

# SOME CONSIDERATIONS ON THE *EX-SITU* MANAGEMENT AND CARE OF GLASSFROG EGG MASSES AND TADPOLES (ANURA: CENTROLENIDAE)

by

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## Resumen

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Se presentan datos relacionados con el mantenimiento, crecimiento y diferenciación de las larvas de anuros de la familia Centrolenidae en condiciones controladas. Se discute la importancia de llevar a cabo este tipo de procesos como herramienta útil en la descripción y comparación de las larvas de los centrolénidos.

**Palabras clave:** Centrolenidae, manejo *ex-situ*, postura, renacuajos.

## Abstract

A methodology is presented for the captive management of anuran larvae of the family Centrolenidae under controlled conditions. The importance to make and enhance this process as a useful tool to describe and compare centrolenid larvae is discussed.

**Key words:** Centrolenidae, egg mass, *ex-situ* management, tadpoles.

## Introduction

One of the tools used in the description of larvae of anurans of the family Centrolenidae has been the

management of egg masses in the laboratory (e.g. **Hoffman**, 2004). However, published details on the proper procedures for captive management of the eggs and tadpoles are scant. Few studies have reported aspects on the

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centrolenid larvae care and management under controlled ex-situ conditions. **Villa & Valerio** (1982) highlighted the importance of maintaining a constant water flow. **Hoffman** (2004) presented similar conclusions, and described how a continuous change of water and the use of materials from the collection site were fundamental in completing metamorphosis in *Cochranella pulverata*. Although **Villa & Valerio** (1982) and **Hoffman** (2004) mentioned several management conditions, they do not report a specific methodology. Authors have not paid the necessary attention to include details on the methodology of their studies, despite mentioning that the animals were held in ex-situ conditions.

Herein we present some simple, low-cost procedures for the care and captive management of Glassfrog egg masses and tadpoles, from the moment of collection to the emergence of froglets, including details on the basic controlled conditions of the enclosures where the tadpoles are raised.

### Collection of postures and their transportation

We suggest collecting various egg masses, including the entire leaf where the mass is found, as well as the male that is taking care of them, in order to determine the species. The eggs should preferably be in an early developmental stage, when the size of the yolk is greater than of the embryo. If they are found in a more advanced state, the movement during transportation can cause them to hatch prematurely and die. The egg masses should be placed in a plastic bag (30 x 20 cm approx.) and spray with water from the stream where the nests were found. Extreme care should be taken in order to avoid contact between the sides of the bag and the egg masses during transportation. Before placing the egg mass in the bag, it is important to take notes of the following characteristics of the egg masses: 1) location of the egg mass (e.g., on the top, underside, or tip of a leaf), 2) height of the egg mass over the water, 3) type of vegetation where the eggs masses are deposited, 4) color of the egg mass (e.g. dark or light eggs, green or white masses), 5) description of the general shape of the egg mass (eg., globular or one-layered), 6) number of masses and the number eggs in each, and 7) position and location of the male with respect to the egg mass.

### Appropriate aquaria (Figure 1).

All aquaria should be at least 25 cm in length and 40 cm in width and fitted with certain basic implements, which are as follows (These supplies are easily found in pet stores):

An air motor that generates the necessary oxygen for tadpoles, as well as creating a small but constant flow of water. The inclusion of two or three pieces of finely porous glass (10 x 10 cm) can facilitate the attachment of algae onto their surface. A small sample of algae can easily grow and constitute a source of food for the tadpoles. If possible, the algae should be obtained at the same site of the egg masses, and can be collected by scraping the surfaces of rocks submerged in the stream. Fish food can be sprinkled in the aquarium before adding water. A water filter keeps the water clean and provides additional flow. A chlorine eliminator will remove the chlorine commonly found in water when it is processed for human use. A few drops of alcian blue in the water will prevent the growth of bacteria and fungi; however, the quantity should be much less than that used for fish in order to allow algae growth (1 drop per 8 gallons is recommended). An aquarium lid or canopy should cover the enclosure completely while at the same time allowing for feeding activities. A thermometer that allows the monitoring of ambient temperature within the aquaria. In some cases the following optional equipment is also useful: A pH monitor and a thermostat. The latter will be useful to increase the temperature of the aquarium in cases where egg masses are from areas with higher temperatures than the ex-situ environmental. In contrast, if the egg masses are from areas with lower temperatures than the ex-situ environment, it is recommended to use a mechanism to cool the water. In both cases, the key is to replicate the temperature conditions of the collection place to prevent mortality.

Centrolenid larvae will look for refuge under substrates in the bottom of the aquarium. While in nature larvae are often found in leaf litter or mud at the bottom of streams; stones of various sizes or sand are more useful in an artifi-



**Figure 1.** Illustration depicting an appropriate aquarium set up and environment for the care and management of centrolenid egg masses and tadpoles.

cial environment to maintain clean water and control of the aquarium conditions.

