Rearing *Xenopus laevis* Life History Stages

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Introduction

The African clawed frog, *Xenopus laevis*, has been studied and used extensively, both at the research and teaching levels. The adult can survive a variety of artificial water conditions including differential pH, salinity, turbidity, and temperature, giving one the impression that all life history stages could be reared and maintained with ease. However, it has been our experience, when using conventional rearing protocols, that many developmental stages displayed stunted growth in terms of size and stage, and were subject to high mortality. Numbers of usable individuals was always unpredictable. Here we report comprehensive methods (using commercially available supplies) for consistently rearing *Xenopus* through the entire life cycle. These methods were developed to support laboratory exercises in an advanced animal developmental biology course and to provide new breeding stock for the department's colony. They have proven to be reliable, cost-effective, and yielded consistent numbers of every life-cycle stage in an abridged life cycle time line.

Materials and Methods

The System, Water Conditions, and Colony. The aquatic system is constructed as a slow flow-through system, with no recirculation. Local city water is passed through a charcoal filter, dechlorinated, and maintained at pH 7.8 with a temperature of 20^{o}C. Seventy adult females and 50 males constitute the colony, which are housed in separate tanks in small room (about 3 m

wide by 4 m long, Figure 1). An additional 150 juveniles at various stages of maturation are also housed in the same room. Every 5 years, eight to ten females are purchased from commercial supply houses and introduced into the colony to circumvent inbreeding.

Injection of Adults. Adults randomly chosen for mating were males with intense black pigmentation on the inside of their front legs, while females were those that had tested oocyte-positive several times. Females were considered oocyte-positive when egg masses were produced after injection with pregnant mare's serum (PMS, Calbiochem) and human chorionic gondadotropin (hCG, Sigma) to induce ovulation. The schedule of injection and concentrations of each hormone are described below. Just prior to injection with hormones, each individual frog was firmly grasped from the dorsal side, with the index finger placed between the rear legs (head pointed into the palm of the hand) and inverted. The head and front legs were then wrapped with a paper towel to control any sudden movements by the frog, which avoided accidents, such as self-injection (Figure 2). (It should be noted that hormones such as hCG are not supplier-screened for hepatitis or other potential human biological hazards.) Adults of each sex were injected at a sub-cutaneous site located on the underside, just above the hip joint, using a 25-gauge needle mounted on a 1-ml syringe (Figure 2). This site was chosen because it is the least intrusive and does not damage the internal organs. Females were injected with 50 International Units (IU) one week prior to mating, then 1000 IU of hCG at mating. Males were injected with 200 IU of hCG 24 hours prior to mating and then 300 IU of hCG at mating. An individual from each sex was then placed together in a 13-liter bucket containing about 9 liters of water. A lid was placed over the bucket so individuals could not escape (Figure 1). The pair was left overnight (about 12 hours) in order for amplexus to occur (Figure 3).

Egg Masses. Egg masses were collected and checked for fertilization (Figure 3 inset), then transferred to 88-liter tanks containing about 70 liters of water (Figure 4). About 2000 eggs were judged to be the appropriate density per tank. Although these numbers seem excessive, it should be recognized that not all eggs are fertilized and the numbers of hatchlings may have to be adjusted according to the following protocols. The eggs were then physically agitated and turned over every 24 hours to avoid fungal and bacterial build up on the surface, which seemed to suffocate the developing embryos. Rearing of all life history stages, except the adult, was under stagnant conditions (no water flow) with a small volume of air bubbled through the water column.

Hatchlings. After about 3 days hatchlings can be observed attaching to the sides of the tanks. It is important that the residual egg masses remain on the bottom of the tanks. The tadpoles appeared to feed on the decomposing jelly, or something else, as an interim food source. In an additional 3 days post-hatching, feeding of tadpoles commenced on an artificial diet of commercially available supplies (described below). Hatchlings approximated about 200 individuals per tank or 70 liters of water volume.

Tadpole "Cocktail" Diet. The composition of the tadpole "cocktail" diet was 25% Nutrifin, 25% Trout Growers Ration, 50% Nasco frog brittle pellets (dry volume/volume/volume). An additional volume of water (50%) was added and blended to the consistency of ketchup. This

was fed to the tadpoles in suspension (50 ml each day) and delivered into the water column with a wide-bore pipette. The cocktail was made up fresh each week.

