Compendium of Axolotl Husbandry Methods

1997

Reprinted from the Axolotl Newsletter, Issue 25

Contents

Amphibian Maintenance Facilities at Sogang University, Korea

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Maintaining a large number of axolotls can be problematic due to the time needed for maintenance and space needed for containers. Here, I would like to briefly describe how we have overcome some of these problems in our axolotl colony facility.

1. Water supply and treatment procedures

We use charcoal-filtered tap water that has been aerated overnight after filtration.

2. Container types and sizes

A. For larvae up to 4 cm in size

We use custom-made compartmentalized cages to prevent cannibalism among small larvae.

B. For larvae larger than 4 cm in size

Animals are housed individually in round plastic food storage containers. The size of the container is dependent on the size of the animal.

C. For adult animals

For large stock animals, we use large aquaria connected to a continuously circulating filtering system (Figure 2). One filtering unit is composed of eight aquaria (in two rows of four each on steel shelves) and one filter box. The aquaria are connected by pipes that lead to a common filter box, located beneath the shelf. Each tank has a separate inlet valve which controls inflow, and each row of tanks has a master flow valve. The filtering system is designed to circulate water through the filter box beneath the aquaria. Within the filter box is a micro habitat which consumes organic materials from food debris or excretion. Except for while feeding and retrieving uneaten food (about 4 - 5 hours a day), water is continuously circulated. The whole system requires complete cleaning only once a year.

3. Food supply

Young larvae (up to 1 month from hatching) are fed freshly hatched brineshrimp.

Larvae and adults are routinely fed chopped beef heart, occasionally supplemented with beef liver.

4. Daily routine

Typically, animals are fed in the morning, and cleaning is done in the afternoon.

A. Small larvae:

The compartmentalized inner cage is slowly raised and put into a clean outer cage with fresh water in it. Any trapped food or droppings are siphoned out.

B. Medium sized larvae:

Water in the plastic container is exchanged for clean aerated filtered water.

C. Adult axolotls:

For the animals housed in the aquaria, uneaten food is retrieved and water circulation is resumed.

5. Lab space used

All animals are in a single room measuring 8 m (L) X 5 m (W) X 2.5 m (H). Room temperature is kept at 20 °C, with12 hours light and 12 hours dark cycle.

6. Time required per day/ week

Feeding and cleaning 20 cages, 100 plastic containers, and the aquaria takes about 1 to 2 hours for two people per day. However, this varies with the number of animals. Keeping stock animals requires no more than 30 minutes, since it is semi-automated.

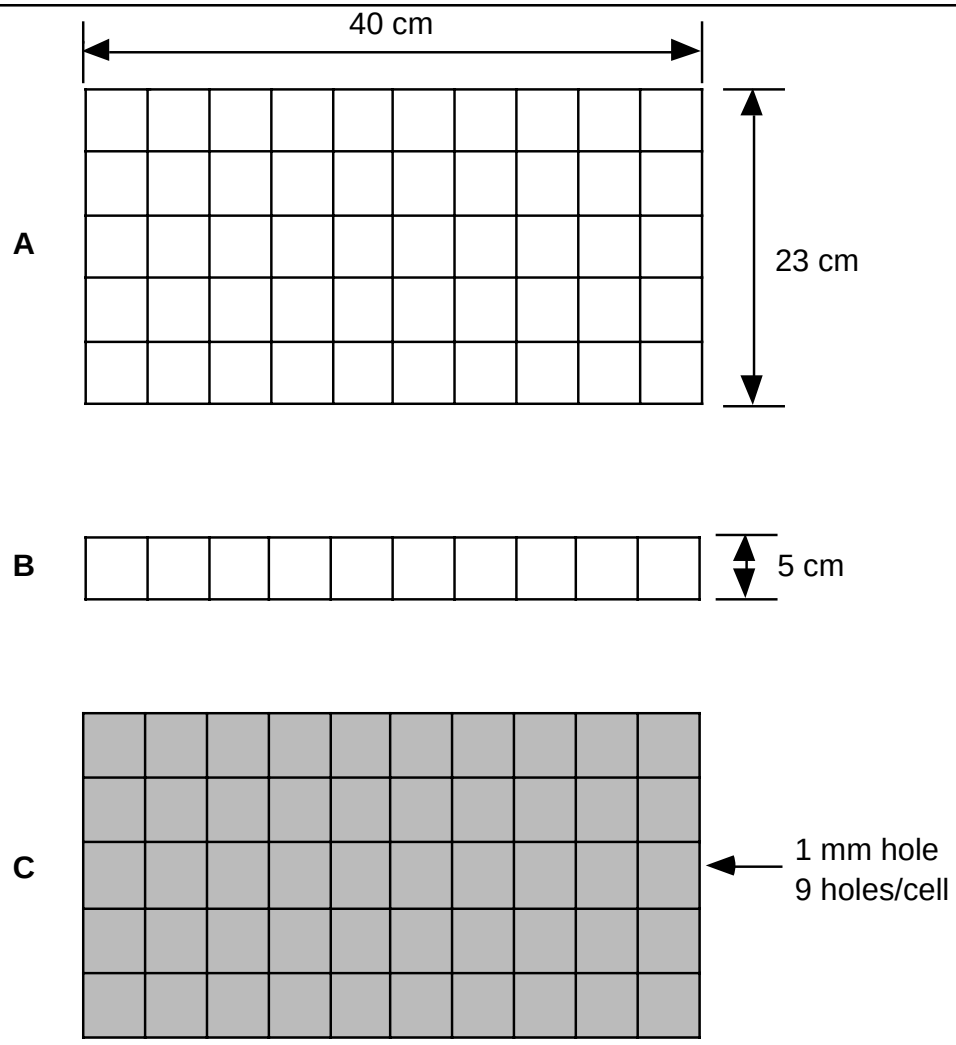


Figure 1. Compartmentalized Cage

(inner cage)

A: top view, B: side view, C: bottom view

<1> inner cage

Material: acrylic plate, 3 mm thick
Dimensions: 40 cm (L) X 23 cm (W) X 5 cm (H) with 50 cells
Cell size: 4.5 cm (L) X 3.7 cm (W) X 5 cm (H)
The bottom plate of the each cell has 9 holes (1mm in diameter)

<2> outer cage

A plastic storage box (44 cm (L) X 27 cm (W) X 11 cm (H)) from a local store is used to hold water.

