LIMITING RESOURCES, DISTURBANCE, AND DIVERSITY IN PHYTOPLANKTON COMMUNITIES

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Abstract. Phytoplankton diversity, limiting resources, and disturbance were studied in two reservoirs, Eagle Mountain Lake (EML) and Joe Pool Lake (JPL), in north Texas, USA, for three summer growing seasons and two winters. Availabilities of phosphorus, nitrogen, and silicon were measured as chemical concentrations, and availability of light was measured as irradiance during the photoperiod, averaged over the depth of the surface mixed layer. A resource was defined as limiting whenever its availability fell below a threshold, treated as a parameter to be fitted. Depth of the surface mixed layer and wind speed were taken as indices of disturbance associated with episodic mixing. Hydrological disturbance was gauged by rainfall, inflow, and the variation of lake level. We found that diversity was strongly and significantly correlated with the number of limiting resources in one lake (JPL), but not the other (EML). The onset of nitrogen and phosphorus limitation during the summer growing season is associated with increased phytoplankton diversity in JPL. Regression modeling detected decreasing relationships between diversity and disturbance indices in one lake (JPL), but concave-down relationships in the other (EML), though the peaks of these relationships did not lie within the usual range of disturbance. Predictive regressions for diversity incorporating both limiting resources and disturbance were partitioned into unique and shared effects of these factors. There was a large unique effect of the number of limiting resources in JPL, but otherwise the shared effects were large relative to unique effects.

Keywords: algae; biological diversity; disturbance; intermediate disturbance hypothesis; limiting resources; nutrients; phytoplankton; reservoirs; resource competition.

INTRODUCTION

Among the factors thought to influence diversity in ecological communities, limiting resources and disturbance have a long history of investigation. Classical theories of resource competition assert that at equilibrium, the number of limiting resources is an upper limit to the number of coexisting competitors, assuming that trade-offs exist in the ability to compete for different resources (Phillips 1973, Tilman 1982). With three or more limiting resources, intransitive competitive relationships might induce chaotic dynamics, making possible the coexistence of more species than limiting resources (Huisman and Weissing 1999, 2001). In either case, the number of coexisting species is expected to increase with the number of limiting resources.

Theoretical perspectives on disturbance and diversity recognize that increasing frequency or intensity of disturbance might increase or decrease diversity, relationships that are unified in the concave down relationship proposed by the “Intermediate Disturbance Hypothesis” (Connell 1978). With little or no disturbance, one or a few highly competitive species dominate and diversity is low. At high levels of disturbance, one or a few highly tolerant species dominate and diversity is also low. At some intermediate level of disturbance, neither type of species strongly dominates and there are more coexisting species.

Investigations of how resources and disturbance influence diversity seem especially tractable for phytoplankton. To a first approximation, limiting resources are easy to enumerate, consisting of light and several essential nutrients (Hutchinson 1961, Tilman et al. 1982), while hydrodynamic and hydrological events produce disturbances linked to phytoplankton community structure and diversity (Reynolds 1993, Sommer et al. 1993). Unlike smaller, prokaryotic microorganisms, morphological taxonomy permits assessment of diversity among phytoplankton. But, similar to other microorganisms, the short generation times of algae allow study over multiple generations, during intervals when either an approach to equilibrium or a number of disturbances might occur.

These opportunities led Interlandi and Kilham (2001) to develop an approach to assess phytoplankton diversity in relation to the number of limiting resources. Their analysis assumes that conditions are at least sometimes sufficiently steady so that superior competitors increase in abundance and inferior competitors decline. This is not a strict assumption of equilibrium conditions, but rather a weaker assumption that the data set includes intervals during which a competitive equilibrium is approached. If only a single resource is depleted during such intervals, theory predicts that a sin-
single competitor will dominate, and thus diversity would become low as equilibrium is approached. If two resources are depleted in availability, theory predicts that two competitors at most will dominate, and so on. Based on this logic, Interlandi and Kilham (2001) count a resource as "limiting" when its availability becomes low enough to limit growth rates of poor competitors. Thus time series of resource availability can be evaluated with respect to a threshold defining limitation: when availability falls below the threshold, it is limiting. The number of limiting resources at each time of observation is then tallied, and the correlation between diversity and the number of limiting resources computed. Data from three lakes in Yellowstone Park (western alpine United States) displayed positive and approximately linear correlations, as expected from classical theory. Interlandi and Kilham (2001) recognized but did not explore the desirability of coupling such an analysis of limiting resources with an analysis of disturbance.

