

**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**

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**Disclaimer:** This manual presents 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. While some government laws and regulations may be referenced in this manual, these are not all-inclusive nor is this manual 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. Commercial entities and media identified are not necessarily endorsed by AZA. The statements presented throughout the body of the manual do not represent AZA standards of care unless specifically identified as such in clearly marked sidebar boxes.

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## 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
<i>Aptenodytes</i>	<i>patagonicus</i>	King penguin	Not listed	Least Concern	Green SSP
<i>Aptenodytes</i>	<i>forsteri</i>	Emperor penguin	Not listed	Least Concern	Red SSP
<i>Eudyptes</i>	<i>pachyrhynchus</i>	Fiordland penguin	Threatened	Vulnerable	
<i>Eudyptes</i>	<i>robustus</i>	Snares penguin		Vulnerable	
<i>Eudyptes</i>	<i>sclateri</i>	Erect-crested penguin	Threatened	Endangered	
<i>Eudyptes</i>	<i>chrysocome</i>	Southern rockhopper penguin	Threatened	Vulnerable	Green SSP
<i>Eudyptes</i>	<i>moseleyi</i>	Northern rockhopper penguin	Not listed	Endangered	Red SSP
<i>Eudyptes</i>	<i>chrysolophus</i>	Macaroni penguin	Not listed	Vulnerable	
<i>Eudyptes</i>	<i>schelegeli</i>	Royal Penguin	Not listed	Vulnerable	
<i>Eudyptula</i>	<i>minor</i>	Little blue penguin	Not listed	Least Concern	Yellow SSP
<i>Pygoscelis</i>	<i>adeliae</i>	Adélie penguin	Not listed	Least Concern	Green SSP
<i>Pygoscelis</i>	<i>antarctica</i>	Chinstrap penguin	Not listed	Least Concern	Yellow SSP
<i>Pygoscelis</i>	<i>papua</i>	Gentoo penguin	Not listed	Least Concern	Green SSP
<i>Megadyptes</i>	<i>antipodes</i>	Yellow-eyed penguin	Threatened	Endangered	
<i>Spheniscus</i>	<i>magellanicus</i>	Magellanic penguin	Not listed	Near Threatened	Green SSP
<i>Spheniscus</i>	<i>humboldti</i>	Humboldt penguin	Threatened	Vulnerable	Green SSP
<i>Spheniscus</i>	<i>mendiculus</i>	Galapagos penguin	Endangered	Endangered	
<i>Spheniscus</i>	<i>demersus</i>	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).

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 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.

#### 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.

## Chapter 1. Ambient Environment

### 1.1 Temperature and Humidity

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.

#### AZA Accreditation Standard

(1.5.7) The animals must be protected from weather, and any adverse environmental conditions.

**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<sub>3</sub>) should be kept at a level below 0.1 ppm and nitrite (NO<sub>2</sub>) levels below 0.5 ppm, although Spotte (1992) lists concentrations 3 ppm as being safe for adult marine fish. Nitrate (NO<sub>3</sub>) 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](http://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)	NH <sub>3</sub> (mg/L)
<b>Antarctic</b>	42–45 (6–7)	7.2–8.2	0	400–600	<0.20	30–34	<1000	<0.10
<b>Spheniscus</b>	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).

**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
<i>King/Emperor</i>				
Exhibit - (per bird for 1 <sup>st</sup> 6 birds)	1.7 m <sup>2</sup> (18 ft <sup>2</sup> )	0.8 m <sup>2</sup> (9 ft <sup>2</sup> )	1.2 m (4 ft.)	6156 liters (1620 gallons)
Each additional bird	0.8 m <sup>2</sup> (9 ft <sup>2</sup> )	0.5 m <sup>2</sup> (5 ft <sup>2</sup> )	---	593 liters (156 gallons)
Short-term holding area <6 mo/per bird	0.8 m <sup>2</sup> (9 ft <sup>2</sup> )	0.5 m <sup>2</sup> (5 ft <sup>2</sup> )	0.9 m (3 ft.)	
<i>All other species (includes program animals)</i>				
Exhibit - (per bird for 1 <sup>st</sup> 6 birds)	0.7 m <sup>2</sup> (8 ft <sup>2</sup> )	0.4 m <sup>2</sup> (4 ft <sup>2</sup> )	0.9 m (3 ft.)	2052 liters (540 gallons)
Each additional bird	0.4 m <sup>2</sup> (4 ft <sup>2</sup> )	0.2 m <sup>2</sup> (2 ft <sup>2</sup> )	---	171 liters (45 gallons)
Short-term holding area (per bird)	0.4 m <sup>2</sup> (4 ft <sup>2</sup> )	0.3 m <sup>2</sup> (3 ft <sup>2</sup> )	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

