

LIMITING RESOURCES AND THE REGULATION OF DIVERSITY IN PHYTOPLANKTON COMMUNITIES

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Abstract. Species diversity is a key concept in ecology, yet the mechanisms regulating diversity in most systems are not completely understood. To address this issue, we analyzed the relationship between phytoplankton diversity and limiting resources (N, P, Si, and light) over two summers in three lakes in the Yellowstone (Wyoming, USA) region. Diversity was highly variable along temporal and spatial axes within lakes. We discovered a strong positive correlation between diversity and the number of resources at physiologically limiting levels. Consistent with resource-competition theory, we found the highest diversity to occur when many resources were limiting. Conversely, the lowest diversity occurred when few resources were measured at limiting levels. Sensitivity analyses demonstrated that threshold levels of resources (below which growth is limited and above which there is saturation for the resource) appear to exist in the natural environment, and that diversity is regulated in part by absolute levels of resources available to phytoplankton in aquatic systems. Threshold levels are generally in agreement with those determined to be limiting in prior physiological experiments. Consistent with previous observations, among-lake comparisons yielded a significant negative relationship between species diversity and system productivity. This result tentatively supports the hypothesis that the proximity of individual phytoplankton plays a role in the strength of competitive interactions. Our results generally suggest that, even in dynamic environments, where equilibrium conditions are rare, resource competition among phytoplankton is a mechanism by which communities are continually structured.

Key words: *community structure; competition regulating community structure; diversity and productivity comparisons; limiting resources; phytoplankton; resource competition; species diversity vs. number of limiting resources; threshold resource levels; Yellowstone region (Wyoming, USA).*

INTRODUCTION

Biodiversity, or the variability of life within biological communities, has been the focus of much research of late, as investigators have begun to realize the importance of this feature in the regulation of ecosystem functioning (Tilman and Downing 1994, Chapin et al. 1997, Yacchi and Loreau 1999). Research has been spurred not only by the clear strength of this relationship, but also by the keen interest of the general public, which understands this issue to be of importance to the well-being of our own species. Interestingly, the most prominent biodiversity research has been that which has sought to discern how community diversity might regulate other ecological properties, like productivity or stability (Tilman and Downing 1994, Drobner et al. 1998, Yacchi and Loreau 1999). This work suggests, and may be guided by, the premise that increased biodiversity is always a benefit to the ecosystem. It has convincingly been argued that high biodiversity allows for quick recovery from disturbance as a result of genetic and phenotypic variation within the community. Other research, which has sought to understand the opposing question, or “What regulates diversity in

communities?” has been extensive (Connell 1978, Sommer 1984, 1993, 1995, Reynolds et al. 1993) but has not been subject recently to the same synthetic treatment. It may very well be that diversity is itself regulated by inherent ecosystem properties that are not necessarily either beneficial or harmful to ecological communities.

Resource-competition theory (Tilman 1982) is a major paradigm for both aquatic and terrestrial ecologists, although the relative importance of resource competition in regulating the structure of ecological communities is a perpetual matter of debate (Reynolds 1997). Despite this controversy, the theory is useful because it makes distinct predictions about community structure, which can be tested in a number of ways (Tilman et al. 1982, Carney et al. 1988, Interlandi et al. 1999). This theory loosely predicts that diversity should be directly proportional to the number of resources at limiting levels within a system. Species richness is directly predicted by the theory, and should exactly equal the number of limiting resources when equilibrium conditions are met. Guided by this theoretical framework, we have attempted to ascertain the connection between species diversity and the number of limiting resources in a set of aquatic systems in the Greater Yellowstone ecosystem, which have been characterized previously (Benson 1961, Kilham et al. 1996,

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Theriot et al. 1997, Interlandi et al. 1999). Populations of individual species of phytoplankton in these lakes appear to occupy separate niches in resource space (Interlandi et al. 1999), so it seemed logical to use this system as a model to look for community-level responses to resource availability via an index of diversity.

Hutchinson (1961) noted that species richness and diversity of phytoplankton communities are often greater than the number of measured limiting resources even when conditions are apparently close to equilibrium. This paradox has been evaluated both theoretically and experimentally (Reynolds 1984, Grover 1990, Sommer 1993, 1995, Siegal 1998). Periodic disturbance has often been shown in both laboratory and field assessments to increase diversity and species richness in phytoplankton communities. According to models considering small-scale transport processes, competitive exclusion theoretically can be delayed indefinitely in systems where phytoplankton abundance is low and interactions between individual phytoplankton cells or colonies are weak (Siegal 1998).

Considering all of these factors, we attempted to see if limiting resources are able to regulate diversity even under dynamic conditions where one might expect disturbance and other forces to overwhelm the limiting factor–diversity signal. First, we assessed the spatial and temporal dynamics of species diversity in three large lakes in the Yellowstone region (Wyoming, USA). Then, we evaluated the empirical data to see if they supported the fluid-transport process model (Siegal 1998) by assessing the relationship between diversity and biomass in phytoplankton communities. Finally, we assessed the relationship between number of limiting resources (N, P, Si, and light are all potentially limiting in the systems) and diversity.

Other resources have been found to limit phytoplankton growth in both natural environments and laboratory culture. These include trace elements such as iron and copper and vitamins like thiamine and B₁₂. While we do not suspect that limitation by such resources is an important factor for phytoplankton in these systems, we lack data to validate this assumption. Therefore, it is possible that the measured number of limiting resources is at times less than the actual number of limiting resources in a given sample.

Our current understanding of the disturbance regimes in these lakes is inadequate for proper evaluation, so we were not able to include a quantitative assessment of the effects of periodic disturbance on the phytoplankton communities studied here. Excellent assessments of this mechanism have been presented by other investigators (Grover 1990, Sommer 1993, 1995).

METHODS

Field collection and sample processing

We collected samples for phytoplankton and nutrient chemistry from Lewis and Yellowstone Lakes in Yel-

lowstone National Park and from Jackson Lake in Grand Teton National Park. The general limnology of these lakes is known from prior work (Benson 1961, Kilham et al. 1996, Theriot et al. 1997, Interlandi et al. 1999). The lakes are all relatively large (11 to 340 km²) and deep (33 to 137 m). The lakes have been characterized as having high silicate and moderate phosphate levels as a result of regional geology, while exhibiting low inorganic-nitrogen levels as a result of dilute atmospheric inputs and nutrient-poor soils in the watersheds.

