Freshwater Snail Feeding Behavior Response to Algae Grown in Excess Nutrient Levels

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August 13, 2018
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Abstract

Nutrient pollution to Earth's freshwater resources is becoming an increasingly important issue that has the potential to impact many ecological communities. Our aim was to understand how the feeding behavior of two species of freshwater snail, *Physa acuta* and *Planorbella campulunata*, is affected by algae grown in increased nutrient levels. Using river water diverted from Maple River in Pellston, Michigan (USA), we grew algae in three different nutrient levels and studied how snails responded to these different diet choices. We found that both species of freshwater snail preferred feeding on algae grown in higher nutrient levels and that competition for the high-nutrient algae occurs between these two species when inhabiting the same area. Our results suggest that anthropogenic nutrient inputs may have substantial impacts on the feeding behaviors of species living in those nutrient polluted environments.

for the purpose of preservation and access in the future.

Signed,
Julia Linder (electronic signature)

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Introduction

Freshwater snails play a large role in freshwater ecosystems as herbivores, prey, and parasite hosts (Hoverman et al., 2011). Like many mollusks, freshwater snails can be valuable indicators of environmental change, as they are often sensitive to changes in their environment (Fortunato, 2015).

One of the main anthropogenic effects that is causing large changes in freshwater systems is the process of eutrophication. Aquatic systems are typically limited by nitrogen (N) or phosphorus (P), and most commonly, marine ecosystems are N-limited and freshwater ecosystems are P-limited (Hecky & Kilham, 1988). Eutrophication refers to the enrichment of nutrients to an aquatic ecosystem, which typically results in excess plant growth, in particular phytoplankton (Smith et al., 1999). While eutrophication can occur naturally, the process is often accelerated and exemplified by human activity, such as pollution inputs from fertilizers, detergents, and sewage (Smith et al., 1999).

Eutrophication can both increase total biomass of phytoplankton species and alter the communities that dominate the ecosystem (Bužančić et al., 2016; Hecky & Kilham, 1988). Eutrophication can lead to a loss of phytoplankton diversity in freshwater ecosystems, despite higher nutrient levels (Bužančić et al., 2016; Ogawa & Ichimura, 1984). This paradox may be explained by the ability of some phytoplankton species to take up more nutrients, thus outcompeting other species with less nutrient-uptake ability (Ogawa & Ichimura, 1984).

Thus, with different algae communities in eutrophic, nutrient-polluted waters, it would be expected that typical consumers of phytoplankton, such as freshwater snails, may be affected by this diet change. Previous studies have shown that freshwater snails living in nutrient-enriched water experience effects such as genomic changes and decreased perception of predation risk (Qiao et al., 2018; Turner & Chislock, 2010). However, little research has been conducted on how the feeding behavior of snails changes when introduced to algae grown in enriched nutrient conditions. The purpose of this study is to determine the effect of algae grown in different nutrient levels on the feeding behavior of freshwater snails. We predict that both freshwater snail species *Physa acuta* and *Planorbella campunulata* will prefer to feed on algae that has grown in high-nutrient conditions compared to lower nutrient levels.

Methods

Snail Collection

We collected snails by hand from the shallow waters of Douglas Lake's South Fishtail Bay in Pellston, Michigan (USA). Living snails of all species were collected and stored in an aerated bucket with algae and plants from the water near their collection. Two days later, we sorted the snails by species. We only used *Physa acuta* and *Planorbella campanulata* in the experiment due to their high abundance.

Experimental Setup and Data Collection

We conducted this experiment at the University of Michigan Biological Station (UMBS) Stream Research Lab on Maple River in Pellston, MI (USA). We diverted water from the Maple River to three streams made from standard rain gutters. Stream A was designated as the control stream, and B and C were designated as the low and high nutrient streams, respectively. We placed 12 two-inch square tiles in each gutter stream. In order to replicate agricultural runoff, we used Jobe's fertilizer spikes (Nitrogen-Phosphorus-Potassium ratio of 6-18-6) as our nutrient inputs for streams B and C. Using mesh bags, we fastened the fertilizer spikes to the head of streams B and C. On day one, stream B received one spike and stream C received three spikes. In order to encourage algae growth, on day twelve we added one spike to stream B and two spikes to stream C for a total of two and five spikes per stream, respectively.

