How I Get Eggs Out of Axolotls

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In an embryological laboratory it is essential to have an assured supply of eggs at all times of the year. Adult axolotls are not available commercially and so I maintain a colony containing at any one time about 50 adults and 50 juveniles. Seasonal behaviour is suppressed as much as possible by keeping the animals at a constant 18°C with a 12 hour light-dark cycle throughout the year. Adult males and females are kept separately in Failsafe Aquaria (Slack, 1983).

Natural matings are usually set up over the weekend. Four pairs are put together on Friday evening in glass tanks measuring 12" x 24". The water temperature is lowered to about 12° with ice and the floor of each tank is covered with rough tiles suitable for spermatophore and egg deposition. In a successful mating spermatophores appear on the Saturday and the female lays eggs on the Saturday night and on Sunday ready for the next week.

In the current series, we have had 24 spawnings from 90 pairs put together which represents a 70% chance of obtaining at least one batch of eggs from four pairs. However this is not good enough for a busy laboratory so I also make extensive use of artifical fertilizations, using a simplified version of the method originally developed at Indiana University.

The female is injected into the dorsal musculature with about

35 Armour units of porcine follicle stimulating hormone dissolved in 0.5 mls sterile water. Hormone purchased from Armour, Sigma and Calbiochem have all been found satisfactory. At 18°C the female starts laying eggs about 20 hours after the injection. When she is laying steadily she is transferred into Steinberg solution and given some stones on which to lay the eggs. The eggs are collected every 20 minutes, drained of fluid, and fertilized with a few drops of sperm suspension. After another 20 minutes they are flooded with 10% Steinbergs. Successful fertilization is shown by the presence of sperm pits and by the rotation of the eggs about 30 minutes after addition of sperm.

The sperm suspension is made by killing a male, removing the vasa deferentia, cutting them into short lengths, squeezing out the contents and suspending in a small volume of 10% Steinberg. The suspension does not last long but the intact vas deferens can be stored in the fridge on a piece of damp filter paper for a few days. During the fertilization the suspension is kept at 10°C and is regularly checked to make sure that it has an adequate density of motile sperm (10-20 in a x 200 microscope field). I find that all males contain active sperm in the vasa deferentia so long as they are in good health. Previous natural matings or hormone injections make no difference, and sperm from the testes are inactive.

27 out of 37 artificial fertilizations in the current series have been successful. I use 2 females each time which represents a 93% overall probability of success. The number of eggs obtained is around 100-300 which is rather fewer than a natural spawning.

The fertility should approach 100% so long as the procedure is properly carried out.

Females are used initially for natural matings and when they refuse to produce eggs by this method they start being used for artifical fertilizations. They are allowed at least 3 months rest after a spawning by either method. Each female used for breeding has a record card and is tagged for identification with a stainless steel ear stud through the tail.

I know that many other workers have difficulties with their artifical fertilizations and I suspect that the main cause is the use of unhealthy animals. The remedy for this must involve attention to diet, space and frequency of water changing. There is also an understandable tendency to select the least healthy males for sacrifice, but in my experience such animals seldom yield good sperm suspensions.

I have found that ovulation in females can also be stimulated by chorionic gonadotrophin and by luteinizing hormone but the number of cases is too small to recommend a standard dosage.

Slack, J.M.W. (1983) A fail-safe aquarium for aquatic laboratory animals. Laboratory Animals 17, 28-31.

DESCRIPTION OF AN AQUARIUM FOR MAINTAINING AXOLOTLS

A recent paper by J.M.W. Slack (A Fail-Safe Aquarium for Aquatic Laboratory Animals, published in Laboratory Animals [1983] 17:28-31) describes a novel method for maintaining axolotls in polypropylene aquaria. The following diagram--from that publication--illustrates its main features:

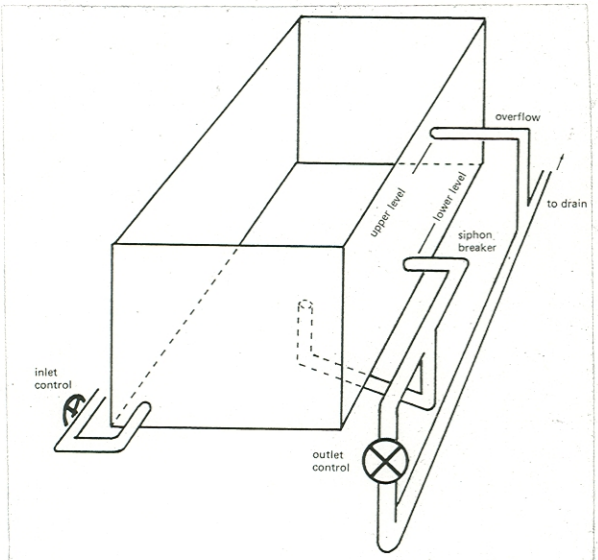

Fig. 1. General view of a FASAQ.

The tank has two water levels: a filled level, assumed when the outlet cock is closed, and a flushing level, assumed when it is open. The filled level is controlled by an overflow. When the outlet cock is opened, the water flows out until its level falls to the siphon-breaker opening. A further fall in the level is then inhibited because air enters the inverted U of the outlet pipe. With the water at the flushing level, the total volume is about one-fifth of the filled volume, and this makes it possible to wash out the debris with a short flush of running water. The water is admitted through an inlet in the front left corner of the tank and, because the outlet is in the center, a vortex is set up with draws the debris down the drain. The presence of animals in the tank may disturb the vortex but, if anything, they accelerate clearing by creating turbulence. After the debris has gone, the outlet is closed and the tank refills to the upper level. The animals are protected from exposure by the siphon-breaker, from flooding by the overflow, and from escaping down the pipes by grills of a suitable mesh. The device is regarded as "fail-safe" because the operator need not take any positive action to protect the animals.

Interested readers might want to consult the original publication or contact Dr. Slack for a reprint.

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