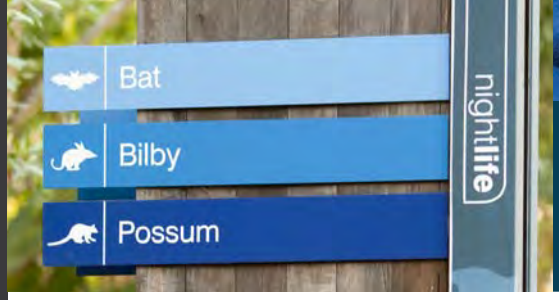
G2.38 Animal food storage and preparation areas should be physically separated from other functions such as the animal hospital, animal holding and staff and visitor food preparation areas.

G2.39 Food should be sourced from reliable suppliers with good biosecurity practices, including appropriate pest management.

G2.40 Written records should be maintained of food sources and delivery dates, or of the sources and delivery dates of food which may pose a biosecurity risk.

G2.41 If the zoo offers whole animal carcases as food items to zoo animals, the carcases should undergo a regular assessment process for possible biosecurity risk. If necessary, the carcases should be scrutinised by the zoo's veterinary service.

G2.42 Collection, pest or stray animals which die within the zoo grounds (other than animals specifically culled for feeding out) should not be fed out to collection animals.



WATER QUALITY AND SUPPLY

Objective

To ensure that water used in enclosures for drinking, bathing, cooling and cleaning, is of a suitable standard for zoo animals and is of low biosecurity risk.

The use of a suitably treated water supply is critical to the maintenance of good biosecurity. Untreated water can spread infectious disease or contaminants and may be a risk to both animals and humans. Effective treatment of surface water to reduce pathogens and contamination is complex and a detailed discussion on water quality and water treatment is outside the scope of this document. It may be necessary to seek expert advice to ensure a safe water supply. A detailed document on water biosecurity for poultry farms, with information applicable to zoo animals can be found at www.daff.gov.au/birds and www.farmbiosecurity.com.au/toolkit.cfm.

RELEVANT AAWS STANDARDS
Section 4: Dietary and water requirements and Section 3: Enclosures
S4.5, S3.24

Guidelines

G2.43 Fresh, clean drinking water should be supplied to all zoo animals.

G2.44 Bowls and equipment used to provide drinking water to animals should be easily and regularly cleaned. They should be positioned so that the risk of contamination and soiling by animals (including pest species) and vegetation is minimised.

G2.45 If drinking water for animals is from a source other than town water, the water should be tested on a regular basis for disease agents and contaminants and treated as appropriate to meet standards suitable for equivalent livestock consumption. The water source itself (e.g. dam, tank or river) should be inspected regularly for contamination such as carcases, rubbish, algal blooms etc. Details of Australian and New Zealand guidelines for fresh and marine water quality (2000); primary industries, and livestock drinking water quality can be found at http://www.mincos.gov.au/publications/australian_and_new_zealand_guidelines_for_fresh_and_marine_water_quality and http://www.mincos.gov.au/__data/assets/pdf_file/0020/316127/wqg-ch4.pdf

G2.46 Water used for cleaning and bathing of zoo animals should be clean and should not contain pathogens or contaminants which pose a health or biosecurity risk to the animals.

G2.47 Where in-house water treatment is being used, the effectiveness of the treatment system should be validated before use. The water treatment system should be maintained and serviced on a regular, scheduled basis, with written records of the service and inspection history.

G2.48 Where in-house water treatment is used, there should be a regular program of testing and recording water quality to demonstrate the effectiveness of the treatment system. Microbiological validation of the efficacy of the treatment system should be conducted regularly.

G2.49 Aquatic and semi-aquatic zoo animals often have very specific water quality requirements. Zoos should be aware of the necessary water quality standards for all species in their care. Regular monitoring and recording of water quality should be performed and water quality should be maintained to appropriate levels. Water quality standards and guidelines for aquatic exhibited animals exist for several Australian states. As an example, water quality standards for captive seals can be found at www.dpi.nsw.gov.au/__data/assets/pdf_file/0011/278075/standards-for-exhibiting-seals-in-nsw.pdf.

G2.50 If humans are in direct contact with water bodies used by animals (e.g. interaction with aquatic animals), water quality should be closely monitored and maintained.

MANAGEMENT OF PEST SPECIES

Objective

To minimise the potential for introduction or spread of disease and contaminants by pest animals.

Pest species include insects, feral rats, mice, cats, foxes, dogs and some bird species. In some situations native animals such as possums may become pests. Any pest species may introduce or spread disease or contamination.

RELEVANT AAWS STANDARDS
Section 4: Dietary and water requirements and Section 5: Health and wellbeing
S4.4, S5.9

Guidelines

G2.51 All zoo enclosures, facilities, waste and rubbish containers should be designed and maintained to limit access by pest species.

G2.52 Feed storage areas should be pest-proof. Feed spills should be cleaned up immediately.

G2.53 Rubbish should be collected and rubbish bins should be emptied frequently, and in an appropriate manner to minimise attraction of pest species.

G2.54 Food presented to zoo animals should be offered in a manner to discourage non-target consumption by pest and other species.

G2.55 Materials within the property that may harbour pest species should be cleaned up on a regular basis and should not be allowed to accumulate.

G2.56 A safe and effective program for the control of pest species should be developed and maintained.

G2.57 A safe and effective trapping and/or baiting program for rodents should be developed and maintained, if necessary.

G2.58 A staff member should be nominated as responsible for pest management (prevention and control) within the zoo. Regular training should be provided.

See also **Food quality and supply.**

MANAGEMENT OF STRAY AND DOMESTIC ANIMALS

Objective

To minimise the potential for introduction or spread of disease and contaminants by stray animals, including domestic species which are not a part of the zoo collection.

Zoo animals are susceptible to a number of diseases that also affect domestic and production animals. Stray domestic animals roaming on zoo grounds may directly or indirectly transmit disease to or from zoo animals.

Guidelines

G2.59 Domestic animals should not have access to zoo grounds unless:

a. they are a part of the zoo collection

b. they are part of public education programs

c. they are disability animals (e.g. seeing-eye dogs accompanying their owners).

G2.60 If domestic animals are brought to the zoo for rescue or rehabilitation purposes, they should be maintained in isolation from zoo collection animals (see also **Section 6 Quarantine**).

G2.61 Perimeter fencing and security measures should be constructed and maintained so as to minimise the opportunity for stray animals to gain access to zoo grounds.

G2.62 The zoo should have a documented procedure for the management of stray animals on zoo grounds.

G2.63 Pets living with staff within the zoo should be confined to the immediate vicinity of the zoo accommodation and should not have access to zoo grounds.

G2.64 Each zoo should have a documented protocol for managing disability animals within the zoo. This may require disability animals to be housed in a designated area within the zoo facility, or may allow disability animals to accompany owners into the zoo grounds, if a risk assessment indicates this poses minimal biosecurity risk.

ANIMAL MOVEMENTS WITHIN ZOO GROUNDS

Objective

To minimise the risk of introduction or spread of disease through animal movements within the zoo. and outside the zoo.

Zoo animals may need to be moved from one enclosure to another for management reasons. Some zoo animals (e.g. elephants, domestic equids, camels, canids and felids) are routinely "walked" within the zoo grounds, outside designated enclosures, for purposes of exercise and interaction. Non-collection animals (e.g. rehabilitation wildlife) may need to be moved around the zoo and between different holding areas. The biosecurity risks associated with these movements should be assessed and managed.

RELEVANT AAWS STANDARDS
Section 2: Security
S2.4, S2.6

Guidelines

G2.65 Managers and animal staff should consider and manage biosecurity risks before moving animals between enclosures.

G2.66 Enclosures should be cleaned, treated, or left empty for designated periods, if necessary, to minimise biosecurity risks when moving animals between enclosures.

G2.67 Rehabilitation wildlife and other non-collection animals (e.g. confiscation cases) entering or leaving the zoo should be physically separated from collection animals at all times. Appropriate quarantine procedures should be undertaken before such animals are permitted to enter the zoo collection (see **Section 6 Quarantine**).

G2.68 Biosecurity risks associated with walking animals outside of enclosures should be considered. Risk management procedures (e.g. choice of times and routes) should be documented.

Details of animal transfers between zoos are discussed in **Section 9 Management of animals, vehicles and equipment during animal transport.**

ZOO ANIMAL FACILITY DESIGN AND CONSTRUCTION

Objective

To ensure that zoo animal facilities are designed and constructed to minimise the risks of introduction or transmission of disease and contaminants and to facilitate biosecurity risk management.

Appropriately designed and constructed zoo facilities, and in particular animal facilities, will greatly aid the prevention and management of biosecurity risks. Well designed and constructed facilities will help zoos to meet many of the guidelines in this Manual. For example, well designed catch-up facilities allow implementation of preventative medicine programs and facilitate investigation, monitoring and treatment of zoo animals. Well designed drainage facilities allow waste products to be managed in a manner which minimises biosecurity risks. Animal facilities must comply with relevant local, state/ territory and federal government regulations with respect to hygiene, sanitation and biosecurity.

RELEVANT AAWS STANDARDS
Section 3: Enclosures; Section 5: Health and wellbeing; Section 8: Capture and restraint and Section 4: Dietary and water requirements
S3.1, S3.3, S3.11, S5.5, S8.2, S4.5

Guidelines

G2.69 The design and construction of zoo facilities should incorporate features that allow for the prevention and management of biosecurity risks.

G2.70 Animal enclosures should be designed and constructed to:

a. prevent animal escape

b. prevent unauthorised access

c. allow adequate staff and vehicle access (for cleaning, removal of substrates, waste, furnishings and animals both alive and dead).

G2.71 Animal facilities, structures and furnishings should be designed and constructed to allow thorough cleaning and disinfection. Attention should be given to areas such as surfaces and drainage.

G2.72 Drainage from enclosures or holding areas should not enter other enclosures or areas that can be accessed by other animals (see **Drainage and waste disposal**).

G2.73 All zoo facilities, enclosures, food storage and preparation areas, waste and rubbish containers should be designed and constructed to prevent access and attractiveness to pest species.

G2.74 Animal feed containers and dispensers should be designed to prevent access by pest species.

G2.75 Appropriate substrates should be chosen that do not harbour or allow the accumulation or growth of disease agents or contaminants and that can be readily cleaned, disinfected or changed as required.

G2.76 Enclosures should be adequately drained to prevent accumulation and stagnation of water likely to attract wild birds, especially in the areas around collection waterfowl.

G2.77 Property and enclosure perimeters should be designed and constructed with the intent to prevent access by stray and pest animals and prevent escape of zoo animals.

G2.78 Facilities used for quarantine, hospital, post mortem examination, isolation and holding should be appropriately designed and constructed.

G2.79 Animal facilities should have appropriate provision for safe capture and restraint.

G2.80 Water filtration and sanitation systems should be capable of minimising contamination, accumulation and transmission of disease agents and contaminants.

G2.81 Animal food storage and preparation areas should be designed and constructed to facilitate appropriate levels of hygiene.

G2.82 Zoo facility design and construction should include the appropriate provision of hand washing or sanitising facilities for visitors and staff.

G2.83 Wash bays for vehicles and equipment should be incorporated into animal facility design and construction, as appropriate.

G2.84 Moats and water bodies should be designed and constructed to allow adequate cleaning, disinfection, drainage and avoid stagnation or accumulation of contaminants.

G2.85 Display and holding facilities for animals should be designed and constructed with physical and/or spatial barriers, as appropriate, to manage risk of disease transmission between animals and people and *vice versa*.

3. Work and hygiene procedures for staff and visitors

Objective

To minimise, through hygiene practices, the risk of introducing or spreading disease or contaminants via movement of staff, volunteers, contractors and visitors.

***Definition*: Staff working with animals – any staff member involved in work activities within the zoo which involves direct or close contact with zoo animals, animal enclosures or animal products.**

An understanding of routes of disease transmission is necessary to ensure that work practices minimise biosecurity risk. There is a risk of disease spread to, from, or between zoo animals through the movement of people, and particularly through the transfer of contaminants via footwear, clothing or equipment. All people who work in direct or close contact with zoo animals, animal products or animal enclosures (whether employed staff, volunteers, researchers or students) have the potential to transfer disease or contaminants between animals. These people may also be at risk of exposure to zoonotic disease from zoo animals or wildlife (see **Section 4 Zoonotic disease risk management**).

Much of the risk of disease transfer through movement of people can be minimised by using good work and hygiene practices. For example, removing organic material and thoroughly cleaning footwear and equipment between one enclosure and another will greatly reduce contaminant transfer, and hence reduce the risk of disease spread.

It is important that staff working with animals have a good understanding of biosecurity risk and management and the routes of disease transmission. Appropriate training, supervision, and written biosecurity guidelines help to ensure good work and hygiene practices. Records of both normal work practices and out-of-ordinary procedures or movements can facilitate tracing if disease concerns develop.

The biosecurity risks associated with movement of people and work equipment between animal enclosures, and outside the zoo will vary depending on the specific circumstances of the zoo and the enclosure. The species, husbandry practices, health status and geographic region, for example, will all influence the biosecurity risks, and will in turn influence the necessary biosecurity management practices. Each zoo is best placed to determine its own biosecurity needs. The guidelines below suggest **best practice** in work and hygiene protocols. It is not expected that all zoos will need to follow all these guidelines on a routine basis, however each zoo should have an awareness of biosecurity management practices, and should actively determine which practices are most appropriate for their unique workplace.

Variations in a staff member's individual circumstances will also influence the associated biosecurity risks. For example, a staff member who has regular contact with domestic animals or wildlife outside of the zoo may have a greater chance of transferring disease or contaminants into and from the zoo. These staff should be aware of these risks and alter their work practices

appropriately (e.g. change of clothing, footwear, excellent personal hygiene before arrival and departure from the workplace).

During times of increased biosecurity risk (e.g. a highly infectious disease situation), work and hygiene practices may need to be altered to manage associated risks. For example, work practices may require full protective clothing which is laundered or disposed on-site, and/or on-site showering before leaving the premises (See also **Section 11 Emergency biosecurity response plan**).

Zoos may also need to consider that different areas of the zoo, or species held may pose differing biosecurity risks and that work and hygiene procedures are best tailored to suit the circumstances of each situation. For example, keepers caring for macaques will likely wear greater personal protective equipment, and practice more rigorous hygiene practices than those caring for macropods in a walk-through exhibit.

RELEVANT AAWS STANDARD
Section 1: Responsibilities
S1.7

ZOO STAFF AND ALL PERSONNEL WORKING WITH ANIMALS

Objective

To minimise the risk of introducing or spreading disease or contaminants by zoo staff and other personnel who have contact with zoo animals.

Guidelines

G3.1 Staff working with animals should be aware of the risk of disease transmission from their person, their clothing and their footwear to animals and humans.

G3.2 Staff working with animals should wear a uniform, or other dedicated work clothing while at work in the zoo and should change out of their uniform or work clothes prior to contact with other animals outside of the zoo.

G3.3 Staff working with animals should wear only dedicated footwear whilst on zoo grounds. Best practice is for footwear to be removed and remain at the zoo site at the end of each work day. Acceptable alternatives are that footwear is thoroughly cleaned and disinfected prior to leaving and on re-entering the zoo grounds. Dedicated work footwear should not be worn whilst working with or when in contact with domestic animals outside the zoo. If zoos allow staff working with animals to wear work footwear outside the zoo grounds, they should be aware of the biosecurity risks associated with these practices and should have measures in place to strengthen footwear biosecurity practices as needed. An acceptable alternative in some institutions will be to designate specific areas or species as “higher biosecurity footwear or practice” areas.

G3.4 If staff work with zoo or wild animals off-site (e.g. animal shipment, *in situ* field work) their uniforms should be laundered and boots should be cleaned and disinfected prior to leaving and returning to the zoo.

G3.5 Zoos should have documented protocols for minimising biosecurity risks associated with staff and other non-zoo personnel working in close or direct contact with zoo animals, enclosures or animal products.

G3.6 Zoo managers should be aware of all personnel, including staff, volunteers, students, researchers and others who have regular close or direct contact with zoo animals, animal products and animal enclosures.

G3.7 Staff working with animals should be trained and, if necessary, supervised to minimise risk of disease or contaminant transmission.

G3.8 Volunteers, students, researchers and other personnel assisting zoo staff with work practices should be instructed and supervised in their work practices to ensure that appropriate biosecurity procedures are followed.

G3.9 Staff working with animals should adopt work practices which minimise transfer of organic material and contaminants into or from enclosures, via their footwear, clothing and equipment. This may include (depending on biosecurity risks) removing organic material from footwear and use of disinfectant footbaths and protective clothing such as gloves, coveralls, dedicated gumboots or disposable footwear covers to minimise the risk of transferring disease and contaminants.

G3.10 Equipment used as part of disease investigations, research or surveys outside of zoo grounds should be cleaned of organic matter and disinfected prior to usage and upon return.

G3.11 Staff working with animals should be aware of biosecurity risks if they have contact with domestic animals or wildlife outside their workplace and should be encouraged to manage these biosecurity risks through appropriate procedures. For example, staff who have contact with animals outside the zoo may need to adopt appropriate hygiene practices, clothing and footwear changes.

G3.12 Staff working with animals should be aware of the biosecurity risks of visiting multiple different enclosures and animals on a single day, and adopt work practices which minimise these risks. For example, work flow through animal enclosures could be made from areas of lower biosecurity risk to those of higher biosecurity risk.

G3.13 Veterinarians should be aware of the biosecurity risks of examining multiple animals (some of which may be diseased) and entering different enclosures on a single day. They should assess each circumstance for its biosecurity risks and implement appropriate precautions, including appropriate personal hygiene and management of clothing and footwear.

G3.14 During time of increased biosecurity risk, work and hygiene practices should be altered to minimise associated risks. Work practices may require full protective clothing which is laundered or disposed of on site, or showering and a full change of clothing when moving from one biosecurity zone to another.

G3.15 During increased biosecurity risk, staff should clean and disinfect footwear when entering or leaving designated management areas.

CONTRACTORS

Objective

To minimise the risk of introducing or spreading disease or contaminants by contractors.

Definition: **Contractor - any external person contracted to perform work on the zoo grounds.**

Contractors such as trades people, maintenance and construction crews and specialist consultants may enter the zoo for work purposes. Because these people often travel between multiple sites, and may not have an understanding of biosecurity and its importance to zoos, they can pose a risk to the zoo's biosecurity processes. Simple precautions, such as scheduling enclosure maintenance when enclosures are empty of animals, can help to reduce biosecurity risks.

Guidelines

G3.16 Zoos should be aware of the biosecurity risks posed by contractors entering enclosures for work related matters and should have documented protocols for minimising biosecurity risks associated with contractors who come into contact with zoo animals, enclosures or animal products.

G3.17 Contractors working within the zoo should not enter enclosures nor have contact with zoo animals, unless it is a necessary part of their work.

G3.18 Zoo managers should be aware of all contractors in the zoo whose work requires close or direct contact with zoo animals, animal products and animal enclosures.

G3.19 Contractors who are in contact with zoo animals, enclosures or animal products should be instructed and, if necessary, supervised in their work practices to ensure that appropriate biosecurity procedures are followed.

G3.20 Enclosure maintenance by contractors should be scheduled, where possible, when enclosures are empty of animals.

G3.21 Any tools used by contractors in animal enclosures should be cleaned and disinfected, if necessary, before and after use at the zoo, and between use in different areas of the zoo.

G3.22 Contractors working in close or direct contact with animals or their products should be briefed on the biosecurity risks they may pose to the zoo, and given assistance by zoo staff to determine appropriate measures to manage biosecurity risks. For example, contractors who have contact with animals outside of the zoo may need to adopt appropriate hygiene and clothing changes prior to their work in the zoo.

G3.23 A standard risk assessment and briefing document should be developed to help manage contractor risk, if these events occur frequently.

G3.24 The zoo should maintain a record of contractors who enter animal enclosures.

G3.25 The entry of delivery personnel into the zoo grounds should be assessed for biosecurity risk.

G3.26 There should be a system in place to allow tracing when delivery personnel enter the grounds (e.g. through delivery dockets and feed company records).

ZOO VISITORS

Objective

To minimise the risk of introducing or spreading disease or contaminants by zoo visitors, in particular through Contact Areas and Interactive Programs.

***Definitions*:**
Visitor - any member of the public visiting the zoo grounds in order to view or interact with zoo animals.
Contact Area - area where there is direct physical contact between zoo animals and the visitor.
Interactive Programs - activities which encourage a visitor to touch, feed and/or have close contact with a zoo animal, either inside or outside the animal's enclosure.
Mobile Zoo - collection animals taken outside the zoo grounds for educational purposes.

In a traditional zoo visitor experience, a physical distance is maintained between visitors and zoo animals, and visitors do not enter animal enclosures. In this situation, biosecurity risks associated with visitors are minimal.

Many zoos now have **Contact Areas** and **Interactive Programs** which allow direct or close contact between visitors and animals, and where visitors may enter animal enclosures. Close contact between visitors and zoo animals may increase the biosecurity risks to the zoo and may also increase the risks of zoonotic disease spread from zoo animals to visitors (see **Section 4 Zoonotic disease risk management**). These types of programs provide valuable educational experience and in most instances the associated biosecurity risks can be minimised through appropriate risk assessment and management protocols.

It is important that all managers and staff working in Contact Areas and Interactive Programs have a good understanding of general biosecurity risks and risk minimisation practices, as well

as the specific biosecurity risks associated with close contact between visitors, animals and their enclosures. The biosecurity risks associated with visitors walking through an open macropod exhibit will differ significantly from those associated with visitors walking through an open primate exhibit. For these reasons, risk assessment and management practices should be tailored to the unique circumstances of each zoo and situation. These guidelines suggest best practice for typical situations.

RELEVANT AAWS STANDARDS
Section 1: Responsibilities and Section 10: Interactive programs
S1.7, S10.2; S10.3; S10.4; S10.5; S10.8, S10.9, S10.10

Guidelines

G3.27 Zoo managers should be aware of the biosecurity risks if the visiting public enter animal enclosures or have contact with zoo animals.

G3.28 Contact Areas and Interactive Programs should be assessed and managed appropriately for their specific biosecurity risks.

G3.29 Zoos should have documented protocols for managing the biosecurity risks associated with visitors (in particular human-animal interaction) in Contact Areas and Interactive Programs.

G3.30 Visitors to Contact Areas and Interactive Programs should be supervised by a staff member who has been trained in, and has a good understanding of, biosecurity risks and minimisation practices. The level of biosecurity supervision should be tailored to the risks of the particular circumstances (e.g. greater supervision with children handling reptiles, or visitors holding koalas than with public walking through macropod enclosure).

G3.31 All staff working in Contact Areas and Interactive Programs should be trained in and have a good understanding of zoonotic risk and management.

G3.32 When selecting species and individual animals for use in Interactive Programs, consideration should be given to minimising biosecurity and zoonotic disease risks.

G3.33 Individual animals used in Interactive Programs should be regularly monitored for biosecurity and zoonotic disease risk.