We allowed the algae to grow for 17 days from the setup date. Approximately every other day, one team member checked and adjusted the stream flow rates to minimize the difference between the three streams. The average flow rates for streams A, B, and C, were 83, 89, and 90 mL/s, respectively. In addition, we used a soft paintbrush to clear detritus from the tiles to ensure algae grew on the tile surface and not on the detritus.

On day 18 we determined there was sufficient algae growth to begin the experiment. We removed nutrient pouches from streams B and C. We marked tiles from stream A with yellow nail polish, stream B tiles with red polish, and stream C tiles with blue polish. Once tiles were marked, we evenly distributed nine tiles from each stream among the three streams in the order of blue(B), red(R), yellow(Y) so that each stream had the identical tile order of BRYBRYBRY. We removed three tiles from each original stream to use for algae analysis.

We designated stream A as the competition stream, so we randomly placed 15 of each *Physa* and *Planorbella* on the tiles. We randomly placed 20 *Planorbella* on the tiles in stream B, and in stream C we randomly placed 20 *Physa.* We ran the snail experiment for three days. We recorded the distribution of snails and consumption of algae twice per day. For distribution, we recorded the number of snails per tile, specifying which color of tile they were found on. For algae consumption, we estimated the amount of algae consumed based on the amount of bare substrate per tile.

Algae analysis

We analyzed the nutrient content and species composition on the blue, red, and yellow tile algae. We scraped the algae from the tiles using a clean toothbrush and making two sets of slurries of algae mixed with deionized water. Chemistry analysis on the nutrient content was conducted by the UMBS chemistry laboratory. The lab digested a nominal mass of sample slurry in 1:1 H2SO4-H2O2 at autoclave conditions for 60 minutes. The resulting digestate was analyzed by ICP/MS for P content(P) and automated colorimetry for N content.

We mixed the slurries used for species composition analysis with a formaldehyde compound to preserve the samples without refrigeration. Dr. Robert Pillsbury analyzed blue-green, diatom, and green algae species densities for the control, low, and medium sample slurries.

Statistical Analysis

We used chi-square goodness of fit tests to determine whether the three tile types (control, low nutrient, and high nutrient) in stream A were equally preferred by *Physa* and *Planorbella,* respectively.

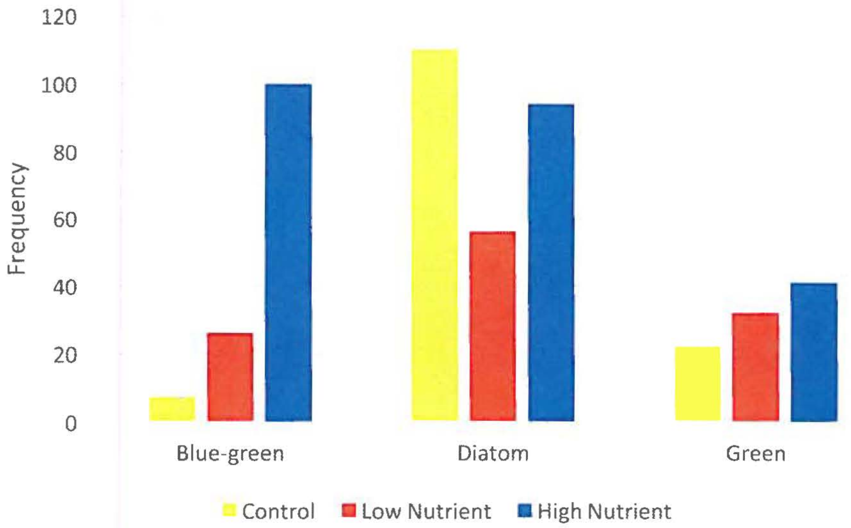
We performed an ANOVA two-factor test with replication for the first two collection times to determine if there was a significant difference in snail consumption patterns between the streams and tiles types. We performed post-hoc Tukey tests to determine exactly where any significant differences in consumption patterns could be found. We chose only to perform ANOVA tests for the first two collection times, as most of the algae had been consumed after this point.

