ESTABLISHMENT OF A FLOW-THROUGH WATER SYSTEM TO MAINTAIN A COLONY OF AXOLOTLS CARRYING THE pi GENE

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We have set up a colony of axolotls carrying the pi gene (Neff et. al., 1987) using a flow-through water system that is inexpensive and labor efficient. Our version of a flow-through system for the care and maintenance of axolotls requires less handling of individual animals (and therefore less stress on the animals) than the conventional system of keeping animals in individual bowls and feeding each animal individually. Our animals are housed in converted laundry tubs, through which there is a constant flow of fresh water so there is no need to change the water of individual animals periodically. Since the animals have a constant turnover of clean water, the need to clean the tubs themselves is reduced. We find that about 3 hours per week of labor is required to maintain up to 64 animals. By contrast, the maintenance of animals in single bowls takes up to 6 hours of labor per week for every 64 animals. Our colony presently houses 40 adult animals and has been in operation for one year.

In order to establish the proper care and maintenance conditions, we experimented with the following parameters: (a) number of animals per tub, (b) water flow rate, (c) water depth in the tubs, (d) feeding, and (e) cleaning. The health and behavior of the axolotls as well as the maintenance of good water quality as measured by cleanliness, neutral pH, and low ammonia levels were used as positive indicators.

The present system, shown in Figure 1, consists of eight white, single-compartment, copolymer laundry tubs (Sears, Roebuck, and Co.: 20 x 20 1/2 x 12 inches) mounted in a double row of 4 tubs each on a rack constructed from 3 by 1.5 inch galvanized angle iron. The bottom row of tubs protrudes forward by 6 inches to allow easier access to the animals and more light to reach into the bottom tubs. The tubs are plumbed with 1 1/4 inch PVC tubing into the room drain. Water level within individual tubs can be controlled by the placement of rubber stoppers in paired drain holes drilled in the PVC pipes set in the internal drain. In order to prevent the fresh water from draining without thorough mixing with tub water, water is drained from the bottom of the tubs. This was accomplished by fitting the drain pipes with a sleeve pipe (2 inch PVC) to within 1 inch of the bottom of the tub. Two 55-gallon, polyethylene holding tanks are mounted on top of the rack. Water is supplied by siphoning water from the bottom of the holding tanks through teflon tubing (Teflon FEP, Cole-Palmer Inst. Co.) and dripping it into the tubs. The flow rate is controlled by selecting the proper tubing size and by varying the length of the tubing. The tubing to the upper tubs has an internal diameter of 1/16 inch and the tubing to the lower tubs has an internal diameter of 1/32 inch. A fiberglass, 250-gallon mixing tank sits at ground level. Twenty-five percent Holtfreters' solution, made up in the mixing tank with tap water, is pumped to the holding tanks as needed.