G3.34 Staff and visitors participating in Interactive Programs or Mobile Zoos should be discouraged from eating, drinking or smoking during the interaction.

G3.35 Hand-washing or disinfection facilities should be available to all participants of Interactive Programs, and they should be made aware of the facilities and encouraged to practice good personal hygiene.

G3.36 If necessary, visitors who enter enclosures or have close contact with zoo animals should receive a briefing beforehand on the biosecurity risks associated with the visit.

G3.37 A standard risk assessment and briefing document should be developed to help manage visitor risk if these events occur frequently.

G3.38 If visitors enter animal enclosures, consideration should be given to their need to wear suitable footwear (robust, enclosed shoes) which can be cleaned and disinfected if necessary.

G3.39 Consideration should be given to the need for visitors who have entered animal enclosures to clean their shoes of organic material and disinfect the soles, using a chemical footbath, prior to leaving the area.

See also **Section 4 Zoonotic disease risk management.**

VEHICLE MOVEMENT WITHIN AND OUTSIDE ZOOS

Objective
To minimise the risk of disease or contaminant spread by vehicle movement.

Vehicles moving into, from and within the zoo can be a route for disease and contaminant transmission. Managing vehicle movements is an important part of ensuring good zoo biosecurity.

RELEVANT AAWS STANDARDS
Section 3: Enclosures
S3.12, S3.13, S3.14, S3.17, S3.18

Guidelines

G3.40 The number of vehicles entering and leaving the zoo grounds should be minimised.

G3.41 Zoos with drive-through enclosures should consider the biosecurity risks of driving vehicles through animal enclosures. These zoos should have a documented protocol for managing and reducing these risks. Documented routes should be established to minimise biosecurity risk.

G3.42 The zoo should have methods to trace all non-zoo vehicles which enter enclosures or transport animals.

G3.43 If vehicles enter animal holding areas, any organic matter or gross contamination, especially on wheels, should be removed prior to entry or exit.

G3.44 If staff or visiting vehicles need to be brought onto zoo grounds, they should only be driven and parked in designated areas, chosen for their low biosecurity risk.

See also **Section 9 Management of animals, vehicles and equipment during animal transport.**

4. Zoonotic disease risk management

Objective

To minimise the risk of disease transmission between animals and people and vice versa.

Diseases that are spread from animals to humans are called zoonoses. Diseases spread from humans to animals are referred to as anthropozoonoses. Zoonoses may be spread through direct physical contact with animals and their products, or indirectly by sharing the same air space. Different zoonoses are transmitted in different ways. Faeces, saliva, urine and birth fluids generally pose the greatest risks. Some animals such as reptiles may carry bacterial contamination on their skin (notably *Salmonella* spp.) which can pose serious health threat to humans, and can be easily transferred if good personal hygiene (washing hands) is not practiced after animal handling.

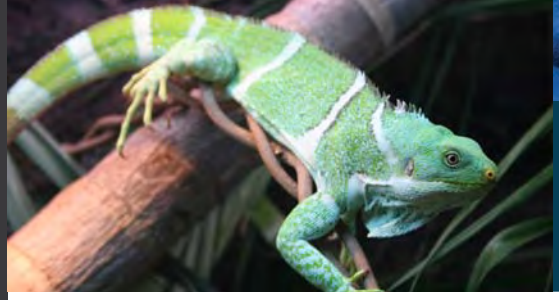
Most zoonotic disease risks can be minimised through appropriate personal hygiene, good work practices, effective quarantine programs and routine disease screening of animals. Many of the work practices outlined in **Section 3 Work and hygiene procedures for staff and visitors** will greatly minimise the risk of zoonotic disease transmission. Hand washing or the judicious use of hand sanitisers is the single most effective personal protection against zoonotic disease spread for both staff and visitors.

In some circumstances, the risks of zoonotic disease transmission are increased. Some species or taxonomic groups of animals have a greater potential to transmit zoonotic disease (e.g. primates and reptiles). Work practices, such as close contact with animals and their products may increase the risk of acquiring, or transmitting a zoonotic disease.

Zoonotic disease risks will also vary according to the specific health profile of the individual animal and human. Children may be particularly susceptible to zoonotic disease risk due their undeveloped immune system and their generally poor personal hygiene (e.g. a tendency to put their hands in their mouth). They are often in closer proximity to the ground (faeces and urine) and are more likely to touch animals, if given the opportunity. Likewise, the elderly and people with a compromised immune system are at greater risk of zoonotic disease. An individual's susceptibility to infectious and other diseases will vary according to their individual health status. For instance, diseases such as diabetes, kidney disease, cancer and immunosuppressive diseases such as HIV-AIDS may alter the zoonotic disease risk for an individual (both animal and human). Because of the large number of factors that influence zoonotic risk, and the variability between zoos, it is recommended that each zoo conduct a site-specific zoonotic risk assessment, in conjunction with human health authorities.

It is important that keepers and other staff working with animals or animal products are aware of both general, and specific, zoonotic risks in their workplace and understand the practices necessary to minimise these risks.

In many situations, the risk to the visiting public of contracting zoonotic disease from zoo animals is minimal due to the physical distance maintained between visitors and animals. **Contact Areas** and **Interactive Programs** which allow direct or close contact between visitors and animals may



increase the risks of zoonotic disease spread. These programs provide a valuable educational experience and most zoonotic risks can be managed with reasonable precautions. However, it is particularly important that all managers and staff working in Contact Areas and Interactive Programs have a good understanding of zoonotic diseases, the particular zoonotic risks in their situation and the appropriate practices to minimise these risks (See **Section 3 Work and hygiene procedures for staff and visitors – Zoo visitors**). Detailed Infection Control Guidelines for Animal Contact can be found at www.health.qld.gov.au/ph/documents/cdb/zoo_guidelines.pdf.

RELEVANT AAWS STANDARDS
Section 1: Responsibilities and Section 10: Interactive programs
S1.1, S1.7, S10.2, S10.3, S10.4, S10.5, S10.8, S10.9, S10.10

Guidelines

G4.1 Zoo managers should be aware of the risks of zoonotic disease spread from zoo animals, enclosures and animal products to staff and visitors.

G4.2 Zoos should develop a documented protocol and management policy (seeking the assistance of human health authorities) for minimising zoonotic disease risks to staff, contractors and visitors. This should address risks associated with direct or close contact with zoo animals, animal enclosures or animal products.

G4.3 Staff working in direct contact with animals and their products should have documented training in zoonotic disease risk management procedures (e.g.: use of personal protective equipment, appropriate hygiene and animal handling) and should have ready access to zoonotic disease minimisation protocols.

G4.4 Zoos with "petting zoos" (close visitor contact with domestic animals) should refer to and develop biosecurity guidelines based on the considerations outlined at www.health.qld.gov.au/ph/documents/cdb/zoo_guidelines.pdf and www.public.health.wa.gov.au/cproot/2222/2/Petting%20Zoo%20Guidelines%202009.pdf

G4.5 Zoos should have appropriate hand washing or hand disinfection facilities available for staff and visitors. Visitors coming into direct or close contact with zoo animals and their products should be encouraged to utilise these facilities.

G4.6 Zoonotic disease awareness and risk management should be part of the zoo animal collection preventative medicine program, with the objective of preventing disease transmission from animals to humans and *vice versa*. These programs should be developed seeking input from a human health professional with knowledge of zoonotic diseases, their prevention and management and should include awareness of anthropozoonotic diseases and their management.

G4.7 Zoos should have a staff health (occupational health and safety) program incorporating appropriate hygiene, education, training and procedures regarding zoonotic diseases. The staff health program should also incorporate, as necessary, pre-employment health and disease screening, vaccination programs and regular ongoing disease screening for staff placed at risk of zoonotic disease due to their work.

G4.8 Suspected or confirmed zoonotic disease in staff or others within the zoo should be reported (through the zoo's occupational health and safety system) to zoo management and/or zoo veterinary staff.

G4.9 If zoo staff are aware, or suspect they have a zoonotic disease, they should advise zoo management.

G4.10 A document detailing the risks of zoonotic disease in a zoo setting should be provided to all staff (and others) who report suspected zoonotic disease, which they can take to their physician.

G4.11 Zoo managers should be aware that the health status of an individual staff member may influence their susceptibility to zoonotic disease. Staff should also be made aware that changes in their health status can alter their risk of zoonotic disease.

G4.12 Staff working with animals should be aware that they may transmit infectious disease to the animals in their care. For example many non-human primates are susceptible to human diseases, including common respiratory tract viruses such as colds and flu. These diseases may be transmitted indirectly through shared air space.

G4.13 Zoo managers and staff should be aware of any taxonomic groups or species in their care with increased zoonotic risk potential. Examples of such taxa include:

a. macaques - Herpes B virus

b. bats - Australian Bat Lyssavirus

c. reptiles - Salmonellosis.

5. Animal health and preventative medicine

Objective

To minimise the risk of introducing or spreading disease or contaminants within a zoo collection, by ensuring good veterinary care, diagnosis, treatment and the development and implementation of an effective preventative medicine program.

A comprehensive animal health and preventative medicine program is a cornerstone of good zoo biosecurity. It minimises the risk of disease entry and spread within the animal collection.

A zoo animal health program consists of both preventative medicine and accurate diagnosis and effective treatment of disease. An effective animal health program is reliant on professional veterinary expertise, excellent animal care and good communication. A preventative medicine and health program requires a detailed understanding of disease, in the zoo setting. Veterinary input is essential for the development and delivery of an effective animal health and preventative medicine program. A comprehensive preventative medicine program will address routine procedures such as appropriate methods of animal identification, quarantine procedures, routine vaccination programs, parasite monitoring and control, nutritional management, reproductive management and contraception, water quality management, pest management, routine testing for selected diseases of concern, health and disease surveillance and investigation of illness or death in all collection animals. Investigation, diagnosis and appropriate treatment of disease requires appropriate veterinary expertise. Significant biosecurity and business risks can occur if lay-staff attempt diagnosis and treatment beyond their capabilities.

Veterinary medicines must, by law, only be prescribed by a registered veterinarian.

RELEVANT AAWS STANDARDS
Section 5: Health and wellbeing; Section 1: Responsibilities and Section 3: enclosures
S5.13, S1.1, S1.4, S3.1, S5.4, S5.9

Guidelines

G5.1 Zoos should engage the services of a suitably qualified veterinarian with relevant experience in the species held. There should be an arrangement for regular veterinary attendance at the facility (to administer the preventative medicine program) and the service should have the necessary professional equipment to deal with zoo animals (e.g. administration of chemical restraint) and there should be a veterinarian available for emergency response at all times.

G5.2 All zoos should establish and maintain a documented preventative medicine and health program, under the supervision of the veterinarian.

G5.3 If the institution is a ZAA member the veterinarian should be encouraged to become a member of the ZAA Veterinary Specialist Advisory Group list serve (http://www.zooaquarium.org.au/Veterinary-SAG/default.aspx).

G5.4 The zoo should be encouraged to become a member of a linked network that enables rapid access to biosecurity information, such as the ZAA list serve and the AWHN (www.wildlifehealth.org.au/AWHN/home.aspx).

G5.5 The veterinarian and key animal management staff should be familiar with the Australian Animal Welfare Strategy-Exhibited Animals (AAWS), the ZAA Accreditation Standards (for Association member zoos), AUSVETPLAN Zoos Enterprise Manual (www.animalhealthaustralia.com.au/fms/Animal%20Health%20Australia/AUSVETPLAN/zoofinal.pdf) and the AUSVETPLAN Wild Animal Response Strategy (www.animalhealthaustralia.com.au).

G5.6 The veterinarian should have knowledge and understanding of the OIE disease list (www.oie.int/animal-health-in-the-world/oie-listed-diseases-2011/), the National and State/Territory Notifiable Disease List and any other diseases considered important to Australia's biosecurity (www.daff.gov.au/animal-plant-health/pests-diseases-weeds/animal/notifiable).

G5.7 The veterinarian should be aware of their state/ territory and national disease notification requirements and have a documented protocol for notification.

G5.8 Animal management staff should be aware of their responsibilities for disease notification and should have protocols for informing the Zoo's veterinary service.

G5.9 The veterinarian should be involved in developing any biosecurity procedures specific to the zoo they support. They should have a good working knowledge of the National Zoo Biosecurity Manual, the individual zoo's biosecurity procedures and any relevant local requirements with respect to biosecurity.

See also **Section 7 Management of sick animals.**

6. Quarantine

Objective

To minimise the risk or introduction of spread of disease or contaminants within or from a zoo collection, by imposing a period of isolation from other zoo animals for newly arrived animals or those suspected or confirmed as suffering from infectious disease.

Quarantine is a period of isolation for newly arrived animals and potentially diseased animals for the purpose of detecting and eliminating (where appropriate) disease. Quarantine is an important component of zoo biosecurity. The quarantine period allows an opportunity for acclimatisation, close observation of animals, animal health checks, preventative medicine programs, permanent identification and confirmation of medical history and provenance.

A zoo's quarantine, hospital, and isolation areas must comply with local, state/ territory and federal regulations. Quarantine of internationally imported zoo animals must also comply with Australia's legislated requirements.

RELEVANT AAWS STANDARDS
Section 5: Health and wellbeing; Section 1: Responsibilities and Section 3: Enclosures
S5.13, S1.1, S1.7, S3.1, S5.4, S5.5, S5.9

GENERAL QUARANTINE PRACTICES

Objective

To minimise, through appropriate work practices, the risk of disease introduction or spread during the quarantine period.

Quarantine practices within zoos are generally applied to animals from four categories:

a) Newly-arrived collection animals

b) Sick/ injured collection animals

c) Wildlife rescue cases

d) Confiscation cases.

Although the principles of quarantine will apply to all four categories, the practical application may differ in each situation. Zoos are encouraged to develop separate, generic quarantine procedures for each of these four categories. Species-appropriate and circumstance-appropriate quarantine procedures can then be developed from these broader procedures.

Barrier keeping procedures (use of work practices which minimise the spread of infectious disease from one animal, group or environment to another) are a vital part of effective quarantine. If carefully utilised, barrier keeping practices can minimise the risks associated with working with different quarantine groups on the same day, or within the same treatment room or facility.

Guidelines

G6.1 Species or circumstance-appropriate quarantine procedures should be developed, documented and implemented when required and should address:

a) isolation of newly acquired animals to provide for examination, treatment, monitoring and acclimatisation

b) physical examination of all animals on or soon after arrival, including performance of appropriate clinical and laboratory diagnostic tests as required

c) veterinary treatment for existing illness, disease or injury

d) a defined, appropriate minimum period for quarantine to ensure animals are free from able disease and

e) veterinary care and treatment as necessary to protect against communicable diseases.

G6.2 Each animal arriving at the zoo, whether a newly arrived collection animal, wildlife rescue case or confiscation case should be assessed for biosecurity risk, including zoonotic risk, by the zoo veterinarian or a Competent Keeper with a strong understanding of biosecurity. An appropriate biosecurity management procedure should be developed for each case. Documentation of the procedure is recommended if the risks are high, or the procedures vary significantly from standard quarantine protocols.

G6.3 If animals arrive at the zoo as part of a planned transaction from another zoo, biosecurity risks and management plans should be developed between both zoos, prior to the transaction (see **Section 9 Management of animals, vehicles and equipment during animal transport**).

G6.4 All biological products arriving at the zoo, such as semen, embryos, feathers and taxonomic preparations, should be assessed for biosecurity risk, including zoonotic risk, by the zoo veterinarian or a Competent Keeper with a strong understanding of biosecurity, and a biosecurity management procedure established for each case.

G6.5 Newly arrived animals or biological products should remain in quarantine until such time as their biosecurity risk has been established and mitigated.

G6.6 There should be adequate and appropriate signage to indicate areas of restricted access and quarantine status.

G6.7 Quarantine work practices should be designed and documented to reduce the risk of cross transmission, introduction, and spread of potential pathogens.

G6.8 All staff working with quarantined animals should be trained and familiar with the zoo's quarantine management protocols.

G6.9 Staff working in quarantine situations should be trained in the principles and application of barrier keeping. In particular, barrier keeping practices should be applied to ensure effective isolation of rehabilitation wildlife cases and confiscation cases from collection animals.

G6.10 The zoo should have physically separate, dedicated holding facilities for the quarantine of **newly-arrived collection animals**.

G6.11 Where a biosecurity risk is suspected, **sick collection animals** and/or their social group should be physically separated from other collection animals. Appropriate facilities should be available for this isolation.

G6.12 If dedicated quarantine or isolation facilities are not available, then protocols for management of **newly acquired or sick collection animals** should be implemented to ensure there is no direct or indirect contact (e.g. equipment, aerosol or drainage) between these and healthy collection animals.

G6.13 If the receiving zoo lacks appropriate facilities for appropriate quarantine of a particular species, then consideration should be given to quarantine occurring at another institution with suitable facilities.

G6.14 If the zoo accepts **wildlife for treatment and rehabilitation**, these cases should be housed, in a physically separate, dedicated facility and managed separately from collection animals. If dedicated wildlife rehabilitation facilities are not available, an acceptable alternative in low-risk situations is to ensure effective isolation of wildlife cases through work practices such as barrier keeping.

G6.15 If the zoo receives and holds **confiscated exotic or native fauna** on behalf of regulatory authorities (often of unknown origin and high biosecurity risk), these animals, and any equipment used to house or care for them, should be maintained and managed in strict isolation from all other animals until an appropriate health assessment and quarantine process has been completed.

G6.16 Keepers designated to care for quarantined, isolated, or confiscated animals should attend to these animals only after fulfilling other responsibilities for collection animals (i.e. work from low biosecurity area to higher biosecurity risk area) and/or should utilise barrier keeping practices.

G6.17 Equipment and tools used in quarantine areas should be dedicated for use only within this area and should be cleaned and disinfected on a regular basis, and at the end of the quarantine period.

G6.18 Footwear protocols within quarantine areas should follow best possible practices. By preference, staff in quarantine areas should wear dedicated footwear which is not worn outside the quarantine area. Alternatively, entry/ exit from quarantine areas should only be made through a footbath containing a suitable disinfectant, used in accordance with manufacturer's instructions, maintained and changed on a regular basis. There should be provision for scraping the soles of footwear before dipping to ensure organic material is removed and the disinfectant makes effective contact with the soles of the footwear. A second alternative involves the use of disposable footwear covers by all staff entering the quarantine area.

G6.19 Facilities for hand sanitation using an appropriate antiseptic should be placed at the entry/ exit to each quarantine area. Hands should be thoroughly cleaned on entry /exit from quarantine areas.

G6.20 Species appropriate quarantine procedures, once established, should be documented and readily available to staff at all times.

G6.21 Waste products, including bedding, food, faeces, urine and water should be assessed for their biosecurity risk and managed and disposed of, using strict biosecurity practices, during quarantine.

G6.22 Biological materials leaving the zoo during the period of quarantine (e.g. diagnostic samples) should be assessed and managed for their biosecurity risk.

G6.23 If the zoo runs a domestic animal ("petting zoo"), these animals should be sourced from low biosecurity risk facilities, risk assessment should occur, and they should be housed and managed to minimise the biosecurity risk to other zoo animals.

VETERINARY CARE AND INVESTIGATION DURING QUARANTINE

A plan of health assessment and preventative medicine for the quarantine period should be developed and carried out under the supervision of the veterinarian. This generally includes physical examination, faecal testing for endoparasites and other appropriate clinical and laboratory diagnostic tests.

Species or taxon-specific protocols for vaccination and disease investigation may be accessed through the ZAA Vet SAG (for Association members).

RELEVANT AAWS STANDARDS
Section 1: Responsibilities; Section 3: Enclosures; Section 5 Health and wellbeing and Section 12: Animal identification
S1.1, S3.1, S5.4, S5.9, S5.13, S12.1

Guidelines

G6.24 Only animals that have undergone appropriate quarantine or disease risk assessment should be allowed to enter the collection.

G6.25 A plan of health assessment and preventative medicine for the quarantine period should be developed and carried out under the supervision of the veterinarian or as described in the quarantine protocol.

G6.26 Complete medical records should be maintained for all newly-arrived collection animals during the quarantine period (see **Section 1 Record keeping**).

G6.27 All newly-arrived collection animals should be permanently identified during the quarantine period. Any existing identification should be confirmed (see **Section 1 Animal identification**).

G6.28 Any treatments required should be determined only by the supervising veterinarian, or by a Competent Keeper in consultation with the veterinarian. Veterinary medicines must, by law, only be prescribed by a registered veterinarian.

G6.29 The cause of death of any animal that dies during quarantine should be established wherever possible. Every animal that dies during quarantine should have a post mortem examination performed under the supervision of a veterinarian and representative tissues should be submitted for histopathologic examination and other specific diagnostic tests (see **Section 8 Animal deaths and post mortem examination**).

G6.30 No zoo animal should be released to the wild, unless deemed as suitable for release as part of rehabilitation or sanctioned recovery programs (with appropriate state/ etrritory or national authority and permits).

G6.31 An animal should not be released from quarantine until all examinations and tests have been completed, the health status of the animal is determined and approval is given by the supervising veterinarian.

Higher level guideline

G6.32 Where possible, blood should be collected and serum banked from animals undergoing quarantine. Either a -70°C freezer or a -20°C freezer (without cyclic defrost) should be available to bank sera. This may provide an important resource for retrospective disease evaluation.

7. Management of sick animals

Objective

To allow the early detection of illness and a prompt response to any potential biosecurity breach.

Appropriate management of sick animals allows timely investigation and diagnosis, which assists in identification of potential biosecurity risks. Early identification of illness allows appropriate, rapid response. Identification of sick animals relies on keeper observation, training and appropriate reporting. Effective management of sick animals relies on experienced veterinary input, with rapid and accurate investigation, diagnosis and treatment. Good record keeping is essential to these processes.

Significant biosecurity and business risks can occur if lay-staff attempt diagnosis and treatment beyond their capabilities.

IDENTIFYING AND REPORTING SICK ANIMALS

RELEVANT AAWS STANDARDS
Section 1: Responsibilities; Section 5: Health and wellbeing and Section 12: Animal identification
S1.5, S5.4, S5.9, S12.4, S12.5, S12.7

Guidelines

G7.1 Animal staff should be trained to recognise signs of ill health in animals held in the zoo's collection and to report their findings appropriately to zoo management and/or the zoo's veterinary service.

G7.2 The condition and health of the animals should be assessed daily by the keepers.

G7.3 The zoo should have a documented procedure for reporting and recording, on a daily basis, all signs of injury or ill health in collection animals.

G7.4 There should be a reporting mechanism that allows this information to be presented to the veterinary service in a timely manner and a documented process for requesting veterinary assistance.

G7.5 Any animal showing signs of illness or injury should receive appropriate and timely attention.

G7.6 Keepers and other animal staff should be aware of their limitations in diagnosing and treating disease and should refer to the zoo's veterinary service for appropriate professional assistance.

G7.7 Keepers and other animal staff should not attempt to interpret signs of illness, reach a diagnosis nor prescribe treatment, beyond the limits acceptable to non-veterinary personnel. The responsibility for diagnosis and prescribing treatment should rest with the zoo's veterinary service.

