

Techniques for obtaining and raising plethodontid salamander eggs

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Introduction

The Plethodontidae is the largest family of salamanders, with over 250 described species. Because it contains both direct developing and metamorphosing taxa, and because multiple transitions among these developmental modes have occurred, the Plethodontidae offers excellent opportunities for comparative developmental studies (reviewed in Wake and Marks, 1993). I have been successful in obtaining and raising eggs from diverse plethodontid species, and here provide protocols.

Techniques

Obtaining eggs

Eggs can be obtained either directly from the field or from gravid females collected in the field. In the temperate zone, most plethodontids are gravid in the spring; however many populations of *Batrachoseps* in California are gravid in the fall. Females are kept in plastic shoeboxes lined with moist paper towels. To induce gravid females to oviposit, I inject them with mammalian luteinizing hormone releasing hormone (LHRH; Sigma catalogue # L-2761) (Verrell, 1989). The LHRH is dissolved in sterilized distilled water and diluted to a concentration of 0.05 mg/ml. I inject all plethodontids with 0.1 ml of the diluted solution intra-abdominally, being careful not to inject into the liver or oviducts. This procedure has induced oviposition in the following species: Desmognathinae: *Desmognathus fuscus*, *D. ochrophaeus*, *D. wrighti*, *D. aeneus*; Hemidactyliini: *Eurycea bislineata*, *Hemidactylum scutatum*; Plethodontini: *Ensatina eschscholtzii*, *Aneides ferreus*, *A. lugubris*, *A. flavipunctatus*, *Plethodon cinereus*, *P. jordani*, *P. dunni*, *P. elongatus*; Bolitoglossini: *Bolitoglossa subpalmata*, *Oedipina uniformis*, *Hydromantes shastae*, *Batrachoseps wrighti*, *B. attenuatus*, *B. nigriventris*, and *B. pacificus*. Success rates of first

injections vary greatly among species, and are highest in animals that have been in captivity only a short time. Repeated injections increase the probability of oviposition. I have the highest success rates in *Batrachoseps attenuatus*, in which over 95% of females lay. Success rates of about 50% have occurred in many Plethodontini. The number of days between injection and oviposition is nearly constant in some species such as *Batrachoseps* (4-5 days) and more variable in others such as *E. eschscholtzii* (5-12 days). Because all plethodontids have internal fertilization and are able to store sperm for long periods, the eggs obtained by this procedure are usually fertile and there is no need for males. Spontaneous laying without injection has been extremely rare in my experience; however, Tomalei Vess and Dr. Reid Harris (pers. comm.) have increased rates of spontaneous laying to 100% in *Hemidactylum scutatum* collected during their migration to breeding sites by placing females outdoors in naturalistic environments.

Raising eggs

All gravid females and eggs are kept in constant temperature environments with ambient light cycles. Most eggs have been raised at 13°C, but I have also successfully raised some eggs at temperatures ranging from 7°C to 17.5°C.

(a) aquatic development (Hemidactyliini and some Desmognathinae): I raise eggs of these species in 10% Holtfreter's solution, changed weekly. Survival to hatching varies among clutches from 0 to nearly 100%, and about half of all clutches have survival rates above 50%.

(b) direct development (Plethodontini, Bolitoglossini, and some Desmognathinae): I raise these on paper towels moistened with distilled water in closed plastic containers. Eggs are rinsed regularly (daily or weekly) with distilled water. Survival to hatching varies among species and clutches. In *Batrachoseps* survival has averaged 75%, the highest found so far in plethodontids. In

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contrast, in some other direct developers, survival to hatching has been extremely low (<10%). Fungal infections may be controlled by washing in a 0.5% peroxide solution (Hanken, 1979). In nature, most plethodontids guard their eggs, and leaving clutches with their mothers may increase survival. However, mothers who are disturbed may eat their clutches. Therefore, if eggs are to be removed over a series of stages, this strategy may not increase the number of embryos obtained.

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