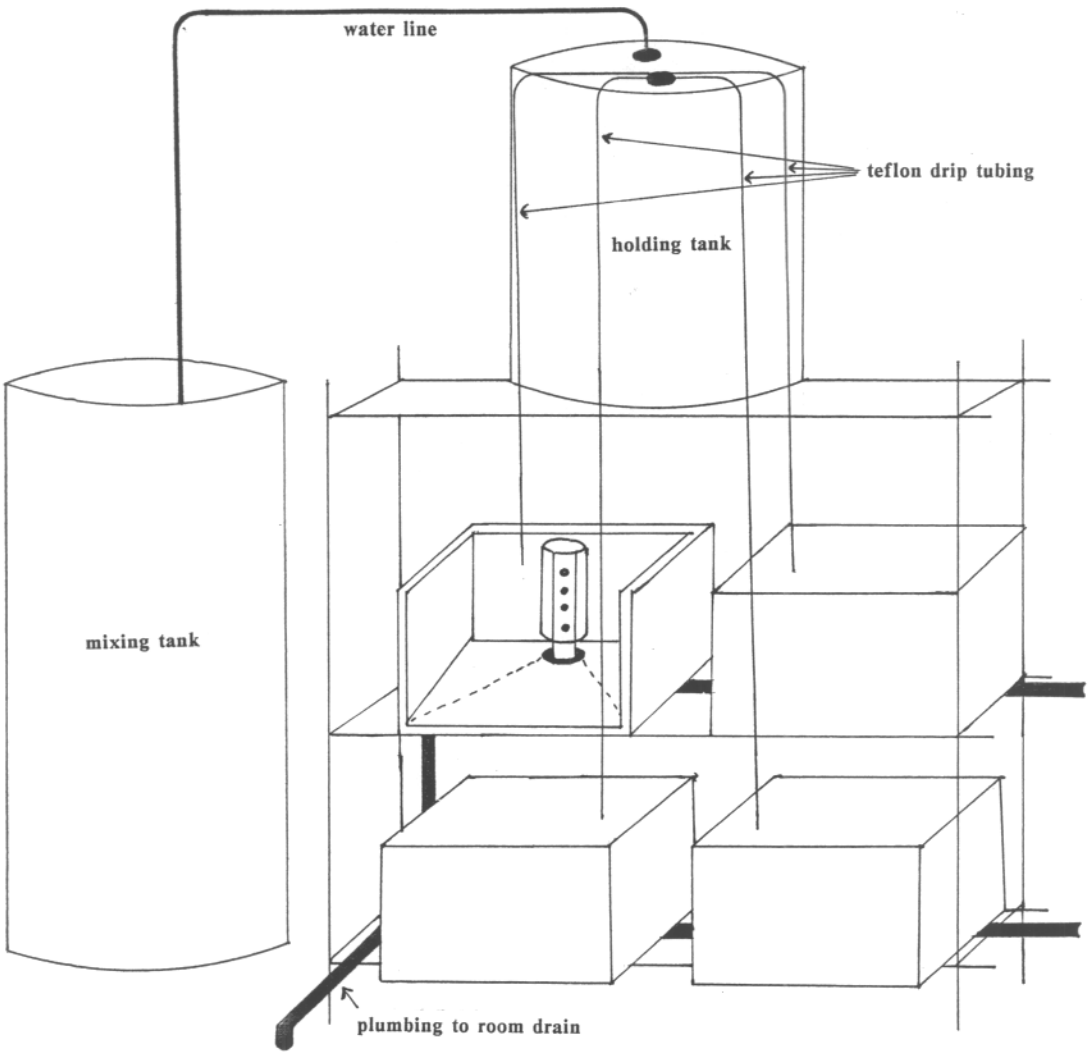

Figure 1. Diagrammatic representation of flow-through water system. One half of the rack and tubs are shown. Top left tub is cut-away to show drainage apparatus. See text for further information on teflon drip tubing specifications.

We found that the following conditions were optimal for the care and maintenance of the axolotls in the flow-through system:

1. # animals/tub	=	adults: 4 to 8 animals (males and females in separate tubs).
	=	young axolotls from the time they are weaned from brine shrimp to 9 months: 8 to 12 animals.
2. water level	=	approx. 5 inches in depth.
	=	approx. 25 liters/tub (approx. 3 to 6 liters/animal).
3. water flow rate	=	approx. 12 to 16 liters/tub/day.

4. cleaning of tubs	=	a water aspiration apparatus consisting of the cut end of a plastic 10 ml pipette end is used to suck up uneaten food and waste from the bottom of the tubs.
	=	adults: every two days.
	=	young animals: daily.
5. cleaning of tubing	=	as needed, 100% Steinberg's solution is injected into the ends of the teflon drip tubing with a 10 ml syringe.
6. water	=	25% Holtfreter's solution made up, in the mixing tank, with tap water with ammonia/chloramine remover and water conditioner (Aquavet, Hayward, CA) added.
7. feeding	=	the animals feed off the bottom of the tubs: soft-moist pellets of salmon food.
		(Rangen Inc., Buhl, ID) are broadcast into the tubs after cleaning.
	=	adults: approx. six 3/16 inch pellets every two days.
	=	young animals: 1/8 inch pellets every day.
8. animal identification	=	mature animals were tattooed (see article by Dave Able this issue).

The most difficult problem to overcome was the maintenance of a constant water flow through the drip tubing. Initially we used Tygon and polyethylene tubing of various sizes. Both of these tubings supported the growth of microorganisms on their internal surfaces, which eventually interfered with the flow rate. Water flow through small diameter tubing periodically stopped unless the tubing was cleaned on a daily or semi-daily basis. We also found that these tubings were very difficult to clean and could only be cleaned by removing the tubing from the system. Teflon tubing, on the other hand, supports less growth of microorganisms and is very easily cleaned without disconnecting the tubing from the system. Saline solution injected into the lower end of the tubing with a syringe reverses the flow and dislodges most of the growth.

We have found that the water flow rate per tub is more important than the water flow rate per animal. A water flow rate that replaces the water in each tub every two days is optimal.

The above system represents an inexpensive and efficient flow-through water method for caring for and maintaining axolotls. Axolotls have been maintained in this system from the young juvenile to the mature adult stage. Several essential technological innovations are incorporated into the overall design: Teflon tubing reduces clogging; soft-moist salmon pellets eliminate the need to feed animals individually; tattooing of animals makes the identification of individual animals within the tubs possible. The basic plan of this flow-through water system can be upgraded and easily upscaled. We suggest the following possible improvements: tubs with larger surface areas and less height, in order to house more animals per rack; continuous mixing of water and continuous water flow control by metered pumping; and automated continuous water quality analysis.

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Reference

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