# Anuran larval rearing

Dr Robert K. Browne, AArk Research Officer, Royal Zoological Society of Antwerp, Belgium, robert.browne@gmail.com
Rachael Antwis, Hon. AArk Research Assistant, Antwerp Zoo, Belgium, rachael.antwis@googlemail.com

The efficient and reliable rearing of healthy larvae and metamorphs is important in the *ex situ* conservation breeding programs for anurans. This is particularly important in the production of large numbers of amphibians for release during rehabitation programmes.

The term "larvae' is a scientific term for the development stage from the egg to metamorphosis in anurans (frogs and toads), and the juvenile stage in salamanders. Larvae have primitive nervous systems and behaviour. Frog and toad larvae are called in Chinese 'ke dou', Spanish 'renacuajo', English 'tadpole', French 'tetrad', Russian 'головастик', Norwegian 'rumpetroll', Dutch 'kikkervisje', German 'kaulquappe'.

Larval rearing techniques for the three orders of amphibian, anurans, caecilians (worm slalmanders), and caudates (salamanders and newts) are as diverse as the reproductive modes in these groups. Similarly, even within anurans, spawn type, egg deposition and larval life stages are highly variable. Some eggs develop in terrestrial nests, are spawned on arboreal leaves over water, and a few anurans even brood eggs and larvae in their mouths or pockets in their skin. However, the majority of anuran larvae develop in flowing or still water bodies.

The highly diverse larval stages of anurans preclude the application of one blanket system to their captive rearing. For example, the Mountain Chicken frog, *Leptodactylus fallax*, has a complicated level of parental care in which the mother feeds unfertilised eggs for her oophagous larvae. Therefore larval rearing of this species would be approached very differently to those of a relatively simpler group such as many of the pond breeding Ranids; knowledge and awareness of the unique requirements of each species is necessary for the development of optimum larval rearing techniques. However, there are general principles for the rearing of anuran larvae in captivity that can then be adapted to many different species.

It is important to simplify larval rearing methods to develop easy-to-use and efficient systems for the mass rearing of healthy tadpoles. Simplified rearing techniques also enable the control of confounding factors during research, consequently enabling even more refinement of the rearing technique.

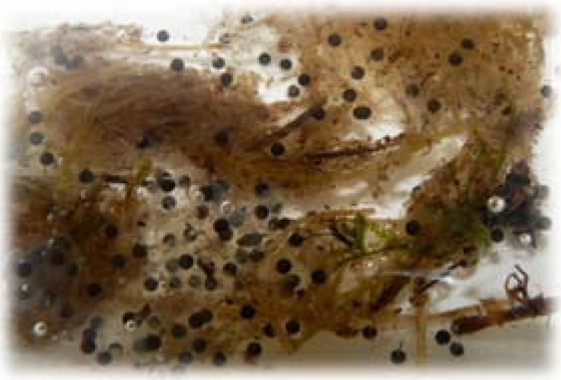
The main factors affecting anuran larval health, growth and development in rearing systems are:

- Eggs and their development
- Density
- Enclosure
- Feed type and feeding
- Temperature
- Aeration
- pH
- Water movement
- Metamorphosis

These factors are strongly inter-related for specifying the optimal conditions for rearing anuran larvae of any species.

When reproducing amphibians consideration must be made of the number of progeny required. Anurans such as the tiny Cuban tree toad *Eleutherodactylus limbatus* lay single eggs, whereas the American bullfrog *Rana catesbeiana* and the Marine toad *Bufo marinus* produce more than 15,000 eggs. Clearly, even with endangered amphibians, all potential progeny from species that lay large egg numbers cannot be cared for. In addition animal ethics requires the least possible number of animals to be kept in conservation breeding programs. This number will be those required for reproduction, the maintenance of genetic variation, and for conservation research.

