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Methodologies for the Care, Maintenance, and Breeding of Tropical Poison Frogs

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The Biodiversity Laboratories at Saint Joseph's University house more than 100 exotic and endangered animal species—including insects, fish, amphibians, and reptiles. Research in the laboratories is devoted, primarily, to understanding the genetic and ecological bases of animal behavior. In addition, a common theme of the work is the development of techniques for the successful care of exotic animals within a laboratory setting. Such techniques may be critical to studies aimed at understanding the life histories of endangered species and saving them from extinction. Current projects to study the biology of tropical poison frogs have methodologies to improve captive care, maintenance, and breeding. In this article, I present these methodologies and their impact on the welfare of captive poison frogs.

Interest in amphibian species has grown in recent years, stemming from evidence of a global decline in wild amphibian populations (Drost & Fellers, 1996; Hero & Gillespie, 1997; Houlahan, Findlay, Schmidt, Meyers, & Kuzmin, 2000; Pounds & Crump, 1994; Wake, 1991). Various factors have been implicated in this decline, including habitat loss, increases in ultraviolet light, fungal pathogens (Berger et al., 1998; Blaustein et al., 1994; Kiesecker, Blaustein, & Belden, 2001); the effects of chemical pollutants such as herbicides (Hayes et al., 2002); acid rain (Bradford, Graber, & Tabatabai, 1994); and the effects of global warming (Pounds & Crump, 1994; Pounds, Fogden, & Campbell, 1999). Although there is some controversy surrounding the exact cause of losses to specific populations, it is clear that changes in environmental conditions are resulting in the declines and extinctions of many amphibians.

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Efforts to understand the losses of amphibians as well as efforts to save them largely depend on developing an understanding of the life history of specific species. Life history parameters can be studied in nature and also in the laboratory where controlled experiments on the effects of specific environmental factors can be conducted. However, laboratory study requires methodologies to maintain and breed different, and often exotic, species under conditions that foster healthy, normal animals. Unfortunately, little information is available about the captive maintenance and breeding of many amphibian species whose needs are complex and often species specific.

Based on concerns about amphibian losses and the lack of information regarding captive care, a project has been initiated in the Biodiversity Laboratories at Saint Joseph's University to establish amphibian colonies and to use the animals in these colonies for detailed life history analysis. The work began with studies on the green-and-black poison frog (*Dendrobates auratus*), an inhabitant of the lowland rainforests of northeastern Costa Rica. Following studies on wild populations in Costa Rica, colonies of *D. auratus* were established in the laboratory. Techniques were developed to house, feed, and breed adult frogs and to maintain and study their tadpoles in captivity. Since the beginning of the project, captive colonies of other tropical poison frogs, including members of the genuses *Epipedobates, Phyllobates,* and *Mantella,* have been established as well, using the same basic methodologies as those for *D. auratus.* In this article, I present the techniques developed for the maintenance of poison frogs in captivity, hoping that they will provide valuable husbandry information for the care and study of these animals in the laboratory.

METHOD

Housing of Adult Frogs

Adult frogs are housed in large (475 L) glass aquaria (see Figure 1). Bricks are placed into each tank to create an aquatic sump beneath the area where the frogs live. On the bricks is a sheet of material sold in hardware stores as a light diffuser for fluorescent lights. This material (from this point known as "platform material"), is made of strong 1.3 cm wide plastic with 1.3 cm spaces to allow water to flow from the sump (underneath) to the substrate (above). Covering the platform material is a tight mesh, plastic screen that prevents the substrate material (soil and sphagnum moss) from falling into the sump. Substrate material, approximately 6 cm deep, covers the entire platform.

The living area for the frogs contains a variety of items used to create a complex environment that mimics the ground layer of a tropical forest. Plants (such as Pothos, orchids, and ferns) are planted directly into the substrate material. Water

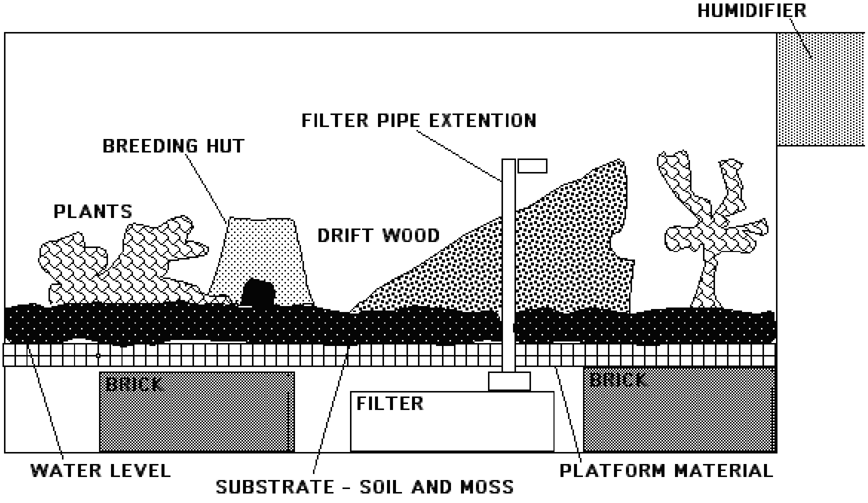

FIGURE 1 Tank design for adult frogs.

from the sump keeps the substrate moist; therefore, only plants that tolerate high moisture levels are used.

