Report as of FY2006 for 2006KY65B: "Lethal and Sublethal Effects of Agricultural Nutrient Pollution on Tadpoles"

Publications

- Conference Proceedings:
  - Earl, Julia E. and Howard H. Whiteman, 2007, Lethal and Sublethal Effects of Nutrient Pollution on Amphibians, in Proceedings of the Kentucky Water Resources Annual Symposium, Kentucky Water Resources Research Institute, Lexington, Kentucky, p 65-66.

Report Follows

Problem and Research Objectives

Agriculture is a major source of non-point source pollution including nutrients such as nitrates and phosphates from fertilizers. Nutrient pollution commonly enters streams and ponds through runoff and erosion (Schlesinger 1997). The influence of such pollution on aquatic environments and organisms is not widely known (Diana and Beasley 1998). Nitrate is toxic to amphibians (Baker and Waights 1994; Rouse et al. 1999), but most studies have concentrated on only a few amphibian species and focused solely on lethal effects (Rouse et al. 1999). Sublethal effects on growth and development may be more relevant for realistic nitrate concentrations (Meredith and Whiteman, in prep.) Most studies also concentrate on constant levels of a toxin, yet because runoff of pollutants is caused by precipitation events that flush chemicals from the terrestrial environment into the aquatic environment, nutrient levels could increase in sharp peaks, or pulses. Such variability could have different effects on amphibians compared to individuals exposed to constant concentrations.

The purpose of this study was to determine the effects of nitrates on larval Cope's Gray Treefrogs, *Hyla chrysoscelis*, and larval American Toads, *Bufo americanus*, and phosphates on larval *Hyla chrysoscelis*, by measuring survival, growth, time to metamorphosis, and developmental stability (as measured by deviations from a normal phenotype). This study examined both the lethal and sublethal effects of these nutrients in constant concentrations and in pulses, to better understand how such pollutants might actually affect amphibians in the natural environment.

Methods

Hyla chrysoscelis and *Bufo americanus* eggs were collected from the Jackson Purchase Area of Kentucky. Larvae from collected eggs were used for three separate experiments. In each experiment, larvae, at hatching, were placed in 100 mL fingerbowls filled with an artificial soft water solution with dissolved Na_2PO_4 for concentrations of 0, 1, 10, 100, and 200 mg/L P-PO_4 for a 15-day LC50 on *Hyla chrysoscelis* and $NaNO_3$ for concentrations of 0, 1, 2.5, and 5 mg/L N-NO_3 for two experiments investigating sublethal affects. The two nitrate experiments investigated the effects of pulses of nitrate on both *Hyla chrysoscelis* and *Bufo americanus*. The concentration of 5 mg/L N-NO_3 was introduced at three different stages: directly after hatching, the middle of the larval period, and directly preceding metamorphosis. In each group, the concentration was decreased every two days to simulate the gradual decline in concentration that would occur naturally in a small pond. Four other treatments were implemented for comparison: one control treatment and three treatments with constant concentrations of 1, 2.5 and 5 mg/L N-NO_3. The *Bufo americanus* experiment was terminated after 32 days, and the *Hyla chrysoscelis* experiment was terminated at metamorphosis. *Hyla chrysoscelis* metamorphs were frozen for analysis of the nitrogen content of muscle tissue with a CHN analyzer. Size, body mass, and developmental stability were assessed for each individual using digital imaging at the termination of each experiment. In the phosphate experiment and the *Hyla chrysoscelis* nitrate experiment, there was one tadpole per fingerbowl, and forty fingerbowls per treatment. In the *Bufo americanus* experiment, there were four tadpoles per fingerbowl and twelve replicates of each treatment.

The concentrations used in these experiments were chosen based on the literature and preliminary experiments. The nutrient concentrations were tested every two to three days using a Lachat Quikchem Flow Injection Analyzer and changed accordingly for static renewal. At experiment termination, tadpoles or metamorphs were weighed, measured and photographed for analysis. Photographs were analyzed using morphometric software.

Findings and Significance

In the 15-day phosphate LC50, there was no significant difference in mortality or growth effects among any of the treatments, indicating that phosphate is not toxic to *Hyla chrysoscelis* at any reasonable concentration. The addition of phosphate did cause a significant increase in the pH of the test water, which, in conjunction with other stressors, may have negative affects within aquatic communities. However, phosphate could also positively affect anuran tadpoles by increasing algal food resources. Because phosphate is a ubiquitous pollutant, further testing using more complex experimental designs is warranted.

In the nitrate pulse experiment for *Bufo americanus*, there was no difference among the treatments in growth or developmental stage. Directional asymmetry was found in both eye width and the distance from eye to nare, but there were no differences among treatments after correcting for the skew. In the *Hyla chrysoscelis* experiment, more extreme directional asymmetry was found in the middle and late pulses in two limb-related traits, indicating that the sudden change in concentration of nitrate decreased the level of developmental stability in the tadpoles. Many scientists disregard traits with directional asymmetry, claiming that only fluctuating asymmetry can be used for studies on developmental stability (Palmer 1994). Recent evidence has shown a shift from fluctuating asymmetry to directional asymmetry with extreme levels of stress (Graham et al. 1993; Lens and Van Dongen 2000). These studies, in conjunction with this one, suggest that traits with directional asymmetry should not be disregarded. Additionally, these results indicate that nitrate has subtle but important effects at low doses. Also, species may be better able to deal with pulses that occur early rather than late in development. Similar results may have been found in the *Bufo americanus* experiment if it had lasted until metamorphosis, so that limb-related traits could be utilized. However, the nitrate concentrations may not have been high enough to induce sublethal effects in this species. Many types of environmental stresses occur in pulses. This study suggests that the temporal regime of stresses may alter the effects of the stress. This type of knowledge will help conservation biologists manage populations and prevent population declines.

References

Baker, J.M. and V. Waights. 1994. The effects of nitrate on tadpoles of the tree frog (*Litoria caerulea*). Herpetological Journal 4: 106-108.

Diana, S.G. and V.R. Beasley. 1998. Amphibian toxicology. IN: Lanoo, M.J., ed. Status and Conservation of Midwestern Amphibians. Univeristy of Iowa Press, Iowa City, IA, 266-277.

Graham, J.H., K.E. Roe, and T.B. West. 1993. Effects of lead and benzene on the developmental stability of *Drosophila melanogaster*. Ecotoxicology 2: 185-195.

Lens, L. and S. Van Dongen. 2000. Fluctuating and directional asymmetry in natural bird populations exposed to different levels of habitat disturbance, as revealed by mixture analysis. Ecology Letters 3: 516-522.

Meredith, C. S. and H. H. Whiteman. Lethal and sublethal effects of nitrate on amphibian embryos and larvae. In preparation for Ecological Applications.

Palmer, A.R. 1994. Fluctuating asymmetry analyses: a primer. In Markow, T.A., ed., Developmental instability: its origins and evolutionary implications. Kluwer, Dordrecht, Netherlands, 335-364.

Rouse, J.D., C.A. Bishop, and J. Struger. 1999. Nitrogen Pollution: an Assessment of its Threat to Amphibian Survival. Environmental Health Perspectives 107: 799-803.

Schlesinger, W.H. 1997. Biogeochemistry: an Analysis of Global Change. Academic Press, New York.