#### 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.

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<sup>®</sup>, fiberglass, grass, Gunitite, ice, cat litter, Nomad<sup>™</sup> 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 Standard 10.3.4).

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.

**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.

#### 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.

**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.

**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.

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.

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<sup>®</sup> (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.

**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](http://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).

#### 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.



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 (Gentoo 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. Gentoo, 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 3 illustrates how a crate can be temperature controlled by lining the bottom with frozen (size) BlueIce®



Figure 4.

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



Figure 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.

**Spheniscus:** The temperature should be kept between 4.4–15.6 °C (40–60 °F) for air and truck travel involving *Spheniscus* 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). *Spheniscus* 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](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.

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).

**Nutrient requirements:** While many items consumed by various species

#### 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.

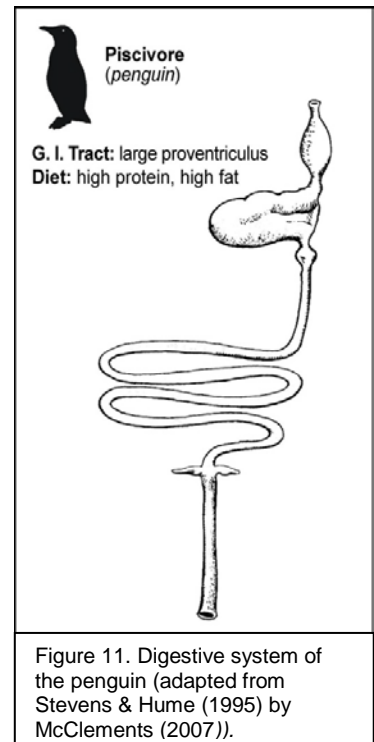


Figure 11. Digestive system of the penguin (adapted from Stevens & Hume (1995) by McClements (2007)).



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 ( $\omega$ -6) fatty acids than fish caught in the marine environment. Generally freshwater fish contain higher concentrations of linoleic (C18:2 $\omega$ -6) and arachidonic (C20:4 $\omega$ -6) acids compared to all other marine fish resulting in a 4–14 times reduction in the  $\omega$ -6 to  $\omega$ -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  $\omega$ -6 and  $\omega$ -3 fatty acids. In contrast, all fish species contain high concentrations of  $\omega$ -3 fatty acids, including docosahexaenoic acid (DHA; C22:6  $\omega$ -3) and eicosapentaenoic acid (EPA) (C20:5  $\omega$ -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<sup>a</sup> 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 <sup>b</sup>
Thiamin, mg/kg	100 <sup>c</sup>
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 <sup>d</sup>
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

<sup>a</sup> 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.

<sup>b</sup> 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.

<sup>c</sup> 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.

<sup>d</sup> 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<sup>®</sup> initiative and Seafood Watch<sup>®</sup>. 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>.

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 3<sup>rd</sup> edition* (Fowler, 1993), and the 5<sup>th</sup> 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.

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

(2.1.1) 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.

#### 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.

#### 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).

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.

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.

**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).

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

### 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.

### AZA Accreditation Standard

**(1.4.7)** Animal records must be kept current, and data must be logged daily.

### 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.

### 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.

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 of 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 tests 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.

**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

(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](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.

### 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](http://www.aazv.org/associations/6442/files/zoo_aquarium_vet_med_guidelines.pdf)).

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.