Husbandry (from hatchling to 5 cm juvenile). Residual food was siphoned from tank bottom once a week to prevent fouling. Fifty percent of the water volume was also removed and replenished with fresh water each week to avoid ammonia and feces build up.

Metamorphic Diet and Conditions. Stage-60 (stages according to Nieuwkoop and Faber, 1968), or those in the process of metamorphosis, and beyond, were transferred to 130-liter tanks containing about 88 liters of water to a maximum of 100 individuals per tank. Segregation according to stage at this phase was important in order to avoid cannibalism of tadpoles by froglets. The diet and its presentation also had to be changed at this stage to Nasco frog brittle. The pellets were homogenized in dry form, to approximate a particle size of 2 - 3 mm, or the consistency of course ground pepper. About 50 ml of dry powder was sprinkled over the water surface daily. Stage-60 or beyond are capable of, and were observed to prefer to ingest solid food using their forelimbs.

Advanced Juvenile and Adult Requirements. Juveniles reaching 5 cm were again segregated and maintained at a density of two or three individuals per liter of water. They were then introduced into the regular flow-through water system and fed a diet of whole Nasco frog brittle pellets. About 10 ml (dry volume) of Nasco Frog Brittle was fed to each individual daily. The density of individuals was held through to and beyond maturity, along with the these dietary regimes.

Sexes. Individuals were subsequently segregated according to sex when they reached about 7 cm in length. Two flaps of tissue on either side of the cloaca distinguished females while males do not have such flaps. Males matured in about 6 months and productive oocyte-positive females (first testing oocyte positive at 12 months) in about 18 months. Oocyte-positive females are injected with PMS and hCG every 3 months to induce ovulation. Although juveniles and adults in our breeding colony are maintained in a flow-through system, there is no reason to believe they cannot be reared and maintained under stagnant water conditions.

Results

Historically, our researchers were committed to using plant particulates [nettle powder or Cerophyl (Cerophyl Labs.)] as the chosen food sources for the laboratory rearing of *Xenopus* larvae. However, these speciality foods became expensive and often limited to particular supplier and availability. Therefore the tadpole “cocktail” diet was formulated. Using this diet, tadpoles grew larger and faster (reaching metamorphic competence in approximately 4 weeks) when compared to the traditional diet of Cerophyl (Figure 5). It should be noted that for our particular teaching needs, around stage 49 was the required target stage. The “cocktail” diet yielded the target stage in about two weeks, whereas the Cerophyl-fed-tadpoles took about a month to reach this stage (Figure 5). The point is that development and metamorphosis occurred in an abridged time line, which was different from our past experiences, and the time line of 2 to

3 months suggested in the literature (Nieuwkoop and Faber, 1968; Wu and Gerhart, 1991). These results had substantial benefits for teaching support and further spin-offs for research endeavours.

Discussion

The purpose for developing new methods was clear: we needed simple, straightforward procedures that worked effectively, consumed a minimum amount of financial and human resources, and revolved around assured supplies. In providing materials for a one-term course, there also had to be consistent, reliable numbers of the appropriate stage. The methods and materials described above fulfilled all these criteria. Most importantly, the resulting abridged time line from oocyte to froglet further allowed for the elaboration of lab exercises, and a "cushion" period, where backup cultures could be started if problems arose. Traditional methods did not allow for such contingencies.

In terms of the colony, very few frogs reached metamorphosis using the plant particulate diets. Those tadpoles that eventually reached competence were small, resulting in extremely small juveniles, which translated into longer rearing periods to the adult stage. Usually, they died during the metamorphic process. There were so few frogs for recruitment back into our colony that each year new females had to be purchased to replenish aging stocks. Using the methods described above, tadpoles grew larger, faster, and there was little mortality through the various stages in the developmental sequence. Males reached maturity in about 6 months. More importantly, females matured, and tested oocyte positive, in about a year and were incorporated into the colony at about 18 months. The time line again was abridged compared to the two to three years suggested in the literature (Nieuwkoop and Fabler, 1968; Wu and Gerhart, 1991).

