A survey of developmental abnormalities in *Fundulus heteroclitus* embryos from Northeast Creek

Brent N. Lawson[1], Ari Dehn[2] and Robert L. Preston[3, 4]
[1] Southern Maine Community College, South Portland, ME 04116
[2] Ari Dehn, University of Maine at Machias, Machias, ME 04654
[3] School of Biological Sciences, Illinois State University, Normal, IL 61790
[4] Mount Desert Island Biological Laboratory, Salisbury Cove, ME 04672

The embryos of Northern killifish can develop immersed in seawater or in air when stranded on rocks or vegetation in estuaries. These embryos are remarkably tolerant to desiccation, thermal and salinity stress. About 6% of these embryos show developmental anomalies, of which the most common anomalies were albinism (1.6%) and microcephaly (0.7%). These data provide a baseline estimate of the rate of occurrence of developmental anomalies and/or mutations in a killifish population from a relatively pristine estuarine environment.

The Northern subspecies of killifish, *Fundulus heteroclitus macrolepidotus*, spawn in Maine in estuaries in June and July during daily high tides[1,2]. The adults may migrate to and from full strength seawater (SW, ~30 ppt) to freshwater (<1 ppt) but tend to spawn in brackish water (~10 ppt)[1,2]. As the tide ebbs, some embryos remain immersed and some embryos become stranded on rocks and vegetation. These stranded embryos are exposed to air for long periods (up to 14 days)[1]. In spite of enduring significant transient desiccation and thermal stress, aerially incubated embryos develop normally and hatch when flooded by high tides after 12-14 days[1,2]. During our ongoing studies on the physiological and molecular mechanisms these embryos employ to resist desiccation stress[1] we noticed that there were a number of developmental abnormalities and/or mutants that appeared in our cultures. Northeast Creek on Mount Desert Island, ME is considered a relatively pristine environment, and animals from this population have been used for a variety of physiological studies as well as for genomic analyses. We felt a preliminary survey of the occurrences and types of embryo anomalies or mutations that spontaneously occur in this population would provide useful baseline information for research, particularly for investigations of embryonic mutations resulting from exposure to environmental pollutants.

Fish were caught in minnow traps at Northeast Creek and kept in aquaria with natural running SW (~30 ppt). Gametes were gently expressed from fish into a beaker with 25 ml of 10 ppt artificial seawater (ASW; Instant Ocean, Mentor, OH). For these experiments 10 ppt ASW was used; as it approximated the salinity at the sites in the estuary at which fish spawn. After 30 min, the embryos were rinsed with 10 ppt ASW, placed on filter paper moistened with 10 ppt ASW in Petri dishes, and cultured aerially in a chamber in which the humidity was equilibrated to that of 10 ppt ASW[1]. Embryos from 7 days post-fertilization (dpf) to 14 dpf were inspected daily for anomalies using a dissecting microscope. Anomalous embryos were classified, based on deformation or alteration of normal embryo body morphology. Typical examples were photographed, and an extensive reference file of the number of types of anomalies was established.

In total, 13667 fertilized embryos were examined from 11 separate batches. The average number of males and females (mean ± S.E.) used per batch was 7 ± 3 and 13 ± 5. Twenty-six individual types of morphological anomalies were identified. The overall mutation/anomaly rate was about 6%. The six most frequent anomalies were (occurrence rate shown in parentheses): albino (1.6%), severe head and body deformation (0.8%), microcephaly (0.7%), only chromatophores but no body development (0.5%), microcephaly and missing tail (0.2%) and reduced coloration with scattered chromatophores (0.2%). A number of rare anomalies such as two-headed embryos (0.07%) were also noted. In general the types of anomalies noted also have been observed in zebrafish and other species. This preliminary survey provides valuable initial baseline data that characterize embryonic development anomalies and possible mutations in the Northeast Creek killifish population.

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