

**Nutrient Limitation of the nuisance, stalk-forming diatom, *Didymosphenia geminata*,
in Rocky Mountain Streams**

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Abstract

The range of some algae has been increasing in recent years due to either internal (genetic) or external (environmental) factors that have been expanding both the nutrient and pH range at which they can survive. Didymosphenia geminata (didymo) is a species of diatom with a silica casing that can form nuisance blooms on stream beds excluding native species of algae. Understanding the water chemistry that allows for the explosive growth of this diatom is necessary in reducing the spread of the carbon rich, extra cellular stalks it produces. We believe that the addition of sulfate and phosphorus will increase both the diatom cell and stalk production. Nutrient diffusing substrates using nutrient enriched agar were placed in flower pots with 12 treatments (including a control) containing various combinations of phosphorus, sulfate, silicate, and nitrogen. One hundred and eight pots in nine blocks were then placed in the East River in Western Colorado for twenty days at which point measures of chlorophyll a, ash free dry mass (organic matter), and visual counts of both cell and stalk were taken from each sample. Our results showed that the addition of phosphorus containing treatments increased the number of didymo cells while the addition of sulfate containing treatments increased the stalk production significantly more than any other nutrient combination. Nitrogen and silicate were shown to have no significant growth effects by themselves. Since the East River is known to be phosphorus limited, we conclude the D. geminata in this water system has few cells but more stalk and so can be considered a nuisance bloom condition that can adversely affect benthic habitat.

Introduction

D. geminata is a diatom, which is a type of single celled algae. Under nuisance bloom conditions, its cells produce copious amounts of extra cellular stalk material that form thick benthic mats (Spaulding and Elwell 2007). In recent years this diatom has produce stalks that are many times the length of the cell itself (see Figure 1). The stalks are excreted by the cell at one end. It is not the diatom cell alone that is responsible for the negative impacts of *D. geminata*, but the massive production of extra cellular stalk (Spaulding and Elwell 2007). This particular diatom is now emerging as an organism with the capacity to impact stream ecosystems on a global scale.

The relationship between nutrient concentrations in the water column and the rate at which nutrients are taken up by algae can be described by a hyperbolic function such as the Michaelis-Menton equation for enzyme kinetics. Nutrient uptake rates become saturated as nutrient concentration increases and this saturation can occur at relatively low concentrations for benthic diatom cells (Bothwell 1989). Saturating the water within a few centimeters of a nutrient diffusing substrate is an approach that can be used to promote algal growth on terra-cotta pots, providing a test of which nutrients may be

limiting algal growth and which might be responsible for causing excess stalk production in diatoms. Alternatively, if the algae are producing stalks in response to depleted nutrient concentrations, then treatments with low stalk production but with diatom cells present may also reveal the nutrient responsible for abnormal stalk production. The Monod model can also be reviewed because it relates algal growth to the external concentration of nutrients in the water (Hauer and Lamberti 2006).

Water chemistry is typically considered a controlling factor for diatom distribution and abundance, particularly nutrient concentrations and pH. One notable factor related to *Didymosphenia geminata* is its ecological tolerance to a broader physical and chemical range than other diatoms. *Didymo*'s distribution has expanded in recent years (Kawecka & Sanecki 2003, Kilroy 2004). The increased biomass and growth rate recently associated with *D. geminata* is thought to be associated with changes in nutrient concentrations in benthic areas where *D. geminata* is found.



