THE EFFECT OF NUTRIENT LOADING ON ALGAL GROWTH AND SUBSEQUENT SNAIL GRAZING BY TWO MICHIGAN SNAIL SPECIES (*Physella acuta, Planorbella campanulata)*

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Abstract

Excessive nutrient loading can have detrimental effects on ecosystems, though nutrient addition in relatively low or appropriate amounts can have beneficial effects instead. We examined the effect of varied nutrient loading on two Michigan snail species (*Physella acuta, Planorbella campanulata*) to determine the effect nutrient loading had on grazing behavior. We also examined the effect on algal cell richness and nutrient capture in each of the three nutrient loading treatments: high-nutrient-added, low-nutrient-added, and control. Snails overall consumed more algal from high-nutrient-added tiles as compared to low-nutrient-added and control tiles, with significant differences existing between consumption of *Physella acuta* alone and *Physella acuta* with *Planorbella campanulata* for both the high-nutrient-added and low-nutrient added treatments (ANOVA $p < 0.05$). Algal communities on the high-nutrient-added tended to have higher cell counts and equivalent but low assimilation of both nitrogen and phosphorus. Communities on control tiles consisted on a much larger proportion of diatoms as compared to blue-green and green algae. The results of this study did not prove significant in most cases, though did point to trends that support the hypotheses that both snail species grazed more on algae grown in a high-nutrient environment.

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Introduction

Nitrogen and phosphorus are considered essential nutrients that control secondary production in many ecosystems, and ratios of these inorganics can have major effects of resulting algal communities (Fink and Elert (2006); Crawley (1983)). Algae are the major source of energy is most aquatic systems and form the basis of most aquatic food webs (Bellringer (2010)). Of the many primary producers reliant on algae, snails are prolific in most freshwater ecosystems and are an important food source for many fish, birds, and small semi-aquatic mammals (DeWreede (2001)). Nutrient availability can have major impacts on the community, including growth and success of primary producers like algae and alteration of the dynamics between algae and primary consumers, which can ultimately result in changes in the food web (Daldorph and Thomas (1991)). Excess nutrients in a system can exacerbate the effect on food webs by promoting growth of toxic cyanobacteria (blue-green algae), potentially leading to the decreased health of a community and lowered biodiversity in areas of high nutrient loading due to runoff from farming and industry (Thomas and Daldorph (1994)).

We were interested in examining the effect of nutrient loading on the grazing behaviors of two freshwater snail species (*Physella acuta, Planorbella campanulata)*, important and abundant sources of food for species in and around Douglas Lake, Michigan. Changes in nutrient enrichment due to anthropogenic factors may have complex effects on freshwater snail populations, including decreased abundance (Smith *et al.* (2012)). While extremely excessive nutrient additions can be detrimental to snail populations, relatively low nutrient loading may prove beneficial in nutrient-limited systems, especially depending on the ratio of nutrients entering the system (Smith *et al.* (2012)).

We grew algae in 3 diversion streams from the east branch of the Maple River in Emmet County, Michigan. Diversions were treated with varied nutrient loading scenarios, and grown algae was then grazed on by *Physella acuta* and *Planorbella campanulata*. We aimed to assess the effect of nutrient loading on a very realistic freshwater stream system to determine if nutrient richness in algal communities grown in the varied treatments had effects on the grazing behavior of the two snail species, hoping to determine whether nutrient loading would prove beneficial or detrimental to the local freshwater snail community.

The stream lab facility where experiment was conducted is on the east branch of the Maple River, the primary outflow of Douglas Lake, making Douglas Lake a suitable and more accessible collection area for the snails used in the experiment than the east branch.

The experiment was conducted at the University of Michigan Biological Station's Stream Research Facility (henceforth called "the stream lab") on the east branch of the Maple River.

We expect snails in all streams to consume more algae from the high-added-nutrient tiles as compared to low-nutrient-added and control tiles. We also therefore expect statistical analyses to show that there were significantly higher levels of consumption on high-nutrient-added tiles in all streams (ANOVA null = the means are not different, alternative = the means are different).

Materials & Methods

During July of 2018, 2 species of snails were collected from South Fishtail Bay on Douglas Lake, Michigan. Wading in the shallow waters, we were able to collect approximately 40 individuals of *Physella acuta*, typically on plant material, and 40 individuals of *Planorbella campanulata,* typically on the silty bottom approaching the macrophyte zone near the drop off. Snails were identified using Burch's guide to land snails around the University of Michigan Biological Station (Burch (1988)).

