Giant Pacific Octopus (*Enteroctopus dofleini*) Care Manual

CREATED BY
AZA Aquatic Invertebrate Taxonomic Advisory Group
IN ASSOCIATION WITH
AZA Animal Welfare Committee

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Dedication:

This work is dedicated to the memory of Roland C. Anderson, who passed away suddenly before its completion. No one person is more responsible for advancing and elevating the state of husbandry of this species, and we hope his lifelong body of work will inspire the next generation of aquarists towards the same ideals.

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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 *Enteroctopus dofleini*

Classification	Taxonomy	Additional information
Kingdom	Metazoa	Animalia
Phylum	Mollusca	
Class	Cephalopoda	Subclass Octopodiformes
Order	Octopoda	
Suborder	Incirrata	
Family	Octopodidae	Superfamily Octopodoidea

(Wülker, 1910)

Genus, Species, and Status

Table 2. Genus, species, and status information for *Enteroctopus dofleini*

Genus	Species	Common Name	US ESA Status	IUCN Status	AZA Status
*Enteroctopus**	*dofleini*	Giant Pacific Octopus (GPO)	Not Listed	Not Evaluated	Species of Interest

*Older literature (prior to 1998) will refer to this species as *Octopus dofleini* (Wülker, 1910), and in some cases *O. dofleini martini*; see Hochberg (1998) for the systematic reference and Anderson (1998) for an overview of the change and announcement to the aquarium community. Older texts also erroneously make reference to the animal as *Octopus apollyon*. Some East Asian researchers do not accept the ICZN taxonomic nomenclature and erroneously refer to the species at it occurs in the northern Sea of Japan as *Paroctopus dofleini* or *Paroctopus asper*.

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* octopus 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 octopus must comply with all relevant local, state, and federal wildlife laws and regulations; AZA accreditation standards that are more stringent than these laws and regulations must be met (AZA Accreditation Standard 1.1.1).

AZA Accreditation Standard

(1.1.1) The institution must comply with all relevant local, state, and federal laws and regulations, including those specific to wildlife. It is understood that, in some cases, AZA accreditation standards are more stringent than existing laws and regulations. In these cases the AZA standard must be met.

The ultimate goal of this ACM is to facilitate excellent octopus management and care, which will ensure superior welfare at AZA-accredited institutions. Ultimately, success in our octopus management and care will allow AZA-accredited institutions to contribute to their conservation, and ensure that these animals are in our future for generations to come.

Reasons to keep octopus: It is reasonable to believe that early public aquaria and many zoos would display octopuses, considering the public's fascination with these animals. Records confirm that early aquariums actually did show octopuses; Lee (1875) writes of octopus in aquaria in Boulogne in 1867 and at Brighton in 1872. He states: "An aquarium without an octopus was like a plum-pudding without plums." It is probably not coincidental that a rise in popularity of octopuses in aquaria coincided with the publication of Victor Hugo's lurid description of an octopus attack in *Toilers of the Sea* (1866). It is interesting to note that these early aquarists had some of the same problems with octopuses (such as their tendency to hide) that beset the modern aquarists.

Today, octopuses continue to intrigue the people in public aquaria all over the world. The reasons for wanting to keep and display octopuses in aquaria are self-evident. Their human-like eyes, alien body shape, and their reputation as the most intelligent invertebrate, all contribute to their popularity. A poll of visitors at one aquarium showed that octopuses are one of the most popular aquarium animals (F. Lighter, personal communication). The question, "Where is the octopus?" is asked at many aquariums and zoos throughout the day.

The giant Pacific octopus (GPO), *Enteroctopus dofleini*, is the species most displayed in American aquariums and zoos (Carlson & Delbeek, 1999). The plural of octopus is octopuses. The word "octopus," which means "eight footed" is from a Greek root, so the Latinized ending of "octopi" is not generally used though appears (erroneously) in the dictionary as well. Octopus caretakers have set the precedent of calling them octopuses all over the world. Giant Pacific octopuses (GPOs) used to be called by the scientific name *Octopus dofleini*, named after a German professor named Doflein. In 1998, Eric Hochberg reassigned the species, putting the GPO into genus *Enteroctopus* with the other giant octopuses (GPO being the largest) of South America and Southern Africa (Hochberg, 1998). This genus shares some other features than size, like the number of gill filaments. The pronunciation is "Enter-octopus doe-FLINE-ee."

Natural history of *Enteroctopus dofleini*: The giant Pacific octopus is the world's largest species of octopus. However, weight estimates greater that than 100 kg (221 lb) are questionable. Tracing the literature indicates the larger numbers were estimates and from photographs (High, 1976). The numbers used were not actual weights, exaggerated, and unfortunately became widely referenced. Weights like 71kg (157lb) is a more realistic upper size limit for this species (Cosgrove, 1987). Most adult animals will never reach these extreme sizes and can be expected to mature at 20–30 kg (44–66 lb) (Hartwick, 1983). Given their rapid rate of growth and relatively abbreviated life spans a 10 kg (22 lb) GPO can only be expected to live about 1 year on exhibit, before the onset of senescence and imminent mortality. There is no species of octopus larger than a GPO. A *Haliphron* sp. was discovered and has been deemed larger than GPOs, but the largest specimen of *Haliphron* was only 70 kg (154 lb).

Figure 1. A 96 kg (210 lb) GPO collected in the 1930's. Original photographer unknown: Image courtesy of R. Anderson

The GPO's natural range encompasses the North Pacific Rim, from Japan through Alaska to Southern California, in water as deep as 300 m (985 ft); they don't live in deep water and therefore are not found in the deep, open ocean. In their shallow range, they are generalist feeders. Some specialize on whatever food is available near their sheltering den using different methods to access different shellfish prey species (Anderson & Mather, 2007). Consuming primarily crustaceans and mollusks, the octopus should have a variety of feeding tactics to be successful on such hard-shelled prey. Suckers located on their arms are able to create tremendous force in prying apart shells, and their arms are limber and long enough to reach the most elusive prey. They are able to puncture exoskeletons with their beaks, but on particularly difficult ones, the octopus will use secretions to soften the surface, after which the octopus radula is used to literally drill through the weakened shell. Through this hole the octopus can secrete venom-like saliva that paralyzes the prey and begins to breakdown the connective tissue for ingestion. Typical prey includes crabs and scallops, other bivalves, snails, shrimp, fish, and other octopus.

Octopus are preyed upon by large fish, sharks such as spiny dogfish and sixgill sharks, and marine mammals such as river otters, sea otters, harbor seals, sea lions, and even killer whales (Hart, 1980; Haley, 1978). The list of octopus predators includes other octopuses.

Octopuses are generally bottom dwellers that move most efficiently by pulling themselves over rocks with their arms. They are able to swim quickly in short bursts over relatively short distances by means of jet propulsion. Water enters the side openings of the mantle and is pumped out through the siphon, producing a jet-propelled backwards motion. Octopuses spend much of their time in the safe refuge of a den.

Like most octopods, and cephalopods in general, the skin of *E. dofleini* is capable of adapting to match its environment as a form of camouflage. While *E. dofleini* does not show the same range of color change as some cephalopods such as the flamboyant cuttlefishes, their pappilose skin combined with the ability to match texture and color can serve as an effective way of disguising the animal. The wide array of colors, patterns, and textures created by the skin of cephalopods is a fascinating topic and the reader is advised to consult Hanlon and Messenger (1996), Hanlon (2007), Allen *et al.* (2010), and Mäthger *et al.* (2012) for a more thorough treatment of the subject

GPOs are relatively long-lived compared to other octopus species. The life span of *E. dofleini* is 3–4 years, and to at least 5 years in colder Alaskan waters. In contrast, tropical octopus' life spans are much shorter. For example, *Octopus briareus* lives only 10–17 months. Because of their longer lifespan, GPOs make good aquarium animals, giving the aquarist more time to display the animal and become acquainted with its behavior and habits.

Senescence: Octopus "old age" (senescence) occurs at the end of a mature octopus' natural life span and may last for a month or more. Poor water quality (e.g., lack of dissolved oxygen, low pH, pollutants), incorrect temperature, collecting stress, and disease can also cause signs of senescence and even death in aquarium-housed octopuses.

The process of senescence is driven by secretions from the optic gland, which triggers ripening of the reproductive organs, inactivation of the salivary and digestive glands, and cessation of appetite, which then normally followed by death after reproduction. Activation of the optic gland appears to be affected by environmental factors such as light, temperature, and nutrition, which ultimately influence reproduction and life span. The behavioral aspects of senescence have been less remarked upon, but have been casually known for millennia. Aristotle stated: “the females after giving birth… become stupid, and are not aware of being tossed about in the water, but it is easy to dive and catch them by hand.” (Balme, 1991).

There are four primary indicators that senescence is occurring (Anderson et al., 2002):

1. Loss of appetite and lack of feeding, leading to subsequent weight loss.
2. Retraction of the skin around their eye (i.e., hollow eyes).
3. Increased activity that is undirected and uncoordinated; lack of “attention.”
4. Sloughing necrotic tissue.

Many researchers and aquarists have noted the loss of appetite in both male and female octopuses at this stage, and it is fairly well reported. It has been posited that such fasting is what may inevitably lead to the animal’s death.

Figure 2. This large male GPO is senescent: He has white sores along one arm and at the end of his mantle, he has “Hollow Eyes,” and he is careless about the position of his ligula. Photo courtesy R. Anderson

Figure 3. Severe mantle lesions (white patches) on a senescent GPO. These lesions, colloquially termed "butt-burn," are typical of senescence. At such advanced stages of senescence where the animal is unattractive to public display curators and veterinarians should work together to determine if the animal should be held off-exhibit and offered supportive care or whether it would be more humane to euthanize the specimen. Photo courtesy of B. Christie

The occurrence of white lesions on a senescent octopus has been the subject of controversy and discussion. A young octopus, in good condition and in good water quality, can sustain an injury that may cause such lesions. However, Hanlon (1983) remarks that the skin deteriorates during the 2nd–4th week of senescence for both male and female *O. briareus*. "Skin damage usually leads to infection only in old animals" (Mangold, 1983, p. 359). Van Heukelem (1977) states that the healing processes of octopuses are shut off during senescence, so skin injuries may become secondarily infected. It appears that the primary cause of such lesions is the shutting off of the immune system.

In addition to the above behaviors, it should also be noted that GPOs, especially male specimens, might be prone to autophagy during senescence (Anderson et al., 2002). Autophagy, as it occurs in cephalopods, involves an animal amputating, though not necessarily eating its own limbs. Should this occur, euthanasia is recommended for the specimen (Chapter 6.6, & 6.7 for further information).

Similar behaviors: Two other conditions in octopuses are similar to senescence: stress and the effects of oxygen deprivation. Stress may cause an octopus to lose its appetite much like senescence, and may also cause it to have sores on its body from bad water conditions. Assessment of the age versus life span of the octopus will enable the aquarist to tell the difference between stress and senescence.

Oxygen deprivation, such as is experienced under collecting or shipping stress, may cause an octopus to appear "brain dead." It will lose its appetite and not behave normally. It may not even recognize its arms and eat them, also known as autophagy. Unfortunately the condition is terminal.

Regulating agencies: Currently *E. dofleini* is not regulated at the federal level in either the United States or Canada, and the species is not listed on Appendices I, II, or III of CITES. The species is not listed on the United States Endangered Species Act (ESA). There is no formal regulatory agency that controls this species except at the local level pertaining to collections, or when moving live specimens across national borders (namely from Canada into the United States). See Appendix G for an overview of the import process.

- In Washington State (USA), *E. dofleini* is classified as shellfish under statute RCW 75.08.080 for purposes of collection and subject to sport-fishing regulations. Specimens may also be collected by scientific permit or by commercial fishermen.
- In Oregon (USA), collection of *E. dofleini* is controlled by sportfishing regulations (bag limit of 1 octopus per day), and commercial fishing regulations (harvest also permitted with possession of a commercial fishing license). Specimens may also be collected by scientific permit.
- In California (USA), sport-fishing regulations (§ 29.10 California Fish and Game) permit the collection of octopus (all species) by hand, or hook and line. Specimens may also be collected by scientific permit.
- In Alaska (USA), targeting *E. dofleini* is only allowed with a Commissioner's permit, and only issued for the Alaskan peninsula and other gulf waters excluding Cook Inlet and Southeast Alaska (Woodby, et al., 2005). Specimens may also be collected by scientific permit.
- In all four US states where *E. dofleini* occurs there are a number of marine reserves, protected areas, National Wildlife refuges, National Marine Sanctuaries, and other areas where collection of any live animals is strictly forbidden or by special permit only. Institutions mounting field expeditions are strongly encouraged to consult local wildlife authorities prior to engaging in any live collection.
- In British Columbia (Canada), collection of *E. dofleini* is regulated by the Oceans and Marine Fisheries Branch of the Ministry of Environment of British Columbia. Recreational fishermen may take octopus by hand only; the use of any sharp pointed object is forbidden. Collection is also allowed by permit.

Conservation status of octopods: Octopuses are discussed generally in this section due to limited specific information on *Enteroctopus dolfleini*. The condition of other octopus species is considered relevant and a catalyst for further investigation.

While the population dynamics of many species of octopus remain poorly understood at best, other less specific conservation efforts worldwide (on other species sharing octopod habitats) will doubtlessly have positive impacts on these species until their conservation status is more fully understood. Coral reef areas harbor entire ecosystems of incredible biodiversity; as such marine protected areas and other ecosystem-based approaches afford an obvious measure of protection for many species of octopus (and even cephalopod assemblages as a whole) (Wagner, 1999). Octopus and shellfish harvesting techniques may be doing irreparable damage not just to the targeted animals, but also to corals, a keystone species in the tropical ecosystems where many of the world's octopod species are found (Wagner, 1999). There is already some very compelling direct evidence that marine protected areas have had some positive effects on *Octopus tehuelchus* populations (Navarte et al., 2006). Other ecosystem-based conservation efforts that may be of benefit to octopus populations include pollution control and prevention programs. Several populations of octopus worldwide have been found to bioaccumulate heavy metals and other toxins owing to their tropic level interactions (Seixas et al., 2004; Seixas, 2003; Seixas & Pierce, 2005; Miramand & Guary, 1980; Anderson, 2003).

The conservation status of octopods, excerpted and modified from Aquatic Invertebrate Taxonomic Advisory Group (AITAG) Regional Collection Plan (RCP) (Mohan, 2008):

> Worldwide, nearly all octopod fisheries are artisanal in nature, though there is some evidence that some are verging on over-exploitation. In terms of fishing pressures, female octopods are particularly vulnerable owing to the high fecundity and the uncommon measure of parental care exhibited by these species relative to other invertebrates (Navarte et al., 2006). There are already some well-founded concerns that *Octopus mimus* populations in Chile are in desperate need of management to prevent imminent overfishing, and that stocks of *Octopus cyanea* at two separate locations in Tanzania are facing similar exploitation ((Defeo & Castilla, 1998; Guard & Mgaya, 2002). These species are not maintained in AZA facilities.
>
> The famed mimic octopus of Indonesia is getting even more difficult to find than it was before (Caldwell and Shaw, 2002). It was never easy to find, only in certain specific mudflats in undeveloped areas and it is considered rare anywhere. It looks like its numbers

are rapidly decreasing as it is caught by local fishermen and sold to tropical fish stores and aquariums, in spite of its poor survival in captivity. It has never reproduced in captivity." There is some disagreement on the status of this species. Hemdal (personal communication) can find no statistics to support decreasing abundance. He believes that the "mimics" people are looking for are just females that "color up" under certain conditions. Males and quiescent females can be misidentified as "long arm mud octopus."

There are few statistics to support whether a cephalopod is endangered or not. But one octopus species may have been eradicated or locally gone extinct. The Caribbean small-egged pygmy octopus, *Octopus joubini*, cannot be found anymore where it was once common (J. A. Mather, personal communication). Other pygmy octopuses in the Caribbean may be a different species as they have large eggs (*O*. *mercatoris*). Locally some bays where *O*. *joubini* used to be common are now bereft of that species, whether due to human activities (pollution), climate change, or over-collecting (excerpted from Anderson, 2008, with permission).

Despite the artisanal nature of most octopod fisheries, management of these fisheries is beginning to be employed worldwide. The Portuguese artisanal fishery has benefited from a holistic management structure involving research into spawning ecology, education, and regulation of benthic trawling (Pereira, 1999). In what may be an indicator of future trends, a commercial fishery and subsequent management framework has been proposed, but not yet fully implemented for *Octopus vulgaris* in South Africa (Oosthuzien, 2003).

The giant Pacific octopus, *E. dolfeini*, is a prominent exhibit animal at many aquatic facilities (Delbeek & Carlson, 1999). It is common from California to Alaska, and while routinely harvested (mostly as bycatch), populations are typically stable with localized overfishing reported in a few regions. Collection is by permit only.

The GPO is not listed by the US Endangered species Act, and has yet to be evaluated with regards to conservation status by the International Union for Conservation of Nature (IUCN). The species is not listed on any of the CITES appendices. *E. dofleini* is considered a species of interest by the Aquatic Invertebrate Taxon Advisory Group (AITAG) of the AZA; it is not an SSP animal.

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: One important component of closed system maintenance of GPOs is the refrigeration unit. It is recommended that these animals be kept at temperatures of 6–12 °C (42.8–53.6 °F); 10 °C (50 °F) is ideal. Prescott & Brosseau (1962) claim that prolonged exposure to temperatures above 12 °C will cause death in GPOs, although at one aquarium the water may be over 13 °C (55.4 °F) for a month in the summer with no deleterious effects observed. However, octopuses in flow-through systems may be less prone to high temperature effects than in a closed system. While some aquaria have maintained *E. dofleini* successfully in closed systems at warmer temperatures (12.5–14 °C [54.5–57.2 °F]), these higher temperature regimes are not recommended for long-term health and survival. Of institutions surveyed in 2012 (n=33) 85.0% were maintaining their GPOs at 6–12 °C (42.8–53.6 °F), with the majority of these (61.2%) being kept at 9–11 °C (48.2–51.8 °F). It should also be noted that animals sourced from populations in Washington or British Columbia will be accustomed to warmer temperature regimes (average ~9–11 °C [48.2–51.8 °F]) than those from Alaskan populations (average ~6 °C [42.8 °F]).

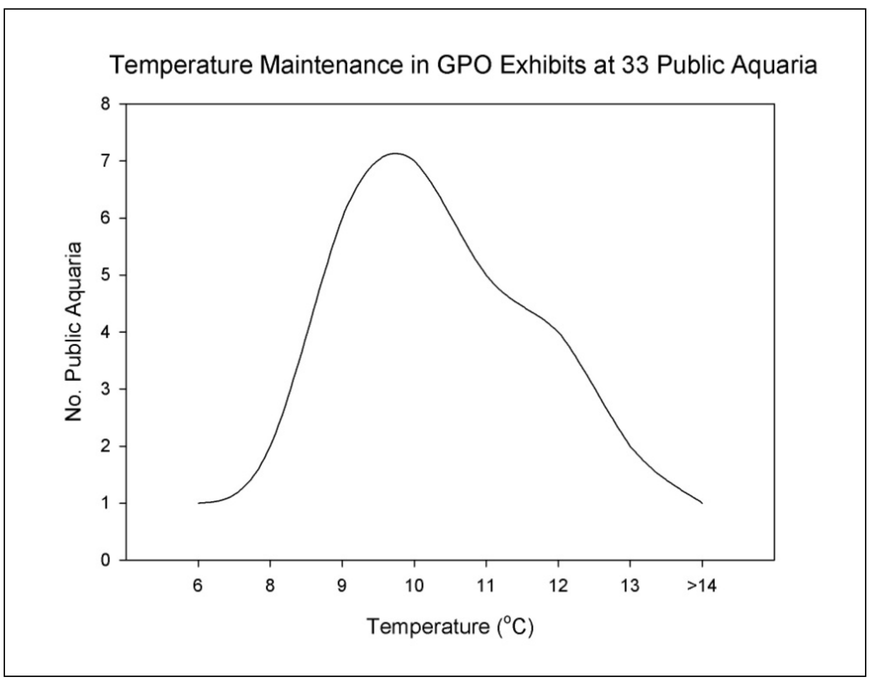


Figure 4. Frequency of mean temperature in *Enteroctopus dofleini* exhibits at 33 public aquaria. Data collected in 2012 survey conducted by the AITAG.

Life support: As stated above, refrigeration units are a critical piece of life support system (LSS) equipment for facilities desiring to maintain *E. dofleini* in closed systems. These units typically consist of a chiller (refrigeration unit consisting of a compressor and condenser coils) that supplies coolant to a heat exchanger (draws heat from seawater into coolant, which returns to the chiller). This apparatus is regulated by a controller, which turns the chiller on or off depending on the temperature of the water being cooled. Centralized chillers with a re-circulating cold water or glycol loop often offer advantages in redundancy and efficiency (especially in facilities with multiple cold water exhibits). Freestanding combined chiller/heat exchanger units are a more economical alternative, and are used successfully in many facilities with adequate alarm/sensing equipment. For these closed system cold-water exhibits, which are dependent on more (relatively) complex LSS, it is recommended that chiller mechanisms be redundant and appropriately sized (or over-sized) for the tank in question.

Refrigeration units should also have no harmful heavy metals in contact with water. Several companies make reasonably priced units designed for aquarium saltwater use, with stainless steel or titanium heat exchangers. There is not complete agreement regarding the use of stainless steel as being appropriate for cephalopods, as stainless is an alloy that often contains significant amounts of nickel and zinc. When stainless steel is used it should be of grade 316, but even then there is no absolute guarantee of safety. Titanium is considered the safest material to use—particularly in closed systems—and is utilized by 81% of institutions keeping GPOs according to a 2012 survey conducted by the authors. Open or semi-open systems may be less of a risk given the dilution and the water is more constantly refreshed. Units with copper, brass, or bronze components (e.g., cupronickel or plated bronze) in the heat exchanger should not be used due to toxicity risk. Care should be taken to ensure that the heat exchanger is not simply plated, as the coating can become compromised over years of service and will eventually leach heavy metals into the water. Daily inspections are necessary to avoid failed seals and leaking coolant water into the tank water.

When sizing a chiller for a GPO tank it should be noted that chillers which use water as the coolant typically operate no colder than 10 °C (50 °F). As no chiller/heat exchanger combination can boast 100% efficiency, a water-cooled system may not provide the chilling capacity needed to maintain a GPO. Chilling systems that use ethylene glycol or a similar chemical refrigerant will be needed to get a closed-system down to ideal temperatures.

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.

Due to the relatively low temperatures at which this species needs to be maintained, specialized LSS equipment is required: namely chillers, pumps, and heat exchangers. Due to the expense of this equipment, only few aquaria will install wholly redundant life support systems, instead relying on spare parts, alarm systems, and continuous checks by aquarists, managers, and LSS staff. Backup systems for octopus exhibits commonly service the entire aquarium (i.e., emergency generators, redundant compressors on chiller units, etc.). It is recommended that facility and animal care staff check temperature and unit functions, even ones with computerized monitoring and controls. Preventative maintenance is important, and users should consult the operations and maintenance (O&M) manuals for every piece of equipment in service to ensure that parts replacements, cleaning, and calibrations are carried out as recommended by the manufacturers. Regular inspections (walkthroughs) should be done at minimum twice daily and anything unusual reported so that minor issues may be dealt with before they become problems.

The critical point of LSS for facilities with open systems is the array of pumps and water distribution valves needed to deliver seawater to the exhibits. In these cases the maintenance of temperature in the exhibit will depend solely on a constant input of clean seawater, and pumping systems should be equipped with flow sensing elements and an alarm system. The tank should also be equipped with temperature monitoring devices, which may also (ideally) be connected to an alarm system.

Regardless of whether aquaria use open or closed systems it is highly advised that an automated telephone paging system be included in the design if 24-hour staff is not present. Such an alarm system should call at least two key staff members in the event of an emergency (i.e., reduced flow, elevated temperatures). Emergency power generators provide an extra layer of security. Automatic transfer switches restore power in a matter of seconds for key LSS such as chillers and pumps. Computerized systems will set alarms and trigger automated paging of emergency personnel in the event of a transfer to emergency power.

The probes of temperature sensing equipment should be maintained by continuous assessment of their performance and replacement when necessary. The reading of electronic probes/thermocouples should be compared with readings of tank temperature taken with an independent thermometer periodically (ideally on a daily basis). Probes that can be removed from the tank water or thermowell should be periodically tested with warm water of a known temperature, as should alarm/paging systems. Probes or temperature controllers failing to maintain accurate and precise readings should be replaced or repaired immediately.

1.2 Light

Careful consideration should be given to the spectral, intensity, and duration of light needs for all animals in the care of AZA-accredited zoos and aquariums. Cephalopods possess sophisticated visual systems, far advanced over most other invertebrates, with the possible exception of the Arthropoda. Nautiloids possess well-innervated retinas without lenses that function like a pinhole camera, while octopods, cuttlefishes, and squids possess a much more highly developed eye (Ruppert & Barnes, 1994). The eyes of these taxa are analogous in both form and function to the eyes of vertebrates in that they contain a lens, iris, and cornea, which serve to focus light on a highly innervated retina (Ruppert & Barnes, 1994). It is because of this highly developed eye that the octopods have extremely good visual acuity used in hunting and predator avoidance. Cephalopods have been shown to have excellent night vision (Allen *et al*., 2010) and contrast perception, though they are color-blind (Mäthger *et al.*, 2006).

There is a common misconception among many cephalopod keepers that these species cannot see red light. Some have displayed octopods under a reverse day-night cycle in the hopes of taking advantage of their nocturnal predilections. Messenger et al. (1973) showed that octopods are indeed color blind, and as such are not able to discern one color light over another, but the absorption spectrum of the eye of *Octopus* spp*.* is nearly identical to that of man (though slightly shifted towards the lower frequency blue colors, as is typical of marine organisms). Because of this, octopods see nearly the same spectrum of light as humans, though without the ability to discriminate colors. Given these facts, the practice of exhibiting octopods under a reverse lighting cycle with red lights should be avoided, as it will severely disrupt circadian rhythms of the animals being maintained.

The effects of seasonal variation in terms of lighting have scarcely been studied with regards to octopods. Several aquaria have maintained specimens in open system exhibits allowing for natural variation in temperature cycles (as described above), though there is little information available on the effects of seasonal lighting schemes. Lighting in an aquarium setting should not be intense, as the GPO is a largely nocturnal species tending to be rather reclusive in the daylight. The light in an octopus exhibit is more for illumination so viewers can see the animal. It is not necessary to keep them in light so dim that it impairs visitor's opportunity to see the animal.

A cursory survey with a photosynthetically active radiation (PAR) meter indicates some typical light levels at the water's surface, though light needs will vary depending on the shape of the exhibit, rockwork present, and type of animal containment (e.g., lids, barriers) in place. A general guideline in lighting shallow exhibits (<1 m [3.2 ft] depth) would be to target approximately 40–75 µmol m^{-2} sec^{-1} (~2–4k Lux) (Christie, unpublished data). Deeper exhibits (>1 m [3.2 ft]) will achieve the desired effect at PAR intensities of 75–150 µmol m^{-2} sec^{-1} (~4–8k Lux) (Christie, unpublished data). Despite these data, the level of lighting is subjective and the response of the animal as demonstrated by its behavior should be accounted for when changing/modifying lighting. Lighting intensity in terms of PAR decreases exponentially with increasing water depth, so manipulation of lighting levels is most easily achieved by moving the light source further away from or closer to the water's surface.

As light emitting diode (LED) aquarium lighting is becoming more popular it is worth noting that some aquarists have seen negative reactions in cuttlefish kept under such lighting at intense levels (B. Siegel, personal communication). Intense LED illumination produces comparatively higher proportions of polarized light over traditional lighting technologies, and the cephalopod eye has long been known to discriminate light polarized into different planes (i.e. Taskai and Karita, 1966; Saidel et. al., 1983). Remarkably, cephalopods can not only perceive polarized light, but can use it to produce patterns on their skin (Shasar and Hanlon, 1997) which some species use to signal their conspecifics (Boal et. al., 2004). Specimens of *E. dofleini* have been kept under subdued LED lighting in at least two different aquaria without negative reaction, but if maladaptive behavior is seen in relation to lighting this factor should be considered when troubleshooting the issue.

Finally, it is important to note that most cephalopod species are extremely sensitive to intense lighting, often inking or displaying other maladaptive behaviors when subjected to such stress. As public aquaria are open to many visitors care should be taken to minimize the effects of light pollution. Camera flashes can be especially powerful and disturbing to cephalopods, and have been implicated in inking events or excessive bumping into the exhibit, which can compromise the integument of the animal. Individual animals may respond differently to light, so observe each animal, and adjust the lighting to suit the proclivities of individual animals as needed. If flash photography is permitted at your institution, management should strongly consider installing signage to restrict or discourage flash photography at cephalopod exhibits.

Figure 5. Aquarium signage to prevent undue stress to the GPO through flash photography. Photos courtesy of B. Christie

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 is the most important aspect to consider when housing GPOs or any other cephalopod. Oestmann (1997) lists water quality as one of two primary components for cephalopod care, the other is monitoring. Many of the water quality parameters suggested here for GPOs are universal to all cephalopods.

Water chemistry levels for octopus are generally maintained similar to many marine invertebrates. Though some cuttlefish have been shown to have tolerance of high levels of nitrate the following levels are accepted as preferred. A good detailed reference for general aquarium water quality analysis may be found in Spotte (1992). The facility wishing to keep a GPO should have an established program of water chemistry analysis and at minimum the ability to monitor ammonia (NH_3), nitrite (NO_2), nitrate (NO_3), temperature, and pH. Many institutions have discontinued nitrate testing due to the carcinogenic reagents, though newer methods that do not involve cadmium in the reagent are now available. In cases where institutions do not quantify nitrate the aquarist is advised to be diligent in the removal of organics in the system through foam fractionation (protein skimming) and gravel siphoning to indirectly mitigate nitrates. Institutions test water chemistry at various intervals ranging from weekly to monthly, though it should be noted that longer intervals are usually indicative of very well established systems that are constant in every other regard.

Nitrogenous compounds: When nitrogenous compounds, especially ammonia and nitrite, rise above acceptable levels in an established system it may be an indicator of accumulated uneaten food (especially in the octopus midden), detritus accumulation, or excessive feeding. A rise in nitrates or a drop in pH below normal parameters may indicate the need for water change. The aquarist responsible for a GPO is advised to consider water quality data in determining the frequency and size of regular water changes to maintain proper pH levels and keep nitrates low. As with any closed seawater system frequent backwashes of sand filters and proper tuning of foam fractionators will remove appreciable amounts of organics from the system.

Ammonia is most commonly measured as ionic ammonia (NH_3), though ammonia nitrogen (NH_3-N), ammonium (NH_4), and total ammonia nitrogen (TAN) are commonly measured by spectrophotometers and colorimeters used by most facilities. For the purposes of this discussion the ammonia levels cited will be approximately applicable to ionic ammonia (NH_3) or ammonia-nitrogen (NH_3-N). Conversions between these species of aqueous nitrogen compounds may be found in Spotte (1992) if needed.

Ammonia levels should be undetectable in the aquarium, and should not exceed 0.02–0.05 mg/l (ppm). This is standard practice in fishkeeping, but is especially important with GPOs due to their unique physiology. The species is known to excrete metabolites as ammonia (47%), urea (15%), and the balance (presumably) as protein-complexed nitrogen (Potts, 1965). Potts (1965) also determined that extra-renal nitrogen elimination was a greater factor than renal excretion. Given the porous nature of the microvillus epidermis of cephalopods the implications of danger resulting from excess ammonia in the ambient seawater are obvious. Potts (1965) also determined (under experimental conditions) that severe respiratory distress including cessation of ventilation can occur in *E. dofleini* when serum ammonia concentrations increase too rapidly.

Nitrite (NO_2) is especially toxic to freshwater fishes as it binds with hemoglobin to form methamoglobin inhibiting formation of oxyhemoglobin and thus impairing respiration. In cephalopods the interaction of nitrite with hemocyanin has yet to be defined, though prudence dictates maintaining this ion at similarly low levels as with ammonia. Nitrite is best kept less than 0.020–0.050 mg/L and should not exceed 0.100 mg/l though it is often undetectable in established aquaria.

Nitrate (NO_3) levels have been anecdotally linked to stress in cephalopods, and in some cases levels excessive nitrate-nitrogen have been shown to cause acute distress and severe inking in some cuttlefish species (Hanley et. al., 1998). Most other cuttlefish species and octopods have not shown similar acute distress, but nevertheless nitrate should be maintained as low as possible and less than 20 mg/l (ppm) in closed systems. In 2012, 90.6% of surveyed institutions (n=33) responded that they maintain their nitrate levels at 20 mg/l or under (with many striving to keep levels <10mg/l). When nitrate levels exceed this limit a large water change (or series of water changes) is indicated to effect a 50–70% reduction. Heterotrophic denitrification systems, while complex, were in use to good effect at one institution (no longer in existence) on cuttlefish and squid culture systems, but to the authors' knowledge neither heterotrophic or sulfur-based systems have yet to be employed in a public aquarium for *E. dofleini* husbandry.

Phosphorus: Orthophosphate (PO_4) is generally kept low in tanks housing invertebrates, but has only been conclusively shown to be problematic to scleractinian corals as it displaces magnesium in skeleton formation. In cephalopods there is no evidence as of yet to suggest that phosphate levels affect the health or well-being of aquarium-housed cephalopods. Preliminary investigations at one aquarium have shown that feeding response does not negatively correlate with orthophosphate, indicating that increased levels do not affect appetite, the primary indicator of good health in cephalopods kept in aquaria (Christie, unpublished data). That being said, orthophosphate, like nitrate, is an indirect indicator of dissolved organic carbon levels, and reducing concentrations wherever possible will only serve to maintain stable water quality. Both lanthanum chloride and granular ferric oxide media have proven to be safe for use with GPOs should an institution wish to mitigate this ion in their exhibit water.

pH: The pH levels are best kept between 8.1–8.3, analogous to natural sea water. GPOs will tolerate slightly lower pH without detriment, though it is important to remember that in closed systems carbonates and bicarbonates are essential for the reduction of ammonia to nitrite and subsequently nitrate by nitrifying bacteria in the biofilter, and octopods produce extreme quantities of waste. In addition pH is a good indirect indicator of appropriate alkalinity and carbonate levels. Low pH may be an indication of high levels of dissolved organic carbon (DOC). Among 33 institutions surveyed in 2012, a total of 90.7% of institutions keeping GPOs maintained their pH in the 7.9–8.3 range. Stability of pH is most important, as rapid fluctuations (up or down) will cause massive disruptions in acid-base regulation, thus affecting oxygen uptake (Pörtner, 1994). Rapid changes in pH have been documented as causing acute mortality in cephalopods (Hanlon *et al.*, 1983). When adjusting pH the aquarist should not exceed ±0.2 units day^{-1} as this level of change is generally well tolerated by squids (Hanlon *et al.*, 1983), which tend to be among the most delicate of cephalopods in captivity.

As carbonate ions may be depleted in seawater by vigorous nitrification activity it may be necessary to raise pH before water changes are required to keep nitrogenous compounds in check. Cephalopods generally have shown no adverse reaction to standard bicarbonate buffer additions. Buffer may be added as needed to maintain a stable pH, a common practice being to add 1:2:12 sodium tetraborate to sodium carbonate to sodium bicarbonate, or 1:6 sodium carbonate to sodium bicarbonate. Sodium bicarbonate alone may also be used but its efficacy in maintaining a constant pH is less effective than the aforementioned mixtures.

Table 3. Water Quality Parameters for *E. dofleini*.

Parameter	Ion	Units	Ideal Levels	Abnormal Levels	Dangerous Levels
Ammonia	NH_3	mg/l	0.00	0.05–0.09	>0.10
Nitrite	NO_2^-	mg/l	0.000	0.050–0.099	>0.100
Nitrate	NO_3^-	mg/l	0–19	>20	>50–75
Phosphate	$PO4^{3-}$	mg/l	0.00–0.25	0.25–2.50	n/a
pH			8.00–8.30	7.70–8.00	<7.70
Temperature		°C	6–12	13–14	>14
Salinity		‰	28–33	15–27; 34–36	<15, >36
Oxygen		% Sat	85–95	75–85; 95–100	<75; >100
Copper	Cu^{2+}	mg/l	0.00	0.01–0.03*	>0.03

* Copper is dangerous to cephalopods in any concentration but the margin of error for most spectrophotographic analysis is roughly 0.01-0.03 mg/l

Salinity: Salinity is an important factor in the maintenance of cephalopods in aquariums. Studies of wild populations of *E. dofleini* have shown a strong influence of coastal salinities on distribution. Hartwick et al. (1984) raise the question as to whether a physiological intolerance to low salinity shapes distributions of octopods in the wild, noting trends within their research and prior published accounts of a strong negative correlation between rainfall and octopus abundance in the Sea of Japan. Alaskan populations were found at normal full-strength seawater concentrations from 32–33‰ (Scheel, 2002). Data from 33 institutions surveyed in 2012 showed that 54.8% of aquaria were maintaining their GPOs salinity at 31–32‰ with a total of 93.5% of respondents striving to maintain an overall range of 31–35‰. In the aquarium rainfall events are only of concern to coastal facilities utilizing open-system tanks, but the need for diligent monitoring and adjusting of salinity is nonetheless important. As with any marine tank salinity should be measured often to prevent increase in salt concentration through evaporation.

Life support systems should also be checked frequently (at least twice daily) for malfunction that may introduce excessive concentrations of freshwater and depress salinity. Such failures are most common in protein-skimmer wash-down mechanisms, where a timer or valve failure will slowly introduce freshwater to the system. While mechanical failure is something to be vigilant against, most incidents of accidental freshwater overfilling occur through human error: a hose left filling a tank can have disastrous consequences. The lower limit of salinity tolerance for *E. dofleini* is 17‰ (Hartwick, 1983); below this animals are subject to extreme stress and the exposure will prove lethal given sufficient time. Higher salinities may not be ideal for the species either; Scheel (2002) reports that wild populations in Alaska avoid habitats with salinities in the 33–35‰ range. It is thus recommended that salinities be maintained slightly less than that of full strength seawater (i.e., 27–33‰).

Heavy metals: Water treatment is necessary with most municipal water sources. Copper, zinc, and other components need to be removed before use in invertebrate systems. Carbon filtering alone is usually inadequate in remediation of elevated heavy metal levels in the source water and thus deionization or reverse osmosis treatment of the source water is important step. EDTA (ethylenetetraaminediacetic acid) has also been shown to be safe for use with cephalopods at a rate of application of 1 mg EDTA per mg absolute (mg/l x total liters) ionic copper. Once chelated with EDTA heavy metals are less toxic and less biologically available but still present in solution, activated carbon will then be more effective in removing the metal-organic compounds from solution.

Trace Elements: Little is known about the interaction with trace elements in seawater and cephalopod physiology. Many institutions add iodine/iodide or a general trace element solution to their closed systems as a matter of protocol, though there is a paucity of published literature on the subject. One area where trace elements are of critical importance, however, ins in the rearing of paralarvae and juveniles. Hanlon *et al.* (1989) showed that strontium is critical (>8mg/l) for the development of calcareous structures (namely the statolith, required for proper balance and orientation) in young cephalopods.

Bacterial flora: Public aquaria and zoos routinely measure levels of coliform bacteria in certain exhibits (i.e., marine mammals, sea turtles, etc.). Few, if any, aquaria routinely attempt to quantify the total number of aerobic bacteria present in seawater, though such estimations are possible (see Hemdal, 2006). Specific bacterial counts, while possible, are not actually necessary in husbandry; though the aquarist should bear in mind that cephalopods in aquarium systems have been shown to have over 100 times the levels of bacteria present on their epidermis as their wild counterparts (Oestmann et al., 1997). As such, wounds or other trauma to the skin are much more readily colonized by opportunistic bacteria and present an additional risk of infection. A number of experts in cephalopod husbandry (i.e., Oestmann et al., 1997; Anderson, 1995; Forsythe, 2002) have recommended the use of ultraviolet sterilization (UV) in the LSS of cephalopod tanks. UV sterilization will dramatically reduce the bacterial flora in a closed-system tank and is highly advised as a design component of any GPO exhibit. Of aquaria surveyed in 2012 (n=33) 35.5% used UV sterilization and 12.9% used ozone on their octopus exhibits, a total of 48.4% of institutions employing some form of sterilant technology in the LSS design.

Cycling: Cycling of new tanks should be done before adding an animal such as a GPO with a high level of waste production. In closed systems, ammonium chloride (NH_4Cl) is commonly used as an ammonia source to feed the biofilter and grow sizeable populations of nitrifying bacteria such as *Nitrosomonas spp.* and *Nitrobacter* spp*.* (among others) to process ammonia and nitrite, respectively. It is advisable to start with additions of ammonium chloride, and gradually increase the dosage until a 1.0 mg/l concentration of ionic ammonia is achieved. As GPOs have a high waste production, a tank should be considered ready to receive animals when a 1.0 mg/l ammonia level is reduced to <0.05 mg/l ammonia and <0.050 mg/l nitrite within 24 hours for several consecutive days at the target temperature. Supplementation of carbonates by using a buffer mixture during the chemical cycling process is advisable for maximum effectiveness.

Octopus ink fouling: One major problem in keeping octopuses in closed system tanks is the possibility of the animal inking and polluting their water. The ink has not been identified as toxic, but interferes with oxygen transmission at the water-gill interface (Oestmann et al., 1997). At least one report has directly attributed the death of aquarium-housed *Octopus bimaculoides* directly to ink clogged around the ctenidia (gills) (Bennett & Toll, 2011). See Chapter 6.7 for information on resuscitation of animals with intramantle ink clogging the gills. Should an animal ink in a closed system tank, most of the water should be changed as soon as possible, especially in smaller systems.

Red octopus (*Octopus rubescens*) ink seems more viscous than that of a GPO, and doesn’t dissipate as quickly. In cases where large octopods have inked their tanks some have noted little or no ill effects to the animal though in other cases the authors have seen water quality degrade rapidly. The degree to which a closed system is affected in such a case will be a function of the system volume, the amount of ink released, and the life support systems in place. Activated carbon and aggressive protein skimming will sequester and remove octopus ink, respectively, although without a water change the efficacy of carbon filtration is diminished as it is quickly plugged up and rendered incapable of further adsorption.

Water exchange: Regular water change rates vary greatly depending on tank size and aquarist general strategy of smaller and more frequent changes or less frequent and larger changes. One reference point in the closed system environment is to change a 500-gallon tank at least once a month by replacing 25–33% of the water, though this may take the form of smaller more frequent exchanges. Anderson (1995) reports several water exchange regimens that have been successful in maintaining GPOs in closed systems. At one aquarium, a 33% water change is conducted every 2 weeks on closed systems. Another zoological institution has exchanged 80% of their exhibit water semi-annually with success, and yet another aquarium, approximately 5–7% of the exhibit water is exchanged per week.

Larger tanks and open systems can mean far fewer water changes. A regular water change schedule will provide a reliable means of preventive maintenance. Water test results falling outside of desired ranges necessitate the need for a water change or other corrective action. Any unusual events, high turbidity, or odors will always call for further investigation and possibly additional water changes. Overall there are many ways to successfully maintain water quality and the institution keeping GPOs will have to devise methods that work best for their situation.

Of 33 institutions surveyed on their husbandry practices for GPOs in 2012, 50.0% of aquaria with closed-system LSS performed weekly water changes. By way of comparison a significant portion (34.6%) performed more than one water change per week, most of these being daily backwashes and gravel siphons averaging less than 5% of the total system volume. The average size of regular water changes performed in closed systems was between 10–30% of the total system volume. A few facilities performed water changes as large as 50% regularly, though as would be expected these larger water changes were performed less frequently.

One important factor to consider in water exchanges for cold-water animals is the replacement water temperature. If a source of chilled clean seawater is unavailable, it is advisable to slowly add new seawater when refilling so as not to temperature shock the animal. Temperature variations of 1–2 °C (33.8–35 °F) when refilling are normal and generally do not produce signs of distress (e.g., dramatic color change, increased ventilation, inking) in GPOs housed in a closed system. More dramatic increases in temperature (>2–4 °C [35–39.2 °F]) may unduly stress the animal and should be avoided except in emergency situations.

Emergency ammonia/nitrite remediation: The suitability of ammonia sponges (i.e., Amquel®, Amquel+®, Fritz Ammonia Remover®, etc.) in emergency situations to mitigate ammonia and nitrite is largely unexplored but not completely contraindicated in octopus systems. There are anecdotal reports of octopods and cuttlefishes inking when these chemicals are added to a system, though they have been used at some institutions without visible distress (B. Christie, personal observation). It is impossible to say if these incidents were caused by the addition of the chemical itself or the elevated ammonia/nitrite they were added to counteract, though prudence is dictated in the use of such chemicals. As the microvillus epidermis of cephalopods makes them incredibly sensitive to any and all chemicals in the ambient seawater the use of any chemical additive should be done cautiously and only when absolutely necessary. In most cases aggressive artificial cycling with ammonium chloride and bicarbonate additions will preclude the use of such emergency measures and a series of water changes when practical is a better alternative strategy.

Gas exchange: Air exchange can be best made relevant to aquatic systems in terms of dissolved oxygen and turnover rate of water in LSS. Aeration can be achieved by an interruption of the water at the water surface, use of bioball-type media or falling water into a filter bed (i.e., a standard trickle filter), or injection of air into the filter stream through a venturi mechanism such as those employed on most foam fractionators. The temperatures at which GPOs are kept works in favor of maintaining high dissolved oxygen levels, as gases are more soluble in cold liquids than in warm. Though aeration is necessary, aquarists should be cautioned against having excessive surface area exposed to ambient air (e.g., grossly over-sized trickle filters or large air interfaces at a sump) as this will place a higher demand on the heat exchanger/chiller units.

