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Captive Reproduction of the Orange-legged Monkey Frog (*Phyllomedusa hypocondrialis*), and Development of a Protocol for Phyllomedusine Frog Reproduction in the Laboratory

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INTRODUCTION

The orange-legged monkey frog (*Phyllomedusa hypocondrialis*) is a small, neotropical hylid that ranges east of the Andes mountains in South America, from Colombia, Venezuela, the Guianas, and Suriname, southward to Argentina, Bolivia, Paraguay, and southeastern Brazil (Frost, 1985). Among others studying the reproductive biology of these frogs, Budgett (1899) made observations in the Paraguayan Chaco, while Pyburn and Glidewell (1971) studied them in Colombia.

Phyllomedusine frogs, with few exceptions, oviposit on vegetation above bodies of water (Duellman and Trueb, 1986). In the subfamily Phyllomedusinae, frogs in the genera *Phyllomedusa, Phrynomedusa, Phasmahyla,* and *Hylomantis* all create funnels around their egg masses by rolling the leaf that eggs are oviposited upon around the clutch. Working from the leaf's bottom to the top, the frogs fold the edges of the leaf or leaves together with their feet while laying eggs (Pyburn and Glidewell, 1971; Pyburn, 1980; Duellman and Trueb, 1986). The edges of the leaves stick together with adhesive residue from the egg laying process. The clutch is commonly concealed from view by the cylindrical leaf funnel. Egg development usually takes from seven to 13 days, after which tadpoles hatch and "slide" through the funnel, falling out of the lower opening into the water below (usually a temporary pool of stagnant water). In *P. hypocondrialis*, a preference for slightly drier, more arid environments is in contrast with many other phyllomedusines. Eggless capsules, filled with water, are deposited alongside the eggs to help hydrate developing larvae (Pyburn, 1980).

Owing to the relative ease of reproducing *P. hypocondrialis* in captivity, a protocol for reproducing phyllomedusine frogs in the laboratory can be made using this species as an example. In captivity, phyllomedusine frogs are best reproduced via simulation of the seasonal weather patterns of the habitat from which they come (especially if the adults are wild caught). Most of the areas inhabited by these frogs experience seasonal change through some form of a "wet season" and a "drier season." The simulation of these conditions has led to the successful reproduction of phyllomedusines in the laboratory.

MATERIALS AND METHODS

My frogs have been housed in tall vivariums with screen tops and/or side screen doors to allow for ample ventilation. To maintain some degree of humidity and moisture for species inhabiting damper habitats, half of the screen top was covered by a piece of thin glass and the enclosure was misted with clean, fresh water once every three or four days. The exact size of the vivarium can vary according to how many frogs it is to contain. A group of 15 adult *P. hypocondrialis* was accommodated in an enclosure that measured 46 x 46 x 76 cm (l x w x h). Sturdy plants were provided with leaves strong enough to easily support the weight of an adult, female frog. A shallow water dish (about 2.5 cm in depth and 7-12 cm in dia.) with clean, dechlorinated, dechloramined water was available to the frogs at all times. The substrate consisted of seedling orchid bark at a depth of about 3-5 cm. Fluorescent light bulbs, intended for plant growth or simulation of sunlight, were placed above the enclosure and main-

tained on a 12L:12D photoperiod by means of a timer.

Small crickets, shorter than the width of the frog's mouth, were provided every three to five days, in a proportion of four to six crickets per frog. The crickets were placed into "feeding dishes" that measured 10-15 cm deep and 10-15 cm wide. The dishes were made of a substance like smooth glass from which crickets could not escape. Once every eight to 10 feedings, the crickets were dusted with Herptivite® reptile vitamins, and every 16 to 18 feedings with Rep-Cal® calcium supplement. Nighttime temperatures were 20°-22°C. Daytime temperatures of 24°-26°C were attained through the use of an incandescent light bulb above the enclosure.

In pre-reproduction conditioning of the adult frogs, it was important to allow the enclosure to dry a bit to achieve a lower humidity. Misting of the enclosure was terminated. Fresh, clean water was always available in the water dish to avoid desiccation. To enhance "dry season" simulation, temperatures in the enclosure were gradually raised from regular daytime temperatures to about 29°-32°C over the course of two weeks. Methods for temperature increase can include the use of a hanging lamp with a 100W bulb being slowly lowered closer and closer to the top of the enclosure (over the course of the two weeks). Alternatively, a heating pad, with a thermostat, attached to the underside of the enclosure, can be adjusted to gradually increase in temperature. Food was made available at all times. Once the higher daytime temperatures had been achieved, the conditions were continued for another 20 days while the frogs gained weight.

