

Instructions for the Care and Feeding of Axolotls

by A. J. Brothers, I.U. Axolotl Colony

Temperature: Axolotl eggs and larvae are best raised at a temperature between 18 and 20°C. Adults can be kept in breeding condition throughout most of the year by keeping them at 18°C. The development rate of eggs and larvae can be controlled by modulating the temperature (the tolerance range is 8° to 24° C.). They should not be kept at the temperature extremes for extended periods, i.e. more than 2 to 3 days. This enables an entire spawning to be used for embryological procedures or detailed observations when screening for mutant characteristics.

Water supply: Use a 20% Steinberg's solution, pH = 7.0-7.4 for eggs and larvae until they are approximately 1 month of age. Be careful to avoid evaporation of the solution in the bowls containing larva. Concentration of the salts above the level found in 100% Steinberg's solution will damage the gills. Thereafter the larvae may be kept in a solution of 20-25% modified Holtfreter's solution (Holtfreter's solution with 51 mg MgSO₄ .7H₂O added per liter of solution). We now routinely use 50% modified Holtfreter's (50% Holtfreter's solution + 102 mg MgSO₄ .7H₂O/liter) for most of the adult animals in the colony, and the juveniles (2 months of age or older). Some laboratories keep axolotls in dechlorinated tap water. The tap water is dechlorinated by adding a few crystals of sodium thio-sulfate per gallon of water (¼ teaspoon/60 liters). This solution is allowed to set at least 6 hours before using it for animals. WARNING--In some areas of the country the tap water is not suitable for axolotls. This can be due to several reasons and in several instances the tap water has proven to be lethal to the animals. Also, there is no guarantee that tap water which can be safely used for the animals will remain so continuously. It is therefore STRONGLY suggested that either appropriate measures be taken to monitor the safety of the water supply or that distilled water be used for these solutions. If a problem of epidermis shedding by adult animals is encountered then it is suggested that the animals be kept in a solution equivalent to 50% modified Holtfreter's solution.

Eggs and developing larvae are kept in large shallow finger bowls (diameter of about 19 cm. or 7.5 inches). The water level should be kept shallow in order to allow proper aeration (use approx. 1½ inch depth water). Avoid crowding--allow about 50 eggs or larvae per bowl. When the larvae begin to hatch carefully remove the discarded jelly capsules with a wide mouthed pipette. Newly hatched larvae should also be removed to another bowl (water depth should also be kept at approx. 1½ inch depth in this bowl). At times some larvae may be delayed in hatching. In those instances the jelly coats may be removed by gently puncturing the capsule with forceps, allowing the larvae to escape. Newly hatched larvae should be fed within 24 hours.

For maximum growth only 20-30 recently hatched larvae should be kept in each shallow finger bowl. The water depth is kept at about 1½ inches in **order** to circumvent the possibility that the larvae will swallow air and also to ensure an adequate oxygen supply. At about 1 week post hatching, decrease the number per bowl to no more than 20. By the time the larvae are 1 to 1½ months of age (approximately 2 cm in length) there should be no more than 10 per bowl (preferably 5-6). Larvae between 2-2½ cm and 6 cm in length are kept in individual finger bowls (diameter of about 4 inches or 10 cm). Larvae larger than 6 cm.

are kept in individual squat fish globes and by the time the animals are 9 to 10 months of age they should be placed in gallon size squat fish globes (water depth is about 7 cm.).

Food: After hatching, the larvae are fed freshly hatched brine shrimp--drain the shrimp free from the brine that you use for the hatching (shrimp) medium before you add them to the bowl of axolotl larvae. The shrimp are left in the bowl for about 1-2 hours. Then remove the dead shrimp, change larvae to clean water and feed again. The larvae should be fed 3-6 times a day--7 days a week. When the larvae reach a length of 2 cm (about 1 month of age) they should be started on a diet of young (or baby) beef liver or beef heart. Use liver/heart which has had the connective tissue removed. Chunks of about 1-3 inch thick are kept frozen. Very thin slivers are easily obtained when this partially frozen liver is sliced with a sharp knife. The larvae are fed individually by dangling a small sliver of liver in front of each larva until it grabs the end of the piece and swallows it. Attempt to get each larva to eat 3 to 6 pieces per day--7 days a week. Let them digest their food for about 1 hour after feeding before changing the water. The diet may be supplemented by feeding dejellied infertile axolotl eggs and small Xenopus larvae to the axolotls. Rana pipiens tadpoles are a delicacy for juvenile and adult axolotls. Small axolotl larvae may have difficulty digesting these tadpoles however. Juveniles and adults enjoy the axolotl cookies (recipe given later). Well rinsed earthworms are also a valuable food supplement.

