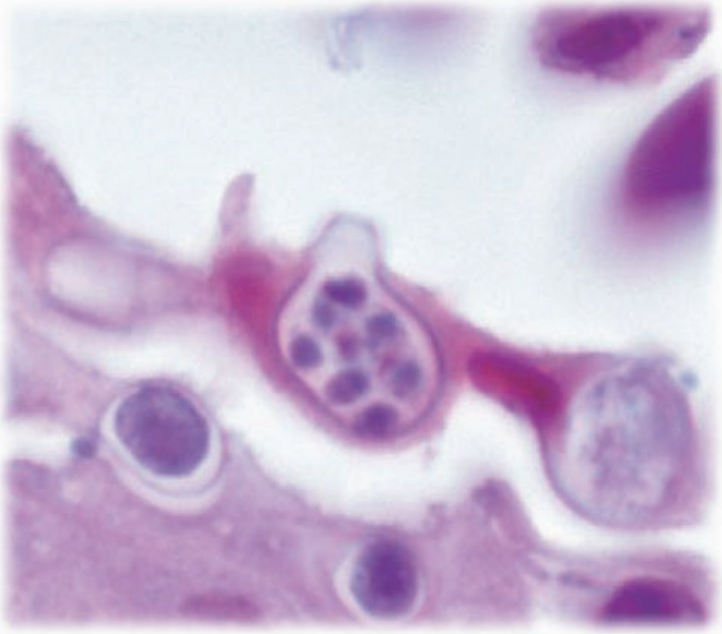
Biosecurity and Permanent Isolation of Ex Situ Conservation Populations

An extract from:

A Manual For Control of Infectious Diseases
in Amphibian Survival Assurance Colonies
and Reintroduction Programs

Version 2.0: updated 2017



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A contribution of the IUCN/SSC Conservation Breeding Specialist Group in collaboration with Amphibian Ark, San Diego Zoo, and Zoo Atlanta

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Pessier, A.P. and J.R. Mendelson III (eds.). 2017. A Manual for Control of Infectious Diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs, Ver. 2.0. IUCN/SSC Conservation Breeding Specialist Group: Apple Valley, MN.

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This project was supported by grant LG-25-08-0066 from the Institute of Museum and Library Services. Any views, findings, conclusions or recommendations expressed in this publication do not necessarily represent those of the Institute of Museum and Library Services.

A Manual for Control of Infectious diseases in Amphibian Survival Assurance Colonies and Reintroduction Programs

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Proceedings from a Workshop:
16–18 February 2009
Zoological Society of San Diego

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CHAPTER 4

BIOSECURITY AND PERMANENT ISOLATION OF *EX SITU* CONSERVATION POPULATIONS

4.0 INTRODUCTION

The example of chytridiomycosis as an infectious disease introduced worldwide by anthropogenic means and resulting in devastating amphibian population declines has highlighted a need for improved biosecurity in facilities that keep captive amphibians. Amphibians are routinely moved globally for use as laboratory research subjects, as pets, as educational or display animals and as part of conservation and breeding programs. These movements can increase the risk that amphibian pathogens will be moved to new locations as has been demonstrated in recent studies of amphibian imports and movements (Fisher and Garner, 2007; Schloegel et al., 2009; Schloegel et al., 2010; Martel et al., 2014) and by the documented introduction of the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) to wild populations of the Mallorcan midwife toad by the activities of a captive breeding program (Walker et al., 2008).

Implementation of biosecurity practices that reduce the potential for introduction of amphibian infectious diseases to new locations are the responsibility of all institutions that maintain or move captive amphibians. In addition, good biosecurity practices help to reduce the risk posed by infectious diseases on the success and sustainability of captive amphibian programs.

The major concepts of amphibian biosecurity are:

- There are risks of infectious disease associated with programs that keep amphibians in captivity outside of the native geographic range of the species or species assemblage (e.g., Panamanian frogs brought to the United States). Similar risks occur anytime animals from multiple geographic regions are mixed in one captive amphibian facility (a mixed or cosmopolitan collection).
- The simplest and least expensive way to reduce these risks is to maintain captive amphibians only within the native range of the species and to avoid creation of cosmopolitan amphibian collections.
- If captive amphibians must be kept outside of their native range or within a cosmopolitan collection biosecurity practices that reduce the risk of disease transmission are necessary.
- Captive breeding programs or survival assurance colonies that intend to reintroduce amphibians to the wild should maintain those animals in permanent isolation (e.g., dedicated rooms or buildings) away from amphibians that originate from outside the native range of the species. Husbandry practices such as the use of dedicated footwear, protective clothing, dedicated tools and equipment, and following specific work-flow patterns reduce the risk of

introducing non-native pathogens to amphibian collections. Facilities located within the natural range of the species and that do not keep amphibians from outside the natural range are the best example of permanent isolation and require the least amount of expense and effort.

- Use of relatively simple husbandry routines and practices reduces the risk of introducing and spreading infectious diseases within an amphibian facility. This is regardless of the role of the species (e.g., education, pet, or as a survival assurance population).
- Procedures for disposal of solid waste and amphibian facility wastewater are considered whenever captive amphibians are held outside the native range of the species or whenever captive amphibians are housed in a cosmopolitan collection that keeps amphibians from different geographic regions (inside or outside the native range).
- Sources for food and water in the facility are scrutinized for the potential to introduce amphibian pathogens.
- Ideally, facilities are: pest-proof; amphibian-proof (for escape of captives from the facility or for entry of or contact with free-ranging native amphibians); designed for automation in feeding, watering and cleaning; and easy to clean and maintain.
- Ideally, facilities provide for the unique environmental needs of amphibians in regard to environmental temperature, humidity, lighting, and water quality. These criteria are essential to amphibian health and are the subject of several expert reviews (Browne et al., 2007; Pramuk and Gagliardo, 2008),

4.1 WHAT IS "BIOSECURITY"?

Biosecurity can be defined as "the protection of the environment and its native species from exotic pathogens." However, the management of *ex situ* populations of amphibians for conservation purposes requires additional considerations. Therefore, in this document biosecurity refers to measures that:

- Protect native species in the natural environment from pathogens that are carried by captive amphibians (especially if captive species are held outside of their natural range or are exposed to other amphibians from outside their natural range).
- Protect captive specimens from pathogens present in the native amphibians (whether facility is in-range or out-of-range).
- Protect captive specimens from pathogens present in other captive amphibians in the collection.

We will never achieve 100% biosecurity in any *ex situ* amphibian population.

- The opportunities for pathogen movement are significantly reduced by identifying vectors and husbandry practices that present potential risks and designing protocols to remove or reduce these risks.
- Implementing and maintaining appropriate biosecurity is a never ending process of risk assessment (i.e., probability of pathogen transfer occurring by any identified vector/practice weighed against the likely severity of the consequences) and subsequent risk reduction.

A realistic level of appropriate biosecurity can be achieved with protocols that are simple and inexpensive to put into practice. Significant investment in facilities and equipment is only required to achieve the highest level of biosecurity—for example, when working outside of the natural range of a species intended for eventual reintroduction to the wild.

4.2 BIOSECURITY DEFINITIONS

Program located inside range of species: The facility (or facilities) holding the amphibian species or assemblage of species is physically located inside the geographic distribution of the species. *Examples:* 1) A building or modified shipping container with Kihansi spray toads (*Nectophrynoides asperginis*) located at the rim of Kihansi Gorge, Tanzania; 2) A collection of multiple species of locally captured amphibians housed in an educational center for visitors inside a national park or natural reserve.