At the stream lab, the experiment was conducted in 2 stages: stage one involved algae being grown in varied nutrient conditions, and stage two examined the effect of grazing behavior on the varied algal communities of two snail species. We utilized 3 diversions of the river water, direct from the east branch, in rain gutters to use as experimental streams. Flows of each stream diversion were recorded at each observation time and adjusted to keep them approximately equivalent around 1000 mL/s. In each diversion, we placed 15 ceramic tiles for algae to be grown on over the course of approximately 14 days.

In stage one, each stream was a different nutrient-loading treatment, with the first stream being a control (having no added nutrients), the second having low added nutrients, and the third having high added nutrients. The nutrients used in this experiment were Jobe's® Fertilizer Spikes, and each spike had a ratio of Nitrogen:Phosphorus:Potassium of 6:18:8. The control stream did not have any spikes added, the low added nutrient stream had 2 spikes added, and the high nutrient stream had 5 spiked added. Spikes were placed in a mash bag and clipped to the top of the stream near the inflow pipe. Tiles for algae were placed far enough away from the inflow pipe for nutrients to be distributed by the inflow and for the algae on the tiles to not be affected by the current of the inflow.

In stage two, after algae had been growing for approximately 2 weeks, the water from all stream diversions was drained and the tiles were temporarily removed so any residual algae on the sides of the gutters could be wiped away. Tiles were then marked with nail polish on the side to indicate their stream diversion of origin and subsequent algal nutrient level. After cleaning the diversion gutters, tiles were evenly distributed to the 3 diversions again (4 of each tile in each stream) and 'control' water (having no added nutrients) was piped in. The 12 tiles in each stream were ordered in order groups of 3: high-nutrient-added, low-nutrient-added, control from upstream to downstream, and the remaining 3 tiles from each treatment were saved for later analysis. Algal communities grown in each treatment were assessed by counts in four random fields of view under a 400x microscope.

After the flows had been adjusted to make them approximately equivalent, snails were placed in the streams, randomly distributed on the algae tiles. 20 *Physella acuta* were placed in stream C, 20 *Planorbella campanulata* were placed in stream B, and 15 of each species (for a total of 30 snails) were placed in stream A to assess potential effects of competition. Snails were allowed to graze for approximately 2.5 days, at which time the majority of tiles in all streams had been depleted of algae. Intermittently over those 2.5 days, the number of snails on each tile was recorded and the percent algae consumed of each tile was estimated.

Statistical analyses were run to assess the differences in consumption across the three streams and within each stream for the first two observation times, 21 hours post-start and 27 hours post-start. Analyses were not conducted for later observation times because it was assumed that a significant or relevant pattern would not be discernible between the tiles, as the majority of tiles had a significant portion of their algae already grazed. A two-way ANOVA assessed differences in the consumption of the 3 treatments of tiles across all 3 streams. One-way ANOVAs were used to assess consumption of each treatment group of tiles across the three streams, as well as the consumption across nutrient levels within a stream.

Tile samples were also lab analyzed for total nitrogen and total phosphorus present. A nominal mass of sample slurry was digested in 1:1 H_2SO_4-H_2O_2 at autoclave conditions for 60 minutes. The resulting digestate was analyzed by ICP/MS (P) or automated colorimetry (N).

Results

Across time in each stream, there was a noticeable trend for the preferential choice of high-nutrient-added algae, though statistical analyses did not return a significant p-value in any case (ANOVA $p > 0.05$ for all tests comparing consumption within a stream across 3 nutrient levels). However, graphical representation of the data across time in each stream does suggest that there may be a preference in all streams for consumption of high-nutrient-added algae. In Figures 1-3, the polynomial regression curves for each nutrient level in streams A (Figure 1), B (Figure 2), and C (Figure 3) are shown.