All samples were collected during the summers of 1996 and 1997. Samples were taken at the lake outlets in May, before ice out, and at one main pelagic sampling station in each lake throughout the summer. Collections were made on a weekly basis from both Yellowstone and Jackson Lakes. Samples were collected every 10–12 d in Lewis Lake. Water was sampled by means of a nondestructive peristaltic pump. A hose attached to a Hydrolab sonde (Hydrolab, Austin, Texas) with a depth sensor was lowered to the sample depth and the water was pumped into 4-L carboys on the boat. Samples for individual phytoplankton counts were taken every 5 m from the surface down to 25 m in Lewis Lake, 30 m in Jackson Lake and 50 m in Yellowstone Lake. Samples for water chemistry were taken from 5 and 25 m in all lakes. An additional sample for water chemistry was made during most outings at 45 m in Yellowstone Lake. Midsummer samples for water chemistry were also made at 15 m in Jackson and Lewis Lakes. Light penetration was measured during many trips with a LI-COR light meter (LI-COR, Lincoln, Nebraska) (cosine corrected, flat-plate quantum sensors), and during all excursions with a Secchi disk. All samples taken were measured discretely, and no composite samples were analyzed for phytoplankton counts or chemical measures as part of this field survey.

Samples for phytoplankton counting and identification were fixed within an hour of collection with Lugol's solution. Phytoplankton were enumerated using the inverted microscope technique, typically within 1 wk of sampling. We settled 25 mL of water for counts of samples from Yellowstone and Lewis Lakes, and 10 mL for samples from Jackson Lake. The abundance of picoplankton including small cyanobacteria was estimated at a magnification of 400× by counting 3–10 random view fields in the chamber. Other small algae including flagellates and diatoms <10 μm in diameter were counted at 400× over an entire viewing strip (one diameter length) of the settling chamber. All larger phytoplankton were counted at 100× by scanning the entire chamber to count all organisms in the subsample. Aliquots of all Lugol's-preserved samples from each excursion were pooled together and additionally preserved in 2% formalin for later certification of taxonomic identity and cell-size estimates.

Water for dissolved-chemical analysis was filtered within a few hours of collection by means of syringe

filtration through 0.4- μm polycarbonate filters. In 1996 samples for all dissolved-chemical analyses were frozen immediately and subsequently shipped to Drexel University in Philadelphia (Pennsylvania, USA) for later analysis. In 1997 subsamples of filtered water for phosphate and silicate measurements were refrigerated and analyzed within 1 wk, while subsamples for dissolved inorganic-nitrogen analyses were frozen and analyzed in Philadelphia as in 1996.

Dissolved phosphorus in the lakes was measured as soluble reactive phosphate (SRP) by the molybdenum blue-ascorbic acid method. Silicon was measured as soluble reactive silicate (SRSi) after sample dilution by the molybdenum blue method. Dissolved inorganic nitrogen (DIN) was measured as nitrate plus nitrite by cadmium reduction followed by nitrite quantification by the sulfanilamide method (APHA 1995). Ammonia was not measured as it has generally been found to be a negligible component (<5% total DIN or below detection levels) of the DIN pool in the pelagic zone of these lakes (S. J. Interlandi, S. S. Kilham, and E. C. Theriot, *unpublished data*).

Data analysis

Light levels in the lakes were calculated using a function based on sample depth, measured Secchi depth, and mean monthly surface irradiance values obtained from the National Renewable Energy Laboratory's solar radiation database². As we had taken many concurrent measurements of light penetration via light and Secchi depth, we modeled light at individual depths based on an empirical nonlinear relationship between light attenuation and Secchi depth in the study lakes (Interlandi et al. 1999). The function used to estimate light availability,

$$\text{Light} = \text{IRR} \times e^{-\{[0.979 - 0.342 \times (\ln(\text{Secchi}))] \times (z)\}} \quad (1)$$

results in a value for daily light dose in units of watts per square meter, where IRR is a measure of daily surface irradiance (in watts per square meter), z is sample depth (in meters) and Secchi is the measured Secchi depth (in meters). Daily light dose has been found to be a critical metric in assessments of competition for light as a resource among phytoplankton (Sommer 1994).

Biomass estimates for individual species were based on cell-size measurements and subsequent calculations of biovolumes made on periodically archived samples. Total community biomass was calculated as the sum total of all species-specific biomass estimates for each sample. Calculations of biomass and subsequent calculations of species diversity were made for a total of 712 individual samples.

Species diversity was calculated as a modified Simpson's index:

$$\text{Diversity} = 1 / \sum p_i^2 \quad (2)$$

where abundance is based on total biomass of each species rather than number of individuals and where p is the percentage of total community biomass for an individual species (i). This eliminates the problem of variation in cell size among different species and reflects the true contribution of individual species to phytoplankton community composition. We use Simpson's diversity index as it is numerically analogous to resource-competition theory, which predicts that the number of species that can coexist at equilibrium is equal to the number of resources limiting their growth. If one species exists on its own, the Simpson's index value is 1. When two species exist at equal biomass, the index value is 2, and so forth. As such, the diversity index should increase in direct proportion to the number of resources limiting in the system. A second component of resource-competition theory, which confounds the direct diversity-limiting resource relationship, is the "resource-ratio hypothesis." This states that the taxonomic identity and relative abundance of coexisting species depends on the ratios of the resources that are limiting. This means that multiple species can coexist at unequal proportions of biomass, resulting in a Simpson's diversity value lower than the total number of species.

In order to discern a relationship between the number of limiting resources at a given time and place and the species diversity associated with that spatial-temporal regime, we developed an algorithm (Eqs. 3–7) that would calculate the number of resources at limiting levels:

$$\begin{aligned} \text{if } N_{\text{conc}} \leq \text{TNL}, \quad \text{then } N_{\text{condition}} = 1; \\ \text{otherwise, } N_{\text{condition}} = 0 \end{aligned} \quad (3)$$

$$\begin{aligned} \text{if } \text{Light} \leq \text{TLL}, \quad \text{then } \text{Li}_{\text{condition}} = 1; \\ \text{otherwise, } \text{Li}_{\text{condition}} = 0 \end{aligned} \quad (4)$$

$$\begin{aligned} \text{if } P_{\text{conc}} \leq \text{TPL}, \quad \text{then } P_{\text{condition}} = 1; \\ \text{otherwise, } P_{\text{condition}} = 0 \end{aligned} \quad (5)$$

$$\begin{aligned} \text{if } \text{Si}_{\text{conc}} \leq \text{TSiL}, \quad \text{then } \text{Si}_{\text{condition}} = 1; \\ \text{otherwise, } \text{Si}_{\text{condition}} = 0 \end{aligned} \quad (6)$$

No. limiting resources

$$= N_{\text{condition}} + \text{Li}_{\text{condition}} + P_{\text{condition}} + \text{Si}_{\text{condition}} \quad (7)$$

where "conc." stands for concentration and TNL, TLL, TPL, and TSiL are the threshold limiting levels for DIN, light, SRP, and SRSi, respectively. ("Condition" refers to the environmental state of the resource in terms of phytoplankton physiology (i.e., a condition where the resource is above saturation for growth = 0 in the algorithm, while a condition where the resource can limit growth = 1.) Threshold levels were defined