Driftwood and small rocks are added to the living area to act as hiding places for the frogs and as surfaces for the plants to grow. A pipe from an underwater filter (in the sump) carries running water to the living area and creates a small waterfall. Running water increases the humidity of the tank and seems to encourage breeding behavior.

The top of each adult tank is covered with glass to maintain high humidity levels. The adult frogs are maintained at 22 to 25°C under fluorescent lights on a 12:12 light–dark (LD) cycle.

Feeding of Adult Frogs

Adult frogs are fed a variety of live foods three to five times per week, including flightless fruit flies (*Drosophila melanogaster* for smaller frogs and the slightly larger *Drosophila hydei* for the larger frogs) and small pinhead crickets. The *Drosophila* stocks are maintained in mass culture in glass milk bottles on Carolina Instant *Drosophila* Media (Formula 4–24; Carolina Biological Company, Burlington, NC). Crickets are obtained from a cricket distributor. All insects are dusted with a multivitamin powder prior to being offered to the frogs.

In addition to adult flies and crickets, a system has been devised to provide the frogs with *Drosophila* larvae, which may increase the fat content of their diet. To accomplish this, certain *Drosophila* stocks are raised in large (2 L) plastic soda bottles that contain *Drosophila* Instant Media. Approximately 10 days after adult flies have been placed into the soda bottles (at 25°C), the large, third-instar larvae climb up the sides of the bottle to prepare for pupa formation. At this point, the soda bottles are cut with a razor blade, removing the top and bottom of the bottle. This creates a plastic tube, covered with larvae that can be placed directly into a frog tank. The frogs climb in and around the tube, feeding on the larvae.

A second step in the larvae methodology increases the efficiency of the process. The bottoms of the plastic soda bottles (removed in the process just described and that still contain *Drosophila* media and hundreds of larvae) are placed into glass aquaria. Clean plastic tubes (created by cutting smaller, 1 L soda bottles) are placed directly into the food and soon become covered with larvae. These plastic tubes are removed and placed into the frog tanks for feeding. The collection of larvae continues in this fashion for about 1 week, at which point new 2-L bottles are ready to cut, and the entire process begins again.

Breeding Adult Frogs

Increasing the humidity of the adult enclosures (using humidifiers attached to the adult tanks) encourages calling in the males and breeding behavior. Breeding and egg deposition typically occur in the "breeding huts" located in the adult enclosures. Breeding huts are made of plastic flowerpots (or other plastic containers), turned upside down, with a small doorway to allow the frogs to enter. Each breeding hut contains a plastic petri dish that holds plastic leaves (from plastic plants sold for fish tanks). Female frogs deposit eggs on the surface of these leaves.

Egg clutches are removed from the adult tanks as soon as they are discovered. Petri dishes containing eggs are moved to small (7.6 L) aquaria where the eggs are observed until hatching. Aquaria used to hold eggs contain a small amount of water and have a glass cover to create a humid environment and are maintained between 25 and 30 °C. Developing tadpoles are easily seen through the clear gelatinous eggs and show distinct signs of movement when they are close to hatching. Tadpoles fall into the water in their petri dishes when they hatch and then are moved into the tadpole tanks (see following).

Tadpole Care and Study

A common method for raising poison frog tadpoles involves placing individuals in small containers holding small amounts of water. The tadpoles are fed in these containers. Clean water is maintained by pouring off old water each day and replacing it. However, because of the large numbers of frogs maintained in the Biodiversity Laboratories and the requirements of a project to monitor the effects of water temperature on growth, a new methodology needed to be developed. To this end, special tadpole tanks were developed that allow large numbers of tadpoles to be raised, in small groups or in total isolation, under conditions in which air and water temperature can be carefully controlled, water circulates and is filtered, animals are easily fed, and the growth of individual organisms can be monitored. Water circulation needs to be high.

Each tadpole tank (see Figure 2), is created from a standard 114 L breeder aquarium (91 cm long × 46 cm wide × 43 cm deep). Bricks placed into the tank raise up the tadpole holding area sufficiently to allow for a large volume of water to be held in the tank. A sheet of platform material placed on the bricks supports the tadpole containers. The containers used to house individual tadpoles are opaque plastic freezer boxes (10 cm × 10 cm × 13 cm). Containers used to hold small groups of tadpoles are small plastic buckets (18 cm in diameter). Tadpole containers are attached to the platform material using silicon aquarium cement. Thin slits (1 to 2 cm long) are cut near the base of each container to provide for water flow without allowing the tadpoles to escape. Larger holes also can be used and then covered with fine mesh (from a fish net) if greater water flow is required.