At about 5 to 6 months of age young axolotls are switched to a diet of chow/liver mixture. This is prepared from 20 lbs ground (all connective tissue removed) calves liver. This type of liver can usually be obtained from a commercial packing house on special order. It should be kept nearly frozen during the grinding process and used immediately. Mix 72 oz. Gainesburgers (pulverize in a Waring blender before mixing) with the liver. Disposable gloves are worn while working with and mixing the liver-chow mixture. This mixture is then packed into small finger bowls (about 4 inches diameter). Fill each bowl about $\frac{1}{2}$ full and cover with aluminum foil. Freeze immediately. A few hours before use the bowl may be removed from the freezer (-20°C) and allowed to thaw slowly in a refrigerator (6°C). The mixture is used while still in a partially frozen state--small portions are scooped up with a metal spatula. Each animal should receive about 4 to 6 portions. Let the animals digest their meal for about 1 hour before changing the water. The water must be changed after each feeding and must be kept clear at all times. Juveniles are fed six days per week; adults are fed every other day (3 days/week).

Cleaning procedures: The bowls of juvenile and adult axolotls are washed once/week (or sooner, if dirty) with a weak disinfectant. We use "CDQ" (Brulin; Indianapolis) at a concentration of about 1cc/cup water. Rinse thoroughly before the animal is put back into the bowl. At each time the water is changed the axolotl bowl is scrubbed with Ajax and a net puff, and rinsed thoroughly.

Metal spatulas should be washed thoroughly and dipped into a solution of 70% ethanol before use.

The axolotls should be divided into small groups for feeding and daily care routines. Each group should have its individual spatula(s) and collander. These procedures will cut down on possible cross-contamination of the animals throughout a colony in case of a disease outbreak. The collander is immersed in the disin-

fectant solution (~ 5 inches deep in a plastic dish pan) before use and between changes for each animal.

We use large plastic containers (garbage cans from Sears Co. (Cat. #11A-63753L2) are very handy) for water containers for our colony. These are scrubbed with disinfectant every week.

Table of Main Symptoms of Diseases and Treatment

<u>Stages Affected</u>	<u>Brief Description</u>
Larva and juveniles affected (but can also affect adults)	<p>A. <u>Vorticella infestation</u>: Colonies appear to cluster on rachis of the gills during the 1st stages of infection.</p> <p>Treatments: a.* 100% modified Holtfreter's soln or 100% Steinberg's soln, for 3-5 days (cover the bowls to prevent evaporation), plus (in cases of heavier infestation)- b. Dilute sodium hydroxide immersion 10 minutes for each of 3 days.</p> <p>Since we now keep all larvae in 20% modified Holtfreter's soln we have not encountered this problem with vorticella infections for several years. However several months ago during some remodeling involving the building duct system we did encounter a problem with a colonizing vorticella. The best treatment in this instance proved to be a combination of the treatments given above with Nitrofurazone (2 days), followed by Methylene blue and Acriflavin treatment (2 days).</p>
Large juveniles and adults.	<p>B. <u>Mold infections</u>: This can be a particularly onerous problem during/after vorticella infection. (1) Tropi-Cure (General Developments Corp., Milwaukee, Wisconsin 53217) immersion. 1 ml/500 ml modified Holtfreter's soln. Immerse larva for 10-15 minutes. Adults should be immersed for 20-30 minutes. This immersion procedure should be followed for 3-5 days. Keep the animals in 100% modified Holtfreter's soln for 3-5 days. (2) Some molds, particularly unicellular molds are best treated by treatment with Potassium permanganate. Gently swab the infected area. (We use cotton tipped swabs for gill areas and gauze pads for areas on the body).</p> <p>A. <u>Aeromonas infection</u> - Symptoms: small red splotches over body infectious and lethal. Described by Dr. Lou DeLanney.</p> <p>Treatment: Tetracycline injection (please refer to Liquamycin instructions in next section) also use of Cosa - terramycin in the water. Do <u>NOT</u> house axolotls in same room with <u>Rana pipiens</u> etc.</p> <p>B. <u>Actinomycetes infection</u> (Red Plague) - Symptoms: diffuse redness over entire body. Lethal within 24 hours</p>

after this symptom appears. Highly infectious Believed to be transmitted upon contact with other salamanders and amphibians from wild populations.

Treatment: Gentomycin injections plus nitrofurzone (2 days) followed by Methylene Blue - Acriflavin treatment. All adult and large juveniles (8 months or older) should be injected and treated simultaneously. Repeat the Gentomycin injections every 48 hours until 3 injections have been given to each animal.

C. Salmonella - Shigella infections - Symptoms: lack of appetite, listlessness, maybe some diarrhea. Infectious. Treatment: All adults and large juveniles should be treated simultaneously. Gentomycin has proven to be the most effective antibiotic. Use Nitrofurazone (2 days) followed by Methylene Blue - Acriflavin (3 days) in the water. For very severe cases, after the animal is injected wrap its body loosely in damp paper towels and put it back into the bowl with about 1 inch of modified Holtfreter's soln. Place the animal in a 8-10° incubator. It is advisable to keep the animal chilled for several days (3-5).

