Synchronous hatching in *Fundulus heteroclitus* embryos: Production and properties

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Fundulus heteroclitus spawning has been observed at the recession of high tides. Eggs have been found positioned among rocks at the spring tide level, suggesting aerial development. Eggs and sperm were harvested from *F. heteroclitus* captured at Northeast Creek, Bar Harbor, ME. Oocytes are collected from one to three females and immediately placed in 10% artificial seawater (ASW) with sperm from one to three males. Gametes were gently swirled together then deposited on a piece of Whatman #1 filter paper in a 66 mm plastic petri plate moistened with 10 ppt artificial seawater. The petri plates were placed in an incubator over water at 20° C. In later experiments, the incubator water was replaced with 10 ppt ASW to prevent water vapor diffusion from the bath into the petri plates. The eggs were staged frequently according to descriptions of Armstrong and Child[1], and our results parallel those of these authors, as seen in Figure 1. Development continues for 14-17 days until hatching occurs. For embryos grown in moist air, development seems to arrest around stage 35, and hatching can be induced at this stage by flooding the petri plate after this stage. Hatching times following flooding are variable, with 50% hatching occurring as quickly as 15 minutes and as long as nearly 7 hours.

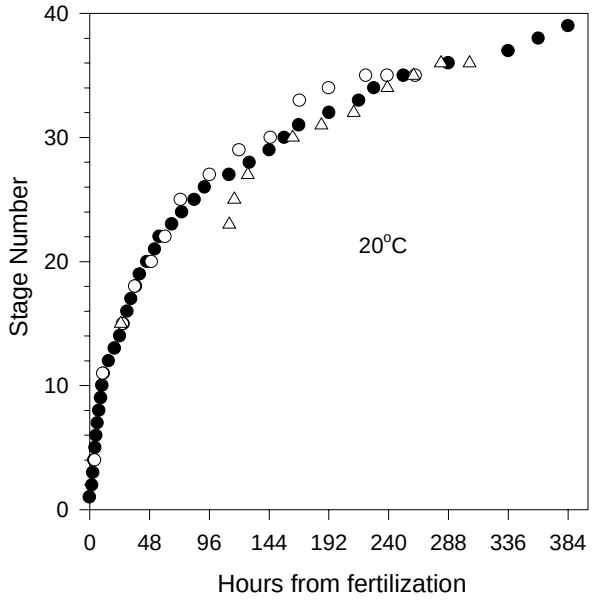

Figure 1. Comparison of published stage data (filled circles, redrawn from Armstrong and Child) and two batches incubated in air as described (open symbols).

Some batches were incubated submerged in isotonic water, with the conditions otherwise the same. These embryos developed at slower rates or failed to undergo development at all. Six batches of air-incubated embryos averaged 92 ± 3.9 percent hatching, while four batches incubated under 10 ppt ASW showed only 11 ± 12 percent hatching. The submerged hatchlings developed more slowly, taking about 10 days more to reach hatching stage, and were much smaller in size than the aerial incubations. When the length of 6 aerial hatchlings was measured with a calibrated occular micrometer, they averaged 3.76 ± 0.201 mm, while a matched set of 6 submerged hatchlings averaged 2.97 ± 0.015; the 21% decrease in length is highly signifigant ($P<<0.01$) It is likely that the eggs raised under water were hypoxic, as suggested by Amberson and Armstrong[2]. It seems possible that those eggs were driven into glycolysis, and were consuming more substrate (the Pasteur effect) than the aerial embryos. With fixed resources, this may result in smaller body size.

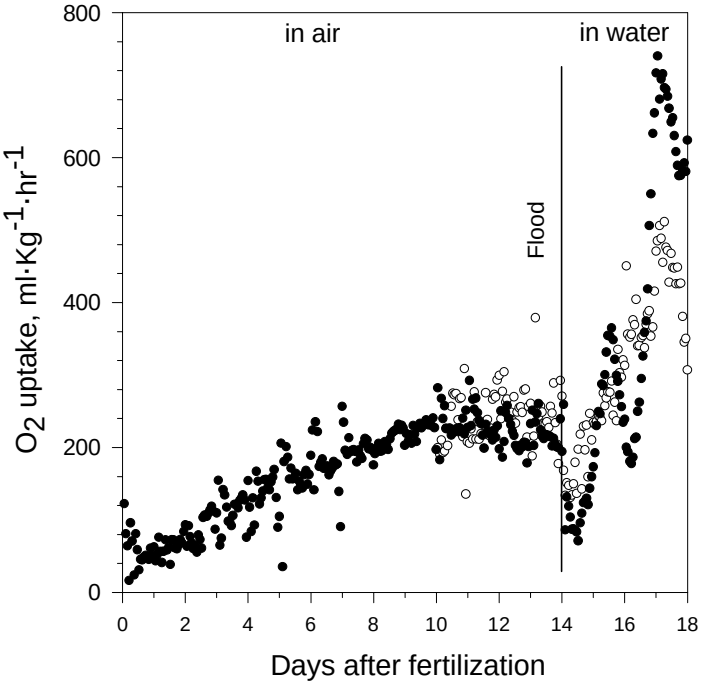

Figure 2 Respiration of developing *Fundulus* embryos in moist air and after flooding. Filled points are one complete run, open points are second run starting at day 10. Decrease in rate after day 16 probably due to hatchling death.

Oxygen consumption of eggs and hatchlings was measured from fertilization to day fourteen as a matched set of embryos placed in the incubator was being staged. Oxygen consumption was measured by methods previously described, with the input air equilibrated with 10 ppt ASW. In one experiment, eggs were fertilized and placed immediately on moist filter paper in the respirometry chamber. After 14 days of development, enough isotonic SW was added to cover the eggs, and recording was continued for another week. A second experiment used eggs incubated outside of the respirometry chamber for 10 days before starting recording. The results are similar, as seen in Figure 2.

We conclude that *F. heteroclitus* may undergo an aerial development in its natural habitat, with eggs being laid above the reach of water until the next spring tide in about 14 days. Flooding at this time would trigger hatching. However, this cannot be the only mode of development, as spawning occurs on many high tides, not only spring tides (Salinas *et al*., this volume.) The method of fertilization and aerial maturation of gametes harvested from captured breeding fish is successful. The technique will allow for studies on the fish at any life stage, as well as the production of large numbers of embryos at the same stage, which is a requirement for studies such as those reported by Preston *et al*. in this volume.

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[2] Amberson, W. R., and P. B. Armstrong. 1933. The respiratory metabolism of Fundulus heteroclitus during embryonic development. *J. Cell. Comp. Physiol.* 2:381-397

[3] Kidder, G. W. III and A. Ball. 1999. Oxygen consumption of the killifish, *Fundulus heteroclitus. Bull. Mt. Desert Isl. Biol. Lab.* 38:20-21