Report for 2001KY2441B: Developmental stability as an indicator of amphibian population health and environmental degradation

- Other Publications:
  - Meredith, Christy and Howard Whiteman, 2002, Lethal and sublethal effects of increasing nitrate concentration on Ambystoma mexicanum embryos and larvae. Annual Sigma Xi Poster Competition, Murray State University.

Report Follows:

Problem and Research Objectives

One of the most important, yet most difficult, tasks associated with conservation of any organism is the identification of populations subject to stress before such stress has a detrimental effect (Clarke 1995). This is particularly true of amphibians; the global decline of amphibians is considered a disturbing indicator of environmental degradation because it may forebode of cascading ecological effects, as well as raising health concerns about human populations (Wyman 1990, Wake 1998). Amphibians are ideal biological indicators, because their semi-permeable epidermis and complex life cycle expose them to multiple stressors in both aquatic and terrestrial environments (Wyman 1990). Because of this, amphibians should be among the first vertebrates affected by anthropogenic stressors in either of these environments (Stebbins and Cohen 1995). Furthermore, some of the same stressors affecting amphibians are known to have negative effects on other species, including humans (e.g., PCBs, UV light, etc.; Wake 1998, Carey 2000). Biologists thus need an early-warning system that could identify environmentally-stressed animals before the stressor causes population and/or regional harm. Such an indicator should be able to measure stress-induced effects before drastic changes in morphology take place which would subsequently decrease the organism's survival and reproductive abilities. One such indicator is obtained by measuring developmental stability (DS), the ability of an organism to develop normally under a range of environmental conditions (Waddington 1942, Clarke 1995). The objective of the current project was to utilize developmental stability as an indicator of amphibian stress and habitat quality from temporary ponds in Kentucky that vary in land use, water quality and other anthropogenic disturbance.

Methodology

During 2001 we continued our collection and imaging analysis of bullfrog (*Rana catesbiana*) larvae and 12 eastern newt (*Notophthalmus viridescens*) adult males. After transporting amphibians to MSU, each individual was anesthetized using tricaine methylchloride (MS-222), and measured for snout-vent length (mm) and mass (g). Each individual was then photographed with a Pixera Professional digital camera connected to a PC. After imaging was complete, animals were submerged in aged water to revive them and released back to their pond of capture. Measurements of DS concentrated on morphological structures directly related to amphibian fitness. Each individual was measured three separate times in order to statistically analyze measurement error (Palmer 1994). Temperature, pH, conductivity, dissolved oxygen, and alkalinity was measured at each pond with portable meters and orthophosphates and nitrate/nitrite were measured using a Lachet Nutrient Analyzer at MSU's Hancock Biological Station (HBS). Currently statistical analyses are being conducted to correlate habitat variables with levels of asymmetry in each species.

Experiments were modified slightly from the grant proposal. We reared *Ambystoma mexicanum* embryos and larvae under various nitrate concentrations. We used this species, an endangered Mexican salamander, because we were concerned that utilizing embryos from spotted salamanders, *A. maculatum*, would have been unwieldy and might lead to spurious results. The latter species has large, gelatinous egg masses in which cutting single embryos for experiments is difficult and can lead to increased mortality. *A. mexicanum* is closely related to *A. maculatum* as well as other Kentucky congeners (*A. tigrinum, A. opacum, A. talpoideum*) and thus provides an indicator of potential native species response. In addition, *A. mexicanum* is utilized extensively by developmental biologists and can be readily reared in the laboratory, allowing for its potential future use in toxicology labs in university, government (EPA) and corporate settings. We reared embryos

in various levels of nitrate, a common agricultural pollutant, and assessed mortality as well as sublethal effects. Surviving larvae were then moved to the same or different nitrate treatment to determine the effects of increasing, decreasing, or constant levels of nitrate on larval growth and development . After rearing for three weeks, larvae were photographed as above and currently await analysis for asymmetry.

Principal Findings and Significance

All field images have now been measured, and we are currently analyzing the results statistically. Our preliminary analyes of bullfrog tadpoles showed significant correlations with anthropogenic stress, as described in last year's report, and our updated data thus far do not refute this result. Our experimental results showed that salamander eggs are fairly robust against increasing concentrations of nitrate, whereas larvae are highly susceptible at nitrate levels commonly experienced in natural farm ponds. Mortality rate was much higher in larvae than eggs at the same nitrate concentrations, perhaps because nitrate affects metabolic processes that are not yet functioning in developing embryos. In addition, both embryos and larvae showed significant sublethal effects, in terms of time to hatching, size at hatching, and larval growth rate. We will be analyzing the images of larvae from this experiment for asymmetry this summer, and may conduct a short-term experiment with *A. maculatum* larvae to confirm the effects of nitrates on native species of salamanders.