

Primary Research Paper

## Resource requirements of four freshwater diatom taxa determined by *in situ* growth bioassays using natural populations from alpine lakes

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### Abstract

We performed a series of *in situ* batch culture experiments to assess the resource requirements of common diatom taxa in alpine lakes of the central Rocky Mountains of North America. While physiological data are available on the resource requirements of some of these taxa, it is unclear whether intraspecific generalizations can be made across aquatic systems due to the potential development of ecotypes. In these experiments, we used amended lake water for a culture medium and natural diatom populations. Growth kinetics were determined for *Asterionella formosa* Hassall, *Fragilaria crotonensis* Kitton, *Staurosirella pinnata* (Ehr.) Williams and Round and *Tetracyclus glans* (Ehr.) Mills. *Staurosirella pinnata*, a historically abundant alpine diatom, had very low N and P requirements. *Asterionella formosa* and *F. crotonensis*, generally considered meso- or eutrophic species, exhibited low P requirements if N and Si were in moderate supply. *Tetracyclus glans* had the highest Si requirement. These experiments reveal that the recent changes in diatom community structure in these alpine lakes may be driven by changes in nutrient supply. We suggest that local diatom taxa and a natural culturing medium should be used to obtain more representative algal physiological data from a particular area.

### Introduction

Diatom fossil records from several western alpine lakes of North America reveal shifts in community structure over the past century (Drake & Naiman, 2000; Wolfe et al., 2001; Saros et al., 2003). In some cases, these shifts occur simultaneously with the initiation of fish stocking (Drake & Naiman, 2000). However, in other systems, such as those in the Colorado Front Range and the Beartooth Mountains of Montana and Wyoming, this is not the case. In these lakes, the relative abundances of typical alpine diatom taxa, such as *Staurosirella pinnata*

(Ehr.) Williams and Round, have declined while those of taxa such as *Asterionella formosa* Hassall, *Fragilaria crotonensis* Kitton, and *Tetracyclus glans* (Ehr.) Mills have increased (Wolfe et al., 2001; Saros et al., 2003; Wolfe et al., 2003). Saros et al. (2005) demonstrated that nitrogen (N) enrichment via enhanced atmospheric N deposition is the primary causal factor behind the recent increases in both *A. formosa* and *F. crotonensis* in these two areas. However, the specific resource requirements of the common diatom taxa in these oligotrophic alpine systems are poorly understood. A refined ecophysiological database is clearly desirable for explicit

interpretations of the regional diatom paleoecological record.

Field studies in alpine areas, as well as laboratory experiments with temperate ecotypes of these diatom species, have provided some insight into N, phosphorus (P), and silica (Si) requirements. In lakes of the Greater Yellowstone Ecosystem, Interlandi et al. (1999) found through field observations that *F. crotonensis* is a high N:P and Si:P specialist requiring a relatively low-light environment. *Asterionella formosa*, although normally considered a meso- or eutrophic taxon, is also a high Si:P specialist (Tilman et al., 1982), but its N requirements remain unknown. Physiological data for taxa, such as *T. glans* and *S. pinnata*, in alpine lakes are sparse.

The physiological requirements of an algal taxon may be more accurately assessed when individuals are acquired from the area of interest, as diatoms can develop several distinct ecotypes over time and hence a particular species could have different resource requirements depending upon its system of origin (Guillard & Kilham, 1977; Lewis et al., 1997). Minimizing the length of time in culture may also improve the accuracy of physiological data because when diatoms are in culture for extended periods of time and sexual reproduction is not induced, cells can fall below the size of sexual induction and still survive, unlike under natural conditions (Round, 1993). As growth rate is a size-dependent process (Reynolds, 1984), this can also affect the growth kinetics of a taxon. In addition, the resource requirements measured for a given diatom taxon collected from a certain area may differ depending upon experimental conditions, such as the type of medium as well as incubation conditions, both of which should mimic natural conditions as closely as possible.