**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

### AZA Accreditation Standard

(2.4.1) The veterinary care program must emphasize disease prevention.

### 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.

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 interdigital, 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 Celdyne shows promise in accurately counting white blood cells. Chemistry profiles should include assays for glucose, alanine aminotransferase (ALT), asparagine 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 *Spheniscus* 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 spheniscid 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 primaquine is not available, mefloquine (Tavernier et al 2005, Willette et al., 2009) can be used. Prophylaxis can be attained using mefloquine, primaquine 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 Spheniscid 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 hemagglutinin-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 is 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 glands 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 move 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 from 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 from 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	<i>Spheniscus magellanicus</i>	Voluntary semen collection method (n=1 male)	O'Brien et al., 1999
	<i>Eudyptes chrysocome</i>	Voluntary semen collection method (n=6-14 males)	Waldoch et al., 2007, 2012
	<i>Aptenodytes patagonicus</i>	Voluntary semen collection method (n=1 male)	O'Brien & Robeck, 2013
	<i>Spheniscus demersus</i>	Voluntary semen collection Method (number of males not specified)	Unknown
Semen characterization & preservation	<i>Spheniscus magellanicus</i>	Short-term chilled storage, long-term cryostorage (n=1 male)	(O'Brien et al., 1999)
	<i>Spheniscus magellanicus</i>	Short-term chilled storage, long-term cryostorage (n=7 males)	2012–2013 unpublished
	<i>Eudyptes chrysocome chrysocome</i>	Semen characterization only (n=14 males)	Waldoch et al., 2007, 2012
	<i>Aptenodytes patagonicus</i>	Short-term chilled storage, long-term cryostorage (directional freezing method)	O'Brien & Robeck, 2013
Artificial insemination	<i>Spheniscus magellanicus</i>	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 &amp; Sirpenski, 2005)

Species	Month											
	JAN	FEB	MAR	APR	MAY	JUN	JUL	AUG	SEP	OCT	NOV	DEC
Emperor						A	A	A				
King					NA	NA	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	NA	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 &amp; 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 unlaidd 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<sup>®</sup>, 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<sup>®</sup> 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<sup>®</sup> brand, large, or Sky-Kennel<sup>®</sup> 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<sup>®</sup>, 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<sup>®</sup> 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 to 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 by 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).

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

**(1.6.1)** The institution must have a formal written enrichment and training program that promotes species-appropriate behavioral opportunities.

#### 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 refurbishing 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-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).

### 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.

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 *Spheniscid* 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 is 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 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 in 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<sup>®</sup> (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](http://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](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 be 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.

### 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,

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.

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<sup>®</sup> (SSPs), Studbooks, and Taxon Advisory Groups (TAGs)].

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):

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>).
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.

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.

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

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,

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.
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.

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.

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.
6. Domestic animals should be transferred in accordance with locally acceptable farm practices, including auctions, and subject to all relevant laws and regulations.
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.

Examples of documentation include ZIMS records, “Breeding Loan” agreements, chain-of-custody logs, letters of reference, transfer agreements, and transaction documents

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.

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.iucnsscrg.org/index.php>).
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.

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.

## 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.
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.

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.

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 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). 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.

### Acknowledgements

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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<sup>®</sup> 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	
	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
<b>Total</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>	<b>100</b>
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 Institution	King		Rockhopper		Gentoo	Humboldt		African		Magellanic	Little Blue	
	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 10<sup>th</sup> 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](http://www.zoologix.com)

9811 Owensmouth Avenue  
Suite 4  
Chatsworth CA 91311-3800  
info@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](mailto: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](mailto: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)