Aeration methods: Direct aeration by bubbling air through the water should never be performed in an octopus system as octopods have developed embolisms as a result of contact with bubbles in the water column. If aeration is necessary it should be performed in a sump or header tank, and only in the exhibit itself with caution. Octopuses have been documented using air sources in play behavior, though there has been at least one case where a GPO has suffered minor subcutaneous emboli from prolonged contact with direct aeration (Wood, 1999). If compressed air is used it should be supplied by a regenerative or belt-driven blower (or aquarium air-pump on small scales) designed for aquaculture or aquarium use; compressors designed for other uses are not oil-free and may contaminate the airstream with hydrocarbons. Intake filters should be mounted where clean air free of exhaust or other contaminants can be drawn and air filters should be changed regularly.

Dissolved oxygen: Though many aquarists measure dissolved oxygen regularly there is little empirical evidence to define best practices for cephalopods, in general as long as dissolved oxygen (DO) is measured at 85–100% saturation the oxygen levels can be considered adequate. As percent saturation is a function of temperature, oxygen should always be measured as such, or converted from mg/l O_2 to eliminate errors of interpretation. It should be noted that it is not uncommon for microbial activity in an established system to depress DO to as low as 70% saturation in the hours following a normal feeding so measurements should be taken before such events.

As in fishkeeping extreme levels of oxygen (greater than 100% saturation) can be problematic or imminently lethal to cephalopods. If dissolved oxygen is measured at greater than 103–105% saturation an immediate evaluation of the LSS components should be undertaken. Infiltration of air on the suction side of a pump or cavitations of an impeller are the two most common causes of super-saturation in aquaria. Gas entrainment may have many potential sources and bubbles in a sump or other non-exhibit area may be sucked into pump intakes with similar results. At one aquarium, oxygen levels approaching 100% saturation have also been noted to produce behavior akin to agitation or irritation that ceased when the DO was slightly lowered (P. Carlson, personal communication). Studies have shown that in the wild the animals routinely survive in habitats with DO levels as low as 70% (Scheel, 2002). Higher average levels in closed system aquaria are recommended to ensure complete nitrification and proper functioning of LSS. The take home message is that systems should be monitored closely for conditions that may impact oxygen levels.

Turnover: In open systems or semi-closed systems the amount of water input in the system will only need to be sufficient to flush out accumulated wastes and maintain temperature, in closed systems this become much more complicated. Water exchange rates in terns of turnover will vary widely depending on the specifics of an individual life support system. LSS for octopods should be designed to process enormous quantities of waste for a given amount of biomass, but the actual turnover rate may vary anywhere between 1–5 times per hour with equal efficiency in nitrification. The average turnover rate in GPO exhibits from institutions surveyed in 2012 (n=33) is between 1–3x per hour in 65.4% of aquaria; 6.9% of facilities had less than 1x per hour turnover and 13.8% had greater than 4x per hour turnover. Systems designed in a simple loop where a single process stream moves water from tank, though filtration components will need greater turnover to achieve the same nitrification as systems with more complex process streams. In any case the turnover rate will be a function of the surface area available for biofiltration, temperature, total system volume, level of foam fractionation, etc.

Table 4. Frequency of use of various life support systems (LSS) equipment on *Enteroctopus dofleini* exhibits at 33 public aquaria

Type of LSS Equipment	Percent Aquaria Employing	No. Aquaria Employing
Sand filtration	41.90%	13
Foam fractionation (protein skimming)	80.60%	25
Fluidized bed	12.90%	4
Trickle filter	41.90%	13
Bioreactor: Kaldness media	3.20%	1
Canister filters: pleated filters	35.50%	11
Canister filters: filter socks	22.60%	7
Ozone	12.90%	4
Ultraviolet sterilization	35.50%	11
Activated carbon	22.60%	7
Other media (Phosban, GFO, et cetera)	12.90%	4
Undergravel Filtration	19.40%	6
Bead filters	6.40%	2
Deep sand bed	3.20%	1

(AITAG, 2012)

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.

Sensitivity: It is worth noting, at the beginning of this section, that the term hearing in the conventional since is more applicable to terrestrial animals. Aquatic and marine animals are much more sensitive to vibrations in the ambient water column due to the density of water as compared to air and its inherently higher vibration transmission efficiency, exposure of skeletal elements to the water (in some taxa), and possession of various sensory apparatus not found in terrestrial vertebrates, among other factors. As such, vibration detection in aquatic animals is not directly analogous to hearing in land animals; though we will use the term here to encompass the wider range of sensory input resulting from vibration experienced by cephalopods. It is also worth noting that in cephalopods the statocyst is the predominant organ used in vibration detection and maintenance of equilibrium, and is in many ways analogous, though not homologous, to the semicircular canals and associated structures of the inner ear in vertebrates.

The question of octopus hearing has a long and contentious history. Traditionally this species, and cephalopods at large, were once considered deaf (Hubbard, 1960; Wells, 1978; Corner & Moore, 1980; Moynihan & Rodaniche, 1982). Interestingly enough, it was postulated that cephalopod deafness was an evolutionary adaptation to the "booming" vocalizations of odontocete whales, though this theory was rather quickly refuted (Moynihan, 1985; Reid et al., 1986; Taylor, 1986).

Hanlon & Budelmann (1987) reviewed the literature arguing that behavioral, morphological, and physiological evidence all pointed towards the fact that cephalopods were likely able to detect vibrations analogous to what would be considered hearing in terrestrial animals. Despite the disagreement over the cause of supposed deafness in this taxon, continued research eventually showed that the octopus statocyst was indeed sensitive to vibrations between 10–200 Hz (Williamson, 1988). Packard et al. (1990) established that octopus hearing is most sensitive in the lower frequency ranges, namely those sounds less than 10 Hz (the lower limit of human hearing is 20 Hz, for comparison). Recent studies have shown that cephalopod hearing may yet be even more complex than previously thought, and more analogous to hearing in teleost fishes (Mooney *et al.* 2010).

While the mechanism of hearing is quite different than terrestrial species, the detriment of noise may play an even greater role in animal welfare and stress mitigation in aquatic and marine animals. While there is no published reference indicating noise-related stress specific to cephalopods, several investigations have determined noise stress in other marine species (predominantly teleost fishes) to be detrimental to overall health (Banner & Hyatt 1973; Lagadere, 1982; Anderson et al., 2007). These effects may include increased mortality and higher stress (as measured by plasma cortisol levels) (Anderson et al., 2007). Decreases of reproductive viability and survival have also been noted in both fishes and invertebrates (Banner & Hyatt, 1973; Lagadere, 1982).

Nearby noise: As described above, the hearing range of cephalopods is confined to very low frequencies, 0–10 Hz for discriminatory hearing, and 10–100 Hz for non-discriminate detection of vibration. As such most noise present in the nearby environment caused by machinery or other sources will likely be far above the range of hearing in this taxon. Of course, the pumps and chillers associated with the LSS necessary to keep any aquatic animal alive will contribute some vibration to the water column, but these sounds as well are typically high-frequency sounds well beyond the sensitivity range of cephalopods. That notwithstanding, care should be taken in any instance to ensure that pumps are tightly fastened or seated on appropriate rubber or neoprene padding to minimize vibrations and that tanks housing cephalopods are insulated from any other nearby machinery or sources of vibration.

Further research: While the limits of hearing have been explored in recent decades, the effect of noise or sound stress in the Cephalopoda is relatively unknown. While the frequencies of pump noise or other vibration inherent in aquarium environments should be well above the threshold of octopod statocyst detection (i.e., "hearing") it is unknown whether that vibration is detected by other sensory apparatuses or could otherwise contribute to stress in an aquarium setting. It is also unknown to what extent periodic low frequency sounds such as visitors pounding on the tanks or footfalls may be perceived by octopods. While preliminary experiments have been conducted (Christie, unpublished data) it is as of yet unknown whether octopods can discriminate between different patterns of low frequency sounds; though it is clear they can be conditioned to discriminate between higher and lower frequency sounds (Packard et al., 1990; Christie, unpublished data). The role of hearing in the ecology of *E. dofleini* in the wild also merits further study, especially considering the long-standing presumption of deafness and other speculation into the role of their sensory faculties.

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. Animals should be presented in a manner reflecting modern zoological practices in exhibit design (AZA Accreditation Standard 1.5.1). All animals must be housed in enclosures and in appropriate groupings which meet their physical, psychological, and social needs. (AZA Accreditation Standard 1.5.2).

AZA Accreditation Standard

(1.5.1) Animals should be presented in a manner reflecting modern zoological practices in exhibit design, balancing animals' functional welfare requirements with aesthetic and educational considerations.

AZA Accreditation Standard

(1.5.2) 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.

Species appropriate behaviors: Octopuses are known for their exploratory behavior. Octopuses are also known for spending large amounts of time tucked into a den with reduced levels of stimulation. The relative amounts of time spent doing specific behaviors are still not clearly understood. Still the known behaviors are relevant to habitat design and enrichment measures offered.

Social structure: GPOs are the largest species of octopus and simply housing one individual requires at bare minimum a 500-gallon system (preferably 1,000 gallons or more). Housing multiple octopuses in a small space will lead to aggressive behavior and the sex ratio of the collection does not appear to affect the level of aggression. This is generally attempted in only 10,000 gallon or larger systems though even at this scale one animal may kill the other.

Exhibit size: A survey of 33 public aquaria keeping GPOs in 2012 revealed that 33.3% of institutions had exhibits between 1000–1500 gallons, 26.7% under 1000 gallons, and 20.0% had exhibits between 1500–4000 gallons. Four of the surveyed institutions (13.33%) have GPO exhibits in excess of 4000 gallons. At least five facilities maintain their GPO in a tank that shares life support with other exhibits; in these cases the mean size of the habitat was 523 gallons with a mean total system volume of 1283 gallons.

A 500-gallon system is a general minimum though it is important to note that adequate filtration capability is the most critical consideration for long-term health and well-being. There is little information to suggest a minimum size needed for psychological well-being, though most aquarists familiar with GPOs will attest that regardless of tank size enrichment or interaction may be important to mitigate self-destructive behavior or escape attempts. One zoological institution's behavior data indicates that octopus behavior and responses to enrichment were unchanged when housed in a 500-gallon tank vs. a 1,500-gallon tank (with the same GPO). Enrichment and appropriate stimuli may be as simple as periodic *ad libitum* feedings of live prey items or as complex as prey puzzles and other interactive enrichment devices, this is covered in depth in Chapters 8.1–8.2.

Exhibit elements: A den is an important feature to make available for a GPO. It does not seem to need to be a space that completely conceals the animal. Behavior studies provide an indication that the ability to press arms into a corner of 2–3 sides provides a den-like setting that enables the animal to rest and hang for long periods of time by choice.

Females protect themselves and their eggs even further by piling rocks up to block the entrance to the den. This situation would seem to make it impossible to display such animals in public aquariums, but this is not the case if displays are creatively designed with the above considerations in mind. Since GPOs frequently grow to 60–80 lbs in an aquarium setting, the display den should not be much bigger than the volume needed for an octopus that size. The opening should face down-slope, so the den should be elevated a bit and facing down, since octopuses like to look out from their dens at a downward slope (J. Cosgrove, personal communication). If there is a likelihood of a female being kept, there should be a hard ceiling to the den, so she can attach her eggs to it. Adult female octopuses are very reclusive. To show the inside of an octopus's den, creative exhibitory can include a dark nook in the public area with either one-way glass or tinted glass to view the back of the den. Exhibit designers need to remember that the den should be dark for the octopus's comfort, and in order to see into the den, that part of the public area should also be dark. Note that octopuses are colorblind, and that use of red light will not be beneficial in this case.

The den is considered to be the most important aspect of any exhibit design. There is compelling evidence that this is even a critical factor in octopus recruitment in the wild as well. GPO populations surveyed in Alaska strongly preferred habitats with large boulders (Scheel, 2002), it may be safely assumed the key factor being suitable dens that support larger octopus populations. In *O. bimaculoides* competition for suitable dens has been found to be the means by which dominance is exerted and social hierarchies formed (Cigliano, 1993).

Figure 6. Six views of GPO exhibits at public aquaria. Top left: A former GPO exhibit; aquarist shown for scale. Top right: A GPO swims amongst numerous rockfish and other invertebrates. Middle left: GPO exhibit with a small male specimen. Middle right: A GPO in a cylindrical exhibit (photo by J. Marvin). Bottom left and right: Two views of a GPO exhibit with acrylic shift doors to allow staff to control access between halves of the exhibit. Photos courtesy of B. Christie, except where noted.

Both red octopuses and GPOs have been kept successfully in closed system aquariums. There is much more experience and literature on keeping GPOs because of their popularity in public aquariums. The systems for keeping GPOs in closed systems in two public aquaria are described in detail in Prescott

& Brousseau (1962) and in Bronikowski (1984). Obviously, since a GPO grows to such a large size the tank needs to be sized accordingly, and a closed system octopus tank needs to be larger than an open system tank to maintain oxygenation and waste removal. One aquarium maintained a 500 gallon display tank as part of a 2500-gallon system and has kept up to a 39 kg (86 lb) animal in it (Bronikowski, 1984).

Octopus exhibits at one North American aquarium: One aquarium well known for GPO displays has displayed octopuses in three tanks more recently that were different from previous versions. The first was an octagonal tank about 800 gallons made of all glass panels with a den in the middle. The tank was 1.8 m (6 ft) across. This tank was difficult to perform standard husbandry practices on, but was practical for exhibiting the animals.

The next version of a GPO tank was nearly ideal from a maintenance and animal health viewpoint. This was a triangular tank custom-made of fiberglass with one blank backside against a wall and two unequal sides glazed with sheets of acrylic, 3.3 m (11 ft) and 4.2 m (14 ft) long. The windows were unequal because there was a .6 m (2 ft) section of one window close to the wall that was cut out for a water-level drain sump (screened of course to prevent octopus escapes). The water was approximately 1.2 m (4 ft) deep. Overall, it was nearly2.4 m (8 ft) high, giving .9 m (3 ft) of air above water level. This was evidently enough to keep an octopus from crawling out, as none ever did, although one escaped through the screen of the water outlet into the sump.

There was planking 1.2 m (4 ft) wide just above water level for keeper and interpreter access. This was accessed by a captain's ladder outside and over the drain sump. There were several wooden steps down to the deck. To lift heavy rocks in and out of the tank, and also to lift heavy octopuses, an I-beam was installed on the ceiling that had a traveling trolley and an electric winch. This could be used to lift animals in and out or used to weigh octopuses, was done weekly in a public demonstration. The first octopus in this tank grew 2.7% a day by weight over 4 months. Thereafter his food was reduced. Incidentally, the exhibit provided an area where octopus food, growth, and weight were discussed. The octopus's weekly weight was recorded there.

Inlets to this tank were provided by PVC piping under the wooden deck and down inside a 30 cm (12 in.) PVC pipe in the center that helped support the deck and provided décor, as it was painted to resemble a stressed wood piling. The water inlets were adjustable and emerged from the piling at the bottom. The outlets were adjusted toward the front corner of the tank near the surface, and hence the octopus mostly rested there in full sight. Unfortunately, this was one of the failures of the tank: the octopus rested on the front mullion where the two acrylic panes came together, a space about .3 m (1 ft) wide. Because of this the octopus was not always clearly visible. This tank could have been constructed of all acrylic with no fiberglass structural elements, thus making the octopus more visible. However, in this instance, it would have been expensive.

Figure 7. Triangular octopus tank featuring a 43 kg (95 lb) GPO; individual shown for scale.
Photo courtesy of R. Anderson

The most current GPO tank at this aquarium is a 3-meter (10-foot) vertical cylinder connected by a .9 m (3 ft) horizontal cylinder to a 1.2 m (4 ft) vertical cylinder (see Figure 8). Each vertical cylinder has artificial rockwork made of gunnite concrete at the back of the tank. The large cylinder has a ladder behind the scenes that is used for tank access by divers, feeders, and interpreters. The rockwork has slanting ledges for water access.

The water in all three parts of the tank is 1.5 m (5 ft) deep as measured from the outside floor with an additional 152 cm (30 in.) of cylinder above the water level. Husbandry staff requested 91 cm (36 in.) of air above the water but the design staff cut it down to 76 cm (30 in.). Consequently, three GPOs have crawled out of this tank. Two survived a brief exposure to air (one was a senescent male), but one female did not survive and crawled “out of the frying pan into the fire,” so to speak, and she died at a particularly inopportune time in the aquarium’s octopus program.


Figure 8. Octopus exhibit at a North American aquarium. Photo courtesy of R. Anderson

This tank was originally designed so that a single octopus could be trained to crawl back and forth through the horizontal cylinder between the vertical cylinders, thus giving enrichment to the public, but this has not proven practical. In addition, this tank was mandated to be a “multi-species” tank, to hold wolf eels, rockfish, and sculpins along with various invertebrates, in spite of the staff’s caution that GPOs would eat the fish. The octopuses did eat some of the fish, which horrified the interpreters, but provided enrichment to the GPOs. The octopuses also forced the wolf eels from their dens into the small cylinder where they looked out-of-place. To keep the wolf eels in the large cylinder, a porous barrier was placed in the horizontal cylinder. This also provided an opportunity to put another GPO in the exhibit. Typically a female was in the smaller vertical cylinder and a male in the larger. The barrier is typically removed with fanfare on Valentine’s Day to allow the GPOs to mate. To prevent the octopuses from crawling out of the small cylinder we now place a circle of plywood on it covered with Astroturf most of the time except during feeding.

This tank has various fiber-optic lights coming from the

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.

cracks in the rockwork, which are largely ineffective, and about 10 adjustable water inlets. It has filtered seawater, unfiltered seawater, and 40 gpm recirculated seawater. It does not have additional filtration or sterilization. For acclimating females to males and vice versa, water direction can be reversed through the horizontal cylinder. An air pump installed in the system allows air bubbles to come out of the tank for enrichment. The outlet to the female's tank runs to the bottom of Elliott Bay to attract males to the area.

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.

The same careful consideration regarding exhibit size and complexity and its relationship to the octopus's overall well-being must be given to the design and size 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).

Off-Exhibit holding: Where it is necessary to house a GPO off-exhibit, whether it be a holding, quarantine, or hospital situation the primary concern is in providing adequate filtration and chilling capabilities, although a den or some form of structure should still be provided to ensure psychological well-being. This need not be naturalistic, and with smaller octopods often takes the form of a terracotta flowerpot. Five gallon HDPE buckets with the metal bale removed or short sections of 15–30 cm (6–12 in.) ID PVC pipe work exceptionally well for large animals. As these animals are especially light sensitive off-exhibit holding tanks should have comparable subdued lighting and day/night cycles as an exhibit would have.

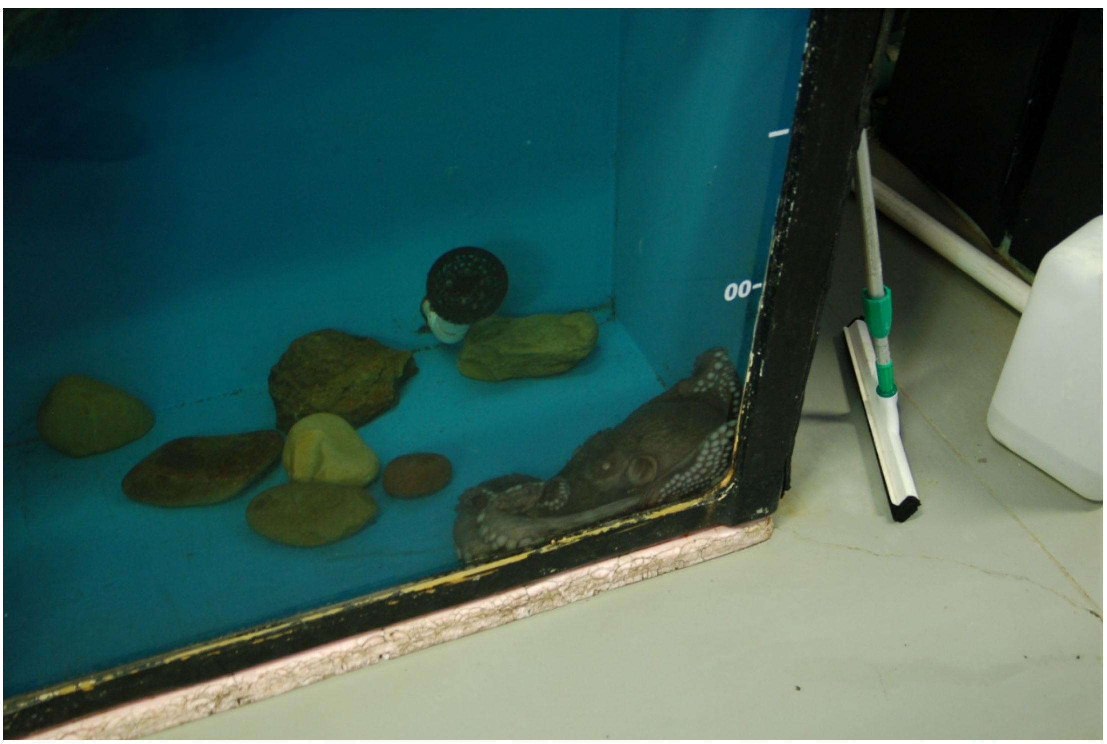


Figure 9. A senescent GPO in a typical holding/quarantine tank. The tank is smaller than a standard exhibit tank and sparse in décor, but rocks and a den (not pictured) are made available to the animal. Photo courtesy of B. Christie

Environmental variability: Introducing objects for GPOs to explore has been a well-established practice (Rehling, 2001). How often, how elaborate, and frequency or intensity of the stimulation is still under debate and study. It is clear that introducing objects whether simple or puzzle-like stimulate a natural exploratory behavior. Studies with *Octopus vulgaris* have shown that animals spend significantly more time on the glass wall of an aquarium looking out when an outside stimulus is present (Byrne et al., 2002). The ability to induce exploratory or curiosity behavior with stimuli is well accepted; but the generalization of the mammal-model of enrichment does not directly translate. Over stimulation for a species that spends a great deal of time hiding in a den merits consideration when planning an enrichment regimen or changing the habitat in which it lives.

2.2 Safety and Containment

Animals housed in free-ranging environments should be carefully selected, monitored and treated humanely so that the safety of these animals and persons viewing them is ensured (AZA Accreditation Standard 11.3.3).

AZA Accreditation Standard

(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 selected, monitored, and treated humanely at all times.

Escape capabilities: There has been a large amount of anecdotal evidence collected regarding the escape capabilities of GPOs. Octopuses, in general, are strong animals and GPOs are especially forceful due to their size. GPOs also are able to squeeze themselves through any hole that is large enough to fit their chitinous beak through; the rest of their body lacks any hard parts so it can squeeze through in a variety of shapes. There are instances where GPOs have crawled out of their tanks during the night. The animal generates a great deal of mucous in an effort to not dry out. Some animals crawl back into their own tank, some can be revived once put back in the tank, though many do not survive. Owing to these facts, a GPO system must have any holes and escape routes sealed. Many institutions have installed locks on the lid of the system that stop the octopus from getting out but also stop anyone else from getting in.

Figure 10. Containment strategies for octopus exhibits. Top left: A 2-piece acrylic lid with stainless steel latches. Top right: A tank with extensive Astroturf on the lip and down the sides to prevent octopods from being able to grip the surfaces. Bottom: A GPO tank. Note structural beams and tank struts covered with Astroturf, and acrylic shift doors (left). At right, note the fiberglass lid with all internal surfaces coated in Astroturf. Photos courtesy of B. Christie

Figure 11. A GPO exhibit (left) with views of the sliding acrylic lid for containment (top and bottom right). Note at top right the plastic mesh and epoxy preventing the animal from crawling behind the fiberglass backdrop. Photos courtesy of R. Drinnen

Figure 12. An open topped octopus exhibit. Left: The exhibit from the public area. Note the flash photography signage. Right: The open topped tank (top) and measuring tape showing the combination of 1 foot of clearance and Astroturf to prevent the animal from being able to grip the tank's exterior is sufficient for containment. Photos courtesy of J. Frey Sr.

Animal exhibits and holding areas in all AZA-accredited institutions must be secured to prevent unintentional animal egress (AZA Accreditation Standard 11.3.1). Pest control methods must be administered so there is no threat to the animals, staff, and public (AZA Accreditation Standard 2.8.1). 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.

> **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.

Containment strategies: Specific containment strategies generally fall into one of three major categories; some facilities utilize a combination of some or all of these:

1. **Use of a secure, locking lid to prevent escape**. This may be made of fiberglass, acrylic, or any other material suitable for exposure to the humid and wet conditions. The lid can either be bolted and latched in place, or weighted down to prevent escape. It is worth noting that a full-grown GPO could lift a weighted lid. Care should be taken to ensure that the types of materials used are suitable for contact with a marine aquarium even if they will not be directly in contact with the seawater.
2. **Use of a material that will not adhere to octopus suckers**. Astroturf is a popular choice as it is cheap, durable, and readily available at any hardware store in multiple colors. Astroturf-type material inhibits the animals from sticking to the substrate and also acts as a deterrent as the animals seem to dislike the sensation of the material against their epidermis. It is important to pay attention to the backing of the materials when installing, as some types have a more durable mesh backing than others. High-quality outdoor carpeting can also be used to similar effect, though it tends to trap more water (leading to mold growth) and is more expensive per square foot.
3. **Incorporating a high vertical surface.** A smooth vertical wall all around the tank can be used to prevent escape (though where this is incorporated it is usually coated with Astroturf as extra insurance). The amount of vertical space needed will vary across different species and size ranges. A vertical clearance of at least 1 meter is *usually* sufficient for containment of most specimens of *E. dofleini*.

Exhibits in which the visiting public is not intended to have contact with animals must have a guardrail/barrier that separates the two (AZA Accreditation Standard 11.3.6). As octopods are aquatic, there will obviously be glass or acrylic separating the animal from the public. If an animal is kept in an open-topped exhibit it should be constructed such that the public cannot reach the water's surface.

> **AZA Accreditation Standard**
>
> **(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.

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 11.2.4). In the event of a catastrophic emergency (hurricane, tornado, flood, et cetera) procedures should be available to ensure the functionality of the LSS and prevent release of non-native species. AZA Accreditation Standard 10.2.1 mandates that warning mechanisms and back-up systems must be available for critical LSS. With the strict temperature tolerances of GPOs and their extreme waste output the chilling systems and pumps that maintain flow across the biofilter should be considered necessities and should be tied to a backup generator or have the ability to be connected to emergency power quickly. As with most aquatic animals, if power or LSS is compromised, feeding should cease until the system is restored to normal and the filtration can handle the additional input of organics to avoid catastrophic water quality degradation. In the event of a catastrophic loss of power that cannot be restored in the near future, rising floodwaters, or other similar situation that will result in the imminent

> **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.

death of a captive GPO that cannot be relocated to adequate holding facilities the institution should consider euthanasia to prevent protracted suffering of the animal. Techniques for the euthanasia of cephalopods are discussed in Section 6.7 of this manual.

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). Staff should be aware of the procedures for restoring power to critical LSS, and for resetting critical chillers and equipment in the event of an emergency. If a fire should break out in the vicinity of the tank or its LSS and it is safe to do so staff should be trained in procedures for closing the venturi injecting air into the protein skimmer to avoid concentration of carbon monoxide and other gases in the aquarium water. As is the case with most aquatic animals, octopus escapes are only dangerous to the escapee, and no special training for security personnel is required.

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).

Emergency contact protocols should be developed for problems that may arise. These will vary from institution to institution, but all should address the issues of alerting the appropriate husbandry personnel (typically a manager) who can act as a liaison with veterinarians, life support staff, facilities staff, and outside emergency response (EMS, et cetera) as needed. Call lists for these key positions should be available to all staff, and it should be known by all departments within an institution whom to alert if an emergency situation arises.

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 extremely unlikely event an octopus bite occurs and the victim is unconscious or unresponsive then the EMS system should be activated and institutional protocols for a staff medical emergency carried out. Most octopus bites, however, will simply require first aid (see recommendations below) and nonemergency medical attention.

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.

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

(11.2.5) 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.

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.

Octopus bites: All octopods have venomous-like saliva, and specimens of *E. dofleini* are capable of inflicting a painful bite. The “venom” is a cocktail of cephalotoxin and a host of other digestive/proteolytic enzymes that is not deadly to humans. In the event of a bite simple first aid followed by medical attention will often preclude any serious danger to the bitten individual.

Octopods are not considered to be a dangerous animal *per se,* but like any animal they could potentially bite a keeper. They are mildly venomous and all staff should be familiar with basic first aid response to an envenomation and institutional accident/incident reporting protocols.

One needs to always be cautious in cleaning or working in an octopus tank to avoid being bitten by the animal. The red octopus is especially prone to biting humans and while GPOs seem much less apt to bite their handlers, bites have occurred (Norman, 2000). It may be that since GPOs are so much larger than other octopuses, their handlers have a greater respect for their strength and large beaks.

Published accounts indicate that the swelling and pain of an *Octopus rubescens* bite may be alleviated in as little as one minute with application of heat but headaches, weakness, and potentially even necrosis may persist after the injury (Anderson, 1999). While pain and swelling may be diminished after 1 min, if possible the wound should be kept under hot water (as hot as the victim can stand) for 15–30 minutes. These typical first-aid measures for an octopus bite will be familiar to most aquarists as being similar to the response to stings by lionfish (*Pterois* and *Dendrochirus* spp.) or other fishes of the family Scorpaenidae. The bitten staff member should seek medical attention after any bite and institutional accident reporting/recording procedures should be completed.

It is also worth noting that in some cases octopus bites have shown exceptionally poor healing persisting for months after the incident. In at least one case of an *Octopus cf. vulgaris* bite necrosis and slow healing due to secondary infection has required surgical excision and debridement of tissue months after the bite (Aigner et al., 2011). Aigner et al. (2011) also recommend renewal of tetanus vaccines following an octopus bite if the victim's immunization is not current.

Animal attack emergency response procedures must be defined and personnel must be trained for these protocols (AZA Accreditation Standard 11.5.3).

Typical First Aid for an Octopus Bite*

1. Initiate institutional incident response (e.g., radio call, envenomation alarm, etc.).
2. Determine if emergency medical attention is necessary; activate EMS (call 911) if this is the case.
3. Submerge the affected area in hot but not scalding water (hot as victim can stand) for 15–30 min.
4. Bandage wound and control bleeding if necessary.
5. Seek medical attention.
6. Complete institutional accident reporting procedures.

*This only applies to bites from species of *Enteroctopus* or *Octopus* spp.; bites from species of the genus *Hapalochlaena* (blue-ring octopuses) are potentially fatal and should be treated as life-threatening emergencies.

If an octopus bite occurs, 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 (AZA Accreditation Standard 11.5.3). As *E. dofleini* is not generally considered a venomous or especially dangerous animal emergency drills may be as simple as reviewing the institutional animal attack protocols and reporting procedures. Documentation for such drills and for documentation of incidents involving animal bites need be no more complicated than standard institutional records kept for incidents involving other animals in compliance with AZA Accreditation Standard 11.5.3.

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.

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.

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.

Fasting and logistics: A GPO should be fasted at least 3 days before shipping. All travel logistics should be pre-arranged and staff should be made well-aware of the shipping plan ahead of time (K. Wong, personal communication). All necessary customs and import documents should be in hand before international shipments, and fees should be pre-paid or a check should be made out from your institutions to the applicable USFWS/Customs inspectors (see Appendix G for further info on international shipping). If shipping via air-cargo the flight path and weather should be monitored closely prior to packing an animal for shipment (K. Wong, personal communication).

Moving animals: Equipment usually used to move these animals short distances (i.e., from quarantine tank to exhibit tank, or exhibit tank to shipping container) usually include trashcans with lids, ice chests, or other similar containers capable of moving a specimen of this size and an appropriate amount of seawater. The most convenient way of moving a specimen is to have it conditioned to sit in a laundry basket or similar mesh structure that can be removed and placed in a smaller container of seawater. Other methods include coaxing the specimen into mesh bags, large nets, or buckets that can be closed with a lid before pulling it out of the water.

Pieces of Astroturf, burlap, scrub pads, rough autoclave gloves, or filter floss are useful to have on hand in order to pry wandering arms loose or deter the specimen from clinging to the tank or other object. Carts or other means of conveyance are usually safer and easier than trying to carry the animal and a large volume of seawater over longer distances.

Containers for shipping GPOs range from multiple plastic bags held in cardboard shipping boxes with foam cooler inserts to large plastic barrels and/or wooden crates depending on the size of the specimen being shipped. Strong sturdy containers are important so check how well the container holds up to pressure, that it is not cracked, and that it is watertight at several layers. Even exceptionally large specimens (up to 20 kg [44 lb]) can be shipped long distances given a sufficient sized container and appropriate amount of seawater. Coolers work well for small animals, and barrels are used for larger specimens; though it is recommended that they be placed inside a square box to prevent air cargo employees from rolling them about in the warehouse (K. Wong, personal communication). Pre-chilling of containers is critical to eliminating excess heat before shipping (K. Wong, personal communication).

Containers used to ship GPOs are generally small enough to be accepted as cargo by all major airlines without special arrangements. Special arrangements are seldom necessary for shipping as the maximum size of crates is unlikely to exceed the size limitations of major carriers. Some airlines will, however, refuse to categorize aquatic specimens as "live animals" which can lead to potentially lethal delays in shipping. The institution sending cephalopods is advised to clarify all details of shipping with the airline or freight company prior to shipment.

Where non-stop flights are not available, it may also be advisable to contact aquarium professionals in connecting cities beforehand to inquire about the possibility of emergency assistance should unforeseen delays occur. The ability to provide chilled clean seawater, ice packs, or both may be necessary should the shipment be delayed beyond 48 hours or more. It is also worth noting though that a properly packed container can often maintain its temperature for well over 24 hours if not exposed to extreme ambient temperatures, and should only be opened en route in extreme circumstances or if necessary for customs inspections. The equipment must provide for the adequate containment, life support, comfort, temperature control, food/water, and safety of the animal(s).

Equipment for GPO Shipping:

- Large plastic shipping bags (3 mm or thicker plastic with welded seams)
- Rubber bands sufficient for bags (at least 3 per bag)
- Nets
- Laundry baskets
- Patches of Astroturf, scrub pads, or filter floss
- Mesh bags
- Oxygen cylinder
- Oxygen regulator, manifold, tubing and diffusers
- Shipping box (pre-chilled) with appropriate foam insulation (very small specimens only)
- Wooden crate and/or plastic barrel (pre-chilled) with appropriate insulation (large specimens)
- Ice packs (wrapped to cover sharp edges)
- Cart/dolly for moving specimen
- Supply of clean, chilled seawater
- Packing tape
- Shipping documents (see Appendix G for international shipments)
- Checks or cash for customs agent inspection fees (if applicable)
- Magnesium chloride stock solution, 75 g/l (optional)

Safe transport also 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 among all affected 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 (AZA Accreditation Standard 1.5.11).

Safety: Safe transport also 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. Unless extraordinary delays in shipping occur, the value of opening a sealed insulated container for inspection, water quality analysis, or maintenance mid-transit outweigh any perceived benefit. Planning and coordination for animal transport requires good communication among all affected 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.

Usually two to three aquarists will be needed to effectively catch and handle a GPO. Given the weight of this species, and the accompanying volume of seawater, at least two people will be needed to physically move most specimens. The use of a sturdy wheeled cart or pallet jack is advisable when longer distances need to be traversed with a container laden with seawater and the animal. The animal is usually caught simply by scooping it into a large net (rubberized nets are less abrasive to their delicate epidermis), or by coaxing the animal into a container such as a bucket or laundry basket. The animal can then be removed from the water and placed directly into the shipping container, or into a barrel of chilled seawater.

As when moving any large animal, an experienced manager or senior staff member should act as team leader when catching/moving specimens of *E. dofleini*. The team leader should ensure that adequate equipment is present and brief other staff members as to their specific roles. Generally staff may need to be divided into those who coax the specimen into the net, and those who will actually catch and lift the specimen. If the tank is in an odd position (i.e., on a platform) it may be necessary to have additional staff below to hand-off the animal and complete the transfer from tank to transport container, or vice-versa.

Emergency situations: Should a large octopus gain access to a staff member while being moved, the arms should be peeled back gently, and in more urgent cases contact with an irritating tactile stimulus such as Astroturf or filter floss will usually cause them to withdraw. Once an octopus is *en route* delays can prove fatal; packaging should be sufficient to ensure temperature regulation and water quality (i.e., pH, nitrogenous compounds, DO) within acceptable levels for 24 hours. Shipments from commercial collectors to European institutions using the same methods are routinely able to maintain water quality and temperature for 36–48 hours. In an extreme case a well packed shipment from Vancouver, BC to Dallas, TX that was lost by the carrier *en route* preserved a 3 kg (6.6 lb) GPO in excellent condition with only a 1 °C (33.8 °F) rise in temperature (despite summer temps exceeding 40 °C [104 °F]) and minor pH degradation for over 72 hours.

In any case shipments lost or delayed in transit should be aggressively pursued with the carrier and located as soon as possible. If receipt of the animal or a return to the sender is not possible within a reasonable time frame it is advisable to seek assistance from local aquaria or zoological parks that may be able to water change and re-oxygenate the shipment or house the animal temporarily. Additionally, the authors are aware of at least one instance where a GPO had been stranded by an automobile accident during an overland transport. In such a case alternate transit arrangements should be made immediately, and if the integrity of the transport container is compromised, the animal should be immediately transferred to chilled seawater with sufficient oxygen.

Lost shipments have been found by having a detailed description of the package available or by having a unique colored portion of the box (a neon pink crate stands out in a cargo warehouse). Having a duplicate copy of all the shipping details within the box has facilitated the delivery of a shipment that lost its label.

Sedation: Mild sedation of many cephalopod species with magnesium chloride is a common practice for shipping. A stock solution of MgCl at a 75 g/l concentration diluted 1:9 with seawater will provide mild sedation for the animal during transport. See Chapter 6.7 for further information on anesthetics.

3.2 Protocols

Transport protocols should be well defined and clear to all animal care staff.

IATA live animals regulations: IATA Live Animals Regulations are provided for octopus. The General Container Requirements for Aquatics (CR 50–60) and Container Requirement 51 are the relevant sections and the pertinent wording is as follows:

> Container construction material requirements should be water-resistant fiberboard, insulating material, plastic or wood, expanded polystyrene or Styrofoam. Thick walled polystyrene or Styrofoam of adequate strength and no cracks have proved very effective over many years.
>
> The outer container can be constructed of fiberboard, wood, wood products or any plastic material of adequate strength. Purpose-built containers made of expanded polystyrene or Styrofoam must be of adequate strength. Sturdy fiberboard (sometimes referred to as corrugated cardboard) must also be of adequate strength to hold the weight.
>
> The innermost container is stated to be strong plastic polyethylene bags. The bags are to be fastened by twisting the top and folding the twisted part so that it can be sealed with elastic bands. It is best to have at least a bag within and bag and even newspaper or additional bags to reduce the complete puncture and loss of water. Bags may be heat sealed, but generally are not used because of possible physical inspections and the inability to add more oxygen or reseal in route if a delay or emergency arises.
>
> Further insulation and cushioning is recommended with expanded polystyrene container or sheets on all sides and top and bottom. Alternatively, compressed newspaper or other fibrous material sandwiched between paper. The regulations do state that containers that conform in principle to the above meet IATA standards.

Shipping GPOs: GPOs can be shipped to other parts of the world (Prescott & Brosseau, 1962; Bronikowski, 1984). As with all aquatic and marine organisms, the animal should be fasted prior to transport to prevent passage of waste during shipping. Unlike when shipping terrestrial animals food is not recommended lest the water the animal is being transported in will quickly foul. Overall the protocols of shipping a GPO should be constructed to minimize production of metabolites and other wastes that could foul the water in transport.

Once the bag is filled with oxygen and sealed, it is placed in an appropriately-sized cardboard box that is lined with 2.5 cm (.98 in.) thick Styrofoam (polystyrene) for insulation. At least two ice packs are added to keep the shipment cold (more in warm weather), and the box is sealed. The ice packs are usually wrapped in newspaper to prevent their sharp edges from puncturing the plastic bag.

The packaging will not only maintain temperature, but also keep the specimen in darkness and insulated from ambient noise in transit. If properly packaged the temperature should not rise more than 1–2 °C (1.8–3.6 °F) during transport over 24 hours. As defined above, the ideal temperature for this species is 10 °C (50 °F), with 6–13 °C (42.8–55.4 °F) being acceptable ranges.

Too often an animal shipment has been bumped from a flight, or shunted into a too cold or hot cargo warehouse for storage before its flight. Boxes should always be labeled clearly with the shippers' and shippees' names, addresses, and phone numbers, and the fact that they contain live animals. It is important to list cell phone numbers or other 24-hour access numbers, and not office numbers in case of a problem developing overnight or in the early morning.

If an animal is packed into a container for more than 36–48 hours and cannot be delivered to its final destination very soon the receiving institution should plan for the animal to be removed to a holding tank at a nearby public aquarium (if facilities are available); or to have the water exchanged with clean, chilled seawater and to have the icepacks and oxygen refreshed before repacking. This option is unlikely to be available often, but is worth consideration in the planning phase as a last-resort effort should a shipment go wrong.

Acclimation: Upon arrival the bag containing the octopus should be floated in its new tank for temperature equalization, usually about 30 minutes. Another good practice is to add tank water to the bag during the acclimation, to allow the animal to adjust slowly to any changes in water chemistries. After this has been accomplished, the animal should be allowed to crawl out into the new tank and the bag water should be discarded, as it will be laden with wastes accumulated during the trip. Animals should be acclimated to within about +/- 1 °C (1.8 °F) and 0.2 pH units before being transferred from bag to receiving tank.

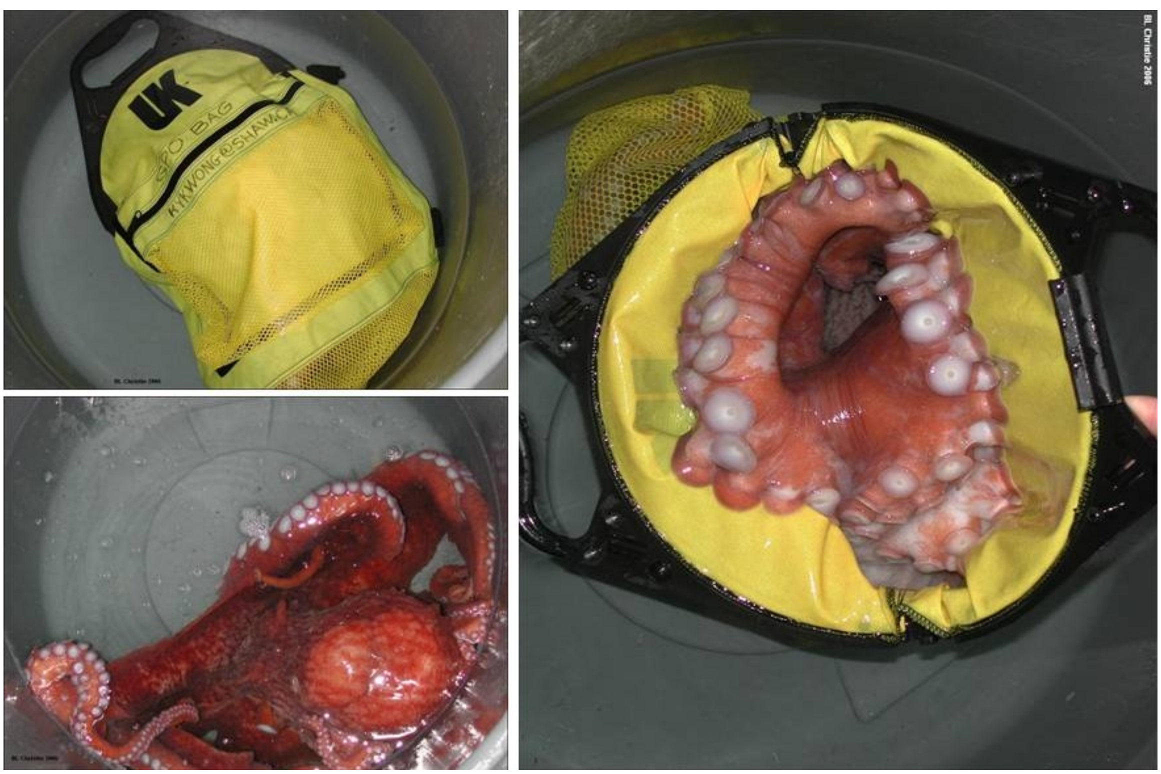


Figure 13. A GPO in a collecting bag as would be typical for transport in the field or for short-term moves (e.g., from quarantine to exhibit, or from exhibit to holding). Top left: Animal enclosed in bag, submerged in water in a suitable container (55 gal trashcan pictured; coolers are preferable for trips over 15–20 min). Right: The bag being opened and the animal released. Bottom left: The animal free swimming after release. Photos courtesy of B. Christie

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. It has long been established that specimens of *E. dofleini* are best kept singly in an aquarium setting as they are solitary animals and will react aggressively towards conspecifics, which has even been documented in chance encounters in the wild (Anderson, 1995; 2008 & Courtney, 1978).

Some facilities have attempted to keep multiple specimens together, though as mentioned above in Chapter 2.1, it should only be attempted in tanks exceeding 10,000 gallons and even then there is no guarantee that severe issues with aggression will not be encountered. Multiple specimens can be kept in the same system, though in different enclosures divided by shift doors or other devices. If such a tank is designed, the ability of octopods to bypass these shift measures should be carefully considered, as mentioned in the discussion of octopus escape in the Introduction and Chapter 2.2.