Results

Algae species composition

We recorded the presence of five blue-green algae taxa, eight diatom taxa, and six green algae taxa in the three different algae samples. While there was no obvious difference in species richness among the three algae samples, there was notably more blue-green algae found in the high-nutrient sample (n=100) than the low nutrient sample (n=26) and control sample (n=7) (Fig.1). A similar relationship of increasing numbers with increasing nutrient level was seen for green algae, but to a lesser extent (Fig.1).

Figure 1 Frequency of blue-green algae, diatom algae, and green algae in control, low nutrient, and high nutrient streams



Algae N and P composition

Algae phosphorus content increased with increasing nutrient levels in the water (Table 1). In contrast, algae nitrogen content decreased with increasing nutrient levels (Table 1).

Table 1 Phosphorus and Nitrogen amounts (per gram of algae) in control, low nutrient, and high nutrient algae samples

Nutrient conditions	ug Phosphorus/g algae	ug Nitrogen/g algae
Control	6.239649742	42.03539823
Low	9.932001419	29.89130435
High	13.7069848	12.53333333

Snail consumption of algae

In all three streams, snails consumed a larger percent of the algae from the high nutrient tiles faster than algae from the low nutrient and control tiles (Figs. 1,2,3). *Planorbella* in Stream B had a faster grazing rate on all nutrient levels (Fig.3) than *Physa* in Stream C on all nutrient levels (Fig. 4).

For the first two collection times, the pattern of snail grazing between the streams was significantly different (F(3,36)=21.36, MSE=4648, $p<0.001$). For the first time period, post-hoc Tukey tests showed that the high nutrient and low nutrient tiles were grazed significantly differently between Stream A and C ($p<0.005$; $p<0.01$). For the second time period, post-hoc Tukey tests showed that there was a significant difference between the grazing of high nutrient tiles Stream C and Stream A and Stream C and Stream B ($p<0.001$ for both). All streams were grazed significantly different for low nutrient and control tiles ($p<0.05$ for all).

Figure 2 Percent algae consumed by *Planorbella* and *Physa* in stream A over time on control, low nutrient, and high nutrient tiles

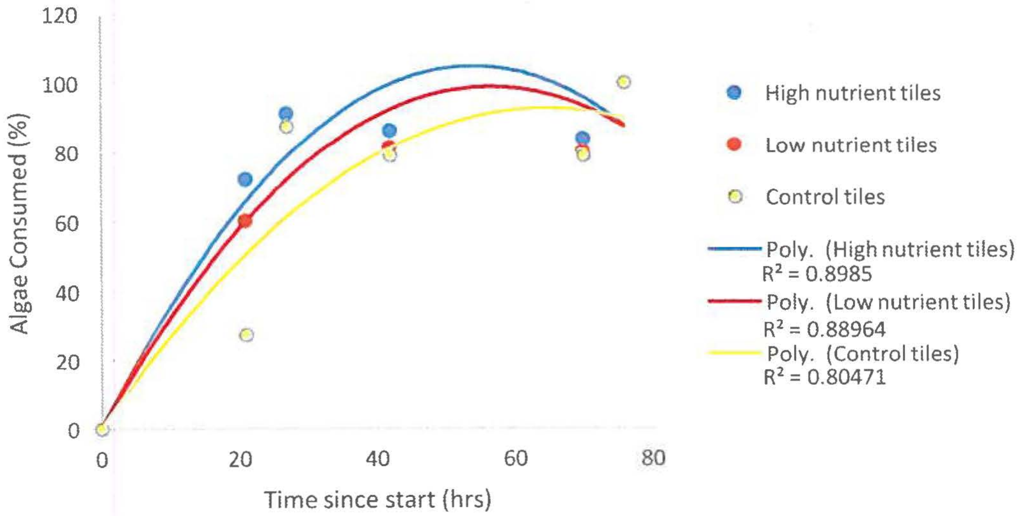


Figure 3 Percent algae consumed by *Planorbella* in stream B over time on control, low nutrient, and high nutrient tiles