Pharmacokinetic 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<sup>®</sup>, 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](http://www.lyonusa.com)
3. Trex<sup>®</sup> 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<sup>®</sup> 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](http://www.autoelex.com) (For USA distributor see also Lyon Technologies).
7. Betadine<sup>®</sup> Solution, Purdue Products L.P., One Stamford Forum, Stamford, CT 06901-3431 USA. <http://www.betadine.com>
8. PDI<sup>®</sup> Iodine Duo-Swab<sup>®</sup> Prep and Scrub SwabStick, PDI, Two Nice-Pak Park, Orangeburg, NY 10954 USA. <http://www.pdipdi.com>
9. Plexiglas<sup>®</sup> Acrylic Sheet, Altuglas International, Arkema Inc., 100 PA Route 413, Bristol, PA 190007, USA. [www.plexiglas.com](http://www.plexiglas.com)
10. The Original Cooler Brooder, Avey Incubator, PO Box 279, Hugo, CO 80821 USA. [www.aveyincubator.com](http://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<sup>®</sup> Grip Ultra Shelf Liner, Kittrich Corporation, La Mirada, CA. Con-Tact shelf liner is widely available at kitchen and home stores.
13. Kendall Sovereign<sup>®</sup> 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](http://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](http://www.lyonusa.com)
15. Animal Intensive Care Unit, Lyon Technologies, Inc., 1690 Brandywine Avenue, Chula Vista, CA 91911 USA. [www.lyonusa.com](http://www.lyonusa.com)
16. Pedialyte<sup>®</sup>, Abbott Laboratories, 3300 Stelzer Road, Columbus, OH 43219-3034 USA. <http://pedialyte.com>
17. Mazuri<sup>®</sup> Vita-Zu Bird Tablet w/o Vitamin A, Land O' Lakes, PO Box 64101, Saint Paul, MN 55164-0101 USA. [www.mazuri.com](http://www.mazuri.com)
18. Enfamil<sup>®</sup> Poly-vi-sol<sup>®</sup> Infant drops with iron, Mead Johnson Global Headquarters, 2701 Patriot Boulevard, Fourth Floor, Glenview, IL 60026 USA. [www.enfamil.com](http://www.enfamil.com)
19. Tegaderm<sup>®</sup>, 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 <sub>1</sub>
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<sub>1</sub> (thiamine) powder to the diet prior to feeding. The powder can be made by grinding 100 mg B<sub>1</sub> 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 <sub>1</sub>
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® <u>with iron</u>

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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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<sup>®</sup> 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 <sup>®</sup> drops SID
251–500 g /8.8–18 oz. (a.m. weight):	0.15 cc Poly-vi-sol <sup>®</sup> drops SID
501–750 g/ 18–26 oz. (a.m. weight):	0.20 cc Poly-vi-sol <sup>®</sup> drops SID
751–1000 g/ 26–35 oz. (a.m. weight):	0.25 cc Poly-vi-sol <sup>®</sup> 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<sup>®</sup> Poly-vi-sol<sup>®</sup> 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 <sub>1</sub>	0.5 mg
Vitamin B <sub>2</sub>	0.6
Niacin	8 mg
Vitamin B <sub>6</sub>	0.4 mg
Vitamin B <sub>12</sub>	2 mcg

Children's poly-vitamin drops with iron: One zoological institution has used Enfamil<sup>®</sup> Poly-vi-sol Infant Drops with Iron

Vitamin	Amount per 1 mL
Vitamin A	1500 IU
Vitamin C	35 mg
Vitamin D (choliciferol)	400 IU
Vitamin E (d-alpha-tocopheryl succinate)	5 IU
Thiamin (as thiamin HCl)	0.5 mg
Niacin (as niacinamide)	8 mg
Vitamin B <sub>6</sub> (as pyridoxine HCl)	0.4
Iron (as ferrous sulfate)	10 mg

Children's Multi-vitamin: One zoological institution uses My First Flintstones<sup>™</sup>

Vitamin	Amount per tablet
Vitamin A	1998 IU
Vitamin C	60 mg
Vitamin D (D <sub>3</sub> )	400 IU
Vitamin E	15 IU



Thiamin (B <sub>1</sub> )	1.05 mg
Riboflavin (B <sub>2</sub> )	1.2 mg
Niacin	10 mg
B <sub>6</sub>	1.05 mg
Folic Acid	300 mcg
Vitamin B <sub>12</sub>	4.5 mcg
Sodium	10 mg

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Contents of Mazuri<sup>®</sup> 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