Here, we further develop methods for analyzing relationships between diversity and number of limiting resources for phytoplankton and incorporate effects of disturbance on diversity. We apply computationally intensive procedures to (1) estimate resource thresholds optimizing the correlation between diversity and number of limiting resources and (2) determine the statistical significance of this correlation and of relationships with disturbance. We apply the analyses to phytoplankton of two warm temperate reservoirs in north Texas, USA, to ask: How strong is the association between diversity and number of limiting resources? How strong is the association between diversity and disturbance, and does it support the Intermediate Disturbance Hypothesis? How do the associations of diversity with the number of limiting resources and disturbance compare to one another, and can unique associations of these factors with diversity be disentangled?

METHODS

Field sites and sampling

Joe Pool Lake (JPL) and Eagle Mountain Lake (EML) are located in the Dallas-Fort Worth metropolitan area (north Texas, USA) and have been subjects of previous studies of nutrient and plankton dynamics (Sterner 1994, Sterner and Grover 1998, Grover et al. 1999, Grover and Chrzanowski 2000, Chrzanowski and Grover 2001a, b). JPL (32°38' N, 97°0' W) is a mesotrophic reservoir with a surface area of 3620 ha, mean depth of 7.2 m, and water residence time of 2.5 yr. EML (32°53' N, 97°30' W) is a eutrophic reservoir with a surface area of 3653 ha, mean depth of 6.1 m, and water residence time of 0.5 yr. The two lakes are in different watersheds and are not hydrologically linked. In both lakes, surface water temperatures vary from approximately 7°C in winter to >30°C in summer. In cool weather, the entire water column is mixed, but neither lake displays persistent seasonal thermal stratification. During warm weather, short-lived surface layers 4–8 m deep may form, 0.5–1°C warmer than water below, but these often do not persist over the scale of sampling in this study (2 wk). During warm weather, both lakes have reduced dissolved oxygen concentrations in a layer about 2 m above the sediments, with a temperature drop of 2–3°C in this bottom layer.

Each lake was sampled from March 1998 to October 2000 at a single station near the deepest part. Depth at the sampling position was usually 10–12 m in EML and 12–16 m in JPL. Samples were taken every two weeks when water was warm (>16°C) and monthly otherwise. Depth profiles of photosynthetically active radiation were determined at meter intervals with a LI-COR model LI 185B light meter equipped with a spherical quantum sensor (LI-COR, Lincoln, Nebraska, USA). Irradiance above the water (Ia) was determined using a shroud to prevent light reflected from the water from reaching the spherical sensor. Depth profiles of temperature and dissolved oxygen were determined at meter intervals with a YSI model 33 meter (Yellow Springs Instruments, Yellow Springs, Ohio, USA). From these profiles, the depth of the surface mixed layer was defined by a 0.5°C temperature drop within 1 m. A Van Dorn sampler (6 L; Wildlife Supply, Saginaw, Michigan, USA) was then used to collect samples at three depths near the top, middle, and bottom of the surface mixed layer. These three samples were combined to create a pooled mixed layer (PML) sample. This procedure was repeated three times to obtain triplicate PML samples at each sampling event.

Laboratory procedures and measures of diversity and resource availability

Within six hours of collection, aliquots (50 mL) were subsampled from each PML sample and preserved with Lugol's iodine and formalin for identification and enumeration of algae. Growth rates of algae during this holding time were low (always <0.5 d⁻¹) as estimated from dilution cultures (Chrzanowski and Grover 2001a), and biomass changes would thus be <15%. Larger aliquots (500 mL) were also subsampled, filtered (GF/F filters), and frozen for later determinations of dissolved nutrients. Soluble reactive phosphorus (SRP), soluble reactive silica (SRSi), ammonium, and nitrite were determined with conventional colorimetric methods (Strickland and Parsons 1972). Nitrate was reduced by shaking with spongy cadmium (Jones 1984) and analyzed as nitrite with final concentrations corrected for reduction efficiency and ambient nitrite concentration (Wetzel and Likens 1991).