In this study, we developed a series of *in situ* bioassays to explore the resource requirements of diatom taxa found in lakes of the central Rocky Mountains. These batch culture experiments were incubated in a lake located in the Absaroka–Beartooth Mountain region, with experiments conducted in July of both 2001 and 2002. Culture organisms were isolated from lakes in the area and lake water from systems with very low nutrient concentrations served as the culture medium.

## Methods

The Beartooth Mountain Range comprises much of the Absaroka–Beartooth Wilderness and is located on the southwest Montana–Wyoming border, directly adjacent to the northeast entrance of Yellowstone National Park. There are over 600 permanent alpine lakes in this region whose watersheds are highly sensitive to atmospherically deposited N owing to sparse vegetation, shallow soils, and large expanses of unbuffered, crystalline bedrock. Lakes in this region are highly dilute, with generally low Si, N and P concentrations (Saros et al., 2003).

Batch culture experiments were developed to explore the N, P, and Si requirements of four diatom taxa. For these bioassays, lake water collected from the Beartooth Mountain region, rather than artificial medium, was used to closely imitate the natural chemical environment in which these diatom assemblages grow. Lake water with very low initial concentrations of Si, N, and P was used as the base for this medium. Nutrient concentrations were determined prior to use of water in the experiment (APHA, 1995). Phosphorus was analyzed as soluble reactive phosphorus (SRP) by the ascorbic acid method. Nitrate + nitrite was measured by the cadmium reduction method, and Si was measured by the molybdosilicate method. The experimental water was passed through a 0.2  $\mu\text{m}$  membrane filter (Criti Cap<sup>®</sup>, Pall Corp., East Hills, NY) to remove all phytoplankton, zooplankton, and most bacteria before use in the experiments.

Water for the 2001 bioassays was collected at a depth of 8 m from Emerald Lake (Park Co., WY) because it had very low initial concentrations of Si, P, and N (Table 1). Concentrations of trace elements were not determined as part of this study. In 2002, nutrient concentrations were higher in Emerald Lake due to late ice-off, hence the most suitable water for the 2002 field bioassays was found at a depth of 2 m in Beartooth Lake (Park Co., WY). This water had high initial concentrations of Si and N and was diluted to lower the levels of these two nutrients. A dilution factor of 3:1 of distilled:Beartooth water was used for water in the Si series and of 2:1 of deionized:Beartooth water for the N and P series. Different diluents were used because of the limited availability of

Table 1. Conductivity, pH, and initial concentrations of Si, P, and N in experimental water used in the bioassays for 2001 and 2002

Lake	Dilution	Year	Conductivity ( $\mu\text{S cm}^{-1}$ )	pH	Si ( $\mu\text{M}$ )	SRP ( $\mu\text{M}$ )	$\text{NO}_3^-$ ( $\mu\text{M}$ )
Emerald	None	2001	11.0	6.00	$\sim 5.00$	$\sim 0.00$	$\sim 0.00$
Beartooth	2:1	2002	8.0	7.00	–	$\sim 0.00$	$\sim 0.10$
	3:1	2002	6.0	6.80	$\sim 4.00$	–	–

Dilution factors for 2002 experiments were 2:1 (deionized  $\text{H}_2\text{O}$ :Beartooth  $\text{H}_2\text{O}$ ) and 3:1 (distilled  $\text{H}_2\text{O}$ :Beartooth  $\text{H}_2\text{O}$ ). Detection limits for SRP and  $\text{NO}_3^-$  were 0.02 and 0.01  $\mu\text{M}$ , respectively. (–) denotes data not reported because nutrient was not tested as a limiting nutrient in that water.

deionized water; the distilled water was acceptable for the Si series because it contained undetectable amounts of Si but a small amount of N. This small amount of N was not an issue for the Si series, as both N and P were added to excess in these treatments (see below). In addition, a greater dilution was used in the Si series due to a relatively high initial concentration of this nutrient.