A 6,500 gallon closed system has recently been installed at one aquarium with two separate sides divided by rockwork and three acrylic shift doors that can potentially be used to house two adult GPOs, or an octopus and a menagerie of fishes. With such a specialized system the animals can be kept separate, thus avoiding most of the challenges described above, though in general octopods should only exhibited singly by most institutions.

4.2 Influence of Others and Conspecifics

Mixed-species tanks: It is typical for GPOs to be exhibited with sea anemones and various echinoderms that are found in the same cold-water habitats as the octopus. It is also becoming more common to house different fish species along with the octopus. Common fishes housed with GPOs include rockfishes, sculpins, and other cold-water teleosts found in the same geographic range as *E. dofleini*. The GPO does not appear to suffer any negative effects from being housed in a mixed species exhibit, to the contrary tank-mates offer a form of enrichment by providing stimuli not found in a monospecies display. It should be well understood, however, that any animal housed with an octopus may one day become a prey item. As such aquarium managers should consider whether these fishes are to be accessioned as part of the animal collection, or simply considered feeder fish.

Many temperate invertebrates and some fishes can be added to an octopus display. Species of *Urticina* (formerly *Tealia*) sea anemones, sea cucumbers, sea stars (other than sunflower stars), plumose sea anemones, chitons, and sea urchins can all be used. In addition some fish such as rockfish, sea perch, sculpins and flounders can be added. These are all animals that a GPO might encounter in the wild. Note that no valuable animals should be added, as GPOs may catch and eat them (Anderson, 1991). At best, there is an uneasy truce between a well-fed octopus and some species of fish. Species that are commonly kept with GPOs are summarized in Table 5, and the frequency with which these accessory animals are displayed with GPOs in public aquaria is displayed in Table 6.

Two species of sea stars should be avoided. The sunflower star (*Pycnopodia helianthoides*) has been implicated in many fish and invertebrate mortalities, especially in closed systems. It likely releases an undescribed toxin into the water. The slime star (*Pteraster tesselatus*—"snot star" in the vernacular to aquarists) releases copious amounts of clear mucus when disturbed. Most other sea stars are probably safe for the octopus tank.

GPOs have eaten a number of different fishes in their displays, even dogfish sharks (*Squalus acanthias*). Animals to replace those eaten should be collected or bought and shipped, sometimes at considerable expense. This list was compiled to help other aquariums exhibiting GPOs decide what auxiliary animals they can safely keep in their display.

Figure 14. Mixed species exhibits are possible, with caution and the knowledge that any animal placed in a tank with a GPO may eventually become prey: the remains of a sculpin that was eaten after having been housed in a tank with a GPO. Photo courtesy of R. Anderson

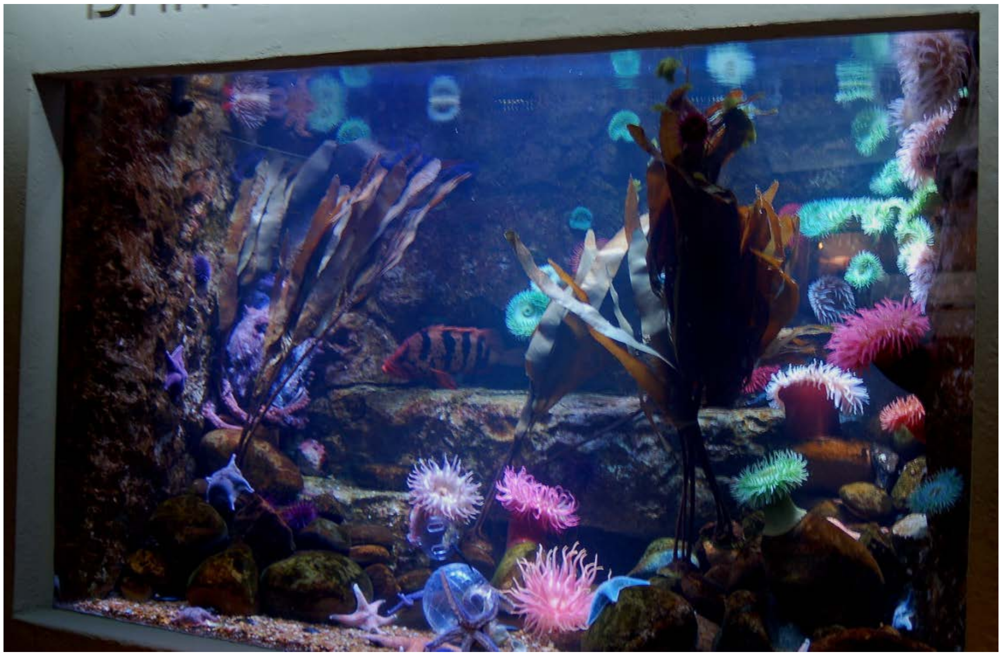


Figure 15. A beautiful multi-species GPO display featuring a densely-stocked tank with anemones, starfish, urchins, and teleost fishes. Photo courtesy of B. Christie

Table 5. Compatibility of selected fishes and invertebrates with *Enteroctopus dofleini*, adapted from an earlier survey by R. Anderson (2003a)

Species	Inst. A	Inst. B	Inst. C	Inst. D	Inst. E	Inst. F	Inst. G	Inst. H	Inst. I
Live sponges	X								X
Sea anemones								X	
Urticina spp.	X		X	X			X		X
Green anemones							X		
Cribrinopsis fernaldi	X								X
Plumose anemones				X	X		X		X
Hydrocorals									X
Sea pens									X
Tubeworms	X								X
Small chitons	X						X		X
Gumboot chitons	X								X
Limpets	X						X		X
Small snails	X						X		X
Nudibranchs	X								X
Sea stars*	X		X	X	X	X	X	X	X
Sea urchins	X		X				X		X
Sea cucumbers	X			X					X
Feather stars	X								
Tunicates	X								X
Elasmobranchs*		X							
Sculpins*								X	X
Schooling rockfish		X	X	X					
Benthic rockfish*		X		X			X	X	X
Greenlings									X
Surf perch*				X					
Salmon*									X
Wolf eels*									X

*Indicates fish that have been eaten in a GPO tank. Sunflower stars have been implicated in the deaths of both fish and invertebrates in closed systems.

Table 6. Accessory species kept with *Enteroctopus dofleini* at 33 aquariums and zoos from survey data collected by the AITAG (December, 2012).

Accessory Species	Percent of Institutions Displaying	No. of Institutions Displaying
Porifera	8.3%	2
Cnidaria: Anemones*	83.3%	20
Cnidaria: Sea Pens	0	0
Mollusca: Chitons	41.7%	10
Mollusca: Gastropods	16.7%	4
Mollusca: Limpets	25%	6
Crustaceans (Spot Prawn)	8.3%	2
Echinodermata: Urchins	29.2%	7
Echinodermata: Starfish	75%	18
Echinoderms: Sea Cucumbers	8.3%	2
Teleost Fishes	25%	6

*Taxa in bold are most prevalent tank-mates (i.e., those kept in >40% of displays).

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.

GPO introductions generally result in fighting and heavy inking. Some institutions have introduced animals in large tanks where they could avoid each other. It can be a highly risky practice that has not been effectively worked out at this point. Current recommendations, as stated above, are to house these animals singly unless an attempt at breeding is desired.

Chapter 5. Nutrition

5.1 Nutritional Requirements

A formal nutrition program is recommended to meet the nutritional and behavioral needs of all octopuses (AZA Accreditation Standard 2.6.2). Diets should be developed using the recommendations of nutritionists, the Nutrition Scientific Advisory Group (NAG) feeding guidelines (http://www.nagonline.net/Feeding%20Guidelines/feeding_guidelines.htm), and veterinarians as well as AZA Taxon Advisory Groups (TAGs), and Species Survival Plan® (SSP) Programs. 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.

> **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.

The nutritional requirements of aquatic animals (excepting marine mammals) have traditionally not received nearly the same attention as the feeding of larger terrestrial animals within the AZA. Aquatic animals are radically different from most terrestrial species whose diets are well-understood. The diversity of feeding niches exploited by the animals under the purview of the Aquatic Invertebrate TAG is likely the widest any animal management group is likely to encounter. As such, there is no established framework or guidelines for the nutritional requirements of Giant Pacific octopus, *Enterooctopus dofleini*, though there is a good amount of growth and metabolic information in the literature. These data, coupled with the practical experiences of aquarists who have maintained *E. dofleini* present a fairly comprehensive picture of the species' dietary needs in an aquarium setting.

In addition, the vitamin requirements of Giant Pacific octopus are unknown. Facilities primarily feed thawed seafood and vitamins degrade in frozen seafood. Animals with fish-based diets benefit from supplements of vitamin E and thiamin (Crissey, 1998). Even though octopuses have a more crustacean-based diet, adding supplements to the diet merits consideration and testing.

Prey selection in the wild: Scheel & Anderson (2012) recently published a detailed study of the diet of *E. dofleini* in the wild based on identifying 35+ species of prey from nearly 7000 individual animals found in the middens of octopus dens from four sites ranging from Seattle to Alaska. Hartwick et al. (1981) had earlier published similar results from two sites in Vancouver, British Columbia identifying 24+ species of prey from nearly 3500 midden remnants. The data from Scheel & Anderson (2012) is summarized in Table 7 to show the general preferences of *E. dofleini*. The table is a gross oversimplification of the complex data presented by Scheel & Anderson (2012), which also identified local specializations and feeding proclivities of different populations of *E. dofleini*. Octopuses in general are highly opportunistic feeders, their diet in the wild is highly variable and they adapt well to utilize the most available food sources.

Table 7. General diet preferences of wild *Enteroctopus dofleini* as determined by midden contents from four sites along the Northern West coast

Prey Type	Total	Species	Percentage	Dominant Genera
Crustaceans	3944	13	58.13%	*Metacarcinus, Cancer* spp.
Bivalves	2714	16	40.00%	*Pododesmus* sp.
Gastropods	38	3	0.56%	*Euspira* sp.
Polyplacophorans	18	2	0.27%	*Tonicella* sp.
Echinoids	14	1	0.21%	*Strongylocentrotus* sp.
Teleosts	4	n/s	0.06%	Various
Other Taxa	53	n/s	0.78%	Various
Total	**6785**	**35+**	**100.00%**	

Adapted from Scheel & Anderson (2012)

Growth: There exists a fair amount of data on the growth of *E. dofleini* in aquariums and in the wild. It should be noted here that growth in octopuses is highly variable between populations and even between individuals. The growth of cephalopods is reviewed in Forsythe & Von Heukelem (1987) and more recently in Semmens et al. (2004). Semmens et al. (2004) showed that standard growth models (Asymptotic, Von Bertalanffy) for populations of *Octopus vulgaris* from the Mediterranean region varied wildly. Thus a smaller octopus may not always prove to be a juvenile, and may enter senescence sooner than anticipated. Similarly an animal may grow to a very large size quickly and remain as such for a long period of time; there has not been shown to be a relationship between speed of growth and the onset of senescence. In general, the greatest gains in mass will be seen in juvenile specimens, and they will have a greater caloric need in relationship to their size than an adult. The variation in the mass to age relationship makes it difficult to specifically predict growth. Cephalopods, in general, have incredible conversion efficiency rates and do not store fat. Any appreciable growth is a sign that the minimum nutritional requirements are being met.

Differences in growth of adult *E. dofleini* specimens (n=10) in one aquarium grew on average 1.2–1.9% of their body weight per day over their entire life span (Anderson, 2003b). Hartwick et al. (1981) studied wild specimens (n=15) of *E. dofleini* for periods ranging from 36–210 days and found their growth rate to average 0.58% per day (range 0.1–1.8% per day). The sample size in the Hartwick et al. (1981) study contained animals ranging from 2.9–17.9 kg (6.3–39.4 lb); unsurprisingly, the greatest increases in mass were seen in the smallest (and presumably youngest) animals. Hartwick et al. (1981) also reported on the growth of aquarium-housed *E. dofleini* (n=6) at the one aquarium for 40–60 days; these animals had an average daily growth rate of 0.54% body weight per day (range 0.6–1.1%).

Cephalopods, in general, and octopods, in particular, have been known to make astonishingly efficient use of food as described by feed conversion rates. The feed conversion rate of these animals averaged 60.6% and ranged between 39–87% as reported by Hartwick et al. (1981). Robinson (1983) reported similar growth rates for aquarium-housed specimens (n=6), finding an overall growth rate of 0.62% per day. These studies are summarized in Table 8, Overall the mean growth between these reports is 0.97% per day, which corresponds well to the guideline given by Anderson (1995) that well-fed, aquarium-housed specimens will, on average, grow about 1% per day.

Table 8. Growth rates of *Enteroctopus dofleini*

Species	**Individuals (n=)**	**Growth* (% day^{-1})**	**Life Stage**	***In situl Ex situ***	**Notes**	**Reference**
Enterooctopus dofleini	5	1.90	Adult	*In situ*	50% Crab Diet	Anderson, 2003b
Enterooctopus dofleini	5	1.20	Adult	*In situ*	Other Diet	Anderson, 2003b
Enterooctopus dofleini	15	0.58	Mixed	*Ex situ*		Hartwick et al., 1981
Enterooctopus dofleini	6	0.54	Adult	*In situ*		Hartwick et al., 1981
Enterooctopus dofleini	6	0.62	Adult	*In situ*		Robinson, 1983

*Mean = 0.97% day^{-1}

Metabolism: There is a fair amount of metabolic data on cephalopods, though the metabolic rates of *E. dofleini* have not been thoroughly investigated across all life history stages or from various populations. At present, there is one study published concerning the metabolism of *E. dofleini*, however to present a more complete picture of the subject, data from a taxonomically closely related species (*Enteroctopus megalocyathus*) and a geographically similar species (*Octopus rubescens*) are presented and discussed as well. Metabolic studies on immature *E. dofleini* showed optimal growth on a low fat diet of 3% body weight every 3 days at temperatures of 7–9.5 °C (44.6–49.1 °F) (Rigby & Sakurai, 2004). This same report also detailed the energy budget for *E. dofleini*, and Onthank (2008) presented data from *O. rubescens* as well as a comprehensive review of the pertinent literature. Immature *E. dofleini* were found to devote 26.5% of their energy to base metabolism and 39.1% to growth with 5.6% lost in feces (Onthank, 2008; Rigby & Sakurai, 2004). By way of comparison *O. rubescens* was found to utilize an average of 63% of their available energy for metabolism and average of 38.7% for growth (averaged from results of two different experimental groups).

Overall these data can be adapted to determine that *E dofleini* requires 12.69 kcal/kg/day for metabolism and growth (at 9.5 °C [49.1 °F]), *E. megalocyathus* requires 11.00 kcal/kg/day (at 17 °C [62.6 °F]), *O. rubescens* requires 8.59–14.76 kcal/kg/day (at 11 °C [51.8 °F]), and *Octopus vulgaris* requires 13.36 kcal/kg/day (at 20 °C [68 °F]) (Onthank, 2008). These data is summarized in Table 9, and adapted to provide values in kcal and percentages for use in nutritional planning. The Onthank (2008) data were also extrapolated to provide a simple graphical representation of energy required for base metabolism and growth in four octopus species in Figure 16.

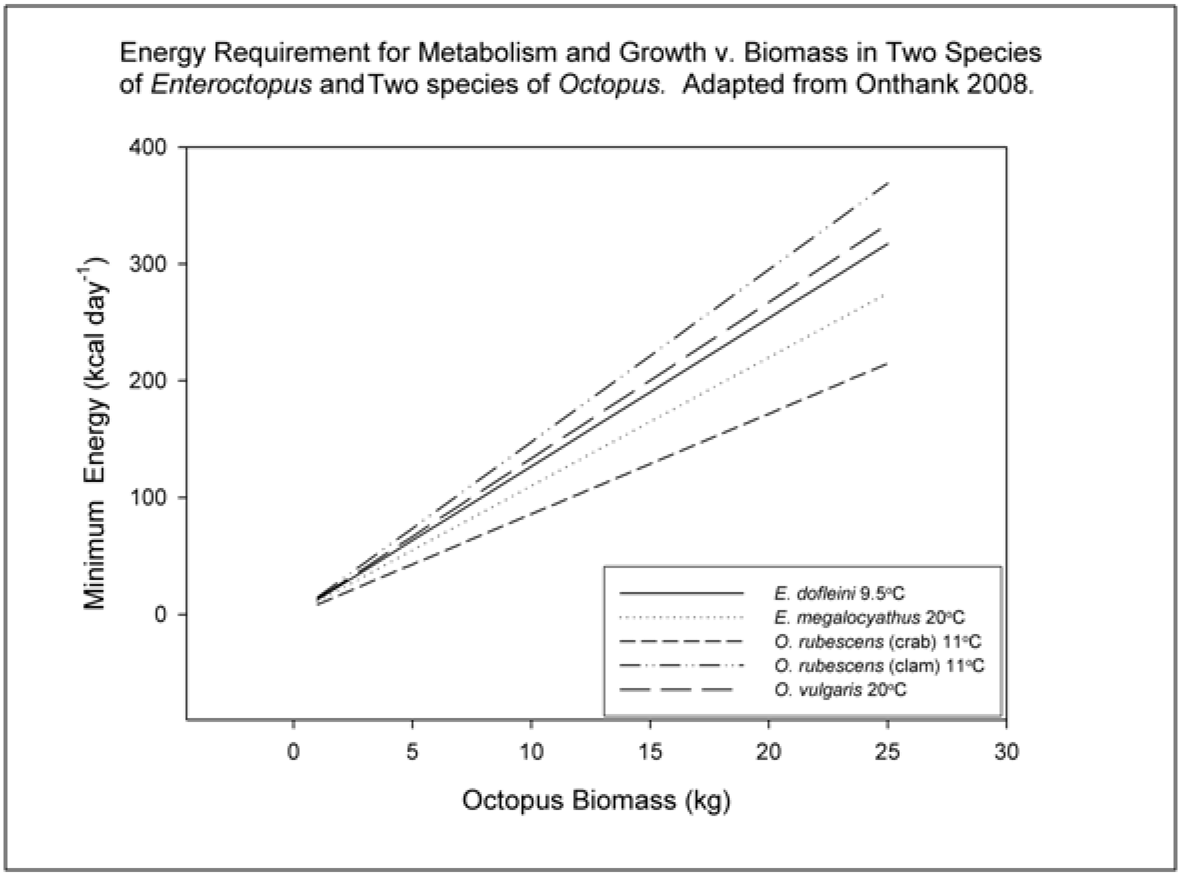


Figure 16. Minimum energy requirements for growth and metabolism in four species of octopus. The data represented by this graph is from Onthank (2008), and is theoretically extrapolated over a arbitrary range of biomass from 0–25 kg (0–55 lb). *O. rubescens, E. megalocyathus*, and *O. vulgaris* do not reach these sizes, and this is presented solely for physiological comparison. This does not account for energy devoted to reproduction, or energy lost as waste; and this appears artificially linear as it is independent of growth rate: natural growth will be much greater in younger individuals.

Nutrients and diet selection: Specific micronutrients required for *E. dofleini* are unknown, though there are some data to suggest appropriate feed items based on macronutrients (i.e., protein, fat content, etc) meet their needs. Multiple studies have shown that crustacean diets are ideal for growth of the species, which is not surprising, since crabs comprise the majority of their diet in the wild. Distinct differences were found in the amount of energy available to animals fed a crustacean diet versus those fed bivalves in *O. rubescens* (Onthank 2008) and in *E. megalocyathus* (Perez et al., 2006). Anderson (2003b) noted slightly higher (though not statistically significant) growth in aquarium-housed *E. dofleini* fed 50% crab in the diet versus those fed exclusively other types of seafood. Rigby & Sakurai (2004) found that ideal growth in juvenile *E. dofleini* was achieved at a feed rate of 3% body weight every 3 days on a low fat diet. Overall a clear trend is seen here that low fat food (e.g., crustacean protein) is preferable to a high fat diet.

Studies suggest that diets low in lipids and high in protein are most beneficial to the growth of *O. vulgaris* (Garcia and Gimenez, 2002; O'Dor et al., 1984). O'dor et al. (1984) suggests this to be due to a lack of emulsifier in the digestive tract preventing octopods from making efficient use of fats and oils in their diet. Lee (1995) states that cuttlefishes and squid metabolize protein almost exclusively and optimal growth in these taxa requires >50 g protein per MJ energy. These studies, coupled with the growth and metabolism information presented above suggest protein content as the most important dietary factor for growth and development of octopods.

Overfeeding is generally not a concern for the health of the animal, as octopuses have high conversion efficiency of feed and do not store fat, devoting this energy instead to rapid growth. It has been common practice in some aquaria and in laboratory settings to feed *ad libitum* or to satiation. While this would seem contrary to good husbandry practice for most other animals, feeding to satiation in octopods should be considered an acceptable feeding strategy, with caveats. In large open systems feeding to satiation or *ad libitum* may be of little concern, however, in closed systems large nutrient inputs could rapidly cause water chemistry problems. High rates of feeding will also lead to high rates of growth, therefore, institutions should consider the size of octopus their exhibit can sustain so that an animal does not outgrow its tank. Overall feeding ration should be determined by the growth rate and health of the individual, but in closed systems this needs to be balanced against water chemistry and size.

Digestive anatomy: Octopods have a powerful beak capable of cutting and biting, and a radula similar to that of the Gastropoda used for rasping and drilling into prey. The location of the buccal mass in a sinus instead of solidly based in the musculature allows octopods to manipulate the beak with great dexterity and open challenging prey. The esophagus is muscular and terminates in a crop before attaching to the stomach and caecum. The gastrointestinal system in octopods is flanked by two paired glands, the anterior salivary gland and the digestive gland. The salivary gland connects to the buccal mass and produce cephalotoxin used to paralyze prey and other proteolytic enzymes. The digestive gland connects to the caecum and stomach via a single duct and produce a slew of digestive and proteolytic enzymes. These compounds are extremely efficient in dissolving and incorporating lean protein into growth, though as mentioned above, they are not accompanied by emulsifiers so there is little to no utilization of lipids by octopods. The caecum turns 180° (as in gastropods and some other molluscs) and connects to a rectum which empties wastes back out through the siphon in an excurrent water flow. The digestive anatomy of *E. dofleini* is pictured in Figure 18 below for general reference.

Nutrition of paralarvae and juveniles: The best nutritional and feeding practices for paralarvae and juveniles of *E. dofleini* are largely unknown. There is a paucity of published information, but what literature exists suggests that the same basic nutritional concepts apply; as stated above Rigby and Sakurai (2004) found low-lipid diets to provide the best growth rates. The need for good nutrition is critical for paralarvae and juveniles as their growth rates are exponential and rapid when compared to most other animals (sometimes up to 10% body weight per day). Feeding practices for paralarvae and juveniles is described in greater detail in Chapter 7 as they are still highly experimental and most facilities will only house adult GPO specimens.

Table 9. Metabolic information and energy budgets for seven species of octopus, adapted from Onthank (2008). The data are further extrapolated to provide corresponding information in nutritional calories (kcal) and in percentages for better practical application to the husbandry of aquarium-housed specimens.

Species	Temperature	Energy Consumed	Metabolism	Energy Lost in Urine	Energy Lost in Feces	Energy for Growth	Unaccounted Energy	Assimilation Effeciency	Notes	Reference
	°C	(J/g/day)	(J/g/day)	(J/g/day)	(J/g/day)	(J/g/day)	(J/g/day)			
Enteroctopus dofleini	9.5	80.96	21.48	-	4.57	31.63	-	-	Immature Specimens	Rigby and Sakurai, 2004
Enteroctopus megalocyathus	17	147.72	39.94	1.28	1.18	6.07	-	99%	Crustacean Diet	Perez et. al., 2006
Octopus rubescens	11	33.03	26.46	0.01	0.96	9.49	-3.89	96%	Crustacean Diet	Onthank, 2008
Octopus rubescens	11	63.89	30.57	0.02	2.94	31.20	-0.84	94%	Bivalve Diet	Onthank, 2008
Octopus vulgaris	20	67.92	38.52	0.01	9.06	17.38	-	87%		Petza et. al., 2006
Octopus cyanea	20	83.96	40.47	-	3.69	40.80	1.26	96%		Van Heukelem, 1976
Octopus maya	20	76.64	27.95	-	3.08	45.61	-	96%		Rosas et. al., 2007
		(kcal/kg/d)	(kcal/kg/d)	(kcal/kg/d)	(kcal/kg/d)	(kcal/kg/d)	(kcal/kg/d)			
Enteroctopus dofleini	9.5	19.35	5.13	-	1.09	7.56	-	-	Immature Specimens	Rigby and Sakurai, 2004
Enteroctopus megalocyathus	17	35.31	9.55	0.31	0.28	1.45	-	99%	Crustacean Diet	Perez et. al., 2006
Octopus rubescens	11	7.89	6.32	0.00	0.23	2.27	-0.93	96%	Crustacean Diet	Onthank, 2008
Octopus rubescens	11	15.27	7.31	0.00	0.70	7.46	-0.20	94%	Bivalve Diet	Onthank, 2008
Octopus vulgaris	20	16.23	9.21	0.00	2.17	4.15	-	87%		Petza et. al., 2006
Octopus cyanea	20	20.07	9.67	-	0.88	9.75	0.30	96%		Van Heukelem, 1976
Octopus maya	20	18.32	6.68	-	0.74	10.90	-	96%		Rosas et. al., 2007
		(J/g/day)	(% of Diet)	(% of Diet)	(% of Diet)	(% of Diet)	(% of Diet)			
Enteroctopus dofleini	9.5	80.96	26.5%	-	5.6%	39.1%	-	-	Immature Specimens	Rigby and Sakurai, 2004
Enteroctopus megalocyathus	17	147.72	27.0%	0.9%	0.8%	4.1%	-	99%	Crustacean Diet	Perez et. al., 2006
Octopus rubescens	11	33.03	80.1%	0.0%	2.9%	28.7%	-11.8%	96%	Crustacean Diet	Onthank, 2008
Octopus rubescens	11	63.89	47.8%	0.0%	4.6%	48.8%	-1.3%	94%	Bivalve Diet	Onthank, 2008
Octopus vulgaris	20	67.92	56.7%	0.0%	13.3%	25.6%	-	87%		Petza et. al., 2006
Octopus cyanea	20	83.96	48.2%	-	4.4%	48.6%	1.5%	96%		Van Heukelem, 1976
Octopus maya	20	76.64	36.5%	-	4.0%	59.5%	-	96%		Rosas et. al., 2007

5.2 Diets

Experience with the species in an aquarium setting has shown that the most favorable food source is live crabs, as it is in the wild. GPO's preferred crab is the Dungeness crab, *Metacarcinus (=Cancer) magister*. The Jonah crab (*Cancer borealis)* or the blue crab (*Callinectes sapidus)* provides helpful substitutes on the East coast and Southern U.S, respectively. One can transfer octopuses to a diet of raw seafood, such as herring, smelt, squid, fish fillets, shrimp, or clam meat. When formulating the diet incorporation of a significant portion of crustacean or other lean protein in the diet is important for the reasons listed above.

Removing shells may aid in minimizing the need to clean the bottom of the tank of crab remnants, which are visually unattractive and may contain bits of uneaten food, which may foul the tank. Food without shells is usually eaten completely, and if not, can be netted out of the tank. Feeding can be accomplished by spearing a piece of food onto the end of a thin plastic feeding stick touched to the octopus, which will grab it. Feeder sticks can be easily made from long knitting needles, hard air line tubes, acrylic rod, or PVC welding rod. Octopus will refuse food once satiated which is estimated to be about 2% of the octopus' body weight per day.

Table 10. Approximate Nutritional Content of Common Finfish and Shellfish Seafood Items Common to Public Aquaria and Zoos.

Seafood Name	**Scientific Name**	**Calories** (kCal/100g)	**Protein** (%)	**Fat** (%)	**Carbohydrate** (%)	**Moisture** (%)	**Ash** (%)
Anchovy	*Engraluis mordax*	132	15.23	7.28	1.26	72.83	3.4
Atlantic Herring	*Clupea harengus*	147	15.99	9.18	0	72.16	2.76
Blue Runner	*Caranx crysos*	189	19.44	12.32	0	63.79	4.54
Bonito	*Euthynnus alletteratus*	116	20.88	3.56	0.11	72.46	2.99
Butterfish	*Peprilus tricanthus*	112	15.11	5.73	0	70.87	8.37
Capelin	*Mallotus villosus*	92	14.27	3.88	0	79.82	2
Flounder	Pleuronectidae spp.	97	19.6	1.5	-	79.4	1.5
Mullet	*Mugil cephalus*	105	15.25	1.33	-	81.96	0.84
Pacific Mackerel	*Scomber japonicus*	86	17.56	1.69	0	77.37	3.39
Salmon (Alaskan)	*Oncorhynchus gorbuscha*	151	19.73	7.94	0.01	70.68	1.64
Sardines (Pacific)	*Sardinops sagax*	91	17.09	2.45	0	76.76	3.75
Sardines (Spanish)	*Sardinella aurita*	112	20.59	3.29	0	69.5	6.68
Smelt (Night)	*Spirinchus starksi*	90	15.26	3.21	0	79.08	2.71
Smelt (Silver)	*Hypomesus sp.*	97	17.25	3.05	0	77.64	2.17
Smelt (Lake)	*Osmerus mordax*	99	13.32	5.04	0	80.2	1.6
Silversides	*Menidia menidia*	143	16.66	8.28	0.24	71.96	2.86
American Lobster	*Homarus americanus*	78	15.8	1.2	-	81.2	2.7
Atlantic Oyster	*Crassostera virginica*	66	8.59	2.36	-	82.2	1.4
Atlantic Surf Clam	*Spisula sp.*	92	15.87	0.87	5.15	76.41	1.7
Blue Crab	*Callinectes sapidus*	101	16.42	0.77	-	78.83	2.06
Dungeness Crab	*Cancer magister*	101	19.3	0.87	-	78.1	1.5
Krill (Pacifica)	*Euphausia pacifica*	78	14.5	1.82	0.82	80.44	2.42
Krill (Superba)	*Euphausia superba*	106	13.56	5.55	0.67	77.71	2.51
Mussel, Blue	*Mytilus spp.*	86	11.8	2.1	-	79.9	3.5
Shrimp (gulf)	*Penaeus spp.*	97	18.1	0.95	-	80.1	1.25
Squid (Calimari)	*Loligo opalescens*	75	14.59	1.6	0.38	82.08	1.35
Whelk	*Busycon spp.*	91	18.5	1.9	-	77.5	-

Compiled from data in McRoberts (2011), Krzynowek & Murphy (1987), and from the U.S. Food and Drug Administration www.fda.gov/food

Of 33 institutions holding GPOs surveyed in 2012, the largest portion (42.4%) fed their animal 3 times per week, while 30.3% of aquaria fed daily. Of these survey respondents, 18.2% fed to satiation, 12.1% fed a specific body weight percentage per day (mean amount was 2.5% animal mass per day), however, the majority of institutions (72.7%) responded that they use a best judgment approach in feeding their animals. More than 50% of responding institutions supplement their GPO diets with live foods.

Seasonal fluctuations in the weight of *E. dofleini* have been observed in the wild, and in facilities that utilize open-system tanks with natural seawater, or sophisticated temperature control to simulate seasonality this may be seen in aquarium settings as well. In such cases feeding regimens may need to be adjusted to correlate with temperature, season, or directly with the measured growth of aquarium-housed specimens (Robinson, 1983). One aquarium switches from feeding three times per week to feeding every other day in the summer when temperatures are warmer to meet the nutritional needs of their GPOs (J. Guthridge, personal communication). The faster growth season for wild *E. dofleini* is mid-summer to mid-winter, with slower growth until water temperatures warm again (Robinson, 1983). In constant temperature closed systems such changes will likely not take place, and growth will be relatively constant year-round.

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). Most seafood wholesalers will provide a variety of seafood, though care should be taken to ensure that products are harvested sustainably. The nutritional analysis of the food should be regularly tested and recorded. Contact the AITAG for further information on sustainable seafood and nutritional analysis resources.

Diet formulation: As described above in Chapter 5.1 GPO's diet in the wild is predominantly crabs and bivalves, but they may be fed a diet of raw seafood in aquarium settings. As stated above low-lipid diets are ideal, and as much lean invertebrate protein (i.e., crab meat, clam, shrimp, squid) should be offered as possible. This does not preclude the use of fish protein. Many types of fish will be readily eaten by GPOs, though the use of high-fat species (e.g., anchovy, herring, blue runner, silversides, etc.) should be minimized in favor of feeding leaner fishes (e.g., mullet, flounder, mackerel). Of 33 institutions surveyed in 2012, 75.0% fed greater than 50% invertebrate protein to their GPO, with the balance being fish protein. A variety of clams, oysters, mussels, crabs, and lobsters are available, and should be fed with fish protein to ensure a varied diet. Live foods such as dungeness crabs, blue crab, American lobster, etc. should be offered as often as is practical and economical. A summary of nutritional information for common seafood products used in aquaria is presented in Table 10. A survey of the foods most commonly fed to GPOs is presented in Table 11.

One way to empirically assess the suitability of food items is to consider the protein to lipid ratio (P/L), this is a commonly applied metric in aquaculture to ensure suitable fatty acids for larval fish development. In Table 10 above, the shellfish feed items have a P/L ratio of 12.04±7.2. The finfish items, by comparison had a P/L of 5.21±3.7 as most contain much higher amounts of fats and oils. Four of the finfish items from the table above have a P/L composition falling within the standard deviation of the shellfish items, namely bonito, pacific sardines, Spanish sardines, silver smelt, flounder, mullet, and pacific mackerel (see Figure 17). Variety in the diet of any animal is important, but these data suggest a few finfish protein sources that may be better suited to being staple items in the diet of captive octopods, with high fat items ideally being offered more sparingly.

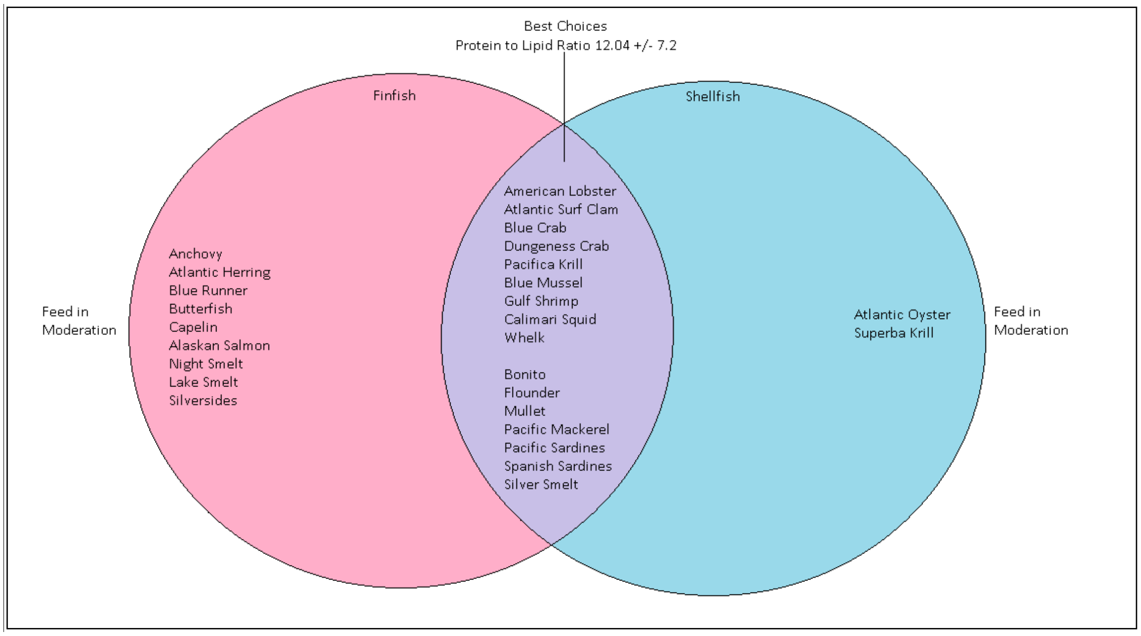


Figure 17. Venn Diagram Showing Suitability of Finfish and Shellfish Seafoods according to Protein to Lipid Composition in Relation to the Approximate Natural (Crustacean & Mollusc Dominant) Diet of *Enteroctopus dofleini*.

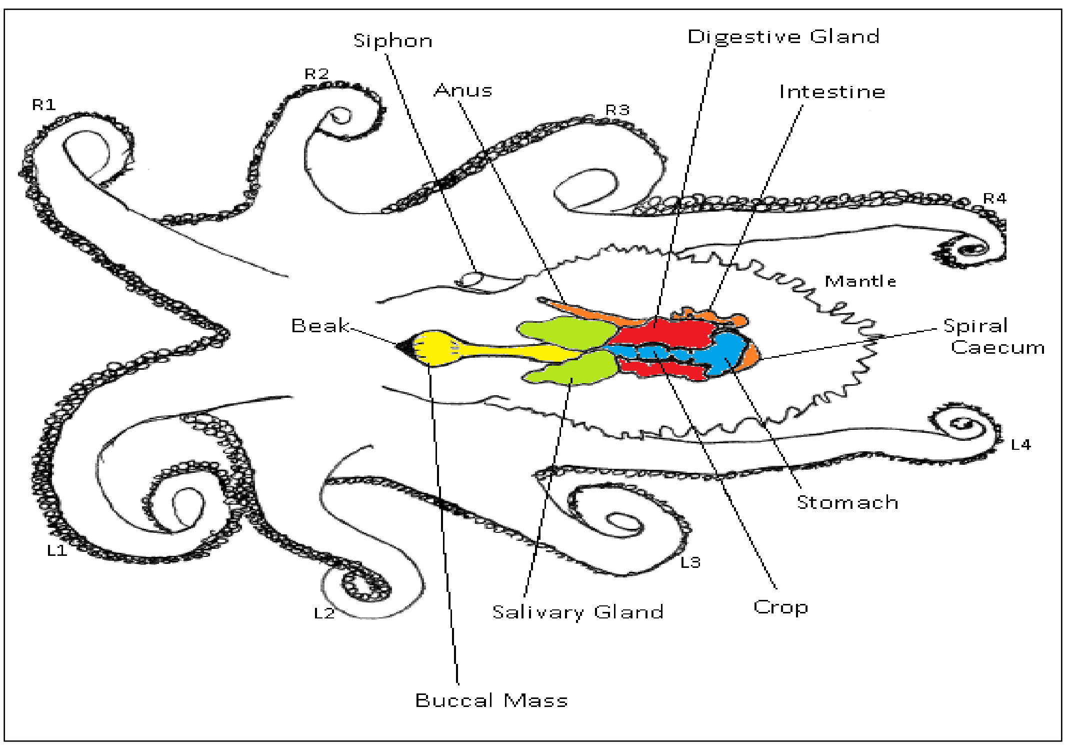


Figure 18. Digestive Anatomy of *Enteroctopus dofleini*. Re-drawn from a Figure by N. Johnson in Onthank (2008).

Table 11. Seafood types most commonly fed to *Enteroctopus dofleini* in aquarium settings. Data from a 2012 Survey of 33 institutions holding GPOs conducted by the AITAG. Items in **BOLD** are most prevalent (i.e., used in >50% of aquaria).

Seafood	Percent Institutions Feed	No. Institutions Feeding Item
Anchovy	13.30%	4
Herring	**70%**	**21**
Mackerel	**50%**	**15**
Bonito	23.3%	7
Salmon	30%	9
Silversides	40%	12
Shrimp	**93.3%**	**28**
Sardines	26.7%	8
Clam	**70%**	**21**
Geoduck	0%	0
Mullet	6.7%	2
Mussels	23.3%	7
Oysters	6.7%	2
Lobster	6.7%	2
Dungeness crab	40%	12
Blue crab	43.3%	13
Crayfish	6.7%	2
Capelin	**73.3%**	**22**
Smelt	26.7%	8
Flounder	0%	0
Squid	**66.7%**	**20**
Crayfish	13.3%	4
Striped bass	3.35%	1
Mahi	3.35%	1
Saury	3.35%	1
Scallops	6.7%	2
Jonah crab	10.05%	3
Cod	6.7%	2
Live clam	6.7%	2
Hard-boiled eggs soaked in fish blood/vita chem	3.35%	1
Atlantic rock crab	3.35%	1
Razor clam in shell	3.35%	1
Gel diet	3.35%	1

AZA Accreditation Standard

(2.6.1) Animal food preparation and storage must meet all applicable laws and/or regulations.

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 octopus 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.

Any AZA aquarium or zoo will undoubtedly already have protocols in place for the handling and preparation of seafood. As such it is beyond the scope of this manual to describe in detail specific protocols that should be in place, but in the interest of seafood safety a few general guidelines are presented here. If the aquarium or zoo housing octopods also houses mammals then USDA standards will already be in place and supersede all other practices.

In general frozen seafood should be kept solidly frozen and free of frost or freezer-burn. Packaging should be kept tightly sealed and a procedure should be in in place for rotation of stock so that the oldest seafood is used first. Seafood should be stacked away from freezer walls and ceilings so the efficiency of the freezer is not compromised.

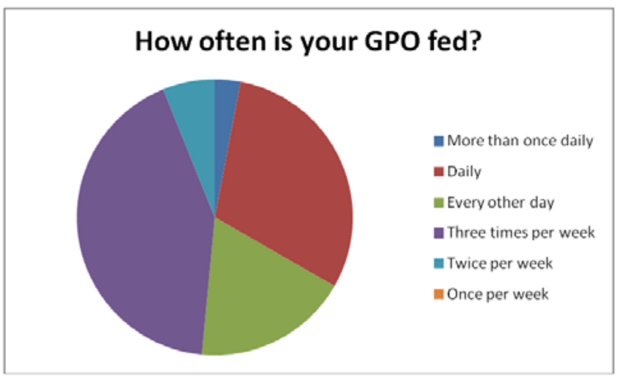
Thawing and handling of seafood should be done in a way to minimize microbial growth. Seafood should preferably be thawed slowly in a refrigerator. Seafood should not be thawed under water due to severe leaching of nutrients (Crissey, 1998). When handling care should be taken to keep the product cold (<4.4 °C [<40 °F]) at all times, and seafood should not be left out in the ambient air for extended periods of time during preparation.

Prepared seafood should be stored in a refrigerator in a container with a lid and the thawed date should be affixed to the outside. Food not used within a 24 hour period from thaw should be discarded (Crissey, 1998), as should any item that falls to the floor or other unsanitary surface. Finfish products that do not have clear eyes or have an especially fishy odor should never be used. Kitchens (including all food prep surfaces, sinks, and floors), utensils (knives, cutting boards, etc.), and dishes should be cleaned and sanitized after every usage.

If plants are used for animals enrichment, 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 octopus. 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.

5.3 Nutritional Evaluations

The best way of ensuring that an octopus' dietary needs are being met is if the animal is showing marked growth. Growth in aquarium settings should average about 1% per day, and at this pace animals will double in size every 70 days. General health and appearance will also indicate that the animal is being fed adequately. If the animal is being underfed, growth will cease and self-destructive behavior or escape attempts may be more likely. The animal will lose weight and be more prone to skin lesions that are slow to heal. Lethargy may be apparent, and coloration may not be as vibrant as normal. If too much fat or oil is present in the diet it will be evident by floating feces in the tank, as much of these compounds pass through the animal's gastrointestinal tract undigested. In summary a steady growth, either measured or visually apparent, as well as good coloration and general health may be taken as signs of adequate nutrition.



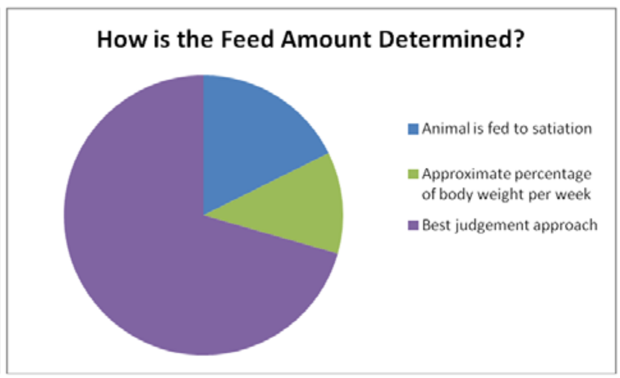


Figure 19 & 20. Frequency of feed times at 33 facilties surveyed in 2012 by AITAG and different feeding schedules successfully used with *Enteroctopus dofleini* at 33 faciltities.

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, however, 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 and to any emergencies (AZA Accreditation Standard 2.1.1). In some instances, because of their size or nature, exceptions may be made to the twice-monthly inspection requirement for certain institutions (e.g., insects only, etc.). Veterinary coverage must also be available at all times so that any indications of disease, injury, or stress may be responded to in a timely manner (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) that were updated in 2009 (http://aazv.affiniscape.com/associations/6442/files/veterinary_standards_2009_final.docx).

> **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.

Cephalopod disease and pathology is still in the very early stages of development; clinicians and aquarists are just beginning to gain an understanding of treatments and disease causing agents. The subject has received a scholarly treatment in Scimeca (2006); veterinarians and husbandry professionals unfamiliar with cephalopods are highly advised to consult that reference for questions of diagnosis, treatment, clinical methods, and formulary. Links to the Aquatic Invertebrate Taxon Advisory Group (AITAG) their Veterinary Liaison can be found on the AZA Animal Program search page.

Histopathology and diagnostic resources: A variety of companies that provide histopathology and other laboratory services are available if these services are not available within the holding institution. These laboratories and their associated pathologists are familiar with a wide range of taxa usually considered "exotic" to veterinarians, including many invertebrates. Contact the AITAG Chair to learn more about recommended companies.