Program located outside range of species: The facility (or facilities) holding the amphibian species or assemblage of species is physically located outside the geographic distribution of the species. *Examples:* 1) Kihansi spray toads (*Nectophrynoides asperginis*) located in a zoo in the USA; 2) wild-caught salamanders from southern Mexico (e.g., Chiapas State) being maintained in a facility in central Mexico (e.g., Michoacan State).

Cosmopolitan facility or institution: Any facility (e.g., room or building) or institution (e.g., university or zoo) that maintains species of amphibians from different geographic regions—i.e., species that do not co-occur in the wild. The reality is that most amphibian programs in the world qualify as “cosmopolitan” but this does not mean that they cannot develop adequately secure programs toward amphibian conservation and reintroductions.

Isolated facility or institution: Any facility (e.g., room or building) or institution (e.g., university or zoo) that is dedicated to only a single species or assemblage of co-occurring amphibian species. These sorts of programs usually are focused on a single species, such as at the US Fish & Wildlife Service Wyoming toad facility (*Anaxyrus baxteri*) at Saratoga National Fish Hatchery. In some restrictive cases, this concept may be applied to separate populations, rather than actual species.

4.3 COSMOPOLITAN COLLECTIONS AND RISKS OF DISEASE FOR WILD AMPHIBIANS

Pathogens that are native (or endemic) to one population of amphibians often cause only a mild or even undetectable illness in those animals. This is because the population has been exposed to the pathogen over time and has developed adaptations to limit the pathogen's harmful effects. However, when introduced to new (naïve) amphibian populations the same "non-native" pathogen has the potential to cause more severe disease.

Traditional zoo amphibian collections typically are "cosmopolitan", keeping animals from different geographic locations and different sources (e.g., wild-caught; captive-born; obtained from a commercial animal dealership) all within the same facility. These collections increase the risk of introducing non-native amphibian pathogens to naïve species or geographic locations because:

- Direct or indirect contact between animals in cosmopolitan collections can facilitate the transmission of pathogens between animals from different locations (e.g., frogs from Central America are exposed to frogs from Australia and become infected with a pathogen that is native only to frogs from Australia).
- If animals infected with a "non-native" pathogen are later reintroduced to the wild they will also introduce the "non-native" pathogen to a new location (e.g., Central American frogs infected with an Australian pathogen are reintroduced to the wild in Central America).
- Amphibians brought to a new geographic location can introduce non-native amphibian pathogens to native amphibians in the new location (e.g., frogs from Central America infected with a pathogen native to Central America are brought to the United States).

For these reasons, efforts should be made to ensure that programs that keep amphibians in captivity are not contributing to the movement of amphibian pathogens.

<u>The simplest and least expensive ways to reduce the disease risk of moving "non-native" amphibian pathogens to new locations are to:</u>

- Establish and maintain captive (*ex situ*) amphibian populations within, or as close as possible to, the native range and habitat of the species that is the subject of a conservation program (e.g., maintain Honduran species in Honduras and Australian species in Australia).
- Avoid establishing captive breeding programs of amphibians intended for reintroduction to the wild inside a cosmopolitan amphibian collection (within the natural range of the species or outside the natural range of the species).

Because this is not always possible, measures to increase biosecurity are necessary for many captive amphibian programs.

4.4 LEVELS OF BIOSECURITY

Biosecurity measures are specific husbandry, staff work-flow and veterinary procedures that reduce, but do not completely eliminate, infectious disease risks from within an amphibian facility.

The reasons for implementation of biosecurity practices in captive amphibian facilities are to:

- Prevent "non-native" amphibian pathogens from leaving a captive facility and becoming introduced to new locations. This can occur when amphibians have been removed from their native range for any purpose.
- Prevent amphibian pathogens from entering a captive population or from being transmitted between animals within a facility. For example, it is not desirable to introduce amphibian chytrid fungi into a healthy amphibian collection or to spread infection with the fungus throughout an amphibian collection.

The types of biosecurity practices needed to achieve these goals is determined individually for each amphibian facility and for each amphibian conservation program. In cosmopolitan amphibian collections, biosecurity practices are determined for each species kept in the facility.

In this manual, guidelines for two levels of biosecurity are defined based on the degree and types of infectious disease risks that are identified in a captive amphibian program.

- The basic protocol is **"BEST PRACTICES"** which requires neither special equipment nor facilities, but outlines simple common-sense practices for hygiene and prevention disease spread for ALL captive amphibians.
- For *ex situ* populations that are intended for eventual use in reintroduction programs, a few extra biosecurity measures are required to reduce risks of introducing pathogens into the wild. This protocol is termed simply **"ISOLATION"**, as the simple act of physically separating a population of animals greatly reduces the majority of risks of disease spread among captive animals.

The decision to use either BEST PRACTICES or ISOLATION protocols is aided by a simple risk assessment and decision tree (see below).

Risk Assessment Decision Tree for Biosecurity

Information Required

The decision tree for biosecurity risk assessment (Figure 4.1) requires three types of information:

1. The role of a species or species assemblage in a conservation program
2. The location of the amphibian facility in relation to the geographic origin of the species or species assemblage
3. Is the captive facility “cosmopolitan” or “isolated” (see Section 4.2)

These criteria are explained in detail below (after the Decision Tree).