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**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<sup>®</sup> Vita-Zu Bird Tablet w/o Vitamin A [www.mazuri.com](http://www.mazuri.com)  
(See table above for contents)

My First Flintstones [www.bayercare.com](http://www.bayercare.com)  
(See table above for contents)

Enfamil<sup>®</sup> Poly-vi-sol<sup>®</sup> Infant drops [www.enfamil.com](http://www.enfamil.com)  
(See table above for contents)

Onset HOB0<sup>®</sup>  
Pendant temp/light datalogger [www.onsetcomp.com/products/data-loggers/ua-002-64](http://www.onsetcomp.com/products/data-loggers/ua-002-64)

## Appendix N: ISIS Physiological Blood Values

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### Blue Penguin (*Eudyptula minor*)

Samples contributed by 8 institutions.

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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 <sup>a</sup>	High Sample <sup>b</sup>	Sample Size <sup>c</sup>	Animals <sup>d</sup>
White Blood Cell Count	*10 <sup>3</sup> cells/μL	2.93 - 34.60	13.39	12.00	1.98	39.40	220	138
Red Blood Cell Count	*10 <sup>6</sup> 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 <sup>3</sup> cells/μL	0.55 - 19.83	6.74	5.59	0.03	24.80	219	137
Lymphocytes	*10 <sup>3</sup> 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

<sup>a</sup> Lowest sample value used to calculate the reference interval.

<sup>b</sup> Highest sample value used to calculate the reference interval.

<sup>c</sup> Number of samples used to calculate the reference interval.

<sup>d</sup> Number of different individuals contributing to the reference interval.

\* Sample size is insufficient to produce a valid reference interval.

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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.

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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 <sup>a</sup>	High Sample <sup>b</sup>	Sample Size <sup>c</sup>	Animals <sup>d</sup>
White Blood Cell Count	*10 <sup>3</sup> 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 <sup>3</sup> cells/μL	0.00 - 10.50	4.82	4.30	1.30	16.40	52	21
Lymphocytes	*10 <sup>3</sup> 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

<sup>a</sup> Lowest sample value used to calculate the reference interval.

<sup>b</sup> Highest sample value used to calculate the reference interval.

<sup>c</sup> Number of samples used to calculate the reference interval.

<sup>d</sup> Number of different individuals contributing to the reference interval.

\* Sample size is insufficient to produce a valid reference interval.

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**Gentoo Penguin (*Pygoscelis papua*)** Sample Selection Criteria:

Samples contributed by 12 institutions.

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- 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 <sup>a</sup>	High Sample <sup>b</sup>	Sample Size <sup>c</sup>	Animals <sup>d</sup>
White Blood Cell Count	*10 <sup>3</sup> cells/μL	3.63 - 22.38	11.36	10.68	1.60	28.00	372	131
Red Blood Cell Count	*10 <sup>6</sup> 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 <sup>3</sup> cells/μL	2.41 - 16.31	7.49	6.98	1.15	20.00	370	130
Lymphocytes	*10 <sup>3</sup> 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

<sup>a</sup> Lowest sample value used to calculate the reference interval.

<sup>b</sup> Highest sample value used to calculate the reference interval.

<sup>c</sup> Number of samples used to calculate the reference interval.

<sup>d</sup> Number of different individuals contributing to the reference interval.

\* Sample size is insufficient to produce a valid reference interval.

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\_combined\_Conventional\_American\_units\_2013\_CD.htm l" in *ISIS Physiological Reference Intervals for Captive Wildlife: A CD-ROM Resource.*, International Species Information System, Eagan, MN.

## Humboldt Penguin (*Spheniscus humboldti*)

Samples contributed by 21 institutions.

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### 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 <sup>a</sup>	High Sample <sup>b</sup>	Sample Size <sup>c</sup>	Animals <sup>d</sup>
White Blood Cell Count	*10 <sup>3</sup> cells/μL	6.16 - 49.88	23.53	21.99	1.37	74.50	2191	468
Red Blood Cell Count	*10 <sup>6</sup> 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 <sup>3</sup> cells/μL	3.33 - 30.22	14.03	13.26	1.05	42.40	2183	468
Lymphocytes	*10 <sup>3</sup> 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

<sup>a</sup> Lowest sample value used to calculate the reference interval.