Consequently, excessive eggs and larvae should be euthanized appropriately at each stage of the egg/larval life stage. Eggs and larvae can be euthanized by cooling in water in a fridge, then freezing. The number of eggs hatched should be about 50% of the juveniles required. If mortalities from the egg, during the larval stage to metamorphosis are higher than this further husbandry research of each stage; egg hatch, larval growth and development, and metamorphosis are needed.



## Eggs and development

Amphibian eggs vary in size from 6mm in the Chinese giant salamander *A. davidianus* to only mm in many species. They may be laid individually, in clumps, in foam, in strings or carried in pockets in their parent's backs. Now extinct species even brooded the eggs in their parent's stomachs. With good eggs hatching rates should be above 95%. Hatching time can be only a day or two or up to several months.

Left: The moderate sized eggs of Red Eyed Tree frogs *Agalychnis callidryas* need substantial amounts of oxygen. The eggs elevated above water by the leaf are developing faster than those submersed in the surrounding water.

However, no matter what the egg type there are two main factors that determine hatching rates and time. These are ambient oxygen levels and temperature. With species that do not exhibit parental care optimum temperature and oxygenation must be provided. As the eggs develop oxygen demands increase until hatch.

Right: The eggs of *Agalychnis callidryas* have high oxygen demand shown by the extensive gills within the egg capsule and the presence of symbiotic algae.

Many amphibians lay their eggs in gelatinous masses up to 10–20 cm in diameter, posing problems for diffusive oxygen delivery. In axolotyls *A. maculatum* egg masses oxygen is supplied by diffusion and by production by specific algal symbionts. Diffusion alone is not adequate to deliver sufficient $O_2$ to the innermost embryos at late developmental stages. In the light, egg masses had a net oxygen production and became hyperoxic and during the dark the eggs were hypoxic (Pinder and Friet 1994).

**The optimum incubation temperature varies among amphibians. The tolerance for temperature variation during incubation is somewhat proportional to the temperature variations expected in nature.**

**With most anurans the hatch rate is constant over a temperature range with a rapid decline in hatch rate below the optimum minimum and above the optimum maximum. However, the period to hatch varies considerably within the optimum temperature range (Goncharov et al. 1989).**

## Density

The density at which the tadpoles are maintained is critical in determining the frequency of water changes, and feed type and feeding frequency. Rearing densities can be broadly categorized as low, medium or high; the specifics of each of these systems are summarized in the table below.

| Density | Low | Medium | High |
|---|---|---|---|
| Number of tadpoles per litre | 1-5 | 5-15 | 15-80+ |
| Enclosure type | Tank | Tank | Cup/ Tray |
| Frequency of water change | 3-7 days | 1-2 days | Daily/Every 12 hours |
| Feed: frequency | 1-2 days | Daily | Every 12 hours |
| *Feed: mixture | Fish flakes/Lettuce | Fish flakes/Spirulina | Fish flakes/Spirulina |

* see footnote page 3.

## Enclosure

The choice of enclosure for tadpole rearing largely depends on the density at which the larvae are reared, which in turn is largely dependent on the life history of the particular species. It may be necessary to maintain tadpoles singularly in small cups, for example for many members of members of the *Dendrobatidae*, or in the case of cannibalistic species such as the Pacman frog *Ceratophrys ornate*. Tadpoles of most aquatic stream and pond breeding species are housed in aquarium tanks with an aerator for oxygenation.

***Above*: Aquarium containing larvae in a medium density rearing system.**

Some tadpoles from oligotrophic environments such as mountain or rainforest streams are adapted to very low quality feed and slow growth and development. With these species rich feed often does not elicit faster growth and shorter metamorphosis time. These species are best kept in tanks with aeration and low feed regimes, often simply the detritus in the tank can be sufficient for development. They can then be maintained with the minimum effort with very infrequent water changes. Other specialised larvae also require special conditions. For example, some species such as stream dwelling tadpoles of Natal ghost frog, *Heleophryne natalensis*, require flowing water and rocks for gripping with their mouthparts for correct development.