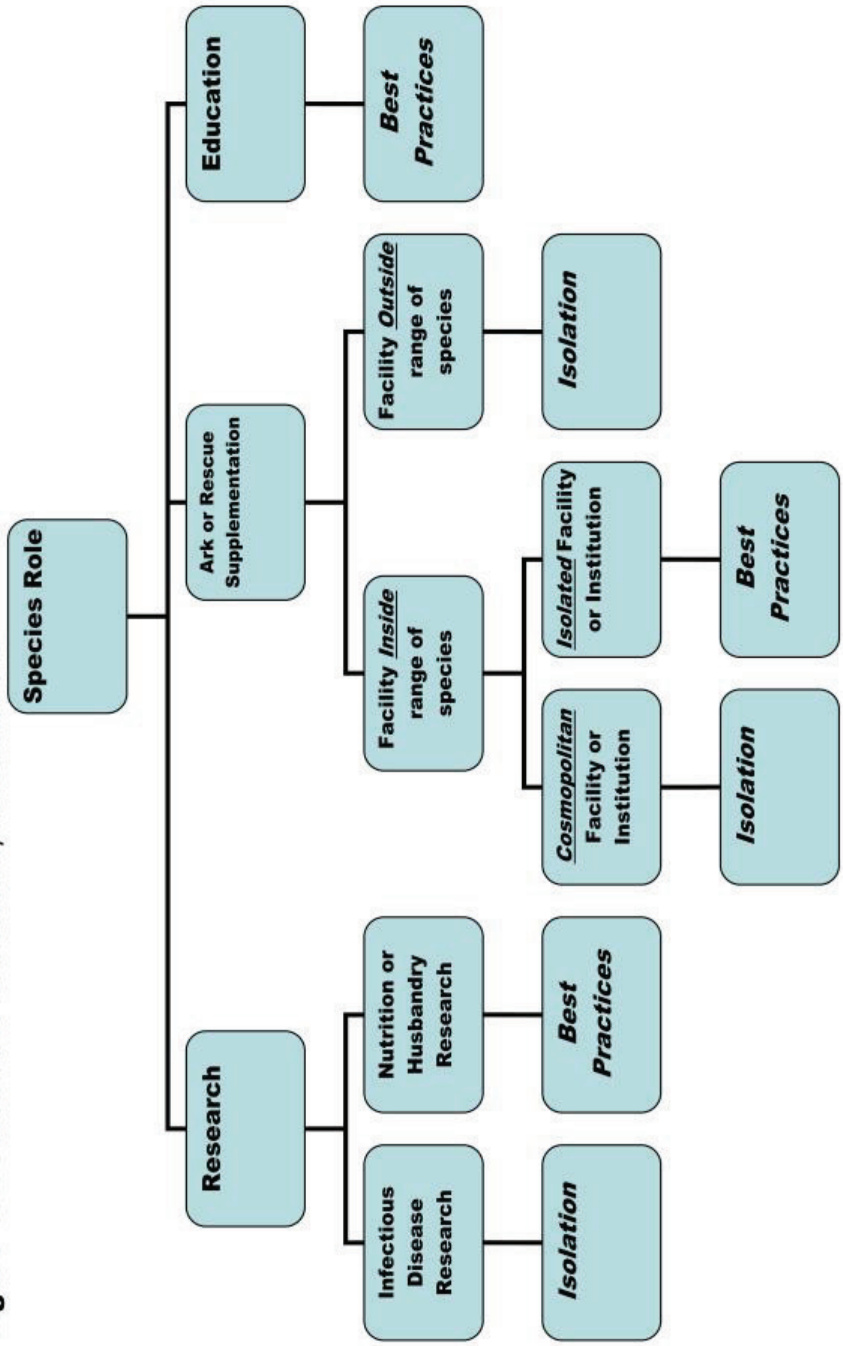


Figure 4.1: Decision tree for biosecurity risk assessment.

1. The 'role' of the species or species assemblage

The most common species roles for captive amphibians, as defined by the CBSG/WAZA *Ex Situ* Amphibian Planning Workshop (Zippel, Lacy and Byers 2006) and by Amphibian Ark are listed below, with the addition of a category for large-scale commercial operations.

Roles for Captive Amphibians:

- Ark, Rescue or Supplementation
- Conservation Research
- Conservation Education
- Amphibian Farming and Mass Production

Ark, Rescue, or Supplementation:

All of these categories have the intention of reintroducing the animals or their progeny to the wild.

Ark—An amphibian species that is extinct in the wild (locally or globally) and which would become completely extinct without *ex situ* management.

Rescue—An amphibian species that is in imminent danger of extinction (locally or globally) and requires *ex situ* management as part of the *recommended* conservation action.

Supplementation—An amphibian species for which *ex situ* management benefits the wild population through breeding for release as part of the *recommended* conservation action.

Conservation Research:

These animals have no prospect of reintroduction to the wild**,** but are used for specific applied research projects that contribute to the conservation of that species, or a related (surrogate) species, in the wild.

Examples of this kind of research include:

- Development of techniques for captive breeding.
- Disease research (e.g., control or treatment of chytridiomycosis).
- Nutrition and development of captive amphibian diets.

Conservation Education:

These animals have no prospect of return to the wild. These are amphibian species that are used only for educational purposes—primarily in zoos and aquariums—to inspire and increase knowledge of visitors, in order to promote positive behavioral change. These animals are those typically held in a cosmopolitan zoo collection but may also include amphibians that are "flagship" or "ambassador" species used to raise

awareness of amphibian conservation issues (e.g., an endangered Wyoming toad removed from the captive breeding population to be used as a display animal). Occasionally, amphibians used for educational purposes are released back into the wild. If this is the case, the animals should be considered as Ark/Rescue/Supplementation for purposes of the risk assessment.

Amphibian Farming and Mass Production:

These animals are reared intensively and in large numbers for food, use as laboratory animals, and for the pet trade. This group of animals has unique disease risks because of the sheer number of animals housed, the common use of outdoor enclosures (allows for escape of animals and contact between native amphibians and farmed amphibians) and the potential for transmission of pathogens in cosmopolitan species situations (e.g., a amphibian or reptile dealership that sells animals from different geographic regions).

2. The location of the amphibian facility

The location of the facility holding captive amphibians is a very important component of the biosecurity risk assessment. The most important considerations are the following dichotomies:

- The facility is within the natural geographic range of the species or species assemblage or near the site of original collection.

OR

- The facility is outside of the natural range of the species or species assemblage, or distant from site of original collection.

3. Is the facility cosmopolitan or isolated?

- The facility is limited to keeping species or species assemblages from within the natural geographic range (isolated).

OR

- The facility maintains species or species assemblages from outside the natural range (e.g., cosmopolitan zoo collection).

Examples Using the Risk Assessment Decision Tree

The following scenarios are provided to guide readers in the use of the Risk Assessment Decision Tree for Biosecurity (Figure 4.1).

Example 1:

American bullfrogs (*Lithobates catesbeianus*) housed in a zoo exhibit in the United States that educates the public about wetland ecosystems. There are no plans to breed this species in captivity or release offspring back into the wild.

Using this information in the decision tree:

- These bullfrogs are not part of a captive breeding or conservation research program and they (or their offspring) will never be released back into the wild. Therefore, have an EDUCATION role.
- Animals with an EDUCATION species role are managed in captivity using the BEST PRACTICES level of biosecurity.
- The BEST PRACTICES biosecurity guidelines (see Section 4.4) include information on how to prevent introduction of infectious diseases carried by the bullfrogs into local wild amphibian populations to or other amphibians that are in the zoo collection. This would also be true if the exhibit mentioned in this example were located outside of the United States (e.g. Europe or Australia), however, considerable scrutiny of biosecurity practices for waste and wastewater disposal (see Sections 4.14–4.15) and aspects of facility design related to preventing escape of captive animals or entry of native wild amphibians (see Section 4.18) is warranted because of significant risks associated with the introduction of "non-native" amphibian pathogens to new locations (see Section 4.3 above).

Example 2:

Wyoming Toads (*Anaxyrus baxteri*) are an endangered species from the western United States that are the subject of a captive breeding program that produces tadpoles for reintroduction into the wild. One facility in the captive breeding program is a zoo located 20 miles (32 km) from a lake where the toad is known to naturally occur. The zoo is a mixed cosmopolitan institution that also keeps a small number of other amphibian species from around the world for educational purposes.

Using this information in the decision tree:

- The Wyoming toads are part of a captive breeding program that aims to reintroduce animals back into the wild. Therefore, these toads have an Ark, Rescue or Supplementation role.
- The zoo in this example is inside the native range of the Wyoming Toad.
- The zoo in this example is a "mixed" facility or institution that also keeps other amphibian species from outside the natural range of the Wyoming Toad.
- Animals with a Ark, Rescue or Supplementation role that are housed in a "mixed" facility or institution are kept in an ISOLATION level of biosecurity. In this situation this is true even though the animals are kept in a facility that is within the native range of the toad. Additional precautions in the ISOLATION biosecurity level (see Section 4.4) help to ensure that toads released to the wild have not been exposed to "non-native" pathogens that circulate in other amphibians kept in the mixed facility.

A second facility that houses Wyoming Toads for the captive breeding program is located 15 miles (24 km) from a lake where the toad is known to naturally occur. This

facility is dedicated to breeding only Wyoming toads and no other amphibians are kept in this facility.

- Because the facility in this example is within the native range of the Wyoming Toad and because the facility is dedicated (or "Isolated") to the Wyoming Toad, the animals can be kept under the BEST PRACTICES level of biosecurity (see Section 4.4). The BEST PRACTICES biosecurity level still includes "common sense" recommendations to reduce the risk that important amphibian pathogens (e.g. amphibian chytrid fungi) are not introduced or spread in the captive population.

Example 3:

Kihansi Spray Toads (*Nectophrynoides asperginus*) are extinct in the wild in their native range of the southern Udzungwa Mountains of Tanzania. Captive survival assurance populations of the spray toad have been established at a zoo in the United States. There are future plans to use offspring from the captive population to reintroduce the spray toad to Tanzania after mitigation of environmental factors in the native range.