Algae were identified morphologically and enumerated with an inverted microscope (Margalef 1969), with 200–400 individuals counted for dominant taxa, with smaller numbers enumerated for less common taxa; total counts per sample ranged 1000–3000 individuals. For most samples, 2–4 diameter transects of the sed-
imementation chamber were counted at 100×, and 10–50 fields were counted at 400×, with additional magnifications used for some samples. Of 167 taxa identified, 67% (112) were identified to species level and 99% to genus level (165); two size classes of otherwise unidentified nanoflagellates were enumerated. Identification generally followed Dillard (1989) for chlorophytes, Hustedt (1930) and Germain (1981) for diatoms, Desikachary (1959) for cyanobacteria, and other works for various taxa, though nomenclature followed recent taxonomic revisions (e.g., Komárek and Anagnostid 1986, Anagnostidis and Komárek 1988). For all samples collected in 1998, 10–30 individuals (cells or colonies) of common taxa were measured with an ocular micrometer, and biovolume was calculated (Margalef 1969). For less common or rare taxa, biovolumes were estimated from occasional measurements or from literature. From biovolumes and cell densities of each taxon, carbon mass density was calculated using the rough conversion of 0.2 pg C/μm² (Rocha and Iszidis 1986, Anagnostidis and Komárek 1988). For a/l recent taxonomic revisions (e.g., Komárek and Anagnostid 1986, Anagnostidis and Komárek 1988). For a/l recent taxonomic revisions (e.g., Komárek and Anagnostid 1986, Anagnostidis and Komárek 1988), a(l - eKz2) (T (z, t) (z, t) dz dt)

\[ I(z, t) = \frac{1}{z_{\text{mix}}} \int_{0}^{T} \left( \alpha l_{\text{sky}}(t) e^{-Kz} \right) dz dt \]

\[ DLD = \frac{1}{z_{\text{mix}}} \int_{0}^{T} I(z, t) dz dt \]

where \( I(z, t) \) is irradiance at depth \( z \) and time \( t \), \( z_{\text{mix}} \) is the depth of the surface mixed layer, \( T \) is the length of the photoperiod, \( l_{\text{sky}}(t) \) is the irradiance above the water at time \( t \), \( \alpha \) is the proportion of this irradiance transmitted through the water surface to depth 0 (i.e., \( \alpha = I_{\text{sky}}(t)/I(0, t) \)), and \( K \) is the diffuse attenuation coefficient for photosynthetically active radiation.

The components of DLD were calculated for each sampling event as follows: The shallowest depth at which a drop of 0.5°C in 1 m was observed in the temperature profile was taken as an estimate of \( z_{\text{mix}} \) to account for the intermittent thermal stratification noted above. If no temperature drop of this magnitude was found, the depth to the sediments was taken to estimate \( z_{\text{mix}} \). Measured values of \( I(z, t) \) from the depth profile were transformed to natural logarithms and regressed against \( z \), taking the slope to estimate \( K \). Transmittance of irradiance at the water surface, \( \alpha \), was estimated from our measurements as the mean value of \( I_{\text{sky}}(t)/I(0, t) \); \( \alpha \) was assumed to be constant because no systematic variation was evident, and a value of 0.75 was applied to both lakes. The value of \( I_{\text{sky}}(t) \) integrated over the photoperiod was estimated from meteorological data for the expected irradiance over the course of a day, at the time of year corresponding to the sampling event. Only a single irradiance profile was available for each sampling event, so only one estimate of DLD was computed.

The estimate of \( I_{\text{sky}}(t) \) integrated over the photoperiod used here assumes clear-sky conditions. Therefore it overestimates on cloudy days, which are uncommon during warm weather in north Texas, but more frequent during cool weather. This systematic error inflates our estimates of DLD and compresses their variance because cool season estimates suffer more inflation from neglecting clouds.

**Measures of disturbance**

Many studies of disturbance in planktonic systems have focused on changes in depth or stability of the surface mixed layer (Chorus and Schlag 1993, Rojo and Alvarez Cobelas 1993, Flöder and Sommer 1999). Mixed layer depth determined (in meters) during sampling events was thus included as an index of distur-
bance. Depth of the mixed layer is strongly influenced by wind, so wind speed data were obtained from continuous air monitoring stations maintained by the Texas Commission on Environmental Quality. For each lake, the nearest station with a continuous record during the study period was used; these stations were within 15 km of the lakes. Reported 24-h mean wind speeds were averaged for the 10 d prior to each sampling event, as an index of preceding wind disturbances.

In reservoirs and natural lakes with high throughput, inflow events influence phytoplankton species composition and diversity (Reynolds and Lund 1988, Zohary et al. 1996, Hambright and Zohary 2000). Hydrological data were obtained from the Tarrant Regional Water District for EML and the U.S. Army Corps of Engineers for JPL. Reports include daily lake level (elevation, in meters above sea level), and from this the standard deviation in lake level over the 10 d prior to each sampling event was calculated as an index of preceding hydrological disturbance.

