Analysis of the Preference of Two Snail Species (*Physa acuta* and *Planorbella campanulata*) for Algae Grown in Varying Nutrient Conditions of Nitrogen and Phosphorous

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The various interactions within communities can represent a balance that is quickly upset by changes in abiotic factors and we investigated to preference of snails on algae subjected to varying nutrient conditions during growth. We grew algae in 3 varying levels of phosphorus and nitrogen using fertilizer and introduced two species of snails, *Physa acuta* and *Planorbella campanulata*, to observe their preference when given the option of all three algae samples. The various algae samples had increasing levels of phosphorus and decreasing levels of nitrogen when grown in increasing levels of phosphorus and nitrogen. The three samples also resulted in differing species composition due to the varied nutrient levels. While not statistically significant, and observed trend was the preference of both snail species for the algae grown in higher nutrient conditions. These results did not support our hypothesis that the algae grown in higher nutrient levels would contain higher levels of both phosphorus and nitrogen, but did support increasing levels of phosphorus only. These results, though not statistically significant, gave some level of support to our hypothesis that both snail species would prefer algae grown in higher nutrient conditions due to nutritional quality and decreased energy used in grazing. This knowledge could be further used to prevent and control algae blooms with the increasing levels of nutrients entering our watersheds from a variety of sources.

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Abstract

The various interactions within communities can represent a balance that is quickly upset by changes in abiotic factors and we investigated to preference of snails on algae subjected to varying nutrient conditions during growth. We grew algae in 3 varying levels of phosphorus and nitrogen using fertilizer and introduced two species of snails, *Physa acuta* and *Planorbella campanulata*, to observe their preference when given the option of all three algae samples. The various algae samples had increasing levels of phosphorus and decreasing levels of nitrogen when grown in increasing levels of phosphorus and nitrogen. The three samples also resulted in differing species composition due to the varied nutrient levels. While not statistically significant, and observed trend was the preference of both snail species for the algae grown in higher nutrient conditions. These results did not support our hypothesis that the algae grown in higher nutrient levels would contain higher levels of both phosphorus and nitrogen, but did support increasing levels of phosphorus only. These results, though not statistically significant, gave some level of support to our hypothesis that both snail species would prefer algae grown in higher nutrient conditions due to nutritional quality and decreased energy used in grazing. This knowledge could be further used to prevent and control algae blooms with the increasing levels of nutrients entering our watersheds from a variety of sources.

Keywords: Snail, Algae, Fertilizer, Grazing Preference

Introduction

The waters of the Great Lakes region and around the world are commonly inhabited by two co-existing organisms: snails and algae. Both are many times seen as a nuisance to humans and usually appear together due to the snails common diet of algae. Algae growth is common in areas of high phosphorus levels which leads to eutrophication (Correll, 1999). Increased phosphorus levels can come from a variety of sources but the most common are sewage runoff and farm and animal waste (Correll, 1999). We were interested in observing whether two species of snails, *Physa acuta* and *Planorbella campanulata*, would exhibit a preference for algae grown in varying nutrient conditions. *P. acuta* is a snail species that is common around the United States and across the globe in many different environments (NatureServe, 2018). *P. campanulata* is a more specialized species found in ponds, lakes, and slow streams in the Northeast and Midwest United States (Cordeiro & Bogan, 2012). We grew algae in water contaminated with varying levels of phosphorus and nitrogen, which would affect the algae both in species composition and nutrient composition based on optimal growth conditions.

We hypothesized that both species of snails would prefer to eat from algae grown in high nutrient conditions as there would be more algae with a more nutritious composition and therefore the snails would have to exert less energy to consume the same amount. We also introduced competition between the two snail species to observe whether one species would monopolize the high nutrient algae while the other was pushed to less desirable food sources. We hypothesized that *P. campanulata* would be more likely to eat from the higher nutrient algae while *P. acuta* would be found more on the medium and low nutrient algae. Since *P. campanulata* are only found in select environments that would encourage large amounts of algae growth such as slow moving, shallow water, they would be forced to find algae of similar nutrients to survive. On the other hand, *P. acuta* is found in a large variety of environments, and therefore algae conditions, and would be more likely to survive on the algae of lesser nutrients.