² URL: <http://tredc.nrel.gov/solar/>

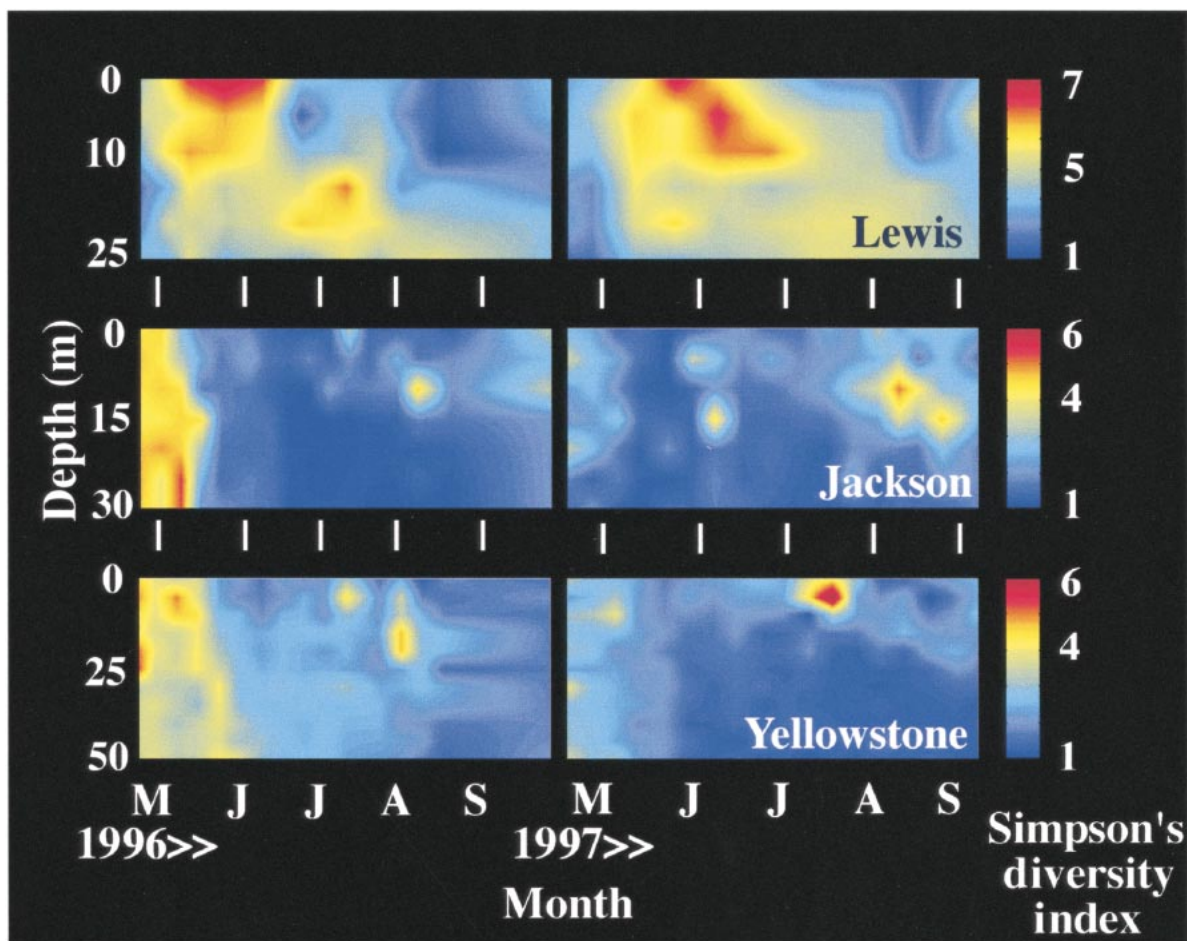


FIG. 1. Phytoplankton species diversity in 1996 and 1997 in three large lakes of the Greater Yellowstone ecosystem. Diversity was calculated by Simpson's index on a biomass basis. Color indicates depth-time variation in this index based on 712 discrete phytoplankton samples; red denotes high species diversity; blue denotes low species diversity.

as those concentrations above which nutrient-saturated growth for the individual resource for *all* species in the system should occur. The number of limiting resources was calculated for 221 total samples.

The relationship between diversity and number of limiting resources was then assessed by correlating Simpson's index with the number of limiting resources determined by Eq. 7. All correlation analyses for which we report significance were performed with nonparametric tests since biomass, relative biomass, and diversity values tended to be heavily skewed toward the low end of their ranges. Neither log transformations nor Box-Cox transformations served to normalize the data. Generally, we report Spearman's rank correlation coefficients and the statistical significance associated with those correlations. Data were analyzed statistically with JMP 3.2 for the Macintosh (SAS Institute 1997).

In order to perform these analyses, the threshold values for the algorithm had to be determined empirically. To ascertain threshold levels associated with these re-

sources, we first looked for likely ranges of these values using physiological data on phytoplankton from laboratory experiments. To estimate threshold limiting levels, we used data from published sources (Eppley et al. 1969, Tilman and Kilham 1976, Ahlgren 1977, Tilman 1977, Gotham and Rhee 1981, Rhee and Gotham 1981, Tilman 1981, Mechling and Kilham 1982, Rhee 1982, Grover 1989, van Donk and Kilham 1990, Taylor 1994) and data from our own previously unpublished experimental work on diatom clones from these lakes. We included all available kinetic data on species and congeners of species that have been found in the study lakes. The threshold values we sought were those where the transition from resource-limited growth to resource-saturated growth occurs along the resource gradient. Using Monod growth curves (Monod 1950), we arbitrarily set the concentration of the resource at $0.85 \times \mu_{max}$, the maximum growth rate, for the least efficient species in the data set as our first approximation of the threshold level for each of the four resources. We used these levels as threshold values

in the algorithm and computed the rank correlation coefficient between Simpson's diversity index and the number of limiting resources.

Although these experimental data provided us an estimate of saturating-resource levels, it was difficult to determine actual environmental threshold levels without extensive experimental work on these natural communities. Laboratory experiments have often been performed in synthetic media that can differ from particular aquatic environments in terms of major ion chemistry and physical condition. Resource-assimilation efficiencies can be affected by properties like pH, temperature, conductivity, and alkalinity—the combinations of which are unique to most aquatic systems. Nutrient-addition experiments using natural communities in lake water may better estimate local threshold levels (Auer et al. 1986, Grover et al. 1999) but the kinetic parameters determined with these experiments are measured with a high degree of uncertainty. Furthermore, to estimate threshold levels for all local species, it would be necessary to perform these experiments frequently over several years, in order to capture all major species under limitation by each available resource. Grover et al. (1999) used this approach, and were able to characterize a number of species for N and P kinetics, but a complete analysis of critical levels for all potentially limiting resources using this technique has not yet been possible.