Silica, N, and P were all tested as limiting nutrients in these experiments (Table 2). To ensure that the changes in growth rates in each treatment were a result of the limiting nutrient and not a consequence of other resources being depleted, all nutrients were added to excess except the limiting one (Si = 150  $\mu\text{M}$ , P = 5  $\mu\text{M}$ , N = 18  $\mu\text{M}$ ). Silica was added in the form of  $\text{Na}_2\text{SiO}_3$ , N as  $\text{NaNO}_3$ , and P as  $\text{NaH}_2\text{PO}_4$ . Nutrient-amended water was added to untreated 300-ml cell-culturing flasks. Spectrophotometric analyses of the flask wall revealed that they were transparent to 88% of 380–700 nm (photosynthetically active radiation, PAR) and 50% of 320 nm (UV-B).

In addition to varying nutrient concentrations, light intensities were also manipulated by placing zero, one, or two layers of fine mesh screen on top of the culture flasks producing high-, moderate- or

low-light intensities, respectively (Table 2). Because light intensity can affect nitrate use by phytoplankton (Rhee & Gotham, 1981), light intensity was manipulated in the nitrogen treatments. Each bioassay treatment and blank were run in quadruplicate, resulting in  $26 \times 4 = 104$  total samples per experiment.

An inoculum of common diatom taxa from the area was created by sampling the phytoplankton of several lakes. Diatoms were collected with an 80- $\mu\text{m}$  mesh phytoplankton net as well as from surface sediment samples, which were a good source of tychoplanktonic species. Samples were microscopically examined to determine species composition and density. Diatom taxa selected for this study were species typical of alpine regions whose relative abundances have recently declined (e.g., *S. pinnata*) as well as species whose relative abundances have started to increase over the past few decades (e.g., *A. formosa*, *F. crotonensis*, and *T. glans*). *Tetracyclus glans* is likely synonymous with *T. lacustris* Ralfs (Patrick & Reimer, 1966).

A mixture of species from the collected algal samples was added to an aliquot of unamended

Table 2. Experimental design of nutrient bioassays, with each limiting nutrient tested in the series of concentrations indicated

Limiting nutrient	Series of nutrient additions ( $\mu\text{M}$ )					Nutrients added to excess
Si (unshaded)	5	10	15	30	150	P & N
P (unshaded)	0	0.05	0.10	0.25	5.0	N & Si
N						
Low-light	0.05	0.10	1.0	5.0	18.0	P & Si
Moderate-light	0.05	0.10	1.0	5.0	18.0	P & Si
High-light (unshaded)	0.05	0.10	1.0	5.0	18.0	P & Si

experimental water to create the inoculum, which had about 6000 ind. ml<sup>-1</sup> of each species. The addition of 1.0 ml of this inoculum to each 300-ml flask resulted in an initial cell density of ~20 cells ml<sup>-1</sup> of each species.

Bioassays were incubated in Beauty Lake (Park Co., WY), which is situated at 2857 m above sea level. Beauty Lake has a maximum depth of 50 m, an average depth of approximately 28 m, and a surface area of 0.36 km<sup>2</sup>. The 1% attenuation depths for 320, 380 nm, and PAR were 2.32, 6.16, and 22.8 m, respectively, as measured with a PUV 501B profiling ultraviolet radiometer (Biospherical Instruments Inc., San Diego, California, USA). The culture flasks were divided by light treatment and randomly placed on four 60 × 70 cm racks that were constructed from 2.54 cm PVC pipe. Flasks and any screen material were placed between two pieces of 3.2 mm Lucite<sup>®</sup> plastic sheeting, which were secured to the PVC frame. The racks were anchored from each corner and suspended at approximately 3 m beneath the lake surface by a 41 cm, round Styrofoam buoy. Accounting for the light that was absorbed by the plexiglass layer and culture flask, mid-day exposures in the high-, moderate-, and low-light intensity flasks were approximately 1170, 878, and 527 μeinsteins m<sup>-2</sup> s<sup>-1</sup>, respectively, of PAR.