A rain chamber was used to simulate the rainy season. The plexiglass (acrylic) rain chamber for this experiment measured 1.22 x .61 x 1.07 m (this large size is not necessary for *P. hypocondrialis*, which will reproduce in a chamber of the dimensions previously described for a housing enclosure). A screen top was provided for good ventilation. The bottom of the chamber was plumbed with an intake vent through which water passed to a mechanical filtration unit (filtering particulates from the water). The water reentered the chamber through one of two exhausts: the first returned the water directly to the water reservoir in the bottom of the chamber, while the second transferred the water to sprinkler heads that sprayed down through the chamber from the top. Ball valves (made of PVC) allowed for the transition of the water flow between the two exhausts. Additionally, PVC pipe, 1.3 to 2.5 cm in diameter, was used for all water transport to and from the filtration unit and the sprinkler heads. Bulkhead fittings were used at the water intake site and the exhaust site flowing directly into the water reservoir from the filter. A Lifeguard® canister filtration unit was used, powered by a March MDXT-3® external water pump. Four ceramic plant pots, 10.2 cm in diamater, were placed upside down on the bare bottom of the chamber to act as stands for a "false bottom." A sheet of plastic "egg crating", exactly the dimension of the inside of the chamber, and covered with soft, mesh screening, was placed on the flower pots, effectively creating the new bottom level of the chamber. The water level was shallow, being only 6 mm above the mesh screen. This false bottom prevented frogs from being swept through the flow of water, generated by the filtration process, and drowned. The shallow water depth of only 6 mm helped to further reduce the chance of drowning.

Plants were grown hydroponically and were placed in ceramic plant pots with their roots surrounded by aquarium gravel. These plants and pots were set on the false bottom. Plants with leaves approximately two to three times the length, and no more than three times the width of the female frogs, were used. Additionally, flexible leaves were important so as to allow for funnel formation. New plants were carefully washed, especially the leaves and roots, to ensure no unwanted chemicals entered the system. Several species of plants were offered so as to provide multiple options in leaf type for female frogs looking for oviposition sites. Fluorescent lights, intended for plant growth, were on a 12L:12D photoperiod, which was controlled by a timer. The lighting unit was hung from the ceiling above the rain chamber. Water temperatures were no cooler than 16°C and no warmer than 21°C. The filter was allowed to run 24 hours per day to maintain water quality and chemistry. When frogs were absent from the chamber, the sprinklers were activated every two or three days to maintain appropriate moisture levels in the gravel for the plants. Frequent water changes of the water reservoir

were made to ensure "fouled" water would not be rained down upon introduced occupants. Clean water chemistry and quality were of particular importance to the successful usage of the rain chamber.

After all preconditioning was completed with a group of frogs, they were introduced to the rain chamber and allowed to acclimate, without disturbance, for one or two days. All introduced females had attained considerable body weight from the heavy feeding schedule prior to being introduced. A daytime temperature reduction of about 5°C was important in accompanying the rain cycles. Daytime temperatures in the rain chamber did not exceed 23°-24°C. Rain cycles were initiated approximately one hour before the timer turned the lights off at the end of the second or third day after introduction to the rain chamber. It was important not to blast the frogs with "strong rain downpours" as stress would result, hindering the likelihood of a successful reproductive attempt. Light showers were always preferred. The first days of raining were short with showers continuing only for one or one-and-a-half hours after the lights had gone out. Over the course of the following several days, the rains were initiated at the same time, but they were allowed to continue for longer and longer periods after the lights had gone off each day. The lengthening of the rain cycle increased to five hours of rain including the one hour prior to the lights turning off. The length of this cycle was maintained until completion of the attempt.

Careful examinations of the rain chamber were made daily for funnels. Funnels were culled by clipping the leaves from the plant and removing them from the chamber daily. Figure 1 depicts a newly created funnel. This examination was collaborated with extensive observations, after the lights had gone out, to monitor which animals were calling, when they called, which females appeared swollen and "ripe" with eggs, and which animals engaged in amplexus. These observations allowed for an approximation of when funnels would be present and which spent females were to be removed.