Using this information in the decision tree:

- The spray toads are part of a captive breeding program that aims to reintroduce animals back into the wild. Therefore, these toads have an Ark, Rescue or Supplementation role.
- The breeding facility for the spray toads is considerably outside of the native range of this species. In this situation, the ISOLATION level of biosecurity is necessary to prevent the introduction of "non-native" amphibian pathogens to the spray toad populations. If "non-native" pathogens are introduced to the spray toads, there is a risk that these pathogens will also be introduced back to wild amphibian populations in Tanzania as part of the spray toad reintroduction effort.

4.5 SUMMARY OF BEST PRACTICES AND ISOLATION BIOSECURITY LEVELS

Best Practices

BEST PRACTICES are recommended for all captive amphibians, and specifically for those that:

- Are maintained in "long term isolation" because they have an ARK/RESCUE/SUPPLEMENTATION role, but are kept in a facility that is within the native range of the species or species assemblage and does not maintain or have contact with amphibians from outside the native range.

OR

- Have a CONSERVATION RESEARCH or CONSERVATION/EDUCATION role with no intention of returning the animals or their progeny to the wild. These animals can be within or outside of the native range. If these animals will be returned to the wild they should be considered to have an ARK/RESCUE/SUPPLEMENTATION role.

BEST PRACTICES will reduce the following types of risks of infectious disease in a collection:

- Acquisition of infectious diseases that:
    - Have a negative impact on the health of individual animals.
    - Interfere with the success of captive breeding or the sustainability of a captive amphibian program.
    - Have a negative impact on the validity of any research that is conducted with a species.
- Inadvertent introduction of a non-native amphibian pathogen to a new location (e.g., frogs from the United States brought to a facility in Australia introduce a non-native *Ranavirus* to Australia).

Isolation

ISOLATION criteria are recommended for those animals that:

Have ARK/RESCUE/SUPPLEMENTATION role and they or their progeny are likely to be returned to the wild

AND

- Have been removed from their native range for *ex situ* conservation efforts (e.g., native to India and incorporated into *ex situ* conservation program in the Australia).

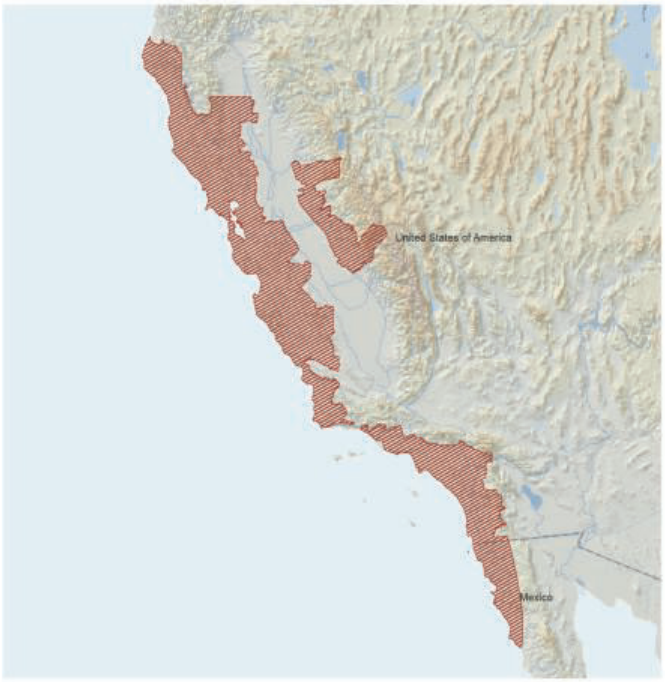
OR

- Are in *ex situ* conservation programs in the native range, but could be exposed to amphibians from outside the native range (e.g., a zoo in Germany that has an *ex situ* conservation program for native German species, but also keeps amphibians from other regions such as Kenya or the United States).

Mixing of individuals that come from allopatric populations of the same species may also require ISOLATION under some circumstances. For example, consider the distribution maps (below) for the hylid frog *Anotheca spinosa* and the plethodontid salamander *Aneides lugubris.* These are examples of species with naturally occurring disjunct populations. In such cases, one must consider the concept of “inside” vs. “outside” the range of the species with special care, taking into account the separate populations that may have differing disease profiles in the wild. Similarly, in such cases,

it may be advisable to maintain in isolation colonies deriving from different, disjunct, portions of the overall range of the species.

Distribution of the hylid frog *Anotheca spinosa* in Central America.



Distribution of the plethodontid salamander *Aneides lugubris* in western North America.

(source: www.iucnredlist.org)

ISOLATION will reduce the following types of risks of infectious disease in a collection:

- All risks covered by BEST PRACTICES.
- Additional safeguards to ensure that animals involved in ARK/RESCUE/SUPPLEMENTATION roles do not become infected with non-native pathogens. The great risks presented by non-native amphibian pathogens are discussed in Section 4.3.

Change in Biosecurity Level

Animals must be maintained at the security level appropriate for their role. It is wise to maintain animals at the highest level of biosecurity (BEST PRACTICES or ISOLATION) necessary for the current as well as any future anticipated role(s) of the captive population.

- This is most important for animals that are kept in ISOLATION because they or their progeny will be returned to the wild. If these animals (or their progeny) are maintained with BEST PRACTICES, they are at higher risk for acquiring a non-native amphibian pathogen and introducing this pathogen into wild amphibian populations.
- If the role of the species changes (e.g., from ARK/RESCUE/SUPPLEMENTATION to CONSERVATION/RESEARCH or EDUCATION, the biosecurity level can be decreased (e.g., ISOLATION to BEST PRACTICES), but it is not appropriate to change the role of these animals back to the category of ARK/RESCUE/SUPPLEMENTATION role at a later time.

If animals previously maintained with BEST PRACTICES must be considered for an ARK/RESCUE/SUPPLEMENTATION role, the process of disease risk assessment is complicated, expensive and time-consuming and may not result in animals that are suitable for release into the wild (See Chapter 3).

4.6 BIOSECURITY PRACTICES

An outline of specific practices for the BEST PRACTICES and ISOLATION is given below in Table 4.1 and details of these practices are provided in subsequent sections. The major difference between the BEST PRACTICES and ISOLATION is the simple act of isolating the latter animals. The process of long-term isolation is described in detail in Section 4.8.

The specific husbandry practices described in subsequent sections accomplish one or more of the following goals:

- Prevent non-native amphibian pathogens from becoming introduced to new geographic locations and amphibian populations (“Nothing gets out”). This protects wild amphibian populations from new infectious disease risks.

- Prevent amphibian pathogens from being introduced into healthy captive amphibian populations ("Nothing gets in"). This is important for the sustainability of captive populations, the success of captive breeding and rescue programs and for maintaining animal welfare standards.
- Prevent amphibian pathogens from becoming transmitted between different animals within a captive amphibian facility ("Nothing gets around inside"). If infectious disease outbreaks occur within a facility, these husbandry practices can minimize the number of animals that will become affected.

Table 4.1—Outline of Husbandry Practices for BEST PRACTICES and ISOLATION.