The flasks were incubated for 6–7 days at ~12 °C (average epilimnetic temperature, which varied by ±3 °C), on a 15:9 light–dark cycle. To determine if nutrients were depleted over the course of the incubation, 125 ml of water was removed from each flask at the end of the experiment and filtered through a 0.4-μm polycarbonate filter for nutrient analysis, by the methods described above. The rest of the sample was preserved with Lugol's iodine for subsequent identification and enumeration of each taxon.

The density of diatoms in each flask was determined in triplicate by settling 50-ml aliquots of water in an Utermöhl-style chamber and examining through a Nikon TS–100 inverted microscope at 400× magnification. Empty diatom frustules were not included in the counts. The average density of each diatom species was determined in every treatment flask by averaging the triplicate counts. Growth rates (μ) were then calculated for each taxon with the following equation:

$$\mu = \frac{\ln(F) - \ln(I)}{T} \quad (1)$$

where  $F$  is the final cell concentration (cells ml<sup>-1</sup>) in the flask,  $I$  is the initial cell concentration (cells ml<sup>-1</sup>) (i.e., concentration in the inoculum), and  $T$  is the incubation time ( $d$ ). Using the nonlinear fitting component of JMP statistical software V5.0 (SAS Institute Inc., Cary, NC, USA), Monod growth curves for the bioassays were constructed by plotting nutrient concentration (μM) vs. growth rate ( $d^{-1}$ ) from each flask, and maximum growth rates ( $\mu_{\max}$ ) and half-saturation constants ( $K_s$ ) were calculated based on the relationship:

$$\mu = \mu_{\max} \frac{K_s}{S + K_s} \quad (2)$$

where  $S$  is the concentration of limiting nutrient.  $K_s$  represents the nutrient concentration at which an organism reaches one-half the maximum growth rate, with a lower  $K_s$  value indicative of a lower requirement for the nutrient and theoretically a better competitive ability for that resource. The initial slope of the growth curve ( $\mu_{\max}:K_s$ ) was also determined; it summarizes the relative competitive ability of a species for a resource, with higher ratios indicative of better competitive abilities (Healy, 1980).

## Results

Monod growth curves from the *in situ* batch culture experiments were constructed for *A. formosa*, *F. crotonensis*, *S. pinnata*, and *T. glans* (Fig. 1) and growth kinetic parameters were calculated for each nutrient (Table 3). Nutrient analyses conducted on water collected from each sample at the end of the experiment indicated that nutrients were never depleted by more than 15%. Due to high levels of variation in cell growth in certain treatments, growth curves could not be constructed for each of the four species for each limiting nutrient (Table 3). The standard errors in these experiments for  $\mu_{\max}$  were relatively low; however, the errors associated with the  $K_s$  values were much larger because of the limited data points at lower nutrient levels. As pointed