All animals in the collection, including specimens of *E. dofleini*, should be inspected twice monthly, a visual assessment is usually sufficient. Any problems or unusual behaviors should be brought to the attention of managers and veterinarians immediately and may require a more in-depth inspection, which may entail removing the animal from the water briefly.

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). Procedures should include, but are not limited to: a list of persons authorized to administer animal drugs, situations in which they are to be utilized, location of animal drugs and those persons with access to them, and emergency procedures in the event of accidental human exposure.

> **AZA Accreditation Standard**
>
> **(2.2.1)** Written, formal procedures must be available to the animal care staff for the use of animal drugs for veterinary purposes, and appropriate security of the drugs must be provided.

Drugs for cephalopods: Cephalopods in general are extremely sensitive to chemicals. The knowledge of pharmacology for these animals is scant, and still being adapted from the standards of fishkeeping. Often cephalopods are not able to withstand the same dosages of drugs that fishes will, or will be able to tolerate them for a shorter duration. No doubt as time passes our knowledge of invertebrate medicine will grow as our knowledge of fish medicine has over the past couple of decades. Extreme caution is thus indicated in the application of any drug or chemical to cephalopods.

Administration: Most of the below listed drugs in Table 12 are commonly administered to octopods as a bath treatment. Cephalopods have a microvillus epidermis that is but a single cell layer thick, and have enormous capacity for direct absorption from the ambient seawater. A reservoir that will hold sufficient water (usually 20–30 gallons for a GPO) and is capable of maintaining temperature (i.e., an insulated cooler or vessel inside a walk-in refrigerator) should be selected for the treatment. Long duration treatments (i.e., those persisting for more than a few hours) may need to be administered directly to the entire tank (though this is much less economical) or in a smaller holding system (e.g., hospital or quarantine tanks). Holding/hospital tanks, or coolers, barrels, etc. used for immersion treatments need to have a secure lid to prevent the animal from escaping, and contain enough water to completely submerge the animal. In general only water-soluble drugs should be used in medicated baths for octopods; ethanol (which is most commonly used as a solvent) is an anesthetic agent and any interactions have yet to be investigated.

Injections in octopods can be difficult, but are possible. The simplest method of administering injectable drugs to an octopus is intramuscular (I.M.). The most common site for an I.M. injection in cephalopods is in the thick musculature of the upper arm (see Figure 21). If the animal is conditioned to sit in a net or laundry basket this is quite easily accomplished; previous conditioning to interact with aquarists is also helpful. Any gauge needle may be used depending on the viscosity of the injection and the size of the animal; larger specimens, especially senescent individuals, often do not seem to notice or care that they being injected, even with needles as large as 18 gauge.

Intravenous injections are also possible with octopus, though much more complicated. The gills and major hemolymph carrying vessels can be accessed in an octopous, though anesthesia and a minor procedure are required. To access a suitable site for I.V. injection in an octopus one needs to cut the median pallial adductor musculature and invert the mantle (Scimeca, 2006). While this procedure seems rather invasive animals recover from it with no observable detriment (Scimeca, 2006). In addition Scimeca (2006) also reports that arterial and venous catheters have been placed for short durations with good recovery rates, such procedures are common in physiological studies of all cephalopod taxa.

Storage and disposal: Storage of drugs used in the animal collection should be done according to directions of the institutions veterinarian, manufacturer's recommendations, any state or local laws, and according to the institution's written policies. Disposal of drugs, or of water containing drugs should be carried out in accordance with all federal, state, and local regulations.

Safety: Staff should be fully trained in all chemical and drug handling procedures, and have access to MSDS information where applicable. Staff should have access to, and use personal protective equipment where appropriate. At minimum the use of latex exam gloves is recommended when handling animals or water containing chemical agents. Of the drugs listed in Table 12 there are a few known hazards that staff should be aware of, though the manufacturer's directions and warnings should be understood before using any product. Formalin is a known carcinogen. Malachite green is a respiratory poison, teratogen, and suspected carcinogen. Nitrofuran drugs (e.g., nitrofurazone, furazolidone, nifurpirinol) are carcinogenic, genotoxic, and mutagenic. Chloramphenicol has been known to cause aplastic anemia in some humans, a fatal and irreversible condition. Tetracycline compounds that have degraded in aqueous solution are nephrotoxic to humans. Always consult the warnings and cautions when using any drug, and aquarists should take care to follow veterinarian's instructions precisely for administration and disposal.

The safety of the animal should also be considered; when any antibiotic compound is applied to the tank water the biofilter may be compromised. Aggressive water chemistry analysis should be conducted during any antibiotic treatment. Ammonia, nitrite, and pH should be checked at least daily during treatments.

Table 12. Pharmacology for cephalopods. The following chemotherapeutics have been used with varying degrres of efficacy in octopuses or other cephalopods. This excludes anesthetics and methods of euthanasia. This should not be considered a comprehensive formulary, as the science of invertebrate medicine is rapidly evolving.

Drug	Concentration	Application	Frequency / Duration	Notes	Reference
Acriflavine	-	-	-	Dosage not specified	Forsythe et. al., 2002
Amikacin Sulfate	-	-	-	Dosage not specified	Forsythe et. al., 2002
Atabrine	-	-	-	Dosage not specified	Forsythe et. al., 2002
Calcium Hypochlorite	-	-	-	Dosage not specified	Forsythe et. al., 2002
Ceftazidime	20 mg/kg	I.M.	q 3 d		Christie, Unpub. Data
Cefotaxime	-	-	-	Dosage not specified	Forsythe et. al., 2002
Ciprofloxacin	125 mg/l	Bath	20 min s.i.d.		Christie, Unpub. Data
Chloramphenicol	10 mg/kg	I.M.			Forsythe et. al., 2002
Chloramphenicol	75 mg/kg	P.O./I.M.	q 12 h x 6 d		Scimeca, 2006
Chloramphenicol	100 mg/kg	P.O.	s.i.d. x 6 d	Concentration for Vibriosis	Forsythe et. al., 2002
Enrofloxacin	10 mg/kg	P.O. q	q 8-12 h		Scimeca, 2006
Enrofloxacin	5 mg/kg	I.V.	q 8-12 h		Scimeca, 2006
Enrofloxacin	10 mg/kg	I.V.			Harms et. al. (2006)
Enrofloxacin	2.5 mg/l	Bath	q 4-6 h		Scimeca, 2006
Furazolidone	2 mg/l	Bath	24 h		Christie, Unpub. Data
Furazolidone	10 mg/l	Bath	1 h		Christie, Unpub. Data
Furazolidone	50 mg/l	Bath	10 min		Scimeca, 2006
Gentamicin Sulfate	20 mg/kg	I.M.	q 24 h		Scimeca, 2006
Hypersalinity	to 115 ppt	Bath	-	Brief Dip, Time n/s	Forsythe et. al., 2002
Hyposalinity	to 0 ppt	Bath	-	Brief Dip, Time n/s	Forsythe et. al., 2002
Itraconazole	5 mg/kg	P.O.	s.i.d. x 14 d		Harms et. al., 2006
Kanamycin	25 mg/l	Bath	60min q 3 d		Christie, Unpub. Data
Kanamycin	-	-	-	Dosage not specified	Forsythe et. al., 2002
Malachite Green	-	-	-	Dosage not specified	Forsythe et. al., 2002
Metronidazole	100 mg/l	Bath	16 h		Scimeca, 2006
Minocycline Hydrochloride	-	-	-	Dosage not specified	Forsythe et. al., 2002
Neomycin Sulfate	-	-	-	Dosage not specified	Forsythe et. al., 2002
Triple Antibiotic Ointment	-	Topical	-		Forsythe et. al., 2002
Nalidixic Acid	13 mg/l	Bath	6h q3d		Christie, Unpub. Data
Nifurpirinol	1-2 mg/l	Bath	b.i.d. 10-30 d	Lethal to squid > 0.1 mg/l	Forsythe et. al., 2002
Nitrofurazone	1.6-3.2 mg/l	Bath	Constant	Lethal to squid	Forsythe et. al., 2002
Nitrofurazone	25 mg/l	Bath	60min b.i.d.	Lethal to squid	Scimeca, 2006
Nitrofurazone	1.5 mg/l	Bath	72 h	Lethal to squid	Scimeca, 2006
Nitrofurazone / Furazolidone	4 mg/kg	Bath	24 h	1:1 mixture, 2mg/l of each	Christie, Unpub. Data
Oxolinic Acid	0.5 mg/l	Bath	24 h		Christie, Unpub. Data
Oxolinic Acid	10mg/l	Bath	1h s.i.d.		Christie, Unpub. Data
Oxolinic Acid	25mg/l	Bath	15 min b.i.d.		Christie, Unpub. Data
Oxytetracycline HCl	10 mg/kg	I.M.	s.i.d.		Forsythe et. al., 2002
Piperacillin	-	I.M. or I.V.	-	Dosage not specified	Forsythe et. al., 2002

Pharmacokinetics: While little has been published on the use of chemotherapeutics in cephalopods even less is known of the physiological interactions of chemicals *in vivo*. Pharmacokinetics for many different antibiotics have been studied in species of value to aquaculture, including some invertebrates (namely shrimps). To date there is a single published investigation of an antibiotic in cephalopods; Gore et al., (2005) studied the pharmacokinetics of enrofloxacin (Baytril®) in the cuttlefish *Sepia officinalis*. The Gore et al., (2005) study found that enrofloxacin does not distribute itself intracellularly to the high degree that it does in mammals, and that it has rapid clearance rates compared to well-studied fish models (trout) with half lives of 1.81 hours following I.V. administration at 10 mg/kg and 1.01 hours following a 5 hour bath at 2.5 mg/l. The same investigation also found that enrofloxacin was not metabolized to ciprofloxacin (Gore et. al., 2005). Based on their results Gore et. al. (2005) suggested that bath concentrations of 2.5 mg/l or I.V. administration at 5 mg/kg should be sufficient to attain acceptable minimum inhibitory concentrations of enrofloxacin in the hemolymph of the cuttlefish, but due to the rapid clearance rates they suggest considering twice daily administration of such treatments. The same study also investigated the effect of oral enrofloxacin administration at 10 mg/kg P.O. with a limited sample size, with promising results (Gore et. al., 2005).

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.

Figure 21. Typical injection site for IM injections in specimens of *E. dofleini*. Animals conditioned to sit in baskets or nets and accustomed to human interaction can be easily injected in the musculature of an arm. Photo courtesy of B. Christie

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 an animal record keeping system 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

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.

institution (AZA Accreditation Standard 1.4.5) as well as be duplicated and stored at a separate location (AZA Accreditation Standard 1.4.4).

AZA Accreditation Standard

(1.4.7) 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.

Record keeping: Records for octopus should include water chemistry analysis, a record of quarantine and any prophylactic treatments administered. Date and source of acquisition, all relevant medical procedures performed. Behavioral information should also be recorded, as well as maintenance of critical life support systems. The institution keeping a GPO should be encouraged to keep records of feeding amounts and types, enrichment activities, morphometrics (i.e., weight and growth), and other factors.

The care of octopus does not fall under the purview of the UDSA, and the species is not on the endangered species list so federal regulations regarding permitting, recordkeeping and reporting do not apply. No formal documentation is required when transporting or maintaining *E. dofleini*, unless required at the state or local level. The institution should check with their state and local wildlife management authorities to ensure they are in compliance with all reporting regulations.

6.2 Identification Methods

Ensuring that octopuses are identifiable through various means increases the ability to care for individuals more effectively. Animals must be identifiable and have corresponding ID numbers whenever practical, or a means for accurately maintaining animal records must be identified if individual identifications are not practical (AZA Accreditation Standard 1.4.3).

AZA Accreditation Standard

(1.4.3) Animals must be identifiable, whenever practical, and have corresponding ID numbers. For animals maintained in colonies/groups or other animals not considered readily identifiable, the institution must provide a statement explaining how record keeping is maintained.

Animal identification: As with most aquatic invertebrates, there are few practical methods commonly established for tagging octopods. Identification of individual specimens in a collection is usually easily managed as their inherent predilection towards interspecific aggression and cannibalism dictates they be housed in separate tanks. Should reproduction in aquaria be attempted in the species, the two animals would be temporarily introduced before being isolated again, in which case they could be identified visually via sexual dimorphism (see Chapter 7 for further information on reproduction). Thus specimens of *E. dofleini* in aquaria may be easily tracked as individuals for recordkeeping purposes without the use of tags or other identifying marks.

Invasive tagging methods applied to the mantle of octopuses are often ripped out by the animal- leaving a gaping wound (Barry et al., 2011). More secure tags (e.g., Peterson tags) that are bolted onto the mantle have been used successfully in some studies, though Barry et al., (2011) found necrosis at the site of application of such devices. Visible-implant elastomer (VIE) tags consist of brightly colored plastics that may be injected under the skin to create distinct markings. These VIE tags are much less invasive than other methods and have been shown effective in *E. dofleini* (Barry et al., 2011, Brewer and Norcross, 2012). This method seems well adapted for marking of large octopuses in a laboratory or aquarium setting if it is deemed necessary.

It should also be noted here that a recent study has used individual body patterns to allow for photoidentification of individual species in *Wunderpus photogenicus*, but this method is not practical for use in *E. dofleini* (Huffard et al., 2008). The use of passive integrated transponder (PIT) or other RFID tagging methods has yet to be utilized, and may be of promise if tagging or marking of an octopus is deemed necessary by institutional protocol or experimental design.

Maintenance of group records: Contrary to recordkeeping practices in most zoos, the possibility of individually accessioning animals in aquaria is usually the exception, rather than the rule. Maintenance of large groups of animals, such as is typical of fishes and invertebrates, presents a unique challenge to those responsible for institutional recordkeeping. Since the current practice in public aquaria trends towards maintenance of single specimens (as described in Chapters 4.1, 4.2, and 4.3), individual identification is almost always possible with octopods (though this may not be practical with other cephalopod taxa).

One notable exception would be in cases where reproduction in an aquarium has taken place. In these cases, excessively large numbers of larvae would need to be managed as a group. Groups can be periodically inventoried (e.g., monthly, every 60 days) until the larvae reach a manageable physical size and stable population size and they can be better tracked.

AZA member institutions must inventory their octopus population at least annually and document all octopus acquisitions and dispositions (AZA Accreditation Standard 1.4.1). Transaction forms help document that potential recipients or providers of the animals must adhere to the AZA Policy on Responsible Population Management: Acquisitions, Transfers and Transitions by Zoos & Aquariums (ATT Policy) (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 AZA-accredited institutions must abide by the AZA ATT policy (Appendix B) and the long-term welfare of animals should be considered in all related decisions. All species 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.

Animal transfers with GPOs are exceedingly rare between institutions, though they do occasionally occur. Owing to their short life spans these animals are usually acquired from collectors and held until their natural death a year or two after acquisition. Each accredited institution undoubtedly has their own form that is utilized when an animal transfer takes place; though a sample generalized animal transaction form can be found in Appendix K.

6.3 Transfer Examination and Diagnostic Testing Recommendations

The transfer of animals between AZA-accredited institutions or certified related facilities due to AZA Animal Program recommendations occurs often as part of a concerted effort to preserve these species. These transfers should be done as altruistically as possible and the costs associated with specific examination and diagnostic testing for determining the health of these animals should be considered.
As veterinary medicine for invertebrates is still a science very much in its infancy there are little available diagnostic options, though this will likely change in the future. One promising research method that has great potential for development into a clinical technique is that of screening cephalopod hemolymph for apicomplexan sporocysts (Gestal et al., 2007). Specimens of *E. dofleini* have also been documented as having been infected with *Aggregata* spp*.* though this is rare in aquarium-housed specimens.

Until clinical methodology is developed cursory visual examinations are the best tool for health assessment of octopods. Estimates of size and weight of the animal along with a general inspection is recommended. The primary indicator of good health in cepahlopods is a robust appetite, specimens displaying inappetence may be compromised. Body condition and general health can be assessed by inspecting the mantle, arms, and suckers. Of special note are the presence of lesions, eye clarity and lack thereof, and signs of the onset of senescence (see Chapter 6.7 and the natural history information in the introduction for more detailed information on senescence). Strong sucker adhesion, color, and general alertness are also indicators of good general health.

Normal health parameter values that are commonly assessed in terrestrial animals (blood and urine values, et cetera) are unknown in cephalopods. Because their morphology is highly plastic (e.g. size is a function of growth, which is a function of diet, and neither strictly correlate to the age of a given animal) length/weight relationships are difficult to define and not necessarily indicative of good health.

6.4 Quarantine

AZA institutions 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 2.7.1). All quarantine, hospital, and isolation areas should be in compliance with AZA standards/guidelines (AZA Accreditation Standard 2.7.3; Appendix C). All quarantine procedures should be supervised by a veterinarian, formally written and available to staff working with quarantined animals (AZA Accreditation Standard 2.7.2). If a specific quarantine facility is not present, then 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. Local, state, or federal regulations that are more stringent than AZA Standards and recommendation have precedence.

AZA Accreditation Standard

(2.7.1) The institution must have holding facilities or procedures for the quarantine of newly arrived animals and isolation facilities or procedures for the treatment of sick/injured animals.

AZA Accreditation Standard

(2.7.3) Quarantine, hospital, and isolation areas should be in compliance with standards/guidelines contained within the *Guidelines for Zoo and Aquarium Veterinary Medical Programs and Veterinary Hospitals* developed by the American Association of Zoo Veterinarians (AAZV), which can be obtained at: http://www.aazv.org/associations/6442/files/veterinary_standards_2009_final.docx.

AZA Accreditation Standard

(2.7.2) Written, formal procedures for quarantine must be available and familiar to all staff working with quarantined animals.

Facility: If at all possible new specimens of *E. dofleini* should be quarantined in a dedicated quarantine tank remote from the display exhibits; though due to the size of this species, the highly specialized LSS required, and the security measures needed to prevent escape, it may be necessary to quarantine the animal on exhibit at some facilities. If an institution does not have a suitable remote quarantine tank, care should be taken to prevent cross-contamination between the octopus exhibit and other tanks, especially if there has been any previous indication of health issues. See Chapters 1.3, 2.1, and 2.2 for a thorough discussion of housing and water quality requirements for the species.

A new octopus should only be placed in a tank formerly occupied by a conspecific that has died if the death is clearly the result of a natural process (i.e., senescence). If there are unusual circumstances in an animal's death the tank should remain empty until veterinary staff have completed all pathology work to rule out an infectious process or until the tank is chemically disinfected and re-cycled. See Chapter 1.3 for detailed information on artificial cycling of a tank, and Chapter 6.5 for information on disinfectants and sterilants.

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. Keepers should be designated to care only for quarantined animals if possible. If keepers must care for both quarantined and resident animals of the same class, they should care for the quarantined animals only after caring for the resident animals. Equipment used to feed, care for, and enrich animals in quarantine 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 use with resident animals.

AZA Accreditation Standard

(11.1.2) Training and procedures must be in place regarding zoonotic diseases.

Zoonotic disease is discussed in greater detail below in Section 6.5, but the risks when working with octopods are minimal. No special protective equipment (masks, gowns, et cetera) are needed when working with quarantine animals, though if an aquarist also works with exhibit (non-quarantine) animals any clothing that becomes wet when working with quarantine animals should be changed. Wetsuits, boots, and waders used in quarantine should never be used when servicing exhibit tanks. Footbaths are employed by some facilities (primarily at institutions that quarantine terrestrial animals in the same building) but are of limited value in preventing the spread of disease among aquatic animals. All equipment used in quarantine should be properly cleaned and sanitized after use, disinfection techniques are discussed in greater detail below in Section 6.5.

Quarantine durations span of a minimum of 30 days (unless otherwise directed by the staff veterinarian). If additional mammals, birds, reptiles, amphibians or fish of the same order are introduced into their corresponding quarantine areas, the minimum quarantine period must begin over again. However, the addition of mammals of a different order to those already in quarantine will not require the re-initiation of the quarantine period.

Octopuses, like all animals, do carry internal parasites. Currently little is known about the host-parasite interactions that may exist, and parasites are usually only identified upon necropsy. There is little information to suggest that common parasite fauna have a detrimental effect on animal health. Methods of identifying parasite burden from live animals have yet to be identified, and the safety of any antihelminthic compounds for use in cephalopods has yet to be determined. A list of common octopus parasites is presented in Chapter 6.7 alongside descriptions of diseases identified.

During the quarantine period, specific diagnostic tests should be conducted with each animal if possible or from a representative sample of a larger population (e.g., birds in an aviary or frogs in a terrarium) (see Appendix C). A complete physical, including a dental examination if applicable, should be performed. Animals should be evaluated for ectoparasites and treated accordingly. Blood should be collected, analyzed and the sera banked in either a -70 °C (-94 °F) freezer or a frost-free -20 °C (-4 °F) freezer for retrospective evaluation. Fecal samples should be collected and analyzed for gastrointestinal parasites and the animals should be treated accordingly. 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.

A tuberculin testing and surveillance program must be established for animal care staff as appropriate to protect both the health of both staff and animals (AZA Accreditation Standard 11.1.3). 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 should be permanently identified by their natural markings or, if necessary, marked when anesthetized or restrained (e.g., tattoo, ear notch, ear tag, etc.). Release from quarantine should be contingent upon normal results from diagnostic testing and two negative fecal tests that are spaced a minimum of two weeks apart. Medical records for each animal should be accurately maintained and easily available during the quarantine period.

> **AZA Accreditation Standard**
>
> **(11.1.3)** A tuberculin (TB) testing and 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.

Husbandry, and most importantly LSS should be essentially the same as for animals on exhibit, see section 2.1 for a discussion of suitable off-exhibit holding facilities for *E. dofleini*. All of the known parasites for *E. dofleini* are discussed in Section 6.7, and chemotherapeutics published as having been used successfully on cephalopods are discussed above in Section 6.1 – though it should be noted that thereis a paucity of information on the use of antihelminthics in cephalopods, and no known treatment regimens have been developed for the Dicyemida. Identification techniques are discussed in Section 6.2. There are, as yet, no vaccines commonly used in cephalopods, though as invertebrate medicine continues to evolve this may change. A more thorough discussion of zoonotic disease is contained below in Section 6.5, but in general the keeping of cephalopods does not necessitate TB testing for staff.

Behavior in quarantine: Behavior and growth during the quarantine period should be carefully noted to ensure the health of the animal. Self-destructive or stereotypical behaviors such as jetting into the tank or escape attempts should be carefully monitored while in quarantine, and stimulus offered to counteract such maladaptive behaviors (see Section 8 for detailed information on octopus enrichment).

Release from quarantine: An animal should be released from quarantine when it has completed a minimum 30 day observational and/or prophylaxis period, is eating reliably, has good color, and is free of external lesions and not showing any maladaptive behavior.

If a GPO should die in quarantine, a necropsy should be performed on it and the subsequent disposal of the body must be done in accordance with any local or federal laws (AZA Accreditation Standard 2.5.1). Necropsies should include a detailed external and internal gross morphological examination and representative tissue samples form the body organs should be submitted for histopathological examination (see Chapter 6.7).

> **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.

Necropsy procedures and pathology information are listed in Chapter 6.7; laboratories familiar with cephalopods for histopathological analysis are detailed in Chapter 6.1. Common causes of death for *E. dofleini* are detailed below in Section 6.7, but it should be mentioned that death of an non-senescent animal in an aquarium is exceedingly rare. Instances where a young animal has died usually involve escape from the tank and subsequent desiccation, exceptionally poor water quality, or trauma from suction of unprotected pump inlets.

6.5 Preventive Medicine

AZA Accreditation Standard

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

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).

Preventative medicine: Visual observation of animals should occur daily as part of normal husbandry procedures. Any changes or irregularities need to be noted and discussed with animal care staff and veterinarians. In the United States there are currently no diagnostics required by law for pre-shipment physicals or during the quarantine period. Morphometrics are important to gauge growth, which is the best indicator of general health and wellbeing. If possible, weights should be collected initially upon arrival at the institution and annually thereafter so growth can be assessed. Fecal samples may be collected and examined by direct smear, but fecal flotation procedures for cephalopods and indices of parasite larvae have yet to be developed for the taxa.

Unlike most fishes growth is highly variable in cephalopods (see Chapter 5.1) and not directly correlated to age class, thus standard fisheries metrics (i.e., length-weight; size-age) are not available for *E. dofleini*. Weights can be taken at several times during the animal's residence at an institution to assess growth or lack thereof. Care should be taken to minimize the level of disturbance to the animal during such procedures, conditioning to sit in a laundry basket or net will facilitate removal from the tank for such measurements (see Chapter 8.1).

Octopus stress: All animals are subject to stress. This may be as simple (neurologically speaking) as a mussel closing in reaction to being touched or as complex as a human reacting to a difficult job situation (Mather & Anderson, 2007). With the alleviation of the stressors, the animals return to a normal baseline of physiology or behavior.

When animals in zoos are subject to life-threatening stress, they react to it. Octopuses show visible signs of distress and suffering, such as loss of appetite or skin sores. For the purposes of brevity, and for the understanding of non-psychologists, the term "stress" here is used to mean stress and distress. Octopuses don't behave normally under stressful situations. Researchers looking at the behavior of octopuses can't depend on normal behavior or reactions in stressful situations (Mather & Anderson, 1993; Sinn et al., 2001). Several researchers looking at lateralization in octopuses found that senescent animals would not behave normally, as they had no appetite (Byrne et al., 2002). Since stress may lead to lack of appetite, the behavior will not be normal. All food choice observations such as Anderson & Mather (2007) will be skewed if an octopus observed is senescent or stressed.

Secondly, with the advent of more public aquariums worldwide, and with GPOs being the most exhibited octopus species in public aquariums it behooves such aquariums to show animals in the best of all conditions, which is what the public expects (Levy, 1998; Carlson & Delbeek, 1999). All zoos and aquariums currently have an obligation to present and keep healthy animals, particularly nowadays when such institutions have the obligation of propagating rare and endangered animals.

Stressed animals are not healthy animals and as such will not breed as readily as healthy animals. For example, a mated female GPO allowed to lay eggs at one aquarium brooded her eggs to hatching, but upon her death 2 weeks after the eggs hatched it was found she had laid only about half the eggs inside her ovaries. This was determined by counting those laid and those found unlaid still in her ovaries upon necropsy.

There are several classic signs of stress in octopuses, particularly in GPOs and it may be difficult to tell if an animal is senescent or stressed from some other condition. Senescence is caused by age and will usually be seen in large old males and sometimes in females if they survive after their eggs hatch. In GPOs senescence is evidenced by lack of appetite, increased activity, large white lesions (see Figures 2, 3, 21), particularly on the posterior end of the mantle (euphemistically called "butt burn" in the aquarium trade), and withdrawn shrinking skin around the eyes, giving the eyes a "hollow-eyed" look. The "hollow eye" condition is from the skin withdrawing around them as the animals lose weight.

All of these signs are also signs of stress except the sunken eyes, and stress due to water quality issues may give an octopus small white spots all over its skin. The usual and most common sign of stress in an octopus is lack of appetite in an octopus that should normally be hungry. An aquarium-housed subadult GPO can eat an impressive amount. One young male at one aquarium gained a mean 2.7% of his body weight per day over 4 months of being weighed weekly. This is a very healthy appetite that neither sick nor stressed animals nor senescent ones will have. If a sub-adult octopus has no appetite it is either sick or senescent.

Octopus endocrinology: Another way of measuring stress in octopuses is more practical and quantifiable. This is by measuring the adrenal glucocorticoid stress hormone, corticosterone, in octopus feces. The landmark study by Larson & Anderson (2010) looked at hormones measured in the feces of GPOs housed in one aquarium. They found measurable amounts of the stress related hormone corticosterone, and the reproductive-related hormones estrogen, progesterone, and testosterone in GPO feces. The levels of corticosterone found within the octopus feces were related to observed stressors in the octopus's life and/or life stage history.

Larson & Anderson (2010) first established a baseline of corticosterone within GPO feces (ca. 20 ng/g feces). They then stressed an adult female by injecting her with adrenocorticotropin hormone (ACTH), a vertebrate adrenal hormone that cause the adrenal glands to release the stress related glucocorticoids, corticol or corticosterone, depending on the species. The octopuses' responses to ACTH were then measured by collecting all voided feces for 72 hours to see if her physiology was similar to vertebrates, i.e., would she also produce one of the glucocorticoids in response to ACTH. The octopus did respond to the ACTH by producing a spike of corticosterone of 100 ng/g in the feces. They then also stressed the female on two different days by removing her briefly from her tank and by giving her an injection of saline to see if she would respond to external stressors by producing one of the glucocorticoids. These two stressors resulted in corticosterone spikes of 50 ng/g feces and 60 ng/g feces, respectively. It is interesting to note that both vertebrates and the invertebrate octopuses use similar hormones; these are animals whose heritages diverged more than a billion years ago.

Injuries will certainly cause stress in a GPO but to what extent hasn't been investigated yet. Hartwick et al. (1978) found that in a wild population 50% of the octopuses had missing or shortened arms that they attributed to predation by octopus predators. It has long been dogma that an octopus could regrow a missing or injured arm. Furlong & Pill (1985) even proposed that divers could cut off an arm of a live octopus for harvest since it would grow back. The truth is somewhat different. It will only grow back in juvenile animals. It will grow back partially in sub-adult animals, and won't grow back at all in adult animals. Thus the practice of harvesting octopus arms should be discouraged as such re-growth doesn't occur in practicality.

Management of senescent animals: A detailed description of senescence in octopods is presented in the Natural History Section of the Introduction and below in Chapter 6.7. It is important to note that the semelparous life style of octopods is finite, and once senescence begins the animal is in the terminal phase of its life and death is imminent. Individual animals are highly variable, and senescence may persist for as little as 2 weeks or as long as 6 months (Anderson et al., 2002; B. Christie, personal observation). In many cases, aquaria may induce the "laboratory artifact" by unnaturally prolonging the animal's demise. Supportive care may also extend the animal's life unnaturally; antibiotics can mitigate epidermal lesions that would otherwise not heal due to the lack of immune function, and some animals may continue to feed for extended periods of time while senescent, prolonging the inevitable. Scimeca (2006) recommends considering the age of a cephalopod versus its natural life span before undertaking any extensive diagnostic or treatment regimen, which is especially applicable to cephalopods. More often than not a specimen of *E. dofleini* will reach a point in aquarium settings where it is humane to euthanize the animal. A review of methods of euthanasia for cephalopods is presented by Anderson (1996) and in Chapter 6.7.

As stated in the Chapter 6.4, 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. Keepers should be designated to care for only healthy resident animals, however if they need to care for both quarantined and resident animals of the same class, they should care for the resident animals before caring for the quarantined animals. Care should be taken to ensure that these keepers are "decontaminated" before caring for the healthy resident animals again. Equipment used to feed, care for, and enrich the healthy resident animals should only be used with those animals.

AZA Accreditation Standard

(11.1.2) Training and procedures must be in place regarding zoonotic diseases.

Zoonotic risks are minimal as with most aquatic and marine species. Water-borne agents like *Mycobacterium marinum* and other bacteria are always a risk particularly if open wounds are not protected from contact with the tank water. While exceptionally rare there have been a significant number of reported cases of *M. marinum* infections of humans in the literature (Edelstein, 1994; Aubry et al., 2002) including cases directly linked to aquarium exposure (Lewis et al., 2003). In most cases an aggressive antibiotic regimen is successful in treatment, though immunocompromised individuals are at greater risk from *M. marinum* (Lambertus & Mathison, 1988; Hanau et al., 1994; Parent et al., 1995).

The only well-documented potential danger of metazoan parasite transmission from cephalopods to humans is that of anasakid nematodes (Pozio, 2008), which can only occur in cases of undercooked seafood. As such, there is no potential metazoan parasite transmission risk in a zoo or aquarium setting though other factors must be considered to safeguard the animals and participants in any type of animal interaction.

Disinfection procedures: Any aquarium maintaining marine life undoubtedly already has disinfection/biosecurity procedures in place to prevent cross contamination. As such the relevant methods to aquarium and aquaculture are given a cursory presentation here, and not described in detail. Common methods of disinfection for nets, scrub pads, and other tools include soaking in chemical solution or application via a pump sprayer. Common disinfecting solutions in aquariums include sodium hypochlorite (bleach), quaternary ammonium compounds, and potassium monopersulfate (Virkon-S). Clinical disinfectants such as clorhexidine (Nolvasan), and alcohols are also used in some facilities. Due to its low cost and proven efficacy most aquaria utilize bleach in various concentrations, which may be rinsed with water or neutralized with sodium thiosulfate (STS). In order for any disinfecting solution to work properly equipment needs to first be free of oils or other organic compounds. Nets or equipment with mucous or other organic fouling should be washed thoroughly with detergent or dish soap prior to disinfection. A selection of common disinfectants for aquarium and aquaculture use is presented in Table 13.

Table 13. Selected standard methods for disinfection of equipment and tanks in aquariums and aquaculture facilities. Compiled and adapted from Floyd (2001), Noga (2010), and manufacturer's instructions for the compounds listed.

Active Ingredient	Trade Name	Concentration	Contact Time
Sodium Hypochlorite	Bleach	10 mg/l	24 hrs
Sodium Hypochlorite	Bleach	100 mg/l	2–3 hrs
Sodium Hypochlorite	Bleach	200 mg/l	30–60 min
Sodium Hypochlorite	Bleach	10% v/v	60–120 sec
Chlorhexidine Diacetate	Nolvasan	30 ml/gal	60 sec
Quaternary Ammonium	Roccal-D	15 ml/gal	10–20 min
Quaternary Ammonium	Simple Green-D	60 ml/gal	10 min
Potassium Monopersulfate	Virkon-S	1% w/v	10 min
Ethanol	Grain Alcohol	70% v/v	15 min
Isopropyl Alcohol	Rubbing Alcohol	85% v/v	15 min

Cross-contamination: Whenever possible staff should be dedicated to quarantine areas to minimize risk of cross-contamination. If an aquarist should have to work both with quarantine and exhibit animals in the same day they should be instructed to complete all of their work with the exhibit animals before beginning work with quarantine tanks. If it is absolutely necessary to work with an animal or tank in a quarantine area before returning to work elsewhere the aquarist should scrub their arms up to the elbow with soap and hot water, and use a hand sanitizing gel. Any items of clothing that become wet while working with quarantine animals should be changed before working elsewhere, and items such as rubber boots, waders, wetsuits, gloves, et cetera should be dedicated to use in quarantine areas and never used elsewhere in the aquarium.

Contrary to standard practice with terrestrial animals footbaths are of limited benefit in preventing disease transmission with fishes and aquatic invertebrates. The use of latex exam gloves when handling animals, especially quarantine or hospitalized animals is a good practice, but there is little practical zoonotic concern with cephalopods. Gowns and masks are similarly of limited benefit with fishes and aquatic invertebrates due to the lack of zoonoses that the aquarist may encounter with this taxa, as described above.

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).

Also stated in Chapter 6.4, a tuberculin testing and surveillance program must be established for animal care staff, as appropriate, to protect the health of both staff and animals (AZA Accreditation Standard 11.1.3). Depending on the disease and history of the animals, testing protocols for animals may vary from an initial quarantine test, to annual repetitions of diagnostic tests as determined by the veterinarian. To prevent specific disease transmission, vaccinations should be updated as appropriate for the species.

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

(11.1.3) A tuberculin (TB) testing and 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.

Aquatic and marine animals are not prone to infection with *Mycobacterium tuberculosis*, and as such TB testing is not needed for staff working with cephalopods. Exposure to *Mycobacterium marinum*, which is a ubiquitous marine bacterium often present in the aquaria may cause false-positive results on a standard tuberculin skin test. A false positive induced by exposure to *M. marinum* may be disproven by further diagnostics. Most institutions consider TB testing for staff that work solely with aquatics to be unnecessary, unless those staff also work with mammals.

Vaccinations: Vaccines for aquatic and marine animals in general are extremely limited, and none have been evaluated for use in the Cephalopoda. In public aquaria, formalin-killed *Vibrio anguillarium* vaccines designed and FDA approved for use in salmonid fishes have been extrapolated to a number of other teleost fishes with good effect. The use of formalin-killed *Vibrio* vaccines has yet to see use in any invertebrates, though preliminary experiments in echinoderms were conducted at one aquarium to little apparent effect against *Vibrio*-induced wasting in starfish (B. Christie, unpublished data).

6.6 Capture, Restraint, and Immobilization

The need for capturing, restraining and/or immobilizing an animal 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.

Typical capture equipment for octopods include nets, mesh dive bags, laundry baskets, and 5-gallon buckets. Equipment used for temporary holding typically includes 5-gallon buckets (for small specimens), large coolers, (clean) trashcans dedicated for animal use, plastic barrels, or large heavy-duty bags (3 mil or greater). Due to their flexibility and dexterity coupled with eight arms, octopods cannot be restrained in a stretcher similar to other large aquatic animals. Should an octopus need to be restrained for any purpose other than a gross external examination, chemical sedation will most likely be necessary.

The use of standard large animal immobilizing drugs has yet to be evaluated in cephalopods, and methods of administration common to zoos (e.g., dart guns, etc.) are not applicable to aquatic animals. There are a number of anesthetics which have been used in sedation of cephalopods for handling, the most common being a 75 mg/l stock solution of magnesium chloride mixed 1:3 or 1:4 with the ambient seawater (Messenger et al., 1985; Scimeca, 2006) to lightly sedate the animal for handling. A detailed discussion of anesthesia in cephalopods is presented in Chapter 6.7.

No specialized training for staff is needed to capture and restrain an octopus, though the authors have found it helpful to make sure every person involved knows their specific responsibility when handling any large animal. See section 3.1 for detailed information on capturing/moving large octopods.

6.7 Management of Diseases, Disorders, Injuries and/or Isolation

> **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-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. Giant Pacific octopus 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. Octopus 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.

The most common medical issue with all cephalopods is senescence, which is described in greater detail in the natural history section of the introduction. Octopods are semelparous (terminal spawners), and as such, their decline and death in an aquarium setting is inevitable. The process of senescence in octopods is described in detail by Anderson (2002). Senescence starts after mating, at which time females will guard the egg clutch until hatching and males seem to "wander" without direction; these behaviors are presented in aquarium settings as well (Anderson, 2002). Specimens of *E. dofleini* may live as short as a couple weeks in a condition of senescence, or for months (Anderson, 2002). Anderson notes that a typical senescence lasts over a month, and one of the authors has observed a senescence period close to 6 months before death. While senescent male octopods make good display specimens as they constantly move about the enclosure, females will guard and tend to their egg clutch; both sexes will typically refuse to feed (Anderson, 2002).

Typical clinical presentations of senescence include anorexia, retraction of the integument around the orbits (hollow eye appearance), uncoordinated movement, increased undirected activity, and white lesions on the body that will not heal (Anderson, 2002). It should also be cautioned that these same symptoms might manifest as a result of poor water quality, temperature stress, or other disease process (Anderson, 2002). Females will typically lose as much as 50–71% of their body weight due to their anorectic behavior while guarding eggs (Anderson, 2002). The risk of secondary infections in lesions is great as cephalopods immune systems seem to shut down during senescence. Often euthanasia is indicated at some point during this process, further discussion of which is presented later in this section.

Reporting of abnormal behavior or signs of possible clinical significance should follow individual institutional policies and procedures. Abnormal behavior should be reported to the appropriate front-line manager/supervisor who can effectively coordinate with veterinarians and/or curators. Any abnormal behaviors should also be recorded in accordance with institutional record-keeping procedures.

Hospital facilities: Due to the incredible chemical sensitivity of cephalopods, their high production of toxic metabolites, and propensity for escape hospital facilities for *E. dofleini* will need to provide equal filtration capacity, space, and security against escape. Hospital tanks need to be pre-cycled and capable of supporting the animal. See Chapters 1.1–1.4 for information on environmental parameters and Chapters 2.1 and 2.2 for space and security requirements.

Diseases in octopods: The appearance of white lesions on the mantle of a senescent octopus is typical and will occur in most specimens at the end of life. A young octopus, in good condition and in good water quality, can sustain an injury that may cause such lesions. However, Hanlon (1983) remarks that the skin deteriorates during the 2–4 week senescence of both male and female *O. briareus*. "Skin damage usually leads to infection only in old animals" (Mangold, 1983). Van Heukelem (1977) states that the healing processes of octopuses are shut off during senescence, so skin injuries may become secondarily infected with *Aeromonas*, *Vibrio* and *Staphylococcus* bacteria. It appears that the primary cause of such lesions is the shutting down of the immune system.

Antibiotics (including enrofloxacin and ceftazadime) have been given (as I.M. injections) to senescent GPOs and other octopods during early senescence to manage wounds of the epidermis with some apparent efficacy, though as the animal is terminal at this point it is difficult to gauge the extent of the benefit.

Oxygen deprivation, such as is experienced under collecting or shipping stress may cause an octopus to appear brain dead. It will lose its appetite and not behave normally. It may not even recognize its arms and eat them. These conditions usually will not appear in a young or mature animal, and the condition is terminal. Prolonged periods of stress may lead to anorexia and wasting in octopus specimens, and ulcerations of the epidermis may develop if the condition persists long enough.

Viral diseases: Viruses are present in enormous quantities in natural seawater, and it is likely to assume that there are any number of viral diseases that may infect octopods, though even in teleost fishes that have been cultured and intensely studied for decades knowledge of viruses is rare. Viral infections of the musculature of *O. vulgaris* have been reported, but little else is known of such pathologies in cephalopods (Farley, 1978). Scimeca (2006) notes that reporting of such viruses in invertebrates is not yet common.

Bacterial infections: Bacteria are present in high numbers in natural seawater, and given the extremely thin epidermis of cephalopods can easily cause integumentary infections that quickly progress to septicemia. Oestmann et al. (1997) noted that the amount of bacterial flora on the epidermis of aquarium-housed cephalopods is over 100 times greater than on their wild counterparts. It therefore stands to reason that aquarium-housed animals are at greater risk from even minor abrasions or damage to the integument.

A number of bacteria have been isolated from diseased octopuses at a now unoperational aquarium. These species include *Aeromonas caviae, A. hydrophila, Cytaphaga* sp*., Pseudomonas putrifaciens,* and *P. stutzeri* (Forsythe, 2002). *Vibrio* spp. are also ubiquitous to the marine environment and a number of species have been reported from the Octopoda; these include *V. alginolyticus, V. carchariae, V. costicola, V. cholera, V. damsel, V. fluvialis, V. natrigenes, V. parahaemolyticus V. pelagius* (biovar 2) and *V. splendidus* (biovar 2) (Forsythe, 2002). Diseased wild octopus (*O. vulgaris*) in Spain were shown to be colonized by *V. lentus* (Farto et al., 2003). Harms et. al. (2006) found *V. vulnificus* in a diseased cuttlefish in captivity. A number of other *Vibrio* spp*.* have been cultured from dead octopus in seafood screening, but these species are not in association with any reported disease conditions and likely represent normal flora found on the epidermis of these animals in the wild.

Fungal infections: Fungal infections in the marine environment are relatively rare compared to freshwater, and none have been reported in *E. dofleini*. A *Fusarium* sp*.* infection has been reported as a secondary infection in *Nautilus*, and *Cladosporium* spp. have been documented in several octopods (Scimeca, 2006; Polglase, 1980; Polglase et al., 1984). Harms et. al. (2006) reported on the surgical excision of a *Cladosporium* lesion from the mantle of a cuttlefish. Avenues of antimycotic therapy in cephalopods are presently unexplored; Harms et. al. (2006) report that itraconazole was administered to a cuttlefish at 5 mg/kg orally for 14 days following surgery but was not effective. The lack of efficacy may have been due to lack of absorption, insufficient duration of treatment, or as a result of insufficiently aggressive excision of the fungal lesion in the animal (Harms et. al., 2006); while inconclusive, this dosage is included here as the only published result of antimycotic therapy in cephalopods.

Protozoan parasites: Protozoan parasites have been a problem of concern in the culture of *Octopus* spp. Forsythe (2002) and Forsythe *et al.* (2006) report *Ichthyobodo* sp*.* as having parasitized *O. bimaculoides*, and *Aggregata* spp. have been relatively well documented as causing disease in octopods, and have also been reported in *E. dofleini* (Gestal et al., 2002a; Gestal et al., 2007; Mladineo & Jozić, 2005; Scimeca, 2006; Poynton et al., 1992). Gestal et al. (2002b) report that *Aggregata* sp*.* infections are responsible for malabsorption in octopods; leading to significant declines in serum protein levels, and eventually emaciation and death. Protozoan infections in teleost fishes are usually controlled with applications of ionic or chelated copper, high concentrations of formalin, or chloroquine diphosphate; though all of these are unsuitable for use on cephalopods. Forsythe (2002) reports using aggressive ultraviolet sterilization compounded with filtration to 1µm as being beneficial in controlling protozoa in the laboratory when broodstock are carefully screened and quarantined.

Animal parasites: As with all animals, *E. dofleini* is also host to a number of animal (and mesozoan) parasites. Mesozoans are a unique group of parasites with characteristics of both protozoans and animals, once thought to be degenerate flatworms (Platyhelminthes). They are vermiform and almost all of the species known are parasites of the renal appendages (i.e., kidneys) of cephalopods, as such they are little known to most pathologists as they are seldom encountered. Mesozoans are the dominant parasite group in both *E. dofleini* (see Table 14), and in members of the genus *Octopus* at-large (see

Figure 22). Dicyemids have complex life cycles, and seem to do no harm to their hosts; there is no known treatment or method of confirming infection outside of a necropsy.

Other incidental animal parasites have been reported for *E. dofleini*, including nematode larvae of the genus *Hysterothylacium* in the musculature (Ishikura et al., 1993). A harpactacoid copepod, *Amplipedicola pectinatus*, has also been described from the gills of a GPO (Avdeev, 2010).