Methods and Materials

This experiment was conducted at the University of Michigan Biological Station's Stream Lab, Pellston, MI, US (45°33'52"N 84°45'04"W) which diverts water from the Maple River for experimentation. We created three imitation streams by placing hosing carrying stream water into three separate rain gutters. The gutters had a cap on both ends to allow sufficient water depth and the end cap was topped in a mesh screen to direct water flow exiting the gutter. 15 ceramic tiles lined the bottom of each gutter and were placed in direct contact with each other to encourage algae growth on the tiles for easy transfer as opposed to the walls of the gutter. Tiles were placed towards the middle of the length of the gutter to avoid turbulence from the water entering the system. We introduced extra quantities of phosphorus and nitrogen using Jobes fertilizer spikes for robust tomatoes which contained a 6-18-6 proportion of NPK. One stream was the control and contained no fertilizer spikes, one stream contained one spike in a mesh bag attached to the side of the gutter, and the third stream contained 3 spikes in an attached mesh bag, with one and two spikes added to the second and third streams, respectively, after 12 days to encourage algae growth. Shade screens were placed on top of each stream to mimic realistic stream conditions with canopy cover but were removed after seven days to encourage algae growth.

Water flow was measured every other day to ensure equal flow going into each stream by measuring the amount of water leaving each stream within a 10 second time period. A soft-bristled paint brush was used to remove detritus from the gutters that entered through the unfiltered water input when water flow was measured. This ensured that the algae was able to grow directly on the tiles and was able to be removed from the gutters later. After 17 days all of the tiles were removed and the edges marked with nail polish to indicate which stream they originated from. The gutters were then brushed clean and the tiles replaced in a repeating pattern so each stream contained four of each tile for a total of 12 tiles. Three tiles from the original streams were kept for analysis.

Two species of snails, *Physa acuta* and *Planorbella campanulata,* were collected from Douglas Lake, Pellston, MI (45°33'38"N 84°40'14"). After the tiles were replaced in the streams, snails were placed in each of the streams. 15 *P. acuta* and 15 *P. campanulata* were evenly placed on the tiles in stream A to model competition, 20 *P. campanulata* were placed evenly on the tiles in stream B, and 20 *P. acuta* were placed evenly on the tiles in stream C. We then placed shade screens over each stream to create optimal conditions for snail survival. We returned multiple times over the next three days to record how many snails were present on each tile and the percent algae consumed for each tile.

One of the tiles from each original stream were used to conduct chemical analysis to determine the nitrogen and phosphorus content of the algae. The algae was brushed off and mixed with deionized water to form a slurry. A nominal mass of each sample slurry was digested in a 1:1 ratio of 2 mL each of H_2SO_4 and H_2O_2 at autoclave conditions for 60 minutes. The digestate was then analyzed using ICP/MS for phosphorus content and automated colorimetry for nitrogen content. A second tile from each stream was used to analyze species composition. Algae was brushed off of the tile and mixed with deionized water and formalin to form a slurry. 4 random sample views of each algae sample were analyzed for algae species composition using a palmer cell at 400x magnification.

An ANOVA was performed on the percentage of algae eaten from each stream for each type of algae tile 21 and 27 hours after introducing snails. An ANOVA was also performed on the percentage of algae eaten from each type of tile for each stream 21 and 27 hours after introducing snails.Tukey's test used to determine which differences were statistically significant at an alpha level of 0.05.

Results

The algae that was grown in the control stream containing no added nutrients had the lowest level of phosphorus and the highest level of nitrogen while the high nutrient stream with five added nutrient spikes contained algae with the highest level of phosphorus and the lowest level of nitrogen (Table 1). The algae grown in water conditions with only two fertilizer spikes contained intermediate levels of both nitrogen and phosphorus when compared to the other algae samples.

Nutrient Conditions	µg P/gram algae	µg N/gram algae
Control	6.24	42.04
Low Nutrient Added	9.93	29.89
High Nutrient Added	13.71	12.53

Table 1: Measurements of Nitrogen and Phosphorus levels in algae sample grown in three different nutrient condition using varying amounts of 6-18-6 NPK fertilizer spikes in water at University of Michigan Biological Station Stream Laboratory in Summer 2018.

Analysis of the algae grown from each nutrient condition revealed a mixture of blue-green algae, green algae, and diatoms. The most blue-green algae cells were observed in the high nutrient added sample while the highest number of diatoms were seen in the control sample. The high nutrient added sample had the largest number of total algae cells observed while the low nutrient added sample contained the least (Table 2).