<3> setting

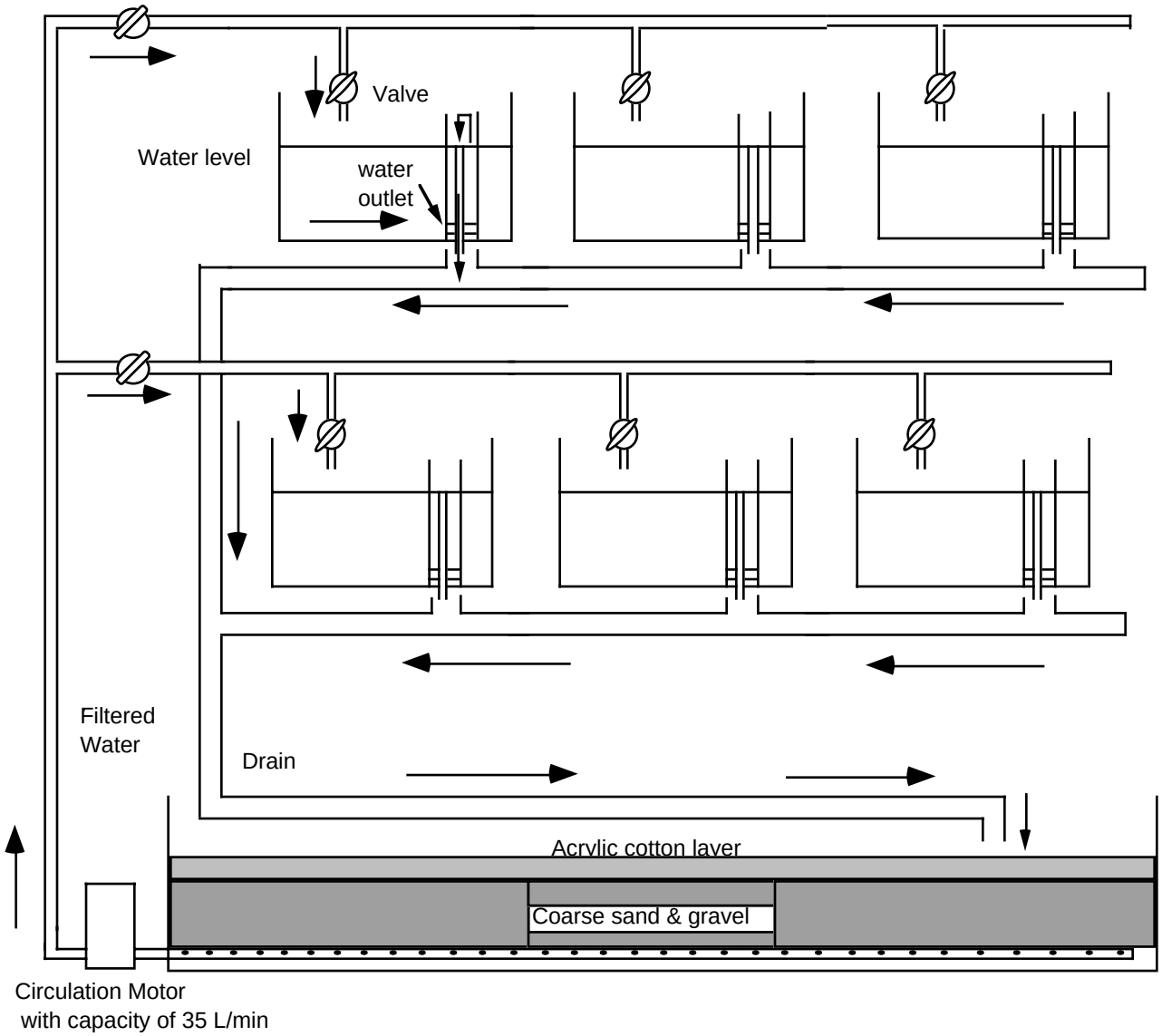
The outer cage is partially filled with filtered water, and the inner cage is slowly lowered into it. The water level is adjusted to a depth of 3.5 cm. Larvae are placed into individual cells. Once set, this unit can be stacked or put onto shelves for better ventilation and lighting.

<1> Aquarium

Material: glass plate 5 mm thick (oven-tempered glass plate with a hole of 3 cm in diameter in the bottom for overflow pipe) Dimensions: 40 cm (L) X 40 cm (W) X 30 cm (H) for 6 - 8 animals

<2> Filtering system

Components: PVC pipe, PVC valve, PVC filter box, electric water pump
Filter box dimensions: 180 cm (L) X 30 cm (W) X 30 cm (H) for 8 aquaria



Pipe size: inlet water pipe (3 cm OD), drain water pipe (7 cm OD)
inside pipe in aquarium (3 cm OD), outside pipe in aquarium (7 cm OD)
→ : direction of water flow

Figure 2. Filtering System for Adult Axolotl

Axolotl Larvae Housing Methods

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Water supply and treatment

Axolotls are housed in two locations: the breeding rooms (located away from the general lab) and a more general access room located in the laboratory. In the breeding rooms, the water is purified through a carbon filter system and stored in a large tank. The water is pumped through lines to each animal container. There is no routine treatment of the water other than filtering with carbon filters.

The general animal room houses various stages of axolotls, embryonic through adult. All axolotls in this room live in Holtfreter's solution, a buffered salt solution adapted from that used by the Indiana University Axolotl Colony. The embryos live in 20% Holtfreter's solution, and both the larvae and adults are housed in 40% Holtfreters solution. The water for the embryos and larvae is purified through the Millipore filter system. The adult's water supply comes from distilled water supplied from the building's filtering system.

Bowl types and sizes

General animal room in laboratory:

Prehatchlings (embryos) are kept in clear plastic containers obtained from the TriState Plastics company.

Size: 5" x 7" x 3 1/2" (W x L x H)

How many animals per container: The smaller the number of eggs per container the better for development and hatching. We put approximately 20-30 per container.

How much 20 % Holtfreter's solution: The maximum amount of liquid this container holds is 1L. We keep approximately 400-500 mls of 20% Holtfreter's in each container of this size.

Larvae are kept in clear plastic cups purchased at the local grocery store, Albertsons.

Brand Name: Solo Cups.
Size: 3 1/2" x 2 3/4" (D x H).
Cost : $1.00 per package of 10 cups.

Size and number of larvae housed: One animal per cup, approximately 1.5 cm to 6 cm long.

How much 40% Holtfreter's solution: We keep approximately 150 mls of 40% Holtfreter's in each cup.

Secondary container: According to UCI animal protocols, experimental animals are required to be housed in a secondary container to prevent any loose animals in the laboratory.

We use two different types of secondary

Modified Holtfreter's Recipe

from the IU Axolotl Colony as communicated to the SVB Lab by S. Duhon 10/4/94

Recipe is for modified 40% Holtfreter's. Dilute 50/50 with filtered water for 20% concentration.
Use 20% for embryonic (prehatchling) stages
Use 40% for everything else

For 45 gallons:	2 tablespoon	$MgSO_4 \cdot 7H_20$
	2 1/2 Teaspoon (or 1 scant tablespoon)	$CaCl_2$
	1 teaspoon	KCl
	240 cc	NaCl
	27 grams	Trizma (7.4) fish grade

Dissolve the above recipe in milliQ H_20 to volume of 3000 mls. This stock makes enough solution to fill 9 Nalgene carboys (5 gallons each x 9 = 45 gallons total).

To make 40% Holtfreter's: Add 300 mls of Holtfreter's stock to a 5 gallons of milliQ H_20.

To make 20% Holtfreter's: Add 150 mls of Holtfreter's stock to 5 gallons of milliQ H_20.

containers to hold our clear plastic cups: clear plastic sweater-type boxes and longer, white plastic mouse cages.

Clear Sweater boxes:
Size: 9 1/2" x 12" x 3 1/2" (W x L x H) Each holds 9 cups.
White boxes:
Size: 10" x 16" x 5" (W x L x H)
Each holds 12 cups.

Larger larvae–adults: Larvae bigger than 6 cm and large adult axolotls that are not used in the breeding colony are kept in the animal room in the laboratory.

Company name: TriStar Plastics
Catalog number: (Part Number) 395C
Size: 3 1/2" x 7" x 3" (W x L x H)

How many animals per container: 1
How much 40% Holtfreter's: The maximum amount of liquid the container holds is 600 mls.

Basement Breeding Rooms:
Container size: 24" x 24"
Number of animals per container: 1 (when not breeding)
Company container purchased from: Home Depot
Cost per container: $25.00
How many per room: 9 containers per room.

Temperature

The breeding room is kept at 16-18°C. The second room is kept between 18–20°C

Food supply

Young axolotl larvae (from just after hatching to 4 cm) have a diet consisting exclusively of baby brine shrimp. Axolotls love brine shrimp and readily eat them. As the larvae grow, we mix frozen A*DULT* brine shrimp into their diet.

Older axolotl larvae (4 cm and up) are fed a mixture of fresh baby brine shrimp and frozen adult brine shrimp. However, some people begin to feed their animals fish pellets at this stage. We have a supply of fish pellets that are soft and moist, easy to crush and easily broken down in the water. We try to get them to eat fish pellets as soon as possible in order for them to grow up quickly.

Adult axolotls are fed fish pellets exclusively. We use various sizes and forms of pellets. The pellets range in size from 2.4 mm to 4.0 mm and come in a variety of textures varying from soft-moist pellets to a dryer and

Sources of food

Fish pellets: Rangen Inc.
115- 13th Ave. S
PO Box 706
Buhl ID 83316-0706
800-657-6446

Soft Moist pellets: 1.6 mm $34.00 for 40 lbs; 2.4 mm $31.50 for 40 lbs
Trout pellets (harder): 4.0 mm $13.50 for 50 lbs; 4.8 mm $27.50 for 100 lbs
Freight cost: $13.49 per box; *Handling fee:* $5.00

Brine shrimp: Bayou Aquatics.
1908 S Lake Place
Ontario, CA 91761
909-947-4575

We use brine shrimp eggs and adult frozen shrimp grown in Utah.