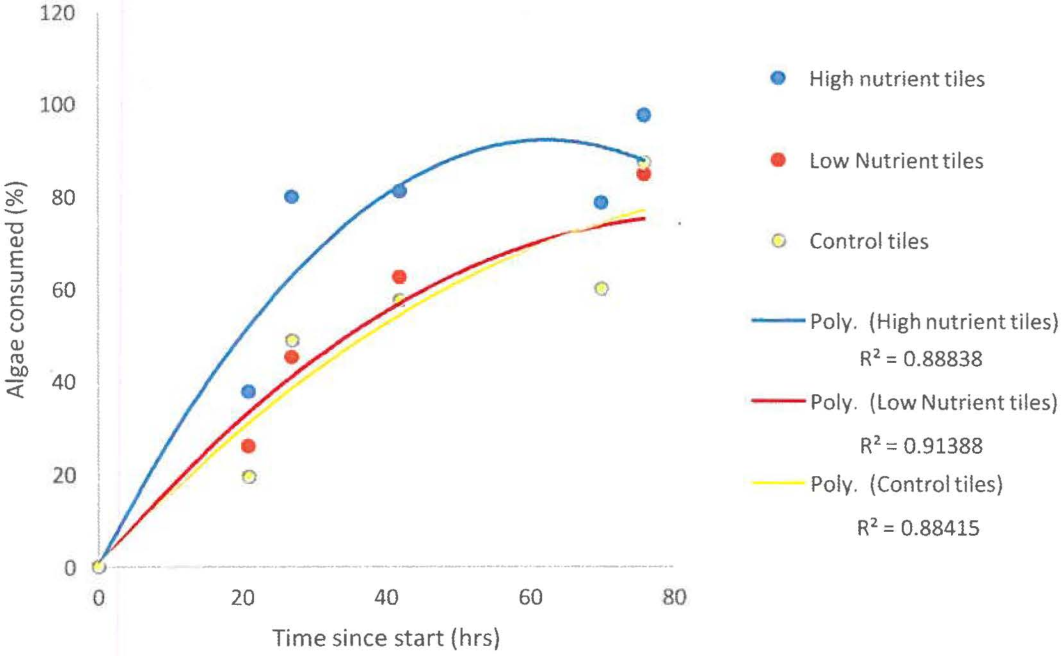
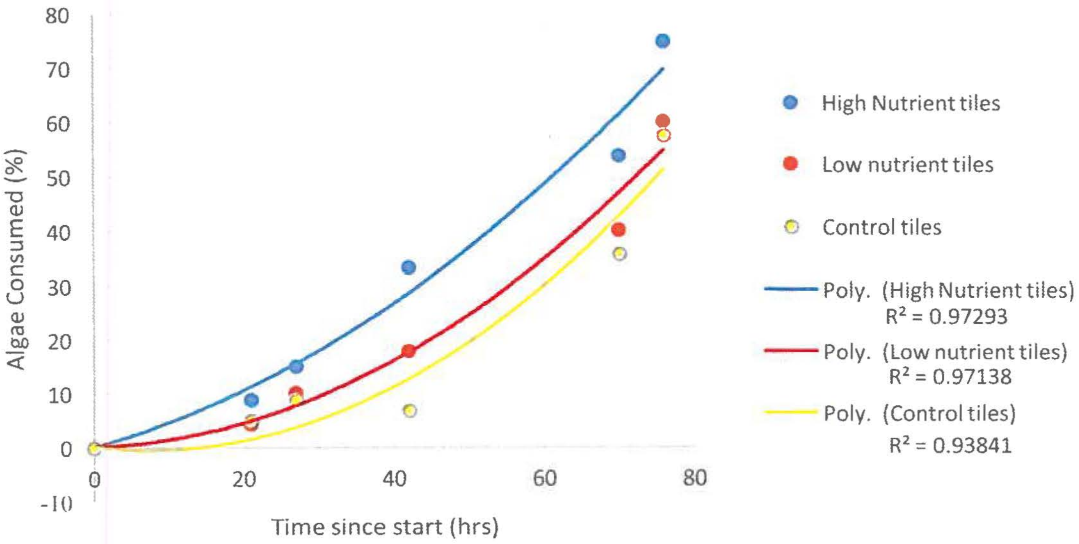