Using our first approximation of threshold levels, it appeared that among all discrete samples there was indeed a significant correlation between diversity and the number of limiting resources. Knowing this, we then wanted to better determine the actual threshold levels for resources in these lakes. In order to do this we employed a sensitivity analysis to look at how changing the set threshold level altered the computed correlation between diversity and number of limiting resources. We systematically varied the set threshold level for one resource at a time, from zero to well above the likely threshold range. The rank-order correlation coefficient for the diversity-limiting resource relationship was recalculated each time we changed the threshold level in the algorithm. If perfect correlation were to exist between these two parameters (as resource theory can predict at equilibrium), then we would expect to see unimodal responses of the correlation coefficients to variation in the set threshold levels. The correlation should weaken as the algorithm is "fooled" into choosing threshold values farther and farther away from the real environmental threshold levels. If no relationship between diversity and number of limiting resources existed, then we would not expect to find such a response. Since we had already discerned a significant correlation between diversity and number of limiting resources using default thresholds set by physiological data for individual algal clones, we felt it was appropriate to then use this indirect analytical tech-

nique to determine actual environmental threshold levels in these lakes.

We varied each of the threshold levels (TNL, TLL, TPL, TSiL) individually while holding the others constant, and looked at how this variation affected the significance of the correlation between diversity and the number of limiting resources. The resource levels that were associated with a peak in the function of diversity vs. number of limiting resources within the predicted threshold range indicated the actual environmental threshold levels for those resources. Clear local maxima existed for all resources when treated to this sensitivity analysis, and these levels were set as the threshold concentrations for the remainder of the analyses.

RESULTS

Spatial-temporal variability in phytoplankton diversity

In most instances phytoplankton populations exhibited high diversity early in the season while nutrients were relatively high and snowmelt was still entering the lakes through stream flow (Fig. 1). This pattern was strongest in 1996, which was a year in which run-off was extremely rapid. The spring of 1996 was followed by a very warm and dry summer (Interlandi et al. 1999). In contrast, in 1997 we observed a more prolonged period of snowmelt followed by a cool, wet summer with frequent snow squalls through July. Midsummer short-term maxima in diversity occurred periodically in Jackson and Yellowstone Lakes probably as a result of disturbances either from deep mixing events or precipitation. Lewis Lake generally exhibited the highest diversity and had the most prolonged periods of high diversity.

Diversity-biomass relationship

Of the three study lakes, Lewis Lake had both the lowest biomass and the highest species diversity in 1996 and 1997 (Table 1). In 1996 Jackson Lake had the highest biomass and the lowest diversity, while Yellowstone Lake exhibited these properties in 1997. By measure of mean annual biomass estimates and mean species diversity, it appears that the negative relationship between biomass and diversity is real and significant (Spearman's $\rho = -0.8286$, $P = 0.0416$, $n = 6$ lake-years). This relationship also held when we looked at diversity vs. biomass for all individual samples in the study (Table 2). The negative relationship did not always hold, however, when we looked at samples within an individual lake. For all samples from Lewis Lake, where biomass was always relatively low, we actually found a slight but significant positive relationship between biomass and diversity.

Scatter plots of the diversity-biomass data help to illustrate the nature of the relationship (Fig. 2). In Yellowstone and Jackson Lakes we saw that very high

TABLE 1. Phytoplankton biomass and diversity in three large lakes of the Greater Yellowstone ecosystem (Wyoming, USA).

Year	Lake	Biomass ($\mu\text{m}^3/\text{mL}$)	Simpson's diversity index
1996	Lewis	155,278 (8,364)	3.65 (0.15)
	Jackson	911,872 (35,303)	2.22 (0.12)
	Yellowstone	238,183 (10,462)	2.86 (0.07)
1997	Lewis	128,593 (7,830)	3.72 (0.15)
	Jackson	564,632 (37,035)	2.08 (0.07)
	Yellowstone	642,094 (23,462)	1.99 (0.06)

Note: Data are means with 1 SE in parentheses. Interestingly, Yellowstone Lake, which has been assumed to be much less productive than Jackson Lake, actually had the highest mean phytoplankton biomass of the three lakes in 1997.

biomass levels occurred only when species diversity was low (but not vice versa). Alternative, we saw that the only instances where diversity was high in these lakes (especially Yellowstone) were times when biomass was low. The plot for samples from all lakes illustrates this relationship as well. High diversity only occurred where biomass was low. In contrast, low diversity occurred in many instances independent of the biomass level. In Lewis Lake we saw no such obvious pattern, suggesting that, within the range of relatively low biomass levels, this relationship may be less ecologically relevant. Plots of samples from Jackson and Lewis Lakes also seem to suggest a possibly more com-

TABLE 2. Biomass–diversity relationships in samples from three lakes in the Greater Yellowstone ecosystem during 1996 and 1997.

Lake	Spearman's rho	P
Lewis	0.1837	0.0364
Jackson	-0.2701	<0.0001
Yellowstone	-0.3877	<0.0001
All lakes	-0.4561	<0.0001

Note: Nonparametric tests were used because of skewed distributions in diversity data that could not be normalized.

plicated pattern of high diversity at moderate biomass levels and low diversity at the extremes. This pattern was reminiscent of predictions made by the intermediate-disturbance hypothesis (Reynolds et al. 1993, Sommer 1995), but we were not able to evaluate this connection further.

The generality of this biomass–diversity relationship should be interpreted with some caution, for while the data set is substantial, samples from within lakes are not completely independent, and this assessment is for only a small number of lakes. Environmental conditions within lakes do vary considerably, spatially and temporally, so a degree of independence among samples certainly still exists. More rigorous analyses of data from many aquatic systems would be needed to verify this pattern as a general property of lake ecology.

Resource thresholds and the limiting resource–diversity relationship

First approximations of threshold levels from laboratory experimental data (Fig. 3) were higher than those determined as threshold concentrations for resources