Figure 1: Scanning electron micrograph of *D. geminata* cells and their mucopolysaccharide stalks. The stalks that are produced are many times the length of the cell itself. Image credit: Spaulding and Elwell 2007

Unlike stalks of many other diatoms (Hoagland et al. 1993), the stalks of *D. geminata* are large, carbon rich, and may contain secondary compounds that make them resistant to decay and, perhaps, herbivores (Spaulding and Elwell 2007). In the East River near the Rocky Mountain Biological Laboratory (RMBL) in western Colorado, the stalk, but not necessarily the cells, of *D. geminata* produced during the summer (June-August) may persist through the fall and winter until they are removed by high spring flows (Brad Taylor, *personal observation*). The degree to which internal (genetic) and external (environmental) change initiates the high level of stalk production is unknown, yet determining the mechanisms of stalk production is crucial for determining ecological impacts and control of the organism. However, in some rivers where *D. geminata* has been forming nuisance blooms in recent years, the stream ecosystems have been impacted by anthropogenic nutrient input. Nuisance algal blooms are typically directly related to external nutrient addition (Schindler 1977, Anderson et al. 2002).

Nutrients are often limiting resources in ecosystems and an environmental template of geological, climatic, and ecological features ultimately delimits their availability (Flecker et al. 2002). The goal of this study is to increase nutrients above ambient concentrations by an order of magnitude thereby alleviating limitation, in order to observe the differing effects of nutrient abundance on algal growth. Nutrient diffusing

substrata will be used to deliver nutrients to algae in situ and test whether *D. geminata* growth or stalk production is nutrient limited, and which nutrients are responsible for that limitation. Silicate is often a limiting nutrient for diatoms (seagrant.gso.uri.edu) and primary producers in the East River are in general P limited (Taylor et al. *in revision*). Sulfate is a micronutrient that is found in the stalk of *D. geminata* which has the potential to limit stalk growth (Gretz 2000).

The objective of this study is to evaluate the response of algal standing crop accrual as measured by chlorophyll *a* and ash-free dry mass (AFDM), to nutrient additions in the East River. Point source nutrient manipulations allow testing of response *in situ* without the artificiality of enclosures. In situ enrichments of substrates (clay flowerpots) provide a realistic test of algal nutrient limitation. Relative to the control treatment, particular nutrients or combinations thereof are hypothesized to change the abundance of either *D. geminata* cells or stalks.

Methods and Materials

Study System

This study was conducted near Gothic, Colorado in the East River, a stream with documented nuisance blooms of *D. geminata*. These blooms cover many areas of the stream bed and collect sediment so the alga appears brown. The organism has a texture similar to wet cotton and is not particularly slippery like many other algae growths. Although the river's current velocity and depth vary, only relatively similar sites were used for this experiment to standardize the conditions.

Nutrient-diffusing substrata methods

Nutrient-diffusing substrata (i.e, small terra-cotta pots) were placed closely within a twenty meter section of the stream with similar water velocity, depth, and canopy cover to control for other factors that can affect algal abundance. The clay pots provided a hard surface similar to an epilithic (rock) habitat. The pots were periodically checked every few days to ensure they remained upright, intact, and free of any debris. The terra-cotta pots were retrieved after a twenty day time period and sampled for chlorophyll *a* and cell counts, as well as visual estimates of *D. geminata* stalks.

Samples for chlorophyll analysis were completed by pipetting a known volume of algal slurry onto a small glass fiber filter and determined by a Turner AU-10 fluorometer. The samples were not ground due to the potential for sample loss. The samples were shaken to homogenize them for the chl-*a* and AFDM measurements. Cell counts and visual estimates were made in the laboratory using a compound microscope.

Materials and Supplies Needed

One hundred and eight terra-cotta pots were used as well as equipment to measure nitrate, silicate, sulfate, and phosphorus levels in the stream. Samples were taken but these samples will be analyzed at Dartmouth College in Hanover, New Hampshire. Multiple plexi-glass strips were used as bases on which I attached clay pots using silicon glue. Marine silicon adhesive was used, because it is non-toxic and does not contain fungicide. Chemicals used included potassium phosphate (KH_2PO_4), sodium nitrate (NaNO_3), sodium silicate pentahydrate ($\text{Na}_2\text{SiO}_3 \cdot 5\text{H}_2\text{O}$), and sodium sulfate (Na_2SO_4).