Laboratory culture was so successful that the residual numbers (both larvae and adults) not required for teaching support, were sold for research purposes. Judging from the demand, it became apparent that many Canadian universities w4ere experiencing similar rearing difficulties. At the present time we support several ongoing research programs on campus, at a considerable cost savings to the researcher. The spin-off was considerable in terms of the funds generated to aid in colony operations.

Rearing *Xenopus* life history stages under stagnant water conditions provides anyone the opportunity to start a small, self-sufficient, sustainable colony. Even the developmental stages can be reared successfully without much trouble. The protocols listed above offer the potential for a small colony to act as a reservoir for both teaching and research programs. This is especially true of smaller organizations, with minimal budgets and limited facilities.

References

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Wu, M., and Gerhart, J. 1991. Raising *Xenopus* in the laboratory. Methods in Cell Biology, 36:3-18.

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Figures

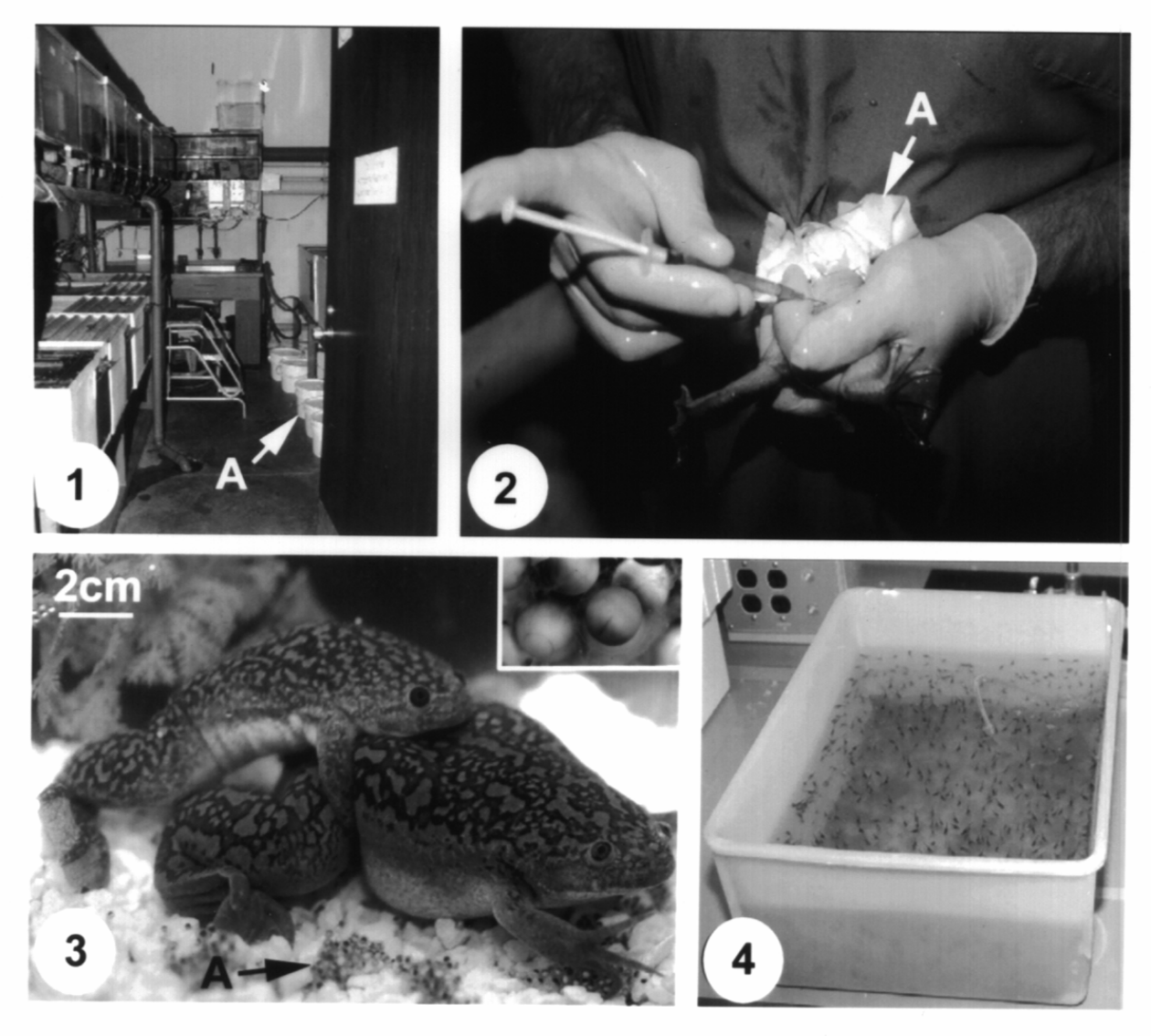

Figure 1. The frog room. Adults are housed in the large lower tanks and juveniles in the upper right hand rung. Note the pails (A) on the floor, which contain mating pairs.

Figure 2. Hormone injection of a female shows the injection site and paper towel containment (A).

Figure 3. *Xenopus* (male is dimorphically smaller) pair in amplexus. Note the egg masses (A). Inset shows a collected egg mass with various stages of development ready for culture.

Figure 4. Culture tank (recycled rat cage) showing the water volume, air supply and tadpoles.

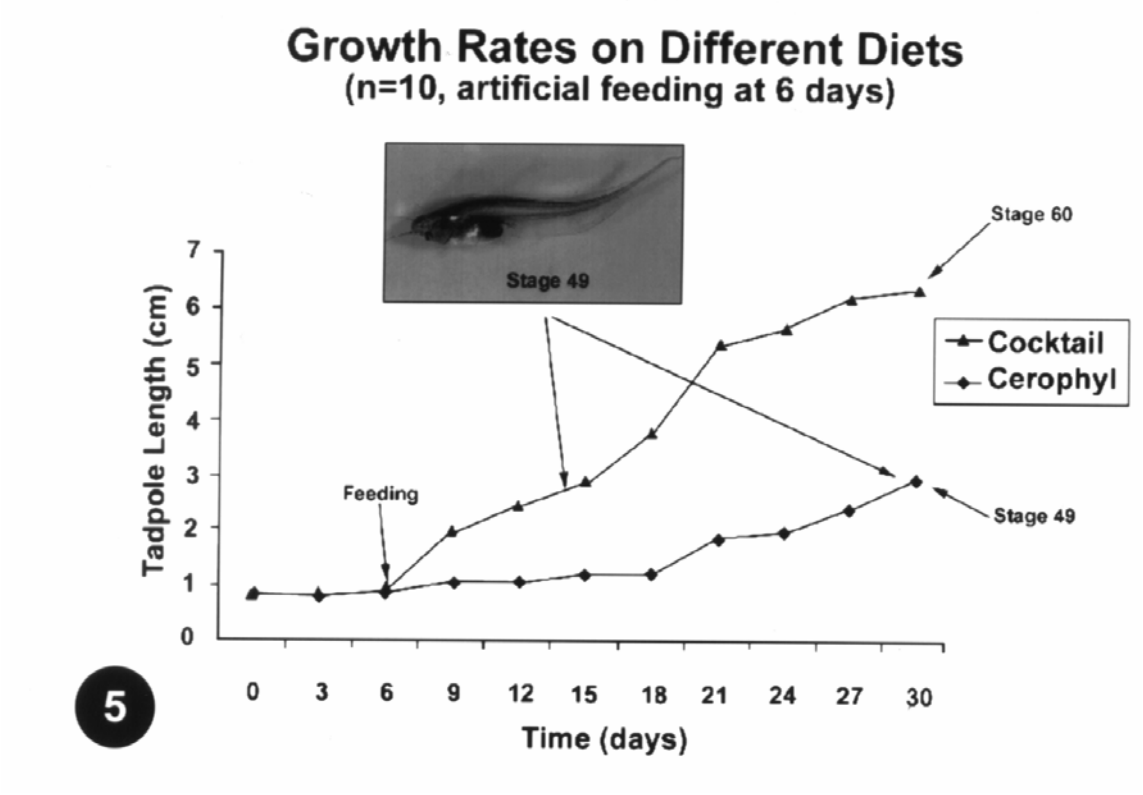

Figure 5. Graph showing the size and developmental stage of tadpoles fed on Cerophyl and "cocktail" diets for 30 days.