SO PRO80
Ocean Star International
PO Box 643
Snowville, UT 84336 USA

Frozen adult brine shrimp: $9.70 per 21 lbs
Brine shrimp eggs: $32.00 per 32 oz container

harder pellet for the adults.

Daily routine

Researchers are responsible for the maintenance of their own research animals. However, in general all embryos and larvae are cleaned daily. The larvae are fed at a minimum of 1 hour and maximum of 2-3 hours prior to cleaning. Adult animals are fed and cleaned three times a week in both the general animal room and basement breeding rooms.

Lab Space Used

Basement rooms: The university has designated a floor to house all research animals in the building. The SVB lab has two rooms on this floor.

The SVB lab has obtained special permission to house some research animals inside a small room within the laboratory. The temperature of this room can be adjusted as desired.

Time required per day/week

The total time required to clean each researcher's batch of pre-hatchlings and larvae is approximately 30 minutes. It takes a few minutes to feed each batch of animals, and this is done at least 1 hour before cleaning, but no more than 2-3 hours, in order for them to get a chance to eat. We do not leave food in the animals' containers after cleaning. This helps to cut down on the number of animal deaths from bacterial or fungal infections caused by dirty containers.

Adult animals both in the general animal room in the laboratory and in the basement breeding rooms are fed and cleaned three times per week. It takes approximately 1 1/2 hours to feed and clean both basement rooms. It also takes 1 1/2 hours to feed and clean the adult animals in the laboratory. This work is mainly done by undergraduate researchers and by workstudy students. We believe that this is the optimal amount of cleaning that the adults need to remain healthy. We have very few bacterial and fungal problems with our adults.

Cleaning Techniques

General access room in laboratory:

The animal is placed in a fishnet set in a secondary container filled with the proper solution. Each cup, plastic box, and lid is scrubbed with a dishcloth in hot water and rinsed briefly with distilled water. The container is refilled with the proper solution, and the axolotl is placed back in its container.

All utensils that come into contact with the animal,the dishcloth, net, and secondary container, are briefly dipped in bleach and rinsed off thoroughly. This bleach solution is changed daily.

Bleach Solution: 1 cap full of bleach in 3 L of distilled water.

Holtfreter's solutions are kept in 5-gallon Nalgene carboy containers. We have separate containers for the prehatchling (20% Holtfreter's), larvae (40% Holtfreter's solution with milliQ H_20), and adult (40% Holtfreters with DI H_20) solutions. Each week every Nalgene carboy is rotated, and the used container is cleaned.

Cleaning protocol

- Rinse each piece with hot water and scrub with sponge.
- Soak for 5 minutes in diluted bleach solution.
- Soak for 5 minutes in diluted Sodium Thiosulfate solution. (5 Tbs. per full sink)
- Rinse thoroughly with tap water 5 times
- Rinse thoroughly with milliQ water
- Let air dry completely.

The animal room floor is swept and mopped daily with a diluted bleach solution and air dried. Weekly, the sinks are cleaned with scouring powder (Comet) and rinsed thoroughly. Table counters are rinsed with 70% EtOH after each use.

Basement cleaning protocol

The basement animal room is cleaned three times per week. Each container is drained and cleaned with a sponge. Then the tanks are filled with fresh carbon-filtered water. There are no special solutions added to the water. There are no chemicals used to clean the animal containers. Sponges are cleaned by dipping them in a diluted bleach solution, rinsed thoroughly, then air dried.

Brine shrimp collecting protocol

Brine shrimp eggs are collected four times per week: M, W, F and Sat. In general, the protocol is as follows:

- 2.5 mls of Utah brand brine shrimp eggs

- 30 mls of rock salt
- 1 ml of Pen/Strep
- 1 L of MilliQ H_20.
- 1 L bottle
- 1 glass rod with a rubber stopper

This solution is air-bubbled in a 23°C water bath for 48 hours.

The next step is to separate the brine shrimps from the egg shells. It is important to insure that all egg shells are separated from the brine shrimp, because the axolotls cannot digest the egg shells and will die if allowed to ingest them.

Step 1:

Pour contents from the bottle into a gravy strainer. Keep one end of the gravy strainer tilted by propping it up. Allow contents to settle and separate, usually 5 minutes. What happens is that the egg shells will float to the top while the live brine shrimp will sink to the bottom corner of the gravy strainer. Using a brine shrimp collecting net, gently pour our the live brine shrimp from the bottom of the strainer into the brine shrimp net. Do not collect the top part of the solution, this contains the brine shrimp egg shells. This step allows most of the egg shells to be discarded. Pour out the rest of the solution that contains the shells into the sink and rinse out the gravy strainer thoroughly. Invert the net into the gravy strainer and rinse out net with 40% Holtfreters, collecting the shrimp and solution into a clean gravy strainer.

Step 2:

Utensils:

- Large Plastic petri dishes with 90% of the side covered with black electrical tape. (This creates a window.)
- Transfer pipettes
- Light source

Brine shrimp are attracted to light. We use this phenomenon to further separate the brine shrimp from the egg shells by creating an environment with only one light source, the window. The shrimp will swim toward the light, and the egg shells will sink to the bottom of the petri dish. Place the light source approximately 12 inches away from the petri dish. Fill the petri dish with some 40% Holtfreter's solution. Using a transfer pipette, suck up some brine shrimp from the gravy strainer. Gently and without creating waves, pipette out the brine shrimp into the middle of the petri dish. The shrimp will swim to the side of the light. After some minutes, a clump of orange brine shrimp will collect at the bright end of the petri dish. Using a separate transfer pipette, suck up this orange clump of brine shrimp. Using the light or a white paper towel as the background, inspect the transfer pipette to see of there are any egg shells in the pipette. You should just see orange brine shrimp swimming in the pipette. An egg shell will be brown. Continue this process until all the shrimp is collected. You can create as many petri dishes as needed. We use two or three petri dishes. Dispense the brine shrimp in a cup until all the shrimps are collected. When finished the clean brine shrimp solution is poured into a clean brine shrimp net. The net is inverted into a 1L beaker and rinsed out with diluted rock salt solution. The brine shrimp is covered and an airline is placed inside the beaker. The shrimp is aerated until needed.

Diluted Rock Salt Solution:

50 mls of Saturated Rock Salt solution
450 mls of milliQ H20.

Saturated Rock Salt Solution:

500 mls of milliQ H20
Rock Salt, enough to supersaturate the water

When brine shrimp are needed, pour the proper amount into a cup. Using a brine shrimp net, pour the contents from the cup into the net. Invert the net and rinse out contents from the net into the cup with Holtfreter's solution (the proper concentration depends on the type of animal being fed). The brine shrimp live longer when swimming in a rock salt solution; however, when feeding the animals we remove the rock salt solution and place the shrimp in Holtfreter's solution. This way, we do not change the content and salt concentration in which the axolotls live.

Corwin Lab Axolotl Protocols

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Reagents

Axo Water

Distilled water, dechlorinated tap water, or commercially available spring water can be used. Deionized distilled water is not recommended due to expense and lack of trace nutrients.

400% Holtfreter's Stock (HFR)

KCl	2.30 g
$CaCl_2.2H_2O$	4.29 g
$MgSO_4.7H_2O$	8.90 g
NaCl	126.72 g

Packets can be prepared ahead of time and stored in Ziploc bags at room temperature in a dry place. Dissolve salts in axo water and adjust volume to 8L. For 100% HFR, dilute 1:4 in axo water; for 50% HFR, dilute 1:8; for 20% HFR, dilute1:20.

0.07% Benzocaine Stock (10X)

Ethyl p-aminobenzoate (Sigma # E-1501)	0.7 g

Dissolve in 1L distilled water (takes several days at room temperature with stirring). Store at room temperature.