G7.8 A veterinarian should be available, including a 24 hour emergency service, to respond to reports of illness or injury in the zoo's animals.

INVESTIGATION PRIORITIES AND TRIGGER POINTS

Certain events may signal the need for action, investigation or implementation of higher level biosecurity practices. In order to ensure good biosecurity practices, zoo managers and veterinary staff must be aware of priorities for investigation and must understand which events may be considered as trigger points, which require urgent and prioritised investigation.

Each zoo should develop a Zoo-specific document recognising likely disease issues which would require prioritised action and investigation. Facilities and resources will vary between institutions, and if necessary, priority of investigation should be given to:

a. exotic collection animals, animals in quarantine and animals under extended or lifetime quarantine surveillance (as defined by AQIS)

b. any sudden or unexpected death

c. mass illness or mass death (mass morbidity or mass mortality events)

d. illness with evidence of infectious disease

e. any unexplained deaths or mass morbidity or mortality event in wildlife within, or in close proximity to, the zoo grounds.

RELEVANT AAWS STANDARDS
Section 1: Responsibilities and Section 12: Animal identification
S1.1, S12.7

Guidelines

G7.9 Unexpected and unexplained illness or death of zoo animals (including deaths suspected to be a result of infectious disease) should be assessed for biosecurity risks to animals and humans, including those outside the zoo. Any recent movements of animals, within or outside the zoo grounds, should be taken into account when assessing biosecurity risk.

G7.10 All signs of illness and all deaths in zoo animals should be reported to zoo management or the zoo's veterinary service, recorded in the zoo's official recording system and investigated by zoo veterinarians or other suitably qualified staff.

G7.11 Unexpected or unexplained illness and death in wildlife within the zoo grounds should be reported to zoo management or the zoo's veterinary service, recorded in the zoo's official recording system and investigated by zoo veterinarians or other suitably qualified staff.

G7.12 Relevant authorities should be contacted if there is any suspicion of an emergency disease or an unexplained morbidity or mortality event.

G7.13 Any biosecurity risks resulting from a disease incident should be addressed with an Emergency Biosecurity Response Plan (see **Section 11**).

8. Animal deaths, post mortem examination and carcase disposal

Objective

To investigate and determine the cause of animal deaths, so as to identify and therefore minimise biosecurity risks.

To minimise or eliminate the spread of disease or contamination via animal carcases.

The death of any animal which is under AQIS imposed conditions, such as extended post entry or lifetime quarantine surveillance, must be reported to AQIS as stipulated in their conditions.

The disposal of carcases must comply with local, state/ territory and federal regulations including environmental compliance requirements. The carcases of imported animals under post entry quarantine or extended quarantine surveillance must be disposed of as directed by AQIS.

ANIMAL DEATHS AND POST MORTEM EXAMINATION

Guidelines

G8.1 Each zoo should have a documented procedure for the investigation of collection animal deaths.

G8.2 Each zoo should have a documented procedure for retrieval, storage and disposal of animal carcases, which minimises biosecurity risks.

G8.3 All collection animal deaths should be reported without delay to the appropriate authority (either the Zoo's veterinary service or Zoo management).

G8.4 All deaths of animals within zoo grounds should be reported on a daily basis to the Zoo's veterinary service or Zoo management.

G8.5 Dead animals within the zoo grounds should be handled and processed (including post mortem examination and disposal) using methods that minimise the risk of disease transmission to animals and people and also reduce any opportunity for scavenging.

G8.6 Collection, pest or stray animals which die within the zoo grounds should not be fed out to collection animals.

G8.7 Animals culled within the zoo grounds should not be fed out to other collection animals, unless the veterinary service has assessed the risk of transmissible diseases and the implications of state/ territory restrictions on swill feeding to minimise disease transmission.

G8.8 Dead collection animals should be removed from their enclosure as soon as observed (and it is safe to do so). In rare instances there may be a social/ behavioural benefit in allowing the carcase to remain for a period of grieving.

G8.9 Dead pest, stray and wild animals within the zoo grounds should be collected and brought to a designated area for inspection, post mortem examination (if deemed necessary) or disposal as soon as possible.

G8.10 A thorough post-mortem examination should be conducted on all dead collection animals to determine the cause of death.

G8.11 If immediate examination is not possible, dead animals should be stored in a designated cool room or refrigerator until post mortem examination or disposal. If examination is delayed, it may be necessary to freeze the carcase. This should be done only after consultation with the zoo's veterinarian.

G8.12 Carcases should be placed in leak-proof, labelled plastic bags or containers until post mortem examination or disposal.

G8.13 The refrigerator, cool room or freezer should not be used to store animal or human food stuffs and should be located in an area away from live animal housing, and all food storage and preparation. The storage facility should have sufficient capacity to hold all carcases prior to examination and/ or disposal and should be cleaned and disinfected regularly.

G8.14 Post mortem examinations should be performed by a veterinarian whenever possible. Other, appropriately trained staff can perform post mortem examinations, collect samples and record observations. However, interpretation of post mortem examination findings is the responsibility of the veterinarian.

G8.15 If resources are limited, priority of post mortem investigation should be given to animals that fit the categories outlined in **Section 7 (Investigation Priorities and Trigger Points)**. A thorough post mortem examination, by the zoo's veterinary service, should be conducted in all cases falling into these categories.

CARCASE DISPOSAL

Guidelines

G8.16 Transport and disposal of carcases should use methods that minimise biosecurity risk and minimise the opportunity for scavenging.

G8.17 If carcases leave the property for disposal, procedures should be followed to ensure that the carcases are suitably contained (e.g. rip proof plastic bags).

G8.18 Carcases should be collected regularly from the property.

G8.19 The vehicle collecting carcases should not enter the area of the zoo which houses collection animals.

G8.20 All containers used for collecting dead and storing dead animals must be washed and disinfected before re-use.

9. Management of animals, vehicles and equipment during animal transport

Objective

To minimise the risk of introduction and spread of disease or contaminants during movement of animals between zoos.

This Manual addresses best practice for transfer of zoo animals within Australia. International transfer of zoo animals is subject to complex requirements which are outside the scope of this document.

Transfer of animals from one zoo to another poses biosecurity risks. Infectious disease may be introduced from another zoo or the animal, vehicle or equipment may be exposed to infectious disease or contaminants during transport. Appropriate biosecurity measures will help to prevent the spread of disease or contamination from one zoo to another. Disease transfer can occur via people, vehicles, equipment and transport crates. Appropriate protocols should be followed at every step of the transfer, by all involved in the operation, in order to minimise biosecurity risks.

In the majority of animal transfers, the sending zoo is responsible for boxing and transport to the receiving zoo. The sending zoo is responsible for ensuring that all involved are fully aware of the biosecurity requirements and that the appropriate protocols are implemented. The receiving zoo is responsible for managing post-arrival biosecurity risks, primarily through appropriate quarantine procedures. Best practice involves both zoos discussing and planning biosecurity management and other aspects of the transfer, well in advance of the event.

RELEVANT AAWS STANDARDS
Section 11: Transportation and Section 12: Animal identification
S11.1, S11.2, S11.3, S11.4; S11.5, S11.6, S11.7, S11.8, S11.10, S11.11, S12.8

Guidelines

G9.1 Zoos should have a plan for biosecurity management during animal transfers.

G9.2 All transport crates, equipment and, if necessary, vehicles, used to transfer animals between zoos should be thoroughly cleaned and disinfected before and after use.

G9.3 Staff accompanying animal transfers should employ the highest biosecurity work practices and personal hygiene, at minimum meeting protocols for zoo quarantine management (See **Section 6 Quarantine**).

G9.4 A Competent Keeper and/ or veterinarian may be required to accompany some animal transfers, to assist in management of biosecurity and other concerns.

G9.5 Zoos should be aware of and comply with relevant state/ territory requirements for movement of animals in general and the movement of animals between particular states/ territories.

G9.6 Waste products, including bedding, food, faeces and urine should be managed with strict biosecurity practices during transfer. If necessary, these products should be securely bagged until arrival, and disposed of through the receiving zoo's established biosecurity management processes.

G9.7 If zoo animals are transferred using commercial transportation companies, best practice requires that zoo animals are not transported in vehicles containing other (domestic) animals. If it is necessary to transport zoo animals in the same vehicle as domestic animals, an appropriate biosecurity risk assessment should be undertaken.

See **Appendix 1: Roles and responsibilities for sending and receiving zoos in zoo animal transactions**.

10. A Zoo-specific Biosecurity Plan

Objective

To provide a detailed framework for each individual zoo that allows rapid identification of biosecurity breaches and minimises the risk of introducing or spreading a disease within the collection,

To provide detailed contingency plans for biosecurity breaches.

Guidelines

G10.1 Individual zoos are encouraged to develop Zoo-specific biosecurity procedures, which should incorporate and build on the guidelines presented in this Manual and clearly demonstrate the biosecurity arrangements in place at the zoo.

G10.2 In developing these Plans, detailed consideration should be given to minimising the risk of disease entering into, spreading within or escaping from a facility.

G10.3 These Plans should align with any National (AUSVETPLAN and AAWS Standards) and local contingency and management plans, including those for zoonoses or incidents that may impact upon human health.

G10.4 Minimum areas for inclusion in a Zoo-specific Biosecurity Plan are:

a. a health program for all animals held at the facility;

b. inspection, testing and quarantining of newly arrived animals;

c. control of pest, wild and stray animals;

d. hygiene procedures for staff and visitors;

e. isolation of sick animals;

f. drainage and waste disposal and;

g. ensuring machinery and equipment does not introduce pests or disease.

11. Emergency Biosecurity Response Plan

Objective

To adopt high risk management procedures and thereby increase biosecurity protection in the event of a suspected outbreak of an emergency disease or serious endemic disease.

During any outbreak of an emergency animal disease, specific operating procedures will be available from Animal Health Australia in accordance with AUSVETPLAN (www.animalhealthaustralia.com.au/programs/eadp/ausvetplan/ausvetplan_home.cfm).

Guidelines

G11.1 Each zoo should establish and document a clear Emergency Biosecurity Response Plan for use if an emergency animal disease alert is raised (e.g. an unusual increase in mortality or illness).

G11.2 The Emergency Biosecurity Response Plan should include protocols for work practices, restriction on animal, staff and visitor movement and should detail the agencies and authorities which need to be informed.

References and other reading

Australian and New Zealand guidelines for fresh and marine water quality (2000); primary industries, and livestock drinking water quality
http://www.mincos.gov.au/publications/australian_and_new_zealand_guidelines_for_fresh_and_marine_water_quality
and
www.mincos.gov.au/__data/assets/pdf_file/0020/316127/wqg-ch4.pdf

Australian Animal Welfare Standards and Guidelines: Exhibited Animals (Zoos)

AUSVETPLAN

www.animalhealthaustralia.com.au/programs/eadp/ausvetplan/ausvetplan_home.cfm

AUSVETPLAN Wild Animal Response Strategy

www.animalhealthaustralia.com.au

AUSVETPLAN Zoos Enterprise Manual

www.animalhealthaustralia.com.au/fms/Animal%20Health%20Australia/AUSVETPLAN/zoofinal.pdf

Australian Wildlife Health Network

www.wildlifehealth.org.au/AWHN/home.aspx

Beale, Fairbrother, Inglis and Trebeck 2008. One biosecurity: a working partnership. The independent review of Australia's quarantine and biosecurity arrangements report to the Australian government. Commonwealth of Australia Barton ACT, Australia 298pp.

Infection Control Guidelines for Animal Contact

www.health.qld.gov.au/ph/documents/cdb/zoo_guidelines.pdf

National Farm Biosecurity Manual Poultry Production

www.daff.gov.au/birds
and
www.farmbiosecurity.com.au/toolkit.cfm

National Notifiable Disease List

www.daff.gov.au/animal-plant-health/pests-diseases-weeds/animal/notifiable

OIE disease list

www.oie.int/animal-health-in-the-world/oie-listed-diseases-2011/

Petting Zoo Guidelines

www.public.health.wa.gov.au/cproot/2222/2/Petting%20Zoo%20Guidelines%202009.pdf

Vertebrate Pests Committee Guidelines

www.feral.org.au/guidelines-for-the-import-movement-and-keeping-of-exotic-vertebrates-in-australia/

Water Biosecurity Manual for poultry farms

www.daff.gov.au/birds

Water quality standards for captive seals

www.dpi.nsw.gov.au/__data/assets/pdf_file/0011/278075/standards-for-exhibiting-seals-in-nsw.pdf.

ZAA Veterinary Specialist Advisory Group list serve

http://www.zooaquarium.org.au/Veterinary-SAG/default.aspx

Abbreviations

AQIS	Australian Quarantine Inspection Service
AUSVETPLAN	Australian Veterinary Emergency Plan, an agreed management plan and set of operational procedures, which would be adopted in the event of an emergency animal disease outbreak in Australia. The procedures are briefly outlined in the Summary Document and details are given in the individual Disease Strategies. The manuals are written with specific reference to certain animal industries where a greater than normal risk of harm could be expected from an emergency disease outbreak. The Enterprise Manual for zoos (zoological gardens, circuses and animal theatres) forms part of the Australian Veterinary Emergency Plan, or AUSVETPLAN Edition 2.
AWHN	Australian Wildlife Health Network ("The Network")
AAWS	Australian Animal Welfare Strategy
CBSG	Conservation Breeding Specialist Group
CVO	Chief Veterinary Officer

DAFF	Australian Government Department of Agriculture, Fisheries and Forestry
IUCN SSC	International Union for the Conservation of Nature, Species Survival Commission
NZBM	National Zoo Biosecurity Manual
OIE	Office International Des Epizooties (World Organisation for Animal Health)
WHO	World Health Organisation
ZAA	Zoo and Aquarium Association The Association (previously known as ARAZPA) was established in 1990 to link zoos and aquariums in Australia, New Zealand and the South Pacific in a cooperative regional network for wildlife conservation. The Association now links over 70 institutions, all working together to protect and conserve the world's wildlife. Their mission is: "To harness the collective resources of zoos and aquariums to conserve biodiversity in the natural environment". Association member institutions support the principles outlined in the World Zoo and Aquarium Conservation Strategy, and aim to further develop zoos and aquariums as centres of excellence in wildlife conservation, environmental education and research. The Zoo and Aquarium Association's Accreditation Program offers a framework for assisting zoos and aquariums to achieve established Association standards of zoo and aquarium operation. The general standards of relevance to all aspects of zoo and aquarium operation include general operations, collection management, animal husbandry, animal health care, education and conservation. Many of these accreditation standards are relevant to zoo biosecurity.
ZAHRG	Zoo Animal Health Reference Group is a committee formed by DAFF to represent zoos on Animal Health issues and comprises members from the following institutions: DAFF, the Association, ZAA Veterinary SAG, Australia Zoo, Zoos South Australia, Perth Zoo, Sea World Australia, Taronga Conservation Society Australia and Zoos Victoria. AWHN provides secretariat for this group.
ZAA Vet SAG	ZAA (previously ARAZPA) Veterinary Specialist Advisory Group The ZAA Veterinary Specialist Group was established for the promotion of communication and collaboration between veterinarians employed by ZAA member institutions. By this means the group aims to enhance the ability of ZAA institution vets to remain informed of and respond to zoological veterinary issues and to provide a collective voice for consultation with ZAA and other organisations when the need arises. The group is convened as an electronic discussion group which is open to all veterinarians working for ZAA member institutions and has been running since 2001.

Definitions

Animal staff: all employees and volunteers coming into contact with zoo animals, includes keepers, veterinarians, veterinary nurses and education staff.

Animal Transaction: acquisition or disposal of an Animal by a Facility. Does not include short-term removal from the Facility for temporary purposes such as veterinary treatment or to give a presentation off-site.

Anthropozoonosis: a Disease spread from humans to animals.

Barrier keeping: use of work practices which minimise the spread of infectious disease from one animal, group or environment to another.

Biosecurity: the set of precautions taken to minimise the risk of introducing an infectious disease into an animal (or human) population.

Biosecurity Plan: a plan that minimises the risk of Disease or infectious agents, chemical and environmental contaminants entering into, spreading within or escaping from the Facility.

Biosecurity Zone: distinct management zone within the zoo, based on differing levels of biosecurity risk. Creating different biosecurity zones allows for more effective risk management and planning.

Contact Area: refers to those areas in which there is direct physical contact between animals and people (usually the public).

Disease: any condition suffered by an Animal such that normally accepted parameters of health are not met.

Domestic Animal: is a species the keeping of which is not restricted in the relevant Australian state or territory.

Drive-through Enclosure: an enclosure into which Vehicles containing members of the public may enter.

Enclosure: any accommodation or structure in which an Animal is contained or can be contained. Includes the grounds of a Facility surrounded by a Perimeter Fence or contained by a building.

Exhibit: any Enclosure or Facility used to display Animals to the public.

Exhibition Purposes: public display, conservation, public education and public entertainment or other prescribed purposes.

Facility: any premises used for Animal Exhibition Purposes, and includes:

any land or place (whether or not wholly or partly built upon or covered by water);

a tent, stall or other structure, whether permanent or temporary; and a Vehicle.

Furniture: any structure or thing within an Enclosure that the Animal has access to. This includes perches, shelter, troughs, ropes, pools, Enrichment toys, trees, vegetation and logs.

Government Authority: a federal, state or territory regulatory body responsible for pest control, conservation, or regulation of Animal exhibition and/or Animal welfare.

Health Program: a preventative and curative program for the care of Animals.

Individual Permanent Identification: a marker that allows a specimen to be distinguished from conspecifics by a third party and includes:

a. ear tags, leg bands and micro-chips;

b. drawings or photographs for specimens that have unique, readily distinguishable, permanent markings and/or colourations;

c. physiological traits such as unusual or unique physical traits that are permanent and are not likely to manifest in a conspecific in such a way as to render such identifier ineffectual.

Interactive Program: activities supervised by one or more Keepers which encourage a member of the public to touch, feed and/or have close contact with an Animal, either inside or outside the Animal's normal enclosure. It is not considered to be an Interactive Program when members of the public enter a designated walk-through animal enclosure such as a macropod walk-through or a walk-through aviary.

Isolation: the segregation of an Animal from its conspecifics for veterinary, husbandry or introduction purposes.

Keeper: a person employed or engaged under the direction of the Operator or the Operator's appointed agent who has a responsibility towards an Animal or group of Animals.

Management Area: A group of enclosures and associated facilities which are managed as one area, with respect to biosecurity risks e.g. feeding, cleaning and waste management procedures.

Off-exhibit Enclosure: any Animal Enclosure, other than a Short-term Enclosure, that is not an Exhibit. This includes, but is not limited to, quarantine and Isolation Enclosures, holding areas, Animal health facilities, and breeding Enclosures.

Operator: any person or organisation who has overall responsibility for the Facility.

Perimeter Fence: a permanent structure that discourages unauthorised entry to a Facility and acts as a barrier for Terrestrial Animals.

Quarantine: a period of isolation, for the purpose of detecting and eliminating infectious disease.

Restraint: any method, (whether physical, chemical or behavioural) of preventing an Animal from performing an act or movement.

Staff: all persons who have been given a level of responsibility associated with the zoo and includes, but is not limited to, keepers, volunteers, researchers, students and contractors. (*NB this definition is drawn from the AAWS definition but differs slightly in wording*).

Staff working with animals: any staff member involved in work activities within the zoo, which involves direct or close contact with animals, animal enclosures or animal products.

Substrate: the material that covers the ground or floor, for example bedding or litter placed on the flooring of a cage, box, stall or Enclosure, or the soil or grass covering of an outdoor ground surface.

Vehicle: a means of transport, including, but not limited to, a car, truck, bus, aircraft, boat, trailer, train, and tram.

Veterinarian: a registered veterinarian.

Visitor: any member of the public visiting the zoo grounds in order to view or interact with zoo animals.

Wild Animal: An animal that is free-living and not confined to a Facility by an Enclosure, a leash or by management practices,

Zoo: includes fauna parks, sanctuaries, aquaria and marine parks holding native and/or exotic species.

Zoonosis/ Zoonotic Disease (Plural Zoonoses): Diseases that are transmissible between Animals and humans.

All definitions cover the singular, plural and all variations of the word.

APPENDIXES

Appendix 1: Roles and responsibilities for sending and receiving zoos in zoo animal transactions.

Task/ action	Responsibility	Staff responsible
Pre-shipment quarantine (where required)	Sending Zoo	Veterinary and/ or keeping staff
Pre-shipment health checks	Sending zoo	Veterinary staff
Cleaning of crates, boxes, bags before use	Sending zoo	Keeping staff
Cleaning machinery and equipment, vehicles, trucks, forklifts etc. Before loading/use	Sending zoo	Keeping and/ or maintenance staff
Disinfecting footwear and hands at start and conclusion of work	All involved including contractors	Each person involved in the animal transport
Dedicated clean clothes and boots	Both zoos	Each person involved in the animal transport
Post arrival quarantine procedures and preventative medicine programs	Receiving zoo	Veterinary staff
Post arrival health checks	Receiving zoo	Veterinary staff
Disposal of waste material, food, bedding in animal crates	Receiving zoo	Keeping staff
Cleaning of crates, boxes, bags after use	Receiving zoo	Keeping staff
Cleaning machinery and equipment, vehicles, trucks, forklifts etc. after unloading/transport	Receiving zoo	Keeping and/ or maintenance staff

Appendix 2: An example of the biosecurity self audit checklist.

A biosecurity self audit checklist for continuous improvement, which zoos can download and adapt to their requirements, is available from www.zooaquarium.org.au

An example of one page is inserted here.

2.0	Property Management	Guideline reference	YES	NO	N/A	CORRECTIVE ACTION
2.1	Perimeter and animal enclosure security					
	Does the property have a secure perimeter fence or otherwise well defined boundary establishing a clearly defined biosecurity zone?	G2.5				
	Are entrances to the property able to be closed and locked to vehicle and foot traffic? Are entrances locked during all non-visitor hours?	G2.6				
	Are all animal enclosures appropriately constructed and secured to prevent animal escape?	G2.7				
	Is each enclosure individually and permanently identified with a unique name, number or alphanumeric code for identification purposes?	G2.6				

Subclinical avian malaria infections in African Black-footed Penguins (Spheniscus demersus) and induction of parasite recrudescence

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SUBCLINICAL AVIAN MALARIA INFECTIONS IN AFRICAN BLACK-FOOTED PENGUINS (*SPHENISCUS DEMERSUS*) AND INDUCTION OF PARASITE RECRUDESCENCE

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ABSTRACT: The subclinical and clinical *Plasmodium elongatum* and *Plasmodium relictum* infections of captive-reared African black-footed penguins (*Spheniscus demersus*) were evaluated in nine adult and 29 juvenile penguins in the Baltimore Zoo (Maryland, USA) during summer 1988 and winter 1989. Two diagnostic methods were used: Giemsa-stained thin blood films, and subinoculation of penguin blood into 1-day-old Peking ducklings. Chloroquine and primaquine treatment was applied to all parasitemic juvenile penguins. Twenty-nine parasite-free, juvenile penguins were monitored for parasitemia by Giemsa-stained thin blood films every two weeks for 26 weeks of their first outdoor exposure. Eighteen of 29 penguins experienced naturally acquired malaria; 14 were infected with *P. elongatum*, three with *P. relictum*, and one bird had a mixed *P. relictum* and *P. elongatum* infection. Eleven of 18 juveniles became parasitemic again after chloroquine and primaquine treatments. Based on Giemsa-stained thin blood smears and subinoculation of penguin blood into 1-day-old ducklings, performed in a mosquito-free environment in winter, nine adult penguins had no evidence of *Plasmodium* spp. infection. After dexmethasone-induced immunosuppression, four of six of these nonparasitemic adult penguins were found to be infected with *P. relictum* by the blood inoculation method.