When assessing consumption by nutrient level across streams, there was a significantly higher amount of consumption of nutrient rich algae (grown in the high-nutrient-added and low-nutrient-added treatments in stage one of the experiment). At 21 hours hours post-start, there was significantly more algae consumed from the high-nutrient-added treatment in stream A (*Physella acuta* with *Planorbella campanulata)* as compared to stream C (ANOVA: $p = 0.004$). At 21 hours post-start, there was also significantly more algae consumed form the low-nutrient-added treatment in stream A as consumed in stream C (ANOVA: $p = 0.005$). There was not a significant difference in the consumption of control algae consumed betweens any stream. Similar differences existed at 27 hours-start, with significantly more high-nutrient-added algae consumed in streams A and B as compared to stream C (ANOVAs: stream A to C, stream B to C both $p < 0.001$). There was also a significant difference in the consumption of control algae between all streams (ANOVAs: all $p < 0.001$).

Algal community composition did not tend to differ between the three treatments (high-nutrient-added, low-nutrient-added, control). Replicate counts were not recorded so statistical analysis was not performed on the algal community count data. Algal counts are summarized in Table 1. Figures 4-6 summarize the proportion of different algal types on tiles from each of the three treatments.

High-nutrient-added tiles contained approximately equivalent levels of nitrogen and phosphorus, and both low-nutrient-added tiles and control tiles control much more nitrogen than phosphorus (Table 2).

Discussion & Conclusions

High-nutrient-added algae tended to be consumed at a higher rate than both the low-nutrient-added and control algae: overall, snails in all three streams collectively consumed 66% of high-nutrient-added algae, while 54% and 53% of low-nutrient-added and control added was consumed. This may suggest that high-nutrient-added algae was preferentially consumed as compared to low-nutrient-added and control algae. Statistical analyses were not conducted on this data as there were not replicates, though these simple data may point to a trend that requires future study to determine significance. These trends are reiterated by Figures 1-3, which show that high-nutrient-algae ("Blue Tiles") were consistently grazed more than the other two treatments of algae, and the polynomial regression curve is also consistently above the curves approximating the other two treatments of algae.

The apparent trend for preferential consumption of high-nutrient-algae may be explained by many factors, though none can be definitively proven by this study. A possible explanation for the consumption pattern in streams A (*Physella acuta* and *Planorbella campanulata)* is competition between the two species; *Physella acuta* was present in higher numbers on low-nutrient-added and control algae tiles at 21 hours post-start as compared to *Planorbella campanulata*, though the sample size is too small to make significant conclusions about this trend.

Figures 1 and 2 display similar regression curves in which the data was best explained with negative polynomial curves. This suggests that stream A (Figure 1) and stream B (Figure B) had similar consumption rates, likely due to the presence of the seemingly voracious grazer, *Planorbella campanulata*. This also may suggest that snails in streams A and B were faster grazers, approaching 100% algal consumption several hours prior to stream C. That may mean that *Planorbella campanulata* was the faster grazing species since it was common to both streams A and B and the consumptive rate of *Planorbella campanulata* alone (in stream B) was approximately the same as the rate of consumption in A, while the consumption rate in stream C (*Physella acuta)* was significantly lower.

In Figure 3, the data points for consumption across time in stream C (*Physella acuta)* were fit to a polynomial regression curve with a much different shape when compared to Figures 1 and 2. The regression curve was approximately linear, and was positive while the regression curves for the other two streams were negative, approaching 100% consumption much quicker than stream C. This may suggest that *Physella acuta* grazed at a slower rate than *Planorbella campanulata*. It may also suggest that the rate of digestion was slower for *Physella acuta* than *Planorbella campanulata,* since *Physella acuta* did not graze 100% of the algae until several hours after snails in streams A and B.

Though a lack of replication excludes the possibility of statistical analyses, algal communities appeared to be rather different between the three treatments (Table 1). Control tiles had a relatively large proportion of nitrogen with minimal amounts of phosphorus, and low-nutrient-added and high-nutrient-added tiles contained increasing amounts of phosphorus (micrograms P per gram algae), suggesting that the taxa in the lower nutrient treatments are phosphorus limited: they are capturing more phosphorus as it becomes available (Table 1). Additionally, high-nutrient-added tiles contained lower masses of nutrients and a higher number of algal cells, suggesting that algal taxa on those tiles were not capturing nutrients effectively, likely because they did not need to in the highly nutritious environment (Tables 1-2). Nitrogen may also be less mobile in the system than phosphorus, an inorganic known to be noth limiting and rare in most aquatic systems (Cornell (1999)). These results are consistent with work by other students at UMBS in 2011 who determined that the east branch of the Maple River was phosphorus limited (Stoll (2015)). High-nutrient-added tiles contained a high proportion of cyanobacteria, or blue-green algaes, which are known to be harmful in high quantities due to their production on cyanotoxins (Bláha *et al.* (2009)).