Anesthetic HFR Solution (0.007% Benzocaine)

400% HFR	500 ml
benzocaine stock (10X)	200 ml
HEPES (free acid)	2.384 g
ddH_20 to 2L final volume	

Check pH and adjust to 6.8 - 7.0 with 1N NaOH. Autoclave if desired (not necessary for anesthesia of less than 18 hours or for amputations) and store at room temperature.

Brine Shrimp

NaCl	22.5 g
brine shrimp eggs	1.5 g

(Bonneville Artemia International Inc., Salt Lake City, UT has given best results. Various brands from pet shops have been less satisfactory.)

Packets can be prepared ahead and stored in small Ziploc bags at room temperature. 18-24 hours before feeding, mix with 1L axo water in glass or clear plastic container until salt has dissolved. Aerate and incubate 6-12 inches from incandescent light source.

Hatching Axo Larvae

Keep eggs in the solution in which they were shipped, at room temperature and out of direct sunlight, until hatching begins.

As axos hatch, transfer them to 20% HFR using a wide-bore pipet. (We cut the tips off plastic transfer pipets using a razor blade or scalpel.) For eggs containing healthy axos which have not hatched within 24 hours of the rest of the batch, gently tear open the egg membrane using two No. 5 forceps to release the axo. (Otherwise axo will be permanently bent.) Transfer to 20% HFR.

The next day, transfer the larvae to 50% HFR. Axos need to eat daily at this stage, but if there are too many brine shrimp they will kill the axo larvae. (There will be a lot of brine shrimp in the gills of the dead larvae.) Therefore, feed a few brine shrimp at a time, and clean out the dish when the axos' bellies are full. Be sure egg shells have been removed from the brine shrimp.

After 7 - 10 days, increase the amount of brine shrimp given, and decrease feeding frequency to every other day.

Older Larvae

Place larvae into containers of fresh 50% HFR when they are received. (We use shallow 4 - 10 cup Rubbermaid refrigerator dishes without the tops.) Larvae less than 1.5 cm long can all be placed in the same container. If brine shrimp are available, feed new larvae as soon as they have recovered from shipping (when movements become rapid). Otherwise, start a fresh batch of brine shrimp immediately and feed the next day.

Feed every 2 - 3 days, being careful to remove brine shrimp egg casings before placing in axo dishes. Clean a few hours after feeding, when bellies are full. Remove any dead (unresponsive and no heartbeat) larvae as soon as possible. "Sick" larvae can sometimes be

saved by transferring to small containers of 100% HFR. They can be returned to 50% HFR after recovery (usually takes several days).

When axos get to be 1.5 - 2 cm long, separate them to avoid nipping and cannibalism. They can be housed in Rubbermaid 12-oz. shallow containers without lids, plastic deli dishes, or other similar containers which have not been used for toxic chemicals. These can be kept in stackable plastic or wire closet organizing bins which provide airflow space between levels, to save bench space. As the axos grow, transfer to larger containers. They should have sufficient space to swim around, and the level of 50% HFR should be sufficient that they can stay completely submerged without having to duck their heads.

"Stressed" axos (recently operated or amputated, sick, or after long periods of anesthesia) should be kept in 100% HFR during recovery.

Feeding

The day before feeding, prepare brine shrimp as specified in **Reagents**.

Remove airstone from 18 to 24-hour-old brine shrimp. Allow 10-15 minutes for shells and unhatched eggs to settle. Brown casings will be seen floating on brine surface and at bottom of container. Pink brine shrimp move in jerks and congregate close to the light source.

Wet a fine fishnet and place in an empty container below the level of the brine shrimp container. Position rigid end of a siphon tube in the shrimp container near the swimming shrimp congregation and away from egg casings. Start siphon by applying suction to the other end of the tube until fluid level extends below the bottom of the shrimp container. Allow fluid to flow through the net into the empty container so the brine shrimp collect in the net. Avoid transfering egg casing material. When sufficient shrimp have been collected, stop the siphon.

Avoid feeding egg casings to newly hatched larvae (less than 3 months old) because they can not process the casing material. For small larvae, we transfer shrimp to a second container of salt solution (22.5g/L axo water) and allow any egg casings to settle a second time in front of the lamp before collecting the shrimp for feeding.

Rinse the shrimp collected in the fishnet with axo water to remove the salt, then transfer a small amount to each axo dish using a plastic transfer pipet or spatula. When all axos have been fed, rinse inverted net to remove any remaining shrimp. Clean siphon of shrimp and salt deposits. Clean brine shrimp container by rinsing with tap water and wiping any residue with a paper towel. Invert to drain, and turn off incandescent light.

Allow several hours for axos to feed (bellies should be swollen with pink mass in gut), then discard any uneaten brine shrimp and clean the dishes.

Larger axos (older than 4 months) can be fed small pieces of liver. Clean dishes 30 minutes to 2 hours after the axo finishes feeding. (If disturbed too soon, axos will regurgitate, but if left too long the uneaten liver will really stink.)

Cleaning

Transfer axos to holding dish containing 50% HFR, avoiding debris as much as possible. This can be done using a wide-bore transfer pipet to suck up the axo head-first (fit should not be tight, but also not loose enough to allow the axo to turn around). Alternately, pour off as much dirty HFR and debris as possible and then gently pour the axo into the holding dish. (Axos larger than 3 cm or so may try to jump out; be prepared for this. If the axo escapes, keep it wet with 50% HFR until it can be scooped up and returned to a dish.) For very small axos, pour dirty axo water through a clean brine shrimp net to contain any escapees.

Discard any dirty HFR remaining in the dish, and scrub out residue with a clean paper towel. Use a separate paper towel for each axo dish, and clean the newest axos last to avoid transferring any diseases. Refill the axo dish with fresh 50% HFR and return the axos. Wipe out the holding dish after each use.

Between cleanings, check for evaporation (a salt ring around the dish above the surface of the HFR). If this occurs, clean as above but rinse dish with axo water (not HFR) to dissolve the salt before adding fresh HFR.

Anesthesia

Prepare anesthetic HFR as specified in REAGENTS section. Larvae are sensitive to both the pH of the solution and the concentration of benzocaine. For small larvae (less than 6 months after hatching), further dilute anesthetic HFR solution with 100% HFR.

The axo should be completely immobile within 10-15 minutes of immersion (it can be

turned onto its back without twitching). It should recover completely within 20 minutes of removal from the anesthetic solution, and its heart rate should remain steady at approximately 1 beat/sec throughout the period of anesthesia. If the heart rate drops, discontinue anesthesia, remove axolotl to 100% HFR and allow it to recover.

Keep the axo in 100% HFR for a period of time (several hours for short periods of anesthesia, or during the period of regeneration or healing if axo has been operated on.) Feed and clean according to usual schedule. Axos can be fed as soon as they have recovered from anesthesia.

If euthanasia is necessary, add 0.4 g NaCl to 100 ml stock Benzocaine (0.07%). When salt has dissolved, place axo in solution until heart stops beating completely.

Acknowledgments

These procedures are based on work by the author as well as on past work by Paula Borden, Jay Jones, and Kenneth Balak in the Jeffrey Corwin Laboratory at the University of Virginia, and on important advice received from Karen Crawford and from the staff of the IU Axolotl Colony over the past twelve years.

Axolotl Housing and Care at St. Mary's College of Maryland and What is an Axo-condo?

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Our Colony

At St. Mary's College of Maryland we routinely maintain a breeding group of about 10 adults around 2 years old, 12-15 animals around a year old and anywhere from 100–300 larvae. Animal care is performed by research students working in my lab or, when funding is available, an animal caretaker. Holiday times, exam times, odd times, we all (family members included) pitch in to feed and clean.

Food and Water

All animals are fed three times a week, and their Holtfreter's solution is changed within 3 but not more than 6 hours after feeding. To prevent cannibalization, young larvae are reared individually, in 8 oz. Sweetheart Æ ice cream and food cups, S308, (approximately $50.00/1000) in 25% Holtfreter's solution. Larvae are fed fresh-hatched brine shrimp and then switched to frozen brine shrimp as soon as they will self feed. Larger larvae are maintained on frozen brine shrimp and adults are fed small cubes of fresh thawed beef liver. Larger animals are housed in 64 oz. polyethylene disposable Fisherbrand Æ multi-purpose containers (11-840E) or larger polystyrene mammal animal boxes. Matings are set up and newly fertilized embryos are maintained in 10% Holtfreter's solution to minimize exogastrulae in the developing embryos. We make up 20 liters of 100% Holtfreter's solution at a time and then dilute it as needed with house distilled water. Because of temperature fluctuations in our science building, a cold room set at 18°C is used to house the animals.