Laboratory Work

Terra-Cotta Pot Preparation

Terra-cotta flower pots were used for this experiment as a base for the algae to grow on. A 9.5 mm hole was drilled through the bottom of each of the 55 mL, 79 cm² pots with a masonry drill bit. Prior to the experiment, the flowerpots were soaked in deionized water for two days to diffuse out any residual chemicals or nutrients in the clay. The pots were dried, glued onto plexiglass strips, and marked according to the nutrient amended agar they contained.

Nutrient Amended Agar

Using a glass beaker a 2% agar solution was prepared by adding 10.2 g of agar to 0.51 L of deionized water. The agar was heated to 85-90°C and stirred using a stirring hotplate until thoroughly dissolved. The nutrient compounds were added to the dissolved agar mixture using the chart below as reference.

Target Solute	Compound	Proportion Target Solute: Salt	Volume of Solvent (L)	Concentration (M)	Salt Added to Agar (g)
N	NaNO ₃	0.144	1	0.25	21.25
N	NH ₄ Cl	0.663	1	0.25	132.25
P	KH ₂ PO ₄	0.228	1	0.5	68
Si	Na ₂ SiO ₃	0.212	1	0.5	61
SO ₄	Na ₂ SO ₄	0.225	1	0.5	71.89

Figure 2: Amount of solute (nutrient) added to create nutrient amended agar. Note that both nitrogen compounds must be added to the same agar batch to reach the desired Molarity. Combinations of target solutes may also be made from this table.

Four pots with approximately 3 cm between them were glued onto three separate strips of plexi-glass thereby constituting an experimental block (i.e., 12 treatments) that were placed near one another in the stream. The order of the pots on the plexiglass strips were randomized. The nutrient treatments used were nitrogen (N), phosphorus (P), silicate (Si), and sulfate (SO₄), as well as the following combinations: N+P, P+Si, P+SO₄, N+Si, N+SO₄, P+Si+N, and P+SO₄+N (12 treatments including the control with no nutrients added). Once the silicon dried, the agar mixtures were funneled into the appropriate pots. The pots absorbed some of the agar, but it was made sure that the pots were completely full because if the structure contained any air it may float. Finally, a small rubber stopper was placed over the opening on the top of the flowerpot.

Terra-Cotta clay pots and saucers contain large quantities of iron, calcium, and aluminum (which bind phosphorus) and they can irreversibly sorb large quantities of phosphorus (e.g. Brown et al. 2001). This could have been corrected by a standard curve created if the nutrient release rate was tested for these pots. However, this was not done because the amount of nutrients added to the agar amended flower pots was far over ambient levels and so was not considered relevant for this experiment. This is simply a pitfall of experimental design that can not be avoided within these methods but only noted.

Field Experiment

Installing Plexi-glass Strips

Eight sites were chosen along the East River in Gothic, Colorado. These sites were no more than three meters away from one another. The experimental blocks were positioned parallel to river flow to help prevent sedimentation. Rebar stakes helped give structural support to the set up so that the strip of plexi-glass stayed in place on the river bottom. Rocks were also placed on top of the strips in order to keep them securely on the river bottom even with a strong current. The order of the blocks were also randomized.

Experimental Maintenance

Every other day each set was checked to ensure that the pots did not tip over or damaged in any other way. If the pots had tipped over, they were turned upright again. If the pots were damaged beyond repair (such as ripping off the plexiglass strip or the agar coming out of the pot) they were taken out of the experimental data set. The nutrient levels in the pots were not recharged due to the short length of the experiment. An “extra” block was created to fill in any gaps in the data if any pots broke free of the plexiglass strips.

Algal Sampling and Analysis

Each experimental set was removed from the river after twenty days. Three measures were taken for each pot. Chlorophyll *a* tests were run from sub samples once each pot has been scrubbed of algae. A Turner Trilogy fluorometer was used for analysis. A sub sample was also kept for ash free dry mass (AFDM) where samples were burned in a muffle furnace at 500 °C for four hours. A small sub sample from randomly selected specimens were kept to examine more closely under a compound microscope. The number of cells and stalks were recorded through visual observation.