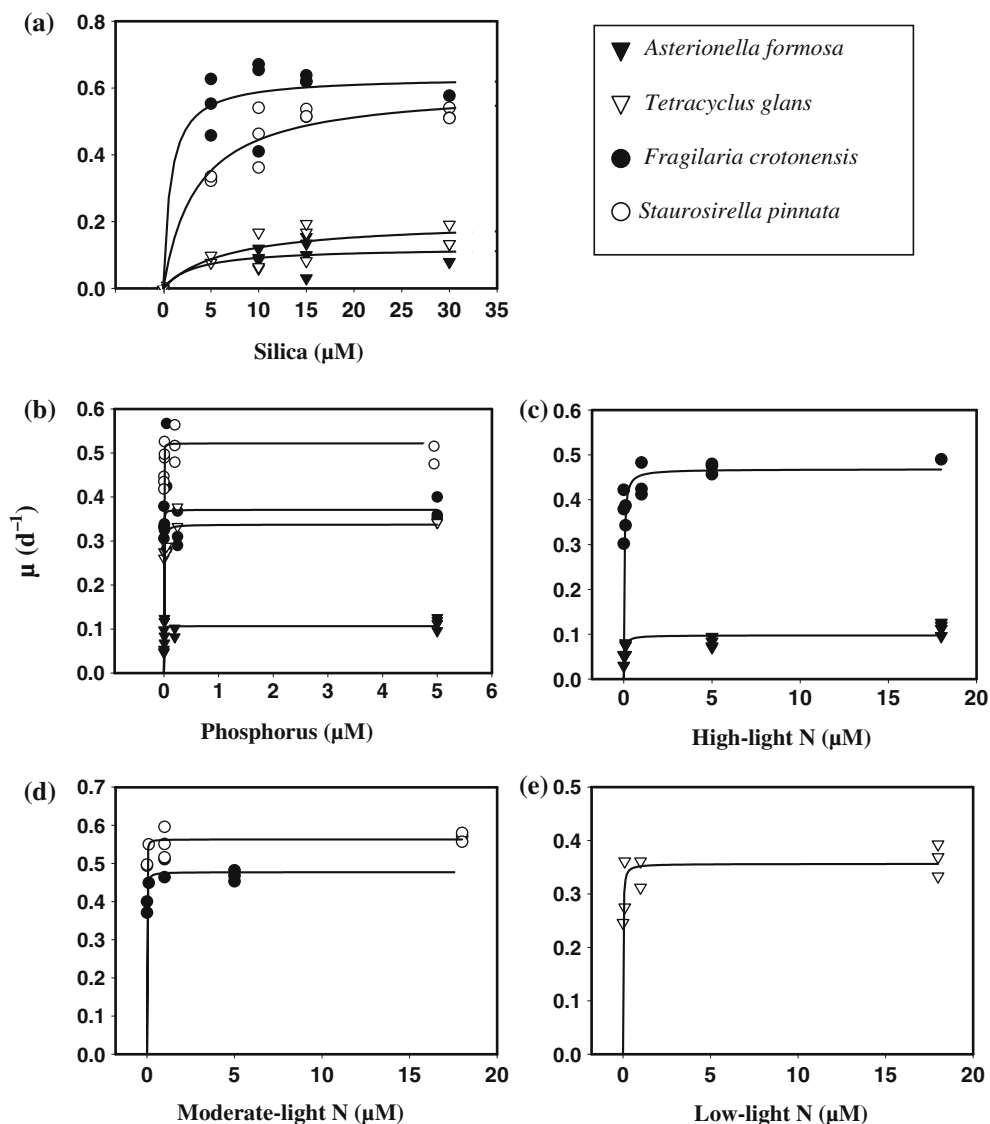


Figure 1. Monod growth curves for *in situ* batch culturing experiments with growth rates ( $d^{-1}$ ) plotted against the following treatments: (a) Si; (b) P; (c) high-light N; (d) moderate-light N; (e) low-light N. Note that in several cases, replicates had identical values and are thus plotted on top of each other.

out by Grover (1989), most measurements of  $K_s$  values have wide confidence intervals due to this problem.

Using  $K_s$  values as an indication of nutrient requirements, *F. crotonensis* has the lowest Si requirement ( $0.78 \mu M$ ). *Staurosirella pinnata* and *A. formosa* have similar Si requirements with  $K_s$  values exceeding  $3 \mu M$ . *Tetracyclus glans* exhibits the highest Si requirement, with a  $K_s$  of  $6.5 \mu M$ . All four species in these experiments have very low P requirements. *Staurosirella pinnata*, *F. crotonensis*

and *A. formosa* all have  $K_s$  values  $\leq 0.0009 \mu M$ . *Tetracyclus glans* has the highest P requirements ( $0.003 \mu M$ ) of the taxa studied in these experiments.

Growth curves were constructed for *F. crotonensis* and *A. formosa* for N at the high-light intensity. *Fragilaria crotonensis* has comparable N requirements ( $0.028 \mu M$ ) to *A. formosa* ( $0.041 \mu M$ ) at this light level. At the moderate light intensity, *S. pinnata* and *F. crotonensis* had similar  $K_s$  values ( $\leq 0.006 \mu M$ ). At the low-light intensity, N growth

Table 3. Growth kinetics measured in the field experiments under each nutrient condition

