

SECTION II: CULTURE CONDITIONS

Axolotls in the Lab

By

Rudolf Brun
Department of Biology
Texas Christian University
Forth Worth, Texas 76129

Probably many of us keeping Ambystoma mexicanum in the lab have gone through the difficult times when they seemed to prefer to sicken or even die rather than cooperate in providing research material. My lab is no exception to this sinister axolotl rule.

When I had the opportunity to start my own collection I followed the rationale of preventing disease by keeping the animals in good spirits (if they have any). How can this feasibly be accomplished?

As anyone knows, good eating is an essential part of happiness. As indicated in several "pet store books" dealing with the care of salamanders, fresh, live earth worms seemed to be the correct diet. Although the liver-trained axolotls were initially frightened by the twisting and turning worms in their glass bowl, they learned quickly how to deal with the new food. The axolotls seemed to like the worms and so did I, because they didn't spoil the water as severely as the liver did. However, the bowls still had to be cleaned at least once a week. Cleaning, of course, means that the animals have to be taken out of the glass bowls either with a net or some other device. Knowing how brittle axolotl skin is, I didn't like these mechanical manipulations. In addition, I learned from a pet store owner that, in his opinion, the integrity of the mucus film on the fish skin is crucial in preventing skin diseases. In order to eliminate the frequent mechanical stress on their skin, I tried to keep axolotls together in a water tank equipped with an undergravel filter system. Pet stores offer a variety of brands. They all use gravel (grain diameter approximate 4-6 mm) to filter the water. Air bubbles provide the necessary water circulation through the gravel. Therefore, an air pump is needed. I have found that the pump "Penn Plax XP 990" has more power than the "Silent Giant" and also has better service support, at least in the Dallas/Fort Worth area.

Currently, I am keeping 65 juvenile axolotls (10-16 cm long) in 24, ten gallon tanks (Fig. 1). Most tanks contain three individuals. I feed two earth worms per individual, three times a week (for adults as well as for juveniles). Sometimes the axolotls regurgitate big uncut worms, perhaps because the swallowed wriggling worms "stimulate" the stomach too much. Cutting the worms solves this problem. It also prevents the worms from burrowing into the gravel. The pieces stay alive sufficiently long enough to be found and swallowed by the axolotls.

The suction necessary to inhale the worms is generated by a sudden wide opening of the mouth. The force of the suction is so strong that, in addition to the worm, some gravel is also sucked into the mouth. This provides the opportunity to observe the phenomenon I'd like to refer to as "selective spitting". The axolotls learn to spit the gravel but retain the worm with

amazing facility. Some gravel, however, might occasionally be swallowed. I have not found this to cause any problems.*

I hesitated to keep several axolotls in one tank because of their habit of displaying aggressive behavior, especially during feeding. The problem was solved by "planting" plastic plants into the gravel of the tanks. This apparently creates some hiding places for the animals and maybe some territorial subdivision of the space available.

Since I keep, in certain ten gallon tanks, one adult male and two sexually mature females together, spawnings occur. It is particularly interesting to see the spawning female climbing in the plastic plants. She sticks the eggs on leaves and branches by touching and wiggling her cloaca. As she moves from one plant to another, the first one can be taken out of the tank without disturbing the female. By turning the plant upside down, the eggs can be picked (with forceps) like fruit from a tree. After harvesting is completed the plant can be put back into the tank. Since there is only one male in the tank, and since the spawning female is easily recognized, no confusion which animals are the parents occurs.

Who is Who in the Tank?

Because several individuals are kept together in the same tank, it is, of course, of crucial importance to recognize each animal with absolute certainty. Since I have several mutants with clear-cut distinguishable phenotypes (wild, white, albino, melanoid, short toes, homozygous eyeless) I keep animals of different phenotypes together (Fig. 2). As mentioned above, I also keep some males and females together. The sex of the animals therefore provides an additional marker. There are, however, other animals of the same phenotype which also have to be individually recognized. These animals might be labeled by a colored thread around their trunk (Fig. 2). The collar is placed behind the gills but anterior to the forelimbs. The animals are individually recognized by the different color of their collar. With some practice the collar will be the right size, not too tight and not too loose that it would be stripped off. The thread sometimes becomes partially covered with mucus. I have not found this to cause any problems to the animals. The thread also might slip under the gular fold, which doesn't seem to bother the animals either.

I have used this marking procedure for only six months. This, of course, is quite a short time. I feel that the tagging of animals can be improved. This contribution will perhaps generate some reactions, comments and suggestions on how this can be accomplished.