The platform material, with attached tadpole containers, is placed on the bricks and weighed down with one to two bricks. Water then is added to the aquarium such that the water level in each tadpole container is approximately 1 cm. As the tadpoles grow, water is added to the aquarium, thereby increasing the water level in each tadpole container.

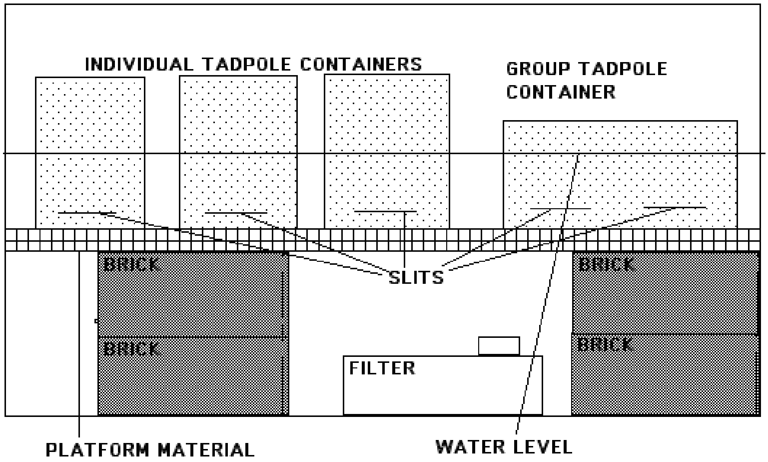

FIGURE 2 Tank design for tadpoles.

Filtration is provided by two to three underwater filters placed under the platform material. The addition of an external filter on one side of the tadpole tank can increase filtration and water flow.

A piece of glass placed on top of each tadpole tank increases humidity. An underwater aquarium heater placed in the bottom of the tank regulates water temperature (typically 22 to 30°C). Full-spectrum fluorescent lights, operating on a 12:12 LD cycle, provide illumination.

Care for the tadpoles in the tadpole tanks is relatively easy. Air temperature, humidity, water temperature, and water cleanliness all remain relatively constant because each individual tadpole container is connected to the large body of circulating, filtered water. Daily care involves feeding the animals and adding water to each tank. The tadpoles are fed a mixture of finely ground flake fish foods that contain fish meal, algae, and spirulina. Each tadpole container holds a few aquatic plants (typically Elodea) that provide substrate for the growing tadpoles and on which the tadpoles may feed. Finally, 10 to 15 guppies are maintained in the large body of water within each tadpole tank. These fish, who cannot enter the individual tadpole containers, help to remove excess food and waste and also act as "mine-shaft canaries," giving immediate indications of problems within any tank.

Although the filtration and circulation of water within the tadpole tank seem to take care of most problems associated with the buildup of toxic materials, excess food occasionally accumulates in the individual tadpole containers. To remove the excess food, the entire platform can be lifted, allowing water from each tadpole container to drain through the slits into the tank. Replacing the platform allows fresh water to flow into each container.

DISCUSSION

The methodologies presented in this article have been successful in the captive maintenance and breeding of tropical poison frogs. The animals in the captive colonies at the Biodiversity Laboratories appear to be healthy and active, the mortality rates are extremely low, and the frogs produce viable eggs that develop into healthy tadpoles and then into healthy frogs.

The creation of the captive colonies of tropical frogs have led to a number of studies on the effects of environmental cues on life history. The initial results of one study, directed at understanding the effects of temperature on survival and metamorphosis in *D. auratus* tadpoles, are tantalizing. The temperatures that led to the highest survival and metamorphosis rates in captivity (22 to 26°C) were similar to the temperatures found in the bodies of water in which tadpoles of this species develop in Costa Rica. Frighteningly, relatively minor increases in water temperature (31°C) significantly lowered survival rates and increased the length of time until metamorphosis. These data suggest that the temperature increases predicted by some global warming models could be disastrous to amphibian populations already under extreme environmental pressure.

Methodologies for the care, maintenance, and breeding of exotic frogs in captivity may hold hope for keeping these, and other, species alive and increasing our understanding of the life histories of these animals in an attempt to save them. Our understanding of the needs of animals in captivity has made great strides in recent years. In addition to meeting basic needs such as food and shelter, environmental enrichment has been shown to have strong effects on the well-being of the captive animals (Seidensticker & Forthman, 1998). Observations of the frogs in the Biodiversity Laboratories indicate that their health and welfare are well served by the conditions described in this article. The highly enriched habitats—containing wood, rocks, flower pots, live plants, and flowing water—offer the animals a complex environment with numerous places to hide. These conditions appear to foster natural, active behavior. The underwater sumps maintain humidity levels similar to those found in the tropics, and the complex food sources maintain healthy, colorful animals. Finally, the frogs exhibit natural behaviors such as calling to attract mates and wrestling to establish and defend territories. All these observations indicate that the large complex habitats are suitable to the success and welfare of these animals in captivity.

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