Key words: Avian malaria, parasite recrudescence, parasite relapse, *Plasmodium relictum*, *Plasmodium elongatum*, African black-footed penguins, *Spheniscus demersus*.

INTRODUCTION

Avian malarial parasites cause significant mortality in captive penguins in open-air colonies (Fleischman et al., 1968). Due to the persistence of pre-erythrocytic and exoerythrocytic schizogonies, even after inoculation of infected blood (Garnham, 1966), the pathology and clinical signs of disease in *Plasmodium relictum* and *Plasmodium elongatum* infections are associated with the exoerythrocytic infection (Fix et al., 1988). Because of the low parasitemia, the destruction of red blood cells usually does not cause clinical anemia in penguins (Cranfield et al., 1990), and penguins often die without detectable parasitemia (Griner, 1974). In such cases, parasites can be detected by the blood passage from an infected donor to experimental domestic ducklings; this causes the amplification of parasites to detectable levels (Herman et al., 1966).

Cranfield et al. (1990) reported that if a penguin survives the first infection with avian malaria, its immune system appears to be capable of reducing the number of parasites to subpatent levels. The recrudescence and relapses of malarial parasites have been reported by many workers in various species of wild birds (Bennett et al., 1976; Pierce and Mead, 1978); however, causes of these phenomena were not given. There are no studies on recrudescence or relapses of *P. elongatum* or *P. relictum* in African black-footed penguins (*Spheniscus demersus*). Our objective was to determine whether African black-footed penguins (*S. demersus*) remained subclinically infected with naturally acquired avian malarial parasites after the parasitemia dropped below detection by blood smear.

MATERIALS AND METHODS

We conducted three experiments: 29 juvenile, captive-reared African black-footed penguins were selected for Experiment I, nine adult pen-

guins were chosen for Experiment II, and six of nine birds from Experiment II were selected for Experiment III. All birds were marked individually by a metal flipper tag with an attached color-coded plastic tape for identification. Juvenile birds selected for Experiment I were hatched in the Baltimore (Maryland, USA) Zoo's hatchery from September 1987 to April 1988, and were kept in controlled mosquito-free conditions until exposure in the open-air colony in early May 1988. The vector-free conditions were maintained according to the protocol of Graczyk et al. (1994a). The 29 penguins were bled every two weeks from mid-May to late October 1988 (Fig. 1) between 1000 and 1100 hr (Stoskopf and Beier, 1979) according to the protocol of Graczyk et al. (1994b). Collection of blood samples and blood processing protocols (slide preparation, staining, examination, parasite identification) followed the procedures of Graczyk et al. (1993). The first three birds diagnosed as parasitemic were transferred from the outdoor exhibition to the vector-free indoor environment. These penguins received chloroquine and primaquine treatment according to the protocol of Graczyk et al. (1994a). Penguins were returned to the main colony in early November 1988. Although in an indoor environment, the three penguins were bled on the same schedule as the penguins in the open-air colony. The same medical treatment also was applied to all the parasitemic penguins in the outdoor exhibition.

For Experiment II, nine adult penguins (6- to 9-yr-old) were randomly selected from the flock of 30 adult birds exposed in the outdoor colony during all summers, and bled every 2 wk during their first outdoor exposure season. Six of nine selected penguins were diagnosed by Giemsa-stained thin film as parasitemic during their first open-air exposure season. Five birds were infected with *Plasmodium elongatum*, and one bird had a mixed *P. elongatum* and *Plasmodium relictum* infection. In December 1988, we tested for avian malarial infections in these adult penguins by Giemsa-stained thin blood smear, and by multiple blood subinoculations (Herman et al., 1966). The subinoculation method was used only when the thin smear method was negative. Two milliliters of jugular blood from each penguin were injected intravenously into each of two ducklings. Ducklings were housed in controlled mosquito-free conditions. After 7 days, 2.0 ml of blood were drawn from each of the ducklings for a blood smear and for a second blood transfer to a second group of 18 1-day-old ducklings. The second group was bled at day 7 post-inoculation (PI) for preparation of thin blood smears. A thin blood smear also was prepared from the first group of ducklings at this time.

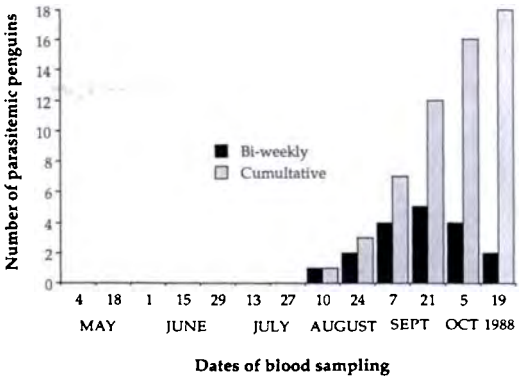

FIGURE 1. Temporal distribution of parasitemia episodes in juvenile African black-footed penguins (*Spheniscus demersus*) during the first outdoor season. Bi-weekly refers to number of penguins parasitemic on a given date when they were bled every two weeks. Cumulative refers to the total numbers of penguins parasitemic up to that date.

In January 1989, six of nine adult penguins from Experiment II were selected at random for Experiment III. Three of the six penguins were parasitemic, and three birds were not parasitemic as determined by Giemsa-stained blood smear during their first outdoor season. One of the three parasitemic penguins had a mixed *P. relictum* and *P. elongatum* infection; two birds were infected with *P. elongatum* (Table 1). All three birds were injected intramuscularly with dexamethasone 21-phosphate (Sigma Chemical Co., St. Louis, Missouri, USA) and 30 mg/per animal of amikacin sulphate (Fort Dodge Laboratories, Inc., Fort Dodge, Iowa, USA) for five consecutive days. Three penguins diagnosed as parasitemic during their first outdoor exposition were injected with 1.0 mg/kg body weight of dexamethasone, and the other three birds received 2.0 mg/kg of dexamethasone. Dexamethasone was used because of its properties for induction of immunosuppression in birds (Isobe and Lillehoj, 1992). Amikacin sulphate was administered to reduce the risk of clinical disease resulting from opportunistic bacterial infection (Brummett, 1983) during the immunosuppression. Seven days after initiation of the dexamethasone treatment, 4.0 ml of blood were drawn from each of the six penguins for the preparation of thin blood smear and for inoculation into two 1-day-old ducklings (2 ml/animal). After 7 days, 2.0 ml of blood from each duckling were drawn for thin blood smears and for inoculation of a second group of 12 ducklings. This procedure also was repeated after 7 days for inoculation of a third group of ducklings. If the thin blood smear from the second

TABLE 1. The results of isodiagnostic blood subinoculation method for nonparasitemic and dexamethasone-immunosuppressed, adult African black-footed penguins (*Spheniscus demersus*).

Parasite species in penguin[a]	Dose of dexamethasone (mg/kg)	Blood passage			Parasite species in ducklings[c]
		First[b]	Second[b]	Third[b]	
P. elongatum	1.0	−[d] −	+ +	ND	*P. relictum*
P. elongatum	1.0	− −	+ +	ND	*P. relictum*
P. elongatum *P. relictum*	1.0	− −	+ +	ND	*P. relictum*
—	2.0	− −	+ −	+ +	*P. relictum*
—	2.0	− −	− −	− −	—
—	2.0	− −	− −	− −	—

[a] Naturally acquired during the first outdoor summer season and diagnosed by Giemsa-stained thin blood film method.
[b] Two one-day-old Peking ducklings used for each blood transfer.
[c] Diagnosed by Giemsa-stained thin blood film method.
[d] −, no *Plasmodium* spp. observed; +, *Plasmodium* spp. observed; ND, not done.

group of ducklings did not contain parasites, a third blood passage was performed (Table 1). The six dexamethasone-treated penguins were bled for blood smears on the same schedule and in the same manner as experimental ducklings.

RESULTS

In Experiment I, during the 26-wk outdoor period, 18 of 29 juveniles experienced malaria, while 11 of 29 juveniles remained nonparasitemic. All avian malaria episodes occurred from 10 August to 19 October with the peak of malaria cases in mid-September (Fig. 1). Fourteen of 29 juveniles were infected with *Plasmodium elongatum*, three with *Plasmodium relictum*, and one with both.

In Experiment I, two of the first three parasitemic penguins which were moved from the outdoor exposition to the mosquito-free environment were infected with *P. elongatum*; one bird had a mixed *P. elongatum* and *P. relictum* infection. All three penguins, nonparasitemic after administration of chloroquine became parasitemic again with *P. elongatum* in ≤4 wk. Fifteen of the remaining 26 juveniles in outdoor exhibition had parasitemia (Fig. 1); eight of them became parastemic again within 4 wk after the initiation of medical treatment. Thus, 11 of 18 juveniles became parasitemic again after chloroquine and primaquine treatments.

In Experiment II, all nine adults were negative for *Plasmodium* spp. by the thin blood smear method. All nine adults were *Plasmodium* spp. negative based on two blood passages to the 1-day-old ducklings.

The blood smears from all six penguins injected with dexamethasone (Experiment III) remained negative for 21 days. Also, the first group of ducklings injected with 2.0 ml of blood from six adult penguins were nonparasitemic at day 7 PI, and remained negative through the experiment (Table 1). Three pairs of ducklings in the second blood passage group became parasitemic by day 7 PI with *P. relictum*, and the blood passage to the third group of ducklings was not done (Table 1); one of the remaining ducklings from this group became parasitemic by day 14 PI with *P. relictum* (Table 1). Based on the blood subinoculation method, *Plasmodium* spp. occurred in four of six dexamethasone-treated penguins. Ducklings inoculated with the blood from two penguins which acquired *P. elongatum* infections during the first outdoor season had only *P. relictum* parasitemia. The ducklings inoculated with blood from the penguin with mixed *P. elongatum* and *P. relictum* infection also had only a *P. relictum* parasitemia.

DISCUSSION

The recurrent recrudescences and relapses of malarial parasites in birds has

been reported by many researchers (Atkinson and van Riper, 1991). We observed that naturally acquired parasites survived chloroquine and primaquine therapy, and under the corticosteroid stimulus they recrudesced into the erythrocytes. Brown (1969) and Garnham (1970) suggested three hypotheses to explain this phenomenon: 1) erythrocytic stages persist and multiply in deep vascular sites and subpatent parasite populations may emerge during the decline of nonsterilizing immunity (Sergent and Sergent, 1956) causing parasite relapses; 2) exoerythrocytic stages continuously release merozoites into the circulating blood, and these allow parasite populations to recover when premunition to them declines (parasite recrudescence); and 3) dormant sporozoites or pre-erythrocytic forms survive in endothelial tissues and later cause parasite recrudescence under a specific stimulus (Griner, 1974). We observed that penguins naturally infected and medically treated for malarial parasites were negative by thin blood smear and blood subinoculation; we observed no parasites after two blood passages to 1-day-old ducklings. However, according to the sensitivity of the Giemsa-stained blood film (Stoskopf and Beier, 1979), and blood subinoculation method (Herman et al., 1966) we should have observed *Plasmodium* spp. if the second hypothesis was true. Therefore, it seems more likely that infected erythrocytes persisted in deep vascular sites in penguins or dormant sporozoites and pre-erythrocytic forms of malarial parasites survived in the endothelial tissues of African penguins. Upon corticosteroid therapy, the parasites may have recrudesced in the penguin hemopoietic tissue, and multiplied in experimentally infected ducklings. Persistence of pre-erythrocytic (post-sporozoite) forms of *Plasmodium relictum* in pigeons (*Columba livia*) has been suggested by Huff (1951).

Based on the positive blood subinoculation results for four of six dexamethasone-treated penguins, we conclude that the invasion of red blood cells is an effect associated with the glucocorticoid-mediated immunosuppression. Dexamethasone-treated birds exhibit a marked decrease in the number of lymphocytes and monocytes (Gross et al., 1979) and decreases of cell-mediated immunity (CMI) (Isobe and Lillehoj, 1992). Rank and Weidanz (1976) demonstrated that immunity to reinfection of chickens with *Plasmodium gallinaceum* occurred in the absence of detectable B-cell function indicating that nonsterilizing immunity (Sergent and Sergent, 1956) is an antibody-independent phenomenon and requires CMI. Thus the effect of dexamethasone on subclinically infected penguins can be explained by debilitating the processes of CMI controlling the pre-erythrocytic parasites in the endothelial tissues. The results of the present study indicate that African black-footed penguins can be subclinically infected with *P. relictum* and clinically infected with *Plasmodium elongatum*.

One striking observation in our study was that penguins which experienced the recrudescence of malarial parasites had been treated for ten days with primaquine. Primaquine inhibits mitochondrial respiration of the primary and secondary liver stages of human malarial parasites (Contacos, 1973). However, pre-erythrocytic and exoerythrocytic stages of *P. relictum* and *P. elongatum* inhabit the endothelial tissue of all organs of penguins (Fleischman et al., 1968). As seen, the parasites may survive the primaquine therapy. Based on our results, we believe that antimalarial therapy based on human infant treatment schedules (Stoskopf and Beier, 1979) is not adequate to eliminate the exoerythrocytic stages of *Plasmodium* spp. parasites.

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REVIEW – STANDARD

Blood parasites of penguins: a critical review

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SUMMARY

Blood parasites are considered some of the most significant pathogens for the conservation of penguins, due to the considerable morbidity and mortality they have been shown to produce in captive and wild populations of these birds. Parasites known to occur in the blood of penguins include haemosporidian protozoans (*Plasmodium, Leucocytozoon, Haemoproteus*), piroplamid protozoans (*Babesia*), kinetoplastid protozoans (*Trypanosoma*), spirochete bacteria (*Borrelia*) and nematode microfilariae. This review provides a critical and comprehensive assessment of the current knowledge on these parasites, providing an overview of their biology, host and geographic distribution, epidemiology, pathology and implications for public health and conservation.

Key words: blood parasite, bird, conservation, pathogen, seabird, vector-borne diseases.

INTRODUCTION

Diseases and parasites may adversely affect breeding success and lead to the mortality of penguins, potentially hampering the viability of their populations (Woods *et al*. 2009). Blood parasites are considered some of the most significant pathogens for the conservation of penguins (Brossy *et al*. 1999; Jones and Shellam, 1999*b*; Levin and Parker, 2011).

Since 1926, when Sir Henry Harold Scott first diagnosed avian malaria as the cause of death of a king penguin captive at the Zoological Society of London (Scott, 1927), a considerable body of literature has gradually accumulated on the blood parasites of penguins, with nearly a hundred publications. The concern that blood parasites could be a conservation threat emerges from the observation of the dramatic impacts of avian malaria outbreaks in captive penguins (e.g. Rodhain, 1939; Griner and Sheridan, 1967; Fix *et al*. 1988; Bueno *et al*. 2010), along with the existence of *Plasmodium* sp. infections in wild penguins (Fantham and Porter, 1944; Laird, 1950; Levin *et al*. 2009) as well as other potentially pathogenic blood parasites (Fallis *et al*. 1976; Jones and Woehler, 1989; Earlé *et al*. 1992; Argilla *et al*. 2013). There are a few documented cases of mortality of wild penguins due to blood parasite infections (Fantham and Porter, 1944; Hill *et al*. 2010; Argilla *et al*. 2013; Cannell *et al*. 2013) and, because there are a number of mass mortality events of wild penguins for which the causes of death cannot be established (Gill and Darby, 1993; Kerry *et al*. 2009; Woods *et al*. 2009), the possibility that blood parasites play a role in some of these events cannot be dismissed.

In this review, we will provide a critical and comprehensive assessment of the state-of-the-art of blood parasites known to infect penguins, providing an overview of their biology, host and geographic distribution, epidemiology, pathology and implications for public health and conservation.

MATERIALS AND METHODS

This review addresses organisms that parasitize blood cells (haemosporidians and piroplasmids), as well as other organisms that can be detected in blood smears (kinetoplastids, spirochetes and nematode microfilariae). All records published in peer-reviewed journals until 01 January 2016 were considered; institutional reports, conference presentations and Ph.D. theses were included when they presented relevant data that could not be found in other peer-reviewed publications. A comprehensive list of the known records of blood parasites of penguins is provided in Appendix 1, and these data are summarized in Table 1. In light of novel evidence and critical consideration of the existing record, a

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Table 1. Summary of blood parasites recorded in each penguin species

Penguin species	*Plasmodium*	*Leucocytozoon*	*Haemoproteus*	*Babesia*	*Trypanosoma*	*Borrelia*	Microfilariae
Aptenodytes forsteri (Emperor)							
Aptenodytes patagonicus (King)	C					W	
Eudyptes chrysocome (Southern rockhopper)	C						
Eudyptes chrysolophus (Macaroni)	C	C					
Eudyptes moseleyi (Northern rockhopper)	W						
Eudyptes pachyrhynchus (Fiordland)		W, R					
Eudyptes robustus (Snares)	W						
Eudyptes schlegeli (Royal)							
Eudyptes sclateri (Erect-crested)							
Eudyptula minor (Little)	W, C	C, E		W	W		
Megadyptes antipodes (Yellow-eyed)	W	W					
Pygoscelis adeliae (Adélie)							
Pygoscelis antarcticus (Chinstrap)	C						
Pygoscelis papua (Gentoo)	C						
Spheniscus demersus (African)	W, R, C	R		W, R		W, R	
Spheniscus humboldti (Humboldt)	C		W				
Spheniscus magellanicus (Magellanic)	R, C						
Spheniscus mendiculus (Galapagos)	W		W				W

Records were classified according to the context in which the diagnosis was established: W, penguins sampled in the wild; R, penguins undergoing rehabilitation; C, penguins captive in zoos; E, penguins infected under experimental conditions.

number of records were revised or were considered inconclusive or questionable; detailed remarks on these cases are provided in Appendix 2. It is worth noting that *Aegyptianella* sp. was considered within the scope of this review, but was not included because it has not yet been conclusively demonstrated to infect penguins (see Appendix 2).

Figure 1 presents a hand-drawn summary of the parasites that can be found in the blood of penguins; these drawings were based on the descriptions and illustrations provided by Fallis *et al.* (1976), Jones and Woehler (1989), Earlé *et al.* (1993), Merkel *et al.* (2007), Yabsley *et al.* (2012), Silveira *et al.* (2013) and Vanstreels *et al.* (2014*a*, 2015*a*, *b*). This figure is not meant as an identification plate, but an illustration of the variations in size and shape of each parasite group. *Haemoproteus* sp. was not included in Fig. 1, because it has not yet been observed in blood smears of penguins. It is possible that *Leucocytozoon* spp. form elongated gametocytes when infecting penguin leukocytes, as occurs in other hosts (Valkiūnas, 2005); however, these parasite forms were never documented in penguins and therefore were not represented in Fig. 1.

To evaluate if there are regions where blood parasites could infect penguins but have yet to be recorded, we juxtaposed the distribution of penguin breeding colonies (IUCN, 2015) with that of records of blood parasites in penguins and of their confirmed or suspected invertebrate hosts/vectors (Figs 2 and 3). The distribution of the following invertebrate hosts/vectors is represented: seabird soft ticks (*Argas* spp., *Carios* spp.) (Dietrich *et al.* 2011), seabird hard ticks (*Amblyomma loculosum, Ixodes* spp.) (Barbosa *et al.* 2011; Dietrich *et al.* 2011; Muñoz-Leal and González-Acuña, 2015), biting midges (*Culicoides* spp.) (Murray, 1975; Spinelli and Martinez, 1991; Tabachnick, 2004; Aybar *et al.* 2010; Guichard *et al.* 2014), black flies (*Austrosimulium* spp., *Cnephia* spp., *Prosimulium* spp., *Simulium* spp.) (Dumbleton, 1963; Hill *et al.* 2010; Argilla *et al.*

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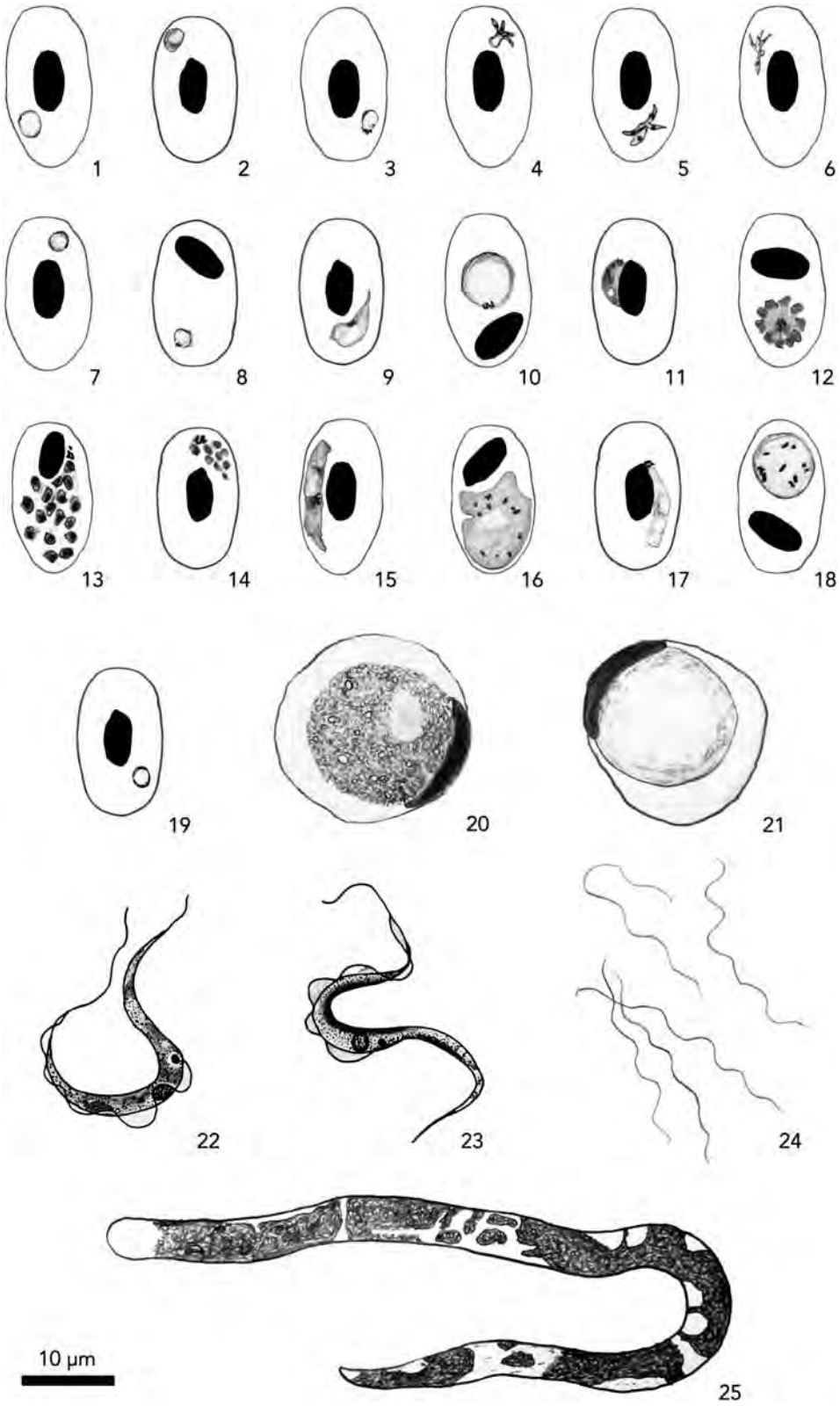

Fig. 1. Overview of the morphology of blood parasites of penguins: *Babesia peircei* (round forms = 1–3, tetrameric forms = 4–6), *Plasmodium* spp. (trophozoites = 7–8, young gametocytes = 9–10, meronts = 11–14, macrogametocytes = 15–16, microgametocytes = 17–18), *Leucocytozoon tawaki* (young gametocyte = 19, round macrogametocyte = 20, round microgametocyte = 21), *Trypanosoma eudyptulae* (trypomastigotes = 22–23), Relapsing Fever *Borrelia* (24), nematode microfilaria (25).