Figure 4 Percent algae consumed by *Physa* in Stream C over time on control, low nutrient, and high nutrient tiles



Stream A competition dynamics

In Stream A, more *Planorbella* were initially found grazing on high nutrient tiles than low nutrient and control tiles (Fig. 5). In contrast, more *Physa* were initially found on low nutrient tiles compared to high nutrient and control tiles (Fig.6).

The difference between the observed number of *Physa* on the control, low nutrient, and high nutrient tiles was not significant (χ^2 (2, N=23)=4.26, p=0.12). Similarly, the difference between the observed number of *Planorbella* on the control, low nutrient, and high nutrient tiles was not significant (χ^2 (2, N=12)=3.5, p=0.17).

Figure 5 Distribution of *Planorbella* in stream A on high nutrient, low nutrient, and control tiles over three days

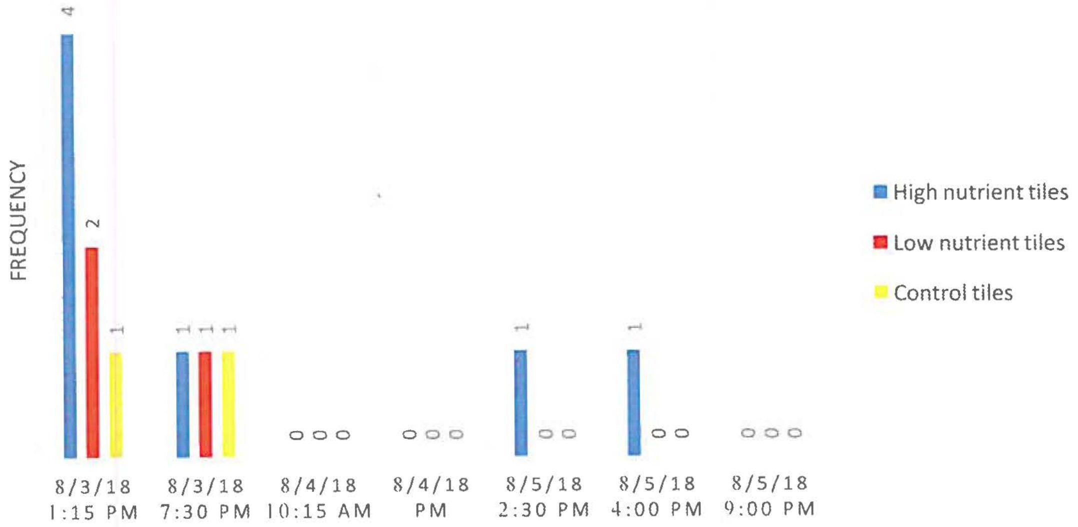
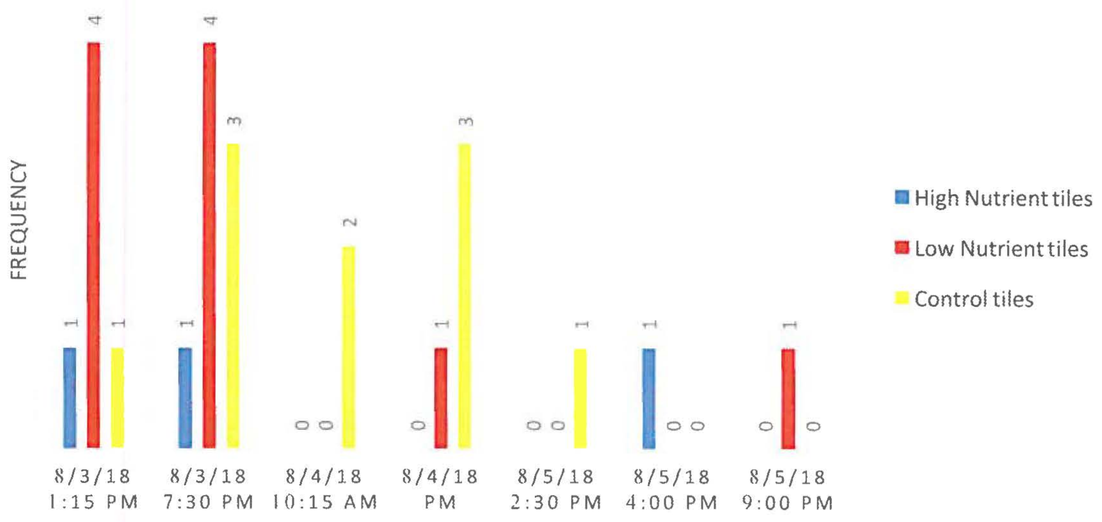