<sup>b</sup> Highest sample value used to calculate the reference interval.

<sup>c</sup> Number of samples used to calculate the reference interval.

<sup>d</sup> Number of different individuals contributing to the reference interval.

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Teare, J.A. (ed.): 2013, "Spheniscus\_humboldti\_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.

## Jackass Penguin (*Spheniscus demersus*)

Samples contributed by 37 institutions.

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### 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 <sup>a</sup>	High Sample <sup>b</sup>	Sample Size <sup>c</sup>	Animals <sup>d</sup>
White Blood Cell Count	*10 <sup>3</sup> cells/μL	4.11 - 39.01	15.34	13.53	0.17	50.40	2105	626
Red Blood Cell Count	*10 <sup>6</sup> 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 <sup>3</sup> cells/μL	1.77 - 21.50	8.48	7.51	0.02	28.70	2084	625
Lymphocytes	*10 <sup>3</sup> 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

<sup>a</sup> Lowest sample value used to calculate the reference interval.

<sup>b</sup> Highest sample value used to calculate the reference interval.

<sup>c</sup> Number of samples used to calculate the reference interval.

<sup>d</sup> Number of different individuals contributing to the reference interval.

\* Sample size is insufficient to produce a valid reference interval.

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Teare, J.A. (ed.): 2013, "Spheniscus\_demersus\_No\_selection\_by\_gender\_\_All\_a  
ges\_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.

## King Penguin (*Aptenodytes patagonicus*)

Samples contributed by 11 institutions.

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### 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 <sup>a</sup>	High Sample <sup>b</sup>	Sample Size <sup>c</sup>	Animals <sup>d</sup>
White Blood Cell Count	*10 <sup>3</sup> cells/μL	2.89 - 22.49	9.40	8.50	0.80	29.80	167	65
Red Blood Cell Count	*10 <sup>6</sup> 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 <sup>3</sup> cells/μL	0.95 - 9.74	4.19	3.90	0.48	11.10	165	64
Lymphocytes	*10 <sup>3</sup> 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

<sup>a</sup> Lowest sample value used to calculate the reference interval.

<sup>b</sup> Highest sample value used to calculate the reference interval.

<sup>c</sup> Number of samples used to calculate the reference interval.

<sup>d</sup> Number of different individuals contributing to the reference interval.

\* Sample size is insufficient to produce a valid reference interval.

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ll\_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.

## Macaroni Penguin (*Eudyptes chrysolophus*)

Samples contributed by 3 institutions.

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### 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 <sup>a</sup>	High Sample <sup>b</sup>	Sample Size <sup>c</sup>	Animals <sup>d</sup>
White Blood Cell Count	*10 <sup>3</sup> cells/μL	3.16 – 19.54	8.70	7.88	1.38	24.00	178	41
Red Blood Cell Count	*10 <sup>6</sup> 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 <sup>3</sup> cells/μL	1.30 – 9.70	4.09	3.61	1.04	12.20	177	41
Lymphocytes	*10 <sup>3</sup> 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

<sup>a</sup> Lowest sample value used to calculate the reference interval.

<sup>b</sup> Highest sample value used to calculate the reference interval.

<sup>c</sup> Number of samples used to calculate the reference interval.

<sup>d</sup> Number of different individuals contributing to the reference interval.

\* Sample size is insufficient to produce a valid reference interval.

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## Magellanic Penguin (*Spheniscus magellanicus*)

Samples contributed by 12 institutions.

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### 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 <sup>a</sup>	High Sample <sup>b</sup>	Sample Size <sup>c</sup>	Animals <sup>d</sup>
White Blood Cell Count	*10 <sup>3</sup> cells/μL	4.79 - 37.51	15.07	13.20	2.30	44.60	908	238
Red Blood Cell Count	*10 <sup>6</sup> 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 <sup>3</sup> cells/μL	1.70 - 20.05	7.42	6.27	0.03	26.20	891	237
Lymphocytes	*10 <sup>3</sup> 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

<sup>a</sup> Lowest sample value used to calculate the reference interval.