Nutrient	Species	$\mu_{\max}$ ( $d^{-1}$ )	$K_s$ ( $\mu\text{M}$ )	$\mu_{\max}:K_s$
Si	<i>S. pinnata</i>	0.62 (0.04)	3.82 (1.089)	0.16
	<i>F. crotonensis</i>	0.63 (0.07)	0.779 (0.973)	0.81
	<i>A. formosa</i>	0.12 (0.04)	3.35 (3.380)	0.04
	<i>T. glans</i>	0.20 (0.06)	6.51 (6.259)	0.03
P	<i>S. pinnata</i>	0.52 (0.17)	0.0003 (0.005)	1736.70
	<i>F. crotonensis</i>	0.37 (0.004)	0.0008 (0.065)	463.80
	<i>A. formosa</i>	0.11 (0.02)	0.0009 (0.003)	118.90
	<i>T. glans</i>	0.34 (0.07)	0.003 (0.008)	112.30
N (HL)	<i>F. crotonensis</i>	0.46 (0.10)	0.028 (0.063)	16.50
	<i>A. formosa</i>	0.10 (0.01)	0.041 (0.038)	2.40
N (ML)	<i>S. pinnata</i>	0.56 (0.12)	0.003 (0.056)	187.70
	<i>F. crotonensis</i>	0.43 (0.11)	0.006 (0.054)	71.0
N (LL)	<i>T. glans</i>	0.36 (0.05)	0.012 (0.032)	29.70

Standard errors for  $\mu_{\max}$  and  $K_s$  are given in parentheses. Light levels in the nitrogen series are denoted by high (HL), moderate (ML), and low (LL).

kinetics could only be determined for *T. glans*; its  $K_s$  value (0.012  $\mu\text{M}$ ) is comparable to those obtained for *S. pinnata* and *F. crotonensis* at the moderate light intensity.

The  $\mu_{\max}:K_s$  ratio for each of the species followed the same trends as the  $K_s$  values. The taxa that exhibited the lowest  $K_s$  values have the greatest  $\mu_{\max}:K_s$  ratio. Therefore, they are predicted to be better competitors for that nutrient. However, this was not the case with *A. formosa*. Although this diatom often demonstrated low resource requirements, it also had the lowest  $\mu_{\max}$  values, which lowered its  $\mu_{\max}:K_s$  ratios and its predicted competitive ability.

## Discussion

The physiological data obtained in this study may allow us to better understand the resource requirements of diatom taxa found in lakes of the central Rocky Mountains. Before considering the N data, it should be noted that algae in the high- and moderate-light treatments may have experienced photoinhibition. According to Kirk (1994), the relative photosynthetic rate decreases at light intensities greater than approximately 500  $\mu\text{Einsteins m}^{-2} \text{s}^{-1}$ . Although these high-light intensities may have slightly inhibited phytoplankton growth, the natural light environment of alpine lakes is very intense in summer and is sig-

nificantly greater than that of most other aquatic systems. Therefore, the diatom communities that are found in high-elevation lakes frequently experience these light intensities, and are adapted to them in both planktonic and benthic habitats. In addition, diatoms in the lakes of the Beartooth Mountains appear to be severely nitrogen limited as demonstrated by Saros et al. (2005). This, coupled with the stress imposed by the light intensity, could account for some of the high variability in data at low nitrogen concentrations. While *A. formosa* appeared healthy in the inoculum, it had relatively low maximum growth rates in all nutrient series (Fig. 1). There may have been an additional factor limiting its growth in these experiments, but both temperature and conductivity were at levels at which this species grows well (Saros et al., 2005).

*Phytoplankton resource requirements.* Little work has been done on the nutrient requirements of small benthic colonial taxa such as *S. pinnata*, though they are generally considered good competitors for nutrients based on their high surface area-to-volume ratios (Reynolds, 1984). *Staurosirella pinnata* is considered tychoplanktonic; however, it was found primarily in the epilimnion during this study. Small colonial *Staurosirella* and *Staurosira* spp. are typically found in alpine or other oligotrophic systems (Douglas et al., 1994, Laing et al., 1994;) and, according to paleolimnological records, have declined in relative abun-

dance in lakes of the Beartooth Mountains in recent decades (Saros et al., 2003).