Figure 6 Distribution of *Physa* in stream A on high nutrient, low nutrient, and control tiles over three days



Discussion

Even though much of our data was not statistically significant, the general trends we observed supported our hypothesis that both *Physa acuta* and *Planorbella campulunata* prefer to feed on algae grown in the highest nutrient levels when offered algae from three different nutrient levels. While there has been research on the harmful effects of blue-green algae, or cyanobacteria, on freshwater snails, the preference we found for snails to feed on the high-nutrient tiles, which had a large number of cyanobacteria compared to lower nutrient tiles, indicates the snails may actually have a dietary preference for blue-green algae (Qiao et al., 2018; Turner & Chislock, 2010). It is also possible that the snails fed on the algae grown in higher nutrient levels due to the increased amount of phosphorus in these tiles, although this algae actually had lower levels of nitrogen. One possibility for this could be that the algae was phosphorus-limited in the control waters, so when it was introduced to a phosphorus source it stored more P than N.

Our results also show that there may have been competition occurring between *Physa* and *Planorbella* in Stream A. When *Planorbella* and *Physa* were grown separately in Streams B and C, they both seemed to prefer the algae grown on the high-nutrient tiles (Figs. 3&4). Although not statistically significant, the observed trends seem to show that *Planorbella* selectively fed on the high nutrient tiles in Stream A (Fig 5), while *Physa* seemed to selectively feed on the low nutrient tiles in the same stream (Fig. 6). This is particularly interesting because in response to *Planorbella* competition, the *Physa* feeds on the next highest nutrient option, which is the low nutrient tiles, rather than the control tiles. This strongly suggests that the *Planorbella* and *Physa* are competing, and the *Planorbella* is the better competitor in this scenario, possibly due to its faster grazing rate.

Since freshwater mollusks are particularly sensitive to environmental changes, are an ideal group of species to study how environmental changes will affect freshwater ecosystems (Fortunato, 2015). Our study shows that anthropogenic nutrient inputs into freshwater ecosystems can have a strong effect on the feeding behavior of native freshwater snails. Since the algae species proportions found in higher nutrient levels are different than natural levels, the input of nutrients means that the diet composition of snails in polluted waters may change significantly. These changes in have the possibility of affecting species growth, reproduction, and competitive ability of these freshwater snails, and possibly other freshwater species that experience the effects of nutrient pollution and eutrophication.

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