Statistical Analysis

An analysis of variance (ANOVA), with presence of N, P, Si, and/or SO₄ as the main factors, will be used to test whether *D. geminata* is significantly affected by nutrient enrichment (Dube et al. 1997). Chlorophyll *a*, Ash Free Dry Mass (AFDM), and cell/stalk counts were the measures of variance. Single nutrient limitation was indicated when just one of the nutrients elicits a positive response but it may be found that the growth of *D. geminata* is co-limited by a combination of the added nutrients.

Results

Through the measure of chlorophyll *a* we found significantly more algae on four treatments that included phosphorus (P, N + P, P + SO₄, P + SO₄ + N) that differed statistically from the control (Figure 1). No other nutrient combinations were shown to be statistically different from the control. Analysis included an ANOVA completed in JMP 5.1 with an additional HOC (Student's *t*) test. One standard error of the mean was included and nutrient combinations with the same letter above it (in Figure 1) were not considered statistically different from one another.

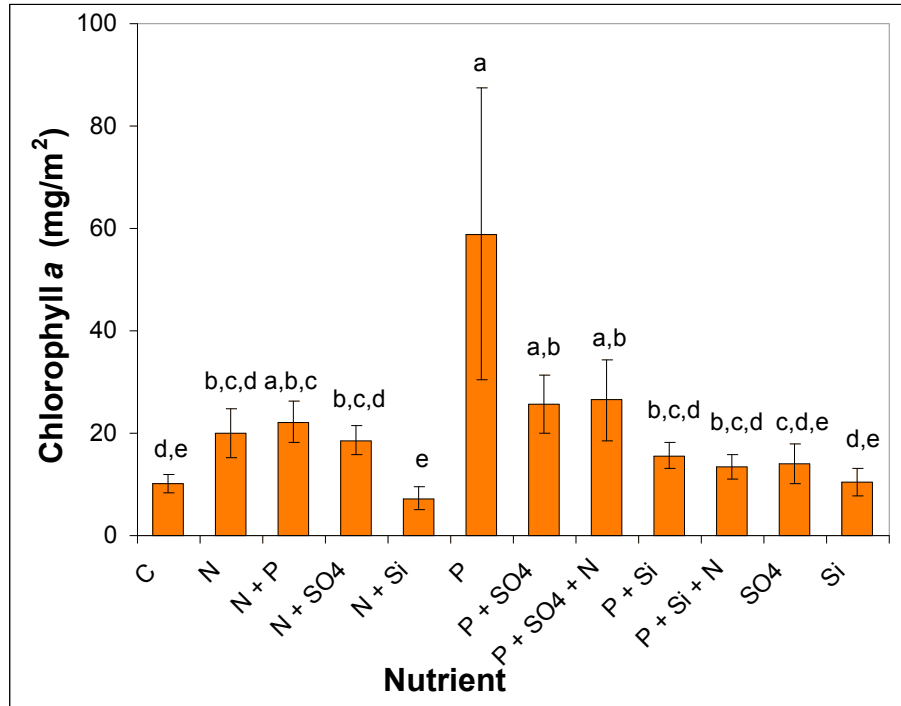


Figure 1: Chlorophyll a measurements for each nutrient combination. Statistical differences from the control was only seen in phosphorus containing combinations of nutrients.

To measure the organic mass that each sample contained we measured the ash free dry mass and found that sulfate containing nutrient combinations had a higher organic content than both the control and all other sample groupings (Figure 2). The organic matter may be either *D. geminata* stalk or other various carbon containing compounds. To tease out this difference, cell and stalk counts were taken from each sample where it was found that there were more *D. geminata* cells in phosphorus containing combinations while there were more stalks in the sulfate samples (Figure 3).