Our results indicate that populations of *S. pinnata* in Beartooth Mountain lakes have very low nutrient requirements. Consistent with its widespread distribution in oligotrophic lakes, this taxon had very low  $K_s$  values for both N and P and is therefore predicted to be a very good competitor for these nutrients. In addition to its low N and P requirements, *S. pinnata* had moderate Si requirements comparable to those determined for *A. formosa*. These data suggest that if concentrations of N or P were to increase, this taxon could remain abundant. However, species with greater  $K_s$  values, particularly within the phytoplankton, could also flourish under nutrient enrichment and reduce light availability to this taxon.

*Tetracyclus glans* is a large, benthic diatom with a restricted biogeographic distribution. It rarely comprises more than 5% of sedimentary diatom assemblages; however, it has recently become more abundant in several lakes of the Beartooth Mountains, reaching 20% in surface sediment assemblages (Saros et al., 2003). Beyond an association with oligotrophic systems, little, if any, information on the physiology of this diatom is available. The bioassays reveal that this taxon has higher Si and P requirements than the other three taxa. Relative to *S. pinnata* and *F. crotonensis*, this diatom has moderate N requirements; however, the light intensities to which these taxa were exposed were not the same. Thus, given this taxon's moderate to high nutrient requirements, *T. glans* is likely to respond to any nutrient addition to these systems. Because *T. glans* is benthic, interactions with sediment biogeochemical processes may allow it to acquire nutrients that are inaccessible to phytoplankton, possibly enabling it to thrive in environments where its nutrient requirements are not met within the water column.

Due to their frequent co-occurrence, *F. crotonensis* and *A. formosa* are commonly mentioned together in the literature. In contrast to *S. pinnata*, these two species have increased in relative abundance in lakes of the Colorado Front Range and Beartooth Mountains (Wolfe et al., 2001, 2003; Saros et al., 2003). Although *F. crotonensis* and *A. formosa* are typically referred to as meso- or eutrophic taxa in the literature, this characterization provides little information on which

nutrients specifically stimulate their growth. In a study of growth kinetics of axenic clones of *F. crotonensis* and *A. formosa* from Lake Michigan, Tilman (1981) showed that both of these taxa had low P and moderate Si requirements. Although *A. formosa* has been previously stimulated by N additions (McKnight et al., 1990) and *F. crotonensis* has been described as a poor N competitor (Interlandi & Kilham, 1998), the specific N requirements of these taxa were hitherto unknown.

With respect to P, *F. crotonensis* has very low requirements for this nutrient and would be considered a very good P competitor, as suggested by Tilman (1981). In fact, the specific P requirements measured in the field bioassays were lower than those observed by Tilman (1981) (Table 4). Additionally, the Si requirement of *F. crotonensis* was also lower than reported by Tilman (1981), but the confidence intervals of these values overlapped, possibly suggesting that the *F. crotonensis* ecotype in alpine lakes may have been better adapted to low concentrations of Si and P than the clone isolated from the Great Lakes. Results of the N bioassays reveal that *F. crotonensis* appears to have moderate N requirements. As *F. crotonensis* was often collected throughout the epilimnion and upper metalimnion of sampled lakes, we infer that the  $K_s$  values obtained from the unshaded bioassays may be most representative of this taxon's natural N requirements, and infer that this diatom has low P and moderate N requirements.

As mentioned above, *F. crotonensis* and *A. formosa* commonly co-occur. From our experiments, many of the same suggestions about the nutrient requirements of *F. crotonensis* can be made for *A. formosa*. Although *A. formosa* exhibits a higher Si requirement, the calculated  $K_s$

Table 4. Comparison of  $K_s$  values for *F. crotonensis* and *A. formosa* from Tilman (1981) and the results of the field batch culture experiments reported here