Funnels collected from the rain chamber were contained in a 1 L glass jar, and were suspended 2.5 cm above clean water. The metal lid to the mason jar was replaced by a piece of cheese cloth. A piece of thread was then tied at

Figure 1. A newly created funnel. Funnels should be culled from the rain chamber daily so as to prevent raining on them and increasing the likelihood of fungal development in the clutch. (Photo by Danté Fenolio).

one end to the cheese cloth, at the other the funnel leaf. The cheese cloth was secured in place by screwing the metal ring top to the jar, over the cloth, into a tight position, thus suspending the funnel over the water. Half of the opening to the jar was taped over to increase humidity for the funnel yet allow for ventilation. The jars were kept at 23°-26°C. The funnel jars were carefully monitored on the seventh, eighth, and ninth days (sometimes longer) for the first falling tadpoles. As soon as a tadpole had hatched and fallen into the water below, the funnel was "artificially hatched" for best results. The funnel was removed from the jar and carefully opened at the seam. Figure 2 depicts an opened funnel where several tadpoles had hatched out, indicating it was ready to artificially hatch. The exposed clutch was lightly misted with clean water from a spray bottle so that hatching tadpoles fell from the mass down onto a predampened ceramic plate. The wriggling tadpoles were left on the plate to struggle

for several minutes and then introduced into a tadpole rearing facility. Tadpoles allowed to struggle on a plate had a higher survival rate than tadpoles allowed to fall directly into the water below.

Tadpole rearing facilities included fish tanks or small, fiberglass fish ponds. Both were equipped with biological sponge filtration units which had been in operation prior to introduction of tadpoles for at least one-and-a-half months. Some living organisms (plants, fish etc.) were present in the facility to establish the filter's biological system. All organisms were removed from the enclosure prior to introduction of tadpoles. An aquatic moss (*Vasicularia* sp.) was included in the enclosures to provide shelter and possibly an additional food source for tadpoles. Water temperatures were maintained at 24°-28°C using submersible aquarium heaters. Water changes of 30-40% were performed every two to three days depending on tadpole densities. Facilities containing high densities of tadpoles (i. e., more than two or three tadpoles per 3.8 L of water) had daily water changes of 20-30%. Crushed fish foods, liquid filter feeding formula foods, or powdered filter feeding foods like Sera Micron® food were supplied twice daily. A fluorescent plant growth bulb on a photoperiod of 12L:12D was set on a timer for the tadpole rearing facility.

As tadpoles neared completion of the aquatic phase, nursery terrariums were prepared (described below). At this late developmental stage, tadpoles had pushed their front arms through the openings in the body wall. They were culled from the tadpole facilities and placed into the nursery. When first introduced into the nursery facility, the froglets may or may not have shed their tadpole mouth part. They were likely to have remained in shallow water for several more days, thus all culled, newly metamorphosed froglets were placed in a shallow dish of clean water containing clumps of *Vasicularia* sp. This dish was situated on the foam or sponge substrate in the nursery tank. Water levels in the dish did not exceed 3 mm depth. This allowed froglets to leave their accustomed watery environment and charge the terrestrial environment of the nursery tank at their discretion. When no new recruits were being added to a nursery tank, the shallow dish of water was removed.

Several 38 L aquaria (50.8 x 25.4 x 30.5 cm) equipped with glass lids covering 3/4 or 4/5 of the top, were used as nursery facilities. The remaining section of each top was covered with soft mesh screen to allow for ventilation. Soft furniture foam or sponge was used to cover the bottom of each enclosure. These substrates were kept moist at all times. They were washed with clean water, soaked in scalding water, and rewashed every three days to maintain cleanliness. Also in the enclosures were hydroponically-grown plants placed in cups that contained water. Lids prevented the froglets from entering the water reservoirs (a small hole in each lid allowed the plant to pass through it). These plants had leaves that were strong enough to accommodate several froglets at once.

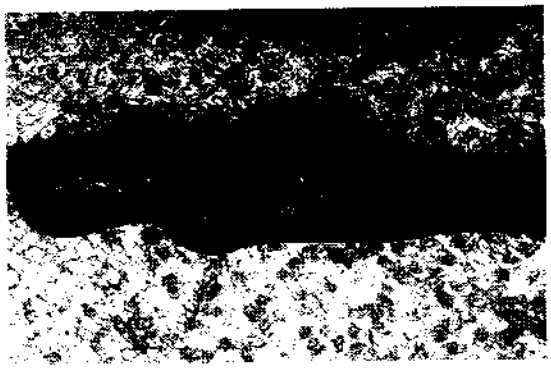

Figure 2. A funnel that was opened in order to lightly mist the eggs with water, which aids the *P. hypocondrialis* tadpoles in hatching. One or two tadpoles have already hatched; they fell into the water contained in a jar that was suspended below the leaf. (Photo by Danté Fenolio).

The nursery tank had the identical lighting and temperature as the housing facility of the adult frogs. Hatchling or very small crickets were the staple of the young froglet's diet, while wingless fruitflies were used as a supplement but not a staple. These food items were dusted with vitamin and mineral supplements (i.e., Herptivite® and Rep-Cal®), on the same schedule as described for adult frogs. One or two crickets were offered to each froglet, daily. Food items were placed into a feeding dish like the one used for adult frogs.