A recent report documents *Kudoa* sp., a myxozoan parasite as having caused myoliquefaction of the musculature of an arm in *E. dofleni* (Yokoyama & Masuda, 2011). This is the first report of a myxozoan parasite of any kind in the Cephalopoda. It is unknown if this infected wild specimen was senescent and the infection was able to progress via lack of immune response or if this is a common infectious disease present in wild *E. dofleini*.

Table 14: Mesozoan and metazoan parasites of *Enteroctopus dofleini*

Parasite Species	Parasite Type	Extraction Site	Reference
Conocyema deca	Mesozoa: Dicymeda	Renal Appendage	McConnaughey, 1957
Dicyemenna abreida	Mesozoa: Dicymeda	Renal Appendage	McConnaughey, 1957
Dicyemodeca anthinocephalum	Mesozoa: Dicymeda	Renal Appendage	Furuya, 1999
Dicyemennea nouveli	Mesozoa: Dicymeda	Renal Appendage	Furuya, 2008
Kudoa sp.	Cnidaria: Myxozoa	Musculature	Yokoyama & Masuda, 2001
Hysterothylacium sp. Larvae	Nematoda: Anisakidae	Musculature	Ishikura et al., 1993
Amplipedicola pectinatus	Arthropoda: Copepoda	Gills	Avdeev, 2010

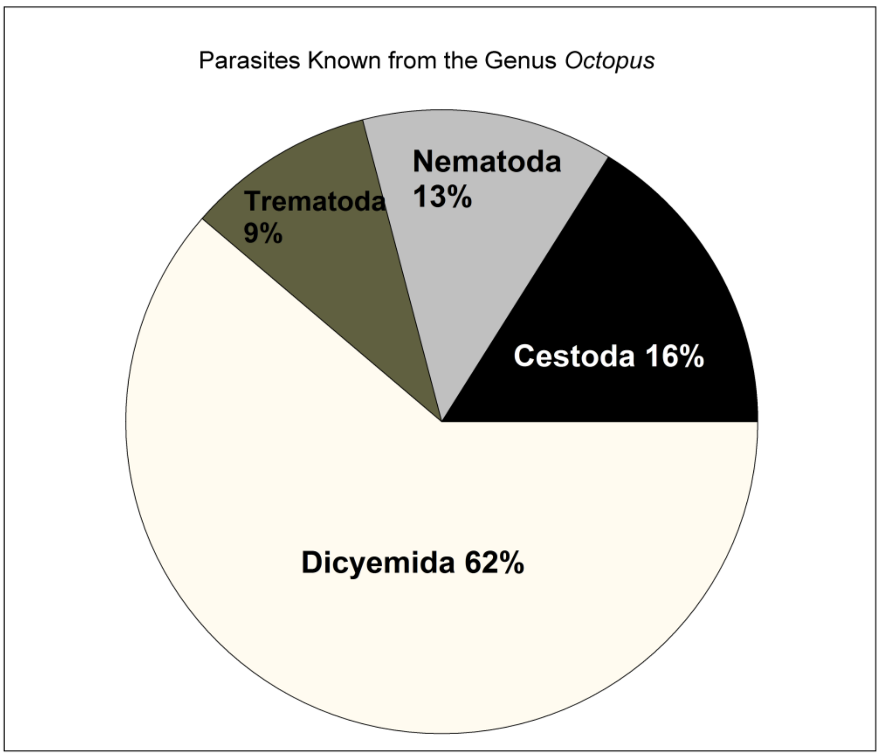


Figure 22. Animal and mesozoan parasite types reported for all species of the genus *Octopus*, adapted from data retrieved from the Natural History Museum of London Host-Parasite Database.

Neoplasia: Knowledge of neoplastic lesions in cephalopods is almost nonexistent. Scimeca (2006) reports that an iridophoroma was included in the (now defunct) Registry of Tumors in Lower Animals (RTLA) of the National Cancer Institute. Tumors in cuttlefish have also been reported, though overall neoplasia in cephalopods appears to be exceedingly uncommon (Scimeca, 2006).

Animal welfare: AZA-accredited institutions must have a clear process for identifying and addressing octopus animal welfare concerns within the institution (AZA Accreditation Standard 1.5.8)

AZA Accreditation Standard

(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.

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.

A myriad of different public aquaria and zoos with a wide diversity of management frameworks and institutional philosophies currently house specimens of *E. dofleini*. As such, it is beyond the scope of this manual, the authors, or the Aquatic Invertebrate TAG to recommend any one framework for the reporting and dissemination of animal welfare concerns and any applicable resolution. All AZA accredited institutions should have protocols in place that address these issues, and staff should be familiar with the process as it pertains to their institution. Serious welfare concerns from employees and even the public-at-large may also be reported to the AZA Animal Welfare Committee. A discussion of the facilities needed to house a specimen of *E. dofleini* off-exhibit for isolation or hospitalization is discussed in Section 2.1, and abnormal behaviors that should be reported immediately to management and appropriately conveyed to veterinarians (following institutional protocols) is displayed below in Table 17.

The semelparous nature and short life span followed by imminent decline after spawning presents a welfare paradigm not common in terrestrial animals. Staff who work with *E. dofleini* should be made well aware, at the outset, of the semelparous nature of the animal and the signs of senescence. Staff should be well aware that when self-destructive behaviors start to occur euthanasia may be necessary, and that in some cases veterinarians and managers may proactively decide to euthanize an animal to avoid a protracted deterioration. It is suggested that all staff working with these animals be reminded of these facts before the onset of senescence, and again after an animal has passed away so that the animal's death does not come as a surprise. Such proactive dissemination of information will help managers and staff avoid confusion between valid animal welfare issues and personal attachments among staff who cannot remain dispassionate about the death of a specimen in their care. Staff who are better trained to recognize the typical behaviors associated with senescence will also be better equipped to differentiate from medical issues that are not associated with the post-spawning decline inherent to the species.

It is also worth noting that recent studies have begun to elucidate the potential for pain in cephalopods, contrary to prior notions that all invertebrates did not possess the capacity to experience pain. This information has begun a change in the thinking of invertebrate welfare, even to the point of cephalopod inclusion under animal welfare laws in the E.U. (Harvey-Clark, 2011). These recent changes, while primarily affecting laboratory specimens, are in the best interest of captive animals as it has been shown that squids do indeed possess nocieptors (nerve cells that are excited in response to potentially damaging stimulus) (Crook *et al*., 2013) which may mean their perception of pain is more analogous to that of higher animals than once thought. Furthermore it has been demonstrated that squid also display long-term defensive responses after minor injuries (Crook *et al.* 2011). See Mather and Anderson (2007) for a more complete review of cephalopod welfare; this reference being part of a special issue of the journal Diseases of Aquatic Organisms (Volume 75(2), 2007) devoted entirely to the welfare of aquatic animals.

Anesthesia and euthanasia: Euthanasia in cephalopods has been a controversial point of discussion in the aquarium community. The semelparitous nature of octopods and the typical pattern of senescence in *E. dofleini* oftentimes necessitate euthanasia as a humane alternative. Senescence in cephalopods has been described by Anderson (2002) and is discussed in greater detail in Chapter 6.7 above. After mating, both male and female cephalopods are in a terminal condition, which will progressively deteriorate until death occurs. Often euthanasia is indicated as an intervention to this eventual decline, especially once lesions appear on the mantle or if severe injury such as autophagy occurs.

Techniques for the euthanasia of cephalopods are reviewed in Anderson (1996), and in the AITAG RCP (Mohan, 2009). The most common practice for specimens the size of *E. dofleini* is freezing, though the use of the local anesthetic benzocaine has also proved efficacious (Anderson, 1996; Barord & Christie, 2007). The use of ethanol is also acceptable, though economically prohibitive for specimens the size of *E. dofleini* (Anderson, 1996). Other methods, such as the use of magnesium salts, have seen widespread usage in anesthetizing and sedating cephalopods, but are less common than other methods for fixation or euthanasia. As with fishes and some other lower vertebrates, physical destruction of the brain is also a humane alternative. In all of the above methods (except brain destruction), euthanasia is achieved by terminal anesthesia. The specifics of each are discussed in greater detail below and in Table 15, and an overview of planes of anesthesia for cephalopods is presented in Table 16.

Ethanol: Ethanol is extremely efficient as an agent of euthanasia in cephalopods. Standard regimes call for immersion in a 2–5% solution for the induction of anesthesia, which may be increased to 5–10% or more for euthanasia (Roper & Sweeney, 1983; Anderson, 1996). Scimeca (2006) recommends a 1.5% ethanol concentration for recirculating maintenance once anesthesia has been induced. Ethanol works extremely well as an anesthetic in all cephalopod taxa, but is usually impractical for use in *E. dofleini* due to its cost and the amount of seawater necessary to hold octopus specimens of this size. While ethanol is extremely effective at narcotizing cephalopods, it has been suggested that repeated use might be potentially dangerous (Messenger et al., 1985).

Magnesium salts: The use of magnesium chloride as a cephalopod anesthetic is well documented in the literature (Messenger et al., 1985). Magnesium sulfate has also been substituted with great efficacy as an anesthetic in *E. dofleini* (Christie, unpublished data). The use of magnesium sulfate requires higher dosages from 150 g/l to 200 g/l, which may place greater osmotic stress on animals in the process (Lincoln & Sheals, 1979; Messenger et al., 1985). Magnesium chloride is most commonly used in cephalopods as a 75 g/l stock solution which is mixed 1:1 with seawater to sedate the specimen (Messenger et al., 1985). Scimeca (2006) recommends the 1:1 dilution of MgCl stock solution for surgical anesthesia, a 1:3–1:4 dilution for mild sedation for handling purposes, and a 1:9 dilution for shipping. Mooney *et al.* (2010) demonstrated with *Doryteuthis pealii* that squids can be anesthetized for up to 5 hours with these methods without harm to the animals. While magnesium chloride works very well in many cephalopod species, it has not shown the same efficacy for *E. dofleini,* particularly for larger specimens (Anderson, 1996).

Benzocaine: The use of the topical anesthetic benzocaine, which is commonly used in fishes, was first suggested as theoretically applicable to *E. dofleini* by Anderson (1996). A decade later, this was evaluated in practice by Barord & Christie (2007). It was determined that dosages (in ethanol solution) greater than 250 g/l are effective at inducing surgical planes of anesthesia in *E. dofleini* within 30 minutes and euthanasia is achieved by terminal anesthesia in less than an hour. The possibility of a synergistic effect between the (water insoluble) benzocaine and the ethanol used as a solvent has yet to be investigated.

Brain destruction: The physical destruction of the brain in fishes and lower animals is an acceptable and exceptionally quick method of euthanasia (American Veterinary Medical Association, 2007). In cuttlefishes and squids decapitation is possible, while in octopods a few quick scalpel strokes between the eyes will rapidly destroy the brain. The use of a sedative prior to making any incision is indicated.

Freezing: While freezing is almost universally decried as an inhumane method of killing vertebrates, including the poikilotherms, it remains one of the preferred methods of euthanizing GPOs (Anderson, 1996). Cold water has been shown as an exceptionally effective anesthetic for many cephalopod species (Andrews & Tansey, 1981). Surgical planes of anesthesia may be achieved by immersion in seawater from 2–6 °C (35.6–42.8 °F), and survivability is good (Bower et al., 1999). These values were for anesthesia induced in tropical species; for the cold-water *E. dofleini* this range would likely be closer to 0–2 °C (32–35.6 °F) for induction. In the case of freezing, prolonged exposure to cold temperatures is essentially the same as euthanasia by terminal anesthesia. Specimens show no apparent discomfort or signs of stress during this process (Anderson, 1996).

Other methods: Pentabarbitone injections have been shown effective in the rapid dispatch of specimens of *E. dofleini* in European aquaria (Slater & Buttling, 2011). The use of controlled substances is beyond the capabilities of most aquarists and can only be administered by a licensed veterinarian.

Table 15. Anesthetics and euthanasia agents for *E. dofleini*

Method	Application	Anesthesia	Euthanasia	Reference
Freezing	Immersion		<2 °C (35.6 °F)	Anderson, 1996
Cold Water	Immersion	2–6 °C (0–2 °F)		Andrews & Tansey, 1981
Benzocaine	Immersion		25–35 g/l	Barord & Christie, 2007
Magnesium Chloride	Immersion	37 g/l	75 g/l	Messenger, 1985
Magnesium Sulfate	Immersion		200 g/l	Messenger, 1985

Ethanol	Immersion	2–5%	5–10%	Roper & Sweeney, 1983
Pentabarbitone	Injection			Slater & Buttling, 2011

Table 16. Stages of anesthesia in the giant Pacific octopus, *Enteroctopus dofleini*

Stage	Plane	Category	Behavioral response
0		Normal	Swimming, movement, and equilibrium normal. Response to visual/tactile stimuli. Ventilation rate normal. Good muscle tone/strong sucker adhesion.
I	1	Light sedation	Depression in response to stimuli. Ventilation, sucker adhesion, equilibrium, muscle tone, and swimming behavior normal.
I	2	Deep sedation	Strong depression in response to stimuli. Decrease in ventilation. Response to positional changes. Muscle tone/equilibrium reduced.
II	1	Light narcosis	Excitement phase, characterized by normal mantle and arm movement, and increased ventilation, followed by marked decrease in response and sucker adhesion.
II	2	Deep narcosis	Total loss of equilibrium. Decrease in muscle tone. Slight response to stimuli. Decrease in ventilation.
III	1	Light anesthesia	Partial loss of muscle tone. Loss of arm/tentacular movement. Near total loss of sucker adhesion. Significant decrease in ventilation.
III	2	Surgical anesthesia	Total loss of muscle tone. Extremely low ventilation rate. Total loss of response.
IV		Medullary collapse	Cessation of ventilation. Branchial hearts in cessation.

Barord & Christie (2007), used with permission, originally adapted from Brown (1993) for cephalopods.

Practices to avoid: The question of euthanasia in invertebrates, and indeed the entire subject of invertebrate medicine, has only recently entered the pantheon of modern veterinary science. Until recently, most methods for the anesthesia and euthanasia have been derived from traditional methods for the relaxation and fixation of museum specimens. As such, there are a number of other methods that persist through historical convention or presumption of validity. Several older texts present methods of fixation that were acceptable in the past for museum specimens, but are inconsistent with modern standards of animal care and overall welfare.

The fixation (i.e. rapid killing prior to preservation) of marine animals in 10% seawater-buffered formalin was long considered the norm for the humane dispatch of a specimen, but should not be considered a viable alternative in the modern aquarium or zoological park. Similarly, some older texts recommend immersion in strong solutions of chromic acid as a method of fixation though this also should be avoided. Immersion of cephalopods in fresh water has also been recommended as a quick and effective way of fixing cephalopods, though this has been found to cause immense distress and should not be used (Rudloe, 1971; Anderson, 1996). The fish anesthetic quinaldine has also been shown to cause distress in octopods (Anderson, 1996).

While many practices of killing for aquatic species evolved from fixation and preservation techniques used for museum specimens, most are not acceptable methods of euthanasia today as they were developed simply as a rapid means of dispatching a specimen with no regard for the welfare of the animal. Modern euthanasia techniques achieve mortality though terminal anesthesia of the specimen, though not all anesthetics are suitable for use in cephalopods.

The most persistent misconception in both aquaria and research facilities working with cephalopods is that the fish anesthetic tricaine methanesulfonate (MS-222) is acceptable for use on cephalopods, though its use should be avoided. MS-222 is effective at anesthetizing and euthanizing cephalopods, though survivability post-sedation is poor (Berk et. al., 2009), and many specimens show signs of distress upon exposure to this chemical (Anderson, 1996). The experience of the authors is consistent with the above findings across cephalopod taxa, though there is variability between and among species (and individual animals) in the vigor of their reaction to the chemical. While MS-222 is highly effective and relatively safe as an anesthetic for teleost fishes and amphibians, its use in cephalopods should be avoided wherever possible in the interest of maintaining the highest standard of animal welfare.

Signs of acute distress in cephalopods: There exist in the published literature a number of criteria for assessing distress in cephalopods. Andrews and Tansey (1981) list violent inking or attempts to climb out of the narcotizing solution within 30 seconds as signs of distress. Messenger et al. (1985) lists escape jetting or inking as overt signals of distress. General agitation and abnormal arm contractions are listed by Anderson (1996) as overt signals of distress in octopods. As discussed above in Chapter 1.3, Potts (1965) found that *E. dofleini* might respond to physiological stressors (specifically rising serum ammonia levels) with cessation of ventilation. In the authors' experience rapid color changes in *E. dofleini* are also likely to accompany any these other behaviors, though this in and of itself is not a severe indicator of physiological or psychological distress. These behaviors should be kept in mind during any clinical procedure involving cephalopods, whether or not chemical anesthetics are involved. See Table 17 below for a compilation of known stress behaviors in *E. dofleini*.

Table 17. Giant Pacific octopus behaviors and associated stress levels

Behavior	Stress	Acute stress	Senescence	Reference
Minor ink release	X			Messenger et al., 1985; Andrews & Tansey, 1981
Violent inking		X		Andrews & Tansey, 1981
Frantic climbing out of water		X		Messenger et al., 1985
Escape jetting	X	X		Potts, 1965; Bennett & Toll, 2011
Cessation of ventilation		X		
Rapid, unusual color change		X		
General lack of color change; poor color	X		X	
Lack of sucker adhesion	X	X	X	
Curling arms over head		X		Oestmann et al., 1997
Unusual arm contractions		X		Anderson, 1996
Visible agitation	X			Anderson, 1996
Minor epidermal lesions	X			
Progressive (non-healing) epidermal lesions		X	X	Anderson et al., 2002
Inappetence	X			Anderson et al., 2002
Anorexia		X	X	Anderson et al., 2002
Autophagy		X	X	Anderson et al., 2002
Sunken eyes			X	Anderson et al., 2002
Egg laying (females)			X	Anderson et al., 2002
Sperm packet deposition (males)			X	Anderson et al., 2002
Egg brooding (females)			X	Anderson et al., 2002
Careless placement of ligula (males)			X	Anderson et al., 2002
Hyperactivity, uncoordinated (males)			X	Anderson et al., 2002

AZA-accredited zoos and aquariums provide superior daily care and husbandry routines, high quality diets, and regular veterinary care, to support giant Pacific octopus 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 giant Pacific octopus both in their care and in the wild. As stated in Chapter 6.4, necropsies should be conducted on deceased giant Pacific octopus 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 histopathological 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) website should be checked for any AZA giant Pacific octopus 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.

Common causes of death in octopuses: The most common causes of death in octopods may be lumped into various broad categories such as senescence, water quality, escape, or trauma. Only in recent years has veterinary medicine progressed to the point of identifying specific disease conditions in cephalopods and identifying efficacious treatment options.

<u>Senescence</u>: As defined above in Chapter 6.7 and in the introduction, senescence is a more precisely defined condition in cephalopods and other semelparous animals than simply the process of growing old —as it is more applied to other taxa. Senescence in cephalopods involves the protracted decline of the specimen during which the behavior will change, lesions often appear on the mantle, autophagy of the arms may result, and the animal is usually (though not always) anorectic. During this time the animal's immune system is completely shutting down and death is imminent. Oftentimes, especially in larger octopod species such as *E. dofleini* this process is evident and precedes either the laying of eggs or the appearance of sperm packets on the tank substrate. Occasionally animals will die with little overt sign of senescence, or when subtle signs are missed by the aquarist.

<u>Water quality</u>: As cephalopods are extremely sensitive to toxins in their surrounding seawater, deaths can occur due to water chemistry issues. More often than not these problems are likely to occur among octopods kept by hobbyists and not public aquaria/laboratories with rigorous water chemistry analysis. Ammonia poisoning in a new tank is a very real possibility if the exhibit has not been cycled or not cycled well enough. Heavy metal toxicity, especially copper, is also a potentially lethal threat. Failure of one or more components could lead to any of the above conditions through inappropriate or incomplete nitrification, metallic ions leaching from damaged heat exchangers, or failure to maintain proper temperature. See Chapter 1.3 for detailed information on water quality and tank cycling.

<u>Escape</u>: Octopods long ago developed a reputation as escape artists when kept in aquarium settings, as is detailed in the introduction. While the escaped specimen may sometimes crawl back into their tank, more often than not the escape of an octopus will result in death by desiccation. In these cases, cause of death is evident, though a thorough water quality analysis is still warranted to rule out stressors that may have contributed to the problem. See Chapter 2.2 for more detailed information on containment procedures.

<u>Trauma</u>: Trauma may occur to an octopus housed in an aquarium in a number of ways. Mantle lesions occurring from contact with the tank wall and autophagy are not uncommon in during senescence (see above), though other injuries occasionally result. Arguably the most common serious trauma encountered in aquarium-housed specimens of *E. dofleini* is that arising from an animal getting around intake screens and into life support piping. If caught early enough the suction of the pumps may be shut down and the animal saved, but if this occurs overnight or when no one is watching the event will likely be fatal. Occasionally, minor damage may result from other sources; it is not uncommon for aquarists to hastily shut a tank lid on an animal to prevent it from exiting the tank. Also at least one GPO and its keeper have been electrically shocked when an octopus wrapped a wet arm around a poorly placed power strip during feeding (B. Christie, personal observation). Cephalopods in the wild have a capacity to rapidly recover from minor injuries to the epidermis or mantle, though captive animals may not always respond as such. Hulet et al. (1979) reported that damage to the fins was a major factor in mortality in laboratory squid specimens owing to secondary bacterial infections. The normal bacterial flora present on the epidermis of captive cephalopods is over 100 times that of their wild counterparts (Oestmann et. al., 1997), so any damage to the epidermis should be carefully monitored and medical intervention initiated if immediate healing is not noted, or behavior (especially feeding) is abnormal following the traumatic event. In some species of octopus fatal, penetrating skin ulcers formed by opportunistic bacterial pathogens have resulted in increased mortality among juveniles housed together simply from contact from other conspecifics (Hanlon *et al.*, 2014).

<u>Intramantle inking</u>: When an octopus releases ink into the surrounding water of its aquarium, the potential for water quality degradation and suffocation exists. Octopus ink adheres to the gills and prevents oxygen transfer, and a recent report of intramantle inking has proven fatal in *Octopus bimaculoides* (Oestmann et al., 1997; Bennett & Toll, 2011). Bennett & Toll (2011) report that successful resuscitation of three unresponsive octopus specimens with intramantle ink fouling has been achieved by removing the affected animals to a vessel with clean, aerated seawater. Once the animals were in clean seawater the mantle musculature was manually massaged and compressed gently to expel ink and stimulate respiration,

followed by aspiration of the ink from the mantle cavity via manual siphon and flushing of the mantle until ventilation resumed as normal (Bennett & Toll, 2011).

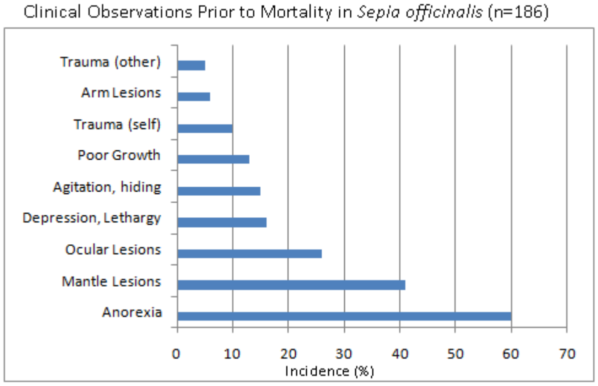
Hemolymph (blood): The cephalopod circulatory system utilizes a system involving a systemic and two branchial hearts to circulate hemolymph. Unlike many invertebrates the cephalopod immune system is closed (i.e., the major organ systems are vascularized and not bathed in a common pool of hemolymph) and bears some similarities to vertebrates in that regard. The more complex, and more efficient, circulatory system likely evolved to suit the extreme metabolic and oxygen demands of cephalopods as high-performance invertebrates. The oxygen-carrying molecule in cephalopods is hemocycanin, a copper-based molecule. True hemapoietic organs have not been identified in cephalopods, but it is believed that immune cells are generated in glands about the optic nerve (Novoa et al., 2002). The immune system consists of a single type of circulating blood cell, the hemocyte, which is non-pahgocytic and as such will not encapsulate and dissolve dead cells, harmful particles, or bacteria. Instead the cephalopods unique physiology relies upon gill tissue to clear foreign bodies from the hemolymph (Oestmann et al., 1997; Sherrill et al., 2000; Scimeca, 2006; Novoa et al., 2002).

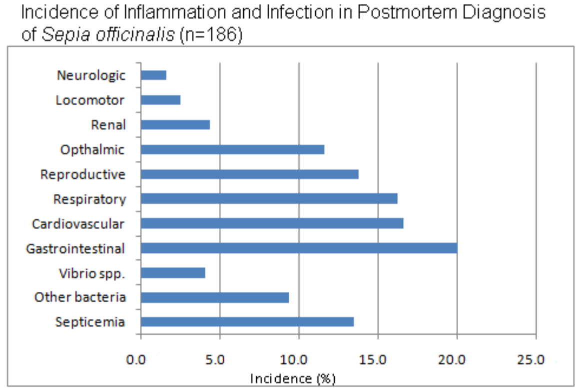
Antibodies are not present in cephalopods, which instead possess lectin-like proteins that hemagglutinate antigenic foreign particles (Sherrill et al., 2000). Acid-base regulation in cephalopods is also yet to be defined for most species, though studies with *Octopus vulgaris* suggest that higher serum pH at the gills stimulates CO_2 offloading and oxygen loading, with lower pH in the tissues having the opposite effect (Pörtner, 1994). Specific ranges for hemocyte concentration, hemolymph biochemistry, electrolyte levels, and metabolomic compounds have yet to be precisely defined for the taxa. Studies in *O. vulgaris* have shown the hemolymph serum to contain 58.7% protein and 17% carbohydrates by dry weight, with fucose, mannose, and galactose being the dominant carbohydrates present (Rögener et al., 1987). Harms et. al. (2006) report on the clinical parameters (electrolytes, hempcyte counts, total solids, protein, glucose, et cetera) of a cuttlefish suffering from bacterial and mycotic infections, though as the animal was diseased those values are not included here. Until such clinical standards are established from healthy cephalopods one aspect that is of diagnostic value in cephalopod hemolymph is the presence or absence of bacteria, as indicated by direct microscopy or special stains. Sherrill et al. (2000) present an example of intralesional bacilli among hemocytes, which indicates a bacterial septicemia in common cuttlefishes, *Sepia officinalis*. Oestmann et al. (1997) notes that septicemia should be suspected when the hemocyctes have aggregated into discernable clumps.

Necropsy procedures: Oestmann et al. (1997) note that cephalopod tissues undergo rapid autolysis, even before death due to high enzymatic activity. As such they note that animals found dead in a tank will likely have little diagnostic value and an animal should be euthanized and a necropsy performed as soon as it likely an animal will not survive (Oestmann, 1997). When specimens are euthanized hemolymph should be collected after immobilization but before the anesthesia becomes terminal (Oestmann et al., 1997). Hemolymph may be examined as described above. Skin scrapings can be taken from external lesions, and swabs may be taken for culture (Oestmann et al., 1997). Skin scrapings not examined as a wet-mount may be air-dried and fixed in methanol. The internal examination should note the condition of the digestive gland and associated diverticula due to their essential role in metabolism (Oestmann et al., 1997). Figure 26 details the internal anatomy of *E. dofleini*. Most commonly the mantle may be opened by a midline incision, though the median pallial adductor musculature may also be cut in order to invert the mantle (Oestmann et al., 1997; Scimeca, 2006).

Internal organ systems should be examined and any samples for histopathology should be fixed in 10% neutral-buffered formalin, or Davidson's fixative, depending on the requirements of the pathology lab to which samples will be submitted. If heavy metal or environmental poisoning is suspected samples of musculature should be frozen for toxicology analysis following Anderson (2003b). The presence of animal parasites should be noted and if identification to species is desired should be fixed in either 70% ethanol (cestodes, acanthocephalans, arthropods, tremadodes) or 70% ethanol with 5% glycerin added (nematodes, dicyemids). In senescent animals the presence of any remaining eggs in females or sperm packets in males should be noted, though it is not abnormal for some of the gamete material to be retained even through protracted senescence.

A list of laboratories for histopathological analysis familiar with cephalopods is presented in Chapter 6.1. There is not currently a large base of knowledge specific to *E. dofleini*, though there is much more pathology data published in cuttlefishes. Sherrill et al. (2000) compiled and analyzed data from 186 cuttlefish necropsy reports spanning 12 years at one zoological institution. Owing to the lack of information specific to *E. dofleini* those data are adapted and presented in Figures 23, 24, & 25. References specific to cuttlebones were significant, but omitted as the structure is not present in octopuses. Many of the clinical signs observed in Sherrill (2002) are congruent with the general characteristics of senescence in cephalopods, as reviewed in Anderson et al. (2002).





Figures 23 & 24. Incidence of significant clinical and postmortem diagnoses in cuttlefish, *Sepia officinalis.* Adapted from data in Sherril et al. (2000).

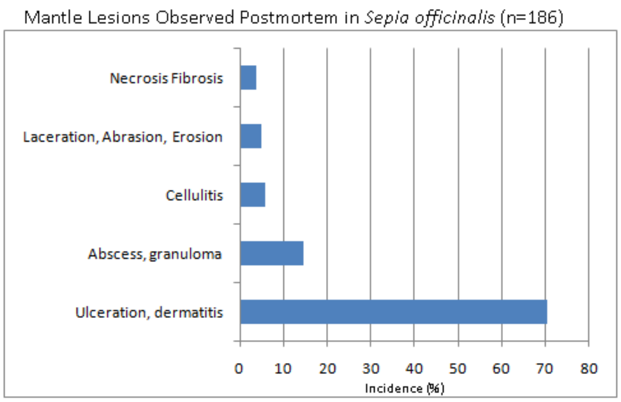


Figure 25. Incidence of significant clinical and postmortem diagnoses in cuttlefish, *Sepia officinalis*, Adapted from data in Sherril et al. (2000).

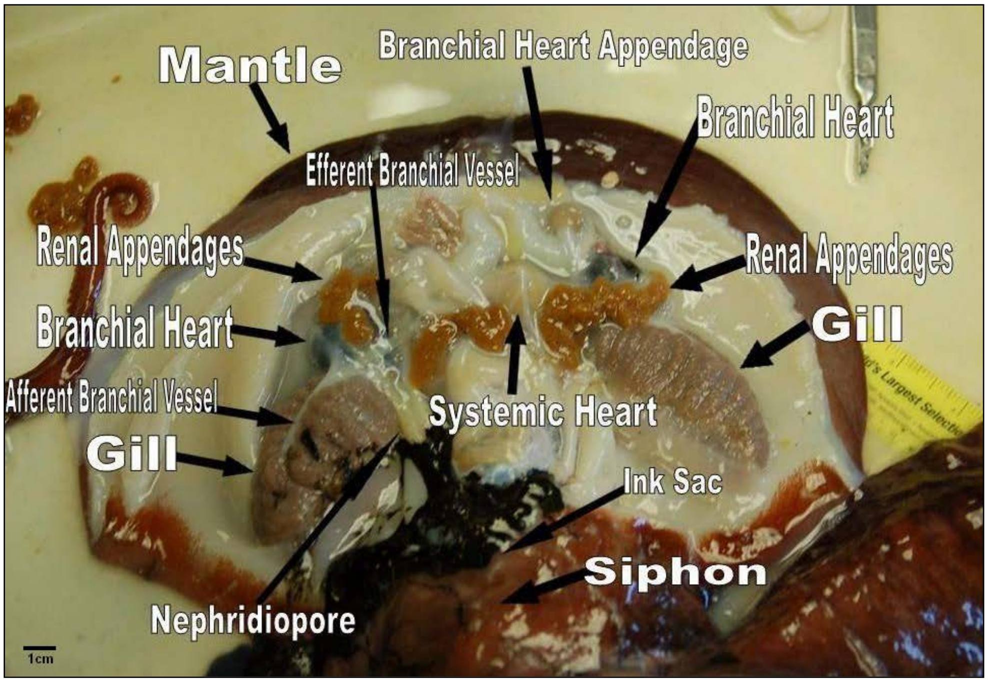


Figure 26. Internal anatomy of *E. dofleini*. Photo courtesy of B. Christie; labels by G. Barord

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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.

Overview: Aristotle was the first to describe cephalopod mating, and despite being kept in laboratories and aquaria for over a century we still know surprisingly little of the reproductive biology of most cephalopod species. The GPO in particular, has only been reared successfully in an aquarium setting to adulthood once, and the care of paralarvae is labor intensive with high mortality (Snyder, 1986; Anderson & Wood, 2012). It is important to learn more of the reproductive behavior of this species in an aquarium setting, but as wild populations are not imperiled and collection of exhibit specimens is sustainable such endeavors are of sheer scientific curiosity and not essential for management.

The giant Pacific octopus is sexually dimorphic and spawning occurs throughout the year, although it may peak at particular times of the year. Sophisticated pre-copulatory behaviors by the male are not uncommon, and include elaborate changes in coloration and aggressive movements towards potential competitors. The male's third arm on the left, called a hectocotylus, is completely void of suckers at the tip, and is used to insert spermatophores into the opening of the female's oviduct. The entire mating process can take 2–4 hours. Approximately 6 weeks after mating, the female lays 20,000–100,000 eggs over the course of several days on the inner side of her rocky den. For the next 5–8 months she tends the eggs, carefully cleaning and aerating them until they hatch. The female does not leave her brood, even to eat, and will die within months after they hatch. The male dies shortly after mating. The typical life span of the octopus is between 3–5 years.

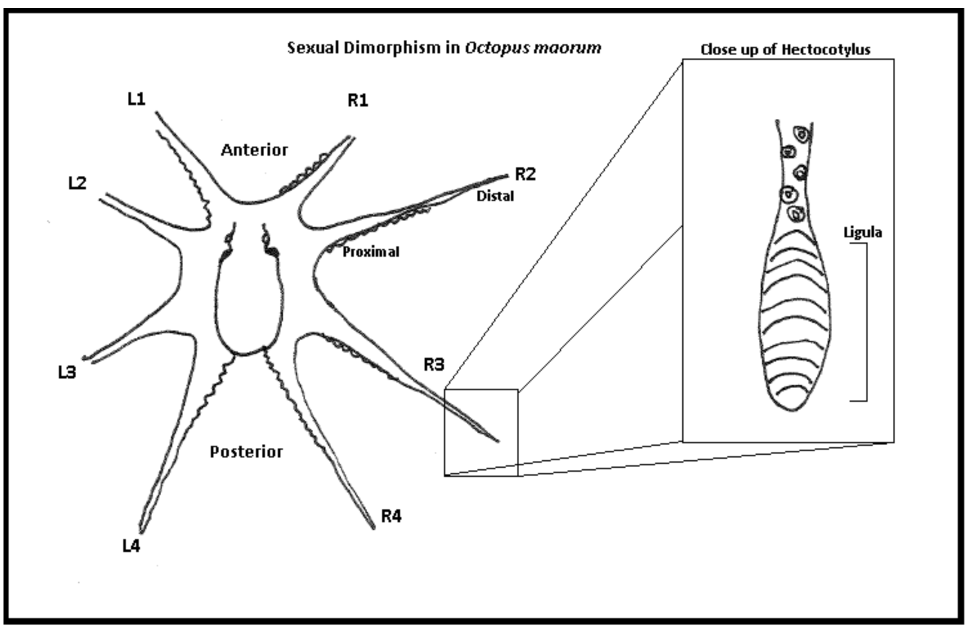

Figure 27. Sexual dimorphism in the octopus. This shows the position of the hectocotylus on the third right arm, bearing the ligula used for spermatophore transfer. Elements of this figure re-drawn from a figure in High (1976) by B. Christie, labeling of arms follows Hanlon & Messenger (1996)

Old age and senescence: In order to understand octopus senescence, it is necessary to first understand the life history of octopuses. Humans are familiar with the reproductive patterns typical of mammals; they can reproduce several times during their life span—they are iteroparous. Almost all octopuses are semelparous; they reproduce once and then die. Salmon, cicadas, and century plants are other examples of organisms that are semelparous.

Reproduction is similar in most octopus species. However, exactly how males find females is not known for sure. They may simply encounter or bump into them by chance or may locate them by "smell" or chemo-sensory means. Evidence for this has been seen at one aquarium, where the effluent water from a female's tank is piped to the to the bottom of the neighboring bay where it seems to draw males. Once a male encounters a female he needs to somehow assess whether she is the same species and whether she is ripe and ready to mate. Mature females typically allow mating to occur but occasionally they will chase off the amorous male.

During mating, the male places a sperm packet—a spermatophore—into one of a female's two oviducts. The male's third arm (either right or left depending on the species) is modified for copulation. The tip of the modified copulatory arm, termed the ligula, lacks suckers and has a groove on its underside for grasping a spermatophore and inserting it into a female's oviduct. Spermatophores in *E. dofleini* may be up to a meter in length, and contain 3.7×10^{10} individual gametes (Hanlon & Messenger, 1996). There is some evidence that the shape of the ligula may also reduce competition by removing sperm from competing males while depositing the spermatophore (Anderson et. al., 2003). Depending on the species octopuses assume one of two positions during mating. Mounted mating is where a male grasps a female from above and distance mating is where a male extends the modified third arm toward and into the body cavity of the female without mounting her. In distance mating the two animals can be as much as a body width apart and hence, the male is able to mate with a receptive female located in a den. Both mounted and distance mating has been observed in *E. dofleini* (Anderson et al., 2003).

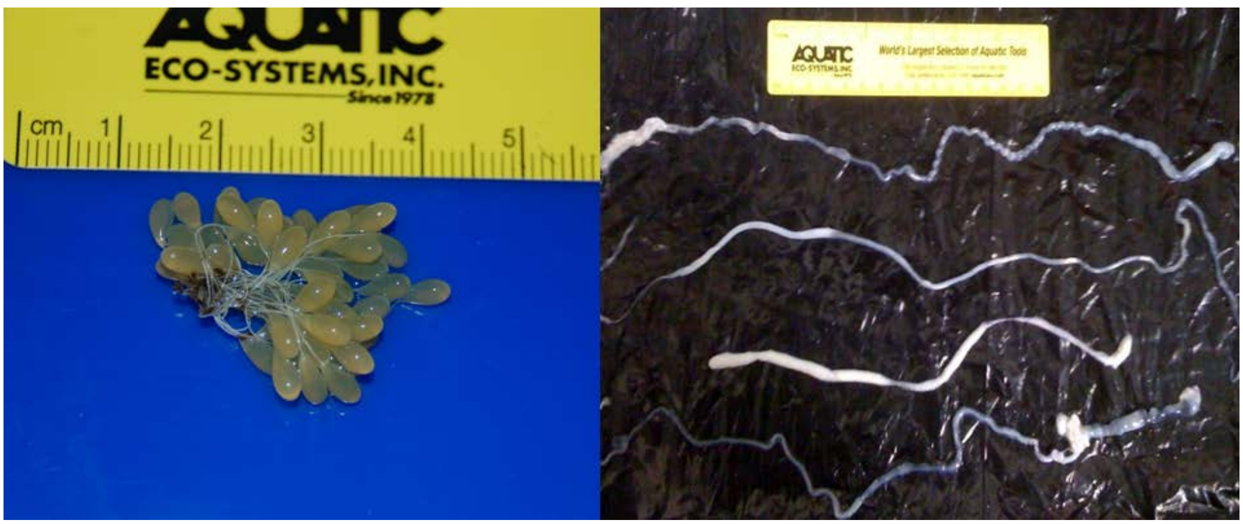

Figure 28. Left: Eggs from *E. dofleini* (infertile). Photo courtesy of B. Christie. Right: Spermatophores from male specimen. Photo courtesy of J. Frey

Anderson et al. (2003) provide the most comprehensive description to date of copulatory behavior in *E. dofleini*, including the first observations of copulation in an aquarium setting (n=4), along with observations from the two aquariums. Overall, they found that the average time of copulation is 245±39 minutes; during mating males were pappilose and reddish brown in color with white spots about the mantle with females being more pale and smooth in appearance (Anderson et al., 2003). The extent of pre-copulatory behavior has not been studied in the wild as it has in other species (i.e., Hanlon & Messenger, 1996), owing to the limitations of SCUBA and the temperatures in which these animals live.

There is no such thing as "safe sex" for octopuses since they are semelparous and die after reproducing as salmon do. Males typically die within a month or two after mating, although they can mate with other females during that time period. Male GPOs only have about 10 spermatophores ready at any one time for transfer to females whereas males of the common octopus may produce several hundred spermatophores. Small species of octopuses may take only a few minutes to an hour to consummate mating. In contrast, four to six hours may be needed for spermatophore transfer in GPOs

After mating, females often take several months to "bulk up" and to find a suitable den in which to lay their eggs. The female typically blocks up the entrance to her small den with rocks. Small-egg species of octopus lay eggs in strings that are attached to the ceiling of the maternal den. In contrast, large-egg species either lay individual eggs in a den or carry a relatively few eggs clutched in the ventral arms and web or under her body. In tropical waters the female broods her eggs without feeding for a month or two before the young hatch. In cold Alaskan waters it may take 8–10 months for a giant Pacific octopus female to brood eggs. The female doesn't eat while aerating and guarding the eggs. She lives off protein metabolism and since octopuses have no fat, she usually shrinks to about half her starting weight during the brooding process and usually dies shortly after the eggs hatch. Studies on brooding females have determined that if she is eaten or is taken away from her clutch of eggs they will all die before hatching due to the presence of egg predators such as sea stars.

The hatchlings of large-egg species are small juveniles that resemble adults. They crawl away and take up a benthic existence just like the adults. In contrast, hatchlings of small-egg species like GPOs are planktonic and are adapted for swimming and feeding in the water column. They are called paralarvae since they do not go through a metamorphosis like other invertebrate larvae (see Young and Harman, 1988 for a detailed description of the terms paralarva, larva, juvenile, and subadult as they apply to cephelopods). The tiny paralarvae have shorter arms and bigger eyes than the adults do and they swim all the time via jet propulsion. They may spend several months in the plankton before settling out onto the bottom as a small juvenile. Depending on body size, adult female GPOs may lay more than 100,000 eggs.

Many animals have life spans that last many years. In general, mollusks other than cephalopods are smaller but often live for many years. Several gastropods and bivalves live for over 50 years. However, cephalopods "live fast and die young," a motto borrowed from the Hell's Angels and applied to cephalopods. Even the GPO, which frequently reaches a size of 50 kg (110 lb), only lives about 3 years on average. The very small sepiolid *Idiosepius pygmaeus* lives for only 80 days. Most other octopuses only live a year, although this depends on size at maturity and temperature that the species lives at. Animals that mature at large sizes and live in colder water tend to have longer life spans.

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 has been achieved with a variety, but not all, octopus and should be investigated further.

At present there is a paucity of knowledge in the basic reproductive biology of most cephalopod species. As all of the cephalopoda brood their eggs outside of the body, the term artificial insemination is more appropriately discussed in terms of *in vitro* fertilization. *In vitro* fertilization techniques have been developed for corals, many species of echinoderm, and for several molluscs of commercial value (i.e., oysters, mussels). *In vitro* fertilization in a cephalopod species was first successfully completed in 1950's in the squid *Ommastrephes sloani pacificus* (Soeda, 1952). In the 60 years since that publication, only 11 other species of cephalopod, all species of squid, have been successfully cultured *in vitro* (Villanueva et al., 2012).

7.3 Pregnancy, Egg-laying/Parturition

It is extremely important to understand the physiological and behavioral changes that occur throughout an animal's pregnancy. The onset of full sexual maturity in cephalopods is also the beginning of the animal's death. Hormones produced by the optic gland stimulate the ripening of the ovaries in females and gamete production in males. After the onset of maturity in females an enormous amount of energy is devoted to egg production; almost all amino acid uptake is shunted to the cells of the ovaries and growth, hemocyte synthesis, and cellular repair processes stop (Wells & Wells, 1977). The proteolytic activity of the secretions of the salivary glands decreases 95%, so that even if senescent octopods were to continue feeding, it is unlikely that they would be able to digest any of their prey (Wells & Wells, 1977). This total reallocation of physiological resources explains the lack of feeding, lack of immune function, and general degradation of senescent animals. Wells & Wells (1977) also state that the rate of protein synthesis in yolk production of female octopods is on par with that of milk protein production in some mammals; an extraordinary physiological feat orders of magnitude above the capabilities of most invertebrates.

Most octopuses will be completely anorexic after egg laying, see the Introduction and Section 6.7 for further information on behavioral changes during senescence. The time from egg-laying to death in octopods varies considerably in captivity, from as little as one week to as long as a year or more. Hatching of fertile eggs has been documented as occurring between 5-13 months from the date they were laid (Snyder, 1986; DeCastro et. al., 2006).

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.". It should be noted that these terms are more applicable to terrestrial animals, and the environment for hatching of eggs/rearing of larvae for an octopus is radically different from birthing facilities for vertebrates.

The female octopus will lay eggs in her den, where the animal spends a good portion of her time. Females prefer an overhang for egg-laying, but will lay the eggs on any vertical surface in an aquarium setting. Eggs may be laid in a single clutch as is typical in the wild, or scattered throughout the tank. The female will attend to the eggs, aerating and cleaning them, until shortly after hatching when the animal dies. Observations of wild octopods show that if the female dies before the eggs hatch the chances of the embryos surviving to the planktonic larval stage are close to zero (Anderson & Wood, 2012); though with meticulous care in captivity it is possible to hatch eggs without the care of an adult female (Gabe, 1974). If fertilized eggs are laid- the paralarvae should be siphoned out of the tank and transferred to a separate tank to be reared (see below, Section 7.5), though optimal tank setups for paralarvae development have not yet been perfected.