<sup>b</sup> Highest sample value used to calculate the reference interval.

<sup>c</sup> Number of samples used to calculate the reference interval.

<sup>d</sup> Number of different individuals contributing to the reference interval.

\* Sample size is insufficient to produce a valid reference interval.

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ll\_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.

## Southern Rockhopper Penguin (*Eudyptes chrysocome*)

Samples contributed by 14 institutions.

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([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 *Eudyptes chrysocome*

Test	Units	Reference Interval	Mean	Median	Low Sample <sup>a</sup>	High Sample <sup>b</sup>	Sample Size <sup>c</sup>	Animals <sup>d</sup>
White Blood Cell Count	*10 <sup>3</sup> cells/μL	2.45 - 19.51	8.30	7.44	1.40	24.20	513	150
Red Blood Cell Count	*10 <sup>6</sup> 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 <sup>3</sup> cells/μL	1.07 - 9.65	4.14	3.60	0.02	14.20	512	149
Lymphocytes	*10 <sup>3</sup> 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

<sup>a</sup> Lowest sample value used to calculate the reference interval.

<sup>b</sup> Highest sample value used to calculate the reference interval.

<sup>c</sup> Number of samples used to calculate the reference interval.

<sup>d</sup> Number of different individuals contributing to the reference interval.

\* Sample size is insufficient to produce a valid reference interval.

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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**

		<b>Mean</b>	<b>S.D.</b>	<b>Min.</b>	<b>Max.</b>	<b>(N)</b>
WBC	*10 <sup>3</sup> /UL	9.440 +	3.319	5.720	12.10	(3)
RBC	*10 <sup>6</sup> /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 <sup>3</sup> /UL	3.607 +	3.540	0.970	7.630	(3)
LYMPHOCYTES	*10 <sup>3</sup> /UL	5.307 +	2.169	3.990	7.810	(3)
MONOCYTES	*10 <sup>3</sup> /UL	0.323 +	0.100	0.210	0.400	(3)
EOSINOPHILS	*10 <sup>3</sup> /UL	0.220 +	0.141	0.120	0.320	(2)
BASOPHILS	*10 <sup>3</sup> /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**

		<b>Mean</b>	<b>S.D.</b>	<b>Min.</b>	<b>Max.</b>	<b>(N)</b>
WBC	*10 <sup>3</sup> /UL	9.186 +	2.265	5.400	11.80	(7)
RBC	*10 <sup>6</sup> /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 <sup>3</sup> /UL	5.301 +	1.972	2.970	8.900	(7)
LYMPHOCYTES	*10 <sup>3</sup> /UL	3.316 +	2.038	0.740	5.430	(7)
MONOCYTES	*10 <sup>3</sup> /UL	0.327 +	0.336	0.074	0.708	(3)
EOSINOPHILS	*10 <sup>3</sup> /UL	0.255 +	0.170	0.054	0.472	(6)
BASOPHILS	*10 <sup>3</sup> /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.

#### **Tissue Checklist**

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
			<b>1</b>	<b>2</b>	<b>3</b>	<b>4</b>
			Sprinkler / mister	Keeper play in habitat	Relocate pm pans	Ice cubes throughout habitat
			W	K	FH	T
<b>5</b>	<b>6</b>	<b>7</b>	<b>8</b>	<b>9</b>	<b>10</b>	<b>11</b>
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
<b>12</b>	<b>13</b>	<b>14</b>	<b>15</b>	<b>16</b>	<b>17</b>	<b>18</b>
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
<b>19</b>	<b>20</b>	<b>21</b>	<b>22</b>	<b>23</b>	<b>24</b>	<b>25</b>
"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
<b>26</b>	<b>27</b>	<b>28</b>	<b>29</b>	<b>30</b>	<b>31</b>	
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	

<b>Week of:</b>	<b>Sunday</b>	<b>Monday</b>	<b>Tuesday</b>	<b>Wednesday</b>	<b>Thursday</b>	<b>Friday</b>	<b>Saturday</b>
	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