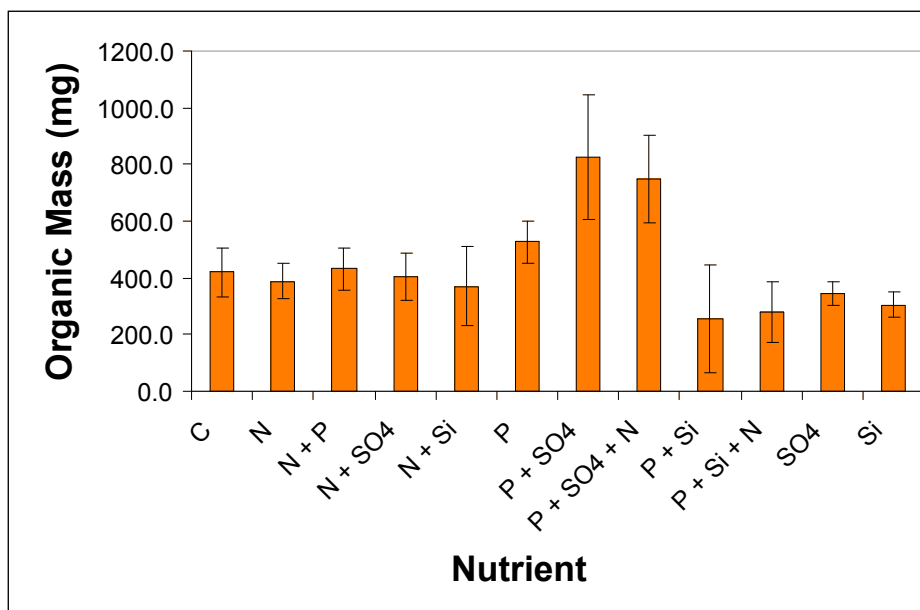


Figure 2: Ash free dry mass (AFDM) measures by nutrient addition. Sulfate containing combinations revealed higher organic mass per sample.