There were a multitude of limitations in this experiment considering this was the first time this sort of experiment was conducted at the stream lab. We struggled to regulate the flows of each stream around 1000 mL/s and were unable to keep the flows approximately equivalent for long. *Planorbella campanulata* are also voracious eaters, as they consumed the vast majority of the algae in streams A and B in less than 2 days - much quicker than we anticipated, resulting is us taking fewer than observations that we originally planned. Additionally, estimation of algal consumption on tiles was also difficult to keep consistent, as we did so independently thus our estimation styles varied and potentially affected the resulting data.

Despite a large portion of our data resulting in non-significance and limitations in experimentation, the results of this study do point to several trends consistent with our hypotheses and expectations. Nutrient loading in freshwater systems is a growing problem, and while it is not yet a major problem in Douglas Lake or its outflow, Maple River, it is a serious issue in farming and industrial communities in areas all over the United States and world at large. Food webs are heavily reliant on primary producers like algae, though in cyanobacteria-heavy systems (a typical result of excessive nutrients in a system), resulting quantities of cyanotoxins may prove harmful to humans, fish, birds, and many mammals (Bláha *et al.* (2009)). Proliferation of blue-green algae in highly nutritious environments can lead to decrease in overall ecological health and could also present dangers to drinking water, aquaculture, and many other economic guilds. Control of point-source and nonpoint source pollutants is essential in ensuring a future with rich biodiversity and safety for humans and animals alike.

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Tables & Figures

Figure 1: The consumption of algae by nutrient level in stream A (*Physella acuta* with *Planorbella campanulata*). A polynomial regression curve best represented the data for all treatments (blue $R^2 = 0.903$, red $R^2 = 0.892$, yellow $R^2 = 0.805$). High-nutrient-added algae is represented as "Blue Tiles", low-nutrient-added as "Red Tiles", and control algae as "Yellow Tiles".

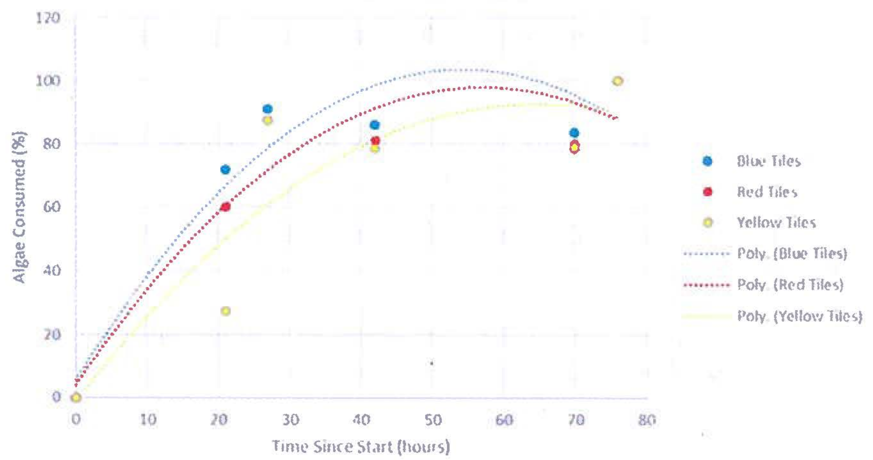

Figure 2: The consumption of algae by nutrient level in stream B (*Planorbella campanulata*). A polynomial regression curve best represented the data for all treatments (blue $R^2 = 0.888$, red $R^2 = 0.914$, yellow $R^2 = 0.884$). High-nutrient-added algae is represented as "Blue Tiles", low-nutrient-added as "Red Tiles", and control algae as "Yellow Tiles".