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.

Octopods do not provide any parental care for their offspring, which are planktonic after hatching and drift in the water column. There have been but a few reported rearing attempts of *E. dofleini* in aquariums. To date, an aquarium-housed hatched GPO has been raised to adulthood at one aquarium. There remains much work to be done to identify methods for rearing of the planktonic paralarvae and appropriate feed items for various size animals. Due to the lack of a comprehensive guide to breeding and rearing the species a general overview of rearing methods is presented here, followed by discussion of the two published attempts to date.

For all intents and purposes octopods can be divided into large egged species and small egged species, the former usually being benthic and the latter planktonic upon hatching. The GPO is a small-egged species, laying 30,000–180,000 eggs per clutch with an average size of 6–8 mm (.2–.3in.) (Anderson & Wood, 2012). By way of comparison, *O. rubescens* lays 4000–45,000 eggs per clutch at 3–4 mm (.11–.15 in.) average size and *O. vulgaris* lays an astounding 100,000–500,000 eggs at 1.5–2.7 mm (.05–.12 in.) average size (Anderson & Wood, 2012). At present *E. dofleini* and *O. vulgaris* are the only octopods with planktonic paralarvae that have been reared in aquarium settings.

Studies have shown that in the wild, eggs will seldom hatch if the female dies prematurely. Egg predators will devour unguarded eggs and even uneaten clutches will suffer deleterious effects from the lack of aeration and cleaning provided by the female (Anderson & Wood, 2012). As with any planktonic organism, a kreisel style tank is necessary for larval rearing, though an optimal design has yet to be determined. There is some degree of sperm storage in octopods, and Anderson & Wood (2012) mention that in general, female octopods which lay eggs within three months of capture may generally be expected to have mated in the wild. There is some evidence that at least two matings may be necessary to fertilize eggs from both clutches, as GPOs in aquariums have produced clutches while retaining infertile eggs (Anderson & Wood, 2012). In GPOs, the incubation period varies, but is about 160 days. The embryos undergo two 180° reversals in orientation in this time (Anderson & Wood, 2012). Cephalopod eggs should be kept submerged when handling, though they are fairly resilient and will not be irrevocably damaged if exposed to air; one study transported cuttlefish eggs up to 8.5 hours out of water (but damp) with no negative effect on hatching, mortality, or growth (Jones et. al., 2009). Data from the rearing attempts of *E. dofleini* paralarvae are summarized in Tables 18 & 20, and described in greater detail in the text that follows.

First rearing attempt, 1974: The first documented intentional captive spawning of a male and female GPO occurred in 1974, and was followed by the first attempt at rearing the paralarvae in an aquarium setting (Gabe, 1974). The female octopus brooded her eggs for approximately 40 days, and hatching was observed between approximately day 155 and day 223 at temperatures between approximately 9-13°C (48-55°F) (Gabe, 1974). The paralarvae were housed in open-system tanks ranging from 10-30 gallons, presumably rectangular in design, and were fed a variety of feeds including ground seafoods (shrimp, mussels), live *Artemia salina* (adults and nauplii), live gammarid amphipods, live sculpin fry, and even egg yolk (Gabe, 1974). Only adult *Artemia* and sculpin fry were taken, and the maximum survival was approximately 40 days (Gabe, 1974).

First successful rearing, 1986: The first—and to date *only*—successful attempt at rearing of *E. dofleini* larvae was presented at the 1986 AAZPA (now AZA) annual conference. A female GPO laid an estimated 20,000–30,000 eggs which were incubated for 5–6 months, both in breeder nets, and by the female herself (Snyder, 1986). Both groups of eggs hatched over a period of about 2 months producing paralarvae 6–8mm in length, of which 200 were collected and reared (Snyder, 1986). Anderson & Wood (2012) noted that the larval rearing was quite labor intensive, requiring approximately 6–8 hours per day for feeding and fastidious cleaning. After 7 months there were 25 surviving animals that had begun to settle, and by 9 months there was one survivor who was transferred to a standard aquarium to take up residence in a clam shell (Snyder, 1986). Snyder (1986) fed a variety of frozen seafood (for lack of an adequate supply of live zooplankton) until the surviving octopus could be transferred to a diet of standard chopped seafood and clams. The surviving octopus (a male) lived to adulthood and reached a peak size of 29 kg before expiring after 38 months (Snyder, 1986).

Other rearing attempts, 2006: Twenty years after the first aquarium presented their results, another aquarium presented a poster at the Regional Aquatics Workshop detailing rearing attempts of *E. dofleini* paralarvae in kreisels of differing designs. While they were not successful in rearing octopus larvae to adulthood, much useful data and photo documentation was produced. The 6.5 mm eggs were produced 4 days after a mating that lasted 6 hours in an aquarium setting (DeCastro et al., 2006). The eggs hatched between days 304 and 397, with the majority hatching between days 375–384 (DeCastro et al., 2006). Survival rates were compared between a traditional kreisel with a sideways rotation and a circular tank with an upwelling type flow from bottom to top coupled with a bubble curtain to keep the larvae off the walls. Survival was greater in the traditional kreisel (80 days) as opposed to the modified kreisel (58 days) (DeCastro et al., 2006). The paralarvae were fed *Mysis relicta* sieved to 850 µm until they grew large enough to take frozen krill and clam shaved on a cheese grater (DeCastro et al., 2006). The work at this aquarium also produced some detailed photographic documentation of egg and larval development, and an annotated chronology of egg development. DeCastro et al. (2006) found that as the eggs developed the eyespots appeared on day 187, chromatophores on day 213, the 1st rotation was noted on day 248, yolk glands appeared on day 268, and yolk sacs were depleted by day 334.

Note on larval feeding: Once the animals are large enough to take frozen seafood that has been finely chopped or shaved feeding of appropriate foods for growth is easier, but providing adequate first feeds for the tiny paralarvae is a greater challenge. Snyder (1986) had some success with frozen seafood, and DeCastro et al. (2006) fed mysid shrimps in the under 1 mm size class, but much is still to be determined to determine the optimal food types and amounts for hatchling octopods. In Hanlon & Messenger (1996) it is mentioned that paralarvae have been observed clinging to the air-water interface via surface tension, possibly in order to feed on the tiny neustonic plankton therein. Anderson & Wood (2012) mention that appropriate first foods are copepods and crab larvae, with *Artemia salina* nauplii being readily available and economical, but suboptimal nutritionally of octopus paralarvae outgrowth. Much work has been done in recent years with attempting to identify novel food sources for syngnathid fishes (e.g., various crustacean larvae, copepod cultures, etc.), and these avenues of research may be useful in future attempts at planktonic cephalopod culture. Providing adequate first foods for octopus paralarvae has also been a major challenge in commercial/academic aquaculture attempts, Uriarte et. al. (2011) summarized the limiting factors in attempts to culture six species of Central/South American octopus which parallel the challenges faced by aquarists attempting to rear *E. dofleini*; the pertinent details of these studies are summarized in Table 19 for cross-reference against the scant data that exist for GPO rearing (Table 18).

Tables 18 & 19. Data from published rearing attempts in *Enteroctopus dofleini* (18) and six species of octopus from Central/South America (19) for comparison.

Species	Temperature (°C)	Brood Time (Days)	Egg Incubation (Days)	Egg Size (mm)	Paralarvae Size (mm)	Max Survival (Days)	Foods	Reference
Enteroctopus dofleini	9-13	43	155-223	-	2.5*	42	*Artemia* + Sculpin Fry	Gabe, 1974
Enteroctopus dofleini	5-9	27	339-349	6.5	6-8	58	*Mysis relicta* + Shaved Krill	DeCastro et. al., 2006
Enteroctopus dofleini	5-9	27	388-349	6.5	6-8	80	*Mysis relicta* + Shaved Krill	DeCastro et. al., 2006
Enteroctopus dofleini	9-13	-	240-271	3.0-3.5	6-8	1150	Wide Variety	Snyder, 1986

*Gabe (1974) measured size as length from the posterior end of mantle until the base of the arms, not the total length of the paralarvae

Species	Life History	Egg Development (Days)	Paralarvae Growth (% day^{-1})	Juvenile Growth (% day^{-1})	Diet	Egg Size (mm)
Enteroctopus megalocyathus	Merobenthic	150	0.33-1.25%	~2%	Crustaceans	7.5-11.5
Octopus vulgaris	Merobenthic	25-32	-	-	-	-
Robsonella fontaniana	Merobenthic	68-71	2.4-2.7%	-	Artemia/zoeae	2.4-2.7
Octopus mimus	Holobenthic	38-68	-	-	Clam/Fish Paste	-
Octopus maya	Holobenthic	45-60	6%	3%	Squid Paste	-
Octopus bimaculoides	Holobenthic	55-85	4-7%	2.6-2.8%	Artemia + Pellets	10-17

Adapted from data in Uriarte et. al. (2011).

Table 20. Foods offered to paralarvae and juveniles of *Enteroctopus dofleini*, from published rearing attempts.

Food Type	Feedings Day^{-1}	Accepted	Life Stage*	Longest Survival	Notes	Reference
Variety Chopped Seafood	n/s	+	PL/JV	36mo.	Particle size adjusted to fit juv growth rate	Snyder, 1986
Fresh Killed Clams	n/s	+	JV	36mo.		Snyder, 1986
Fresh Killed Crabs	n/s	+	JV	36mo.		Snyder, 1986
Egg Yolk	n/s	-	PL	42d.		Gabe, 1974
Ground Shrimp	n/s	-	PL	42d.		Gabe, 1974
Ground Mussel	n/s	-	PL	42d.		Gabe, 1974
Live Gammarid Amphipods	n/s	-	PL	42d.		Gabe, 1974
Adult *Artemia salina*	n/s	+	PL	42d.	Best feeding response @ 100+ per liter	Gabe, 1974
Artemia salina Nauplii	n/s	-	PL	42d.		Gabe, 1974
Live Sculpin Fry	n/s	+	PL	42d.	*Hemilepidotus hemilepidotus*	Gabe, 1974
Frozen *Mysis relicta*	5-6x	+	PL	58-80d.	Shaved through 850 µm sieve	DeCastro et. al., 2006
Grated Clam	5-6x	+	PL	58-80d.		DeCastro et. al., 2006
Grated Krill	5-6x	+	PL	58-80d.	*Euphausia pacifica*	DeCastro et. al., 2006

*PL=Paralarvae, JV=Juvenile

As described in Chapter 5 ideal nutrition for young GPOs is poorly understood. The feeding attempts of the few scarce rearing reports are summarized in the tables and text above, though given the paucity of information it is worth considering strategies that have been effective in rearing other cephalopod paralarvae. Table 21 presents a list of foods that have been employed in the culture of two cuttlefishes, a squid, and an octopus for which the nutritional needs are better understood and success in culture of multiple successive generations have been achieved. Some trends emerge that the aquarist might consider obvious: that live crustacean feeds of the appropriate size are the ideal food source for developing cephalopods. Going beyond the obvious assertions the greater challenge is to identify the optimal crustacean that is the right size, has the right protein and micronutrient composition, and is readily available and inexpensive to culture reliably with minimal effort.

Identifying optimal feeds for each life stage of cephalopods is also of critical importance to their future growth and survival. Domingues et. al. (2001) showed that cuttlefish larvae fed *Artemia* for their first 20 days of life grew at a substantially lower rate later in life than those fed on mysid shrimp as a first food. Uriarte et.al. (2011) also postulated that the condition of octopus paralarvae and juveniles may well be a critical, yet unexplored factor in the culture of numerous octopods in captivity, and it stands to reason that this would hold true for *E. dofleini* as well.

Among the most common first feeds in finfish aquaculture are the adult form and nauplii of *Artemia salina*. *Artemia* is a convenient and inexpensive food source, and has been used to rear some cephalopods in conjunction with other food items (Hanley et. al., 1998), but generally is not considered to provide optimal nutrition, even when further enriched with commercial fatty acid supplements (Navarro and Villanueva, 2000). The Rigby and Sakurai (2004) study exemplifies the need for a low-lipid protein to rear paralarvae of *E. dofleini*, most likely some form of crustacean zooplankton, though the ideal first food has yet to be identified. Growth studies with *Octopus maya* and *O. bimaculoides* show that previously frozen first foods are nutritionally insufficient during the earlier exponential gowth phase of paralarvae, but may be suitable once the animal reaches a certain size (DeRusha *et al.* 1989).

There are likely a number of other factors at play in determining the ideal feeding for young *E. dofleini* such as palatability. Villanueva (1994) found that in *O. vulgaris* and *Loligo vulgaris* paralarvae feeding response was best stimulated by offering decapod zoeae that were 50-100% the mantle length of the paralarvae being fed. Lee et. al. (1991) tested the palatability of numerous prepared diets on *O. bimaculoides* and *S. officinalis*, including numerous commercial aquaculture pellets (2 types of shrimp pellet, chicken pellet, mysid pellets) chicken puree, raw chicken, surimi, and even turkey hot dogs! Overall Lee et. al. (1991) concluded that pelletized foods may be used for maintenance of subadult/adult cephalopods in conjunction with other feeds but that they were unsuitable for growth. Their ingenuity in food items may serve as inspiration for the aquarist looking for novel feeds for the occasional enrichment item for adult GPOs but as of yet do not identify a suitable artificial diet for growth of juveniles. The aquarist experimenting with feeds for juvenile octopods should also be cautioned that palatability does not necessarily equate with nutritional completeness; Castro et al., (1993) noted that *S. officinalis* found surimi highly palatable, but 78% of their study population died for lack of micronutirents in that diet.

When feeding paralarvae of *E. dofleini* the density of prey offered may be as important as the type of food. In the first attempt at rearing GPO paralarvae in 1974 Snyder noted that the feeding response to live *Artemia* was greatest when prey density exceeded 100 shrimps per liter of culture water. Frequency of food offerings is also an area to be explored. DeCastro et al., (2006) fed E. dofleini 5-6x per day with frozen foods, and many studies with cuttlefish culture demonstrate that the greatest success may be had when animals are fed to satiation multiple times per day or *ad libitum* (i.e. Domingues et. al., 2002, Alamansa et. al., 2006, Minton et. al., 2001).

Suggested reading: Hanlon & Messenger (1996) provide the best overview of cephalopod reproductive behavior across a variety of taxa including reproductive behavior. Rocha et al. (2001) review cephalopod mating strategies. Forsythe and Hanlon (1980), Boletzky and Hanlon (1983), Hanlon and Forsythe (1985), DeRusha et al. (1987), and Forsythe et al. (1994) provide a wealth of information on system design, feeding strategies, growth rates, and many other useful topics related to cephalopod culture in closed systems. Uriarte et al., (2011) review the problems limiting octopus aquaculture in Central and South American species, and Anderson & Wood (2012) provide a concise overview of the techniques for rearing octopus in an aquarium setting that an aquarist looking for practical information will find useful.

Table 21. Food items used in the culture of other cephalopod paralarvae and juveniles where nutritional requirements from hatching to adulthood are better understood.

Species	Mortality	Growth	Feed Rate	Food Type	Food Condition	Cephalopod Life Stage	Relative Efficacy	Reference
Sepia officinalis	9%	H	*Ad libitum*	Mysid: *Paramysis nouveli*	Lv	PL	+	Domingues et. al., 2002
Sepia officinalis	0%	H	*Ad libitum*	Shrimp: *Paleomonetes varians*	Lv	JV	+	Domingues et. al., 2002
Sepia officinalis	27%	M	-	Fish: *Atherina sp.*	Lv	JV	-	Domingues et. al., 2004
Sepia officinalis	20%	H	20% BWD	Shrimp: *Paleomonetes varians*	Fz	JV	+	Domingues et. al., 2004
Sepia officinalis	9%	M	20% BWD	Fish (unspecified)	Fz	JV	+	Domingues et. al., 2004
Sepia officinalis	33%	L	*Ad libitum*	*Artemia salina*	Lv	PL	-	Domingues, 2001
Sepia officinalis	-	L	*Ad libitum*	Fish: *Atherina sp.* & gobies	Lv	JV	-	Alamansa et. al., 2006
Sepia officinalis	-	H	*Ad libitum*	Shrimp: *Paleomonetes varians*	Lv	JV	+	Castro et. al., 1993
Sepia officinalis	32%	M	-	Shrimp Pellet	Ar	JV	+/-	Castro et. al., 1993
Sepia officinalis	78%	L	-	Surimi	Ar	JV	-	Castro et. al., 1993
Sepia officinalis	5%	H	-	Shrimp (frozen, unspecified)	Fz	JV	+	Ferreira et. al., 2009
Sepia officinalis	-	H	-	Shrimp: *Paleomonetes sp.*	Lv	JV	+	Ferreira et. al., 2009
Sepia officinalis	-	M	-	Crayfish: *Procambarus clarkii*	Lv	JV	+/-	Ferreira et. al., 2009
Sepia officinalis	-	L	-	Fish: *Sardinia pilchardus*	Lv	JV	-	Ferreira et. al., 2009
Sepia officinalis	H	N	-	Fish Meal Gel Diet	Ar	JV	-	Ferreira et. al., 2009
Sepia officinalis	H	N	-	Shrimp Meal Gel Diet	Ar	JV	-	Ferreira et. al., 2009
Sepia pharaonis	L	H	*Ad libitum*	Mysid: *Mysidopsis sp.*	Lv	PL	+	Minton et. al., 2009
Sepia pharaonis	L	H	*Ad libitum*	Shrimp: *Paleomonetes pugio*	Lv	PL/JV	+	Minton et. al., 2009
Sepia pharaonis	L	H	*Ad libitum*	Fish: *Poecilia reticulata* (fry)	Lv	PL	+	Minton et. al., 2009
Sepia pharaonis	L	H	*Ad libitum*	Fish: *Cyprinodon variegatus*	Lv	JV	+	Minton et. al., 2009
Sepia pharaonis	L	H	*Ad libitum*	Fish: *Poecilia latipinna*	Lv	JV	+	Minton et. al., 2009
Sepia pharaonis	L	H	*Ad libitum*	Shrimp: *Penaeus spp.*	Lv	JV	+	Minton et. al., 2009
Sepia pharaonis	L	H	*Ad libitum*	Crab: *Callinectes sapidus*	Lv	JV	+	Minton et. al., 2009
Loligo vulgaris	-	-	*Ad libitum*	Crab: *Pagurus prideaux* (zoea)	Lv	PL	+	Villanueva, 1994
Octopus vulgaris	-	-	*Ad libitum*	Crab: *Pagurus prideaux* (zoea)	Lv	PL	+	Villanueva, 1994

Symbols: H=high, M=moderate, L= low, N=none; BWD= body weight per day, Lv=live, Fz=previously frozen, Ar=artificial (pellet/gel); JV=juvenile, PL=paralarvae

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. With GPOs, however, this will likely not be a problem in the foreseeable future as breeding in an aquarium is rare, and successful rearing of larval octopods to adults has yet to be achieved. The need to house animals singly also precludes the need for contraceptive techniques. At present there are no known methods of contraception for octopods.

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. There are a number of other ways to go about training a GPO, depending on what the task to be learned is. Food is a good positive reinforcement for having performed the desired task, but the environmental preferences and habits of the octopus can also be used in training or learning. We have seen how food can be a stimulus for learning to open containers. One should take into account octopus behavior both in the wild and normal behaviors seen in aquarium settings to be effective. For example, the Seattle Aquarium recently built a new GPO exhibit with a 3000-gallon triangular tank, which had one side against a wall. The intent was for the octopus to rest/sit in the front corner, where it would be most visible to the public. Based on the knowledge that GPOs like dark dens, light was purposely increased in the back corners and decreased it in the front corner.

Based on the knowledge gained from previous experience that GPOs prefer to sit in front of water currents, both the new water and recirculated water inlets were directed toward the front corner. All GPOs that lived in that tank (separately, of course) chose to sit in the front corner. When octopuses have chosen to sit in a back corner, the light intensity was increased there and if necessary the animals were prodded out of the corner with a bristly brush, thus making it uncomfortable, and prompting a move to the front corner.

Laundry basket conditioning: One specific behavior, which has been utilized in several facilities, is conditioning the animal to sit in a laundry basket or similar container at the water’s surface. The basket is smeared with residue from an oily fish to get the animal’s attention, and with persistence and reinforcement the animal can be conditioned to sit inside the container while it is briefly removed from the water to measure the weight of the animal. This is also very useful for moving animals from exhibit to holding, and vice-versa. It should be noted, however, that GPOs will likely not cooperate after the onset of senescence, even if they could be counted on to perform this trick regularly beforehand.

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 giant Pacific octopus 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 giant Pacific octopus should take into account the natural history of the species, individual needs of the animals, and facility constraints. The giant Pacific octopus enrichment plan should include the following elements: goal-setting, planning and approval process, implementation, documentation/record-keeping, evaluation, and subsequent program refinement. The giant Pacific octopus enrichment program should ensure that all environmental enrichment devices (EEDs) are "giant Pacific octopus" safe and are presented on a variable schedule to prevent habituation AZA-accredited institutions must have a formal written enrichment program that promotes giant Pacific octopus-appropriate behavioral opportunities (AZA Accreditation Standard 1.6.1).

Giant Pacific octopus 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.

Enrichment: AZA's *Connect* magazine summarized enrichment activity with GPOs and is excerpted and modified below (Peters et al., 2005). Environmental enrichment is a critical part of total animal care at zoos and aquariums, and has come about as a result of a long progression of cultural change (Seidensticker & Forthman, 1998). Caretaker innovation, combined with research and visitor interests, has helped highlight its importance in zoo and aquarium environments.

Enrichment has become so important to quality animal care that it is now an industry standard and part of the American Zoo and Aquarium Association's accreditation process. While many have already worked to bring about environmental enrichment in zoos and aquariums, many more will refine and expand its scope and integration into everyday practice. Environmental enrichment is not static: it is not limited to specific activities, such as the introduction of a toy, or to a particular group of animals, such as mammals.

In addition to addressing the specific needs of a species, enrichment sometimes requires addressing the needs of individual animals. Whether adjusting an animal's physical enclosure, changing the substrate or water flow, varying the presentation of food, adjusting the social composition of a group, or decreasing predictability of events, enrichment has brought about more activity, more diverse behavior, and greater utilization of exhibit space by animals (Shepherdson et al.,1993). In addition, the introduction of objects for manipulation and foraging opportunities reduced stereotypic behaviors in bears (Carlstead et al., 1991).

From mammals to cephalopods: Environmental enrichment initially focused on mammals and then spread to birds. Now it has expanded to many other taxa, including cephalopods (Wood & Wood, 1999). The GPO has been singled out mostly due to perceived intelligence, behavioral repertoire, and responsiveness to stimulus.

Data has been collected on cephalopod behavior, specifically giant Pacific octopus, as a result of environmental enrichment. Modifying tank enclosures, water flow, or introducing objects create opportunities for animals to make choices and explore environmental changes. Cephalopods respond through skin color and texture changes, body position changes, and object manipulation (Peters & Powell, 2005).

For GPOs, creating opportunities for exploration may be more important than simply increasing their overall level of activity. Mammalian behavior studies have been done for many years. Cephalopod enrichment behavior studies are increasing and gaining more attention (Anderson & Leontiou, 2005). Current data suggest that having the octopus in an enriched state is a worthwhile enrichment goal rather than focusing on the "best" type of enrichment.

Intelligence: One aquarium and one university have studied how giant Pacific octopuses habituate to new objects (Mather & Anderson, 1999). They presented sub-adult animals (fewer than 10 kg) with a floating pill bottle and observed the animals' reactions.

Most of the octopuses did as expected—grasping the bottle and trying to eat it before finally ignoring it. However, two octopuses surprised the researchers: using their water jet, they gently blew the bottle away from themselves toward the water inlet, only to have it come back at them in the water current. The two animals persisted in this behavior, which has been likened to a child bouncing a ball. This incident was the first report of an invertebrate demonstrating play behavior. Play behavior is a repeated, not necessarily functional behavior, differing from more functional behaviors, and initiated voluntarily when the animal is in a relaxed or unstressed setting (Burghardt, 2004).

There are several methods of determining an animal's intelligence, including measuring and comparing brain mass to body mass. Octopus fare well when using this method. Their brain-to-body ratio is higher on the intelligence scale than fishes and reptiles and on par with some birds. With only 68 million neurons in their brains, compared to the 100 billion in human brains, octopus may not seem so smart. But there's more to this count than numbers. More than half of all nerves in an octopus are in its eight arms, which function with dramatic precision in gathering information and executing tasks.

Laboratory tests provide other ways to measure animal intelligence. Octopuses can learn to go through simple mazes and can be trained to swim to a specific target for food. Yet, they are not consistent in making the "correct" choices to reach the food. This may be explained by the octopuses' natural behavior of varying hunting grounds.

Since brain capacity and classic conditioning tests unreliably measure octopus intelligence, how else can octopus intelligence be noted? Wild populations of common octopuses, *Octopus vulgaris*, have been noted using tools. Octopuses use water to blow out garbage from their dens, much like using a hose to wash down a driveway, seal dens with shell pieces, and navigate with landmarks. They can navigate using landmarks, as in following a triangular hunting route and then swimming directly back to their dens without having the den in direct sight (Mather, 1994).

Case Studies of GPOs: A few years ago, Dr. Jennifer Mather of University of Lethbridge and Dr. Roland Anderson were testing habituation in GPOs at one aquarium. They wanted to learn how GPOs became habituated to new and novel objects. The GPOs were presented with pill bottles weighted so they just barely floated. They received black and white painted bottles with smooth or rough surfaces (sand added to black or white paint). The GPO also received a bottle after a day of fasting, since hunger is known to stimulate exploration in octopuses. Then the actions of the GPO were observed and recorded.

The octopuses predictably grasped the bottle with their suckered arms and passed it to their mouths underneath to see if it was edible. Most of the bottles ended up with scrapes on them from the octopuses' beaks, which are similar to a parrot's beak. When they found it wasn't edible they held onto the bottles for varying periods of time and eventually dropped them. Some octopuses held the bottle up to an hour or more and tried to eat it periodically. In repeated trials over 2 weeks the octopuses became used to the bottle and held it for shorter periods of time.

It turned out that the color and texture of the bottles made no difference in the habituation. Researchers and aquarists may have been imposing their own standards on what might or might not interest an octopus. But another behavior was observed that proved very intriguing. Some of the octopuses, after they cast off the bottle, continued to interact with it. The bottle floated on the surface of the water and was carried by the water inlet back to the octopus, whereupon it blew water jets at it with its funnel, carrying thence bottle back to the octopus. The octopus was not using the forceful jets of water it uses to get rid of an annoyance, but more gentle flows. This continued for more than a half hour. Some octopuses aimed their jets so the bottle went directly back and forth across the water's surface and others directed the bottle in a circle around the tank. Dr. Roland Anderson remembers reporting to his colleague when he first saw this behavior and equated it with bouncing a ball, and indeed they thought this was play behavior.

There are many definitions of play behavior and many examples that we can easily think of. Cats play with a ball of yarn. Puppies play with each other. Parrots grasp a limb and fall over repeatedly. Many defined play behaviors in juvenile animals are practice for catching prey when they are older or mock mating behaviors. But in essence, play behavior is a behavior that has no practical end; it is non-productive. That is what the octopuses were doing and this was the first reported play behavior in an invertebrate. The results of this experiment were recorded for the Journal of Comparative Psychology and it was accepted and well received by the scientific community (Mather and Anderson, 1999).

So-called play behavior heretofore had only been reported in the "higher" vertebrates, like mammals and birds, although there is one report of a turtle playing with a ball. Thus it is associated with higher intelligence, but it is not a measure of their intelligence. Measuring intelligence is much more difficult. Humans have trouble measuring our their own intelligence much less that of animals. Although there are IQ tests, there is a great deal of controversy over their use. The questions in such tests have to be carefully screened to match the culture of the takers and they may not be accurate assessments of the intelligence of some cultures so they can't be used.

Likewise, when one tests the intelligence of an octopus, one has to be careful not to impose human standards on the tests. People can teach an octopus to go to a specific target—to go to a specifically shaped object—to be rewarded with food, but there are problems with this. The octopus statistically learns to go to the target, but is never 100% accurate on such tests. This behavior can be explained using the behavior ecology of the octopus. It is a refuging predator—it refuges in a den and emerges only to get food (or find a mate at the end of its life span). A well-fed octopus rarely leaves its den, much to the disappointment of home aquarists keeping octopuses. As a refuging predator, it won't forage in the same area every day since it knows it got the food there yesterday. Hence it isn't logical to an octopus to go back to the same target day after day to get food in a lab experiment.

Since there are no IQ tests for octopuses, aquarists have to use other methods to measure their intelligence. One method is to compare their brain capacity and size to that of other animals. In terms of brain size to body weight, they compare relatively well. Their brain/body weight ratio is higher than fishes and reptiles and compares well to birds, indicating a good intelligence.

In terms of absolute brain capacity, they don't fare so well, but there is a caveat. The brain of the common octopus (*Octopus vulgaris*) contains about 168 million neurons compared to the 100 billion of a human, a considerable disparity. On the basis of this alone we wouldn't think an octopus capable of higher thought processes, but it turns out that half of an octopus's total neurons are in its arms. Their movements are largely self-directed and activated. Past experiments have shown that if an octopus's arm is cut off, it will go crawling off by itself, will attach to a food item, and even pass the food along the arm's suckers to where the mouth would have been (note that such cruel experiments would not be performed today and researchers are lucky to learn from what was done in an earlier era). But the fact that the arms are self-enervated may free up space in the brain for higher thought processes such as learning.

The other way of measuring octopus intelligence is to observe what they can do. One example noted by Mather (1994) is tool use, clearly an activity of more intelligent animals. Octopuses were reported to use tools in two ways. They use rocks to block up the entrances to their dens – thus making "doors," typically during the daytime – and unblocking them at night when they go out to forage. Granted, this is a pretty simple tool but it meets the definition.

The other method they use is more complicated. Octopuses use water as a tool when they blow it out their funnels. Normally, octopuses take water into their mantles, pass it over the gills for respiration, then blow it out a siphon tube in a water jet. In a secondary use of this water jet octopuses use it for swimming like a jet-propelled submarine. They go into oxygen debt when swimming so they never jet very far before pausing to catch their breaths. In tool use mode, octopuses use water to sweep out their dens, to blow out feces and food wastes and to blow away annoyances. In the wild they blow water jets at non-dangerous fish that get too close to them such as damselfish or parrotfish. In Hawaii, a day octopus (*Octopus cyanea*) was observed in shallow water at low tide blow water jets straight upward at a butterfly that winged above it. The jets erupted in a geyser about 15 cm (6 in.) above the water surface that scared the butterfly away.

Sometimes an octopus's use of water jets in a lab or aquarium can have amusing results. An early researcher set up a target training experiment with common octopuses (Dews, 1959). He devised an apparatus that had to be triggered by the octopus in order to get food. He found that one octopus he tested was not suited for the experiment. "[octopus] Charles was capricious… and sustained control was not achieved." (Dews, 1959). Charles pulled the apparatus so hard each time it broke and furthermore "Charles had a tendency to direct jets of water out of the tank, specifically they were in the direction of the experimenter." (Dews, 1959). One can just see the scholarly, lab-smocked, bespectacled researcher trying to control the octopus and the octopus shooting water at him. This is certainly an apt use of a tool by an octopus.

Another amusing story of octopus water jets and seeming intelligence happened at one aquarium a number of years ago. One nighttime employee reported the giant Pacific octopus on display always shot jets of water at her as she walked by the tank. She felt rather discriminated against, as she was the only one it did this to, possibly a case of human recognition by octopuses (another indicator of intelligence). What was actually happening was that at night the employee would shine her bright flashlight at the water inlet to the tank to ensure proper water flow, disturbing the octopus's midnight slumbers. The octopus then associated the employee with this nighttime disturbance and blew water jets at her.

Another indicator of intelligence is the way they find their way around the landscape of the seafloor. Common octopuses go on extensive forays to find food, ranging tens of meters away from their home den, yet they always find their way back. Mather (1991) investigated how they were able to do this and found they use landmark navigation ("left at the big rock, right at the coral head, then straight down the alley"). Some insects use a simpler form of this such as bees when they signal to others where a good flower is or wasps determining where they laid eggs, but the octopus system is more sophisticated. She observed octopuses on their foraging expeditions and found they could go on a triangular route, indicating that they not only knew where the landmarks were but could recognize the landmarks in the context of the environment and they had to be able to recognize them from different angles, a much more complex system than just recognizing that their den is next to big rock. In fact, further studies have shown that octopods have excellent spatial recognition and exhibit exploratory behavior; even finding their den when their landscape was shifted 180 degrees (Boal *et al.*, 2000).

Octopuses can learn to run mazes in the lab but they are perhaps surprisingly not very good at it, possibly again because of their refuging forager status and perhaps because of another reason. Octopuses have been found to be either left or right handed (another domain of higher intelligent animals). They use predominantly their left or right arms and left or right eyes. In a maze they tend to keep to one side and follow a wall and they have trouble when they have to make a choice of left or right. Perhaps they would have better luck if there were landmarks at each turn of the maze.

About 10 years ago, Mather & Anderson (1993) determined another big indicator of octopus intelligence, that they have individual personalities. Aquarists knew for years that different octopuses have different characters and named them for these. At one aquarium, a giant Pacific octopus tore up her tank, another hid behind her backdrop, and yet another had a proclivity for intense tactile interaction with its keeper. The octopuses that squirted water at certain individuals mentioned earlier are also examples of individual character in octopuses.

Mather and Anderson worked with 44 red octopuses and looked at what they did when staff opened their tank, touched them with a test tube brush and gave them a live crab for food. They found that their behaviors clumped into three categories: aggressive, passive, and shy. An aggressive animal would grasp the test tube brush, a passive animal would shrink away from it and a shy octopus would ink and jet away. Graduate student David Sinn who found similar traits in a different species of octopus and that these traits were long lasting and genetically based (a shy parent would have shy offspring) (Sinn et al., 2001). Sinn has gone on to study personalities in a bobtail squid (*Euprymna tasmanica*) at the University of Tasmania, so personalities, while indicators of intelligence, may be more widespread than currently thought.

The above examples are indicators of octopus intelligence because there are no proper IQ tests for them yet. Their world is so different from a human's—it just may not be possible. It's also difficult for humans to realize how very different they are from us in spite of their similar eyes (an example of convergent evolution) and seeming intelligence. Current theory says that the ancient lineages that led to octopuses and humans diverged more than a billion years ago (Wray et al., 1996).

Enrichment for GPOs: During the last several decades, zoos and aquariums have come to realize (perhaps belatedly) that animals kept in aquarium settings need environmental enrichment for their well-being. Early zoos and aquariums tried to show as many animals as possible; hence, their exhibits were frequently small and bare to permit easy viewing, cleaning and sterilization. Now, we have come to realize that aquarium-housed animals need environmental enrichment for their health and for the education and increased expectations of visitors to zoos and aquariums. Undesirable behaviors such as escape jetting and autophagy can be decreased seemingly as a result of enrichment (Beigel and Boal, 2006), in addition to increasing the overall activity level of the animal, making for a better display (Rehling, 2001).

Shepherdson (1998) defines environmental enrichment as "an animal husbandry principle that seeks to enhance the quality of captive animal care by identifying and providing the environmental stimuli necessary for optimal psychological and physiological well-being." Mensch (1998) points out the importance of exploratory behavior: "housing systems should incorporate enrichment that allows animals to engage in their species-typical patterns of information gathering." Another form of enrichment, behavioral enrichment (or "behavioral engineering"), has been described in terms of modifying and enriching adaptive behaviors. For example, the use of interactive devices decreases abnormal behaviors in primates.

There are three closely related reasons why enrichment is needed for animals in zoos and aquariums based on their behavior in these facilities, plus two others. The first is to maintain healthy activity levels, the second to alleviate space confinement, and the third to change a deviant behavior back to normal behavior. In other words, animals in zoological settings should be physically and mentally healthy. In the wild, animals spend significant amounts of time and energy acquiring resources such as food and mates while at the same time avoiding predators. In a zoo or aquarium environment, food and mates (if available) are often obtainable without much effort and predation pressure is usually non-existent. This can leave animals without the activities that normally take up their time.

It is known that intelligent animals get bored and social animals get bored. Boredom can be reflected in abnormal sleep or rest patterns. However, in a zoo setting, animal boredom is more usually exhibited by destructive behavior, which can be directed toward its surroundings, to the contents of its enclosure, against itself or its cage mates, or its keeper. Close confinement in barren surroundings has classically led to deviant behaviors. Some common symptoms of close confinement are: repetitive pacing in an animal, constant attempts to break its confinement, resting or sleeping abnormally, or engaging in destructive or self-destructive behavior. The simple solution of enlarging an animal's enclosure often brings its behavior back to normal.

A fourth reason for enrichment is to meet the expectations of the public. The design of zoo and aquarium enclosures must be considered from the public's perspective. A sloth may be perfectly happy hanging from a projecting pipe all day, but zoo visitors are more comfortable seeing it in the branches of an artificial or natural tree. More and more zoos and aquariums are emphasizing enclosures that resemble natural habitats for the comfort and expectations of its customers as well as for the comfort of the animals.

The fifth reason for enrichment is to prepare animals for reintroduction to the wild. Popular examples include: Elsa the lion, Keiko the killer whale, whooping cranes and California condors. Many reintroductions are of endangered animals in the hopes of reestablishing the population. Reintroduced animals need to be able to cope with natural environmental conditions and hazards, such as daily or seasonal temperature changes, rain, snow, cold, variations in food supply, parasites etc. They need to be able to cope with competition among cohorts. They may need to migrate at certain times of the year and they need to be able to avoid predators. Hatchery-reared salmon are notorious for being unaware of predators and suffer higher than normal predation loss because of it. Some salmon hatcheries have tried placing a predator in with the salmon to make them better at predator avoidance when they get released into the sea (Berejikian, 1995).

All wild animals live in complex and dynamic environments and all have adaptations to those environments. However, enrichment has traditionally been focused on mammals because of our anthropocentricity, but it is beginning to be applied to reptiles and amphibians, birds and invertebrates (Shepherdson, 1998; King, 1993; Hayes, et al., 1998; Mather & Anderson, 2007). The unspoken rule seems to be to apply enrichment only to animals thought of as social and/or intelligent but until we know which animals enrichment benefits, enrichment should also be applied to animals other than mammals and other vertebrates. Enrichment for all species of animals kept in zoos or aquariums should at least be considered, just as the ethics of animal experiments using invertebrates are now being considered (Mather & Anderson, 2007). Invertebrates, such as octopuses, which make up 95% of the animal kingdom, have traditionally been underrepresented in zoos, aquariums and in scientific research. There is almost nothing written on enrichment for invertebrates, with two exceptions, both on octopuses and Rehling (2000) has started an enrichment notebook on octopuses.

The GPO is the cephalopod most exhibited by zoos and aquariums, probably because it is the largest octopus species in the world (Carlson & Delbeek, 1999). Octopuses in general are popular with the public because of their intelligence, their alien appearance, their ability to change color and texture, and the impression that "when you are watching them, they are watching back." Octopuses have been known for their large brains and intelligence since at least 330 BC when Aristotle wrote his *Historia Animalium*. In recent years, much more has been learned about their intelligence (Anderson, no date). Cephalopods are able to collect large amounts of information about their environment with their well-developed sense organs. Their brain to body weight ratio is higher than that of most fish and reptiles and is the largest of the invertebrates (Packard, 1972). Hence, octopuses in aquariums are prime candidates for an enrichment program.

Determining if a GPO needs enrichment is a complicated question. Considering the five needs required for enrichment when applied to GPOs may offer answers. Humans cannot determine if a GPO is bored. An octopus is a refuging predator (Curio, 1976). In the wild, unless inspired by hunger, sex, irritation, or predators, most octopuses will remain in their dens. Field observations have indicated that *O. cyanea* in Hawaii spend 72% of their time during daylight hours in their dens. *O. vulgaris* in Bermuda spent 88% of their daylight hours in dens. Mather and O'Dor (1991) suggest that wild octopuses minimize risk of predation by minimizing foraging time. Predators have a large influence on the population structure of octopuses. In areas without teleost predators, wild *O*. *briareus* were 100 times more abundant. Evolution and current behavior of octopuses are thought to be largely a response to predation pressure from vertebrates (Packard, 1972; Aronson, 1991).

Zoo and aquarium environments typically lack predation pressure; therefore, one might expect octopuses in aquariums to be at least as active as wild ones. However, at one aquarium there have been many examples of well-fed octopuses that sit all day and night with little activity other than breathing. With a diet of easy-to-capture and easy-to-eat frozen food and no enrichment, aquarium-housed octopuses appear to have little reason for activity.

Octopuses are well known as the grand masters of escape in the animal world and GPOs are no exception. The question is, do octopuses try to escape from their tanks because of boredom and lack of enrichment, poor water quality, lack of food quantity, lack of food quality, or are escapes normal foraging behavior? There are indications that some octopus species are more prone to escape from aquarium settings than others; for example, the California two-spot octopus (Octopus *bimaculoides*) is one that rarely tries to escape. GPOs are well-known escape artists and they are strong. One 40-pound GPO was able to slide a plywood cover off its tank with 66 pounds of rocks on top, and escape. This sort of escape is probably a foraging expedition. The activity pattern of wild octopuses is to stay in a single lair for days or weeks. They only leave it to forage and return to eat or to look for a mate. Hence, attempts to escape from tanks may simply be the octopuses' efforts to go get something to eat or to have sex.

Octopuses also attempt to escape from tanks that have poor water quality, such as not enough water flow, not enough O_2, improper salinity, improper chemical balance, or presence of metals (Wood & Anderson, 2004). Besides the above reasons, GPOs try to escape from tanks where other conditions are not "right," and hence, if conditions were made "right" through enhancement, there would be fewer escape attempts. At one aquarium, there have been fewer escape attempts from tanks with front viewing windows than from those without, indicating that these octopuses like a "room with a view." Perhaps the movement of aquarium visitors is an effective enrichment.

It can't be determined yet whether enrichment can help an aquarium-reared GPO survive better if released back into the wild, because researchers don't know how many of its behaviors are learned and how many are innate. There is some evidence of innateness in GPOs, but octopuses as a whole are difficult to rear, difficult to follow in the wild, and there have been very few cases of aquarium-reared octopuses being released and observed (Anderson, 2000). However, since an octopus can learn, it is likely that the more experiences it encounters while growing, the more successful it will be in later life, and hence, an octopus reared for possible release should have an enriched life.

Good reviews of enrichment ideas for smaller octopuses can be found in Wood & Wood (1999) and Rehling (2000). Anderson & Wood (2001) suggest octopuses can be enriched by use of suitable space, inclusion of a complex environment, including "toys," "complex" food or feeding strategies, and proper den or lair space. Due to their large size and strength, GPO enrichments may be different than those used for small octopus species.

Food and feeding strategies can be an important source of enrichment for GPOs (see Figures 29, 30 & 31). An easy and effective enrichment is simply varying the food given. This is also good nutritional practice. At one aquarium, GPOs have been reared to 97 pounds on a varied diet. GPOs in aquarium settings can live, grow, and reproduce when fed a diet of thawed, frozen, uncooked seafood such as herring, smelt, capelin, fish fillets, clam meat, or squid. However, a simple form of enrichment is to feed a GPO live food, such as crabs, fish, lobsters, clams, or even freshwater crayfish. A recent feeding of a large, live Dungeness crab to a GPO at one aquarium provides a good example. Once the crab was added to the tank, the octopus had to stalk the crab, pounce on it and subdue it in its arms and web. Then, the octopus drilled a hole in the crab's shell, using a radula in combination with a salivary papilla that exudes a dissolving agent. When the tiny hole was drilled, the octopus injected venomous saliva that first paralyzed the crab, and then killed it within minutes. The injected saliva also began the digestive process by loosening the muscle attachments.

There is preliminary evidence that the octopus's saliva affects the crab flesh, making it easier to extract from their thin legs. All of the crab flesh was eaten. The carapace was removed from the body, and the legs were all dismembered and broken at the joints, presumably with the octopus's hard, horny beak or pulled apart after loosening the ligaments holding them together by the injected saliva. Cleaned shell parts were then dropped or blown out of the den to form a midden in front. This process took more than 3 hours. Live clams are also drilled before opening and eating, which can engage an octopus in species-appropriate behavior for hours. As a comparison, one of the puzzle boxes presented by Rehling (2000) was solved in 16 seconds the first time it was introduced, and several commercially produced puzzle boxes were solved in six seconds. Feeding live food is simple and can keep the octopus busy for long periods of time with natural behaviors.

Public aquariums have been creative in supplying live food for their aquarium-housed GPOs. Such foodstuffs have included Maine lobsters and Chesapeake blue crabs. These are not prey items that a GPO would normally encounter or know how to catch and eat. Therefore, they need to learn how to catch and eat the item, in addition to doing the actual feeding. Freshwater crayfish have proven to be a valuable and practical live food for GPOs and other species of octopus large enough to eat them.

Other live foods can be fish or shrimp. The octopus uses a speculative pounce technique called a "webover" (see Figure 28) to catch small, hidden prey by spreading the web between its arms and "throwing" it up, forward, and down over rocks that may house potential prey items. In other words, they use their bodies like a living cast net. The octopus then squeezes out the water from this "balloon" and feels inside with its arm tips to catch small swimming prey. Nontraditional foods may be used to enrich the captive octopus as well- turkey hot dogs, turkey necks, and giblets may be good as the occasional food item to add variety to the diet for the sake of enrichment. And of course, a wide variety of frozen seafoods should be offered to maximize variety alongside the occasional 'treat' such as live crabs, poultry, lobsters, et cetera.