How to Raise Earth Worms and an Estimate of Cost

Raising earth worms is simple. A visit to a local earth worm farm will prove that point. The following brief description of how to raise the worms is a summary of what I have learned from a local earth worm farmer. The worms suitable for raising under laboratory conditions are called Red Wigglers. The

*Ed note: A top layer of larger gravel (over 1 cm diameter) can be added to prevent any ingestion of foreign bodies.

worms - the popular name of which is Nightcrawler-reproduce slowly and seem to require special production and control procedures. The Red Wigglers grow and reproduce well in moistened peat moss. The peat moss is prepared by soaking it for approximately 24 hours in tap water. The water is then squeezed out of the peat moss by pressing it with both hands. This produces correct moistening of the bedding. The peat moss prepared in this way is placed into a wooden container which resembles a big drawer. The height of the peat moss layer should be approximately 5 cm. The worm farm I visited used containers which measured 240 cm x 190 cm x 30 cm (length, width, height). In order to have good breeding, the beds should accommodate about 25,000 sexually mature worms. The worms are fed with grain- or cottonseed-meal. I put the meal into a large metal kitchen sieve. By gently tapping on the sieve a fine layer of meal can be easily broadcast over the peat moss. During the night the worms will come up to the surface to feed. When the meal has disappeared it is time to feed again (approximately every two days). I buy the worm food from an earth worm farm. They use a mixture of different meals, including alfalfa, to fatten the worms. The only space I had available was under the tables! I therefore had to subdivide my worm colony into three containers each approximately 90 cm x 70 cm x 25 cm. Roughly 5,000 worms (5 pounds) were placed into each container. I started to use up the first one, then switched to the next container, and so forth. One container feeds my 72 axolotls for approximately 8 weeks (each animal is fed three times, 2 worms a week). Following exhaustion of the third container, the first container had four months during which it had reestablished a population of at least 5,000 worms. There is a good possibility that up to 100 axolotls can be fed from the described worm colony. The food to feed the worms is inexpensive: 50 pounds would cost approximately \$7. This would bring the cost of feeding 15,000 worms a year to \$28. Since the peat moss has to be replaced, perhaps \$17 has to be added to the \$28. This rough estimate would therefore bring the cost of feeding 100 axolotls on earth worms to a total of \$45 per year. These costs might be considerably lowered if space is available to breed the worms in containers as large as those I have seen in the worm farm. The millions of small white worms will grow much faster if provided with more space.

I feed axolotl larvae larger than 2 cm with these white juvenile worms. The food for raising these larvae to maturity is therefore included in the above estimated costs. I change the peat moss every two months. The used peat moss is removed from the two sides of a compartment and accumulated in the middle part. The new peat moss is placed on either side of the old bedding. The worms will crawl into the new peat moss overnight, so that the old bedding can be removed without losing too many adult worms.

I tried to raise the larvae from one spawning all together in one 10 gallon tank (I hear you all laughing!). In my opinion, this is simply impossible. The larvae had no trouble feeding on the brine shrimp and later on the earth worms; they also fed on each other. There are always some larvae in a spawning which grow considerably faster than their sibs. Not even a jungle of plastic plants can prevent their cannibalism. I therefore raise hatched larvae individually in glass bowls (with a diameter of 10 cm). As mentioned above, I feed brine shrimp only to larvae up to 2 cm of length. After they have reached this size they are fed with small white earth worms. These do not spoil the water. They can be obtained in large quantities from the earth worm colony. The young worms like to burrow themselves into the rest of the meal. This makes it easy to collect them. If they are placed in water, they will assemble into a sphere. This sphere of young worms can then very easily be taken out to feed the small axolotl larvae.

I hope that these few lines provoke some reactions from the experts who have raised axolotls for years. Perhaps we could come to some kind of a generally accepted way of raising axolotls in the lab.

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FIGURES



Fig. 1

Keeping axolotls in tanks equipped with an undergravel filter eliminates cleaning of glass bowls.

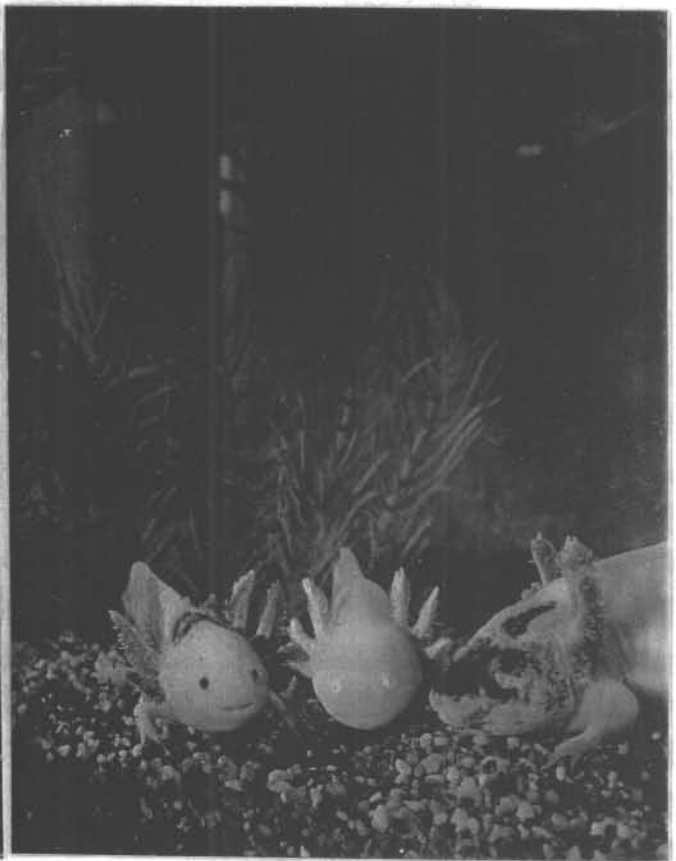


Fig. 2

Three axolotls in one 10 gallon tank: different phenotypes or collars of colored thread are used to distinguish the animals.