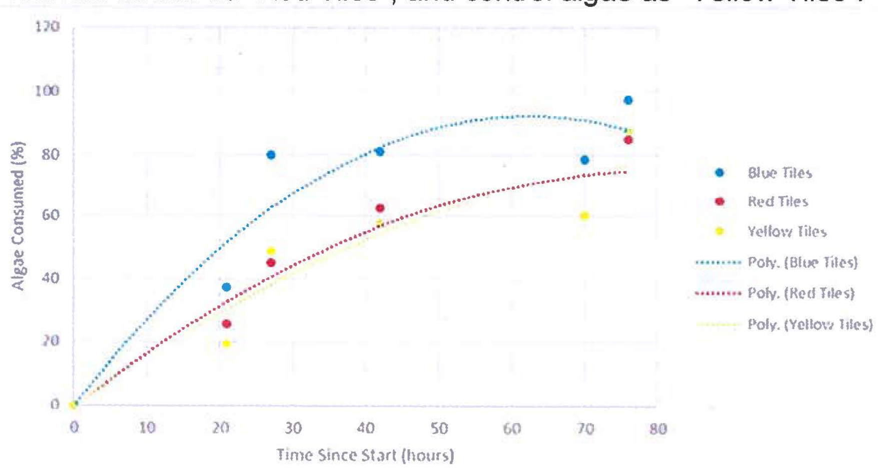

Figure 3: The consumption of algae by nutrient level in stream C (*Physella acuta*). A polynomial regression curve best represented the data for all treatments (blue R^2 = 0.973, red R^2 = 0.972, yellow R^2 = 0.941). High-nutrient-added algae is represented as “Blue Tiles”, low-nutrient-added as “Red Tiles”, and control algae as “Yellow Tiles”.

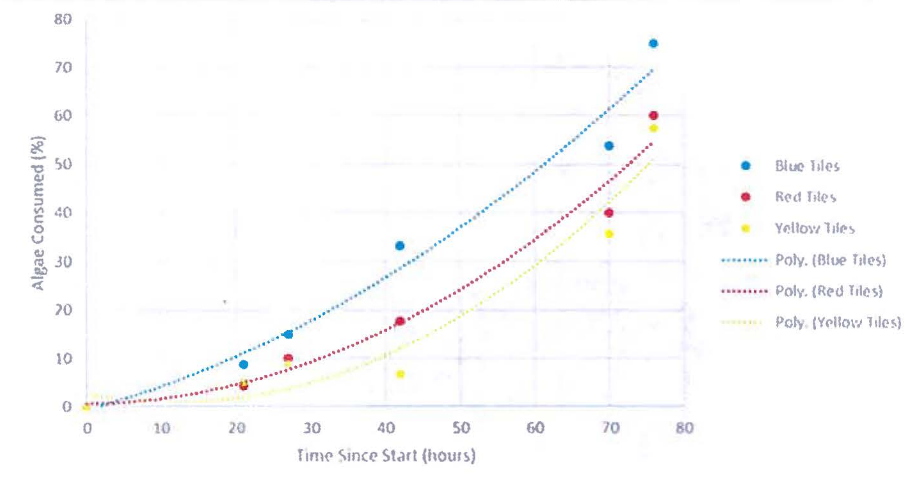

Figure 4: The proportion of algae by algal type on a high-nutrient-added tile.

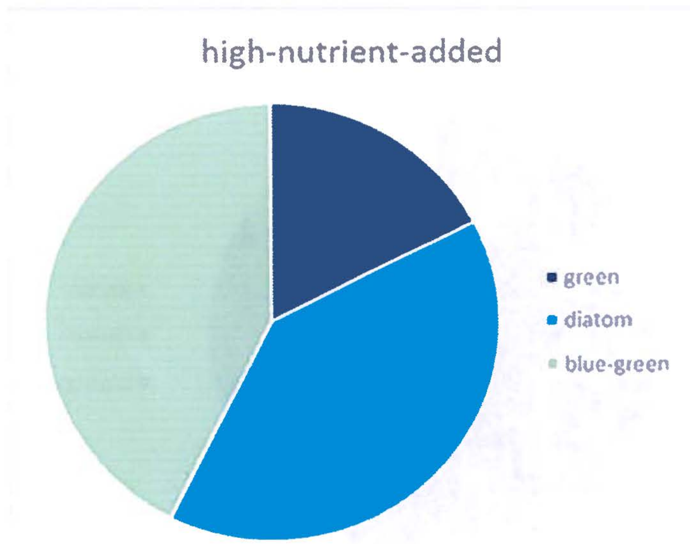

Figure 5: The proportion of algae by algal type on a low-nutrient-added tile.