Practice	Best Practices	Isolation
Long-term isolation of the species or species assemblage (Section 4.8)		+
Dedicated footwear for each long-term isolation room (Section 4.10)		+
Dedicated clothing for each long-term isolation room (Section 4.10)		+
Animals in long-term isolation cared for first in the day (Section 4.11)		+
Dedicated tools and equipment for each long-term isolation room (Section 4.13)		+
Dedicated footwear and clothing for each building (Section 4.10)	+	+
Wash hands or use disposable gloves between EACH enclosure (Section 4.10)	+	+
Follow a husbandry routine that reduces the potential for disease transmission (Section 4.11)	+	+
Clean and disinfect tools between different enclosures (Section 4.13)	+	+
Determine need for special wastewater treatment (Section 4.14)	+	+

Determine need for solid waste disposal (Section 4.15)	+	+
Disease free water and food sources (Sections 4.16 & 4.17)	+	+
Automation of husbandry practices if possible (Section 4.18)	+	+
Quarantine period for new animals entering a collection (Chapter 6)	+	+
Disease surveillance/necropsy of animals that die in collection (Chapter 9)	+	+

4.7 Staff Training and Implementation of Biosecurity Practices

Animal husbandry staff members are one of the most important means (vectors) by which infectious diseases can be transmitted in an amphibian facility. Therefore, proper staff training and good standard operating procedures are the best methods for the control and prevention of infectious disease problems in captive amphibian conservation programs.

Suggestions that can help animal husbandry staff in carrying out biosecurity practices and that minimize the possibility of error include:

- Development and adherence of user-friendly written Standard Operating Procedures (SOPs) that provide an overview of biosecurity practices. These should be customized for each amphibian facility. The SOPs should be easily accessible to staff members. SOPs should be practical and developed with the consideration for the unique husbandry needs of each facility and the amphibian species being housed.
- Review biosecurity practices with new staff members before they begin working with animals. Annual review of biosecurity practices with all animal care staff is also suggested.
- Advanced training in biosecurity measures and good amphibian husbandry practices can be promoted by specialist educational programs for amphibian keepers. For example, the American Association of Zoos and Aquariums has a model course in Amphibian Biology and Management with the goal to “provide a solid background in amphibian biology as it relates to husbandry, breeding, conservation and cooperative programs” (http://www.aza.org/prodev/Amphibians/).
- Provide husbandry staff with the tools and equipment necessary to manage captive amphibian populations at appropriate biosecurity levels for the role of the species that they care for.

- Errors in biosecurity practices will occur. Encourage a work environment where staff members feel comfortable reporting these errors as soon as they occur. If errors are identified early more can be done to minimize their impact.
- When formulating SOPs design procedures that reduce the amount of staff contact with amphibians (automation). These procedures should still allow staff to regularly observe subtle signs of disease or abnormal behavior. Staff should assume that all enclosures have the potential to contain animals with infectious diseases and follow routines that minimize the possibility of disease transmission (see Section 4.11).

4.8 Long-Term Isolation of Amphibians Destined for Reintroduction to the Wild

Amphibians kept in conservation programs that have a goal of reintroducing captive animals or their progeny to the wild should be permanently separated and protected from other amphibians in a cosmopolitan zoo or other amphibian collections in "long term isolation". Programs that have been established within the range country of the species or species assemblages maintained in the facility and that do not maintain any amphibians from outside the range country are the best and most effective examples of long-term isolation.

- Long-term isolation has also been called "permanent quarantine" by the 2006 CBSG/WAZA Amphibian *Ex Situ* Conservation Planning Workshop (Zippel et al., 2006) or "Quarantine 1" and "Quarantine 2" by the 2008 Association of Zoo and Aquariums Amphibian Husbandry Resource Guide (Kast and Hanna, 2008).

- The term "long-term isolation" is used in this manual to distinguish "permanent quarantine" from the quarantine used when adding new animals to an amphibian collection (see Chapter 6).

Long-term isolation is accomplished by:

- Housing only a single species or species assemblage (an amphibian faunal group that naturally occurs together in the range country) in a freestanding building, or inside of an isolated room or rooms within a building. Details for creating facilities for long term isolation are discussed below.

- The greater the physical isolation of a species or species assemblage from a cosmopolitan amphibian collection the simpler it is to establish and maintain long-term isolation and effective biosecurity practices (e.g., a separate building for long-term isolation is better than separate rooms within a building).

↑ separation of isolated populations = ↓ difficulty of maintaining biosecurity

- Animals in long-term isolation are never housed in the same room with amphibians from outside their native range (e.g., A Mississippi gopher frog from the United States should not be kept in the same room as a Kihansi spray toad from Tanzania).
- Preventing indirect contact with amphibians from outside the native range by potential vectors including animal care staff, cages, substrate, water systems, or tools. In other words, nothing that has come into contact with amphibians from outside the native range should come into contact with animals held in long-term isolation. This involves implementation of specific biosecurity practices that are discussed in detail in Sections 4.10, 4.11 and 4.13 below.

4.9 FACILITIES FOR LONG-TERM ISOLATION

Isolated rooms within a building

The development of dedicated rooms for long-term isolation within a cosmopolitan amphibian facility is a cost-effective method for achieving ISOLATION for captive amphibian populations. This approach may be cost effective, but it is not ideal, because of the increased likelihood that cross-contamination may occur during times of water leaks, flooding, or even simple inadvertent violation of quarantine.

- If dedicated rooms are used the potential for a significant error in facility biosecurity is greatly increased. Animal husbandry staff must be committed to the concept and practice of ISOLATION guidelines for staff work-flow, clothing and footwear, and use of tools and equipment.

The rooms used for long-term isolation can be converted from rooms that already exist within the amphibian facility or can be specially constructed. Special and relatively inexpensive (approximately $ 7000 US) construction of modular long-term isolation rooms in otherwise unused space within a zoo has been described in detail from Omaha's Henry Doorly Zoo (Krebs, 2008; See Appendix 3). Long-term isolation rooms should be considered individual units with modifications that prevent the entry or exit of amphibian pathogens.

Specifications for rooms that reduce disease transmission risks include:

- Rooms should be sealed to prevent escape of water or amphibian waste into adjacent rooms (Section 4.18).
- Rooms should be escape-proof and pest-proof (see Section 4.18).
- Rooms and surrounding corridors should be designed to easily qualify for ISOLATION, with regards to staff work-flow, clothing and footwear (Section 4.18).
- If modular rooms are constructed plans should be made for regular maintenance to prevent breakdown of construction materials (e.g., sealants used between walls and floor as water barrier).

- Consideration should be made of air handling and movement within the facility (Section 4.18).

Separate Long-Term Isolation Buildings

As noted earlier, development and construction of separate long-term isolation buildings further reduces disease risk. A successful approach developed by the Australian Amphibian Research Center for creating inexpensive long-term isolation buildings uses modified large cargo shipping containers. Details on construction of these containers can be found online (http://frogs.org.au/arc/container.php). Shipping containers range from 6–12m in length and are outfitted with independent air cooling/heating, water and electrical systems and designed to utilize husbandry space in an efficient manner. It is possible to retrofit containers prior to installation on-site, and therefore they can be installed with less construction support compared to new building construction. These features make shipping containers a flexible and economical option for many amphibian programs. The units can also be built with viewing windows for exhibition purposes in order to increase public support, funding opportunities, and educational opportunities for the general public.

4.10 Husbandry Staff Hygiene and Protective Clothing

Procedures for amphibian facility husbandry staff hygiene and protective clothing are important for the success of biosecurity protocols. This section makes recommendations for amphibians maintained either with BEST PRACTICES or in ISOLATION. Invitation of visitors to biosecure areas should, in general, be discouraged. When necessary, visitors are to follow the same guidelines as regular staff members.