Figure 28. A GPO uses a "webover" prey capture technique—the webbing is spread over an entire rock with fishes or crabs to entrap prey. Photo courtesy of R. Anderson

Figure 29. Live and novel food enrichment. Top left: A GPO named "Baldur" eating a live Dungeness crab, *Cancer magister*. Photo courtesy of S. Palm. Top right: A GPO given the moniker "Devil" contemplates his horde of live clams sitting in his den. Photo courtesy of L. Shaw. Bottom left: A chicken wing that has been eaten by a GPO, a food that would never be encountered in the wild, but is cheap, easily available, and adds novelty and variety to the animal's diet. Photo courtesy of R. Anderson. Bottom right: In many parts of the country crayfish are available live seasonally at the supermarket and make excellent enrichment for a GPO. Photo courtesy of R. Anderson

Octopuses often explore their environment and may even indulge in exploratory play behavior as described earlier. While a complex environment and presence of conspecifics is important, the opportunity for certain kinds of direct interaction (e.g., play, exploration, and manipulation) are the most important. Hence, two additional enrichments that can be done with food are hiding the food so the octopus has to search for it or putting food in a puzzle box. One idea is to release a number of smaller live prey that will hide themselves all over the tank, and let the octopus find them (Wood & Wood, 1999). Even giving a larger crab time to bury or hide increases its lifespan and hence, the predation time. Wood and Wood (1999) also hid food in a play ship—the octopus had to "sink" the ship in order to get the proffered food. Such a demonstration with a large octopus and an appropriately larger toy ship would probably interest the paying customers of a public aquarium by invoking a "sea monster" image.

A simpler version of hiding the food is to put it in a container the octopus has to open. Various species of octopuses have been trained to open puzzle boxes to get at a crab or other food. Although octopuses can frequently get at the food very quickly, but interaction with the puzzle boxes can last much longer as some octopuses hold onto the puzzle box or parts of the puzzle box for hours. At one aquarium a small, dead herring was placed inside a plastic cigar container and the GPO was timed to see how long it took to open it and get the food. Although the octopus never showed signs of habituation to the container since, she never let go, she didn't really show outward signs of learning to open it either, as the mean time to opening hovered around one hour. The point here is not whether the octopus really learned to open the container, but that she remained active during the process.

One way to maintain the octopuses' interest is to smear the outside of a container with food, such as herring fluids. Octopuses taste with their suckers, so the octopus will associate the container with food or assume that there is food somewhere. At one aquarium, aquarists have also used this propensity of tasting with their suckers to facilitate getting large (and strong) octopuses out of their tanks with a minimal amount of stress. Smearing herring fluids on the lip and outer surface of the tank, then let the octopus pursue the "phantom" herring and crawl out by itself. Obviously, this method would work to train the octopus to do other tasks.

Individual octopuses have different temperaments that may even be akin to our own personalities, and octopuses with some of these temperaments make poor display animals (Mather & Anderson, 1993; Sinn, et al., 2001). One reclusive female at one aquarium persisted in squeezing behind a fiberglass backdrop at the rear of a rectangular 300-gallon tank. While it was interesting for staff and volunteers to view an almost two-dimensional, 30-pound octopus (only 2 inches thick), she was not visible to the public.

Was this animal suffering from lack of enrichment? Was she bored or engaging in abnormal behavior? Was she just "shy?" Or was she looking for a secluded spot to lay her eggs in? It is likely the latter explanation, which leads to another aspect of habitat enrichment: do aquariums need to provide a proper den for females to lay eggs in? The answer is probably yes, with qualifications.

One of the goals of environmental enrichment is to bring behaviors of aquarium-housed animals back to those seen in the wild. The problem is that brooding females hide in dens not much larger than themselves. Females protect themselves and their eggs even further by piling rocks up to block the entrance to the den. This situation would seem to make it impossible to display such animals in public aquariums, but this is not the case if displays are creatively designed with the above considerations in mind. Since GPOs frequently grow to 60–80 pounds in aquarium settings, the display den should not be much bigger than the volume needed for an octopus that size. The opening should face down-slope, so the den should be elevated a bit and facing down, since octopuses like to look out from their dens and downslope (James Cosgrove, personal communication). If there is a likelihood of a female being kept, there should be a hard ceiling to the den, so she can attach her eggs to it.

Adult female octopuses are very reclusive. To show the inside of an octopus's den, creative exhibitry can include a dark nook in the public area with either one-way glass or tinted glass to view the back of the den. Exhibit designers should remember that the den needs to be dark for the octopus's comfort, and in order to see into the den, that part of the public area should also be dark. Note that octopuses are colorblind and that use of red light will not be beneficial in this case. The current policy at one aquarium is to release females before they lay eggs so they can successfully reproduce in the wild, but the display of a brooding female can be used to educate the public about alternative life-history strategies, like semelparity. There is no point in attempting any behavioral or environmental enrichment once they have arrived at this life stage, since all their behaviors are directed at keeping their eggs alive.

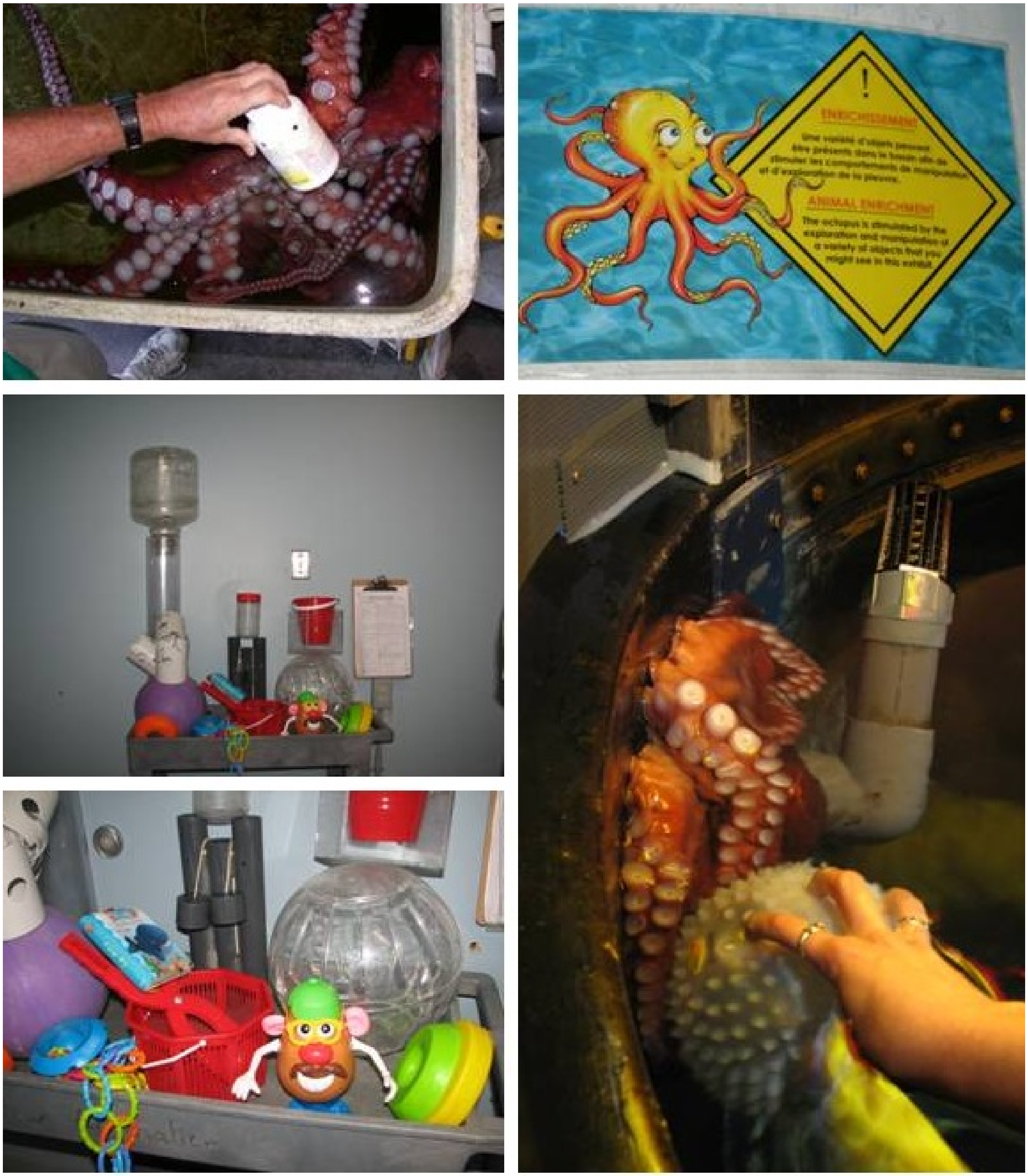

Figure 30. Enrichment for *Enteroctopus dofleini*. Top right: A simple pill bottle offered as a prey puzzle. Photo courtesy of R. Anderson. Top left: Creative graphics to explain enrichment to the public. Photo courtesy of J. Marvin. Middle and bottom left: A selection of prey puzzles and octopus toys used for enrichment. Mr. Potato Head is a popular option at many facilities. Photo courtesy of J. Marvin. Bottom right: An octopus being offered a ball. Many objects with interesting textures may be presented for an octopus to play with; rubbing fish on the surface will increase their interest. Photos courtesy of J. Marvin

A parallel situation is found in males. Mature male GPOs end up in “senescence,” even if they have not had a chance to mate. Isolated aquarium-housed males will frequently pass out spermatophores, 61–91 cm (2–3 ft) long transparent structures that superficially resemble round worms, and keepers of older male GPOs may find these lying on the bottom of an octopus tank. Senescent male GPOs do not behave normally, even in the wild. They don't eat. They rarely sit in dens, and they crawl or swim about aimlessly. In Puget Sound, they are frequently found alive or dead on shore or in river outlets, but they are wonderfully active aquarium display animals until they die. Another opportunity for designers of GPO tanks is to provide enrichment by adding co-inhabitants to the tank. For a more detailed discussion of appropriate tank-mates see Chapter 4.2.

Creative enrichment: Most zoo professionals understand the concept of enrichment but may find it difficult to implement. Enrichment programs need to be species specific or even targeted to an individual animal. A program for a bear does not necessarily work for cheetah or octopus.

The octopus is a fascinating and cryptic creature. It is a solitary animal and hunts by lying in wait for unsuspecting prey. Cuttlefish and squid can be very social and display a myriad of courtship behaviors and complex behavior patterns. Much cephalopod behavior can be perceived as alien and strange. This makes it difficult to apply existing mammalian enrichment programs, yet much has been done.

Initially, cephalopod enrichment involved having an octopus open a jar lid and take out the food within. Since then many institutions have presented various toys or novel items to curious cephalopods with some items selected based solely on their keeper's curiosity (i.e., "See what he does with this!"). While some of this behavior has been documented in scientific papers, most of it is anecdotal and few facilities had any established enrichment protocols for cephalopods.

This practice has changed over time. The Aquatics Department of one zoological institution collected ideas in an Octopus Enrichment Notebook (Rehling, unpublished work). It didn't take long for the word to get out and information began to flow. Ideas that had literally sat on a shelf were circulated and became even more innovative. The wide variety of continuing submissions reflects the needs of the animal species and various facilities. Institutions contributing ideas to the notebook range from educational theme parks to public aquariums and zoos, and academic institutions. This "small" notebook project has grown to include more than 70 entries. Fifty-one facilities around the world hold copies. Now many institutions plan and track enrichment activities for GPOs.

The various enrichment concepts originated from a simple, single list, and is now a large notebook divided into three sections: Environmental Stimulus, Hunting Stimulus, and Interactive/Tactile. The Environmental Stimulus section deals directly with habitat: den design, lighting, water currents, and exhibit textures. One entry identifies weekly habitat change, moving rockwork, and fake kelp.

The Hunting Stimulus section is designed to promote hunting behavior through investigation and food acquisition. Most of the ideas come in the form of "prey puzzles"—devices that need to be solved to obtain the food reward such as opening a door or spinning a knob. These devices are varied, from the simple floating skewer or dog toy, to the more complex acrylic puzzle or "childproof" pill bottle.

The third section, Interactive/Tactile, involves physical contact and non-food rewards for investigations. The curious nature of cephalopods lends itself to investigative activities. Many of the entries are children's toys, which are rich in texture, shape, and movable parts. Other activities involve scheduled physical interactions, such as the presentation of human hands for the specimen to investigate, as well as protocols for crate training.

Other forms of enrichment: No discussion of the practice of enrichment would be complete without mentioning that enrichment for captive animals need not be in the form of toys or feedings. In stark contrast to zoos, aquariums have traditionally exhibited their animals in mixed-species displays with more naturalistic settings. The effect of having fishes, echinoderms, and other animals in a more naturalistic setting certainly contributes towards the psychological well-being of the animal (especially when a tank-mate ends up doubling as a prey item!). See Section 4.2 for further information on housing other species with a GPO. In addition to having other animals displayed with an octopus simple tasks such as cleaning the tank may contribute towards enriching the life of a captive octopus. Any aquarist that has kept an octopus knows that anytime a scrub pad or gravel siphon goes into the tank it is likely to draw the interest of the animal and result in a game of tug-of-war! Such interactions, and many more that are thought of as mere routine acts of husbandry cannot be discounted when discussing the well-being of a captive octopus.

Figure 31. A photo series (left to right, top to bottom) showing a GPO solving a prey puzzle. Food is placed in the hamster ball that is offered, and manipulated until opened. Photos courtesy of J. Marvin

Current and future research: While the enrichment notebook was being developed for cephalopod enrichment, departments of several institutions documented first hand research of these enrichment interactions. Two aquariums have published papers on these enrichment applications, shedding light on a little known topic. Other facilities are also at work doing behavioral studies on invertebrates. These are in many ways establishing a data baseline for understanding GPO behavior in aquariums.

Despite the common practice of exhibiting cephalopods, there is surprisingly little published on "normal" behavior in aquariums. "Are stereotypic behavior traits being observed?" is a standard question asked of a bear or cat keeper. An octopus keeper would be hard pressed to answer this same basic question. Is a normally secretive octopus being too reclusive? Is this a stereotypic behavior or a natural relaxed state? We simply don't know, yet.

Enrichment activities coupled with behavior data are a vital part of good animal care and this field is expanding with continued research, innovation, and education. It is not just a "work in progress," progress is being made. Determining the desired level of enrichment, whether the goal of that enrichment is greater activity, psychological stimulation, simulating life in the wild, or for the comfort of viewing public, and discerning if an octopus that naturally spends most of its life in a den can be over-stimulated, are questions that still need further research. As enrichment is expanding with a generation of more ideas and research, these questions may soon be answered. Work is proceeding with the uniqueness of octopus in mind.

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. The degree of animal and staff contact is not agreed upon among AZA institutions. Many institutions use direct contact with the GPOs as a form of enrichment. Other institutions avoid direct contact because it seems to encourage GPOs to reach outside of the tank and interfere with tank maintenance activity. GPO may or may not bite if the octopus mouth is in contact with the arm. The decision to have intentional direct contact with the animal is for the institution to undertake and decide by weighing in the level of risk that staff and management are willing to accept.

Even if direct contact is avoided there will be contact made at some point due to the natural curious nature of the GPO and the necessity to maintaining the tank. Most tanks do not have the option of shifting nor is it considered necessary. Moving the animal to another tank regularly is not worthwhile given the stress of regularly moving the animal to a different environment or water. When contact is initially made by an arm touching a keeper's arm it can be pealed of by quickly grabbing it with the other hand and firmly pulling (not a jerking action) it away from you and releasing it into the tank. If three or more arms grab the keeper's arm, it generally becomes necessary to get help. As a result it is recommended that anytime the keeper is going to reach into the tank for a prolonged period of time or more involved level of work another keeper needs to be on duty and within hearing range to respond for a call for help.

GPOs are very curious and respond to handling. Octopus will quickly habituate to coming to the top of the tank and reaching out if keepers handle and particularly of hand feeding (rather than using feeding sticks) is practiced. The safety of the keeper and animal needs to be considered in the decision of how much contact to allow with the octopus. Bites or tearing of the octopus' skin can be avoided if the keeper's full attention is on the animal. Training GPOs to move into containers is a practice that has been tried, but needs more time and attention to see if it can become a truly trained behavior.

8.4 Staff Skills and Training

Giant Pacific octopus staff members should be trained in all areas of giant Pacific octopus 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.

In general, most staff who work at an aquarium will be required to have a SCUBA certification, and while such qualifications may be needed for exhibit maintenance it will probably not be required for the behavioral management of GPOs. Certifications in CPR, AED use, and Oxygen Administration are good things for anyone who works around water to have, especially where SCUBA or surface supplied diving is required.

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) If program animals are used, a written policy on the use of live animals in programs must be on file. An education, conservation, and welfare message must be an integral component of all programs. Animals in education programs must be maintained and cared for by trained staff, and housing conditions must meet standards required for the remainder of the animals in the institution. While outside their primary enclosure, although the conditions may be different, animal safety and welfare need to be ensured at all times.

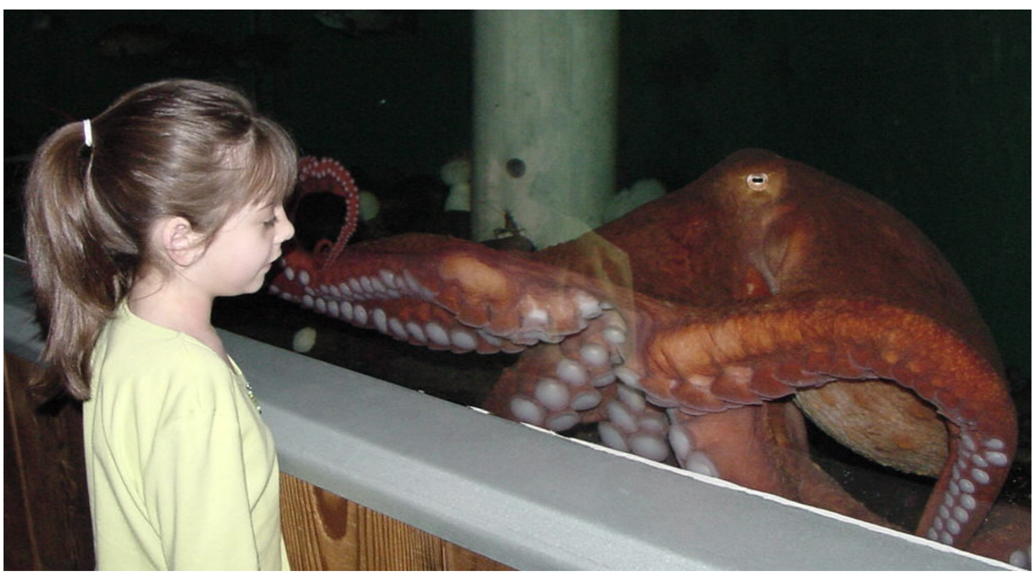

Figure 32. While of immense educational value, the complexities of life support and containment preclude the use of GPOs as program animals except in behind-the-scenes encounters. Photo R. Anderson

The complexity of maintaining closed or open LSS for a GPO makes use of this species as a program animal unlikely, though it is not necessarily precluded. In the case of an aquatic animal, a sufficient size tank and maintenance of water quality (as per the criteria in Chapters 1.1, 1.3, 2.1, and 2.2 of this manual) is needed for the animal's well-being. Small housing similar to shipping set ups are possible, but not recommended for regular programming. It is far more reasonable to utilize GPOs in a program or educational setting in the form of a behind the scenes tour or holding setting, rather than as a dedicated program animal.

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.

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.

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).

> **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.

At present the only known potential zoonotic threat is that of the bacterium *Mycobacterium marinum*. The potential zoonotic threat is discussed in greater detail in Section 6.5 of this manual.

As with any aquatic or marine system, tanks housing cephalopods may have the water fouled to some degree by the presence of lotions, creams, salves, or other cosmetic products on the skin. Furthermore, the demonstrated sensitivity of cephalopods to chemicals through their microvillus epidermis necessitates caution with any form or handling by the general public. Participants in any type of animal encounter involving cephalopods should thoroughly wash their hands beforehand to minimize such risks, and appropriate protocols should be followed afterwards to minimize any cross-contamination within the facility.

Any number of disinfecting solutions may be used for disinfecting tanks, nets, or other tools used with cephalopods. As with any chemical, extreme care should be taken to make sure tanks and other tools have been completely rinsed with fresh water and (preferably) dried before subsequent use with a live animal. Reviews of disinfectants common to aquaria and aquaculture can be found in Noga (2010) and in Wedemeyer (2002) and in Chapter 6.5. In general, common methods of surface disinfection in aquaria include sodium hypochlorite (household bleach) solutions of varying strength (usually at least 50–100 mg/l free chlorine or a 1:100 dilution), potassium monopersulfate (Virkon) solutions of 0.5–1.0% w/v), chlorhexidine (Nolvasan) solutions at 10% (v/v), or quaternary ammonium

> **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.

compounds at 1:200 dilutions (v/v). See Chapter 6.5 for further information.

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

(1.5.2) 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.

9.3 Program Evaluation

AZA-accredited institutions, which 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.

Chapter 10. Research

10.1 Known Methodologies

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.

AZA believes that contemporary GPO management, husbandry, veterinary care and conservation practices should be based in science, and that a commitment to scientific research, both basic and applied, is a trademark of the modern zoological park and aquarium. AZA-accredited institutions have the invaluable opportunity, and are expected, to conduct or facilitate research both in *in situ* and *ex situ* settings to advance scientific knowledge of the animals in our care and enhance the conservation of wild populations. This knowledge might be achieved by participating in AZA Taxon Advisory Group (TAG) or Species Survival Plan® (SSP) Program sponsored research, conducting original research projects, affiliating with local universities, and/or employing staff with scientific credentials (AZA Accreditation Standard 5.3).

There is, at present, no dire need for the conservation of *E. dofleini*, as the species is sustainably harvested, but further research into their biology, behavior, and ecology is no less valuable. GPOs often act as a key ambassador species for the unique ecosystem they represent, which is under threat as are all marine habitats. Octopods are also much loved by the public who visit aquaria and zoos, and research into their behavior allows educators to connect the live animals with the overall conservation message at our facilities. The AITAG can provide guidance in the construction and evaluations of research projects with cephalopods, or contact information for experts in the field upon request. A list of experts from the AITAG who are willing to discuss husbandry issues of invertebrates and direct interested parties to researchers is included in the AITAG Regional Collection Plan; the contact information for the authors can be found via the AZA Animal Program search page.

Research investigations, whether observational, behavioral, physiological, or genetically based, should have a clear scientific purpose with the reasonable expectation that they will increase our understanding of the species being investigated and may provide results which benefit the health or welfare of 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 investigations and these types of programs are strongly encouraged by the AZA.

Much behavioral research has been done with octopods in general, both in terms of learning abilities and more practical applications such as enrichment and conditioning. See the pertinent sections of this work for an overview of the work done to date. Physiological and neurological studies have long used cephalopods for their well-understood nervous system and ease of performing minor surgical procedures. Physiological studies which may warrant further study in an aquarium setting include development of detailed energy budgets and reproductive effort of all life stages of *E. dofleini* so that bioenergetics models may be constructed, and our knowledge of their metabolism expanded. Much work remains to be done in the reproductive biology and aquarium-based larval rearing methodology as well.

There are no AZA SSPs related to *E.dofleini* but it is considered a species of interest to the AITAG. Researchers who are actively working with E. dofleini tend to be concentrated in academia and the fisheries management authorities of the Pacific Northwest (primarily Washington, Canada, Alaska); searching an online database such as Google Scholar® can identify authors who have recently published on the species and may be resources to facilitate collaboration or help guide research programs involving *E. dofleini*. The list of authors affiliated with the AITAG above is also a good starting point when undertaking research, and the projects listed below in Section 10.2 identify research needs where individual staff or institutions may make an impact on the knowledge of the biology of this species.

Research investigations, whether observational, behavioral, physiological, or genetically based, should have a clear scientific purpose with the reasonable expectation that they will increase our understanding of the species being investigated and may provide results which benefit the health or welfare of 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 investigations and these types of programs are strongly encouraged by the AZA.

The AZA Biomaterials and Banking Scientific Advisory Group suggests opportunistic and systematic sampling for study at necropsy to include tissue from most internal organs. In addition, samples of feces frozen and stored at -80C, and recovery of any oocytes or sperm are potential material for study. Much more work will be required before considering regular live animal sampling of tissue, serum or whole blood are done in a public aquarium. The AZA Biomaterial and Banking SAG can be contacted by those interested in guidance and assistance in this kind of work.

At current most of the published research that has come out of public aquaria has been observational or behavioral in nature. Any collection of biometric, behavioral, or veterinary data has potential to be of great use in research programs, see Section 10.2 below for research needs. Tissue samples from deceased animals have been used in determining bioaccumulation (via prey) of pollutants inherent in the surrounding seawater (Anderson, 2003b). The collection and interpretation of other veterinary samples (hemolymph, fecals, et cetera) is completely unexplored for *E. dofleini*, a condition which will likely change soon at the rate invertebrate medicine is advancing. Many behavioral studies have been conducted with captive GPOs, see Chapter 8 for further detail on these studies and for ideas on how these could be expanded into a research training program. The coloration and display capacity of the species also needs investigation. What color patterns can *E. dofleini* produce? Is the species capable of making the passing cloud display known from other octopods? Are color patterns indicative of the “personality” of individual specimens or are there typical well defined patterns for the species as a whole? Can we infer some stereotypical behaviors from coloration? There is much to be learned about this species, and zoos and aquaria have a unique opportunity to answer some of these questions.

AZA-accredited institutions are required to have a clearly written research policy that identifies the types of research being conducted, methods used, staff involved, evaluations of the projects, the animals included, and guidelines for the reporting or publication of any findings (AZA Accreditation Standard 5.2). Institutions must designate a qualified individual to oversee and direct its research program (AZA Accreditation Standard 5.1). If institutions are not able to conduct in-house research investigations, they are strongly encouraged to provide financial, personnel, logistical, and other support for priority research and conservation initiatives identified by Taxon Advisory Groups (TAGs) or Species Survival Plans® (SSP) Programs.

AZA Accreditation Standard

(5.2) The institution must have a written policy that outlines the type of research that it conducts, methods, staff involvement, evaluations, animals to be involved, and guidelines for publication of findings.

AZA Accreditation Standard

(5.1) Research activities must be under the direction of a person qualified to make informed decisions regarding research.

At present, other than the lifelong body of work produced by R.C. Anderson (see literature cited section), there is not a large amount of to draw upon for examples of organized research programs involving *E. dofleini*. Research managers in zoos working with charismatic megafauna typically devote a percentage of their time to organized formal behavioral programs, but this has yet to be employed in many aquaria in any organized sense. Most of the research that has been produced from aquaria has been a result of projects originating from the passion of the animal caretakers themselves. There are currently no AITAG priority conservation initiatives for *E. dofleini*, either *in situ* or *ex situ*, though there are pressing social and wildlife management issues (e.g. the creation of octopus sanctuaries in the Pacific Northwest or reformation of fishing laws) for which an institution could advocate.

10.2 Future Research Needs

This Animal Care Manual is a dynamic document that will need to be updated as new information is acquired. Knowledge gained from areas will maximize AZA-accredited institutions’ capacity for excellence in animal care and welfare as well as enhance conservation initiatives for the species.

Table 22. Research needs for *Enteroctopus dofleini* and knowledge gaps for future investigations

Research Need	Area	Specifics
Metabolism	Nutrition	Establish metabolic rates for all sizes, ages, and life stages. Describe more detailed energy budgets for the species. Quantify amount of energy devoted to reproduction so that bioenergetics models may be constructed. Identify differences in senescent animals, if any.
Intelligence/problem solving	Behavior	Further investigate the problem solving and learning abilities of *E. dofleini*. Fully investigate sensory capabilities of the species.
Hemolymph Composition	Veterinary	Establish standards for hemolymph cell counts, electrolytes, and serum biochemistry. Identify differences in hemolymph in senescent individuals. Identify indicators of good health.

Endocrinology	Veterinary/ behavior	Continue work onto hormones and stress behavior. Identify standards for species and procedures for application in husbandry. Stress hormones in relation to water chemistry, light levels, and noise are undefined and could be useful in husbandry and management of the species in aquariums. This could lead to empirical evidence for stereotypic behaviors, which are difficult to define based on behavior alone.
Parasite ecology	Veterinary	Species and composition of Dicyemid parasites in wild populations of *E. dofleini*. Effects, if any on the host. Could lead to new species descriptions and treatment methods for prophylaxis.
Parasitology/virology histopathology	Veterinary Veterinary	Reporting of any new parasites or viral pathologies found in *E. dofleini*. Identify normal histopathological parameters for healthy animals and senescent animals to aid in necropsy.
Pharmacology	Veterinary	Report drugs used successfully in the species to broaden the knowledge of available chemotherapeutics that are safe for use on cephalopods.
Pre-copulatory behavior	Reproduction	Identify courtship behavior in the wild and in aquariums. Identify pheromones or other chemical cues used by the species.
Copulatory behavior	Reproduction	Complete further investigations of copulatory behavior in the wild and aquariums. These may include behavioral observations, interactions between males and subordinate males, average number of matings per female, etc.
Husbandry of paralarvae	Reproduction	Identify ideal tank design for rearing of paralarvae. Temperature needs, flow rates, water chemistry tolerances, and appropriate feed types and amounts need to be identified.
Growth rates	Nutrition	Collect growth data and feed amounts for aquarium-housed specimens to better describe growth rates in aquariums from a more robust dataset. Construction of growth models from wild populations.
Micronutrients enrichment foods	Nutrition Nutrition	Identify micronutrient requirements, if any, for octopus health and growth. Report novel foods used for enrichment of the species to compliment the standard diet fed to aquarium-housed animals.
Enrichment devices	Behavior	Report novel enrichment devices and prey puzzles to continue the GPO enrichment notebook.

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Appendix A: Accreditation Standards by Chapter

The following specific standards of care relevant to octopuses 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 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.

(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.

Chapter 6

(2.1.1) A full-time staff veterinarian is recommended. In cases where such is not practical, a consulting/part-time veterinarian must be under written contract to make at least twice monthly inspections of the animals and to respond as soon as possible to any emergencies.

(2.1.2) So that indications of disease, injury, or stress may be dealt with promptly, veterinary coverage must be available to the animal collection 24 hours a day, 7 days a week.

(2.2.1) Written, formal procedures must be available to the animal care staff for the use of animal drugs for veterinary purposes, and appropriate security of the drugs must be provided.

(1.4.6) A staff member must be designated as being responsible for the institution's animal record-keeping system. That person must be charged with establishing and maintaining the institution's animal records, as well as with keeping all animal care staff members apprised of relevant laws and regulations regarding the institution's animals.

(1.4.7) Animal records must be kept current, and data must be logged daily.
(1.4.5) At least one set of the institution's historical animal records must be stored and protected. Those records should include permits, titles, declaration forms, and other pertinent information.
(1.4.4) Animal records, whether in electronic or paper form, including health records, must be duplicated and stored in a separate location.
(1.4.3) Animals must be identifiable, whenever practical, and have corresponding ID numbers. For animals maintained in colonies/groups or other animals not considered readily identifiable, the institution must provide a statement explaining how record keeping is maintained.
(1.4.1) An animal inventory must be compiled at least once a year and include data regarding acquisitions and dispositions at the institution.
(1.4.2) All species owned by the institution must be listed on the inventory, including those animals on loan to and from the institution. In both cases, notations should be made on the inventory.
(2.7.1) The institution must have holding facilities or procedures for the quarantine of newly arrived animals and isolation facilities or procedures for the treatment of sick/injured animals.
(2.7.3) Quarantine, hospital, and isolation areas should be in compliance with standards/guidelines contained within the *Guidelines for Zoo and Aquarium Veterinary Medical Programs and Veterinary Hospitals* developed by the American Association of Zoo Veterinarians (AAZV), which can be obtained at: http://www.aazv.org/associations/6442/files/veterinary_standards_2009_final.docx.
(2.7.2) Written, formal procedures for quarantine must be available and familiar to all staff working with quarantined animals.
(11.1.2) Training and procedures must be in place regarding zoonotic diseases.
(11.1.3) A tuberculin (TB) testing/surveillance program must be established for appropriate staff in order to ensure the health of both the employees and the animals. Each institution must have an employee occupational health and safety program.
(2.5.1) Deceased animals should be necropsied to determine the cause of death. Cadavers must be stored in a dedicated storage area. Disposal after necropsy must be done in accordance with local/federal laws.
(2.4.1) The veterinary care program must emphasize disease prevention.
(1.5.5) For animals used in offsite programs and for educational purposes, the institution must have adequate protocols in place to protect the rest of the animals at the institution from exposure to infectious agents.
(2.3.1) Capture equipment must be in good working order and available to authorized, trained personnel at all times.
(2.4.2) Keepers should be trained to recognize abnormal behavior and clinical signs of illness and have knowledge of the diets, husbandry (including enrichment items and strategies), and restraint procedures required for the animals under their care. However, keepers should not diagnose illnesses nor prescribe treatment.
(2.3.2) Institution facilities should have radiographic equipment or have access to radiographic services.
(1.5.8) The institution must develop a clear process for identifying, communicating, and addressing animal welfare concerns within the institution in a timely manner, and without retribution.

Chapter 8

(1.6.1) The institution must have a formal written enrichment and training program that promotes species-appropriate behavioral opportunities.
(1.6.2) The institution must have specific staff member(s) or committee assigned for enrichment program oversight, implementation, training, and interdepartmental coordination of enrichment efforts.

Chapter 9

(1.5.4) A written policy on the use of live animals in programs must be on file. Animals in education programs must be maintained and cared for by trained staff, and housing conditions must meet standards set for the remainder of the animals in the institution, including species-appropriate shelter, exercise, social and environmental enrichment, access to veterinary care, nutrition, etc. Since some of these requirements can be met outside of the primary enclosure, for example, enclosures may be reduced in size provided that the animal's physical and psychological needs are being met.
(1.5.3) If animal demonstrations are part of the institution's programs, an educational/conservation message must be an integral component.

(1.5.5) For animals used in offsite programs and for educational purposes, the institution must have adequate protocols in place to protect the rest of the animals at the institution from exposure to infectious agents.

(10.3.3) All animal enclosures (exhibits, holding areas, hospital, and quarantine/isolation) must be of a size and complexity sufficient to provide for the animal's physical, social, and psychological well-being; and exhibit enclosures must include provisions for the behavioral enrichment of the animals. AZA housing guidelines outlined in the Animal Care Manuals should b e followed.

(1.5.2) All animals must be housed in enclosures and in appropriate groupings which meet their physical, psychological, and social needs. Wherever possible and appropriate, animals should be provided the opportunity to choose among a variety of conditions within their environment. Display of single animals should be avoided unless biologically correct for the species.

(1.5.11) Animal transportation must be conducted in a manner that is safe, well-planned and coordinated, and minimizes risk to the animal(s), employees, and general public. All applicable laws and/or regulations must be adhered to. Planning and coordination for animal transport requires good communication among all involved parties, plans for a variety of emergencies and contingencies that may arise, and timely execution of the transport. At no time should the animal(s) or people be subjected to unnecessary risk or danger.

Chapter 10

(5.3) The institution should maximize the generation of scientific knowledge gained from the animals. This might be achieved by participating in AZA TAG/SSP sponsored research when applicable, conducting original research projects, affiliating with local universities, and/or employing staff with scientific credentials.

(5.2) Institutions must have a written policy that outlines the type of research that it conducts, methods, staff involvement, evaluations, animals to be involved, and guidelines for publication of findings.

(5.1) Research activities must be under the direction of a person qualified to make informed decisions regarding research.

Appendix B: AZA Policy on Responsible Population Management: Acquisitions, Transfers and Transitions by Zoos & Aquariums

PREAMBLE

The Association of Zoos & Aquariums (AZA) was established, among other reasons, "…to foster continued improvement of the zoological park and aquarium profession through the development and regulation of high standards of ethics, conduct, education and scholarly attainments." The stringent requirements for AZA accreditation and high standards of professional conduct are unmatched by similar organizations and also far surpass the United States Department of Agriculture's Animal and Plant Health Inspection Service's requirements for licensed animal exhibitors. Every AZA member must abide by a Code of Professional Ethics (https://www.aza.org/Ethics/). In order to continue these high standards, AZA-accredited institutions and certified related facilities should make it a priority, when possible, to acquire animals from and transfer them to other AZA member institutions or other regional zoo associations and their members.

AZA-accredited institutions and certified related facilities cannot fulfill their important missions of conservation, education, and science without living animals. Responsible management and the long-term sustainability of living animal populations necessitates that some individuals be acquired and that others be transferred or transitioned at certain times. Furthermore, priority for acquisition and transfer activities should be the long-term sustainability of living animal populations among AZA-accredited and certified related facilities, and between AZA member institutions and non-AZA entities with animal care and welfare standards aligned with AZA. AZA member institutions that acquire animals from the wild, directly or through commercial vendors, should perform due diligence to ensure that zoos/aquariums are not creating a commercial market that promotes the taking of those animals from nature and/or that is detrimental to the survival of species in the wild. Animals should only be solicited and acquired from non-AZA entities that are known to operate legally and conduct their business in a manner that reflects and/or supports the spirit and intent of the AZA Code of Professional Ethics as well as this Policy.

In this Policy "AZA member institutions" refers to AZA-accredited institutions and certified related facilities (zoological parks and aquariums). "AZA members" may refer to either institutions or individuals.

Non – AZA entities includes facilities not accredited or certified by the AZA, facilities in other zoological regions, academic institutions, museums, research facilities, private individuals, etc.

I. INTRODUCTION

The AZA Acquisition, Transfer and Transition Policy was created to help (1) guide and support AZA-accredited and certified related facilities in their animal acquisition and transfer/transition decisions, and (2) make certain that all acquisitions and transfers/transitions are compatible with the Association's stated commitment to save and protect the wonders of the living natural world. This AZA Acquisition, Transfer and Transition Policy applies to individual animals, groups/colonies, and specimens (animal parts, materials, and products). More specifically, the AZA Acquisition, Transfer and Transition Policy provides guidance to AZA members to:

1. assure that the health and welfare of individual animals is considered during acquisition and transfer/transition activities,

Acquisition of animals can occur through breeding (births, hatchings, cloning, and division of marine invertebrates = "fragging"), trade, donation, lease, loan, transfer (inter- and intra-institution), purchase, collection, confiscation, appearing on zoo property, or rescue and/or rehabilitation for release.

Transfer/transition occurs when an animal leaves the institution for any reason. Reasons for transfer or transition may include cooperative population management (genetic, demographic or behavioral management), animal welfare or behavior management reasons (including sexual maturation and individual management needs). Types of transfer include withdrawal through donation, trade, lease, loan, inter- and intra-institution transfers, sale, escape, theft. Types of transition include reintroduction to the wild, humane euthanasia or natural death.

"Dispose/Disposing of" in this document is limited to complete and permanent removal of an individual via incineration, burying or other means of permanent destruction.

2. assure that the health and conservation of populations, species, and ecosystems are carefully considered during acquisition and transfer/transition activities,

3. maintain a proper standard of conduct for AZA members during acquisition and transfer/transition activities, including adherence to all applicable laws and regulations,

4. assure that animals from AZA member institutions and certified related facilities are not transferred to individuals or organizations that lack the appropriate expertise or facilities to care for them [*see taxa specific appendices (in development)*], and

5. support the goals of AZA's cooperatively managed populations and associated Animal Programs [Species Survival Plans® (SSPs), Studbooks, and Taxon Advisory Groups (TAGs)].

Attempts by members to circumvent AZA Animal Programs in the acquisition of animals can be detrimental to the Association and its Animal Programs. Such action may also be detrimental to the species involved and may be a violation of the Association's Code of Professional Ethics.

AZA's scientifically-managed Animal Programs, including SSPs, have successfully bred and reintroduced critically endangered species for the benefit of humankind. To accomplish these critical conservation goals, populations must be managed within "carrying capacity" limits. At times, the number of individual animals in a population exceeds carrying capacity, and while meaning no disrespect for these individual animals, we refer to these individual animals as "extra" within the managed population.

This AZA Acquisition, Transfer and Transition Policy will serve as the default policy for AZA member institutions. Institutions may develop their own Acquisition, Transfer and Transition Policy in order to address specific local concerns. Any institutional policy must incorporate and not conflict with the AZA acquisition and transfer/transition standards.

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

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.

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.

> Feral animals are animals that have escaped from domestication or have been abandoned to the wild and have become wild, and the offspring of such animals. Feral animals may be acquired for temporary or permanent reasons.

3. Any receiving institution must have the necessary expertise and resources to support and provide for the professional care and management of the species, so that the physical, psychological, and social needs of individual animals and species are met.

4. If the acquisition involves a species managed by an AZA Animal Program, the institution should communicate with the Animal Program Leader and, in the case of Green SSP Programs, must adhere to the AZA Full Participation Policy (http://www.aza.org/full-participation-in-ssp-program-policy/).

5. AZA member institutions should consult AZA Wildlife Conservation and Management Committee (WCMC)-approved TAG Regional Collection Plans (RCPs), Animal Program Leaders, and AZA Animal Care Manuals (ACMs) when making acquisition decisions.

6. AZA member institutions that work with commercial vendors that acquire animals from the wild, must perform due diligence to assure the vendors' collection of animals is legal. Commercial vendors should have conservation and animal welfare goals similar to those of AZA institutions.

7. AZA member institutions may acquire animals through public donations and other non-AZA entities when it is in the best interest of the animal and/or species.

B. Acquisitions from the Wild

Saving species and wild animal populations for education and wildlife conservation purposes is a unique responsibility of AZA member zoos and aquariums. The AZA recognizes that there are circumstances where acquisitions from the wild are needed in order to maintain healthy, diverse animal populations and to support the objectives of managed species programs, in which case acquisitions from the wild may be a preferable choice to breeding in human care.

Acquiring animals from the wild can result in socioeconomic benefit and environmental protection and therefore the AZA encourages environmentally sustainable/beneficial acquisition from the wild when conservation is a positive outcome.

1. Before acquiring animals from the wild, institutions are encouraged to examine alternative sources including other AZA institutions and other regional zoological associations or other non-AZA entities.

2. When acquiring animals from the wild, both the long-term health and welfare impacts on the wild population as well as on individual animals must be considered. In crisis situations, when the survival of a population is at risk, rescue decisions will be made on a case-by-case basis by the appropriate agency and institution.

3. Institutions should only accept animals from the wild after a risk assessment determines the zoo/aquarium can mitigate any potential adverse impacts on the health, care and maintenance of the permanently housed animals, and the animals being acquired.

> The Lacey Act prohibits the importation, exportation, transportation, sale, receipt, acquisition or purchase of wildlife taken or possessed in violation of any law, treaty or regulation of the United States or any Indian tribal law of wildlife law.
>
> In cases when there is no documentation accompanying an acquisition, the animal(s) may not be transferred across state lines. If the animal was illegally acquired at any time then any movement across state or international borders would be a violation of the Lacey Act.

IV. TRANSFER AND TRANSITION REQUIREMENTS

A. Living Animals

Successful conservation and animal management relies on the cooperation of many entities, both AZA and non-AZA. While preference is given to placing animals with AZA-accredited institutions or certified related facilities, it is important to foster a cooperative culture among those who share AZA's mission of saving species.

> Attempts by members to circumvent AZA Animal Programs in the transfer or transition of animals may be detrimental to the Association and its Animal Programs (unless the animal or animals are deemed extra in the Animal Program population by the Animal Program Coordinator). Such action may be detrimental to the species involved and may be a violation of the Association's Code of Professional Ethics.

1. Any transfer must abide by the Mandatory Standards and General Advisories of the AZA Code of Professional Ethics which indicates that AZA members should assure that all animals in their care are transferred and transitioned in a manner that meets the standards of the Association, and that animals are not transferred or transitioned to those not qualified to care for them properly.

2. If the transfer of animals or their specimens (parts, materials, and products) involves a species managed by an AZA Animal Program, the institution should communicate with that Animal Program Leader and, in the case of Green SSP Programs must adhere to the AZA Full Participation Policy (http://www.aza.org/full-participation-in-ssp-program-policy/).

3. AZA member institutions should consult WCMC-approved TAG Regional Collection Plans, Animal Program Leaders, and Animal Care Manuals when making transfer decisions.

4. Animals acquired as animal feed are not typically accessioned into the collection. There may be occasions, however, when it is appropriate to use accessioned animals that exceed population carrying capacity as feeder animals to support other animals. In some cases, accessioned animals may be transitioned to “feeder animal” status by the local institution as part of their program for long-term sustained population management of the species.

5. In transfers to non-AZA entities, AZA members must perform due diligence and should have documented validation, such as a letter of reference, that the recipient has the expertise and resources required to properly care for and maintain the animals. Supporting documentation must be kept at the AZA member institution.

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.iucnsscrsg.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: The following are recommendations and suggestions for appropriate quarantine procedures for giant Pacific octopuses:

Giant pacific octopuses:

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:
    - i. Public access outside the exhibit/enclosure. Public may interact with animals from outside the exhibit/enclosure (e.g., giraffe feeding, touch tanks).
    - ii. 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:
    - i. 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").
    - ii. 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).
    - iii. 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:
    - i. 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 and 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 and Benefield, 1986, 1988; Wolf and 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 and Rios, 2001), visitors viewed animals three and four times longer while they were being presented in demonstrations outside of their enclosure with an educator than while they were on exhibit. Clearly, the use of program animals in shows or informal presentations can be effective in lengthening the potential time period for learning and overall impact.