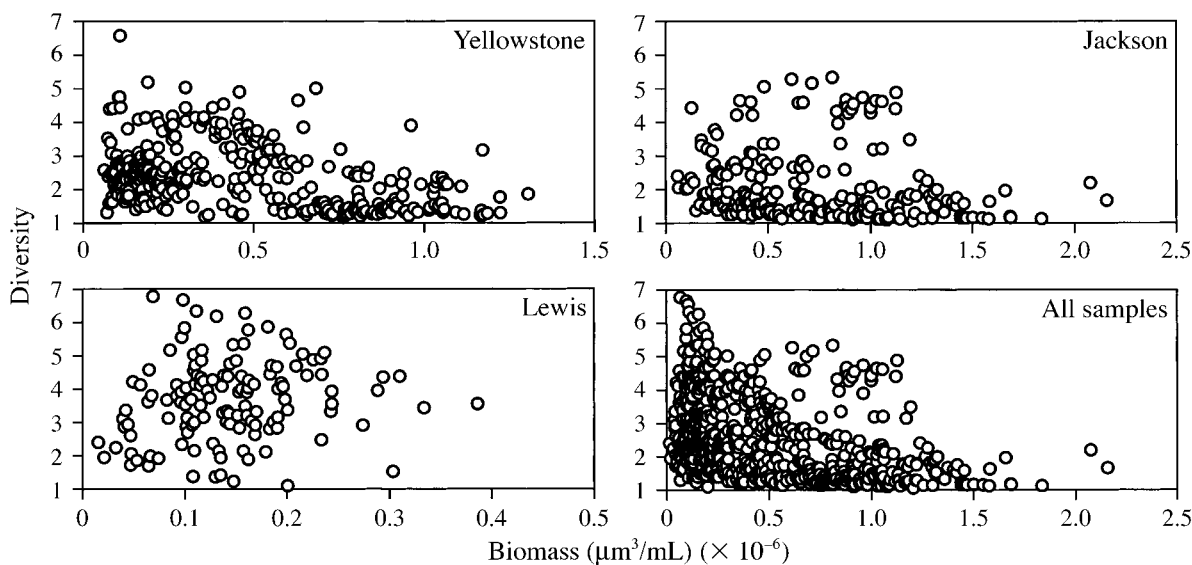


FIG. 2. Diversity as a function of biomass in samples from 1996 and 1997 from three large lakes in the Greater Yellowstone ecosystem. Negative relationships held in samples from Yellowstone and Jackson Lakes and in the pooled samples. This was not the case in samples from the most oligotrophic of the lakes, Lewis Lake. Note that biomass is shown in units of millions of cubic micrometers per milliliter.

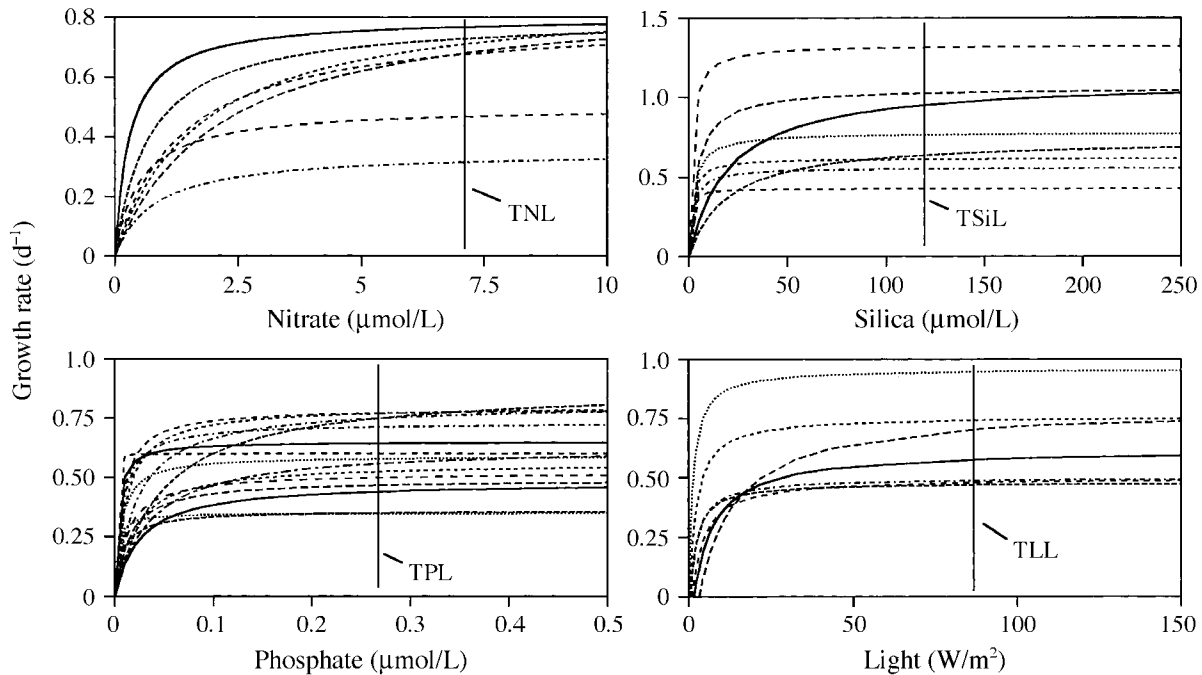


FIG. 3. Limiting-resource threshold estimation using fits of laboratory-determined kinetic data to the Monod equation. Kinetic data are from a variety of published sources, and from our own previously unpublished work. Data are for species and congeners of species found in the lakes of the Yellowstone region. Threshold levels (TNL, TSiL, TPL, TLL), above which nutrient-saturated growth occurs, are approximated as the resource level at $0.85 \times \mu_{\max}$ (the maximum growth rate) for the least efficient species assessed.

in studies of eutrophication processes. Our values correspond to transition levels from nutrient-limited to nutrient-saturated growth, while other surveys have looked to find threshold levels below which positive net growth cannot be sustained (Auer et al. 1986). The value of the threshold for Si limitation ($120 \mu\text{mol/L}$) was much higher than we expected, even considering this factor. As we had mathematically set the estimated threshold resource level at the concentration corresponding to $0.85 \times \mu_{\max}$ along the growth curve, these levels were accepted since they were consistent with our theoretical understanding of resource competition even if they seemed intuitively high to us. The light and phosphorous levels corresponded well to our expectations, although the P threshold was somewhat higher than others might argue is environmentally realistic (Reynolds 1997). The daily light dose threshold of 88 W/m^2 was reasonable, and corresponds to an illumination level of $\sim 180 \mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ for a 12 h:12 h day:night light cycle.

The correlation between diversity and number of limiting resources using these approximate thresholds was found to be highly significant ($n = 221$ samples, Spearman's $\rho = 0.2979$, $P < 0.0001$). We interpreted this correlation as strong evidence for the regulation of diversity by limiting resources for phytoplankton communities in these systems.

We found that variation in the threshold levels of all

resources in the sensitivity analyses altered the computed correlation of the diversity-limiting resource relationship (Fig. 4). For P, light, and Si we found the relationship remained strongest when the threshold levels were at some middle range of resource levels. The functional responses of the correlation coefficient to the varied threshold levels for these resources were unimodal. Also, the peaks in correlation level were strikingly close to values that have been found to be threshold levels for limiting phytoplankton growth in laboratory experiments (Fig. 3) and field assays (Auer et al. 1986, Grover et al. 1999). The correlation behaved somewhat differently when the N threshold was varied because nearly all of our measured dissolved inorganic-N (DIN) levels were lower than $3 \mu\text{mol/L}$. The correlation level reached a plateau at $3 \mu\text{mol/L}$, and remained constant above this value. This is exactly the qualitative response of this algorithm which is predicted at equilibrium by resource theory when measured samples are all below a perfectly known environmental threshold level. Interestingly, this threshold was significantly lower than that determined by the laboratory data. This may be a result of the fact that only species that are relatively good at utilizing N exist in these lakes, because N concentrations in this region are uncommonly low for temperate freshwater systems (Kilham et al. 1996, Theriot et al. 1997, Interlandi and Kilham 1998).