Anesthetic

To anesthetize animals for amputation or treatment we immerse the animals in a 0.007% solution of Benzocaine, Ethyl p-amino-benzoate (Sigma Æ), dissolved in 25% Holtfreter's solution. For convenience, a 0.07% stock solution can be made and stored on the shelf at room temperature. To make this stock solution, Benzocaine must first be dissolved in a small amount of 100% Ethanol before being added to the Holtfreter's solution. Benzocaine also works well for fish and worms, however the concentrations may need to be modified.

The *Axo-condo*

To minimize the amount of space required for my colony in the lab and cold room, I have designed a special rack to house trays of larvae. The standard lunch trays found in most cafeterias accommodate 12 of the waxed paper cups we use to hold larvae. These trays are handy to use when organizing experimental groups or transporting animals and are easy to clean. To acquire a rack designed to hold lunch trays, I went through the company which provides our college with racks. Working over the phone, I was able to design a rack specific to my needs. The "Axo-condo" rack stands 65 inches tall and is 22.5 inches wide and 29 inches deep. It moves easily on wheels and has 11 shelves spaced 5 inches apart. Each shelf holds two trays of 12 animals. In this way we are able to move or store 264 larvae in the floor space of little more than two lunch trays. To discourage animals from hopping out of their cups, plastic ceiling light "egg crate" panels are cut to size with heavy shears and placed over the top of each 12 cups/tray. These covers minimize animal loss and are easily cleaned. The initial cost of the rack in 1991 was about $150 with shipping a surprisingly high additional $150. Regardless and despite the cost, I cannot imagine functioning without this useful cart and could easily use another.

Raising Axolotl Larvae

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I had some 1000+/- hatchlings left over from the 14 January spawnings that I set up for the Cleveland State University Biology Department's Development course. I kept the larvae in groups of 25–30 in cut-off, plastic gallon water/milk jugs. These were fed daily on newly hatched brine shrimp until mid-May. Setting up the brine shrimp (in large finger bowls of saltwater under goose-necked desk lamps) and feeding the larvae only took about 30 minutes/day and that included cleaning a few bowls each time. The difficult part comes in trying to switch over to fragmented salmon chow.

The Biology Department allows me to use one of their cold rooms (15 degrees), and I have that lined with shelves. Some of the shelves are on wheels, and, in addition, I have a hand cart for moving dirty animals out to the sink in the lab for cleaning. Near the sink, I keep a 30-gallon plastic garbage can filled with tap water to allow the chlorine to evaporate overnight. I used to treat the water but no longer bother and have not noticed a difference.

Once the larvae are about an inch long, I isolate them into small and medium-sized plastic butter tubs which are labeled with bits of tape. I have an inexhaustable supply of plastic dishes. My Physical Therapy and Occupational Therapy students are more than happy to bring them into class, and many of the students have axolotl pets for their children.

Once isolated in the small dishes, the larvae either start eating the broken up bits of salmon chow or start shrinking in size. At that time they become lollypops for the adults or begin growing by leaps and bounds. The isolated larvae take lots longer to clean and feed, but are all stored, stacked up on the shelves in the coldroom. I usually spend 4–5 hours every other day cleaning, feeding, or doing experiments.

The animal-care fellow for the Biology Department is now 'into' axeys too. I just gave him 50 adults today to sell to a wholesale guy. The money he gets he uses to buy shrimp eggs or frozen brine shrimp to feed the larval axeys that he raises in large groups up in the animal facility. He then supplies me with as many 3" larvae as I need to run my experiments. This is just a hobby with him, but it is a neat way for me to get the older axolotls off my hands when they are no longer useful for my experiments. My stock of adults is down to about 150 now, but I have plus or minus 500 larvae from 1 to 3 inches long, plus an almost inexhaustable supply from my friendly animal-care fellow.

I keep the salmon chow in another garbage can in the cold room, so there is no problem with roaches. If I'm in a hurry to grow-up some larvae for use, I'll hand feed them strips of beef liver cut from slightly thawed liver stored in freezer on picnic plates in baggies. Many of the staff and students around CSU now have axey pets and feed them this way. When they accidentally get spawns, they usually bring them into me to raise. There will soon be more axolotls in Cleveland than there are researchers!! I contribute them to the classrooms of as many area science teachers as I can reach with my tales about the marvels of axolotls.

I often have 4–6 year adults in my colony and have not noticed a damaging effect of the plastic dishes. The square cut-off plastic gallon milk cartons fit nicely on shelves and are just high enough to prevent isolated animals from jumping out. They hold slightly less than 1/2 gallon water. I can usually clean about 70 adult dishes with my 30 gallons of declorinated water. So any individual adult axey usually gets cleaned at least once every two weeks and fed 2-4 pellets every other day or so.*

Acknowledgments

I learned about all I know about axolotls from spending 6 months in Hugh Wallace's lab in Birmingham, England (Jan –June,'80). He's really great and certainly the most knowledgable person imaginable when it comes to any kind of amphibian. He dug a pond in his back yard and now 'grows his own' newts and frogs.

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* These very infrequent changes are probably possible because the animals are kept at 15°C and in the dark. If in doubt, monitor ammonia levels. The editor.

Raising Axolotl Larvae

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In our experience, the biggest hassle with small larvae has to do with food; Raising live brine shrimp (or any live food animal) is a nuisance. Getting babies to switch food items as they grow is another hassle.

We have experimented with a variety of frozen food — brine shrimp of various sizes, worms of various types. Axolotls love worms and will readily gobble up living ones. Like other amphibians, their visual system is highly sensitive to moving, but not necessarily to dead, immobile prey items. But keeping live worms around is a pain, and they are not always available. We have found that frozen worms are readily accepted by axolotls so long as they are first trained to identify the frozen worms (black or red —available at pet stores) as food. It seems pretty clear that axolotls use odor, as well as visual cues to localize and identify food, and they very quickly learn to associate a particular odor with food. We soak the frozen worms in fresh water to defrost them, then offer them to the axolotl on the end of a forcep. It helps to move the worms around and bring them in contact with the axolotls nose. Within a few trials the axolotls identify dead, limp worms as food, and you no longer have to wave them across their face.

We have applied the same training technique to inducing young larvae, previously raised on live brine shrimp, to accept dead food. The worms need to be cut up a lot smaller (obviously), and the training takes somewhat longer, but it is worth the investment: More larvae survive, grow and thrive if they are encouraged to eat in this way, they develop faster if they are eating on a regular basis, and you don't need to mess with living food. We repeat the training process when it comes time to wean the larvae onto salmon pellets, and in very short order they learn to feed on their own.

Northcutt Lab Larval Rearing Methods: 1996

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Embryos are raised in egg capsules to hatching in large finger bowls of artificial freshwater (AFW) at room temperature (20°C - 28°C over the course of the year).

AFW Recipe:	$CaSO_4$	50 mg/L
	$MgSO_4$	5 mg/L
	KCl	4 mg/L
	NaH_2PO_4	1 mg/L
	in dH_2O	

Feeding of larvae

At stage 43, we begin to feed the axolotl larvae brine shrimp nauplii that we hatch from commercially available encysted eggs (found at any pet store). We raise the brine shrimp according to the instructions on the packet. Before we give the shrimp to the larvae, the shrimp are quickly rinsed in AFW using a fine-meshed net. Larvae are fed daily, and their water is changed every day.

Once the larvae are 10 days old (x = 1.7 cm), we begin to feed them finely chopped tubifex worms on a daily basis, and their water is changed every day. We get tubifex weekly from the pet store, and keep them in tap water in a bowl in the refrigerator.

By the time the larvae reach 4 - 5 weeks (~4 cm), they are fed intact tubifex worms. Water changes continue every day.