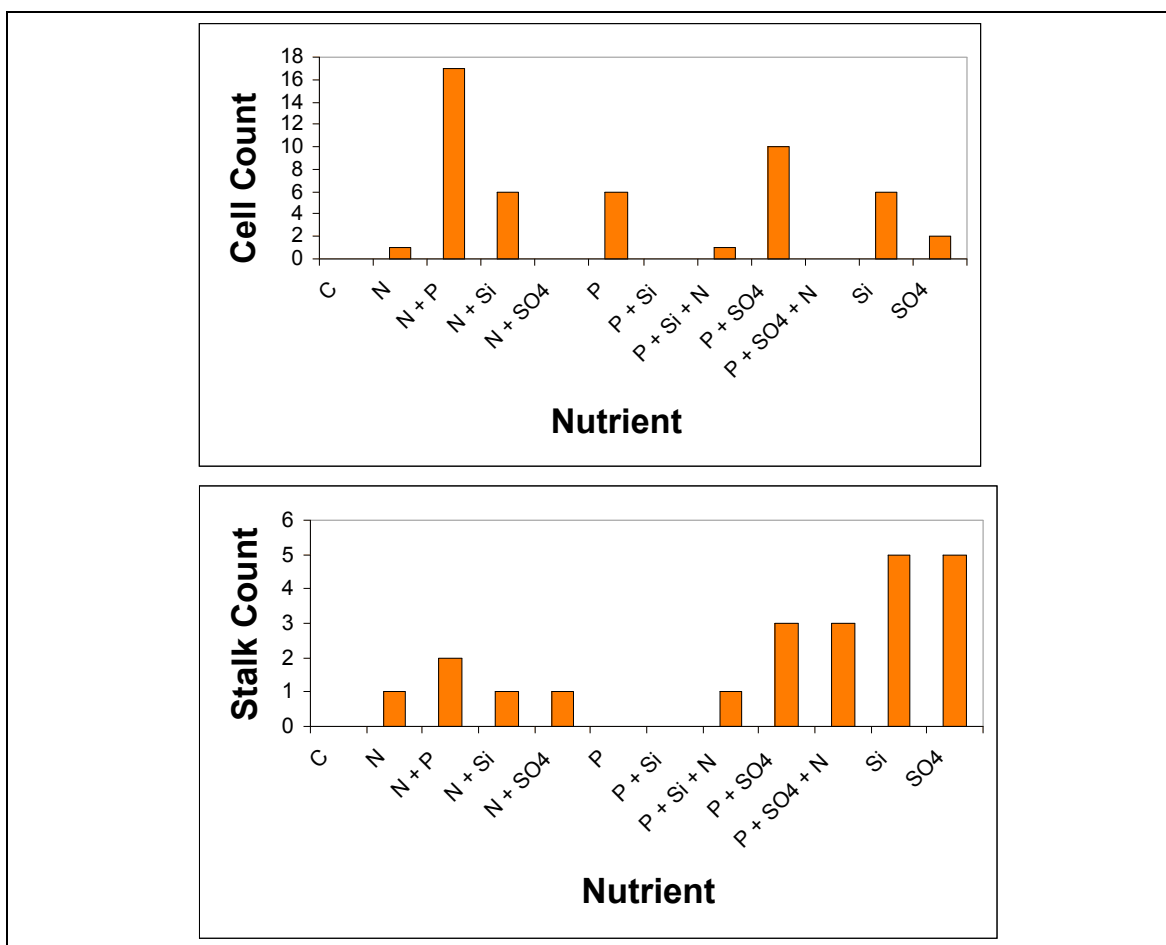


Figure 3: *D. geminata* cell and stalk counts by nutrient combinations.

Discussion

Chlorophyll *a* measurements showed that there was a significant increase in algal growth with the addition of phosphorus to the water column. Cell counts of individual samples confirmed that there were more *D. geminata* cells in particular in these samples compared to other nutrient combinations. Since our study system, the East River in Gothic, Colorado is phosphorus limited we concluded that there may not be many cells and the composition of *D. geminata* was mostly stalk. This was also confirmed with the cell count data as there were far fewer cells found in the control than in phosphorus containing nutrient combinations.

The measure of organic matter, through AFDM, confirmed that the presence of sulfate would increase the extra-cellular stalk growth of this diatom. This may be in part because *D. geminata* puts a large amount of sulfate into its stalk. Through manual stalk counts we found that there was a higher concentration of stalks for sulfate containing nutrient combinations. Neither nitrogen or silicate was found to be an important factor in either cell or stalk growth.

The experiment was short in duration (twenty days) because the question of limitation rather than regulation or control is being addressed. The experiment has been designed specifically to examine the relative differences in nutrient effects within artificial guidelines rather than provide absolute estimates for nutrient addition into the natural stream (Flecker et al. 2002).

The benthic nutrient levels in the East River Valley have been changing in the last twenty years due to the re-emergence of beavers and subsequently the ponds that they create. The beaver ponds sorb both phosphorus and sulfate to the sediment and trap these nutrients so that reaches downstream will be nutrient deprived. By creating nutrient diffusing substrate blocks both above and below beaver ponds we may be able to distinguish a difference in algal growth. Future research of this type will complement this study both by determining if the return of beavers have effected the water chemistry enough to change algal growth and more specifically if more nuisance bloom conditions of *D. geminata* appear below beaver ponds.

The ecological, economical, recreational, and aesthetic impacts of *Didymosphenia geminata* have garnered attention from diverse groups including scientists, policy makers, businesses, economists, anglers, and anyone who uses streams (Spaulding and Elwell 2007). This study addresses the increasing concern of the spread of *D. geminata* by providing an understanding of the nutrients that limit its abundance and stalk production. Determination of limiting nutrients of *D. geminata* will provide more information for the cause and/or control of the nuisance blooms. This and additional research will help to show the effects of stream water chemistry on nuisance blooms and will determine if the problem is stemming from anthropogenic or natural chemical changes in the streams and rivers in which this diatom thrives. Changes might be able to be made in and around streams that are affected or at high risk of *D. geminata* to help prevent the spread and blooms of this diatom.

Acknowledgements

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