#### Location of aquaria and egg masses

Aquaria should be located where they receive natural light but not direct sun light, which will help algae growth. The egg masses are carefully attached to the aquarium lid, using string to secure the leaf to the lid, so that the eggs hang vertically. It is important to recreate the position in which the egg mass was found, whether on the top or underside of the leaf and take care that the mass does not receive direct sunlight. The egg mass must remain moist, which can be achieved by spraying water. Egg masses are sensitive to fungi, thus extreme care should be taken to avoid contamination, especially by reducing handling (McDiarmid & Altig, 1999).

#### Care and management of tadpoles

It must be kept in mind that constant changes of water are important for the maintenance of the centrolenid tadpoles environment (Villa & Valerio 1982; Hoffman, 2004). The changes of water allow elimination of waste that is not captured by the filter, offers additional oxygen, and keeps the water pH around neutral values. We suggest changing the water once or twice a week. Water, either from rain or tap, should be set aside for at least one or two days before, in order to allow the chlorine to settle.

Tadpoles should be fed once a day with small quantities of fish food (0.3 gr approx.); so as to reduce accumulation of residue in the aquarium and maintain the availability of algae. It is recommended not to change the type of food offered once a food source is established, it could increase the mortality rate. The quantity of food provided should vary with tadpole growth rates, sprinkling at first a fine dust, and later fragments of flakes.

Once tadpoles reach an advanced developmental stages (39 or 40 *sensu* Gosner, 1960), they can be placed in a small plastic container with water and stones or any object that allows froglets to climb. During this stage, and in the absence of the air motor, water should be changed every two days to keep oxygenation. Feeding during this stage continues as before. Metamorphosed juveniles can be moved into a terrarium and the feeding changed to fruit flies or small insects.

#### Preparation and preservation

In order to develop a detailed study and evaluation of the characteristics and changes during tadpole growth, continuous observation is required. It is recommended to

generate a developmental series by preserving one or two individuals frequently (once or twice a week or every two weeks depending on the number of larvae). Larvae should be euthanized in a 10% formalin solution, and later they should be changed to a new 10% formalin solution for permanent preservation. Larvae must not be crushed in the container vials because it will cause body deformation and poor preservation. Tadpoles from developmental series must be clearly tagged according to the frequency of preservation, and not crushed altogether in a single vial. See McDiarmid & Altig (1999) for more information on tadpole and egg preservation and storage

#### Comments

Development of larvae in aquaria has been shown to be practical and very useful for those interested in describing larvae that are difficult to observe and to collect in the field or to assign to a given species. The success of the development of the larvae from eggs to juveniles is important because it allows researchers to 1) identify the taxon in question when a parent was not captured, 2) to study certain behavioral aspects at the larval phase, 3) to monitor the development and growth of tadpoles (ontogenetic changes) and 4) to get information on the morphology of the tadpoles for future taxonomic diagnosis and comparisons between species. Its applicability and success depends on the rigor of care and management, which have already been demonstrated by some authors (see Villa & Valerio, 1982; Ibáñez-D. *et al.* 2000; Jaramillo *et al.* 1999; Hoffman, 2004). Unfortunately, those previous studies did not provide the necessary details to allow replication of their methods.

Our study includes six species from high and medium altitude regions of Colombia (*Centrolene buckleyi*, *Centrolene hybrida*, *Cochranella daidalea*, *Cochranella* sp., *Hyalinobatrachium ibama* and *H. colymbiphyllum*), but this method is certainly useful for lowland species. During the learning phase, several errors, including failure in the frequency of water change, changing the type of food during care, inadequate transport, and use of a thermostat too powerful for the aquarium size, were incurred on the management of two species (*C. buckleyi* and *H. colymbiphyllum*), producing the death of specimens.

Continuous observation of the tadpoles is very important during the developmental process. This allows the creation of a temporal record of the changes that occur throughout the care and management of tadpoles (color, ontogenetic change and other characteristics), as well as allowing some insight into certain behavioral patterns which would otherwise be very difficult to observe.

Through experience we have learned to maintain the tadpoles in healthy conditions. This achievement is reflected in the success rate in the growth and differentiation of larvae of three species, with a low mortality rate ( $\pm 20\%$ ) in aquaria where this method has been implemented. An example, with respect of timing of growth and differentiation of tadpoles is the case of *Cochranella daidalea*. We collected a freshly laid egg mass and two weeks elapsed before the larvae hatched (stage 24 *sensu* Gosner, 1960), seven weeks passed from the moment the larvae emerged until they reached stage 26, seven more weeks to observe stage 29, five additional weeks until stage 35 was reached, until the froglet finally emerged after one and a half more months. Obviously, these time periods will vary according to the species, individuals and conditions (*e.g.*, temperature, food and pH), but it is important to remember that it is key to allow the specimens to reach advanced stages of differentiation for a better comparison and description of the tadpoles, a process which requires much time and attention. Descriptions in early stages of development tend to show few differences in characteristics such as the dorsal shape of the body, snout, dorsal and ventral fins and the interorbital distance, not allowing for identification of the larvae of known species.

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