2013), *Culex* mosquitoes (*Culex pipiens*, *Culex quinquefasciatus*, *Culex pervigilans*) (White, 1989; WRBU, 2014), saltwater mosquito (*Ochlerotatus australis*) (Holder, 1999; Snell, 2005; Landcare Research, 2015) and black salt marsh mosquito (*Aedes taeniorhynchus*) (WRBU, 2014).

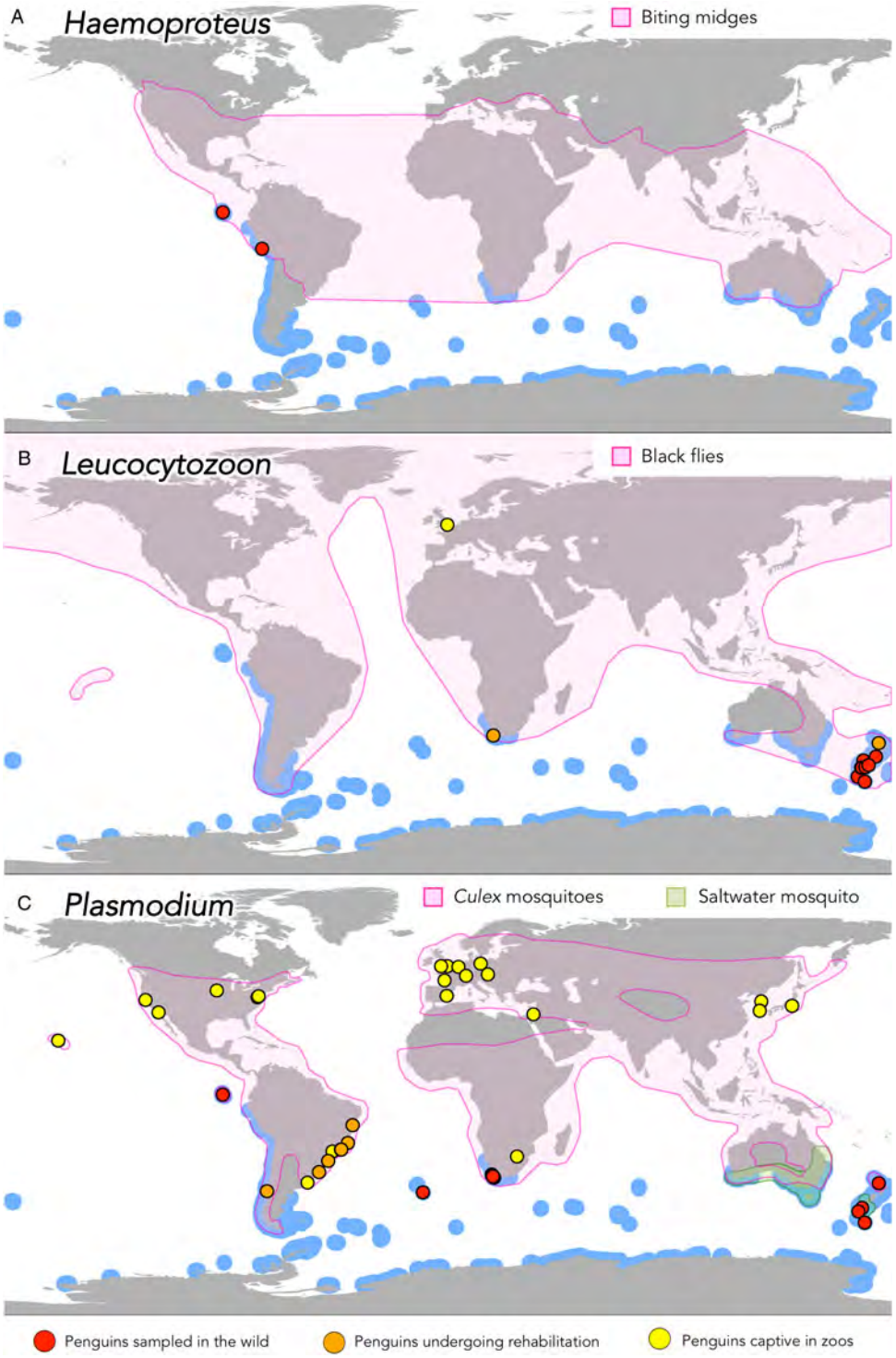

Fig. 2. Geographic distribution of records of haemosporidian blood parasites in penguins in relation to their invertebrate hosts. Blue areas represent the breeding distribution of penguins.

PLASMODIUM (AVIAN MALARIA)

Plasmodium spp. (Haemosporida: Plasmodiidae) are parasites of all tetrapod classes. More than 60 avian-infecting species have been described in five subgenera: *Bennettinia, Giovannolaia, Haemamoeba, Huffia* and *Novyella* (Valkiūnas, 2005; Martinsen and Perkins, 2013). In the avian blood, these parasites can be found in the cytoplasm of erythroblasts and erythrocytes (and occasionally thrombocytes, see Silveira *et al*. 2009) in the form of trophozoites, erythrocytic meronts or gametocytes (Fig. 1); haemozoin granules are present. In the avian tissues, these parasites will invade endothelial cells and macrophages; megalomeronts are absent (Valkiūnas, 2005).

Species recorded in penguins

Five species of *Plasmodium* have been demonstrated to infect penguins through both morphological and

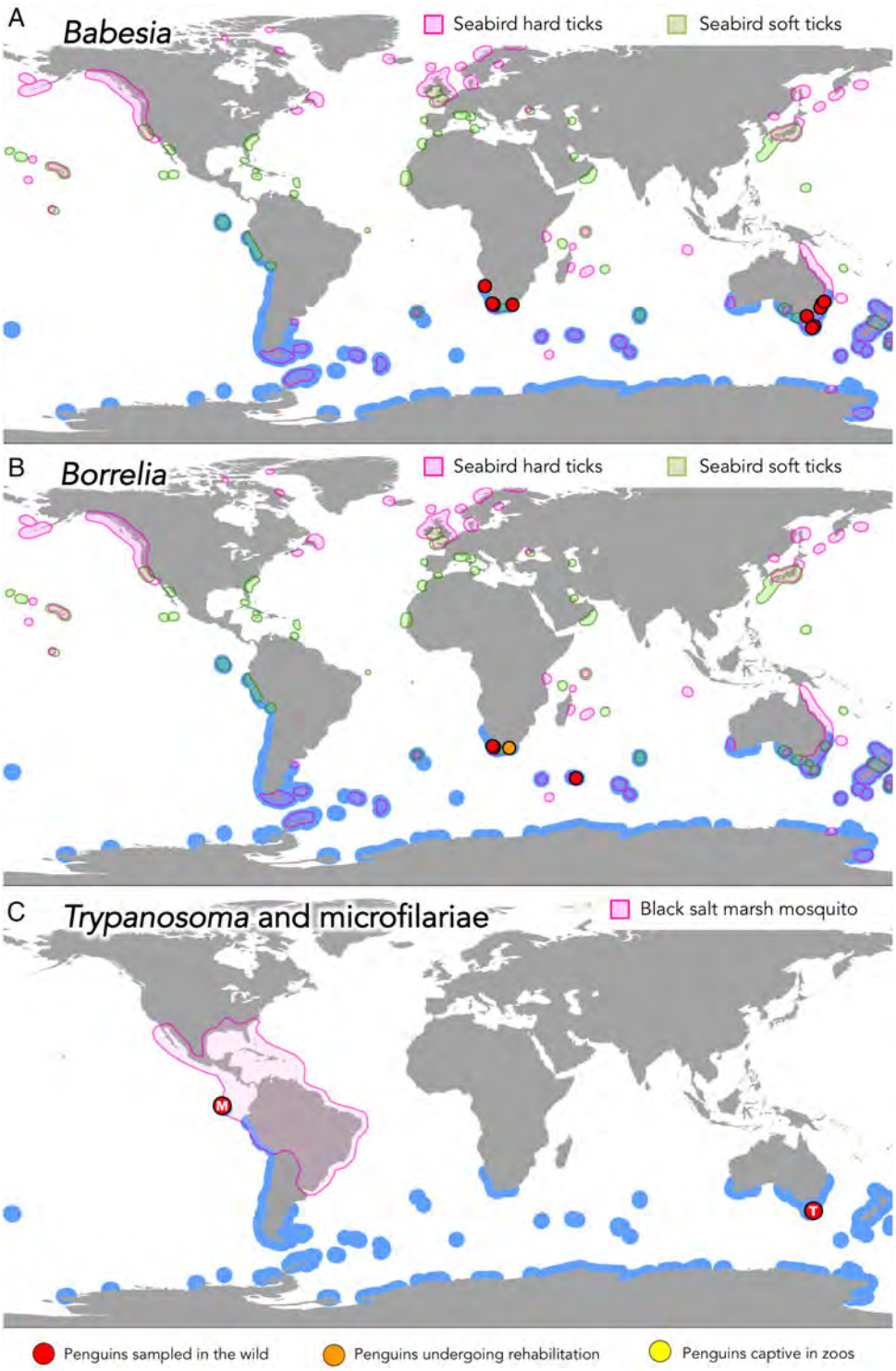

Fig. 3. Geographic distribution of records of non-hemosporidian blood parasites in penguins in relation to their confirmed or presumed vectors. Blue areas represent the breeding distribution of penguins.

genetic evidence: *Plasmodium* (*Haemamoeba*) *relictum* (Fantham and Porter, 1944), *Plasmodium* (*Huffia*) *elongatum* (Huff and Shiroishi, 1962), *Plasmodium* (*Haemamoeba*) *tejerai* (Silveira *et al*. 2013), *Plasmodium* (*Haemamoeba*) *cathemerium* and *Plasmodium* (*Novyella*) *nucleophilum* (Vanstreels *et al*. 2015*a*). Additionally, *Plasmodium* (*Novyella*) *unalis* was identified through genetic evidence (Vanstreels *et al*. 2015*a*). There is a record of *Plasmodium* (*Bennettinia*) *juxtanucleare* infecting penguins (Grim *et al*. 2003), however that report is problematic (see Appendix 2).

Because a number of studies have not conclusively identified all *Plasmodium* lineages that were detected (see Appendix 1), it is probable that many other species of *Plasmodium* have yet to be recorded in

penguins. Additionally, concomitant infection by two or more *Plasmodium* lineages is not uncommon (Huff and Shiroishi, 1962; Fleischman *et al*. 1968*a*, *b*; Herman *et al*. 1974; Sladen *et al*. 1979; Stoskopf and Beier, 1979; Beier and Stoskopf, 1980; Beier and Trpis, 1981; Vanstreels *et al*. 2014*a*, 2015*a*, in press).

Distribution among penguin hosts

Thirteen species have been shown to be susceptible to *Plasmodium* in the wild or in captivity: king (Scott, 1927), Humboldt (Rodhain, 1939), African, northern rockhopper, yellow-eyed (Fantham and Porter, 1944), Snares (Laird, 1950) (see Appendix 2), chinstrap (Rodhain and Andrianne, 1952), little, gentoo (Griner and Sheridan, 1967), Macaroni (Herman *et al*. 1974), Magellanic (Fix *et al*. 1988), Galapagos (Levin *et al*. 2009) and Southern rockhopper penguins (Dinhopl *et al*. 2011). There are only five penguin species in which *Plasmodium* infection was never documented: Adélie, emperor, erect-crested, Fiordland and Royal penguins. These species are inhabitants of remote sub-Antarctic and Antarctic environments, were seldom examined for blood parasites in the wild and were either never maintained in captivity or are generally maintained in vector-free acclimatized enclosures. It is therefore probable that the lack of records in these species is not due to a particular resilience to these parasites, but instead reflects a lack of studies in the wild and/or lack of exposure to environmental conditions that might allow their infection *ex situ*.

Invertebrate hosts

It is well established that avian plasmodia are transmitted exclusively by mosquitoes (Culicidae), particularly *Culex* spp., *Mansonia* spp., *Culiseta* spp. and *Aedeomyia* spp. Additionally, *Aedes* spp., *Anopheles* spp. and *Armigeres* spp. can also be competent hosts in laboratory experiments (Valkiūnas, 2005; Atkinson, 2008*b*).

Studies in zoos indicate that *Culex* spp. play a key role in the transmission of *Plasmodium* sp. to captive penguins, particularly *C. pipiens* (Rodhain, 1939; Raethel, 1960; Grünberg and Kutzer, 1963; Beier and Trpis, 1981), *Culex quinquefasciatus* (=*Culex fatigans*) (Laird and Van Riper, 1981), *Culex tarsalis* (Huff and Shiroishi, 1962), *Culex restuans* (Beier and Trpis, 1981) and *C.* (*Culex*) sp. (Bueno *et al*. 2010). Fantham and Porter (1944) found *Plasmodium* sp. in *C. quinquefasciatus* collected at Saldanha Bay, South Africa, where a wild penguin was found dead with a *P. relictum* infection. The invasive *C. quinquefasciatus* is likely involved in the transmission of *Plasmodium* in New Zealand and in the Galapagos Archipelago (Tompkins and Gleeson, 2006; Levin *et al*. 2009; Levin and Parker, 2011). In the Galapagos Archipelago, however, *Aedes taeniorhynchus* still has to be investigated as a potential host since this species has been recorded in Mexico carrying *Plasmodium* lineages closely related to those identified in Galapagos penguins (Levin *et al*. 2013). In New Zealand, *C. quinquefasciatus* is restricted to the North Island (White, 1989; Holder, 1999) and *Culex pervigilans* is suspected to be responsible for the transmission of *Plasmodium* sp. at South Island and other islands (Holder, 1999; Sturrock and Tompkins, 2008). *Ochlerotatus australis* (=*Aedes australis*) is an invasive species in New Zealand (Holder, 1999; Snell, 2005) that could also play a role in the transmission of avian malarial parasites.

Geographic distribution

Plasmodium relictum was documented in wild penguins in South Africa (Saldanha Bay), New Zealand (Campbell Island, Fouveaux Strait, Tiritiri Matangi Island, Snares Island) and Gough Island (Fantham and Porter, 1944; Laird, 1950), and in captive penguins in Europe (Rodhain, 1939; Fantham and Porter, 1944), North America (Griner and Sheridan, 1967; Stoskopf and Beier, 1979), Hawaii (Laird and Van Riper, 1981), Eastern Asia (Bak *et al*. 1984), South Africa (Penrith *et al*. 1994) and at rehabilitation centres in South Africa (Brossy *et al*. 1999) and Chile (Carvajal and Alvarado, 2009). *Plasmodium elongatum* was documented infecting penguins at zoos in North America (Huff and Shiroishi, 1962; Beier and Stoskopf, 1980), Europe (Dinhopl *et al*. 2011) and rehabilitation centres in Brazil (Vanstreels *et al*. 2014*a*, 2015*a*). *Plasmodium tejerai* was identified in penguins undergoing rehabilitation in Brazil (Silveira *et al*. 2013; Vanstreels *et al*. 2014*a*, 2015*a*) and Argentina (Vanstreels *et al*. in press). *Plasmodium cathemerium*, *P. nucleophilum* and *P. unalis* were reported only in penguins undergoing rehabilitation in Brazil (Vanstreels *et al*. 2015*a*). Additionally, unidentified lineages of *Plasmodium* have been detected in wild penguins at the Galapagos Archipelago (Isabela, Fernandina, Las Marielas and Bartolomé Islands) (Levin *et al*. 2009, 2013), as well as at zoos in North and South America, Europe and Asia, and at rehabilitation centres in South Africa, Argentina and Brazil (see Appendix 1).

All *ex situ* and most *in situ* records of *Plasmodium* spp. infecting penguins are within the distribution range of *C. pipiens*, *C. quinquefasciatus* and *C. pervigilans* or, in Southern New Zealand, *O. australis* (Fig. 2C). The record of *P. relictum* in a Northern rockhopper penguin at Gough Island (Fantham and Porter, 1944) is a surprising exception, considering this is an extremely remote island where mosquitoes are absent (Gaston *et al*. 2003); the only

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neighbouring archipelago, Tristan da Cunha, is also mosquito-free (Medlock *et al*. 2010). A possible explanation is that this penguin was exposed to *P. relictum* while being vagrant in South Africa (see Rollinson *et al*. 2013).

The geographic distribution of *Culex* mosquitoes overlaps with the breeding habitat of penguins in Peru, Chile, Namibia and Australia (Fig. 2C), and it is therefore plausible that wild penguins in these countries may be infected by *Plasmodium* sp. Most sub-Antarctic islands probably do not provide environmental conditions compatible with the transmission of *Plasmodium* spp. Gough, South Georgia, Marion, Macquarie and Tristan da Cunha Islands are reportedly free from mosquitoes (Laird, 1952; Hänel *et al*. 1998; Medlock *et al*. 2010) and the climate of South Georgia, South Sandwich, Bouvet, Amsterdam, Saint Paul, Crozet, Kerguelen and Peter I Islands is likely too adverse for mosquitoes (Medlock *et al*. 2010). It is therefore reasonable to assume that there is no *Plasmodium* sp. transmission in those locations, even if there have been little to no studies on blood parasites of penguins. The Falkland Islands are reportedly free from mosquitoes (Medlock *et al*. 2010), however DNA from *P. relictum* was detected in the blood of a thin-billed prion (*Pachyptila belcheri*) at New Island; because this a pelagic seabird that only comes to land in the breeding season, it is reasonable to suspect that infection occurred on the island (Quillfeldt *et al*. 2010). The harsh climate of Antarctica and the South Shetland Islands probably also precludes the occurrence of *Plasmodium* sp., as corroborated by blood parasite studies in the region (e.g. Laird, 1961; Becker and Holloway, 1968; Jones and Shellam, 1999*a*; González-Acuña *et al*. 2013; Vanstreels *et al*. 2014*b*).

Epidemiology and pathology

In wild penguins, the prevalence of *Plasmodium* sp. varies considerably. *Plasmodium* sp. was detected in the blood smears of 0·7% of African penguins at Dyer Island, 3% at Saldanha Bay, 9% at Stony Point, 11% at Robben Island, and 34% at Dassen Island (Fantham and Porter, 1944; Brossy, 1992; Thiart, 2005). In New Zealand, blood smears revealed *P. relictum* infection in 10% of yellow-eyed penguins at Fouveaux Strait, 10·7% of Snares penguins at Snares Island, and one of two yellow-eyed penguins at Campbell Island (Fantham and Porter, 1944; Laird, 1950). Furthermore, one of five Northern rockhopper penguins from Gough Island was blood smear-positive to *P. relictum* (Laird, 1950). Using molecular methods, Levin *et al*. (2009, 2013) detected *Plasmodium* sp. in the blood of 5·4% of Galapagos penguins, with prevalence varying between 2·1 and 42·9% among islands. None of the wild penguins in which *Plasmodium* sp. was detected had external signs of disease, and parasitaemia was generally low or undetectable in blood smears (Fantham and Porter, 1944; Laird, 1950; Brossy, 1992; Levin *et al*. 2009). Fantham and Porter (1944) detected *P. relictum* in a deceased wild African penguin; however, because the penguin had multiple traumatic lesions it was not clear to what extent avian malaria may have contributed to its death.

It is well established that avian malaria outbreaks in zoos result from local mosquitoes inoculating penguins with *Plasmodium* sp. acquired from the native birds in the surroundings of the penguin exhibit (Beier and Trpis, 1981; Ejiri *et al*. 2009; Bueno *et al*. 2010; Leclerc *et al*. 2014; Dinhopl *et al*. 2015). Because mosquito abundance is markedly seasonal, cases of avian malaria in captive penguins tend to concentrate in spring-summer, particularly late summer (Grünberg and Kutzer, 1963; Griner and Sheridan, 1967; Sladen *et al*. 1979; Beier and Stoskopf, 1980; Vanstreels *et al*. 2015*a*). Mosquitoes are most active in penguin enclosures at night (Beier and Trpis, 1981). At zoos that recorded avian malaria outbreaks, the prevalence of *Plasmodium* sp. in mosquitoes near penguin exhibits is generally low (<5%) (Beier and Trpis, 1981; Bueno *et al*. 2010; Ejiri *et al*. 2009, 2011), and similar results were obtained in studies at locations where *Plasmodium* sp. was reported in wild penguins (Fantham and Porter, 1944).

Outbreaks of avian malaria in permanently captive penguins usually occur suddenly and/or in successive waves. Mortality might depend on the *Plasmodium* species/lineage involved, whether there was prior exposure to *Plasmodium* sp., and on the administration of drug treatment, with between 10 and 83% penguins dying within a few weeks or months (Fleischman *et al*. 1968*a*; Stoskopf and Beier, 1979; Fix *et al*. 1988; Cranfield *et al*. 1994; Graczyk *et al*. 1994*a*; Bueno *et al*. 2010). A similar pattern is observed for penguins kept in temporary captivity while receiving rehabilitation care in South America (Carvajal and Alvarado, 2009; Vanstreels *et al*. 2014*a*, 2015*a*). On the other hand, avian malaria is enzootic to African penguins undergoing rehabilitation, with 30–35% of penguins being positive (blood smears) upon admission (Parsons and Underhill, 2005).