Larval / juvenile density

For the first 3 weeks, approximately 50 larvae are kept in each large finger bowl. After 4 weeks (x = 3.6 cm), the density of juveniles is reduced to 5 - 6 per bowl. By ~7 cm, only 2 -3 animals are in each bowl, and that number is reduced to 1 animal per bowl once the juveniles reach approximately 9 cm (9 weeks). We typically raise 12 -15 animals to adulthood. It takes about an hour or so a day to handle 20 animals including getting their food from the pet store.

Inexpensive and Low Maintenance Facility for Housing Axolotls

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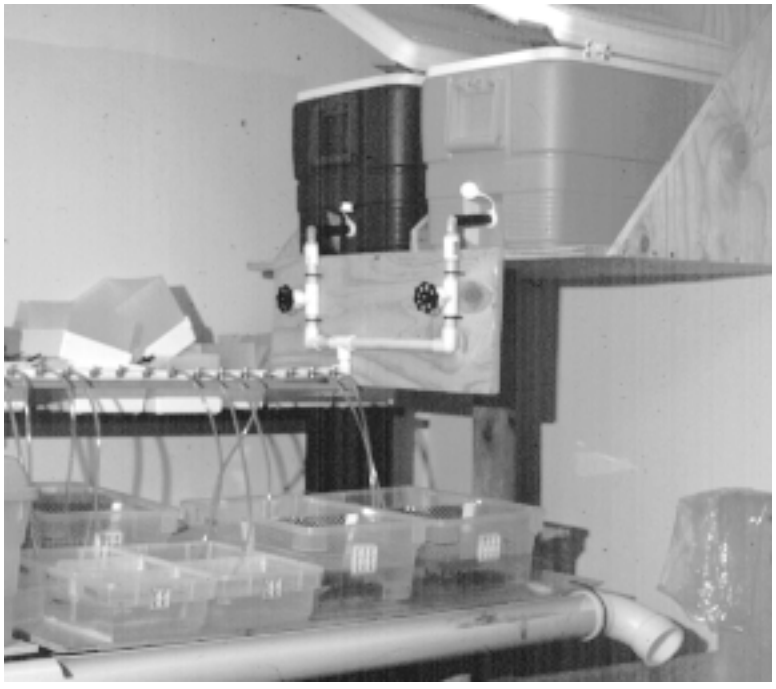
In order to maintain axolotls in the laboratory we have constructed an inexpensive easily maintained system from plastic containers, PVC pipe, rubber tubing, and picnic coolers. Stagnant water and all waste materials are removed by siphoning, and then tanks are flushed with aged tap water. This procedure is performed following feedings every 2–3 days and provides for an efficient low-maintenance method of caring for axolotls. Labeled photographs of the system are included, and a more complete description follows:

Large axolotl tanks: The larger axolotls are kept in clear plastic rectangular containers (22 x 36 x 14 cm) partitioned in half by 8-mm-diameter plastic mesh glued in place with clear silicon sealant. Two adult axolotls are housed in each container. A drain hole was cut and a protective grating was inserted in the front of each container. This drain maintains a constant water depth of about 5.5 cm and the grating prevents axolotls from escaping into the drainage pipe.



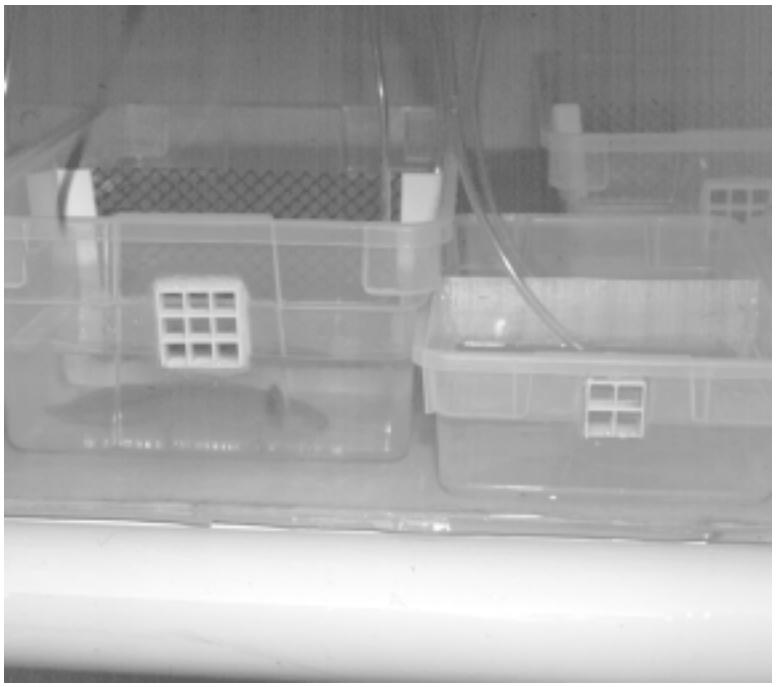
Small axolotl tanks: The smaller axolotls are kept in clear plastic rectangular containers (16x30x8 cm) partitioned in half or thirds by 1 mm diameter plastic mesh glued in place with clear silicon sealant. Two to six axolotls (depending on their size) are housed in each container. A drain hole was cut and a protective grating covered by a 2 mm diameter mesh was inserted in the front of each container. This drain maintains a constant water depth of about 4 cm and the mesh size of the grating prevents axolotls from escaping into the drainage pipe.

Water replacement system: Two large Igloo coolers which hold 40 liters of water each are mounted on a sturdy shelf approximately two feet above the axolotl tank water level. Half-inch PVC pipe connects each of the coolers' drain holes to a valve before converging into a single 0.5" PVC pipe with a series of 3/32" ID adjustable aquarium valves. Clear aquarium tubing (two per tank) supply each axolotl tank with fresh water at a rate of about 4 ml/s when one cooler valve is fully open. The tanks rest on a Plexiglass-covered plywood bench tilted slightly forward. Excess runoff water is collected in a trough cut from large PVC pipe at the front of the bench and is carried to a drain which eventually empties into a sink.

Additional information: We currently maintain 6 adult axolotls (15-19 cm long) in 3 large axolotl tanks and 6 juvenile axolotls (4-10 cm long) in 2 small axolotl tanks; however, the system is easily expanded to accommodate additional axolotl tanks. The Plexiglas covered bench on which the axolotl tanks rest measures 42" wide x 38" deep and resides on top of a lab bench 46.5" above the floor. The shelf on which the water storage tanks rest measures 38" wide x 26" deep and is located 24" from the ceiling. The entire system occupies a space 66" wide x 38" deep x 52" high, not including the sink located conveniently adjacent to the system. The sink serves as both a source of water for refilling the water storage tanks and a drain for flushed or excess runoff water.

Care of the axolotls: The axolotls are maintained at room temperature in ambient room lighting conditions. They are fed 4-5 pieces of frozen beef or pork liver that is slightly thawed (as it is easier to cut when semi-fro-

zen) and cut with a razor blade. The adult axolotls are fed pieces approximately 10 x 10 x 5 mm in size. After feeding the axolotls, their tanks are siphoned to clean out waste material and then flushed with fresh water from the water storage tanks. The water used to refill and flush the axolotl tanks is untreated tap water which has been allowed to age for three days in the water storage tanks (uncovered) in order to remove the chlorine. After cleaning the axolotl tanks, the water storage tanks are refilled using a rubber garden hose attached to the spigot of the nearby sink. The entire feeding/cleaning/refilling procedure takes approximately 45 minutes and is repeated every three days.



Housing and Feeding Larval Axolotls

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1. We house axolotl larvae individually in bowls that have the water changed every other day. We use glass bowls that are 9 cm wide and 3.5 cm deep. They are filled 2/3 full with water (solution: see 5 below).

2. The animals that we house usually range in size from 2 to 4 cm.

3. We feed the animals brine shrimp twice a day at 08:00 and 17:00. Once a week we feed them white worms from a colony that we keep.
I think the simplest way to go is feeding the larvae brine shrimp. They are easy to brew in a separatory funnel that is aerated from the bottom and you get a good hatch in 24 hours.

