

Axolotl Larvae Housing Methods

Cheryl A. Nugas
Susan V. Bryant Laboratory
Developmental Biology Center
University of California
Irvine, CA 92637-2275

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% Holtfreter's 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.

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 ·7H ₂ O
	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 H₂O 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 H₂O.

To make 20% Holtfreter's: Add 150 mls of Holtfreter's stock to 5 gallons of milliQ H₂O.

We use two different types of secondary 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 ADULT 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 harder pellet for the adults.

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

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 pre-hatchling (20% Holtfreter's), larvae (40% Holtfreter's solution with milliQ H₂O), and adult (40% Holtfreter's with DI H₂O) 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 H₂O.
- 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 gravity strainer. Keep one end of the gravity 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 gravity strainer. Using a brine shrimp collecting net, gently pour out 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 gravity strainer thoroughly. Invert the net into the gravity strainer and rinse out net with 40% Holtfreter's, collecting the shrimp and solution into a clean gravity 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 approxi-

mately 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 gravity 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 if 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 H₂O.

Saturated Rock Salt Solution:

- 500 mls of milliQ H₂O
- 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.