Algae Type	Control	Low Nutrient Added	High Nutrient Added
Blue-green	7	26	100
Diatom	110	56	94
Green	22	32	41
Total Algae	139	116	236

Table 2: Various Algae Cell counts found on 4 random samples of algae at 400x magnification grown in three different nutrient condition using varying amounts of 6-18-6 NPK fertilizer spikes in water at University of Michigan Biological Station Stream Laboratory in Summer 2018.

An ANOVA test comparing the percent of algae consumed for each stream for each algae type (control conditions, low added nutrients, high added nutrients) 21 hours after snails were introduced was conducted and resulted in a p-value <0.0001 indicating the various tiles were grazed differently depending on which snail species, or both, were in the stream.

Tukey tests indicated that at 21 hours post-introduction, the high nutrient added tiles and low nutrient added tiles were grazed significantly differently between streams A and C ($p=0.004$ and 0.005, respectively) while the control tiles were not grazed differently between the three streams. At 27 hours post-introduction tukey tests indicate the high nutrient added tiles were grazed differently between streams A and C ($p<0.001$) and between streams B and C ($p<0.001$) but not between A and B ($p=0.250$). For the low nutrient added tiles all streams were grazed significantly differently (A&B $p<0.001$, A&C $p<0.001$, B&C $p=0.001$) as were the control tiles in all streams (A&B $p=0.017$, A&C $p<0.001$, B&C $p=0.014$).

When the tiles within each stream were compared to each other using Tukey tests it was determined that there was no significant difference in the amount that the different tiles were grazed within each stream using an alpha of 0.05. But when a polynomial regression line is applied to the data a trend appears across the streams showing increased grazing of the high nutrient added tiles (blue) with lower grazing of the low nutrient added (red) and control (yellow) tiles (Fig. 1a, b, c).

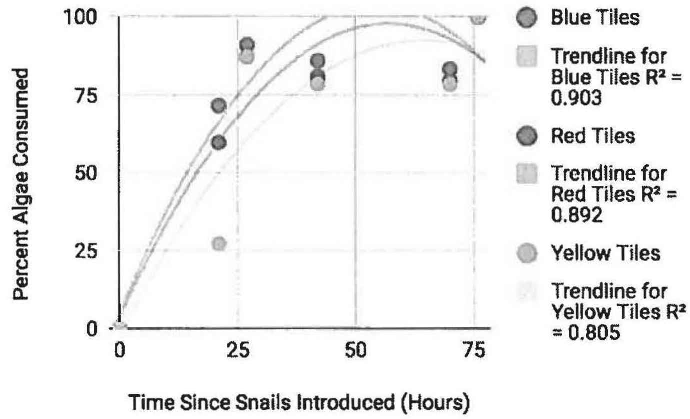

Figure 1a: Percent Algae Consumed in Stream A (competition with *P. acuta* and *P. campanulata*) over time for each tile type (blue=high nutrient added, red=low nutrient added, yellow=control) with a polynomial trendline with a degree of 2.

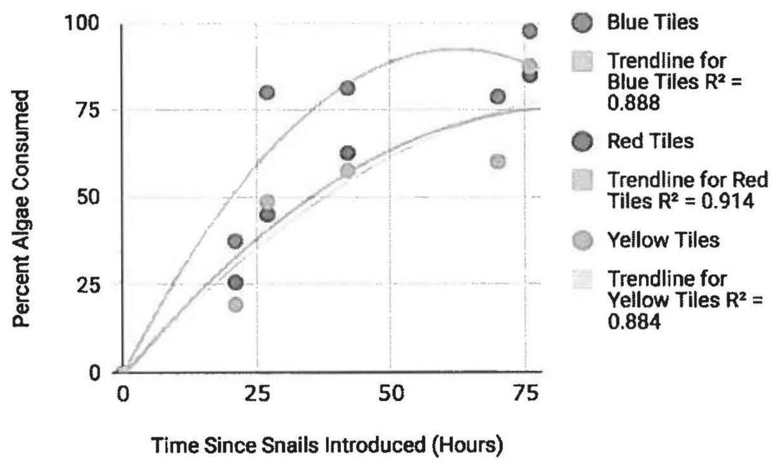

Figure 1b: Percent Algae Consumed in Stream B (*P. campanulata*) over time for each tile type (blue=high nutrient added, red=low nutrient added, yellow=control) with a polynomial trendline with a degree of 2.