Footwear and Disinfectant Foot Baths

- BEST PRACTICES dictates that dedicated footwear should be required for each building that houses captive amphibians. It is important that husbandry staff or visitors not enter animal care areas with footwear that has been used outside of the captive facility (e.g., at their homes or in the field).
- In ISOLATION, dedicated footwear is required for each long-term isolation room. Dedicated shoes or boots can be stored within the isolation room. The goal is to avoid tracking organic material or amphibian pathogens from one long-term isolation room (that contains a different species or species assemblage) to another, or from areas of BEST PRACTICES into areas of ISOLATION. An alternative to dedicated footwear is the use of disposable plastic foot covers (e.g., Shubees™).
- Disinfectant foot baths are used in some animal facilities to clean footwear between animal rooms. Foot baths are only effective if footwear is made of easily disinfected material (e.g., rubber boots) and is not heavily contaminated with soil or other organic material and the footwear is exposed to the

disinfectant for the required contact time. Reviews of the use of disinfectant foot baths are available (Morley et al., 2005; Dunowska et al., 2006).

- Foot baths require a high degree of maintenance to avoid the build up of organic materials that inactivate disinfectants and to avoid evaporation of the disinfectant solution.
- Footbaths might be useful when placed at the entrance and exit of biosecure areas to remove primary matter, and to remind staff that they are entering a biosecure area and should remain mindful of biosecurity protocols.
- Disinfectants for use in foot baths include sodium hypochlorite (bleach), Virkon, and F10. Virkon may have advantages for use in footbaths because it maintains greater activity in the presence of organic materials. (See Chapter 5).
- For most situations use of dedicated footwear rather than footbaths is preferable as disinfectants will only kill certain pathogens (depending on agent used and concentration. Furthermore footbaths may introduce a risk to animals in the facility as there is a possibility that animals (e.g. escapees) could come into contact with residual disinfection material on the floor. There is also no detrimental effect on the environment through the discharge of disinfectants if dedicated footwear is used.

Dedicated Clothing

- For BEST PRACTICES dedicated clothing is required for each building that houses captive amphibians. It is important that husbandry staff or visitors not enter animal care areas with footwear that has been used outside of the captive facility (e.g., at their homes or in the field). Dedicated facility uniforms that are regularly laundered are sufficient. Uniforms should be changed if they become wet or heavily contaminated with organic material.
- For ISOLATION dedicated protective clothing is required for each long-term isolation room. In most circumstances it will be sufficient to have a separate laboratory coat or other coverall that is placed over the staff member's regular clothing or uniform. If the regular clothing is wet, dirty or otherwise contaminated with material from outside the long-term isolation room, a full-change of clothing is required before entering a long-term isolation room. Alternatives include the use of disposable protective clothing for each long-term isolation room (e.g., Tyvek ® jumpsuits or surgical "scrubs").

Hand-Washing and Use of Protective Gloves

- Frequent washing of the hands and arms (up to the elbows) with a disinfectant soap is recommended for husbandry staff members as a standard feature in

programs operating in both BEST PRACTICES and ISOLATION. It is especially important to wash the hands and arms:
    - Before entering each ISOLATION rooms or facilities.
    - In-between working on different enclosures as standard part of both BEST PRACTICES and ISOLATION protocols.
- Disposable gloves should be used when handling amphibians or cleaning enclosures.
    - A new pair of gloves should be worn for each enclosure.
    - Non-powdered gloves should be used or gloves should be thoroughly rinsed before handling animals.
    - A recent study (Cashins et al. 2008) suggested that latex and nitrile gloves might be toxic to some tadpoles. Vinyl gloves were also shown to be toxic if they were not rinsed with water prior to exposure. The observation of toxicity associated with glove use has not been consistent and recommendations for addressing this issue have been published (Greer et al., 2009).
    - Toxicity associated with glove use has **not** been observed in postmetamorphic animals.
    - One experimental study shows increased survival times for the amphibian chytrid fungus (*Batrachochytrium dendrobatidis*) on bare hands that had been repeatedly washed with ethanol and water (Mendez et al., 2008). The implications for disease transmission in a natural setting are unclear and hand-washing was still found to be preferable to continuous re-use of disposable gloves.

4.11 HUSBANDRY ROUTINES

The husbandry routines and work-flow pattern used by amphibian husbandry staff are important for minimizing the potential to move pathogens within a captive amphibian facility including:

- Transmission of pathogens between animals kept at different levels of biosecurity.
- Transmission of pathogens between amphibian enclosures.
- Transmission of pathogens between animals in quarantine (see Chapter 6) and animals in an established amphibian collection or animals held in the ISOLATION level of biosecurity.

Animals that are kept in ISOLATION should be cared for first in the day before taking care of animals maintained by BEST PRACTICES. This is also true when animals intended for ISOLATION are brought into quarantine (see Chapter 6). Animals in ISOLATION should never be housed in the same quarantine room as animals from outside their original host range.

Automation of amphibian husbandry tasks such as feeding and cleaning can reduce direct contact time with amphibians and minimizes possibilities for disease transmission. Suggestions for automation are provided in Section 4.18.

A systematic organized routine for the daily care of a collection is highly recommended. The routine must be specific so that if a problem does occur, each step can be reproduced eliminating areas of risk, and determining where exactly the error occurred or pathogen was introduced. For example, when servicing a room always start at the far end of the room and work towards the door, or always work in a clockwise-rotation around the room. A concise list stating the direction in which to proceed with routine husbandry practices would be preferred and should be clearly stated and posted for all who maintain specimens to see and understand. For example, tanks could be labeled sequentially, and the procedure could dictate that one proceeds from:

Enclosure A → Enclosure B → Enclosure C

- Enclosures that contain amphibians that are least likely to be infected with pathogens of concern (e.g., animals that have been in captivity for a long time or animals that have tested negative for *Bd* or other diseases of concern) should be arranged so that they are serviced first in the directional sequence (e.g., Tanks A–D, of 15 total tanks).
- Enclosures and equipment associated with the enclosures (e.g., lighting and filtration units) should be labeled to clearly identify each unit in the sequence of enclosures.
- If sick or dead animals are found during the husbandry routine they should immediately be removed from the enclosure. Dead animals are submitted for necropsy examination (see Chapter 9). Sick animals are removed for veterinary attention. At a minimum, staff members should wash their hands before returning to complete the directional servicing in that room or facility.
- Incorporate these procedures into the facility Standard Operating Procedures (See Section 4.7) and provide standardized training for daily husbandry staff in their implementation.

4.12 ENCLOSURE SANITATION

Regular cleaning of amphibian enclosures is essential at all levels of biosecurity to reduce build-up of organic materials that may increase the risk of infectious and parasitic diseases in captive amphibian populations. Some general concepts of enclosure sanitation that are important from a biosecurity standpoint include:

- The frequency with which amphibian enclosures should be cleaned increases as amphibian biomass (number of animals) and feedings increase.

- Frequency of water changes is dependent on many factors including availability and use of filtration systems.
- Tanks must be made from materials that allow for easy cleaning and disinfecting. Non-porous materials such as glass, fiberglass, or plastic are recommended. Prior to housing any amphibians, these tanks should be cleaned, disinfected, rinsed, and thoroughly dried. The same procedure should be followed when a tank is emptied and stored. Recommendations for disinfectants to use on tanks and equipment are provided in Chapter 5.
- Equipment such as racks, shelves, counters, and also floors should be constructed of materials that are easy to wash/mop, disinfect, and rinse. A regular cleaning and disinfecting schedule of all exposed surfaces is necessary.
- For animals kept in ISOLATION, it is important to wash equipment and enclosures only in sinks or areas that have been carefully disinfected and are free of contaminated materials from other animals in the amphibian collection. This includes materials from other animals held in ISOLATION biosecurity that are from a different geographic region (e.g., tanks used for animals in ISOLATION from Panama should not be cleaned in the same sink as those from animals in ISOLATION from the United States without complete disinfection). Alternatively, ISOLATION rooms can be constructed with dedicated sinks for each room.