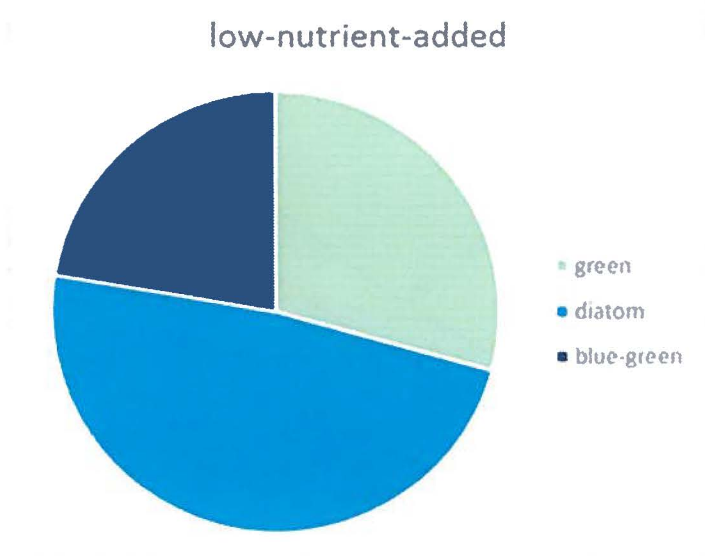

Figure 6: The proportion of algae by algal type on a control tile.

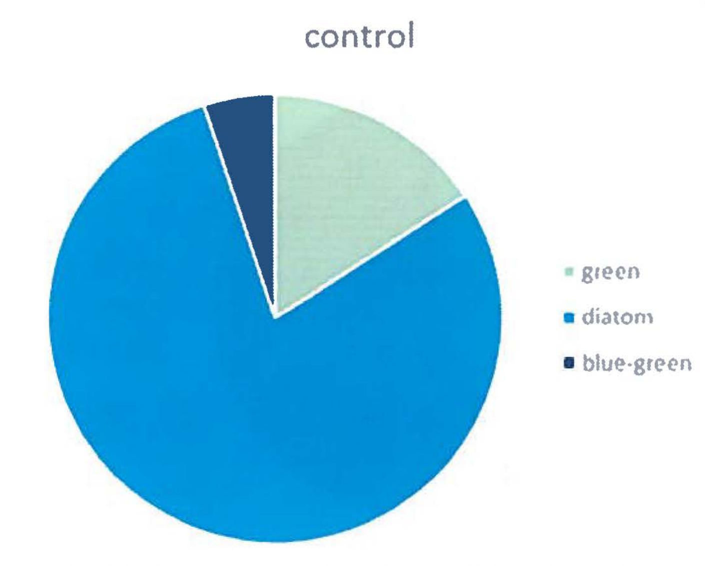

Table 1: Counts of algal communities for each treatment: high-nutrient-added, low-nutrient-added, control. Counts were achieved using a 400x microscope and 4 random fields of view.

algal type	algal taxa	high-nutrient-added	low-nutrient-added	control
Blue-green	Anabaena	0	12	0
Blue-green	Chroococcus	16	0	0
Blue-green	Merismopedia	0	0	4
Blue-green	Oscillatoria	84	14	0
Blue-green	Schizothrix	0	0	3
Total Blue-Green		**100**	**26**	**7**
Diatom	Achnanthes	16	17	13
Diatom	Cocconeis	6	3	6
Diatom	Cymbella	0	4	10
Diatom	Fragilaria	21	11	64
Diatom	Gomphonema	4	0	1
Diatom	Navicula	9	7	3
diatom	Nitzschia	4	0	0
Diatom	Synedra	34	14	13
Total Diatom		**94**	**56**	**110**
Green	Anksitrodesmus	1	2	0
Green	Coelastrum	10	0	0
Green	Cosmarion	0	0	1
Green	Dicteospherium	0	15	0
Green	Mougeotia	24	14	17
Green	Scenesmus	7	3	4
Total Green		**41**	**34**	**22**
Total Cells		**236**	**116**	**139**

Table 2: Total Nitrogen(N) and Phosphorus(P) in each nutrient treatment. A nominal mass of sample slurry was digested in 1:1 H_2SO_4-H_2O_2 at autoclave conditions for 60 minutes. The resulting digestate was analyzed by ICP/MS (P) or automated colorimetry (N).

nutrient conditions	ugP/gram algae	ugN/gram algae
high-nutrient-added	13.71	12.53
low nutrient-added	9.93	29.89
control	6.24	42.04