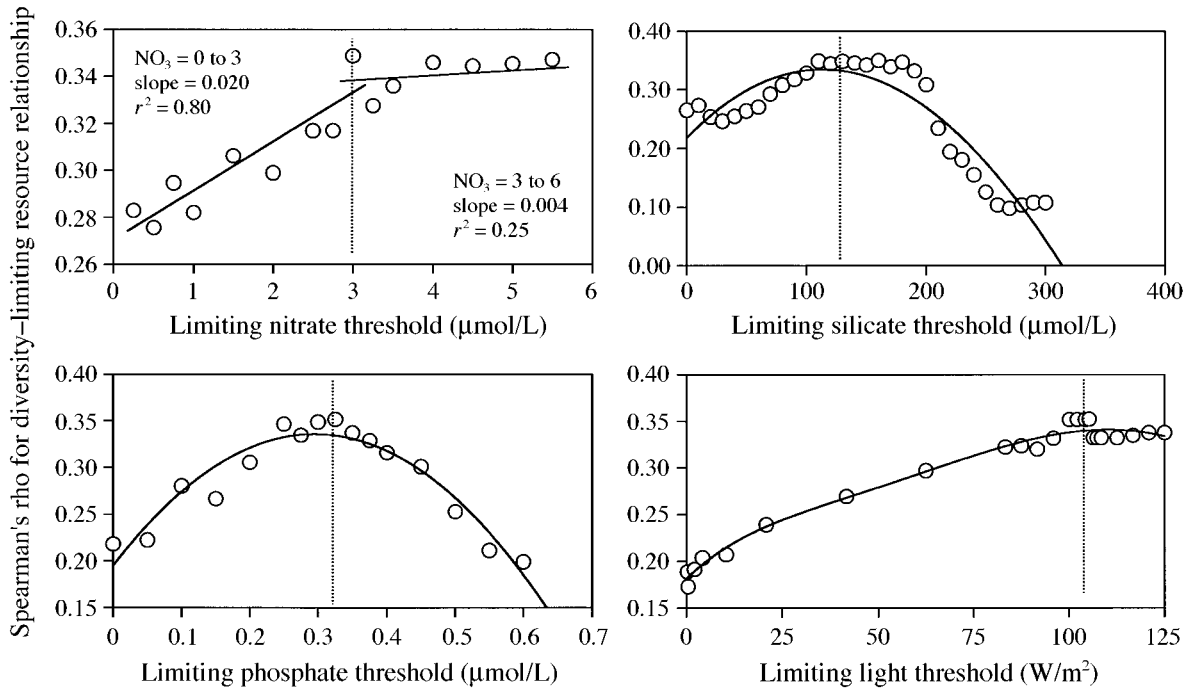


FIG. 4. Results of sensitivity analyses for the limiting-resource algorithm (Eqs. 3–7). Threshold levels for four resources were varied individually within their range in the natural systems to ascertain the effect of set threshold level on the diversity–number of limiting resources correlation. Peaks in relationship strength indicate environmental threshold levels of resources (vertical dotted lines) below which phytoplankton growth was limited and resource competition was occurring. Spearman rank-order correlation was computed throughout the analyses, as numerical transformations were not useful in normalizing data.

When the threshold N levels were varied, we found that the diversity–limiting resource correlation was always significant, but became weaker with a N threshold level below 3 $\mu\text{mol/L}$. The correlation remained relatively strong with higher threshold levels, but did not vary much from the highest correlation value (Spearman's rho = 0.35, $P < 0.0001$, $n = 221$). When the P threshold level was varied, there was a distinct peak in the correlation coefficient at a concentration of 0.32 $\mu\text{mol/L}$. The correlation was insignificant when threshold levels were set below 0.1 or above 0.45, indicating a strong ecological significance of P limitation and competition within this range. When the light threshold level was varied, we found the relationship weakened below 105 W/m^2 with decreasing light level. The relationship also weakened slightly above this threshold level. Varying the set threshold level for Si resulted in a unimodal response of the correlation, but the maximum was not as sharp as when the P threshold was varied. Nevertheless, the regional maximum corresponded well to the higher-than-expected threshold predicted by laboratory assays. The peaks in the threshold levels (N = 3 $\mu\text{mol/L}$, P = 0.32 $\mu\text{mol/L}$, light = 105 W/m^2 , Si = 130 $\mu\text{mol/L}$) indicated real environmental threshold levels for resource limitation in these lakes. As such, these values were used in our further

analyses of the relationship between number of limiting resources and mean diversity.

The correlation between the number of limiting resources and diversity that was illustrated in the sensitivity analyses is highly significant, but the correlation coefficient (Spearman's rho = 0.35) was relatively low owing to the large range of diversity values in the samples. For instance, when threshold levels were set at the values determined by the sensitivity analysis, we found that diversity varied between 1.10 and 3.52 where there was one measured limiting resource, and between 1.48 and 5.34 when there were four measured limiting resources. Histograms of the relative frequencies of diversity levels associated with the number of limiting resources available illustrate this variability, but also demonstrate the trend of increasing diversity with increasing numbers of measured limiting resources (Fig. 5). To get a clearer picture of the importance of this relationship, we analyzed the mean diversity as a function of the number of measured limiting resources, and discovered that this relationship was very strong (Fig. 6). While the slope of the relationship (0.524) is considerably less than the 1-to-1 slope expected from resource competition theory for species richness at equilibrium, the high level of correlation ($r^2 = 0.996$) is an exciting result, nevertheless. The lower-than-ex-

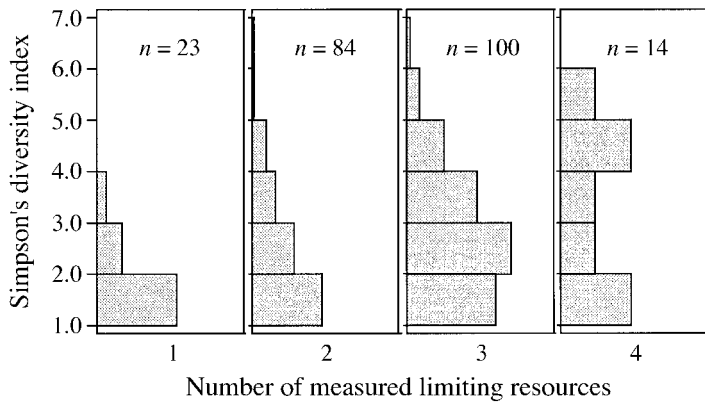


FIG. 5. Relative frequency distributions for phytoplankton diversity associated with samples in which different numbers of limiting resources were measured. Threshold levels for limiting resources are set by peaks in the strength of the diversity-limiting resource relationship determined in the sensitivity analyses. Threshold levels are $P = 0.32 \mu\text{mol/L}$, $N = 3.0 \mu\text{mol/L}$, $\text{Light} = 105 \text{ W/m}^2$, and $\text{Si} = 130 \mu\text{mol/L}$; $n = \text{no. of samples}$.