4.13 EQUIPMENT, ENCLOSURE SUBSTRATES, AND CAGE DECORATION

The tools, equipment and enclosure substrates used in an amphibian facility can be important vectors for the introduction of new pathogens to a captive population or for the transmission of pathogens between enclosures and different groups of animals.

Recommendations for reducing these risks include:

- ISOLATION rooms should have a dedicated set of equipment (e.g., nets, forceps, suction tubing, scrub brushes, sponges etc.). This equipment does not leave the individual isolation room and is never used on a different group of animals.
- For both the BEST PRACTICES and ISOLATION protocols the husbandry staff should assume that all amphibians and amphibian enclosures are a potential source of pathogens that can be transmitted to another enclosure. This is regardless of the presumed health of the animals. For example, an amphibian might appear to be very healthy, but can still act as a carrier of amphibian chytrid fungi.
    - Tools and equipment are cleaned and disinfected between use in different enclosures (e.g., a net used in Enclosure A is disinfected before use in Enclosure B); alternatively, nets or other minor tools may be dedicated per enclosure.
    - Multiple sets of equipment may be necessary (one set of equipment can be disinfected while the other set is in use). Tools are labelled to corresponding tanks or rooms for easy recognition.

    - Guidelines for disinfection of tools and equipment are provided in Section 5.5). Care should be taken not to disinfect or clean enclosures or equipment from one group of animals in the same sink or area as equipment from a different group of animals without first cleaning and disinfecting the sink or cleaning area.
- Enclosure substrates, cage furniture and decorations (e.g., plants and rocks) of any kind are not moved from one enclosure to a different enclosure without disinfection. Some enclosure substrates such as sphagnum moss or soil should be considered disposable and not moved between enclosures. Porous materials such as wood or cork are difficult to disinfect. Guidelines for disinfection of substrates and plants are provided in Section 5.6–5.7.
- Whenever possible tools, equipment and cage furniture should be made of materials that are easily cleaned and disinfected (e.g., plastic, metal, glass).
- Use of natural materials (e.g., soil, gravel, rocks, plants) in amphibian enclosures can be important for supporting normal behaviors (including breeding), reducing stress and for decoration of display enclosures in zoos.
    - Natural materials do have the potential to be contaminated with amphibian pathogens and the source of these materials as well as disinfection should be carefully considered before placement into an enclosure.
    - When selecting natural materials for use in enclosures the likelihood that there has been exposure to native or non-native amphibian fauna, insecticides and fertilizers should be considered.
    - Materials from areas with known amphibian disease problems should be avoided (e.g., plants from the site of an ongoing outbreak of chytridiomycosis). Plants that have been grown hydroponically (in water) or in a dedicated greenhouse (without native amphibian colonization) may reduce disease risks. The use of artificial (plastic or silk) plants can also be considered especially for use in the animals in ISOLATION.
    - Suggestions for disinfection or sterilization of natural materials are found in Section 5.6

4.14 WASTEWATER DISPOSAL

Disposal of wastewater is an important biosecurity consideration in the development and management of captive amphibian facilities. Best practices require that pathogens not be discharged into the environment. The most important biosecurity concern for wastewater disposal is the potential to introduce non-native amphibian pathogens to new geographic locations by discharge of untreated wastewater to the environment.

- For facilities that keep amphibians from outside the native range (e.g., Panamanian amphibians kept in the United States) or that conduct research with

amphibian pathogens wastewater biosecurity measures are needed (details below). This applies to animals under either with BEST PRACTICES or in ISOLATION. It is not acceptable to release wastewater to the local environment.
- A facility that only keeps amphibians from inside the native range (e.g., southern Japanese amphibians kept in southern Japan) usually does not need to consider wastewater biosecurity measures.

Implementation of water biosecurity measures can be complicated and expensive and is a major reason why keeping captive amphibians within the natural range country of the species is strongly preferable. Unfortunately, amphibian wastewater biosecurity is a controversial topic because much of the information needed for evidence-based decision making is simply not available. Organization of a working group to perform a risk-assessment of amphibian wastewater management is sorely needed. This working group should include experts in wastewater treatment (especially experts from intensive aquaculture), sanitary sewer systems, environmental microbiology, amphibian disease, and amphibian facility design/husbandry.

Factors that must be considered for wastewater biosecurity include:

- The amount of water that must be treated (e.g., a small captive breeding facility can accommodate different water treatment methods than a large commercial facility that raises frogs for food).
- The infrastructure of general wastewater (sewage) treatment for a geographic region or country.
- Financial and other resources available.

There are a variety of wastewater treatment and disinfection options available for installation in a captive amphibian facility. Each option has its own advantages, disadvantages, and resource requirements. No water treatment option is absolutely failsafe for removal of potential amphibian pathogens and options are especially limited when large quantities of water effluent must be treated.

Options for wastewater treatment include:

- **Modern municipal wastewater (sanitary sewer) system.** Modern sanitary sewage treatment systems are adequate for disposal of wastewater from many small to medium sized amphibian facilities without additional disinfection and at low risk to wild amphibian populations. For instance, in many areas it is considered acceptable to directly release liquid infectious waste from humans and domestic animals into the sanitary sewer. However, the sanitary sewer option has limitations that need to be seriously considered:
    - It is not acceptable to discharge large amounts of waste or large quantities of infectious agents into the sanitary sewer without disinfection. Very large amphibian facilities (e.g., frog farms), facilities

experiencing outbreaks of infectious disease, facilities that regularly import large numbers of frogs from outside the range country and facilities conducting infectious disease research should take additional measures to disinfect water before discharge into a sanitary sewer.

- It should not be automatically assumed that local sanitary sewer systems are adequate for biosecurity. Local systems should be individually evaluated for effectiveness and reliability. For instance, the actual treatment facility may be adequate, but the plumbing (sewers) that feed the system could be prone to failure and discharge of infectious material into the local environment. In other cases, the local storm drains are connected to the sewage drains and when it rains, the treatment facilities are overwhelmed from storm water and discharge untreated sewage into the environment.

- **Processes for Disinfection or Sterilization of Wastewater.** There are a variety of options for treatment of wastewater to inactivate infectious agents prior to disposal either in a sanitary sewer or to the environment include application of chemicals (sodium hypochlorite "bleach"), physical agents (heat and pressure), ozonation, and ultraviolet radiation. Details and limitations of some of these methods are provided in Section 5.8 and in Appendix 2.
  - Automation of the water disinfection methods in a facility is suggested. Automation minimizes human error; ensures that disinfectant concentrations are appropriate; ensures that contact time with disinfectant is adequate; and makes wastewater treatment simple for animal staff to implement. An automated wastewater disinfection system (AWWDS) for amphibian captive breeding facilities (using sodium hypochlorite) has been described (Robertson et al., 2008). Manual methods for water disinfection using sodium hypochlorite have also been described (Krebs, 2008).
  - Water disinfection systems and protocols for use in large-scale commercial aquaculture are a valuable source of information for system design and implementation. Many of these systems are based on ozonation of wastewater (Schuur, 2003). Large scale amphibian breeding facilities or farms should follow the guidelines of the World Organization for Animal Health (OIE) for disinfection of effluent wastewater in aquaculture facilties (www.oie.int/eng/normes/fmanual/1.1.3_DISINFECTION.pdf).
  - Water disinfection systems should be designed to minimize introduction of toxic chemical contaminants (such as chlorine or chloramines) if wastewater is discharged directly to local environments. For instance, chlorine can be neutralized by treatment with sodium thiosulfate (Browne et al., 2007) or by exposure to UV radiation (Robertson et al., 2008).