4. I usually house 24 to 30 animals at a time, but I have housed as many as 80 during a big experiment. I always house the animals individually.

5. Holtfreter's solution. The recipe I use consists of the following:

To 6 liters of water add:

300 ml Sodium Chloride
6 g Potassium Chloride
12 g Calcium Chloride
32 g Magnesium Sulfate

To another 6 liters of water add 24 grams Sodium Bicarbonate. The 12 liters are mixed together in a carboy. The chemicals dissolve best if the water is hot.

I make 24 liters at a time and store it in a 30 liter carboy I keep another 30 liter Carboy where I mix 1 liter of the above stock solution to 12 liters of water. I used to use a 1:6 ratio of stock to water, but I have found that 1:12 works just as well, and I don't have to mix up the stock solution as often.

White worm colony

I have a 3-pound coffee can that contains potting soil (about 2/3 full). I bury a slice of bread in the dirt once a week (whole wheat) and keep the soil damp. It is important that the bread stay buried or you get mold if it's exposed to air. I started with about 10 worms and in 2 weeks I had a colony. The best way to get the worms for a feeding to the axolotls is to make the soil very wet. They come to the surface and can be picked off. You could feed them to axolotls everyday, but you would need a big supply for a lot of animals. I have a colleague who used to keep a large aquarium full of the worms to feed fish. I think a larva that is 2 cm long could eat six to eight 1.5 cm worms a day. I use the worms primarily for reinforcement during learning experiments. They can last in a bowl of solution for about 24 hours. If they die the salamander won't eat them. It could also become very tedious picking the worms off the potting soil. You can't do it with forceps without picking up some dirt. I always transfer them first to a bowl of clear water using forceps. I tease them away from the dirt (they tend to clump with each other), then I pull them into a stopper and squirt them into their destination.

Axolotl Care at Indiana University Northwest

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At Indiana University Northwest, about two dozen animals are in the axolotl colony at present. Eight of these are proven breeders, and the remaining are from 1995 spawnings. In the present building, care was taken to have all animal care facilities in one location.

The main animal care room is in the shape of an "L" with the long leg measuring about 8 feet by 30 feet and the short leg measuring about 9 feet by 14 feet. In the long leg are two racks to hold cages for warm-blooded animals. These racks have been modified so that each rack shelf is considered an animal room, and each is connected to an air vent leading outside the building. In fact, a different species can be placed on each shelf and still be in excellent shape for anyone's inspection. Such a facility allows the remainder of the room to hold a rack with the axolotl containers on it. In the elbow of the "L" is a large sink and work area, which is used for the warm-blooded animals. At the end of the "L" is a storage room measuring about 10 feet by 10 feet. Tall storage racks on each side hold mouse food, cages, shavings, axolotl bowls, and other equipment.

This large room has a Tork timer (model W202) set for 12 hours light and 12 hours darkness.

Off from the short leg of the "L" are two smaller rooms, one of which measures about 9 feet by 12 feet and the other about 9 feet by 9 feet. Each room has a door, which may be closed and locked if necessary. The smaller room has a still in it (Barnstead Fistreem II) which empties into a 30 gallon holding tank. The tank is equipped with a switch that shuts the still off when full.

The larger room has a sink and work area along one long wall with a table against the opposite long wall. On the table is a 30 gallon plastic tank with spigot (see Cole Palmer HPDE H-06317-71). Distilled water and salts to make a 50% Holtfreter's solution are kept in this tank and used for axolotls, regardless of age. The rack with the axolotls is moved from the larger room into this room for changing and feeding. The bowls are changed in the sink and placed on the table. After the bowls have been filled with solution, the animals are fed pellet food, as used by the I.U. Axolotl Colony. The bowls are then returned to the rack and the rack returned to the larger room. The total time needed for the colony for changing and feeding is about a half-hour.

Larvae are also kept on the rack although tanks for brine shrimp are set up in the still room, where light for the brine shrimp will not bother any other animals.

The smaller rooms have their own lights not associated with the timer in the large room. It was discovered that if the solution tank were kept in the larger room, an algae problem developed with the extra light. In the smaller room with no lights on except for feeding and changing, there is no such problem.

When Biology first moved into this building about 6 years ago, the colony was about 100 animals—all adult and non-breeding. Changing and feeding took a little longer but still went smoothly with this set-up.

Maintaining Larvae

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We use 1x Holfreter's solution made in deionized water. We change the axolotls every other day, but sometimes they go from Friday to Monday without a change. We keep them in *Tupperware* containers usually at least 5 times the size of the animal. We feed them the salmon pellets from *Rangen*, but they like liver more. They are fed 3 times a week. The containers are cleaned when the water gets changed with a bleached and well-rinsed brush.

Our animals are housed on shelves in a small room with a sink. We spend about 2- 3 hours a week on maintenance depending on the number of animals and whether Holtfreter's solution needs to be made.

Simple Brine Shrimp Hatchery

Brent Mundy
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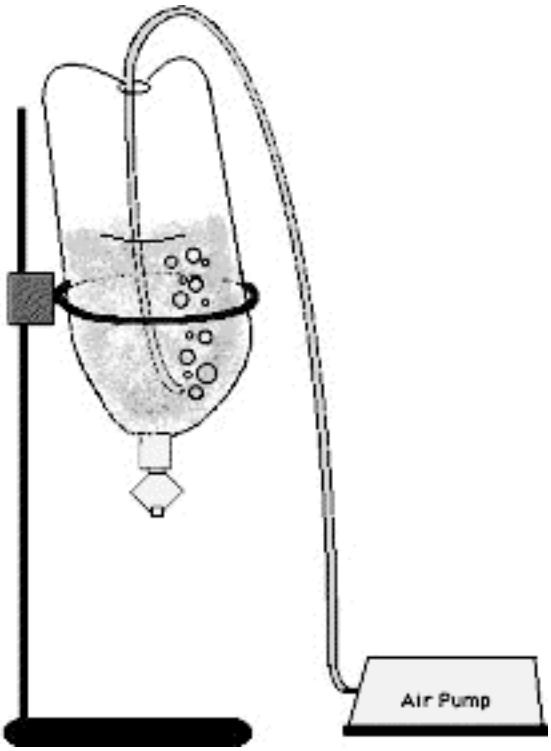
Bloominton, IN 47405

Constructing the Hatchery

Construct a simple shrimp hatchery out of a two-liter clear plastic cola bottle, the pull-up cap from a 32 oz (1 liter) dishwashing detergent bottle (or sports drink bottle), an aquarium air pump, a 3-4 ft (about 1 meter) long piece of tubing that fits onto the air pump outlet to use as an air hose, and a stand of some kind to support the bottle in an inverted position. A heat lamp with a 40-watt bulb is optional.

First, empty and clean the two-liter cola bottle with hot water. Use a sharp knife to cut a hole 1 to 1 1/2 inches (about 3 cm) in diameter in the bottom of the cleaned bottle. Next, rinse the pull-up cap from the detergent bottle very well with warm water. The threads



on the cap and the two-liter bottle should be compatible. Screw the cap onto the two-liter bottle.

This apparatus will be used inverted. The pull-up cap serves as a reclosable drain at the bottom. Fill the bottle half-full of water and draw a line across the bottle at the water line with a permanent marker. Empty the water back out.

Position the air pump so that the air hose reaches the bottom of the inverted bottle. The diagram shows how the setup should look.

Operating the Hatchery

Rinse out the bottle, clean with a brush if necessary, make sure the drain spout is securely closed, and place it in the stand in inverted position. Add about a cup (about 350 ml) of hot tap water through the hole at the top. Use a funnel to add three tablespoons (45 cc) plain (uniodized) table salt —NaCl— to the hot water. Swirl to dissolve the salt. Fill the bottle the rest of the way to the half-full line with cold tap water. Next, add 1/4 - 1 teaspoon (2-5 cc) brine-shrimp eggs (cysts) to the bottle, depending on how many larvae you need to feed. For fewer than 20 larvae, 1/4 teaspoon (2 cc) should be plenty. Swirl the bottle gently to mix the eggs and brine. Place the air hose through the hole so that it reaches the bottom of the inverted bottle. Make sure that the air hose is positioned properly and bubbling vigorously because the shrimp will not hatch well unless the water is agitated continuously.