Approximately 20-25 froglets were housed in each 38 L nursery tank. Froglets were removed from the nursery tank after three to

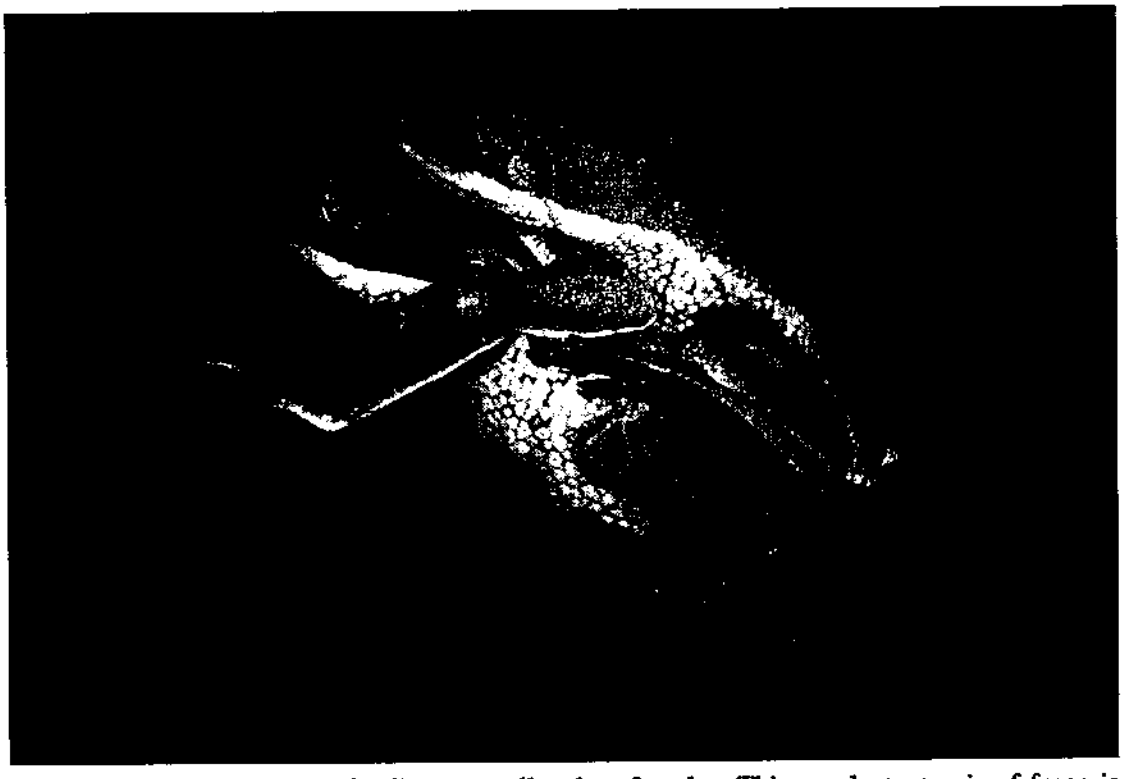

Figure 3. Male *P. hypocondrialis* are smaller than females. This amplectant pair of frogs is searching for a suitable leaf from which they will create a funnel. (Photo by Danté Fenolio).

four weeks, after showing noticeable growth. These juvenile frogs were then placed into enclosures identical to the adult enclosures.

Reproductive data was collected for four attempts at four different times. The size of adult reproductive groups ranged from six (sex ratio = 3.3) to 16 (sex ratio = 8.8) frogs. Females were distinguished from males because of sexually dimorphic size differences; full grown females are almost 1/5 to 3/5 larger than the males. Reproductively-ready males had recognizable, dark nuptial pads on the base of the thumbs. Males also actively called prior to and during rain cycles.

Data collected involved dates of funnel creation, number of days in incubation (the number of days from the funnel creation date to the hatch date), number of eggs oviposited, number of eggs hatched, hatch rate, and the cumulative hatch rate. Defined, the cumulative hatch rate yields a percentage including all funnels where eggs were oviposited, regardless of the number of tadpoles actually hatched (this includes clutches with zero hatchlings). This is in contrast with the hatch rate percentage which only includes funnels that had tadpoles hatch out after a specific number of days (excluding funnels with no hatchlings). The cumulative hatch rate and the hatch rate figures allow for a quick understanding of true hatching percentages overall, contrasted to varying incubation time hatch rates.

RESULTS

Upon initiation of rain cycles, pairs of frogs engaging in amplexus were observed from the first night of the cycles through the 23rd night, when the last female of that group was spent. Figure 3 depicts *P. hypocondrialis* in amplexus. On average, the frogs in amplexus oviposited on their second night together. Competition between males, for ripe females, was noticeable only after a male had mounted a female. There were no observations of males