pected slope can be the result of several phenomena, including nonequilibrium conditions that allow diversity to be higher than the number of limiting resources, and resource-ratio effects that theoretically allow Simpson's index to be lower than the number of limiting resources. Using the nonparametric Kruskal-Wallis test, we also found that the groups of samples at each discrete number of limiting resources were significantly different ($\chi^2 = 28.64$, $P < 0.0001$).

In ecological terms, this result suggests that, on average, species diversity is directly proportional to the number of resources limiting at any given time. Furthermore, it suggests that competition for resources is

acting to directly regulate community composition in concert with other factors, such as disturbance, which are known to enhance levels of diversity.

DISCUSSION

Spatial and temporal variation in species diversity illustrates the importance of sampling frequency in understanding ecological systems. Succession in phytoplankton communities has been well studied with frequent sampling, mainly from the perspective of the dynamics of individual species or groups of species. It is only because of this intensity of sampling that we know as much as we do about phytoplankton ecology. Our data suggest that community-level properties can also be better understood with frequent sampling, particularly in highly dynamic aquatic systems.

While we have focused on community-level parameters in this article, prior research has shown that particular phytoplankton species in these lakes appear to occupy distinct temporal and spatial niches as a function of variability in resource availability. Both experimental research with cultures (Interlandi and Kilham 1998) and empirical correlations of field data (Interlandi et al. 1999) suggest that resources are regulating the relative abundance of diatoms in particular in these systems. It appears that the growth and abundance of individual species in these lakes are segregated within resource space according to their individual physiological requirements for the measured resources. This is further evidence for our contention that resources are regulating community structure, which we have now seen at both the population level and the community level in phytoplankton of these lakes.

Diversity was highly variable in several ways that may not have been expected. There were distinct differences among lakes, which fit into our general understanding of patterns of diversity. Many researchers have found eutrophic lakes to have low diversity and oligotrophic lakes to have high diversity (Wetzel 1983). The negative correlation between diversity and total phytoplankton biomass in our own data was further support for this generality, but in itself does not lead

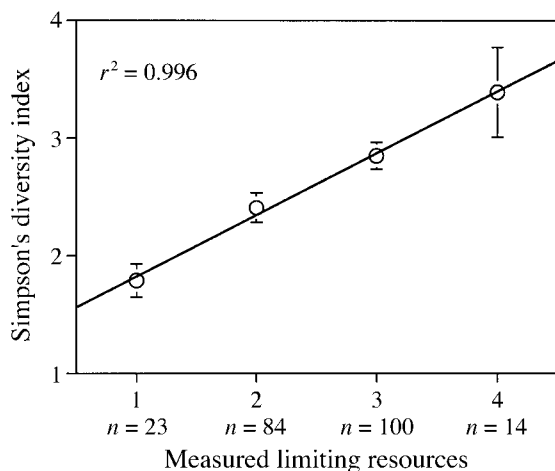


FIG. 6. Simpson's diversity (mean $\pm 1 \text{ SE}$) associated with samples with different numbers of measured limiting resources. Threshold levels for limiting resources are set as in Fig. 5. Linear regression was performed using JMP 3.2 for the Macintosh (SAS Institute 1997); diversity = $0.524 (\text{limiting resource}) + 1.300$. Individual diversity values are highly variable, and not normally distributed. As a result, no exact probability of significance can be associated with this linear relationship, but the correlation between mean diversity and number of measured limiting resources is surprisingly high, nevertheless. Groups of samples are statistically distinct according to the Kruskal-Wallis test ($\chi^2 = 28.64$, $P < 0.0001$); $n = \text{no. of samples}$.

toward an understanding of why this relationship should exist. It has been suggested that eutrophic systems are highly disturbed, and that this results in an inhospitable environment for many species. It has also been suggested that severe light limitation in eutrophic systems can result in lowered diversity, but biomass was always more significant than light level in all additional analytical tests to which we have subjected these data. The idea that transport processes control competitive interactions (Siegal 1998) can be invoked in this case to explain, at least in part, this relationship between biomass and diversity.

Temporal differences in diversity within and between years do, however, seem to be driven to some degree by variability in disturbance regimes. All lakes in 1996 exhibited patterns of high diversity in spring followed by long-term stability in phytoplankton diversity for much of the summer. We suggest that in 1996 low summertime disturbance allowed the lakes to behave much like homogeneous systems at equilibrium. Species diversity in 1997 did not exhibit the same springtime peak in all three lakes and was much less constant through the summer. The summer of 1997 was characterized by colder, more variable weather and by greater precipitation (S. J. Interlandi and S. S. Kilham, *unpublished data*). The resulting variability in light climate and nutrient levels in the lakes may have led to the more dynamic response of the phytoplankton community as measured by species diversity.

In many instances we observed levels of diversity that were greater than we would have predicted. Other researchers have suggested that species richness and diversity in phytoplankton communities above that predicted by resource-competition theory can be achieved as a result of heterogeneity in the environment (Hutchinson 1961, Richerson et al. 1970). Specifically, Siegal (1998) recently mathematically demonstrated that even when water is homogeneous in bulk, it may be heterogeneous from the reference point of individual phytoplankton cells, and that the occurrence or strength of competition is dependent upon the proximity of cells in the fluid. In general, the predictions of Siegal's model suggest that competition will be stronger where cell density is high and unlikely where cell density is low. This is an alternative mechanism which can in part explain why eutrophic systems exhibit lower diversity on average than those systems that are less productive. Given enough time at or near equilibrium, however, even communities in dilute systems will and can exhibit responses due to competitive effects. In the Lewis Lake samples alone, however, we did not see a negative correlation between biomass and diversity, suggesting that, within that lake, mechanisms other than those dominated by fluid mechanics must be acting to regulate community composition.

