Morphological Plasticity of Green Frogs (*Lithobates clamitans*) and Wood Frogs (*Lithobates sylvaticus*) as Indicators of Eutrophication Levels

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Introduction

Eutrophication occurs when excess nutrients are introduced to aquatic ecosystems, resulting in increased primary productivity that initiates bottom-up control via trophic cascades.[4] In early-stage or moderate eutrophication, respiration, death, and decomposition of primary producers depletes dissolved oxygen while still providing resources to tolerant species. In these systems, direct mortality may be low, while indirect effects of eutrophication dominate via changes in trophic or other behavioral interactions. Along with changes in primary production, altered trophic interactions and structural diversity serve as bioindicators of eutrophication[4], yet such sub-lethal population and community effects of eutrophication are poorly understood. Using a mesocosm approach, we assessed the effects of anthropogenic eutrophication on morphological responses in larval Wood Frogs (*Lithobates sylvaticus*) and Green Frogs (*Lithobates clamitans*).

Trophic interactions in our study species in response to environmental conditions and predation risk are reflected in many ways, including morphological plasticity.[1] In many anurans, morphological plasticity in response to predation and food availability alters developmental rates, with metamorphs emerging either larger and later when food is abundant and predation risk is low or smaller and earlier[1] when food is limited and predation risk is high. Predation risk also influences anuran body shapes, most notably muscle height, muscle width[2], tail length, and tail height[1]. Increases in tail muscle conformations and tail length:height ratios are common and advantageous to organisms in high predation risk environments[1], and this project will explore the relationships between eutrophication, primary productivity, predator abundance, and resulting tadpole morphology.

Methods

Exposure Treatment - Wood Frogs

Even without anthropogenic eutrophication, increased solar insolation can replicate spikes in primary productivity created by eutrophication. 10 replicate 20-gallon mesocosms each were established as unshaded and shaded (50% mesh) treatments. 10 larval *L. sylvaticus* were introduced to each mesocosm. After 21 days, survivors (*N* = 30) were measured for eight morphological variables commonly influenced by larval environments. Algal mass, primary productivity, temperature and dissolved oxygen were also sampled during tadpole collection.[5]

Fertilizer Treatment - Green Frogs

15 replicate 20-gallon mesocosms with 16 larval *H. chrysoscelis* were created for each of three levels of fertilization with Osmocote 14-14-14 fertilizer: 0 g/mL, 0.3 g/mL, and 0.6 g/mL of. After 25 days, tadpoles were collected via minnow traps and measured for the same traits as for *L. sylvaticus*. Nitrate, primary productivity, temperature and dissolved oxygen sampling was conducted.[6]

Analysis

Multivariate analyses of variance (MANOVAs) were used to assess the influence of treatments on tadpole morphology, primary productivity, and abiotic variables. Principal Components Analyses (PCAs) was used to visually evaluate differences between treatments effects, as well as to assess variation among mesocosms in the response variables affected by treatments (as determined by MANOVA).[5,6] Linear regression models were then used to characterize relationship between morphological characteristics and other variables associated with mesocosms.

Results

Exposure Treatment - Wood Frogs

All morphological characteristics except one were significantly affected by treatment (p=0.05), with tadpoles from shaded tanks being significantly smaller. Dissolved oxygen and temperature were significantly decreased in shaded mesocosms. However, dissolved oxygen had no significant correlation to any morphological characteristics. Temperature was broadly positively correlated with all morphological characteristics but only statistically significantly correlated with tail muscle height and developmental stage.

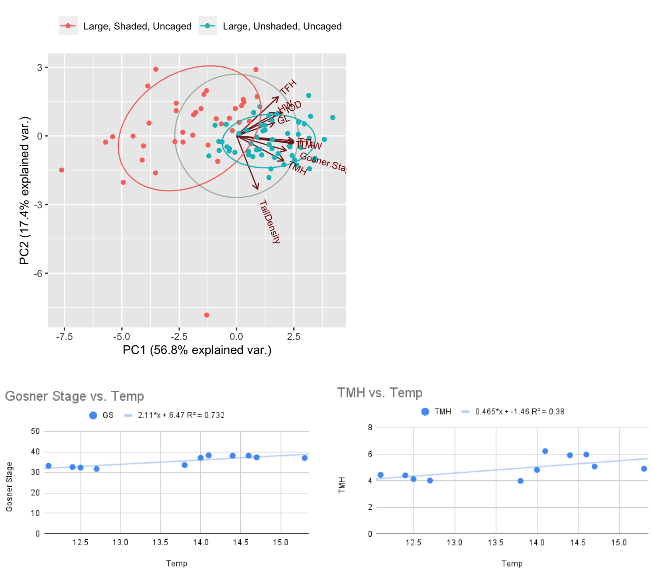

Figure 1. (a) PCA mapping tadpole morphology, and grouped by treatment. (b) Graph showing positive relationship between developmental stage at collection and temperature. (c) Graph shoulding positive relationship between average tadpole tail muscle height and mesocosm temperature.

Fertilizer Treatment - Green Frogs

All morphological characteristics were significantly affected by treatment, with tadpole size and tail fin depth increasing with increasing fertilization. Of four abiotic factors (primary productivity, dissolved oxygen, temperature, and post-treatment nitrate concentration), primary productivity was affected by treatment in a positive parabolic relationship. Primary productivity had statistically significant positive correlations to all morphological characteristics with a positive parabolic relationship.

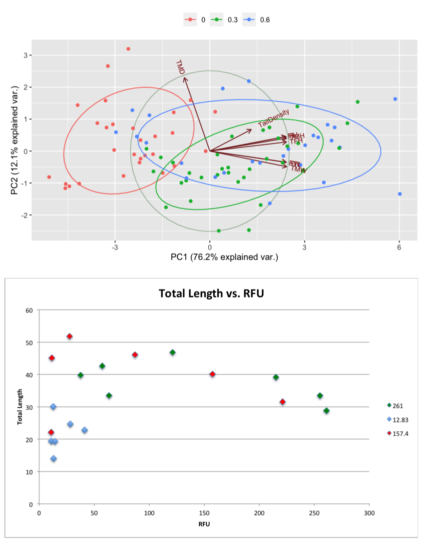

Figure 2. (a) PCA mapping tadpole morphological characteristics, grouped by treatment. (b) Map showing positive parabolic relationship between mesocosm primary productivity and average tadpole total length

Conclusions

We were able to induce significant environmental variation that resulted in significant morphological variation, suggesting our attempts to induce eutrophication were successful. Further, we may have fertilized hypertrophic treatments enough to cause decreases in phytoplankton populations to such extent as to result in the positive parabolic relationship found between fertilization and primary productivity, and between primary productivity and tadpole size and fin depth. However, since we were unable to correlate primary productivity to post-treatment nitrate levels, future study would be needed into data from nitrate levels before and after our tadpole sampling.

Increases in tadpole size and tail fin depth occurring due to increases in temperature in the exposure treatment and due to increased primary productivity in the fertilization treatments could be attributed to increased predation risk due to eutrophication or increased availability of phytoplankton for tadpoles to consume. Future analysis into macroinvertebrate populations before and after tadpole inoculation could help solve that question. Since temperature did not vary significantly with the pre-treatment concentration of fertilizer added, the increase in tadpole size and tail fin depth found in the fertilizer treatments is likely a different mechanism to the increase found in the exposure treatment.

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