Program animals also provide the opportunity to personalize the learning experience, tailoring the teaching session to what interests the visitors. Traditional graphics offer little opportunity for this level of personalization of information delivery and are frequently not read by visitors (Churchman, 1985; Johnston, 1998). For example, Povey (2001) found that only 25% of visitors to an animal exhibit read the accompanying graphic; whereas, 45% of visitors watching the same animal handled in an educational presentation asked at least one question and some asked as many as seven questions. Having an animal accompany the educator allowed the visitors to make specific inquiries about topics in which they were interested.

Knowledge acquisition

Improving our visitors' knowledge and understanding regarding wildlife and wildlife conservation is a fundamental goal for many zoo educators using program animals. A growing body of evidence supports the validity of using program animals to enhance delivery of these cognitive messages as well.

- MacMillen (1994) found that the use of live animals in a zoomobile outreach program significantly enhanced cognitive learning in a vertebrate classification unit for sixth grade students.
- Sherwood and his colleagues (1989) compared the use of live horseshoe crabs and sea stars to the use of dried specimens in an aquarium education program and demonstrated that students made the greatest cognitive gains when exposed to programs utilizing the live animals.
- Povey and Rios (2002) noted that in response to an open-ended survey question ("Before I saw this animal, I never realized that . . . "), visitors watching a presentation utilizing a program animal provided 69% cognitive responses (i.e., something they learned) versus 9% made by visitors viewing the same animal in its exhibit (who primarily responded with observations).
- Povey (2002) recorded a marked difference in learning between visitors observing animals on exhibit versus being handled during informal presentations. Visitors to demonstrations utilizing a raven and radiated tortoises were able to answer questions correctly at a rate as much as eleven times higher than visitors to the exhibits.

Enhanced environmental attitudes

Program animals have been clearly demonstrated to increase affective learning and attitudinal change.

- Studies by Yerke and Burns (1991) and Davison and her colleagues (1993) evaluated the effect live animal shows had on visitor attitudes. Both found their shows successfully influenced attitudes about conservation and stewardship.
- Yerke and Burns (1993) also evaluated a live bird outreach program presented to Oregon fifth-graders and recorded a significant increase in students' environmental attitudes after the presentations.
- Sherwood and his colleagues (1989) found that students who handled live invertebrates in an education program demonstrated both short and long-term attitudinal changes as compared to those who only had exposure to dried specimens.
- Povey and Rios (2002) examined the role program animals play in helping visitors develop positive feelings about the care and well-being of zoo animals.
- As observed by Wolf and Tymitz (1981), zoo visitors are deeply concerned with the welfare of zoo animals and desire evidence that they receive personalized care.

Conclusion

Creating positive impressions of aquarium and zoo animals, and wildlife in general, is crucial to the fundamental mission of zoological institutions. Although additional research will help us delve further into this area, the existing research supports the conclusion that program animals are an important tool for conveying both cognitive and affective messages regarding animals and the need to conserve wildlife and wild places.

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Appendix E: Developing an Institutional Program Animal Policy

Last revision 2003
Re-authorized by the Board June 2011

Rationale

Membership in AZA requires that an institution meet the AZA Accreditation Standards collectively developed by our professional colleagues. Standards guide all aspects of an institution's operations; however, the accreditation commission has asserted that ensuring that member institutions demonstrate the highest standards of animal care is a top priority. Another fundamental AZA criterion for membership is that education be affirmed as core to an institution's mission. All accredited public institutions are expected to develop a written education plan and to regularly evaluate program effectiveness.

The inclusion of animals (native, exotic and domestic) in educational presentations, when done correctly, is a powerful tool. CEC's **Program Animal Position Statement** describes the research underpinning the appropriate use of program animals as an important and powerful educational tool that provides a variety of benefits to zoo and aquarium educators seeking to convey cognitive and affective messages about conservation and wildlife.

Ongoing research, such as AZA's Multi-Institutional Research Project (MIRP) and research conducted by individual AZA institutions will help zoo educators to determine whether the use of program animals conveys intended and/or conflicting messages and to modify and improve programs accordingly and to ensure that all program animals have the best possible welfare.

When utilizing program animals our responsibility is to meet both our high standards of animal care and our educational goals. Additionally, as animal management professionals, we must critically address both the species' conservation needs and the welfare of the individual animal. Because "wild creatures differ endlessly," in their forms, needs, behavior, limitations and abilities (Conway, 1995), AZA, through its Animal Welfare Committee, has recently given the responsibility to develop taxon- and species-specific animal welfare standards and guidelines to the Taxon Advisory Groups (TAG) and Species Survival Plan® Program (SSP). Experts within each TAG or SSP, along with their education advisors, are charged with assessing all aspects of the taxons' and/or species' biological and social needs and developing Animal Care Manuals (ACMs) that include specifications concerning their use as program animals.
However, even the most exacting standards cannot address the individual choices faced by each AZA institution. Therefore, each institution is required to develop a program animal policy that articulates and evaluates program benefits. The following recommendations are offered to assist each institution in formulating its own Institutional Program Animal Policy, which incorporates the AZA Program Animal Policy and addresses the following matters.

The Policy Development Process

Within each institution, key stakeholders should be included in the development of that institution's policy, including, but not limited to representatives from:

- the Education Department
- the Animal Husbandry Department
- the Veterinary and Animal Health Department
- the Conservation & Science Department
- the Behavioral Husbandry Department
- any animal show staff (if in a separate department)
- departments that frequently request special program animal situations (e.g., special events, development, marketing, zoo or aquarium society, administration)

Additionally, staff from all levels of the organization should be involved in this development (e.g., curators, keepers, education managers, interpreters, volunteer coordinators).

To develop a comprehensive Program Animal Policy, we recommend that the following components be included:

I. Philosophy

In general, the position of the AZA is that the use of animals in up close and personal settings, including animal contact, can be extremely positive and powerful, as long as:

1. The use and setting is appropriate.
2. Animal and human welfare is considered at all times.
3. The animal is used in a respectful, safe manner and in a manner that does not misrepresent or degrade the animal.
4. A meaningful conservation message is an integral component. Read the AZA Board-approved Conservation Messages.
5. Suitable species and individual specimens are used.

Institutional program animal policies should include a philosophical statement addressing the above, and should relate the use of program animals to the institution's overall mission statement.

II. Appropriate Settings

The Program Animal Policy should include a listing of all settings both on and off site, where program animal use is permitted. This will clearly vary among institutions. Each institution's policy should include a comprehensive list of settings specific to that institution. Some institutions may have separate policies for each setting; others may address the various settings within the same policy. Examples of settings include:

I. On-site programming
    A. Informal and non-registrants:
        1. On-grounds programming with animals being brought out (demonstrations, lectures, parties, special events, and media)
        2. Children's zoos and contact yards
        3. Behind-the-scenes open houses
        4. Shows
        5. Touch pools
    B. Formal (registration involved) and controlled settings
        1. School group programs
        2. Summer Camps
        3. Overnights
        4. Birthday Parties
        5. Animal rides
        6. Public animal feeding programs

II. Offsite and Outreach
    1. PR events (TV, radio)
    2. Fundraising events
    3. Field programs involving the public
    4. School visits
    5. Library visits
    6. Nursing Home visits (therapy)
    7. Hospital visits
    8. Senior Centers
    9. 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

All 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:
   - Temperament and suitability for program use
   - Husbandry requirements
   - Husbandry expertise
   - Veterinary issues and concerns
   - Ease and means of acquisition / disposition according to the AZA code of ethics
   - Educational value and intended conservation message
   - Conservation Status
   - 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., handwashing 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 numbers 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: Bibliography of Cephalopod Husbandry Articles from Drum & Croaker 1958–2013 & Presentations given at the Regional Aquatics Workshop 2004–2009

The following is a bibliography of cephalopod husbandry and related articles from the aquarium industry publication Drum and Croaker (D&C) within 1958–2013, and presentations given at the Regional Aquatics Workshop (RAW) within 2004–2009 pertaining to similar topics. The articles listed serve to chronicle the history of cephalopod keeping in modern aquaria, and show the advances made in recent decades. These are listed for reader edification only, given the age of some of these articles there may be dated information or taxonomic incongruities and the reader is advised to cross reference information with current literature, authorities on the subject, or the original authors whenever possible. This is especially true where medical advice is discussed: treatments and techniques should be discussed with a veterinarian knowledgeable in aquatic medicine prior to application in a husbandry program. That being stated, however, this is an important portal to the literature for the public aquarist as D&C is freely available where much of the primary literature may not be at the disposal of many facilities.

Bibliography of Cephalopod Related Articles from *Drum and Croaker* 1958–2013:

Anderson, R. C., & Wood, J. B. (2012). Raising Baby Octopuses. *Drum and Croaker*, *43*, 34–40.

Anderson, R. C., & Wood, J. B. (2009). Feeding Octopuses Live Crabs is Good Enrichment. *Drum and Croaker*, *40*, 9–11.

Anderson, R. C. (2008). Novel Foods as Enrichment for Giant Pacific Octopuses (GPOs) *Drum and Croaker*, *39*, 62–65.

Anderson, R. C. (2003). Salmon Return Provides Food for Aquarium Animals. *Drum and Croaker*, *34*, 9–10.

Anderson, R. C. (2001). Name Change of Giant Pacific Octopus. *Drum and Croaker*, *32*, 46.

Anderson, R. C. (2000). The Release of a Captive-Reared Giant Pacific Octopus. *Drum and Croaker*, *31*, 7–10.

Anderson, R. C. (1998). Transferring Giant Octopuses. *Drum and Croaker*, *29*, 39.

Anderson, R. C. (1996). Sedating & Euthanizing Octopuses. *Drum and* Croaker, *27*, 7–8.

Anderson, R. C. (1995). Aquarium Husbandry of the Giant Pacific Octopus. *Drum and Croaker*, *26*, 14–23.

Barord, G. J. (2011). Post-Mortem Analysis of Cuttlebones Provides Critical Insight into the Natural History of Captive Cuttlefish, *Sepia. Drum and Croaker*, *42*, 20–29.

Barord, G. J., & Henderson, R. (2008). Treatment of Negative Buoyancy in a Captive Chambered Nautilus, *Nautilus pompilius*. *Drum and Croaker*, *39*, 68–81.

Barord G. J., & Christie, B. L. (2007). Benzocaine in the Euthanasia of the Giant Pacific Ocopus, *Enteroctopus dofleini* (Wulker, 1910). *Drum and Croaker*, *38*, 8–12.

Burke, J. (2004). Occurrence of the Jumbo Squid*, Dosidicus gigas*, off the Oregon Coast: Its Capture and Life in Captivity. *Drum and Croaker*, *35*, 3–7.

Delbeek, J. C. (2002). Husbandry of the Big-Fin Reef Squid (*Sepiateuthis lessoniana*) at the Waikiki Aquarium. *Drum and Croaker*, *33*, 35–37.

Dempster, R. P. (1958). Shipping Test of the Giant Pacific Octopus *Apollyon* within a Sealed Container. *Drum and Croaker*, *1*, 11–12.

Fields, R. I. (2006). Hatching of *Natutilus pompilius*, in a Closed System Using Artificial Seawater. *Drum and Croaker*, *37*, 42–47.

Moffatt, J. (2006). The Training of a Male Giant Pacific Octopus, *Enteroctopus dofleini*, to Assess Haelth and Weight. *Drum and Croaker*, *38*, 43.

Newberg, C. (2004). Occurrence of the Jumbo Squid*, Dosidicus gigas*, off Oregon Coast: It's Capture and Life in Captivity. *Drum and Croaker*, *35*, 3–7.

Peters, A. (2005). Preliminary Results of Giant Pacific Octopus Behavior Study on Enrichment. *Drum and Croaker*, *36*, 4–8.

Ross, R. (2010). Display, Husbandry and Breeding of Dwarf Cuttle, *Sepia bandensis,* at the California Academy of Sciences *Drum and Croaker*, *41*, 8–16.

Bibliography of Cephalopod Presentations from Regional Aquatics Workshops 2004–2013. (Abstracts of these presentations are published in D&C the year following the meeting.)

Anderson, R. C. (2006). How to Keep Your GPO Happy. Presented at Regional Aquatics Workshop XX. Galveston, TX: Moody Gardens Aquarium.

Anderson, R. C. (2006). Enrichment for Giant Pacific Octopuses. Presented at Regional Aquatics Workshop XX. Galveston, TX: Moody Gardens Aquarium.

Barord, G. J. (2011). Successful Treatment of Infection in *Nautilus pompilius*. Presented at Regional

Aquatics Workshop XXV. Virginia Beach, VA: Virginia Aquarium and Marine Science Center.

Barord, G. J. (2010). Nautilus Husbandry in the 21st Century. Presented at Regional Aquatics Workshop

XXIV. Omaha, NE: Omaha's Henry Doorly Zoo.

Barord, G. J. (2007). The Analysis of Growth Lamellae as They Relate to Age Determination and Stress

in *Sepia pharonis* and *Sepia officinalis*. Presented at Regional Aquatics Workshop XXI. Pittsburgh, PA: Pittsburgh Zoo and PPG Aquarium.

Barord, G. J. (2007). Treatment of Shell Deterioration in Captive *Nautilus Pompilius*. Presented at Regional Aquatics Workshop XXI. Pittsburgh, PA: Pittsburgh Zoo and PPG Aquarium.

Bekiares, N. (2010). *Euprymna scolopes* Husbandry at the University of Wisconsin. Presented at

Regional Aquatics Workshop XXIV. Omaha, NE: Omaha's Henry Doorly Zoo.

Blair, S. (2007). Preliminary Attempts on the Capture, Transport and Husbandry of the Humboldt Squid,

Dosidicus gigas. Presented at Regional Aquatics Workshop XXI. Pittsburgh, PA: Pittsburgh Zoo and PPG Aquarium.

Carlson, B. (2006). Cephalopod Work at the Waikikki Aquarium and Georgia Aquarium. Presented at Regional Aquatics Workshop XX. Galveston, TX: Moody Gardens Aquarium.

Cicitello, E. (2011). Implementing a Husbandry Training Program for the Giant Pacific Octopus *(Enteroctopus dofleini)* at the John G. Shedd Aquarium Presented at Regional Aquatics Workshop XXV. Virginia Beach, VA: Virginia Aquarium and Marine Science Center.

Daw, A. (2006). *Sepia Pharonis* Husbandry Advances. Presented at Regional Aquatics Workshop XX. Galveston, TX: Moody Gardens Aquarium.

DeCastro, E., Trobaugh, D., & Hocking, R. (2006). Reproduction in the Giant Pacific Octopus, *Enteroctopus dofleini*: An Elusive Circle Closing? Presented at Regional Aquatics Workshop XX. Galveston, TX: Moody Gardens Aquarium.

DeWitt, T. G., & Peters, A. (2005). A Real Hole in the Wall: A case Study of *Nautilus pompilius*. Presented at Regional Aquatics Workshop XIX. Long Beach, CA: Aquarium of the Pacific and Cabrillo Marine Aquarium.

Fields, R. (2006). Propagating Nautilus in Artificial Seawater. Presented at Regional Aquatics Workshop XX. Galveston, TX: Moody Gardens Aquarium.

Gibson, J. (2010). Apparent Mimicry Behavior Displayed by the Pharaoh's Cuttlefish, *Sepia pharaonis.* Presented at Regional Aquatics Workshop XXIV. Omaha, NE: Omaha's Henry Doorly Zoo.

Hemdal, J. (2008). The Husbandry of the Striped Octopuses: *Thaumoctopus mimicus* and *Wunderpus photogenicus*. Presented at Regional Aquatics Workshop XXII. Riverhead, NY: Atlantis Marine World.

Kegal, K. (2011). Seattle Aquarium Giant Pacific Octopus Census. Presented at Regional Aquatics Workshop XXV. Virginia Beach, VA: Virginia Aquarium and Marine Science Center.

Lobo, V., Carlos I., E. D. (2006). Oval Squid (Sepioteuthis lessoniana) Husbandry at Oceanario deLisboa. Presented at Regional Aquatics Workshop XX. Galveston, TX: Moody Gardens Aquarium.

Peters, A., & Boedeker, N. C. (2008). In My Eye: Common Cuttlefish, *Sepia officinalis*. Presented at Regional Aquatics Workshop XXII. Riverhead, NY: Atlantis Marine World.

Peters, A., & Powell, D. (2004). Preliminary Results in Giant Pacific Octopus Behavior Studies on Enrichment. Presented at Regional Aquatics Workshop XVIII. Sarasota, FL: Mote Marine Laboratory.

Ramsay, J., & Hellmuth, H. (2007). The Mind of a Mollusk: Behavioral Management of Giant Pacific Octopus (*Enteroctopus dofleini*) at the National Aquarium in Baltimore. Presented at Regional Aquatics Workshop XXI. Pittsburgh, PA: Pittsburgh Zoo and PPG Aquarium.

Rollinson, A., Hall, K., & Coco, C. (2009). Environmental Enrichment and Behavioral Desensitization of the Common Cuttlefish (Sepia officinalis) at the Georgia Aquarium. Presented at Regional Aquatics Workshop XXIII. Newport, KY: Newport Aquarium.

Ross, R. (2010). Displaying and Breeding Dwarf Cuttles. Presented at Regional Aquatics Workshop XXIV. Omaha, NE: Omaha's Henry Doorly Zoo.

Snider, S. (2007). Some Success in Raising Octopus rubescens at Monterey Bay Aquarium. Presented at Regional Aquatics Workshop XXI. Pittsburgh, PA: Pittsburgh Zoo and PPG Aquarium.

Widmer, C. (2009). Is it Possible or Feasable to Display the Giant Humboldt Squid, Dosidicus gigas? Presented at Regional Aquatics Workshop XXIII. Newport, KY: Newport Aquarium.

Appendix G: Procedural Guideline to the Importation of Non-Protected Wildlife Specimens into the United States.

As almost all of the GPOs in aquarium settings today (save for those at a few west-coast institutions) are collected in Canada the issue of international shipping is an inherent logistical task that needs to be undertaken with each new acquisition. As all aquarists know all too well errors in shipping easily prove fatal with marine wildlife, despite the best packing and planning. GPOs, being large animals with high metabolisms, strict temperature requirements, and susceptibility to shipping stress are at even greater risk to shipping delays. Thus it is in the best interest of the animals for all aquarium professionals transporting large octopods to be intimately familiar with the red tape involved, that such delays may be avoided.

One common question that frequently occurs with animal professionals attempting to bring animals into the U.S. is exactly how the process works. The following is a guide for importation of wildlife that do not have special status (i.e., non-CITES animals that are not on the U. S. Endangered Species List or otherwise regulated). This is only meant as a guide, and the authors assume no responsibility for subsequent use in transporting wildlife across national borders.

Importers should always coordinate with state wildlife authorities and the U. S. Fish and Wildlife Service prior to importation of any living specimens. This is provided to simplify the complex and confusing process of import, which many aquarists and curators may struggle with. These procedures are of course subject to change, but at the time of writing these are the basic steps:

1. **Arrange carriage**: The animal shipment is booked as a shipment of live fish with the airline. The shipment must be received in the United States at one of 18 designated ports with USFWS inspectors (see below). An airwaybill number is provided from the carrier at this time. Take care to note this number and the flight information (flight number, times) as it will be needed for the USFWS paperwork.
2. **Obtain a health certificate signed by a licensed veterinarian**: If one is necessary, inquire with USFWS officials as to whether this paperwork is required in your situation.
3. **Obtain an import permit**: If one is necessary, inquire with USFWS officials as to whether this permit is required in your situation (for many public aquaria it may not be).
4. **Complete any state or local import paperwork or permitting/reporting processes**.
5. **Complete USFWS form 3-177:** Once carriage has been booked, complete a Declaration of Importation of Exportation for Fish or Wildlife, USFWS form 3-177. This can be done electronically at the following web address: https://edecs.fws.gov/eDecsHome.cfm. These forms must be filled out by both the importer (consignee) AND the exporter (shipper). Instructions for completing the form are listed at this web address: http://www.fws.gov/le/pdf/3177instructions022011.pdf USFWS will alert you when the declaration has been accepted or rejected.
6. **Pay applicable fees**: Once the declaration of import and export have been completed, fees may be paid electronically at the website listed above. Inquire with local USFWS offices if your institution is unable to pay import fees online with a credit card for other payment options.
7. **Generate commercial invoice:** The exporter (shipper) must generate a commercial invoice for the shipment. This will accompany the shipment but will also be needed on the receiving end for customs purposes.
8. **Notify USFWS inspectors**: No less than forty-eight (48) hours prior to the shipment you should contact the USFWS office at the port of arrival and inform them of your shipment, and answer any questions they may have so they may schedule an inspection. Be ready to provide airwaybill and flight information, location of air cargo facilities, time of arrival, types and number of containers, etc.
9. **Obtain customs clearance**: After USFWS officials have inspected or cleared your shipment you will need to take a copy of form 3-177 stamped as cleared, commercial invoice, airwaybill, and other required permits to the nearest US Customs Bureau (present at most international airports) for final clearance and release of the shipment.
10. **Claim shipment**: With all of this paperwork in hand, you should be able to claim your shipment from the air cargo facility and take the animal back to the aquarium for acclimation.

This process is very complex, and as stated above this is presented simply as a guide to help in planning, and should not replace coordination with state and federal wildlife authorities. Currently the prevailing suppliers of GPOs to public aquaria in Canada are willing to drive the specimens across the border and ship them from within the U.S. for a nominal fee. This greatly streamlines the process from the receiving end; the authors highly suggest considering such a service if octopus specimens cannot be collected locally for your facility.

USFWS Designated Ports of Importation as of 2012

Anchorage, AK
Mail: P.O. Box 190045
Anchorage, Alaska 99519
Physical: 4600 Postmark Drive, Suite NB207
Anchorage, Alaska 99502
Phone: (907) 271-6198 / Fax: (907) 271-6199

Atlanta, GA
4341 International Parkway, Suite #104
Atlanta, Georgia 30354
Phone: (404) 763-7959 / Fax: (404) 366-7031

Baltimore, MD
Mail: P.O. Box 778
Hanover, Maryland 21076
Physical: BWI Air Cargo Complex
Building F, Suite 1500
Baltimore, Maryland 21240
Phone: (410) 694-9590 / Fax: (410) 694-9594

Boston, MA
70 Everett Avenue, Suite 315
Chelsea, Massachusetts 02150
Phone: (617) 889-6616 / Fax: (617) 889-1980

Chicago, IL
10600 Higgins Road, Suite 200
Rosemont, Illinois 60018
Phone: (847) 298-3250 Ext: 110 / Fax: (847) 298-7669

Dallas/Ft. Worth, TX
Mail: P.O. Box 610069
DFW Airport, Texas 75261
Physical: 1639 West 23, Suite 105
DFW Airport, Texas 75261
Phone: (972) 574-3254 / Fax: (972) 574-4669

Honolulu, HI
3375 Koapaka St., #B296
Honolulu, Hawaii 96819-1867
Phone: (808) 861-8525 / Fax: (808) 861-8515

Houston, TX
16639 W. Hardy
Houston, Texas 77060-6230
Cargo Office
Phone: (281) 230-7225 / Fax: (281) 230-7227
Seaport Office
Phone: (713) 673-0805 / Fax: (713) 673-0830

Los Angeles, CA
370 Amapola Ave., #114
Torrance, California 90501
Phone: (310) 328-6307 / Fax: (310) 328-6399

Louisville, KY
601 W. Broadway, Suite 115-A
Louisville, Kentucky 40202
Phone: (502) 582-5989
Fax: (502) 582-5981

Memphis, TN
3150 Tchulahoma Rd., Suite #6
Memphis, Tennessee 38118
Phone: (901) 544-3694 / Fax: (901) 544-3696

Miami, FL
Mail: 10426 NW 31 Terrace
Miami, Florida 33172
Physical: 6105 NW 18th St., Room 405
Miami, Florida 33122
Phone: (305) 526-2994 or 2620 / Fax: (305) 526-7480

New Orleans, LA
2424 Edenborn, Room 100
Metairie, Louisiana 70001
Phone: (504) 219-8870 / Fax: (504) 219-8868

New York, NY
70 E. Sunrise Hwy. #419
Valley Stream, New York 11580
Phone: (516) 825-3950 / Fax: (516) 825-1929

Newark, NJ
1210 Corbin Street, 1st Floor
Elizabeth, New Jersey 07201
Phone: (908) 787-1321 / Fax: (908) 787-1334

Portland, OR
P.O. Box 55206
Portland, Oregon 97238
Phone: (503) 231-6135 / Fax: (503) 231-6133

San Francisco, CA
1633 Old Bayshore Highway, Suite. 248
Burlingame, California 94010
Phone: (650) 876-9078 / Fax: (650) 876-9701

Seattle, WA
19639 28th Avenue South, Bldg A
Seattle, Washington 98188
Phone: (206) 429-2198 / Fax: (206) 429-2673

Appendix H: A Historical Perspective on Escape in Octopods Housed in Aquariums

The following poem was written in response to an octopus escape at the one aquarium.

“The Straying “Topus,”
Tom Hood, 1873

Have you heard of the Octopus—
“Topus of the feelers eight—
How he left his tank o’po’pus
Lumpfish to disintegrate

To the lumpfish tank as sprightly
As the Brighton coach he’d ride;
For two passengers he nightly
Found convenient room inside.

But it happened Mr. Lawler,
Whom the lumpfish ought to thank,
Caught this very early caller,
“Dropt-in” on his neighbor’s tank.

For some weeks the world lumpfishious
Very strangely vanished had;
So the visit was suspicious,
And appearances were bad!

Well for him, this brigand larky
Was not brought before J.P.
(Neither clergy, nor squire-archy)
But to Mr. Henry Lee.

Said he, “Punish on suspicion,
Is a thing I never will—
Catch him in the same position;
Then I’ll send him to the Mill!”

Treadmill is a wear-and-tear case,
And Octopus would you see,
Do four men upon a staircase—
Law, how tired the beast would be!

Appendix I: Results of 2012 AITAG GPO Husbandry Survey

The following data were collected via an email survey to the IRs of the AITAG and to the Aquatic Info professional list-serve in December of 2012. A total of 33 institutions holding GPOs responded.

Part I: Nutrition and Feeding Practices

	Percent	No.
How often is your GPO fed?		
More than once daily	3.00%	1
Daily	30.30%	10
Every other day	18.20%	6
Three times per week	**42.40%**	**14**
Twice per week	6.10%	2
Once per week	0.00%	0
What method is used to determine the feed amount?		
Animal is fed to satiation	18.20%	6
Ad Libitum	0.00%	0
Approximate percentage of body weight per week	12.10%	4
Best judgement approach	**72.70%**	**24**
What types of food are offered?		
Live Foods	51.50%	17
Previously frozen seafood	**100.00%**	**33**
Gel food	3.00%	1
Approximatley what percentage of the GPOs diet is Invertebrate Protein?		
10%	3.10%	1
20%	0.00%	0
30%	**18.80%**	**6**
40%	3.10%	1
50%	9.40%	3
60%	**18.80%**	**6**
70%	**18.80%**	**6**
80%	**18.80%**	**6**
90%	9.40%	3
100%	0.00%	0

Part II: Habitat Construction and Life Support

	Percent	No.
Size of Exhibit (gal)?		
500-750	16.67%	5
750-1000	10.00%	3
1000-1250	23.33%	7
1250-1500	10.00%	3
1500-1750	3.33%	1
1750-2000	3.33%	1
2000-3000	13.33%	4
3000-4000	0.00%	0
4000-5000	3.33%	1
5000-10,000	6.67%	2
10,000+	3.33%	1
Open or closed LSS?		
Open System	13.30%	4
Closed System	86.70%	26
LSS design include a sump?		
yes	65.60%	21
no	34.40%	11
Type of metal used in heat exchanger?		
Titanium	80.80%	21
Stainless steel	19.20%	5
Types of LSS equipment employed?		
Sand Filtration	41.90%	13
Foam Fractionation (protein skimming)	80.60%	25
Fluidized bed	12.90%	4
Trickle filter	41.90%	13
Bioreactor-Kaldness media	3.20%	1
Canister filters: pleated filters	35.50%	11
Canister filters: filter socks	22.60%	7
Ozone	12.90%	4
Ultraviolet sterilization	35.50%	11
Activated carbon	22.60%	7
Other media (phosban, et cetera)	9.70%	3
Undergravel filtration	19.40%	6
Bead Filters	6.40%	2
Granular Ferric Oxide	3.20%	1
Deep Sand Bed	3.20%	1

Turnover rate of LSS?		
Less than 1x per hour	6.90%	2
1x per hour	17.20%	5
2x per hour	31.00%	9
3x per hour	17.20%	5
4x per hour	6.90%	2
5x per hour	6.90%	2
Greater than 5x per hour	13.80%	4
Type of décor in exhibit?		
Natural Stone	16.10%	5
Fiberglass Backdrop	38.70%	12
Epoxy Backdrop	9.70%	3
Cement	35.50%	11

What is the tank itself made of?		
Concrete, sealed	9.40%	3
Concrete, fiberglass coated	3.10%	1
Fiberglass, uninsulated	9.40%	3
Fiberglass, insulated	18.80%	6
Acrylic and fiberglass	34.40%	11
All-Acrylic construction	31.30%	10
Glass	3.10%	1

Part III: Water Chemistry

	Percent	No.
What is your aveerage pH?		
<7.69	0.00%	0
7.7-7.9	15.60%	5
7.9-8.1	43.80%	14
8.1-8.3	46.90%	15
8.3-8.4	6.30%	2
>8.4	0.00%	0
Average Salinity (ppt)?		
<17	0.00%	0
17-20	0.00%	0
20-25	0.00%	0
26-28	6.50%	2
29-30	19.40%	6
31-32	54.80%	17
33-35	38.70%	12
>35	0.00%	0
Average Nitrate Levels (mg/l)?		
<10	50.00%	16
10-20	40.60%	13
20-30	3.10%	1
30-40	9.40%	3
40-50	0.00%	0
>50	0.00%	0
Frequency of Water Changes?		
More than once per week	34.60%	9
weekly	42.30%	11
every other week	7.70%	2
monthly	15.40%	4
Size of Water Changes?		
0-10%	3.40%	1
10-20%	40.80%	12
20-30%	23.80%	7
30-40%	10.20%	3
40-50%	3.40%	1

Average Temperature °C (~°F)		
6 (43)	3.40%	1
8 (46)	68.00%	2
9 (48)	20.40%	6
10 (50)	23.80%	7
11 (52)	17.00%	5
12 (54)	13.60%	4
13 (56)	6.80%	2
>14 (>58)	3.40%	1

Appendix J: A Glossary of Cephalopod Terminology

This is intended to cover some of the terms and acronyms used throughout the manual that are common to the keeping of octopods. This is by no means a complete glossary of phrases encountered in cephalopod biology but should help readers unfamiliar with these animals in deciphering the manual.

Arm: The eight appendages of an octopod are referred to as arms, despite common misconceptions that these structures are termed "tentacles." Octopod arms bear rows of suckers, which differ from the suckers of squids in being stalkless and toothless (without hooks). The third right arm in male octopods bears the hectocotylus.

Beak: The octopus beak is used to tear through flesh as well as crack through crab carapaces.

Biological filtration: A process which utilizes microorganisms, or other living organisms to break down toxic metabolites (and/or their by-products) to less toxic, or non-toxic substances. The classic example in aquaria are bacteria such as to *Nitrosomonas, Nitrobacter,* and *Nitrospira spp*. (among others) which reduce ammonia to nitrites, to nitrates. Common examples of devices that promote biological filtration include trickle filters, bead filters, undergravel filters, and algal turf scrubbers.

Branchial hearts: The paired accessory hearts (in addition to the system heart) in the circulatory system located at the gill apparatuses that pump hemolymph locally at the site of gas exchange to optimize the oxygen transfer.

Cephalotoxin: Cephalotoxin is a neurotoxin present in the venom of many octopods, though not in *E. dofleini.*

Chemical filtration: A process of chemically altering water in order to purify it or remove undesirable substances. Common examples include ozone systems, ion exchange media, and flocculants.

Chromatophores: Part of the system of pigment cells in the integument most responsible for the dramatic color changes and camouflage known in cephalopods.

Closed system: A tank (or series of tanks) with self-contained life support systems that does not rely on a constant influx of clean water. The water is recirculated through the LSS and back to the tankwhich removes nitrogenous wastes, particulates, and organic material.

Ctenidia: The individual filaments of gills.

Dicyemid: Representative of a little-known phylum (Dicyemida) of parasitic mesozoan organisms who's taxonomy and phylogeny is much debated. Dicyemids were once believed to be degenerate flatworms but are currently though to lie outside the kingdom metazoan. They are vermiform (wormlike) and most species only parasitize the renal appendages of cephalopods. GPOs are known to harbor several species of dicyemid parasites in the wild.

Enrichment: An animal husbandry practice that seeks to enhance the quality of aquarium care by identifying and providing environmental stimuli necessary for optimal psychological and physiological well-being. This are often introduced to prevent boredom in animals, stop stereotypic behaviors, or elicit natural behaviors otherwise unexpressed in an aquarium setting. With octopuses enrichment may be used to prevent escape attempts or self-destructive behavior, and to demonstrate the intelligence capacity of the animal to visitors.

Escape jetting: A swimming behavior in which an octopus uses its funnel to rapidly propel it backwards away from perceived danger, often accompanied by a burst of ink to confuse would-be predators.

Funnel: Also sometimes called a siphon, the apparatus used to direct water in and out of the mantle cavity in octopods. Also used in swimming behavior, especially in escape jetting.

GPO: An acronym common in the aquarium industry for specimens of *Enteroctopus dofleini*, derived from the common name: **G**iant **P**acific **O**ctopus

Hemocyanin/Haemocyanin: The oxygen transport protein analogous to hemoglobin in vertebrates. Hemocyanin utilizes copper to bind oxygen and has advantages over other oxygen transport molecules in being able to unload at higher partial pressures of oxygen.

Hemocyte: The single cell type in cephalopod hemolymph. It also functions in immune response, but is not phagocytic like leukocytes in higher taxa. Hemocytes aggregate around foreign particles/pathogens and the clump is removed by the gills.

Hemolymph: The equivalent of blood in molluscs; hemolymph carries oxygen to the cells, transports metabolites and other wastes away from the cells, and functions in immune response.

Hectocotylus: The modified third right arm in male octopods which facilitates transfer of the spermatophore to the female oviduct.

Ink: Ink is used as a defense mechanism when predators are encountered. The ink-defense is most likely a secondary defense if other primary defenses, such as camouflage, are not successful.

Iridophores: Part of the system of pigment cells in the integument that differentially reflect light.

Iteroparity: A reproductive strategy where the organism reproduces more than once, usually at regular intervals, throughout its adult life. Iteroparitous organisms may be either K- or r-selected in terms of brood size and parental investment. Nautiloids are iteroparitous, while most other cephalopods are semelparitous.

Kreisel: Commonly used for jellyfish; tanks that keep planktonic organisms in suspension without touching the bottom or sides are commonly known as kreisels, planktonokreisels, and pseudokreisels. These typically have a curved bottom and shielded water inlets that create a unidirectional laminar flow to keep animals in suspension. Several designs have been used experimentally to raise GPO paralarvae, though the optimal configuration has yet to be determined.

Large-egged octopus: Octopods with larger eggs tend to be less fecund and their paralarvae will immediately start living a benthic lifestyle. The GPO is a small-egged species.

Larvae: See **Paralarvae**

Ligula: The end of the hectocotylyzed third right arm in male octopods used to transfer spermatophores to the female during mating. It is often scoop or paddle shaped, and it is theorized that this morphology may aid in removal of spermatophores from competing males during copulation as well as placement of their own.

Leucophores: Part of the system of pigment cells in the integument that produce white coloration.

LSS: Life Support Systems- the filtration apparatus used in aquaria to maintain water quality, temperature, clarity, and mitigation of toxic metabolites excreted by the exhibit animals. Generally divided into biological, chemical, or mechanical filtration depending on their respective method of action.

Maculotoxin: An extremely potent neurotoxin with effects nearly identical to tetratodotoxin. In cephalopods the toxin is namely found in the venom of the blue ring octopods of the genus *Hapalochlaea.*

Mantle: The muscular coating that envelops the viscera and gills in an octopus and comprises the body proper (as opposed to the arms). The mantle also acts in ventilation by contracting to pump water in order to supply the gills.

Mechanical filtration: A process that physically removes organic or inorganic particles or detritus from exhibit water. Common examples include sand filters, bag filters, filter floss, and foam fractionators.

Microvillus epidermis: Term commonly used to refer to the integument of cephalopods to denote the immense surface area caused by microvilli (microscopic projections or folds). This is analogous in morphology and function to the epithelial lining of the vertebrate gastrointestinal tract, and the resulting surface area is responsible for the inherent hypersensitivity of cephalopods to dissolved toxins in the ambient seawater. The integument also plays a major role in respiration, with some species of deep sea octopods having vestigial gills and relying entirely on integumentary gas exchange

Midden: Also referred to as an "octopus's garden", a midden is the collection of inedible prey remnants that is characteristic of an octopus den. Crab carapaces, bivalve and gastropod shells, fish bones, and other leftover debris are common midden items. These often denote the presence of a den in the wild, and the midden contents can offer a valuable record of the diet and prey handling techniques. Octopus's gardens are always found under the sea, but despite popular misconceptions are not always located in the shade.

Nautiloid: A member of the order Nautilida, the nautiluses.

Octopods: The correct plural form when referring taxonomically to multiple specimens of the order Octopoda, the family Octopodidae, or the genus *Octopus*. Technically 'octopodes' is the correct classical Greek pluralization of the common name, though 'octopods' is acceptable in scientific writing as an Anglicized pluralization of the taxonomic determination.

Octopodes: The correct classical pluralization derived from the Greek workd 'Oktopus' (ὀκτώπους), though 'octopods' is also acceptable in scientific writing as an Anglicized pluralization of the taxonomic determination, and 'octopuses' is generally acceptable in reference to the common name. The terms 'Octopi' and 'Octopii' are incorrect for pluralizing either the taxonomic names or the common names as the word octopus is a derived from the Greek word 'Oktopus' and cannot be pluralized as a Latin noun using '-i' or '-ii'. This mistake, while regrettable, is persistent in common usage and even in the non-malacological primary literature.

Octopuses: Another generally acceptable plural of octopus when referring more generally to the common name 'octopus'. This is an anglicized version of the classical pluaralization. Octopodes—as defined above—is the classical Greek pluralization.

Open-system: An open-system does not rely on filtration in order to sustain the living creatures it houses, but instead has a steady influx of natural water at one point, which overflows from the tank at another point down a drain. Such systems are generally only employed for GPOs in the Pacific Northwest, where access to seawater at the appropriate temperature is to be had.

Paralarvae: As the young of octopods are not true larvae (i.e., they do not undergo a metamorphosis) the term paralarvae is commonly used to describe the planktonic hatchlings of small-egged octopus species. Paralarvae typically are primarily defined as being the first life stage of an organism with a distinctively different mode of life than the adults (e.g. planktonic vs. benthic); this term which does not encompass all of the cephalopod taxa but is true of most octopods.

Prey puzzle: A term commonly used in octopus enrichment to denote a device which needs to be manipulated by the animal to be unlocked (or otherwise opened) to receive a food reward.

Radula: The radula is similar to a human tongue, though a radula has small tooth-like projections on it used to grind up food.

Renal appendage: The cephalopod equivalent of the kidneys in vertebrates. The renal appendage handles the concentration and excretion of metabolites (primarily nitrogenous wastes) from the circulatory system.

Semelparity: A reproductive strategy where the organism reproduces only once in its respective lifetime. Occasionally referred to as terminal spawning, such species tend to produce large numbers of offspring with no direct investment in parental care once hatched. Octopods, squids, and cuttlefish are typically semelparitous, while nautiloids are not.

Senescence: In semelparous cephalopods, senescence defines the terminal condition entered into when the animal reaches full sexual maturity. An animal's entire energy budget is devoted towards reproduction at this point and cellular repair, hemocyte production, salivary enzyme production, and growth cease. Thus the animal cannot feed and is increasingly immunocompromised towards the end of its life, and wastes away after mating. Females devote their final efforts into meticulously aerating and tending their eggs, the only form of parental care known in invertebrates, while males wander aimlessly.

Sepiod: A member of order Sepiida, the cuttlefishes.

Small-egged octopus: Octopus species with small eggs generally are more fecund and the paralarvae are planktonic for a period of time after hatching. These larvae depend on planktonic prey for growth until settlement when the small octopods will adapt a benthic lifestyle typical of adult specimens. GPOs are small-egged octopods.

Speculative feeding: A feeding behavior employed by octopods where they use the webbing between their arms to completely envelop a rock or other benthic feature unaware as to whether prey is located there. The animal then can search for the trapped prey at its leisure. Also called "webover" feeding.

Spermatophore: Also known as a ‘sperm packet’, this is an encapsulated parcel of sperm produced by male octopods and passed to the oviduct of females via the hectocotylus.

Statocyst: The cephalopod sensory organ containing statoliths (calcareous concretions) which is responsible for maintaining equilibrium in cephalopods, and also for vibration detection at low frequencies analogous to hearing in terrestrial animals.

Systemic Heart: The third, centralized heart in the octopod circulatory system. The other two are branchial hearts, located at the gill apparatuses. The three hearts and closed circulatory system represent a highly evolved physiology not seen in many mollusks or lower invertebrates.

Subadult: A cephalopod that has attained all the diagnostic morphological features characteristic of its species, but that has not yet reached sexual maturity. Subadults are preceeded by paralarvae in octopods (termed larvae in some other cephalopod taxa), and succeeded by adults in the course of development.

Tentacle: An elongated, unsegmented appendage which is typically circumoral in invertebrates. The presence or absence of tentacles in cephalopods is largely broken down along taxonomic lines, with octopods having eight arms and no tentacles, sepiods and teuthoids having eight arms accompanied by two tentacles, and nautiloids possessing many tentacles and no arms. The term tentacle is quite often erroneously used in reference to octopuses, who, as a group, are atentacular. In general tentacles are differentiated from arms as having no suckers along a portion or all of their length.

Teuthoid: A member of the order Teuthida, the squids.

Sucker: The suckers are located along the eight arms of the octopus and used to “smell” and investigate its surroundings.

Venom: In cephalopods the term is used to describe a “cocktail” of various proteolytic and other enzymes excreted into prey to predigest the meal before it is consumed. Better described as a proteolytic saliva. Some species (but not *E. dofleini*) also have potent neurotoxins present in the venom-like saliva such as cephalotoxin and maculotoxin. The venom-like “cocktail” of *E. dofleini* contains a mixture of various digestive enzymes that have yet to be precisely defined.

Appendix K: Sample Transaction Form

Animal Transfer Agreement - Sale/Donation/Trade

THIS DOCUMENT SIGNED BY A DULY AUTHORIZED REPRESENTATIVE OF THE INSTITUTION LISTED BELOW (THE "RECIPIENT INSTITUTION") BETWEEN SAMPLE AQUARIUM AND THE RECIPIENT INSTITUTION **MUST BE RETURNED TO THE SAMPLE AQUARIUM BEFORE THE LISTED ANIMALS ARE TRANSFERRED**.

The Sample Aquarium is dedicated to the present and long range welfare of all its specimens and believes it has an obligation to make every effort to guarantee that specimens leaving the Sample Aquarium by sale, donation, or trade will be maintained in a manner acceptable to modern professional zoological park and aquarium standards.

Therefore the Sample Aquarium agrees to the transfer of the following animal(s):

Species: ______________________________

____

____

ISIS #('s): __________ __________ __________

__________ __________ __________

__________ __________ __________

Total Price: ______________________________

To: ______________________________

____

____

____

USDA ______________________________

with the understanding that the recipient will comply with all the animal welfare conditions set forth in this agreement.

1. The recipient will hold, on a current basis, all necessary licenses, permits, and/or permissions to acquire and maintain the animal(s) and will supply copies to the Sample Aquarium prior to the transfer.
2. The animal(s) and the progeny thereof will be housed, fed and, in general, maintained in a manner that will insure their physical and psychological well-being.
3. The animal(s) and the progeny thereof will not be sold, traded, loaned or donated to or used in any stressful or terminal research program.
4. The animal(s) and the progeny thereof will not be sold, traded, loaned or donated to any organization that may use the animal(s) in any animal auction(s).
5. The animal(s) and the progeny thereof will not be sold, traded, loaned or donated to any retail pet shop.

6. The animal(s) and the progeny thereof will not be sold, traded, loaned or donated to any facility for the purpose of providing game for sport or subsistence hunting or such other purpose prohibited by any applicable AZA standards.
7. The animal(s) and the progeny thereof shall be transferred only under conditions and to persons or entities that are in accordance with AZA standards.
8. If the animal(s) are transferred by sale, donation, or trade to another party, the RECIPIENT INSTITUTION will keep and maintain information including, without limitation:
    a. the name and address of the transferee;
    b. the intended use and location where the animal(s) will be kept by the transferee and;
    c. if applicable, the conditions and cause of death, including supporting documentation.

   Further, the undersigned recipient agrees to require the transferee to adhere to the same conditions contained herein relating to the treatment of the animal(s) or its subsequent transfer or death.
9. All shipping charges will be borne by the recipient institution. In the event Sample Aquarium crates are used for shipping, they will be returned promptly to the Sample Aquarium after shipment. Transport charges are to be prepaid unless otherwise arranged between the recipient institution and the Sample Aquarium
10. The animal(s) described herein shall become the property and responsibility of the recipient at the time of delivery to the terminal of an authorized common carrier or loading into the equipment of the recipient's authorized agent for transport. Such transfer is made on an "as is, where is" basis. The undersigned recipient accepts the above named animal(s) from the Sample Aquarium with the understanding that this transaction is contingent upon its adherence to the above stipulations.

Recipient By: ______________________________

Title: ______________________________

Date: ______________________________

Approved by: ______________________________ Date: ____________