Morphological and behavioral embryology and spontaneous diapause in the African killifish, *Aphyosemion gardneri*

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**Synopsis**

A brief description of the morphological and behavioral development of embryos of the African killifish, *Aphyosemion gardneri*, is presented. Most embryos spontaneously entered a period of prolonged, reversible developmental arrest of variable duration at the pre-hatching interval. This is presumed to be an adaptation to the unstable natural habitat of this group of fishes. Aspects of the pre-hatching arrest of *A. gardneri* and the annual *Austrofundulus myersi* are compared.

**Introduction**

The unusual embryology of certain cyprinodontid fishes, characterized by a reversible developmental arrest of variable duration (diapause) at one or more points, has been studied by Peters (1963), Wourms (1972a, 1972b, 1972c), Markoński & Matias (1977), Matias & Markoński (1978) and Markoński et al. (1979). The bodies of water in which these fishes are found are often transitory, subject to unpredictable periods of drying. The ability of eggs to enter diapause when conditions are unfavorable is believed to be an adaptation to these unstable habitats. A population of cyprinodontids may survive as diapausing embryos in mud or under conditions in which not enough water is available to maintain adults. When rains refill the habitat, diapause ends, development resumes and young are hatched into a favorable environment. Young develop rapidly and, depending on the species, may reach sexual maturity in less than 90 days. Spawning then occurs ensuring continuation of the population through the next dry period.

Wourms' work has also revealed that cellular movements in the pre-embryonic interval of these fishes are unlike those described for other fish groups, 'the spatial and temporal dissociation of the processes of epiboly and embryogenesis' being characteristic of many annual cyprinodontid species (Wourms 1972b).

This paper will compare the embryology of *Aphyosemion gardneri*, a cyprinodontid fish found in ponds, rivers and swamps of West and Central Africa (Scheel 1968), with the findings of the above authors, including observations on the developmental arrest of *A. gardneri* embryos. It is part of a larger study on the development of behavior in this species (Kroll 1981).

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Materials and methods

A laboratory stock of *A. gardneri* was established from 7 pairs of fish that were obtained from a local pet dealer and an amateur breeder. Males and females were housed separately in 38 l aquaria (51 cm × 27 cm × 31 cm) that were divided in half by a sheet of non-reflective aluminium. Illumination was provided by fluorescent lights on a 15 L/9 D photoperiod. Water temperature was maintained at 22°C. (±1°C.). Spawning mops were made by tying together approximately 20 strands of green yarn 10 cm in length. Females spawned on the strands of these mops and the eggs were collected for incubation 12-16 h after placing a male in the female’s aquarium.

Five females from the stock were each mated with a different male. During spawning, water temperature was maintained at 22°C. Six eggs were then selected from each of the resulting matings. Newly spawned eggs are coated with an adhesive substance which reduces observability of the otherwise transparent eggs. Observations were conducted either on eggs which contained little adhesive or on eggs from which it was manually removed with watchmaker’s forceps. However, in all cases the zona radiata was left intact. The 5 sets of 6 eggs were each placed in separate flat-bottomed bowls (10 cm in diameter) and covered with 5 cm of water. The embryos in each bowl were from the spawning of a different male-female pair. The bowls were not covered and therefore fresh water was added as necessary to replace evaporational loss. Eggs were incubated at 22°C (±0.5°C.) and illuminated by fluorescent light on a 15 L/9 D photoperiod. All eggs were removed from their bowls with a small pipette and examined daily under compound and dissection microscopes throughout development, using both transmitted and reflected light.

Results

The eggs of *A. gardneri* are spherical with an outer diameter of approximately 1.3-1.6 mm (x̄=1.44, S.D.=0.20, n=43) while the yolk is 1.1-1.3 mm in diameter (x̄=1.20, S.D.=0.10). The surface of the egg is smooth with no specialized attachment structures. One oil droplet 0.3-0.4 mm in diameter (or occasionally several smaller ones) is present in the yolk. If more than one droplet is present, they usually fuse within 24 h of fertilization.

The chronology of events in the development of *A. gardneri* embryos is as follows:

1-2 h. -- Cytoplasm has collected at the animal pole, forming a blastodisc. Cleavage begins, the first 3 of which are synchronous and meridional (Fig. 1a, 1b).

6 h. -- A cap of about 32 cells sits atop the yolk.

20 h. -- The number of blastomeres has increased to approximately 128. As cleavage continues, the blastodisc flattens and epiboly begins (Fig. 1c).

48 h. -- The yolk is entirely covered with blastomeres. During epiboly the formation of an embryonic shield was never observed (Fig. 1d).

60 h. -- A thickened ridge of cells, the embryonic axis, has formed, usually adjacent to the oil droplet (Fig. 2a).

3 d. -- Main divisions of the brain are discernible, especially the large optic vesicles. The heart exists as a straight, non-contractile tube (Fig. 2b).

4 d. -- The eyes are large and the lens apparent. The otic vesicles, olfactory pits and Kupfer’s vesicle have also appeared by this time. Somites are developing and the number observed on day 4 ranged from 6-16 pairs. The heart exists as a straight, non-contractile tube (Fig. 2c).

5 d. -- Growth and differentiation continue. Somite number ranges from 15-24 pairs with few other observable changes from day 4. The embryo is approximately 1.5-2.0 mm in length and is curved over the yolk surface.

6 d. -- Heart contractions are first observed in 10 of the 22 surviving embryos. The rate of contraction ranges from 14 to 35 beats per minute (x̄=25.0, S.D.=6.0, n=10) (Fig. 3). The heart has begun to fold upon itself and the vitelline circulation is well developed. The embryo also begins to exhibit apparently spontaneous contractions of the somitic musculature. The tip of the tail is