Most penguins with avian malaria in captivity are in good body condition and do not present clinical signs, dying suddenly. When clinical signs are present, they are not specific and may include: anorexia, depression, lethargy, weakness, regurgitation, green faeces, hyperthermia, pale mucosae, and dyspnoea (Rodhain, 1939; Griner and Sheridan, 1967; Sladen *et al*. 1979; Stoskopf and Beier, 1979; Bak *et al*. 1984; Fix *et al*. 1988; Bueno *et al*. 2010; Vanstreels *et al*. 2014*a*). Haematology may reveal leucocytosis with lymphocytosis and/or monocytosis (Stoskopf and Beier, 1979; Fix *et al*. 1988; Graczyk

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et al. 1994*a*). Infected penguins often have low parasitaemia (<2%) (Stoskopf and Beier, 1979; Graczyk *et al*. 1994*a*); however, occasionally much higher parasitaemia may be observed, with up to 80% of erythrocytes parasitized and multiple parasites per erythrocyte (Fantham and Porter, 1944; Bueno *et al*. 2010; Vanstreels *et al*. 2014*a*).

Captive penguins deceased due to avian malaria typically present hepatomegaly, splenomegaly, lung congestion and hydropericardium (Rodhain, 1939; Bak *et al*. 1984; Fix *et al*. 1988; Graczyk *et al*. 1994*a*; Grim *et al*. 2003; Ko *et al*. 2008; Carvajal and Alvarado, 2009). Tissue meronts are present in multiple tissues and concentrate especially in the lungs, kidneys, brain, heart, liver and spleen (Rodhain, 1939; Fleischman *et al*. 1968*b*; Bak *et al*. 1984; Fix *et al*. 1988; Graczyk *et al*. 1994*a*; Grim *et al*. 2003; Ko *et al*. 2008; Silveira *et al*. 2013; Vanstreels *et al*. 2014*a*, 2015*a*). Concurrent diseases are not uncommon, and aspergillosis is frequently reported in captive penguins that died from avian malaria (Scott, 1927; Rodhain, 1939; Rewell, 1948; Grünberg and Kutzer, 1963; Griner and Sheridan, 1967; Sladen *et al*. 1979; Fix *et al*. 1988; Carvajal and Alvarado, 2009; Grilo, 2014; Vanstreels *et al*. 2015*a*). Septicaemia (Grünberg and Kutzer, 1963), enteritis/diarrhoea (Scott, 1927; Fix *et al*. 1988), infestation with gastrointestinal helminthes (Rodhain and Andrianne, 1952; Fix *et al*. 1988; Vanstreels *et al*. 2015*a*), clostridiosis (Penrith *et al*. 1994), babesiosis (Yabsley *et al*. 2012), poxvirosis and infestation with lung or liver helminthes (Vanstreels *et al*. 2014*a*, 2015*a*) have also been documented concurrently with avian malaria.

Serological studies

Graczyk *et al*. (1993, 1994*b*) developed an indirect enzyme-linked immunosorbent assay (ELISA) that was extensively used to test penguins for antibodies against *Plasmodium* spp. (Graczyk *et al*. 1994*a*, *b*, *c*, *d*, 1995*a*, *b*, *c*; Botes, 2004; Thiart, 2005; McDonald, 2012; Palmer *et al*. 2013). Seroprevalence for *Plasmodium* sp. was 29–52% in wild African penguin in South Africa (Graczyk *et al*. 1995*a*, *b*), 33% in Gentoo and 58% in king penguins at Kerguelen and Crozet Islands (Graczyk *et al*. 1995*c*), 23–100% in yellow-eyed penguins in New Zealand (Graczyk *et al*. 1995*b*, *c*; McDonald, 2012) and 91–100% in Galapagos penguins at the Galapagos Archipelago (Palmer *et al*. 2013). No Adélie penguins were seropositive at Ross Island, Antarctica (Graczyk *et al*. 1995*c*).

Penguins in captivity or undergoing rehabilitation have also been tested for antibodies against *Plasmodium* sp. using this assay. At a rehabilitation centre in Cape Town, South Africa, oiled African penguins had higher seroprevalence to *Plasmodium* sp. upon admission (62%) than those that had been in rehabilitation for at least two weeks (38%) or those permanently captive (20%) (Graczyk *et al*. 1995*a*). Seroprevalence to *Plasmodium* sp. was 92% in little penguins captive at Napier, New Zealand, and 43% in Magellanic penguins captive at San Diego, USA. Furthermore, a few studies have applied this ELISA to study the epidemiology of avian malaria in African penguins in captivity (Graczyk *et al*. 1994*a*, *b*, *c*) and undergoing rehabilitation (Botes, 2004; Thiart, 2005).

However, several authors have noted that there is a considerable discrepancy between the high seroprevalence to *Plasmodium* sp. detected by this assay and the rarity of individuals with detectable *Plasmodium* sp. parasitaemia in blood smears and PCR tests in the same populations (Sturrock and Tompkins, 2007; Hill, 2008; McDonald, 2012; Palmer *et al*. 2013). Some have interpreted this discrepancy as an indication of inaccuracy of the serological test (Sturrock and Tompkins, 2007; McDonald, 2012), whereas others considered it an indication of poor sensitivity of PCR tests (Palmer *et al*. 2013) or to be due to parasite latency in tissues (Hill, 2008; Palmer *et al*. 2013). It remains to be tested whether or not this assay cross-reacts with *Leucocytozoon* spp., which is plausible considering their shared phylogenetic history and genetic similarities (Cosgrove *et al*. 2006; Martinsen *et al*. 2008). Cross-reactivity with viruses (Greenberg *et al*. 1986) and helminths (Naus *et al*. 2003) have also been shown to occur in serological tests targeting *Plasmodium* sp. in humans. The results of these serological tests should therefore be interpreted cautiously until detailed studies explain the discrepancies in the results of serological and direct diagnosis tests and to determine if cross-reactivity may have influenced the serological results.

Implications for public health and conservation

There is no evidence to indicate that avian-infecting *Plasmodium* spp. can infect humans (Valkiūnas, 2005). *Plasmodium* spp. are recognized as conservation-threatening pathogens due to their well-documented impacts to the Hawaiian avifauna (Van Riper III *et al*. 1986; Atkinson and Lapointe, 2009). The high susceptibility of Hawaiian native birds and penguins is thought to result from a lack of physiological/immune adaptations to deal with the infection, as they did not co-evolve with these parasites (Valkiūnas, 2005). The high morbidity and mortality observed in penguins when they are exposed to avian plasmodia in captivity has led to concern that the introduction of mosquitoes to penguin breeding habitats where they had historically been absent could ensue in substantial morbidity and mortality (Jones and Shellam, 1999*b*; Miller *et al*. 2001; Meile *et al*. 2013). This is acutely concerning as climate change increases the pressure imposed by *Plasmodium* sp. on birds (Garamszegi, 2011).

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In particular, *Plasmodium* sp. may constitute a significant conservation threat to the African, Galapagos and yellow-eyed penguins, three endangered species with relatively narrow geographic distribution (IUCN, 2015) in which infection has already been documented in the wild (Fantham and Porter, 1944; Levin *et al*. 2009). Fortunately the *Plasmodium* sp. lineages detected at the Galapagos Archipelago so far have failed to become established and produce significant disease in Galapagos penguins (Levin *et al*. 2009, 2013); however, this could change if more pathogenic lineages are introduced to the archipelago in the future. *Plasmodium* sp. appears to be enzootic in African and yellow-eyed penguins; however, these species' populations are already declining due to a variety of environmental impacts and pathogens (Crawford *et al*. 2011; King *et al*. 2012), and avian malaria could synergize with these existing threats. Furthermore, penguin populations at other areas with relatively warm climate such as Peru, Chile and Tristan da Cunha and Gough Islands could also become at risk if mosquitoes become successfully established near penguin breeding habitat, particularly near freshwater deposits associated with human communities. Other populations of penguins that have relatively narrow geographic distributions, such as Fiordland and Snares penguins could also be at risk, since mosquitoes are already present in their breeding habitat (Fantham and Porter, 1944; Laird, 1950).

LEUCOCYTOZOON (LEUCOCYTOZOONOSIS)

Leucocytozoon spp. (Haemosporida: Leucocytozooidae) parasitize exclusively birds. There are approximately 40 recognized species, one in the subgenus *Akiba* and the remaining in the subgenus *Leucocytozoon*. In the avian blood, young gametocytes and gametocytes of these parasites can be found in the cytoplasm of erythrocytes and mononuclear leucocytes (Fig. 1); there are neither erythrocytic meronts nor haemozoin granules. In the avian tissues, these parasites will invade endothelial cells, macrophages and hepatocytes; megalomeronts may be developed, which are very large and thick-walled (Huff, 1942; Valkiūnas, 2005).

Species recorded in penguins

Leucocytozoon (*Leucocytozoon*) *tawaki* was described from Fiordland penguins (Fallis *et al*. 1976; Allison *et al*. 1978). When Earlé *et al*. (1992) and Peirce *et al*. (2005) observed similar parasites in other species of penguins in Europe and South Africa they did not hesitate to attribute these records to *L. tawaki*. On the other hand, other authors have documented leucocytozooids in yellow-eyed penguins at locations in New Zealand but preferred not to comment on the species involved (Hill, 2008; Hill *et al*. 2010; Argilla *et al*. 2013). Phylogenetic analyses indicated these lineages from yellow-eyed penguins belonged to the subgenus *Leucocytozoon*, and that there might be at least two distinct phylogenetic groups: cluster A is limited to Enderby Island and might be more pathogenic than cluster B, which was detected at Enderby, Campbell, South and Stewart Islands (Argilla *et al*. 2013). It is unclear whether these phylogenetic clusters are variations within the same morphospecies or correspond to different species, and their relationship to the *L. tawaki* originally described in Fiordland penguins is also not clear.

Distribution among penguin hosts

Leucocytozoon spp. have been detected in wild Fiordland (Fallis *et al*. 1976; Allison *et al*. 1978) and yellow-eyed penguins (Hill *et al*. 2010; Argilla *et al*. 2013), as well as in African (Earlé *et al*. 1992) and Fiordland penguins undergoing rehabilitation (Hill, 2008). Additionally, the infection was documented in Macaroni penguins in captivity (Peirce *et al*. 2005). Allison *et al*. (1978) demonstrated that little penguins can develop the infection when forcibly exposed to black flies near *L. tawaki*-infected Fiordland penguins. There is also evidence to suggest that *Leucocytozoon* sp. could infect wild and captive little penguins (see Appendix 2). It is worth noting that Snares, erect-crested and little penguins live in close proximity to populations of Fiordland and yellow-eyed penguins that were found to be infected with *Leucocytozoon* sp., and it is reasonable to presume they are exposed to these parasites in the wild.

Invertebrate hosts

L. (*Leucocytozoon*) spp. are transmitted by black flies (Simuliidae), particularly *Simulium* spp. and *Prosimulium* spp., but also *Cnephia* spp. and *Austrosimulium* spp. (Valkiūnas, 2005; Forrester and Greiner, 2008). *Cnephia* spp., *Simulium* spp. and *Prosimulium* spp. are not present in New Zealand (Dumbleton, 1963), whereas *Austrosimulium australense, Austrosimulium dumbletoni* and *Austrosimulium ungulatum* are abundant and were shown to be competent in the transmission of *L. tawaki* at South Island (Fallis *et al*. 1976; Allison *et al*. 1978; Desser and Allison, 1979). *Austrosimulium ungulatum* is also very abundant in Stewart and South Islands, New Zealand, and could be involved in the transmission of *Leucocytozoon* sp. to yellow-eyed penguins (Hill *et al*. 2010; Argilla *et al*. 2013). On the other hand, *Austrosimulium campbellense* and *Austrosimulium vexans* are thought to be respectively responsible for the transmission at Campbell and Auckland Islands, New Zealand (Argilla *et al*. 2013). *Austrosimilium* spp. do not occur in South

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Africa (Dumbleton, 1963) and thus other simulid flies must be involved in the transmission of this parasite to African penguins (Earlé *et al*. 1992); *Cnephia* spp. and *Simulium* spp. are present in the region (Dumbleton, 1963).

Geographic distribution

Leucocytozoon tawaki is known from South Island, New Zealand (Kaikoura, Jackson Head) (Fallis *et al*. 1976; Allison *et al*. 1978). *Leucocytozoon* sp. has been documented at South Island, New Zealand (Otago Peninsula and Catlins) (Argilla *et al*. 2013) and at Campbell, Enderby and Stewart Islands (Hill *et al*. 2010; Argilla *et al*. 2013). Additionally, *Leucocytozoon* sp. has been documented in penguins undergoing rehabilitation in South Africa (Cape Town) (Earlé *et al*. 1992) and North Island, New Zealand (Auckland) (Hill, 2008), and captive in England (Peirce *et al*. 2005). Because the blood smears examined by Earlé *et al*. (1992) were prepared between 5 and 24 days after admission to the rehabilitation centre in South Africa, it is possible that infection occurred in the wild. The geographic distribution of black flies overlaps with that of penguin breeding colonies in several regions where *Leucocytozoon* sp. has not yet been reported in penguins, such as Peru, Chile, Argentina, Namibia, Australia and New Zealand (Fig. 2B).

Epidemiology and pathology

L. tawaki prevalence is very high in Fiordland penguins at South Island, New Zealand (Jackson Head) (blood smears: 77–94%) (Fallis *et al*. 1976; Allison *et al*. 1978). *Leucocytozoon* sp. prevalence is more variable in yellow-eyed penguins in New Zealand, being lower at South Island (Otago Peninsula and Catlins) (PCR: 11%) and Campbell Island (PCR: 21%), and higher at Enderby (PCR: 66%) and Stewart Islands (PCR: 83%) (Hill *et al*. 2010; Argilla *et al*. 2013). Both Fiordland and yellow-eyed penguins are infected only when they are 3-weeks-old or older, with the infection being acute and disseminated in older chicks then progressing to a subclinical chronic infection in adulthood (Allison *et al*. 1978; Hill *et al*. 2010; Argilla *et al*. 2013). Although prevalence is similar in older chicks and adults (and possibly highest in moulting adults), parasitaemia tends to be lower in adults (Fallis *et al*. 1976; Allison *et al*. 1978). In fact, parasitaemia in adults may be so low as to be undiagnosed or substantially underestimated by blood smears in comparison with molecular methods (Hill *et al*. 2010; Argilla *et al*. 2013). *Leucocytozoon* sp. occurs at low prevalence amongst African penguins undergoing rehabilitation (blood smears: 0·75%) (Earlé *et al*. 1992). Because it is generally accepted that leucocytozooids are not transmitted among birds of different taxonomic orders (Valkiūnas, 2005), it is unlikely that birds other than penguins can serve as reservoirs of infection.

Leucocytozoon sp. can be occasionally pathogenic for penguin chicks. One yellow-eyed penguin chick found dead at Enderby Island ($n = 19$) and two at Stewart Island, New Zealand ($n = 14$), were considered to have died from leucocytozoonosis. Necropsy findings included disseminated petechial and ecchymotic haemorrhage, hepatomegaly, splenomegaly and hydropericardium; megalomeronts were abundant in the liver, spleen, kidneys, lungs and other tissues (Hill *et al*. 2010; Argilla *et al*. 2013). The tissues of an additional seven yellow-eyed penguins were PCR-positive for *Leucocytozoon* sp. at Stewart Island; however, it was not determined whether leucocytozoonosis was the cause of death or not (Hill *et al*. 2010). Furthermore, a juvenile Fiordland penguin found at North Island, New Zealand (Muriwai beach), died during rehabilitation after having been positive to *Leucocytozoon* sp. on blood smears, but it was not possible to determine if leucocytozoonosis was the cause of death (Hill, 2008). The health effects of the infection in African and Macaroni penguins are not known (Earlé *et al*. 1992; Peirce *et al*. 2005).

Implications for public health and conservation

There is no evidence to indicate that *Leucocytozoon* spp. could infect humans (Valkiūnas, 2005). Although *Leucocytozoon* sp. appears to have limited impacts to the health of adult penguins, this can be a considerably pathogenic parasite to penguin chicks and juveniles (Fallis *et al*. 1976; Allison *et al*. 1978; Hill, 2008; Hill *et al*. 2010; Argilla *et al*. 2013). This is particularly troublesome for yellow-eyed penguins, an endangered species that has faced substantial population decrease in the past decades (IUCN, 2015). Yellow-eyed penguin chicks already face a variety of stressors and diseases (Alley *et al*. 2004; Hocken, 2005; Browne *et al*. 2011; Buckle *et al*. 2014), and *Leucocytozoon* sp. might be an additional factor contributing to decrease the species' chick survival (King *et al*. 2012). In the case of African penguins, which are also endangered (IUCN, 2015), additional studies are urgent to bring better understanding on the epidemiology and pathology of this parasite. There is also evidence to suspect that wild little penguin chicks might also die as a result from leucocytozoonosis (see Appendix 2), and therefore an investigation on the occurrence of *Leucocytozoon* sp. in this species, particularly in Western Australia, would be valuable.

HAEMOPROTEUS (HAEMOPROTEOSIS)

Haemoproteus spp. (Haemosporida: Haemoproteidae) parasitize exclusively birds. There are approximately

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150 recognized species, 10 in the subgenus *Haemoproteus* and the remaining in the subgenus *Parahaemoproteus* (Valkiūnas, 2005; Levin *et al*. 2011, 2012; Valkiūnas *et al*. 2010, 2013). These parasites can be found in the cytoplasm of avian erythrocytes, as trophozoites and gametocytes; haemozoin granules (dark-brown staining pigment) are present, but not erythrocytic meronts. In the avian tissues, these parasites invade endothelial cells and macrophages to form exoerythrocytic meronts; megalomeronts may be developed, which are very large and thick-walled (Huff, 1942; Paperna and Gill, 2003; Valkiūnas, 2005).

Species recorded in penguins

Haemoproteus sp. detected in penguins have not been morphologically characterized, hence their identity has not been conclusively established. Phylogenetic analyses indicate however that the lineages identified in Galapagos and Humboldt penguins belong to the subgenus *Parahaemoproteus* and are closely related to lineages found in passerines (Levin *et al*. 2009; Sallaberry-Pincheira *et al*. 2015) (see Appendix 2).

Distribution among penguin hosts

DNA from *Haemoproteus* sp. has been detected in the blood of wild Galapagos (Levin *et al*. 2009) and Humboldt penguins (Sallaberry-Pincheira *et al*. 2015). There is a report of *Haemoproteus* sp. infection in wild little penguins (Cannell *et al*. 2013), however that record is problematic (see Appendix 2).

Invertebrate hosts

Haemoproteus (*Parahaemoproteus*) spp. are transmitted by biting midges *Culicoides* spp. (Ceratopogonidae) (Valkiūnas, 2005; Atkinson, 2008*a*). Eleven species of *Culicoides* spp. have been associated with the transmission of these parasites (Valkiūnas, 2005).

Culicoides pusillus is the only species of its genus that occurs at the Galapagos Archipelago (Sinclair, 2014), whereas a broad variety of species occurs in Peru (Wirth and Felippe-Bauer, 1989; Borkent, 2013). It is worth noting that the *Haemoproteus*-positive penguin identified at the Galapagos Archipelago was sampled at western Isabela Island (I. I. Levin, personal communication), whereas *C. pusillus* has been recorded only at Santa Cruz Island (Sinclair, 2014). Even though DNA from *Haemoproteus* sp. was identified in the blood meals of mosquitoes *Aedes taeniorhynchus* at the Galapagos Archipelago, this was most likely an incidental finding and probably did not correspond to actual infections (Bataille *et al*. 2012). It is not clear which species of biting midges were involved in the transmission of *Haemoproteus* sp. to Humboldt penguins at Punta San Juan (Sallaberry-Pincheira *et al*. 2015), however *Culicoides* spp. are not uncommon in Peru (Tabachnick, 2004; Felippe-Bauer *et al*. 2008) and the coastal range of Peru provides suitable climatic conditions to these insects (Guichard *et al*. 2014).

Geographic distribution

Haemoproteus (*Parahaemoproteus*) spp. were detected in penguins in the Galapagos Archipelago (Isabela Island) (Levin *et al*. 2009) and Peru (Punta San Juan) (Sallaberry-Pincheira *et al*. 2015). The distribution of biting midges overlaps that of penguins breeding in Namibia, South Africa and Australia, possibly providing opportunities for *H*. (*Parahaemoproteus*) sp. inoculation. Similarly, captive penguins in areas of North and South America, southern Europe, Africa, Asia and Oceania could also be exposed (Fig. 2A).

Epidemiology and pathology

The *Haemoproteus*-positive penguins studied by Levin *et al*. (2009) and Sallaberry-Pincheira *et al*. (2015) had no external signs of illness. Considering that no parasites were seen in blood smears and that molecular tests may produce false-positive results if DNA of recently inoculated sporozoites is present in the blood even when infection was not developed (Levin *et al*. 2013; Valkiūnas *et al*. 2014), it is possible that these *Haemoproteus* spp. were not truly capable of infecting penguins and instead represent incidental findings (i.e. abortive infections). The report of lethal haemoproteosis in wild little penguins (Cannell *et al*. 2013) is problematic, and is addressed in detail in Appendix 2.

Implications for public health and conservation

There is no evidence to indicate that *Haemoproteus* spp. could infect humans (Valkiūnas, 2005). *Haemoproteus* spp. are generally considered the least pathogenic of avian haemosporidians; however, there are exceptional cases of lethal haemoproteosis (Atkinson and Van Riper III, 1991; Donovan *et al*. 2008). In the case of penguins, however, it is still unclear whether *Haemoproteus* sp. sporozoites are able to infect and develop in penguin cells (see Levin *et al*. 2013; Valkiūnas *et al*. 2014) and, until this has been conclusively demonstrated, it seems unlikely that these parasites pose a significant threat for their conservation.

BABESIA (BABESIOSIS)

Babesia spp. (Piroplasmida: Babesiidae) parasitize birds and mammals, with 13 avian-infecting species (Peirce, 2000, 2005; Schnittger *et al*. 2012). In the avian blood, these parasites can be found in the

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cytoplasm of erythrocytes either as round forms (trophozoites and pre-gametocytes) or tetrameric elongated forms (meronts) (Fig. 1); there are no haemozoin granules. There is no invasion of tissue cells; however, infected erythrocytes can occasionally be seen in the margin of blood vessels (Peirce, 2000; Chauvin *et al*. 2009; Schnittger *et al*. 2012).

Species recorded in penguins

Babesia peircei was described from African penguins (Earlé *et al*. 1993). It is not clear whether the *Babesia* sp. reported in little penguins in Australia corresponds to *B. peircei* or to a different species (Cunningham *et al*. 1993; Vanstreels *et al*. 2015*b*). The remarkable morphological and/or genetic similarities between *B. peircei, Babesia* sp. of little penguins, *Babesia poelea* (parasite of boobies *Sula* spp.) and *Babesia uriae* (parasite of common murres *Uria aalge*) has led to speculation that these taxa could in fact correspond to a single seabird-infecting species (Peirce, 2000; Vanstreels *et al*. 2015*b*).

Distribution among penguin hosts

Babesia peircei is known from African penguins in the wild and in rehabilitation (Coles, 1941; Earlé *et al*. 1993; Brossy *et al*. 1999; Yabsley *et al*. 2012) and *Babesia* sp. was documented in wild little penguins (Cunningham *et al*. 1993; Vanstreels *et al*. 2015*b*).