However, studies show that it is possible to maintain relatively high densities of tadpoles for some species, without compromising mortality rate and metamorph size. For suitable species high density larval rearing greatly increases the efficiency of the system through;

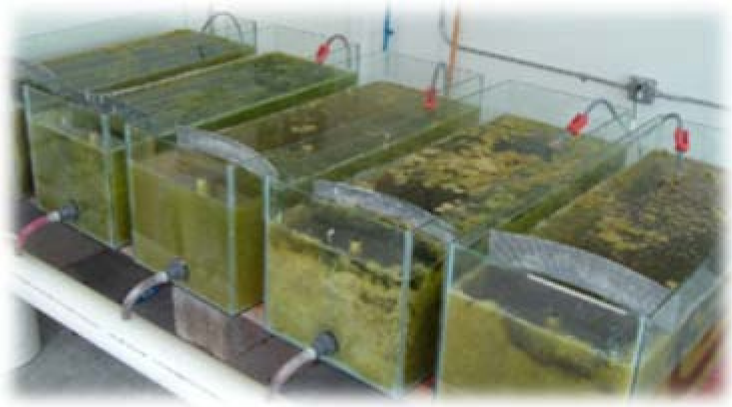
- producing consistently high quality uniform metamorphs,
- shortening rearing time and metamorphosis period,
- using water, feed and space more efficiently.

***Left*: High density tray system for rearing larval toads.**

In the high density system, trays containing small volumes of water can be used to promote high levels of dissolved oxygen in the water, thus ensuring low mortality at high larval densities. High density systems are ideal for some types of larval rearing – and for experiments - as they reduce confounding effects of hierarchies, production of algae and detritus in the tray, and reduction of metabolites and waste. However, high density systems require frequent and reliable water changes at approximately 12 hourly intervals and only suit some species.

### Feed type and feeding

In general, anuran larvae are filter feeders, browsers or detritavores, although some have been known to be highly predatory, such as tadpoles of the African genus *Hymenochirus;* the Dwarf African clawed frogs. Tadpoles require a diet sufficient in nutrients and rich in varying amounts of protein, fats, and carbohydrates to sustain health and a high development and growth rate. A general feed is a mixture of fish food grains or flakes combined with *Spirulina* powder is recommended to provide correct nutrition (see table above) or artificial gel based feed can be produced (see footnote). Exploitative competition between tadpoles can be reduced by supplying abundant feed, but care should be taken to avoid bacterial blooms as a result of over-feeding - indicated by the appearance of milky water. High density systems allow for an easily accessible and plentiful supply of feed. This reduces competition enabling the rearing of healthy metamorphs of uniform size, in a short metamorphosis time and period.



*Left*: In nature many toad tadpoles feed vigorously on fine filamentous algae. Filamentous algae when supplemented with *Spirulina* and fine fish flakes make ideal food for larvae of toads and many other amphibians. Filamentous algae can be grown in outdoors tanks of in cold climates or in a greenhouse shed with transparent roofs or walls.

### Temperature

The range of temperatures for optimal larval growth and development will vary just a few degrees centigrade for some species from climates with fairly constant temperatures to many degrees centigrade, for example with tadpoles from shallow ponds in temperate regions that have high diurnal temperature ranges. However, within the tolerated temperature range often tadpoles prefer high temperatures. This is particularly the case with many tadpoles form temperate climates where they will swarm in very shallow warm water during the day, or rest on the top of warmer dark surfaces.

Tadpoles grow faster as temperatures warm and so the time to metamorphosis can be minimised through the use of warmer husbandry temperatures. However, growth and development are faster at higher temperatures and consequently more feed must be supplied. Both the supply of more food, increased metabolism, and bacterial growth will demand more frequent water changes, especially in the high density tray system.