D. Pseudomonas infection - Symptoms: ulceration of abdominal epidermis. If unchecked the infection will perforate the epidermis/basement lamella into the body cavity. Animals show some loss of appetite. Somewhat infectious.

Treatment: Swab the infected area with potassium permanganate solution. Repeat every 2-3 days for a period of 2 weeks. Injection of Gentomycin will protect against secondary infections. Use the Nitrofurazone (1 day/week) followed by the Methylene Blue - Acriflavin (3 day/week) treatments in the animal's water.

E. Blister Disease - Symptoms: large fluid filled swelling on the top of the head. Wistar strains are more prone to develop this type of blister than the other axolotl strains. Not fatal.

Treatment: Trim away the loose skin and expose the area. Penicillin G powder is sprinkled onto the affected area. The area will heal in 1-2 weeks.

If blisters are small, or do not rupture, they might actually disappear without treatment.

Antibiotics and Agents That Can Be Used Safely For Treatment

1. Injection:

- a. Gentamicin (Schering Corporation) -- antibiotic with a broad spectrum of activity. When in doubt this is the antibiotic of choice.

Dose for a full-grown adult axolotl = 1.8 mg injected intramuscularly. Injection can be repeated every 48 hours if necessary. The dose for smaller animals should be adjusted for the size of the animal.

We have used this antibiotic to treat infections caused by (1) Actinomycetes (very effective), (2) Salmonella - Shigella (very effective) and (3) Pseudomonas (only partially effective).

- b. Liquamycin (Pfizer, Dept. of Veterinary Medicine) -- this is an oxytetracycline hydrochloride which we have found to be effective against infections caused by (1) Salmonella - Shigella and (2) Aeromonas.

Dose for a full-grown adult axolotl = 4.1 mg injected intramuscularly. Injection can be repeated every 48 hours if necessary.

- c. Bicillin Fortified (Wyeth) -- Aqueous suspension of benzathine penicillin G (150,000 I.U./cc) and procaine penicillin G (150,000 I.U./cc).

Dose is a total of 25,000 I.U. Prepare from 2 cc stock plus 10 cc sterile saline. Inject 0.5 μ of this working solution intramuscularly into adult axolotl.

2. Treatments administered in the water.

- a. Cosa-terramycin (Pfizer, Dept. of Veterinary Medicine) -- this is a fortified soluble powder of oxytetracycline hydrochloride.

Dose for all sizes of animals = 250 mg/2 liters. This solution should be changed every 24 hours, preferably fresh solution should be used each 12 hours. Duration of treatment is 3 days.

- b. Nitrofurazone (Eaton Veterinary Laboratories) -- Trade Name is FURACIN, Water Mix, Veterinary.

Dose for all sizes of animals = 250 mg/2 $\frac{1}{2}$ liters. This solution may cause some diarrhea. Duration of treatment is 2 days.

- c. Methylene blue (0.0075%) plus Acriflavin (0.045%) = stock solution. For use dilute 1cc of stock solution to 2 liters of the modified Holtfreter's soln. Duration of treatment is 2-5 days. (This treatment was suggested by Dr. Roy Tassava).

- d. Dilute NaOH 10 drops of 0.1 NaOH per 1 liter. Duration of treatment is 10 minutes.

- e. Potassium permanganate 1 gram/100 cc distilled H₂O. The affected area is swabbed with this solution (gently) and the animal replaced into 50 or 100% modified Holtfreter's solution. The treatment can be repeated every 2nd or 3rd day. This treatment is effective for molds and pseudomonad infections.

Diet Supplements

Axolotl Cookies

Ingredients

calcium phosphate (CaPO₄ .3H₂O) - 5% 5gm/100ml

bone meal may also be used instead of calcium phosphate

desiccated liver - 5%

nettle powder - 1 tsp./liter

finely ground Gainesburger dog food (pulverized in a Waring blender)

gelatin - 5%

1 liter distilled H₂O

Procedure

Mix the gelatin in the distilled H₂O (50gm per liter water). Autoclave the gelatin solution for 15 minutes at 15 lbs pressure. Allow this to cool, then add calcium phosphate, nettle leaves, and desiccated liver. Pour this over ground Gainesburger and spread in flat baking pans, such that the gelatin just covers the Gainesburger. Cool and serve ... Food supplement for juveniles (over 2½ months) and adults.

Synthetic food for axolotls

(reprinted from: Meier, A.H. & L.E. DeLanney, Turtox News, Vol. 40, No. 11, November 1962, pp. 280-281)

Ingredients

Mixture A

1 egg

liver slice 2 X the size of an egg

2 level tablespoons Cream of Wheat (Quick)

1 beef bouillon cube

1 cup water

Beat above thoroughly in blender.

