Aerobic and Submerged Development of Embryos of *Fundulus heteroclitus*

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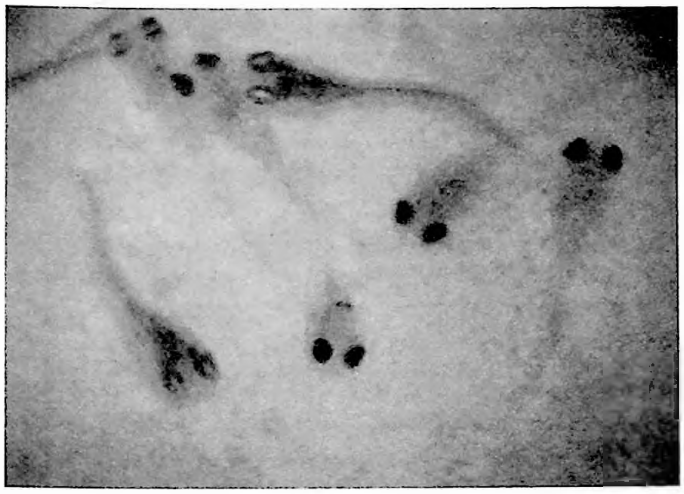

Figure 1. *Fundulus heteroclitus* hatchlings. Length ~ 6 mm.

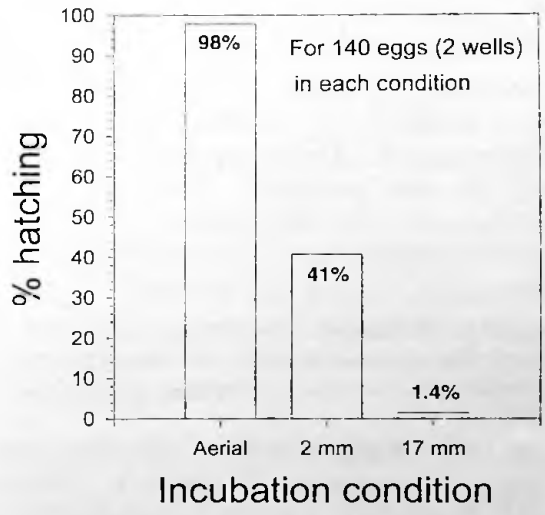

Figure 2. Percent hatching vs. incubation condition. Aerial; in air saturated with 10‰ ASW. 2 mm and 17 mm; under that depth of 10‰ ASW. All at 20° C, without stirring except daily solution changes.

T. H. Morgan[3] showed and this lab and others have confirmed[1] that embryos from *Fundulus heteroclitus* will develop normally in moist air, and hatch upon flooding[2] to produce the fry shown as Figure 1. We now offer some data comparing the results of aerial and submerged incubation under controlled conditions.

Fish freshly caught in Northeast Creek (Bar Harbor, ME) were stripped of eggs and milt into diluted artificial seawater (ASW, 10‰, approximately isotonic to plasma in these fish) and placed on filter paper in 35 mm, 6-well culture plates for incubation at 20° C in an atmosphere equilibrated with 10‰ ASW. Fertilized eggs and embryos were incubated aerially, or submerged in 2 mm or 17 mm of 10‰ solution. (Since the average egg diameter is 1.5 mm, this results in submersion under 0.5 or 15.5 mm of fluid.) In this experiment, 2 wells in each condition contained 70 eggs each. After 8 days of incubation, aerial embryos were flooded for one hour each day until all had hatched or died, while submerged embryos had their water changed. As shown in Figure 2, hatching success is markedly improved with aerobic incubation, and immersion in "deep" water nearly abolishes development at this high embryo density. Since these solutions were not stirred, we postulate that oxygen becomes depleted in the unstirred layers adjacent to the eggs in the submerged conditions, but not in aerial conditions.

We have previously reported[1] that submerged embryos are smaller than aerially-developing embryos, and can now provide conclusive data on that point. A pooled

collection of fertilized embryos was distributed at a lower density (~20/well), such that the oxygen consumption per unit fluid volume is reduced, and more oxygen is available to each embryo. Under these conditions, the effect of water depth is blunted, and many embryos survived to hatch under all treatments. The total length of these embryos was individually measured on the day of hatching (dissecting microscope with calibrated ocular micrometer). Table 1 shows that the embryo lengths were significantly reduced by submersion.

Length of Hatchling, mm		**Replicate wells**					**Summary**	
Condition		1	2	3	4	5		
	Mean (well)	5.95	5.81	5.89	5.93	5.76	**5.87**	**Mean of 5**
Aerial	SE	0.05	0.06	0.05	0.04	0.11	0.03	SE
	N	22	17	20	21	19	99	N
	Mean (well)	5.56	5.69	5.93	5.63	5.50	**5.66**	**Mean of 5**
2 mm depth	SE	0.13	0.08	0.08	0.10	0.06	0.04	SE
	N	17	12	21	17	22	89	N
	Mean (well)	5.24	4.96	5.99	5.25	5.22	**5.22**	**Mean of 5**
17 mm depth	SE	0.07	0.09	0.95	0.15	0.12	0.12	SE
	N	9	9	8	6	10	42	N

Table 1. Summary of length measurements at ~20 embryos/well. Each hatchling in each well was removed from the well and measured on the day of hatching. No well mean was significantly different from the other wells in that condition, and the overall means for each condition were significantly different from the means of each of the other conditions, by t-test (Aerial vs. 2 mm, $P < 0.0001$; 2 mm vs. 17 mm, $P < 0.01$; Aerial vs. 17 mm, $P < 0.0001$)

Since pre-hatching embryos do not feed, their store of substrate is fixed before fertilization. The observation of a difference in embryo size at low oxygen availability suggests that submerged embryos are not converting stored substrate to body mass as efficiently as fully-aerobic embryos. It is possible that anaerobic metabolism, with its greatly reduced ATP/mole substrate, is supporting development in the oxygen-stressed embryos, leading to a "Pasteur effect" decrease in hatchling size. Since sustained whole-organism anaerobic metabolism is unusual in metazoans, this deserves investigation. Lactate accumulation should be observable, either in the medium or within the chorion. It might also be possible to demonstrate an oxygen debt under proper circumstances. These hypotheses remain to be tested.

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1. **Baldwin, J. L, C. E. Goldsmith, C. W. Petersen, R. L. Preston and G. W. Kidder.** Synchronous hatching of *Fundulus heteroclitus* embryos: Production and properties. Bull. Mt. Desert Island Biol. Lab. 43:110-111, 2004.
2. **DiMichele, L. and M. H. Taylor.** The environmental control of hatching in *Fundulus heteroclitus*. J. Exp. Zool. 214:181-187, 1980.
3. **Morgan, T. H.** Experiments with frog's eggs. Biol. Bull. Mar. Biol. Lab., Woods Hole, Mass. 11:71-92, 1906.