### Aeration

In an aquarium tank, the use of an aerator should provide an adequate source of oxygen for the system. In shallow trays, oxygen can be supplied through the large surface area with water being circulated by the movement of tadpoles. However, if the water in the trays is too deep stratification can occur and hypoxic conditions develop, which may kill tadpoles. Depending on species and larvae size unsafe depths without aeration may be as shallow as 1cm.

**Gel based tadpole feed:** Agar-gelatine tadpole feeds incorporate commercial feeds and binders to prevent disintegration. Add 250 g of pulverized rabbit chow (or similar), 20 g granular agar, and 14 g unflavored gelatine to 1 liter of water (Both agar and gelatine are required). Bring to about 100 $^{0}$C and solidify in flat pans. The agar-gelatine feed can then be stored frozen or for up to 14 days refrigerated. Recently hatched tadpoles can first be fed wilted lettuce for several days, then both lettuce and the agar-gelatine diets for several additional days, after which the agar-gelatine diet alone is sufficient. This diet does not cloud the water and requires minimal attention. A full I to 2 day ration administered at one time is not excessive for a single feeding.

## pH

Most tadpoles have optimum growth at a neutral pH. Conditioned tap water should provide the approximately neutral conditions required for most larval development. Often, acidic conditions delay metamorphosis, reduce size at metamorphosis and increase mortality. However the tadpoles of some species, such as the Cape Platanna, *Xenopus gilli,* are highly adapted to develop in acidic water.


## Water movement

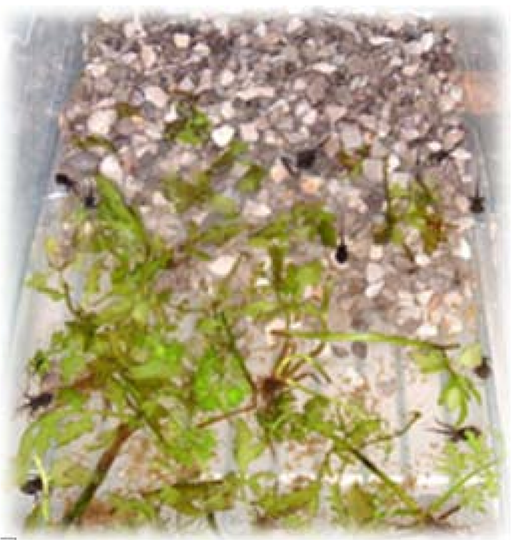
Left: Some species of tadpoles such as Natal Ghost frog *Heleophryne natalensis* need strong flowing water to maintain good health. In this species water flow along a rocky raceway appears to be related to maintaining good mouth structure for feeding.

## Metamorphosis

Metamorphosis is a period of great morphological and physiological change, especially for frogs, which in most species change from an aquatic to a terrestrial life stage. The point of metamorphosis is usually taken to be the emergence of the front legs. Metamorphosis includes dramatic changes in the internal respiratory system, mouth and jaws, absorption of tail, and skeletal structure.

*Above*: Front leg emergance in an Amazonian Milk Frog, *Trachycephalus resinifictrix*, marks the point of metamorphosis.

*Below*: An emergent sloping bed of fine gravel enables metamorphs to adopt a terrestrial life stage without drowning. At this stage feeding is not required but rapid metabolism means frequent water changes to prevent the build up of ammonia.



It is important to remove the metamorphs from the larval rearing conditions at front leg emergence to avoid drowning. Then to maintain them in a container which provides both water and dry land simultaneously, usually through the use of a slanted-bottom tank. Once the tail is fully absorbed the froglets can be moved to suitable tanks and start to be fed.

Goncharov BF, Shubravy OI, Serbinova IA, Uteshev VK. 1989. The USSR programme for breeding amphibians, including rare and endangered species. International Zoo Yearbook. 28:10-21.

Pinder AW., Friet SC. 1994. Oxygen transport in egg masses of the amphibians *Rana sylvatica* and *Ambystoma maculatum*: convection, diffusion and oxygen production by algae. J. exp. Biol. 197: 17–30.