Mixture B

½ pkg. dried yeast or brewer's yeast tablets

1 teaspoon wheat germ

2 level tablespoons gelatin

food coloring (red or green)

1 cup water (cold)

Procedure

Allow Mixture B to set for at least 5 minutes before combining with Mixture A. Heat 1 cup of water to boiling and add Mixture A. Heat to boiling or until curdles are formed in clear liquid; stir occasionally. After Mixture B has been allowed to stand for at least 5 minutes combine the two mixtures. Cool the entire mixture to room temperature by allowing it to stand. Place in refrigerator compartment and check every 5 minutes; stir at each interval. If the floating form is desired, when the mixture is well-jelled about the edges (10-20 minutes) beat vigorously for several minutes with a beater and then replace in the freezer compartment until jelled (about 10 minutes). If the nonfloating form is desired, omit the beating procedure.

HOLTFRETER SOLUTION

<u>Component</u>	<u>Amount</u>	<u>MW</u>	<u>Moles</u>
Solution A:			
NaCl	3.5 gm	58.45	0.0598
KCl	0.05 gm	74.56	0.0007
CaCl ₂	0.1 gm	110.99	0.0009
Glass Distilled H ₂ O	500 ml		
Solution B:			
NaHCO ₃	0.2 gm	84.01	0.0024
Glass Distilled H ₂ O	500 ml		
			<u>0.0638</u> molarity

The solutions should be sterilized separately. When cooled, they should be mixed in 1:1 proportion.

100% Modified Holtfreter's Solution: Holtfreter solution plus 204 mg MgSO₄ .7H₂O per liter.

STEINBERG SOLUTION FOR CULTURING AMPHIBIAN EMBRYO EXPLANTS*

<u>Stock Solution</u>	<u>Formula</u>	<u>per liter</u>	<u>per 500 ml</u>	<u>per 250 ml</u>
17.0% NaCl	17 gm/100 ml	20 ml	10 ml	5.0 ml
0.5% KCl	2.5 gm/500 ml	10 ml	5 ml	2.5 ml
0.8% Ca(NO ₃) ₂ .4H ₂ O	4 gm/500 ml	10 ml	5 ml	2.5 ml
2.5% MgSO ₄ .7H ₂ O	10.25 gm/500 ml	10 ml	5 ml	2.5 ml
1.00 N NCl	(for pH 7.4)	4 ml	2 ml	1.0 ml
Tris Buffer--	Trizma Base (reagent grade)	560 mg	280 mg	140 mg
Glass-distilled water		946 ml	473 ml	237 ml

For surgical operations we use 400 mg Penicillin-G, plus 400 mg Streptomycin sulfate, plus 50 mg Gentomycin/liter. The solution is sterilized by Milpore filtration.

*From Carnegie Institute of Washington Yearbook 56. Annual Report--Director, Department of Embryology, p. 348.

Axolotl Supplies and SuppliersShrimp

Metaframe San Francisco Bay Brand brine shrimp eggs; Metaframe Corporation, Newark, California 94560.

Order from pet food suppliers, e.g., Atlas Pet Food Supply, Inc., 2042 Stout Field, West Drive, Indianapolis, Indiana. \$16.00/15 oz/can.

For hatching directions see separate sheet.

Bowls

(low profile)

Anchor Hocking squat fish globes distributed by Belknap Hardware, Louisville, Ky. We have only been able to get these from retail hardware stores, e.g., Bloomington Hardware, Courthouse Square, Bloomington, Indiana 47401.

Approximate prices: 1 quart @ \$.65, ½ gal @ \$.90, 1 gal (normal adult size) @ \$1.49, 2 gal @ \$1.95.

Shipping jugs

Most insulated "picnic jugs" made of plastic or metal will serve well; e.g., Coleman and Thermos brands. We prefer those with a shoulder spout rather than an outlet at the bottom to prevent accidental release of water during shipping. Present supplier: Family Products, Inc., P.O. Box 1600, Lowell, Mass. 01853. Order Stock #012, one gallon jug with shoulder spout @ \$2.11 plus shipping.

Gentamycin

Gentamycin reagent solution 50 mg/ml in 10 ml vial @ \$22.80, Schering Corp., P.O. Box 92338, Chicago, Illinois 60675.

FSH

Follicle stimulating hormone pituitary (FSH-P) with diluent, 50 mg vial @ \$6.00; W.A. Butler Co., 4140 Fisher Road, Columbus, Ohio 43228.

Powdered Nettle Leaves

Amend Drug & Chemical Co., Irvington, New Jersey 07111.

Dessicated Liver

Bacto Liver, Difco Laboratories, Detroit, Michigan.