Invertebrate hosts

It is generally accepted that hard ticks (Ixodidae) are the most relevant invertebrate hosts of avian *Babesia* spp., but soft ticks (Argasidae) are thought to play a significant role for colonial ground-nesting birds (Peirce, 2000). Hard ticks, particularly *Ixodes uriae*, are the most probable vectors of *B. peircei* to African penguins (Earlé *et al*. 1993; Peirce, 2000), but the soft tick *Carios capensis* has also been speculated to play a role in the transmission (Brossy *et al*. 1999). The hard tick *Ixodes kohlsi* may play a key role in the transmission to little penguins at New South Wales, Australia (Cunningham *et al*. 1993), and both soft and hard ticks were observed on *Babesia*-infected little penguins in Tasmania, Australia (Vanstreels *et al*. 2015*b*).

Geographic distribution

Babesia peircei was documented infecting penguins in Namibia (Ichaboe Island), South Africa (Western Cape and Eastern Cape) and *Babesia* sp. infects penguins in Australia (New South Wales, Victoria and Tasmania) (Cunningham *et al*. 1993; Earlé *et al*. 1993; Vanstreels *et al*. 2015*b*; Parsons *et al*. in preparation). Seabird ticks, both soft and hard, are broadly distributed around the world, overlapping with the distribution of penguins in numerous sub-Antarctic islands, Peru, Chile, Argentina, New Zealand, Antarctic Peninsula and at some locations in the Antarctic mainland (Fig. 3A); the occurrence of *Babesia* sp. in penguins at these locations is therefore plausible.

Epidemiology and pathology

Babesia sp. infects 1·6 to 4·8% (blood smears; Cunningham *et al*. 1993) and 2·7% (blood smears; Vanstreels *et al*. 2015*b*) of wild little penguins in south-eastern Australia. *B. peircei* is endemic at low prevalence in wild African penguins in Namibia and South Africa (blood smears: 1–4%) (Brossy, 1992; Parsons *et al*. in preparation), whereas a higher prevalence (blood smears: 11–15%) was observed in African penguins undergoing rehabilitation (Brossy, 1992). Because it is not known whether penguin-infecting *Babesia* sp. and *B. poelea* are the same species or not, it is not clear if other seabirds can serve as reservoirs of infections for penguins and vice-versa.

The clinical and pathological effects of *Babesia* spp. infections are not clear. Infected little penguins can present mild regenerative anemia, but did not show any evident signs of illness (Cunningham *et al*. 1993; Sergent *et al*. 2004). Brossy *et al*. (1999) considered that *B. peircei* 'does not cause overt clinical symptoms except under stress or in association with other debilitating diseases'. On the other hand, Parsons *et al*. (in preparation) found that *B. peircei*-infected wild African penguins had signs of regenerative response of the erythrocytic lineage and haematological indications of active inflammatory response and hepatic function impairment.

Furthermore, approximately 50% of *Borrelia*-infected African penguins undergoing rehabilitation in South Africa are also co-infected with *B. peircei* (Yabsley *et al*. 2012), which could reflect: (a) transmission by a shared invertebrate host; (b) *Babesia* sp. infections predispose penguins or ticks to *Borrelia* sp. infections or vice versa; or (c) the poor health and immune status of penguins in rehabilitation predispose them to both of these pathogens.

Serological studies

The indirect ELISA designed for *Plasmodium* by Graczyk *et al*. (1993, 1994*b*) was adapted to test penguins for antibodies against *Babesia* sp., and showed that 18–22% of wild African penguins in South Africa were seropositive (47% in oiled birds) (Graczyk *et al*. 1996). However, the limitations and concerns raised regarding the use of this assay to test for *Plasmodium* sp. may also apply to its application for *Babesia* sp.

Implications for public health and conservation

There is no evidence to indicate that avian-infecting *Babesia* spp. can infect humans (Peirce, 2000). There

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is evidence that *Babesia* sp. infections significantly affect the health of penguins (Cunningham *et al*. 1993; Parsons *et al*. in preparation), which is concerning because this pathogen is not uncommon in African penguins, an endangered species whose population has been steadily decreasing (Crawford *et al*. 2011; IUCN, 2015). Epidemiological and pathological studies of *Babesia* sp., particularly in African penguins, will therefore be important to clarify its potential conservation impacts.

TRYPANOSOMA (TRYPANOSOMIASIS)

Trypanosoma spp. (Kinetoplastida: Trypanosomatidae) parasitize all tetrapod classes. Avian-infecting trypanosomatids are considered part of the '*Trypanosoma avium* complex', for which more than 100 species have been described with arguable validity (Molyneux, 1977; Haag *et al*. 1998; Sehgal *et al*. 2001; Votypka *et al*. 2002; Hamilton *et al*. 2004; Zídková *et al*. 2012). These parasites remain free in the avian blood in the form of trypomastigotes, which have a characteristic elongated shape with flagella, a kinetoplasm and an undulating membrane. Trypomastigotes can concentrate in blood vessels of lymphoid tissues and in the bone marrow, however in birds the asexual multiplication probably does not occur in those tissues nor in the myocardium (Diamond and Herman, 1954; Baker, 1956; Baker and Bird, 1968).

Species recorded in penguins

Only one species, *Trypanosoma eudyptulae*, has been reported in penguins (Jones and Woehler, 1989). This parasite has not been reported in other avian hosts.

Distribution among penguin hosts

Trypanosoma sp. has only been reported in wild little penguins (Jones and Woehler, 1989).

Invertebrate hosts

There is no information regarding which invertebrates are involved in the transmission of *T. eudyptulae*. Black flies (*Metacnephia lyra, Simulium* spp. and *Prosimulium decemarticulatum*), mosquitoes (*Aedes aegypti*), louse flies (*Ornithomya avicularia*) and mites (*Dermanyssus gallinae*) have been found to be competent hosts of other avian trypanosomes (Molyneux, 1977; Reeves *et al*. 2007). Of those, *A. aegypti* is absent in Tasmania (Kearney *et al*. 2009), whereas *Dermanyssus* spp. (including *D. gallinae*) and *Ornithomya* spp. (including *O. avicularia*) are present (Domrow, 1979; ALA, 2014). Furthermore, other species of black flies (*Austrosimulium* spp. and *Cnephia* spp.) also occur in Tasmania (Dumbleton, 1963) and could be plausible hosts.

Geographic distribution

Trypanosoma eudyptulae was originally described at a little penguin colony on Marion Bay in Tasmania, Australia (Fig. 3C) (Jones and Woehler, 1989). However, that colony was destroyed during a fire in 1994 and has not been recolonized since (Stevenson and Woehler, 2007; E. J. Woehler, personal communication). Recent efforts to detect this parasite in breeding colonies near Marion Bay have failed (Vanstreels *et al*. 2015*b*). Because this parasite's invertebrate hosts remain unknown, it is difficult to speculate on its potential distribution.

Epidemiology and pathology

Despite having been observed with a relatively high prevalence (blood smears: 15·8%), *T. eudyptulae* was present only with low parasitaemia (often only one parasite per blood smear) (Jones and Woehler, 1989), which suggests chronic infection. Infected penguins presented no external signs of illness.

It is worth noting that Jones and Woehler (1989) obtained blood samples by superficially scraping the skin near the brachial vein on the flipper with razorblades then collecting a drop of blood with a capillary tube or glass slide (E. J. Woehler, personal communication). This method would result in the collection of blood from capillary vessels, as opposed to blood from larger vessels as is obtained through venipuncture. This may be relevant because it has been shown that mammal-infecting trypanosomes tend to concentrate in capillaries rather than larger blood vessels (Hornby and Bailey, 1931; Banks, 1978). It is unclear whether or not avian trypanosomes behave similarly (Holmstad *et al*. 2003), but there is evidence to suggest that these parasites concentrate in the bone marrow of birds rather than in their circulating blood (Diamond and Herman, 1954). For these reasons, it is possible that studies using blood smears from samples collected by venipuncture may have systematically underestimated the occurrence of trypanosomatids in penguins. Molecular methods could also enhance the detection of these parasites in the future (see Sehgal *et al*. 2001).

Implications for public health and conservation

There is no evidence to indicate that avian-infecting *Trypanosoma* spp. can infect humans (Molyneux, 1977). Avian trypanosome infections are not usually regarded as pathogenic, but in some circumstances these parasites may have mild health impacts (Molyneux *et al*. 1983; Merino *et al*. 1996; Sehgal *et al*. 2001). Because of how little is known about *T. eudyptulae*, it is difficult to evaluate the impacts it could have on the conservation of little penguins, if any.

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BORRELIA (LYME DISEASE, RELAPSING FEVER, ANIMAL SPIROCHETOSIS)

The bacteria of the genus *Borrelia* (Spirochaetales: Spirochaetaceae) are classified in three groups: Lyme disease *Borrelia* (LDB), relapsing fever *Borrelia* (RFB) and animal spirochetosis *Borrelia* (ASB) (Olsén, 2007). LDB are often referred to as '*Borrelia burgdorferi* sensu lato', a group that comprises 10 species and numerous unidentified strains, of which *Borrelia garinii* is particularly relevant for seabirds (Olsén *et al*. 1995*a*, *b*; Olsén, 2007). There are 18 species in the RFB group, of which *Borrelia hermsii, Borrelia parkeri, Borrelia recurrentis* and *Borrelia turicatae* are considered most relevant (McDowell *et al*. 2003; Cutler, 2006). Three species are associated with ASB, of which only *Borrelia anserina* infects birds (Barbour and Hayes, 1986; McDowell *et al*. 2003; Olsén, 2007). *Borrelia* spp. are present in the blood in the form of small extracellular helical filiform structures, typically 9–30 µm long and 0·2–0·5 µm wide (Fig. 1); there is no invasion of cells of the blood or other tissues (Barbour and Hayes, 1986; Olsén, 2007).

Species recorded in penguins

Borrelia sp. strains detected in king penguins had a restriction fragment length polymorphism profile identical to that of *B. garinii*, and are therefore thought to belong to the LDB group (Schramm *et al*. 2014). On the other hand, the strains identified in African penguins are phylogenetically most related to *B. parkeri* and *B. turicatae*, both of which are classified as RFB (Yabsley *et al*. 2012). Coles (1941) observed spirochetes in the blood smear of a wild African penguin chick at Dassen Island, and discarded them from being *B. anserina*; considering that RFB were later found to infect African penguins at that region (Yabsley *et al*. 2012), it is reasonable to assume these corresponded to similar strains.

Distribution among penguin hosts

RFB has been documented in African penguins in the wild (Coles, 1941; Parsons *et al*. in preparation) and undergoing rehabilitation (Yabsley *et al*. 2012; Parsons *et al*. in preparation). LDB was recorded in wild king penguins (Schramm *et al*. 2014); it is reasonable to presume that Gentoo, Macaroni and Southern rockhopper penguins breeding near king penguins at Crozet Archipelago (IUCN, 2015) are also exposed to LDB.

Vectors

With the exception of *B. recurrentis*, which is transmitted to humans by lice, all *Borrelia* spp. are transmitted by ticks. LDB are transmitted by hard ticks *Ixodes* spp., RFB are transmitted by soft ticks *Carios* (=*Ornithodoros*) spp., and *B. anserina* is transmitted by soft ticks *Argas* spp. (Barbour and Hayes, 1986; Olsén, 2007; Elbir *et al*. 2013). Additionally, *B. anserina* can be transmitted through the ingestion or inoculation of faeces, fluids, and tissues (Olsén, 2007).

Soft ticks, particularly *C. capensis*, are commonly found on wild African penguins (Clarke and Kerry, 1993), and are likely responsible for the transmission of RFB to those birds (Yabsley *et al*. 2012). On the other hand, the hard ticks *Ixodes kerguelensis* and *I. uriae* are abundant in sub-Antarctic islands and are through to play a key role in the transmission of LDB to king penguins (Olsén *et al*. 1995*b*; Gauthier-Clerc *et al*. 1999; Schramm *et al*. 2014).

Geographic distribution

RFB infects penguins in South Africa (Cape Town, Dassen Island) (Coles, 1941; Yabsley *et al*. 2012; Parsons *et al*. in preparation), and LDB is present in king penguins at Crozet Archipelago (Possession Island) (Olsén *et al*. 1995*b*; Schramm *et al*. 2014). The distribution of *Ixodes* spp. overlaps with penguin breeding habitat in Southern South America, Antarctic Peninsula, South Africa, Australia, New Zealand and at a number of sub-Antarctic islands (Fig. 3B), and LDB strains are broadly distributed in seabirds at a number of these locations (Olsén *et al*. 1995*b*; Olsén, 2007). Similarly, the distribution of *Carios* spp. overlaps with breeding colonies of penguins in the Galapagos Archipelago, Peru, Tristan da Cunha Archipelago, South Africa, Amsterdam and Saint-Paul Islands, Southeastern Australia, New Zealand and Chatham Islands (Fig. 3B).

Epidemiology and pathology

RFB occurs at low prevalence (blood smears: 0·9–1·1%) in African penguins undergoing rehabilitation (Yabsley *et al*. 2012; Parsons *et al*. in preparation); infection is more frequent in chicks (3·6%) than in juveniles (0·83%) and adults (0·14%). As previously discussed, approximately 50% of RFB-infected African penguins undergoing rehabilitation in South Africa are co-infected by *B. peircei* (Yabsley *et al*. 2012).

In only one RFB-infected African penguin studied by Yabsley *et al*. (2012) death was considered to result from *Borrelia* infection; that penguin presented signs of neurological disease (unsteady gait, circling, torticollis) and died after four days. On post-mortem examination, splenomegaly and hepatomegaly were noted and histological findings were consistent with relapsing fever: splenic reticuloendothelial hyperplasia

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with haemosiderosis, lung congestion, and lymphocytic meningoencephalitis. On the other hand, LDB are generally considered non-pathogenic to seabirds (Olsén *et al*. 1995*a*, *b*; Olsén, 2007), and no external signs of illness were observed in LDB-infected king penguins (Schramm *et al*. 2014).

Serological studies

Gauthier-Clerc *et al*. (1999) tested king penguins with a passive haemagglutination commercial kit developed to detect human antibodies against LDB. No additional studies have employed this serological assay to test other penguin species, and it is unknown whether the assay cross-reacts with other *Borrelia* spp. or other spirochetes (see Magnarelli *et al*. 1987).

Implications for public health and conservation

Relapsing fever is a relevant disease for humans worldwide, including in South Africa, but it is presently unknown whether the RFB strains that infect African penguins could be the same involved in any of the few human cases recorded in South Africa (Yabsley *et al*. 2012; Elbir *et al*. 2013). Similarly, Lyme disease is relevant for humans, and seabirds are thought to play a role in the maintenance and transmission of LDB to humans and other mammals, especially at high latitudes (Olsén *et al*. 1995*b*). It seems probable that domestic and synanthropic animals play a much more significant role than penguins in transmitting this infection to humans (Gauthier-Clerc *et al*. 1999; Yabsley *et al*. 2012), however it is plausible that humans entering penguin colonies for research, tourism or guano/egg exploitation, or handling these birds in rehabilitation centers could be at risk of exposure to RFB or LDB.

LDB are generally non-pathogenic to seabirds (Olsén, 2007) and therefore are unlikely to be a conservation threat to penguins. On the other hand, RFB have been reported to cause mortality of an African penguin (Yabsley *et al*. 2012), an endangered species (IUCN, 2015); studies on the epidemiology and pathology of this pathogen in African penguins could therefore help clarifying its conservation significance.

NEMATODE MICROFILARIAE (FILARIASIS)

Microfilariae are early life stages of onchocercid nematodes (Filarioidea: Onchocercidae) that may occasionally be present in the blood. There are 17 genera of Onchocercidae known to infect birds, totaling over 160 species, of which only *Dirofilaria immitis* and *Pelecitus* spp. can also infect non-avian hosts (Sano *et al*. 2005; Bartlett, 2008). In the avian blood, microfilariae are extracellular densely stained linear structures, typically 100–250 µm long and 5–10 µm wide (Fig. 1). Adult worms can be present at a broad variety of organs and tissues such as the skin, subcutaneous connective tissue, air sacs, heart and coelomic cavity (Friend and Franson, 1999; Anderson, 2000; Bartlett, 2008).

Species recorded in penguins

Phylogenetic analysis of microfilariae from the blood of Galapagos penguins revealed this is the same species as the one present in the blood of flightless cormorants (*Phalacrocorax harrisi*) at the Galapagos Archipelago. This parasite was closely related to mammalian-infecting Onchocercidae, but could not be conclusively identified (Merkel *et al*. 2007).

There are other instances in which Onchocercidae have been reported infecting penguins: adult *Paronchocerca straeleni* in the heart of a captive Galapagos penguin in the USA (Chabaud and Ball, 1964; Bartlett and Anderson, 1986), adult *D. immitis* in the heart of a captive Humboldt penguin in Japan (Sano *et al*. 2005), and multiple unidentified microfilariae in the eyelid skin of a Magellanic penguin undergoing rehabilitation in Brazil (Vanstreels *et al*. 2015*a*). In these cases, even though microfilariae were not observed in blood smears, they could have been present in the blood stream at some stage of the infection.

Distribution among penguin hosts

Microfilariae have only been observed in wild Galapagos penguins (Harmon *et al*. 1985; Merkel *et al*. 2007). However, other life stages of onchocercid worms have been documented in captive Galapagos and Humboldt penguins (Chabaud and Ball, 1964; Sano *et al*. 2005) and in a Magellanic penguin undergoing rehabilitation (Vanstreels *et al*. 2015*a*).

Vectors

The following insects have been incriminated in the transmission of avian-infecting onchocercids: biting midges (*Culicoides* spp.), chewing lice (*Austromenopon* spp., *Pseudomenopon pilosum, Trinoton anserinum*), mosquitoes (*Aedes taeniorhynchus, Armigeres subalbatus, Culex* spp., *Mansonia crassipes*), and black flies (*Simulium* spp.) (Anderson, 2000; Bartlett, 2008; Manrique-Saide *et al*. 2008). Ecological modelling suggests that *Aedes taeniorhynchus* is the most probable vector of microfilariae to Galapagos penguins (Siers *et al*. 2010; Bataille *et al*. 2012); this is corroborated by the detection of DNA from nematodes in blood meals of *Aedes taeniorhynchus* (Bataille *et al*. 2012).

Geographic distribution

Microfilariae were documented in the blood of penguins at the Galapagos Archipelago (Fernandina and

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Isabela Islands) (Harmon *et al.* 1985; Merkel *et al.* 2007). However *Aedes taeniorhynchus*, its most probable vector, is distributed in salt marshes along the tropical and temperate coast of the Americas, including Peru, and could transmit onchocercid worms to wild and captive penguins in the region (Fig. 3C). Adult Onchocercidae have been reported infecting captive penguins in the USA (Chabaud and Ball, 1964) and Japan (Sano *et al.* 2005), and microfilariae were reported in the skin of penguins undergoing rehabilitation in southern Brazil (Vanstreels *et al.* 2015*a*).

Epidemiology and pathology

Local prevalence of microfilariae in Galapagos penguins ranges from 5·3 to 50% among locations (blood smears) (Merkel *et al.* 2007; Siers *et al.* 2010). Infection rate was higher in males than in females and was positively correlated to ambient temperature, precipitation and dry-season vegetation, whilst being negatively correlated to elevation and slope (Siers *et al.* 2010). The parasite often occurred with higher prevalence in sympatric flightless cormorants, suggesting this species might act as a reservoir of infection for penguins (Merkel *et al.* 2007). Infection rate of *Aedes taeniorhynchus* at these sites was relatively low (0·15%) (Manrique-Saide *et al.* 2008; Bataille *et al.* 2012).

Parasitaemia varied greatly among individuals, ranging from 0·04 to 12 parasites per low magnification microscope field (10× objective lens) (Merkel *et al.* 2007). With few exceptions in which they cause vasculitis, microfilariae are seldom pathogenic *per se*, and the most significant health implications tend to derive from the adult parasites (Bartlett, 2008). Because the infection site of the adult onchocercids recorded in Galapagos penguins is unknown, it is not currently possible to evaluate the health implications of these infections.

Implications for public health and conservation

The microfilariae detected in Galapagos penguins remain unidentified, but it seems unlikely that it could infect humans since the only onchocercid worm to infect both birds and humans is *D. immitis* (Bartlett, 2008), which produces pulmonary disease in the latter (Simón *et al.* 2005). However, it seems unlikely that penguins play a significant role as reservoirs of infection to humans, considering there is only one documented case of this parasite in a penguin, which is also the only known case of *D. immitis* in a bird (Sano *et al.* 2005). Considering the high prevalence and parasitaemia with which microfilariae were observed in Galapagos penguins, an endangered species (IUCN, 2015), studies to determine the identity, adult infection site and health effects of these worms are urgent to determine their relevance as a conservation threat.

CONCLUDING REMARKS

Blood parasites are frequently studied through the examination of blood smears, which does not require an *a priori* decision on the parasites to be searched for. As a result, the fact that there is a much greater number of studies reporting some parasites but not others – for example, there are more than 50 studies reporting *Plasmodium* sp. but only one report of *Trypanosoma* sp. – suggests an actual difference in how common or widely distributed these parasites are. However, it is also possible that blood smears or blood collection methods perform differently for the detection of different parasite taxa, especially if some parasites tend to produce more acute infections than others or are distributed unevenly in the host's blood vessels (Holmstad *et al.* 2003; Valkiūnas, 2005; Garamszegi, 2010). Furthermore, sampling effort is not evenly distributed worldwide, and this review identifies a number of geographic areas in which future studies could identify blood parasites in wild and captive penguins (see Figs 2 and 3).

Among the blood parasites of penguins, *Plasmodium* sp. and *Leucocytozoon* sp. stand out as the most relevant for conservation, as both have been documented to cause the death of penguins in captivity and/or in the wild. However, other parasites such as *Babesia* sp., RFB and nematode microfilariae could also produce more subtle yet still significant impacts on the health and fitness of wild penguins. It is concerning that these parasites are known to infect wild populations of the three most endangered penguin species: Galapagos, yellow-eyed and African penguins. Considering that climate change is already changing the distribution and epidemiology of avian blood parasites (Garamszegi, 2011), the perspective of increased morbidity and mortality of these endangered penguins is troubling.

It is therefore clear that studies on the diagnosis, ecology, epidemiology and pathology of blood parasites of penguins will be valuable not only in furthering the advancement of parasitological science, but will also be important components of efforts for the conservation of these birds and their environments, especially in tropical and temperate regions.