Most of the shrimp should hatch within 24 to 48 hours. The length of the cycle depends upon the temperature. The hatched shrimp are orange and can be easily seen through the plastic bottle. When most of the shrimp appear to have hatched, remove the air hose and hold the bottle over a clean shallow pan. After most of the gray shells have floated to the surface, open the drain to empty the newly hatched shrimp and brine into the pan. Close the drain before the last of the brine and the shells enter the pan.

Manipulating the Cycle

To shorten the cycle raise the temperature of the system by putting the apparatus in a warmer location or directing a lamp with a low-wattage bulb at the hatchery. Alternatively add more hot water initially to start with a warmer mixture.

To lengthen the cycle place the hatchery in a cooler location or use less (or no) hot water initially.

Troubleshooting

The percentage of shrimp that hatch depends on the temperature and length of the

cycle, on whether the brine has been continuously and vigorously agitated, and on the quality of the shrimp eggs used. If, after checking that temperature and agitation are appropriate, only poor hatches are obtained, consider changing to a different brand or supplier.

Collecting Shrimp to Feed

Place a large coffee filter across the top of a wide-mouthed jar or similar container. Collect live, swimming brine shrimp from the pans by sucking them up in a large pipette (turkey baster). Do not suck up dead brine shrimp from the bottom of the pan. Avoid getting any floating shells by placing the tip of the pipette just below the surface of the water. For easier collecting, place a light at one end of the pan. The shrimp are phototropic and will swim toward the light, conveniently gathering themselves together.

Squirt the shrimp and brine into the filter to strain the shrimp out of the brine. Discard the brine, then wash the shrimp off the filter and into the container with axolotl water. Distribute the shrimp suspended in axolotl water among the bowls of larvae with the pipette. Feed very young larvae just enough to make their bellies orange. Feed larger larvae generously to forestall cannibalism. It may take a few days of feeding and observing the results to get a sense of how much to feed.

Care for Larvae

Small larvae (< 2 inches or 5 cm long) are fed brine shrimp. Change the water and feed them daily. Keep their bowls clean but never use soap. If necessary, use a little baking soda and salt mixed together as a cleaning agent (two parts baking soda to one part salt). Scrub, then rinse thoroughly. As the larvae grow, split them up into additional bowls. Keep similar sized larvae together.

Terrestrial Axolotl Care Sheet (*Ambystoma mexicanum*)

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IU Axolotl Colony
Bloomington, IN 47405

Metamorphosis

Transforming from an aquatic salamander to a terrestrial salamander is very stressful for the axolotl. Once it is noticed that an axolotl is metamorphosing it should be removed from its regular habitat and placed in a covered one gallon container. The container should be large enough for the animal to fit comfortably. A few rocks should be placed in the container for the animal. Add just enough water to the container so that the axolotl's back is covered.

Over the course of a few days, the animal will molt and shed quite a large amount of skin, which will look like black sheets. The skin should be removed on a daily basis. During metamorphosis the axolotl will absorb its dorsal fin, grow eye-lids, absorb its gills, lose its slime coat, and begin breathing air. As the animal progressively becomes more terrestrial, the amount of water that is in the container can be reduced.

After the animal switches over, a period of adjustment will occur. Limbs that were once used for swimming must now learn to walk on land. At first the axolotl will spend most of its time in the water, but as it learns to use its legs, travel on land will become more common. It is not uncommon for a newly metamorphosed axolotl to go off of food for some time. This fast may last for up to two months.

Habitat (Vivaria)

A wide variety of habitats can be created for the terrestrial axolotl. The only steadfast requirements are that the animal has access to water, so that it can totally submerge itself if it so desires, and a dry land area. A very basic design could consist of a 10 gallon aquarium and a large water bowl for the axolotl. Obviously more sophisticated setups can also be created. Below is the outline of an advanced setup:

> A large sturdy aquarium will need to be divided in a 30% water, 70% land split; a piece of glass or Plexiglas can be glued using aquarium silicone cement in the tank in order to make a shore. Slant the partition to make a slope that the axolotl can climb to leave the water. Fill the water portion with an inch of aquarium gravel (Note: gravel should be large enough that the animal cannot ingest it. Put two inches of coarse sand or washed aquarium gravel in the bottom of the land portion of the tank for drainage. On top put sterile potting soil or peat moss. The soil should be loose so that the axolotl can burrow if it chooses. Furnish pieces of bark, rocks, or clay pot shards to make hiding places for the axolotl. You can plant small plants in the soil if you like, but they should be large enough so that the axolotl will not destroy them.

You will need a fitted lid for the tank. The lid will keep live food inside the tank. The lid is also a useful place on which subdued lighting can be placed.

Temperature

Salamanders need a relatively cool environment. The terrestrial axolotl should be kept between 70-75 °F. (15-24 °C.). If possible provide a temperature gradient so the axolotl can thermoregulate by moving back and forth between warmer and cooler areas. This is easily accomplished by designating one side of the tank as the warm side, usually the dry side. A slight gradient of only a few degrees is needed, which can be created using lights.

Lighting

Light is necessary for the regulation of the axolotl's seasonal clock. Do not rely on sunlight, which could raise the temperature of the habitat too much. Use a broad-spectrum light connected to an appliance timer. If you plan to try breeding the axolotl, you may wish to increase and decrease the number of hours of light based on the changing photoperiod of the axolotl's native environment near Mexico City.

If possible, the light should be subdued and indirect. A hiding place should be provided for the animal so that it is able get out of direct light if it wants.

Ventilation

Ventilation is important to prevent the atmosphere inside the tank from becoming foul

and to reduce the growth of organisms in the water and soil. Drafts, however, should be avoided. This can be accomplished by using a screen cover over the tank.

Water Filtration

To keep the water clean, either filter it or do a complete water change every other day. Choose a filter appropriate to the volume of water in the tank.

Food

Larger prey items should be dusted with a vitamin powder that is available for herptiles (found in pet stores). Worms, small spiders, pillbugs, beetles, earthworms, small millipedes, aphids, small moths and other night-flying insects are suitable for more terrestrial axolotls. In winter, crickets, mealworms, white worms, and tubifex worms can be purchased. Mealworms should be fed only as a supplement as their hard outer covering is difficult to digest. Tubifex worms can be fed in a shallow dish with a little water in it.

Feed daily only as much as the axolotls can eat. Leftovers can be left in the tank if they are still alive, but hold off feeding more until they are gone. A wide variety of food will insure a more balanced diet. For the most part, terrestrial axolotls need live food, because they rely on movement to detect their prey. You may be able to train them to eat raw beef or dead prey. Crickets, earthworms, mealworms and the like can be grown at home or purchased, if collecting prey is a problem.

Handling

Handling should be kept at a minimum. If you need to handle an axolotl, be sure your hands are cleaned and free of soap residues. Wet your hands first, then scoop up and cage the axolotl in your two hands without squeezing it. Use gloves (wet them first) or a net if you prefer. Be sure to wash your hands thoroughly when you are done handling the axolotl, since amphibians can carry Salmonella and other diseases.

Mixing Species

A note on keeping different species together in one tank: many species cannot tolerate the toxins produced by other species. Putting them together may result in the deaths of one or more species. The Axolotl Colony has been successful in housing terrestrial axolotls and tiger salamanders of similar size together.

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To 8 liters distilled, dechlorinated tap, or spring water add:

2.3 g potassium chloride
4.29 g calcium chloride
8.90 g magnesium sulfate
126.72 g sodium chloride

For 100% dilute 1:4, for 50% 1:8, for 20% 1:20.

Cheryl Nugas (Holtfreter's stock)

To 3 liters milliQ water add:

2 tablespoons magnesium sulfate
2.5 teaspoons or scant tablespoon calcium chloride
1 teaspoon potassium chloride
240 cc sodium chloride
27 g Trizma (7.4) fish grade

For 40% add 300 mls stock to 5 gallons milliQ water.

Wonsun Kim

Use charcoal filtered tap water that has been aerated overnight after filtration.