



# Lethal and sublethal effects of nutrient pollution on amphibians

Julia E. Earl and Howard H. Whiteman

Center of Excellence in Ecosystem Studies and the Department of Biological Sciences

Murray State University, Murray, KY 42071

## Introduction

Fertilizer application is a widespread practice that greatly affects aquatic ecosystems in a variety of ways. Previous studies show that nitrate toxicity could be a major problem for amphibians at elevated levels, though few studies examine sublethal effects. On the other hand, phosphate has not been evaluated at all for toxic effects. To examine nutrient toxicity, laboratory experiments were conducted to measure the response of American Toad and Cope's Gray Treefrog tadpoles to various concentrations of nitrate and phosphate.



Figure 1. Experimental set-up.

## Basic Methods

- Eggs were collected from western Kentucky.
- Hatchlings were placed in glass dishes (Figure 1).
- All experiments were static renewal.

## Phosphate Experiment

- *Hyla chrysoscelis* tadpoles
- 5 treatments: control and 1mg/L, 10 mg/L, 100 mg/L, and 200 mg/L P-PO<sub>4</sub>
- Experiment terminated after 15 days
- 40 replicates of one tadpole per dish
- PO<sub>4</sub> increased the pH of the water, but had **no effect on the tadpoles** in a two week time period. The concentration of PO<sub>4</sub> had no effect on mass, body condition, SVL, directional asymmetry, fluctuating asymmetry, or mortality.

## Nitrate Experiments

### *Bufo americanus*:

- 4 tadpoles per dish, 12 dishes per treatment
- Experiment terminated after 32 days
- Tadpoles were exposed to one of 7 treatments (Figure 2),

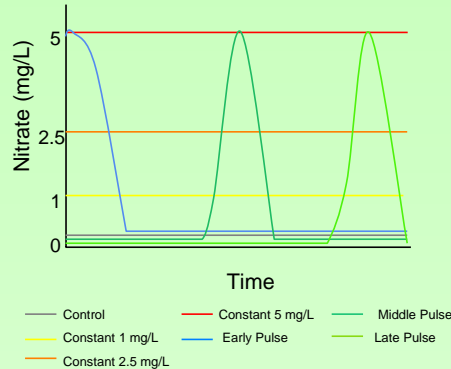


Figure 2. Treatments for nitrate experiments.

including a control, three concentrations kept constant throughout the experiment and three pulses at different points during development. Each pulse involved raising the concentration to 5mg/L N-NO<sub>3</sub> and decreasing it every two days until the concentration was 0mg/L, where it remained during all other times during the experiment.

There was **no difference among treatments** in mass, body condition, SVL, developmental stage, fluctuating asymmetry, directional asymmetry, deformities, or mortality.

### *Hyla chrysoscelis*:

- Dishes were kept in incubators at 29 ° C
- Same treatments as *B. americanus* experiment
- Experiment terminated at metamorphosis

No differences were found among treatments in time to metamorphosis, mass, SVL, body condition, or fluctuating asymmetry.



Figure 3. Leg measurements used in FA analysis.

However, **individuals from the late pulse treatment were found to have more extreme directional asymmetry** in calcaneum length (Figure 3) than other treatments, except for constant 5 mg/L (Figure 4). Treatments also appear to affect mortality, but further analysis is needed.

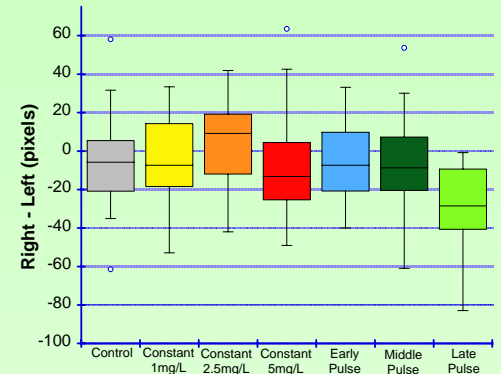


Figure 4. Differences in directional asymmetry among treatments in calcaneum length.

## Conclusions

Phosphate pollution, even at high concentrations, had no effect on *Hyla chrysoscelis*. While it may be benign toxicologically, indirect effects of phosphate pollution, such as algal blooms, may still endanger amphibians.

Nitrate had no effect on *Bufo americanus* at relatively low levels but affected the development of *Hyla chrysoscelis* through directional asymmetry, which may indicate a high level of stress. The late pulse of nitrate occurred during hind limb development, and directional asymmetry in a hind limb trait suggests that the developmental process was disrupted during the sudden increase in concentration of nitrate. Individuals from the early and middle pulses may have had similar disruptions in development but were able to compensate prior to metamorphosis.

## Acknowledgements

Thanks to Beth Kobylarz for great advice, and to my Master's thesis committee: Ed Zimmerer, Claire Fuller, Chris Mecklin, and George Kipphut for support. Funding was provided by KWRR1 104B grant, the Water Science Program, and Hancock Biological Station at Murray State University.