4.15 Solid Waste Disposal

The most important biosecurity goal of procedures for solid waste disposal in captive amphibian facilities is the same as for wastewater disposal—to prevent the introduction of non-native amphibian pathogens to new geographic locations. Therefore considerations for solid waste disposal are most important for facilities that keep amphibians from outside of the native range or that conduct research with amphibian pathogens. Solid wastes from these facilities should not be discharged into local environment without treatment.

Options for disinfection or sterilization of solid wastes prior to disposal include:

- Autoclaving.
- Incineration (not environmentally friendly).
- Alkaline hydrolysis (amphibian tissues or carcasses).

Deep burial or transfers to a modern landfill are also acceptable options for waste disposal without the need for disinfection. The key is to contain infectious material and prevent native amphibians from becoming exposed to this material.

4.16 Sources of Water

The source and composition of water for an amphibian facility is a critically important consideration for the success of any captive amphibian conservation program. Details on water sources, quality and treatment for amphibian facilities have been reviewed in a recent publication (Browne et al., 2007). Items that must be addressed include pH, water hardness, trace elements, presence of potential toxic metals (e.g., copper) and the presence of potentially toxic additives (e.g., chlorine or chloramines in municipal water supplies or environmental contaminants such as pesticides).

- It is important that water used in a captive amphibian facility be free of important amphibian pathogens. This is true for both BEST PRACTICES and ISOLATION programs. If modern municipal water supplies are used as an initial source for facility water, the risk of disease introduction is very low. If natural water sources are used, consideration should be given to disinfection of incoming water prior to use in amphibian enclosures. Potential methods of disinfection include application of physical methods (e.g., heat and pressure) and filtration (see Section 5.8).

4.17 Sources of Food

Food items offered to amphibians in captive facilities are a potential source for introduction of pathogens to a population. This is a concern for animals maintained both with BEST PRACTICES or in ISOLATION. The extent to which food items could be a source of amphibian pathogens is unknown. Most likely food items act as a mechanical vector

for disease transmission (e.g., wild-caught insects or aquatic invertebrates used as food are wet and transfer water with infective zoospores of the chytrid fungi into the captive facility), rather than becoming infected with these pathogens and serving as a long-term source of infection for the amphibian population (Rowley et al., 2007). A clean, reliable and trusted source of food items is desirable for all facilities that keep captive amphibians.

- When possible invertebrate food items should be cultured on-site at the amphibian facility. Aquatic food items (e.g., *Daphnia* or brine shrimp [*Artemia*] should be started from eggs if possible.
- If food items are purchased from a commercial supplier, the amphibian facility should make sure that practices that reduce disease risk are used. Ideally, food items should be reared indoors without the potential for exposure to the elements, amphibians of any kind (wild or captive) or to wild insects. The supplier's facility should be clean and use high-quality materials.
- There are potential benefits to feeding wild-caught food items to captive amphibians (e.g., superior nutrition or the amphibian species has unique dietary requirements and preferences). These benefits must be considered with the disease risks when making decisions for a captive population. Animals kept in ISOLATION should not be fed wild-caught food items from outside of their native range (e.g., Panamanian frogs kept in a survival assurance population in the United States should not be fed wild-caught insects from the United States).
- If wild-caught food items are fed to captive amphibians, these food items should only be collected from areas that are known to be free of pesticides or other chemicals. Some pesticides can persist in the environment for several years, so it is recommended as a general (and admittedly arbitrary rule) that food insects be collected at least 0.5 km away from any areas where pesticides have been applied in the past three years.
- Wild-caught food items should not be collected from locations known to be experiencing outbreaks of amphibian infectious diseases (e.g., do not collect aquatic invertebrates from a stream where amphibians are dying of chytridiomycosis).

4.18 Facility Design and Building Specifications

Amphibians have special requirements for water systems and filtration, climate control and light that are essential for overall animal health and success of breeding colonies in captivity. Complete overviews of amphibian facility design and husbandry standards have been recently published and these resources should be consulted when designing or renovating a facility (Browne et al. 2007; Pramuk and Gagliardo, 2008).

Elements of facility design that are important for biosecurity are highlighted below:

- **Facilities should be pest-proof.** Free-ranging pests (e.g., cockroaches, ants, or rodents) can occur in any captive amphibian facility. Dangers of pests include direct injury or killing of amphibians; destruction of enclosures which allow for amphibian escape; and transmission of amphibian pathogens (either between amphibians within the facility or to wild amphibians outside of the facility). Facility design features to minimize risks from pests include: tight-fitting gaskets on doors and windows; self-closing doors to amphibian rooms; screened floor drains; and addition of baffles to air conditioning units. Physical pest control methods can be used within the facility (e.g., insect traps and fly paper). Chemical pest control methods should be avoided because of the risk of poisoning the facility amphibians.
- **Facilities should be amphibian-proof.** Facilities and enclosures should be designed to prevent the escape of captive amphibians and prevent the entry of wild amphibians from outside the facility. This is most important for amphibians kept in ISOLATION.. The risk in this situation is that non-native amphibians could escape from the facility and introduce non-native amphibian pathogens to native wild amphibians or that native amphibians could introduce new pathogens to non-native amphibians in the facility. BEST PRACTICES dictate that native amphibians could still introduce a pathogen that has been controlled or eliminated in the captive population (e.g., the captive population has been treated and cleared of infection with chytrid fungi, but local wild amphibians could serve as source of re-infection of the captive animals). An example of amphibian-proofing is to add screen coverings to drains in amphibian rooms.
- **Husbandry routines should be automated.** Using automated systems for draining enclosures, adding water to enclosures, filtration and feeding animals are encouraged to minimize keeper/animal contact and reduce probability of human errors. Automation is an important measure that can reduce potential for disease transmission between individual enclosures. For feeding, a funnel can placed through each enclosure lid and secured to allow for the feeding of prey items into the enclosure without the need for the keeper to open, or otherwise contact the enclosures. The funnel neck should be small enough and placed appropriately so that it does not allow the amphibians to escape; the funnel can be capped after prey items are introduced to prevent their escape as well. Details on facility automation can be found at the Amphibian Research Center website (/frogs.org.au/arc/features.php).
- **Air distribution systems.** Airborne transmission of significant amphibian pathogens has not been documented to date. Although the focus of facility biosecurity efforts should be on movement and introduction of pathogens by contact with husbandry staff, other amphibians and substrates and tools, the potential for airborne transmission should not be entirely ignored. For facility design, air distribution systems that prevent air from being forced between rooms are desirable. Shared ductwork between different rooms that hold animals at the ISOLATION should be avoided.

- **Water proofing and sanitation.** Amphibian facilities are wet and humid environments, therefore walls, ceilings, and floors must be waterproof—both to maintain the integrity of the room or building, and to facilitate regular cleaning and disinfection of those surfaces. In addition, seals help to prevent leakage of amphibian wastes from areas containing cosmopolitan collection animals or other amphibians from different geographic regions into rooms used at for animals held at ADVANCED biosecurity levels. Drywall (plasterboard) and cellulose ceiling materials are difficult to disinfect and are not recommended.
- **Design of facilities to accommodate the needs of ISOLATION.** Rooms that hold animals held in ISOLATION are greatly improved by design features such as anterooms that allow for easy change of clothing and footwear prior to entry and by placement of sinks in the individual rooms to eliminate the risk of using communal sinks (used to clean tools and materials from cosmopolitan collection animals or different groups of animal held in ISOLATION).

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