Theoretically speaking, species richness is the response variable we should have used if we wanted to test for exclusion as a result of resource competition

(Sommer 1995). The dynamic nature of the natural environment, however, often precludes complete exclusion, and the measure of species richness, while perfect at stable equilibrium, is less useful for assessments of field data. Furthermore, the number of individuals counted and the sample size will both affect the number of species encountered, and separating this sampling effect from the effects of competition on species richness can be complicated (Goldberg and Estabrook 1998). Diversity allows us to use an index that, while partly a measure of equitability and therefore not directly compatible with resource theory, is primarily a function of the major organisms present in the community. A species representing <5% of community biomass, for example, will have little effect on the diversity index of that community, so species on their way to exclusion do not strongly affect the index value. Sommer (1995) found that a biomass-based diversity index behaved qualitatively like species richness in laboratory experiments carried to steady-state equilibrium conditions. It is likely that this discrepancy between theory and measured response variable is a smaller problem than sampling-related errors in species-richness values might be in our current analysis.

Phosphorus levels varied over a considerably wide range of values from limiting to nonlimiting levels. As a result, we saw a very distinct peak in the strength of the diversity-limiting resource relationship. In the range from 0.2 to 0.4 $\mu\text{mol/L}$, the relationship was highly significant (Fig. 4). This indicates that at concentrations below the level where the correlation maximum occurred in this range, the diversity of the phytoplankton community was being regulated in part by degree of P availability. When P was in a range where it was potentially limiting the growth of many species, it seems that competition for P resulted in a community-level response of lower diversity. Assays of P limitation in freshwater phytoplankton have shown that maximum growth can be achieved by most species when available phosphate is $>0.1 \mu\text{mol/L}$. This finding has been used by some to suggest that since P levels in natural environments are frequently above this level, regulation of growth and competition for P occurs less often than we might expect (Reynolds 1997). This argument is based on the assumption that all soluble reactive phosphorus (SRP) is available. Our sensitivity analysis indicated a community response at a greater measured nutrient level (0.32 $\mu\text{mol/L}$). This is indirect evidence that some SRP is not available for utilization by phytoplankton, and that the actual useable resource level for them is somewhat lower than the measured SRP. Future research concerning nutrients and their effects on communities might benefit from more thorough assessments of the actual bio-availability of P and other resources in aquatic systems (Urnberg and Peters 1984).

The diversity-limiting resource correlation was also sensitive to changes in threshold light levels in the

algorithm. The correlation strength increased with increasing threshold levels up to 105 W/m². Above this level the significance of the relationship slightly weakened. This pattern suggests that environmental threshold levels for light limitation in phytoplankton occur around the peak of 105 W/m². This is roughly equivalent to an experimental light level of 215 $\mu\text{mol photons}\cdot\text{m}^{-2}\cdot\text{d}^{-1}$ with a light:dark cycle of 12:12, and is very close to experimentally determined threshold limiting light levels for diatoms from these lakes grown in culture (data illustrated in Fig. 3).

While competition for light among phytoplankton is conceptually more complex than competition for mineral resources, the mechanisms regulating this process are beginning to be elucidated both theoretically and experimentally (Huisman 1999, Huisman et al. 1999). The empirical connection between light level and the diversity-limiting resource relationship, which we have observed here, seems to generally support the notion that light competition is important for phytoplankton communities, and that light, like nutrients, can alter community structure in the aquatic environment. The role of light in terrestrial plant ecology has often been demonstrated, but the effects of this resource on aquatic communities are still being ascertained. We suggest that light should be evaluated as a resource along with mineral nutrients in most assessments of phytoplankton ecology. Additionally, it must be noted that the availability of light varies seasonally as a result of both changing surface irradiance and light extinction in the water column. Both these factors must be accounted for when attempting to quantify the effects of light as a resource on phytoplankton community structure.

Light also confounds this analysis through its effects on N assimilation and utilization efficiency (Rhee and Gotham 1981). Increased N requirements with decreasing light have been observed in algal cultures caused by the need for greater levels of N-rich photo-pigments that are needed to harvest scarce photons. Discrete threshold levels for N, it could be argued, cannot really exist, even with relatively regular major ion chemistry within a particular lake region. The results of the sensitivity analysis for the N threshold were somewhat inconclusive along these lines, but would be consistent with either a discrete or variable N threshold. The correlation between diversity and number of limiting resources did not vary significantly with a N threshold set above 3 $\mu\text{mol/L}$, so although this was theoretically problematic, the issue did not affect our generalized results. Ideally, empirical data on light-N interactions for many species of algae would be evaluated, and the TNL (the threshold limiting level for dissolved inorganic N) could be determined as a function of light rather than as a direct scalar quantity.

Regulation of diversity by number of limiting resources can be invoked in the development of hypotheses about some as-yet-unexplained patterns of phy-

toplankton diversity in many environmental systems. Specifically, severe limitation by single resources may indeed be a factor behind low diversity in "disturbed systems." For example our results are consistent with the idea that severe light limitation in nutrient-replete conditions in eutrophic lakes can result in low diversity as a result of competition for light. Severe limitation for P could similarly be the cause of low diversity in acidified clear-water lakes, as bio-unavailable forms of P are favored at low pH, while light, Si, and N can remain at higher levels in such circumstances.

Our final analysis of mean diversity vs. number of limiting resources was based on the threshold levels set at the local maximum in correlation significance in the sensitivity analyses for all four resources (N, P, Si, and light). This might seem to bias the final assessment towards finding a significant relationship. We would argue that this is not the case because we had used independent empirical data to set the threshold range, assessed the correlation, found it significant, and only then allowed the sensitivity analysis to fine tune the algorithm and to determine the actual local environmental threshold levels. The improvement is not only the result of numerical manipulation; it is also based on mechanistic properties predicted by theory, which we had already found to be part of the system prior to conducting the final analyses. That these optimum threshold levels are very close to the experimentally determined threshold limiting levels makes our argument stronger still.

We think that the importance of this work is best understood when it is put in context with all of the other research that has sought to elucidate the mechanisms involved in the regulation of species richness and diversity in plant communities. Ultimately, we hope further study incorporates this approach coupled with the approach of studies that have quantified the effects of disturbance on community structure (Tilman 1982, Grover 1990, Sommer 1993, 1995, Reynolds 1997). This would greatly improve our understanding of the relative importance of both resource competition and disturbance in the regulation of biotic communities. It makes no sense to argue that one process is generally more important than another because an extensive body of research has shown that both mechanisms—disturbance and resource competition—most certainly are working in concert in ecological systems. Furthermore, evidence suggests that each process can dominate community regulation in specific circumstances (Tilman 1982, Sommer 1993, 1995). Sometimes equilibrium conditions are nearly approximated, and other times they are not. An ideal study would assess both the disturbance regime and the number of limiting resources together, yet we have not found such a study in the literature. A number of investigators have attempted to answer similar multi-layered questions in small-scale and whole-lake manipulation experiments by looking at the simultaneous effects of predation and

resource availability on producers (Carpenter et al. 1996). We suggest that these kinds of studies would greatly benefit by using a combination of the approaches of Sommer (1993) and the one we have taken here to quantify the strengths of multiple mechanisms in regulating species diversity.

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