Taxa	Nutrient	Tilman (1981) $K_s$ ( $\mu$ M)	This study $K_s$ ( $\mu$ M)
<i>F. crotonensis</i>			
	Silica	1.5	0.78
	Phosphorus	0.011	0.0008
<i>A. formosa</i>			
	Silica	2.2	3.4
	Phosphorus	0.006	0.0009

values for P for these two taxa were almost identical. Alpine *A. formosa* populations have lower P and higher Si requirements than those reported for temperate-lake populations by Tilman (1981). The N requirements of this taxon may be slightly higher than the other taxa tested; however, these are still considered moderate requirements when compared to taxa found in temperate, more eutrophic waters (Grover et al., 1999). Therefore, *A. formosa* appears to be a good competitor for P when Si and N are in moderate to high supply.

**Implications.** The resource physiology data presented here support the results of Saros et al. (2005), in which the growth of *A. formosa* and *F. crotonensis* were only stimulated by N additions, not P or Si. All four taxa investigated in this study have relatively low P requirements; therefore, the recent shifts in diatom community structure are not likely the result of increased P concentrations. With respect to Si,  $K_s$  values for *S. pinnata* and *A. formosa* are very similar, but *F. crotonensis* has a lower requirement. Increased Si inputs, driven by climate change and increased weathering rates, would cause an increase in all three taxa. However, only *F. crotonensis* and *A. formosa* have increased in abundance (Saros et al., 2003). Ambient Si levels could have been initially sufficient for *A. formosa*, *F. crotonensis*, and *S. pinnata* to thrive in these systems. Thus, increased Si loading does not appear to explain the recent community shifts in these three taxa. In contrast, enhanced Si loading is compatible with the increases in *T. glans* populations in recent decades. The Beartooth Mountains have experienced an increase in total annual precipitation over the last century (Western Regional Climate Center, 2001) that may have accelerated weathering rates and the amount of Si transported to surrounding aquatic systems. *Tetracyclus glans* has a relatively high Si requirement, implying that an increased Si supply would increase the relative abundance of this heavily silicified diatom.

Although there are differences in the resource requirements of *S. pinnata*, *A. formosa*, *F. crotonensis*, and *T. glans*, the differences in their  $K_s$  values for each nutrient are very small, suggesting that even subtle changes in nutrient dynamics in alpine lakes may restructure diatom communities. Slight increases in the amount of N deposited to these systems are likely sufficient to allow taxa such as *A.*

*formosa* and *F. crotonensis* to become more abundant. Furthermore, increased precipitation may concurrently be raising N and Si concentrations, as well as altering Si:P, Si:N, and N:P ratios, thus accounting collectively for the recent increases in *A. formosa*, *F. crotonensis*, and *T. glans*.

These experiments also suggest that generalizations cannot be made about the resource physiologies of individual diatom taxa across their distributional ranges, given that intraspecific nutrient requirements appear variable among populations of different origin. Laboratory studies that measure algal growth to a single limiting nutrient under otherwise favorable conditions (e.g., cells grown in a general culture medium within a culture box) likely overestimate growth in environments with non-optimal conditions (Grover et al., 1999). In addition, while *A. formosa* is an indicator of P enrichment in temperate lakes (Reavie & Smol, 2001; Ramstack et al., 2003), Saros et al. (2005) found that the growth of *A. formosa* populations was not stimulated by P additions, but only by N additions, in lakes of the Beartooth Mountains that were co-limited by N and P. For these reasons, the physiological requirements of algae are likely to be more accurately assessed when specimens acquired from the area of interest are used and cultured in a medium that closely mimics natural conditions.

## Conclusions

Considering the Si, P, and N requirements of four dominant diatoms in lakes of the Beartooth Mountains, it appears that all have relatively low nutrient requirements. Therefore, even slight changes in one or more of these nutrients may have profound effects on the structure of algal communities in alpine lakes. The results from this study suggest that the intraspecific resource requirements of diatom taxa can vary among aquatic systems, and that the use of natural diatom populations from the study area of interest may provide more realistic representations of resource requirements than the use of organisms in culture for extended periods of time. Future studies utilizing this approach may expand our understanding of how natural communities respond to anthropogenic disturbances.



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