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APPENDIX 1

Table A1. Published records of blood parasites in penguins

Host, context, location and period of sample collection	Parasite	References
King penguin (*Aptenodytes patagonicus*)		
C – London, England (1926)	*P. relictum*	Scott (1927), Fantham and Porter (1944)
C – London, England (1947)	*P. relictum*	Rewell (1948)
C – San Diego, California, USA (1965)	*P. relictum*	Griner and Sheridan (1967)
C – Praetoria, South Africa (1992)	*P. relictum*	Penrith *et al*. (1994)
C – Bristol, England (year unknown)	*Plasmodium* sp.	Redrobe (2000)
W – Possession Is., Crozet (year unknown)	*Borrelia* sp. (LDB)	Schramm *et al*. (2014)
Southern rockhopper penguin (*Eudyptes chrysocome*)		
C – Baltimore, Maryland, USA (1972–1976)	*Plasmodium* sp.	Sladen *et al*. (1979)
C – Vienna, Austria (2000–2008)	*Plasmodium* sp.	Dinhopl *et al*. (2011)
Northern rockhopper penguin (*Eudyptes moseleyi*)		
W – Gough Island, South Atlantic (year unknown)	*P. relictum*	Fantham and Porter (1944)
Rockhopper penguin (*E. chrysocome* or *E. moseleyi*)		
C – Berlin, Germany (1957–1958)	*Plasmodium* sp.	Raethel (1960)
C – Honolulu, Hawaii (year unknown)	*P. relictum*	Laird and Van Riper (1981)
Macaroni penguin (*Eudyptes chrysolophus*)		
C – Washington, DC, USA (1969)	*P. elongatum*	Herman *et al*. (1974)
C – Bristol, England (year unknown)	*Plasmodium* sp.	Redrobe (2000)
C – Unknown location, England (1999)	*Plasmodium* sp.	Gough *et al*. (2002)
C – Unknown location, England (2005)	*Plasmodium* sp.	Peirce *et al*. (2005)
C – Unknown location, England (2005)	*L. tawaki*	Peirce *et al*. (2005)
Fiordland penguin (*Eudyptes pachyrhynchus*)		
W – Kaikoura, South Is., New Zealand (1975)	*L. tawaki*	Fallis *et al*. (1976)
W – Jackson Head, South Is., New Zealand (1975–1977)	*L. tawaki*	Fallis *et al*. (1976)
W – Jackson Head, South Is., New Zealand (1976–1977)	*L. tawaki*	Allison *et al*. (1978)
R – Auckland, North Is., New Zealand (2007)	*Leucocytozoon* sp., Undetermined[a]	Harvey and Alley (2008), Hill (2008)
Snares penguin (*Eudyptes robustus*)		
W – Snares Is., New Zealand (1947)	*P. relictum*	Laird (1950)
Little penguin (*Eudyptula minor*)		
C – San Diego, California, USA (1965)	*P. relictum*	Griner and Sheridan (1967)
E – Jackson Head, South Is., New Zealand (1977)	*L. tawaki*	Allison *et al*. (1978)
W – Marion Bay and Little Spectacle Is., Australia (1986)	*T. eudyptulae*	Jones and Woehler (1989)
W – Unknown location, New South Wales, Australia (1990)	*Babesia* sp.	Cunningham *et al*. (1993)
W – Lion and Bowen Is., Australia (1991–1992)	*Babesia* sp.	Cunningham *et al*. (1993), Sergent *et al*. (2004)
C – Auckland, North Is., New Zealand (2005)	*Leucocytozoon* sp.[b]	Varney (2006), Harvey and Alley (2008)
W – Tiritiri Matangi Is., New Zealand (2006–2007)	*P. relictum*	van Rensburg (2010)
W – Tiritiri Matangi Is., New Zealand (2006–2007)	Undetermined[a]	van Rensburg (2010)
W – Multiple locations, Western Australia, Australia (2006–2012)	Undetermined[c]	Cannell *et al*. (2013, 2014)
W – Althorpe, Granite, Kangaroo and Troubridge, Australia (2013)	Undetermined[d]	Colombelli-Negrél and Kleindorfer (2014)
W – Cabbage Tree Is., Australia (2012–2013)	*Babesia* sp.	Vanstreels *et al*. (2015*b*)
W – Phillip Is., Australia (2012–2013)	*Babesia* sp.	Vanstreels *et al*. (2015*b*)
W – Maria and Bruny Is., Australia (2012–2013)	*Babesia* sp.	Vanstreels *et al*. (2015*b*)
Yellow-eyed penguin (*Megadyptes antipodes*)		
W – Fouveaux Strait, New Zealand (1929)	*P. relictum*	Fantham and Porter (1944)
W – Campbell Is., New Zealand (1948)	*P. relictum*	Laird (1950)
W – Otago Peninsula, South Is., New Zealand (1997–1999)	Undetermined[e]	McDonald (2012)
W – Codfish and Stewart Is., New Zealand (2005–2008)	*L* (*Leucocytozoon*) sp.	Alley *et al*. (2004), Harvey and Alley (2008), Hill (2008), King *et al*. (2012)
W – Stewart Is., New Zealand (2006–2007)	*L* (*Leucocytozoon*) sp.	Hill *et al*. (2010)
W – Multiple locations, South Is., New Zealand (2008)	*L* (*Leucocytozoon*) sp.	Argilla *et al*. (2013)
W – Campbell Is., New Zealand (2006–2009)	*L* (*Leucocytozoon*) sp.	Argilla *et al*. (2013)
W – Enderby Is., New Zealand (2006–2009)	*L* (*Leucocytozoon*) sp.	Argilla *et al*. (2013)
Chinstrap penguin (*Pygoscelis antarcticus*)		
C – Antwerp, Belgium (1952)	*P. relictum*	Rodhain and Andrianne (1952)

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Table A1. (*Cont.*)

Host, context, location and period of sample collection	Parasite	References
Gentoo penguin (*Pygoscelis papua*)		
C – San Diego, California, USA (1965)	*P. relictum*	Griner and Sheridan (1967)
C – Bristol, England (year unknown)	*Plasmodium* sp.	Redrobe (2000)
African penguin (*Spheniscus demersus*)		
C – Antwerp, Belgium (1936)	*P. relictum*	Rodhain (1939), Rodhain and Andrianne (1952)
W – Saldanha Bay, South Africa (1927–1929)	*P. relictum*	Fantham and Porter (1944)
W – Dassen Is., South Africa (1938)	*Babesia* sp., *Borrelia* sp.[b]	Coles (1941), Bennett *et al.* (1992)
C – Vienna, Austria (1958–1962)	*P. relictum*	Grünberg and Kutzer (1963)
C – San Diego, California, USA (1965)	*P. relictum*	Griner and Sheridan (1967)
C – Baltimore, Maryland, USA (1967–1996)	*P. relictum, P. elongatum*	Fleischman *et al.* (1968*a*, *b*), Herman *et al.* (1968), Sladen *et al.* (1979), Stoskopf and Beier (1979), Beier and Stoskopf (1980), Beier and Trpis (1981), Cranfield *et al.* (1994), Graczyk *et al.* (1994*a*, *b*, 1995*a*), McConkey *et al.* (1996), Grim *et al.* (2004)
C – Yongin, South Korea (1983)	*P. relictum*	Bak *et al.* (1984)
R – Cape Town, South Africa (1990)	*B. peircei*	Brossy (1992), Earlé *et al.* (1993)
R – Cape Town, South Africa (1991)	*L. tawaki*	Earlé *et al.* (1992)
W – Multiple locations, South Africa (1992–1999)	*Babesia* sp.	Brossy (1993), Brossy *et al.* (1999)
R – Cape Town, South Africa (1992–1999)	*P. relictum*	Brossy *et al.* (1999)
R – Cape Town, South Africa (unknown year)	*Plasmodium* sp.	Grim *et al.* (2003)
C – Unknown location, England (1999)	*Plasmodium* sp.	Gough *et al.* (2002)
R – Dassen and Robben Is., South Africa (2004)	*Plasmodium* sp.	Thiart (2005)
R – Stony Point, South Africa (2004)	*Plasmodium* sp.	Thiart (2005)
R – Cape Town, South Africa (2001–2003)	*Plasmodium* sp.	Botes (2004), Thiart (2005), Parsons and Underhill (2005)
R – Cape Town, South Africa (2006–2007)	*Plasmodium* sp.	Sherley *et al.* (2014)
C – Baltimore, Maryland, USA (unknown year)	*P. elongatum*[b]	Beadell and Fleischer (2005)
U – Unknown location, South Africa (unknown year)	*Plasmodium* sp.[b]	Beadell *et al.* (2006)
R – Cape Town, South Africa (2002–2013)	*Babesia* sp., *Borrelia* sp. (RFB)	Yabsley *et al.* (2012), Parsons *et al.* (in preparation)
W – Ichaboe Is., Namibia (2009)	*Babesia* sp.	Parsons *et al.* (in preparation)
W – Dassen, Robben and Dyer Is., South Africa (2010–2012)	*Babesia* sp.	Parsons *et al.* (in preparation)
W – Bird Is., South Africa (2012)	*Babesia* sp.	Parsons *et al.* (in preparation)
C – La Palmyre, France (2013–2014)	*Plasmodium* sp.	LeClerc *et al.* (2014), Grilo (2014)
C – Hilvarenbeek, Netherlands (2013–2014)	*Plasmodium* sp.	Grilo (2014)
C – Basel, Switzerland (2013–2014)	*P. relictum*	Cereghetti *et al.* (2012), Grilo (2014)
C – West Jerusalem (2013–2014)	*Plasmodium* sp.	Grilo (2014)
Humboldt penguin (*Spheniscus humboldti*)		
C – Antwerp, Belgium (1938)	*P. relictum*	Rodhain (1939), Rodhain and Andrianne (1952)
C – Washington, DC, USA (1956)	*P. elongatum, P.* (*Haemamoeba*) sp.	Huff and Shiroishi (1962)
C – Vienna, Austria (1958–1962)	*Plasmodium* sp.	Grünberg and Kutzer (1963)
C – San Diego, California, USA (1965)	*P. relictum*	Griner and Sheridan (1967)
C – Kanagawa, Japan (year unknown)	*Plasmodium* sp.	Yoshio *et al.* (2006), Ejiri *et al.* (2009)
C – Vienna, Austria (2000–2008)	*Plasmodium* sp.	Dinhopl *et al.* (2011)
W – Punta San Juan, Chile (2010–2013)	*Haemoproteus* (*Parahaemoproteus*) sp.[b]	Sallaberry-Pincheira *et al.* (2015)
C – Valencia, Spain (2013–2014)	*Plasmodium* sp.	Grilo (2014)
Magellanic penguin (*Spheniscus magellanicus*)		
C – Des Moines, Iowa, USA (1986)	*P. relictum*	Fix *et al.* (1988)
C – San Francisco, California, USA (1997–2000)	*Plasmodium* sp.	Tollini *et al.* (2000)
R – Salvador, Brazil (1999–2012)	*P. cathemerium, P. nucleophilum, Plasmodium* sp.	Vanstreels *et al.* (2015*a*)
R – Rio de Janeiro, Brazil (1999–2012)	*Plasmodium* sp.	Vanstreels *et al.* (2015*a*)
R – Rio Grande, Brazil (1999–2012)	*P. nucleophilum, P. unalis, Plasmodium* sp.	Cabana *et al.* (2014), Vanstreels *et al.* (2015*a*)
R – Cariacica, Brazil (1999–2013)	*P. cathemerium, P. elongatum*	Vanstreels *et al.* (2015*a*)
C – Jeju Is., South Korea (2005)	*Plasmodium* sp.	Ko *et al.* (2008)
C – São Paulo, Brazil (2007)	*P. elongatum, Plasmodium* sp.[b]	Bueno *et al.* (2010)
R – Valdívia, Chile (2009)	*P. relictum*	Carvajal and Alvarado (2009)
R – Florianópolis, Brazil (2009–2013)	*P. cathemerium, P. elongatum, Plasmodium tejerai, Plasmodium* sp.	Silveira *et al.* (2013), Vanstreels *et al.* (2014*a*, 2015*a*)
R – Niterói, Brazil (2010)	*Plasmodium* sp.	Campos *et al.* (2014)
R – Rio de Janeiro, Brazil (2010)	*Plasmodium* sp.	Campos *et al.* (2014)
C – San Clemente del Tuyú, Argentina (2010)	*P. tejerai, P.* (*Novyella*) sp., *P.* (*Huffia*) sp.	Vanstreels *et al.* (in press)

Table A1. (*Cont.*)

Host, context, location and period of sample collection	Parasite	References
Galapagos penguin (*Spheniscus mendiculus*)		
W – Unknown location, Galapagos (unknown year)	Microfilariae	Harmon *et al*. (1985)
W – Fernandina and Isabela Is., Galapagos (2003–2005)	Microfilariae	Parker *et al*. (2006), Merkel *et al*. (2007), Siers *et al*. (2010)
W – Isabela Is., Galapagos (2003–2005)	*H*. (*Parahaemoproteus*) sp.	Parker *et al*. (2006), Levin *et al*. (2009)
W – Isabela, Fernandina, Las Marielas and Bartolomé Is., Galapagos (2003–2009)	*Plasmodium* sp.	Parker *et al*. (2006), Levin *et al*. (2009, 2013)

Records were classified according to the context in which the diagnosis was established: W, penguins sampled in the wild; R, penguins undergoing rehabilitation; C, penguins captive in zoos; E, penguins infected under experimental conditions; U, samples obtained from penguins in unknown context.
Notes (see Appendix 2): [a] Small round intraerythrocytic inclusions were observed, which could be compatible with early life stages of *Babesia* sp., *Haemoproteus* sp., *Leucocytozoon* sp. or *Plasmodium* sp.
[b] Revised records, which had originally attributed to a different parasite species.
[c] Molecular and morphological evidence produced conflicting results.
[d] Morphological evidence inconclusive, not clear if structures observed were parasites or artefacts.
[e] Molecular testing for *Haemoproteus*/*Plasmodium* produced conflicting and inconclusive results.

APPENDIX 2

Remarks On Revised, Inconclusive Or Questionable Records Of Blood Parasites In Penguins

Plasmodium

Laird (1950) reported to have examined '*E. pachyrhynchus* (drooping-crested penguin) collected at the Snares Islands'; however this taxon was later revised and specimens breeding in the Snares Archipelago are currently considered a separate species, *Eudyptes robustus* (Oliver, 1953). Additionally, some records refer to rockhopper penguins as *Eudyptes crestatus* (Raethel, 1960; Sladen *et al*. 1979; Laird and Van Riper III, 1981), but because this taxon was later split into two species, *Eudyptes chrysocome* and *Eudyptes moseleyi* (Jouventin *et al*. 2006), it is not always possible to determine to which species these records correspond. In the case of the Baltimore Zoo (Sladen *et al*. 1979), records indicate the penguins had been captured at the Falkland Islands (E. Brown, personal communication) and therefore were *E. chrysocome*. It should be noted that although Grilo (2014) did not list which penguin species had been positive at each zoo, this information was obtained through personal communication.

Early reports refer to *P. praecox* or *P. praecox relictum* (Rodhain, 1939; Rodhain and Andrianne, 1952; Grünberg and Kutzer, 1963), which were later revised as synonyms of *P. relictum* (Valkiūnas, 2005). It is worth considering that in some reports the identification of *P. relictum* was based solely on the presence of large round gametocytes and absence of rod-shaped pigment granules and/or no photomicrographs or detailed morphological descriptions were provided (e.g. Rodhain, 1939; Raethel, 1960; Stoskopf and Beier, 1979; Fix *et al*. 1988). Because *P. relictum* is remarkably pleomorphic and many other species of the subgenus *Haemamoeba* share these general morphological characteristics (Laird and Van Riper, 1981; Valkiūnas, 2005), such records should be considered with caution. Records of *P. elongatum* are probably more reliable as this species has a number of unique morphological characteristics (see Valkiūnas, 2005).

Grim *et al*. (2003) reported *P.* (*Bennettinia*) *juxtanucleare* while examining African penguins undergoing rehabilitation at Cape Town, South Africa. However, the photomicrographs and morphological description of the parasites are not consistent, since the fully grown gametocytes of *P. juxtanucleare* should not exceed the size of the nuclei of infected erythrocytes (Valkiūnas, 2005). Genetically, the parasite was identified as *P. juxtanucleare* solely on the basis of highest sequence identity in BLAST search, a method that can be inadequate to identify avian haemosporidians (Valkiūnas *et al*. 2008; Vanstreels *et al*. 2014*a*). Because the gene sequences were not deposited in public databases, it is impossible to conduct further phylogenetic analyses that might contribute in establishing the identity of the parasite. It is therefore more judicious to attribute this record to *Plasmodium* sp. until further information is obtained.

The identity of some *Plasmodium* sp. lineages for which mitochondrial *cytochrome b* gene sequences are publicly available were later revised and found to correspond to different species. The lineage obtained by Beadell and Fleischer (2005) was revised as *P. elongatum* (Valkiūnas *et al*. 2008). The lineage obtained by Beadell *et al*. (2006) was revised as *Plasmodium* sp. (Vanstreels *et al*. 2015*a*). The lineages obtained by Bueno *et al*. (2010) were revised as *P. elongatum* and a lineage of *Plasmodium* sp. closely related to *P. lutzi* (Vanstreels *et al*. 2014*a*).

It is well established that *Leucocytozoon* spp. invade hepatocytes to form tissue meronts, whereas

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Plasmodium spp. does not (Atkinson and Van Riper III, 1991; Valkiūnas, 2005). The preliminary records provided by Alley *et al*. (2004) and Varney (2006) regarding wild yellow-eyed penguins at Otago Peninsula (South Island, New Zealand) and captive little penguins at Auckland Zoo (North Island, New Zealand) are therefore more consistent with *Leucocytozoon* sp. than with *Plasmodium* sp. as originally attributed. There are unconfirmed reports of wild little penguin chicks suspected to have died from avian malaria at Tiritiri Matangi Island (van Rensburg, 2010).

Thiart (2005) found an unusually high PCR prevalence to *Plasmodium* sp. (88–94%) in wild African penguins that was not consistent with blood smear and serological results and with previous studies in the region (see Fantham and Porter, 1944; Brossy *et al*. 1999; Parsons and Underhill, 2005). Because the electrophoresis of amplification products showed multiple bands with inconsistent patterns that often did not match that of the positive control, non-specific annealing of PCR primers is likely to have occurred.

Leucocytozoon

As previously discussed (see '*Plasmodium*'), the preliminary records provided by Alley *et al*. (2004) and Varney (2006) are more consistent with *Leucocytozoon* sp. than with *Plasmodium* sp. as originally attributed. Similarly, the wild little penguins studied by Cannell *et al*. (2013, 2014) at Western Australia may have been infected by *Leucocytozoon* sp. (see '*Haemoproteus*').

Haemoproteus

Vanstreels *et al*. (2014*a*) reported *Haemoproteus* sp. infection in a Magellanic penguin, however later found this to have been a false-positive result due to a laboratory contamination (Vanstreels *et al*. 2014*b*). Sallaberry-Pincheira *et al*. (2015) found two different *Haemoproteus* sp. lineages in Humboldt penguins and considered that one belonged to the subgenus *Haemoproteus* and the other to the subgenus *Parahaemoproteus*. Closer inspection of the phylogenetic tree therein presented, however, reveals that both lineages cluster consistently with lineages of the subgenus *Parahaemoproteus*.

Cannell *et al*. (2013, 2014) reported *Haemoproteus* sp. as the cause of death of ten little penguins found dead on Penguin Island, Western Australia. However, they report that the parasites were present intracellularly in hepatocytes, which does not occur in *Haemoproteus* spp. infections (Valkiūnas, 2005); their histopathological findings are therefore most compatible with *Leucocytozoon* sp. The intraerythrocytic inclusion photographed in that study is compatible with either *Babesia* sp., *Haemoproteus* sp., *Leucocytozoon* sp. or *Plasmodium* sp., and therefore does not allow for conclusive morphological identification. Although the authors were able to obtain DNA sequences from *Haemoproteus* sp., several aspects of the molecular analyses of that study are concerning: (a) the nested PCR test had a high false negative rate (60%) and poor repeatability; (b) 35 thermal cycles were employed in the HaemNFI/HaemNR3 reaction instead of the 20 recommended in the original protocol (Hellgren *et al*. 2004), which could result in co-amplification of other parasites (see Cosgrove *et al*. 2006; Szöllősi *et al*. 2008); (c) no information was provided on the identity/sequence of the positive control, hence the possibility laboratory contamination, which is not uncommon (see van Rensburg, 2010; Vanstreels *et al*. 2014*b*), cannot be dismissed. As a result, the results reported by Cannell *et al*. (2013) should be interpreted judiciously, requiring further investigation to verify the identity of the parasite and the possibility of infection by *Leucocytozoon* sp.

Babesia

The intracellular parasites observed by Coles (1941) were revised by Bennett *et al*. (1992) as corresponding to *Babesia* sp.

Nematode microfilariae

The record of an unidentified filarioid worm in the heart of a little penguin at Kangaroo Island, Australia (Mawson *et al*. 1986), was later revised as belonging to the superfamily Ascaridoidea (Merkel *et al*. 2007), and therefore would not have produced circulating microfilariae.

Aegyptianella

Coles (1941) observed intraerythrocytic inclusions in the blood smear of a wild African penguin and speculated it to be *Aegyptianella* sp.; however, this record was later revised as *Babesia* sp. (Bennett *et al*. 1992). Gough *et al*. (2002) briefly mention *Aegyptianella* sp. infection in a captive penguin in England. However, the fact that avian malaria was confirmed through histopathology in the same bird suggests that the intraerythrocytic inclusions most likely corresponded to early life stages of *Plasmodium* sp., especially when it is considered that chloroquine treatment can lead *Plasmodium* sp. to develop abnormal shapes that may resemble *Aegyptianella* sp. (see Vanstreels *et al*. 2014*a*). We therefore consider there is not sufficient evidence to demonstrate that *Aegyptianella* sp. infects penguins.

Undetermined or inconclusive records

It is possible that the cases of toxoplasmosis reported by Ratcliffe and Worth (1951) in captive Humboldt

penguins actually corresponded to avian malaria, considering the unusual epidemiological and pathological characteristics of those cases and the morphological similarity between *Toxoplasma gondii* tachyzoites and *Plasmodium* spp. tissue meronts (see Fleischman *et al*. 1968*b*; Ploeg *et al*. 2011).

Round intraerythrocytic inclusions were reported in the blood smears of wild little penguins at Tiritiri Matangi Island (Cook Strait, New Zealand) (van Rensburg, 2010) and of a Fiordland penguin rescued at Muriwai beach and treated in Auckland (North Island, New Zealand) (Hill, 2008), and could be compatible with early life stages of *Babesia* sp., *Haemoproteus* sp., *Leucocytozoon* sp. or *Plasmodium* sp.

McDonald (2012) did not observe parasites in blood smears of yellow-eyed penguins in South Island (New Zealand) however obtained conflicting results when employing different PCR and serological tests targeting *Plasmodium* sp., and it was ultimately not possible to determine whether these or other parasites were present or not. Colombelli-Negrél and Kleindorfer (2014) examined the blood smears of little penguins in South Australia and found structures they considered could correspond to *Plasmodium, Shellakia, Trypanosoma, Hepatozoon* or *Leucocytozoon*; upon closer inspection, however, the photomicrographs provided in that report are most compatible with staining artefacts (pseudoparasites).

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