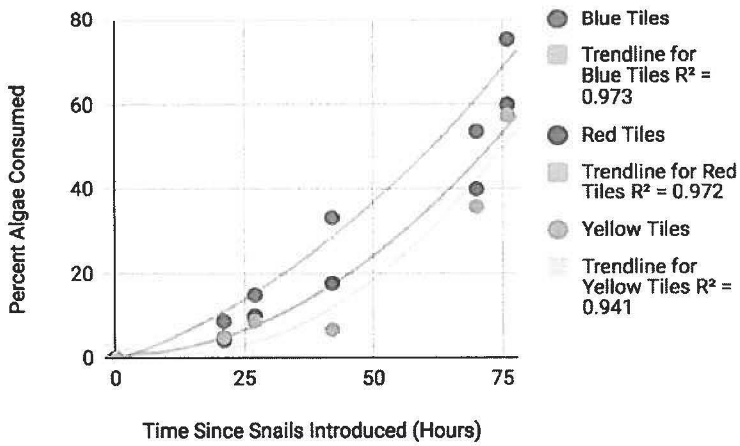

Figure 1c: Percent Algae Consumed in Stream C (*P. acuta*) over time for each tile type (blue=high nutrient added, red=low nutrient added, yellow=control) with a polynomial trendline with a degree of 2.

Discussion

We examined algae growth with varying levels of added phosphorus and nitrogen and the preference of two snail species, *Physa acuta* and *Planorbella campanulata*, for the algae grown in various conditions. We found that with increasing fertilizer amount in the water, the algae contained increasing levels of phosphorus but decreasing levels of nitrogen. We believe this could be due to the fact that certain algal species are able to incorporate phosphate into their cells extremely quickly when provided with excess levels (Stewart, 1974). Increasing the nutrient levels in water systems cause algae numbers to increase due to more available resources (Smith, Tilman, & Nekola, 1999). Algae levels are frequently used as a bioindicator for nutrient levels to their rapid response to changes in phosphorus and nitrogen in water systems (Bellinger, 2010). The decrease in nitrogen levels could be due to different rates of decomposition of dying algae due to the increased number of cells found with the increasing nutrient levels. But it is difficult to determine the exact cause as increasing nutrient levels can change plant size, nutrient content, number/type of plants, or a combination and is thus difficult to separate these effects from confounding each other (Crawley, 1983). The variation in algal species can be seen for our experiment in Table 2 and confirms the effect of varying nutrient levels on species composition and abundance.

We did not observe any statistically significant preference for the high nutrient added algae in any of the individual streams but we did observe a trend in which the high nutrient algae was grazed more than the low nutrient added or control grown algae. Both snails consumed the algae at an unexpectedly rapid rate and due to this, observations closer to the time of snail introduction, that could have provided stronger evidence for our trends, was not recorded. Due to the short time frame in which this study was conducted, there is also the theory that the snails were not drawn to the higher nutrient algae in a significant manner as some snails will not alter their original diet even if other food could yield more growth (Kimberly & Salive, 2012).

Anecdotally, it was observed that the algae within the stream containing *P. campanulata* was grazed much more rapidly than the algae within the stream containing *P. acuta* which could be due to higher energy demands of the former. This can also be observed with the shape of the polynomial trendline in Fig. 1c which shows a slow initial rate of consumption that slowly increases as opposed to the rapid rate of initial consumption in streams A and B (Fig. 1a, 1b) which plateaus as the algae is completely consumed.

The stronger affinity of *P. campanulata* for the algae containing increased nutrients when compared to *P. acuta* could indicate a stronger competitor. This could alter the species composition of snails when algae blooms occur due to the rapid rate at which *P. campanulata* consumes algae, leaving decreased resources for *P. acuta.* This increased consumption rate would also increase organism defacation and cycling of the nutrients, which could contribute to a positive-feedback loop and further increase the algae levels by increasing available nutrients (Guidone, Thornber, and Field, 2010). Further analysis into the competition of *P. acuta* and *P. campanulata* would help to indicate if success of the latter would push the former to local extinction or if the ability of *P. acuta* to survive on low nutrient-containing algae would allow for resource partitioning and the survival of both species. But it has been shown that increasing eutrophication has the ability to decrease community diversity and the full extent to how this impacts environments common to the Great Lakes region where our study was conducted is unknown (Worm and Lotze, 2006).

Due to the similarity of the trends in our results to our expected trends, further data conducted with more observations close to the time of introduction could lead to statistically significant results. A larger system in which grazing choice is not limited simply by the availability of algae would also have the opportunity to produce results with stronger significance. The results from this project could be useful in working to control algae blooms that can produce harmful toxins and to also preserve snail community diversity in certain regions to also allow for food sources for other freshwater organisms.

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