Variation in lake level integrates several disturbance processes and also depends on withdrawals and releases from the reservoirs. We thus considered two components of hydrologic disturbance that could be important on their own: rainfall and tributary inflow. Both disturbances increase reservoir volume and deliver nutrient and sediment loads. As an index of disturbance, rainfall over the 10 d preceding each sampling event was totaled from the reported daily data. As another index of disturbance, total tributary inflow over the 10 d preceding each sampling event was estimated from water budgets. Daily data reports include change in reservoir storage, rainfall, evaporation, withdrawals, and discharge from the reservoir. From these data, daily inflows excluding rainfall were estimated by calculating daily balances on water mass. Following procedures used by the management agencies, daily balances were then corrected to eliminate negative estimates of tributary inflow, presuming these to be erroneous. This adjustment was done monthly, by setting negative inflows to zero, and reducing positive inflows by a proportion that preserved the monthly mean inflow in the adjusted data. In practice, adjusted and raw estimates of tributary inflow were highly correlated. Adjusted daily estimates of tributary inflow were then totaled for the 10 d preceding each sampling event.

In computing disturbance indices, we adopted the 10-d interval for three reasons. First, estimates of algal growth rates for these lakes (Chrzanowski and Grover 2001a) imply median generation times of ~3.8 d, and disturbances at scales of roughly three times the generation time are thought to be especially relevant to phytoplankton diversity (Reynolds 1993). Second, sampling events in each lake were at least 14 d apart, and indices calculated over a 10-d interval are reasonably likely to contain one or more disturbance events, without introducing strong dependence between successive values. Third, exploratory analyses suggested that relationships between diversity and disturbance were similar when disturbance was measured over time scales ranging from 5 to 12 d, providing no reason to reject the a priori scale of 10 d.

Statistical procedures

Here we provide an overview of statistical procedures. Details and sample calculations for some analyses are presented in the Appendix. This study examines the correlation between a measure of diversity, $Y_t$ and the number limiting resources, $X_t$, where $t$ indexes the sampling time ($t = 1, \ldots, N$). A given resource $j$ is counted as limiting whenever its availability falls below a threshold $\theta_j$ (see Appendix Eqs. A.1 and A.2). We use Pearson's product-moment correlation coefficient, $r$, because in contrast to the rank correlation coefficient, $r^*$, it does not require data sorting and so is less computationally intensive. The relationship between diversity and number of limiting resources was approximately linear in the data of Interlandi and Kilham (2001) and appeared to be so in exploratory analyses of our own data.

Three questions naturally arise analyzing the association between diversity and the number of limiting resources. First, what values of the thresholds $\theta_j$ produce the strongest association of diversity with the number of limiting resources? Estimating the best-fit thresholds requires maximizing the correlation $r(Y_t, X_t)$ with respect to the thresholds $\theta_j$, defining limitation for each resource. Second, after obtaining the maximized correlation $r^*(Y_t, X_t)$, is it statistically significant, meaning, unlikely according to a reasonable null hypothesis? Third, what is the uncertainty in the estimated limitation thresholds $\theta_j$ that maximize the correlation?

To estimate the limitation thresholds $\theta_j$, maximizing the correlation between diversity and the number of limiting resources, we apply an exhaustive search algorithm. A given value of the threshold $\theta_j$ partitions the resource availability data into limiting and nonlimiting cases, and the exact value of $\theta_j$ achieving a given partition is not unique. When the resource availability data are ranked, any value of $\theta_j$ separating the same successive resource measurements partitions the data in the same way into limiting and nonlimiting cases. Thus we arbitrarily limit the values of $\theta_j$ to the midpoints between successive ranked values of resource availabilities and define a lowest $\theta_j$ as half the lowest observed availability and a highest $\theta_j$ as the highest observed availability plus the lowest $\theta_j$. There is inherent imprecision in estimating the limitation thresholds. At best, the analysis constrains an estimate to lie in the interval between two successive ranked observations of resource availability.

The set of thresholds defined above for each resource is finite (~$7 \times 10^4$ for our data sets), making it possible to search exhaustively for the combination of thresholds that maximizes the correlation between diversity and the number of limiting resources. Resource avail-
abilities should be examined prior to analysis to ensure that some fall below biologically plausible thresholds for limitation and some above. Fortunately, the literature on phytoplankton growth reviewed by Interlandi and Kilham (2001) suggests that their resource availability data, and ours, satisfy this test for at least some resources (phosphorus and nitrogen).

To estimate the statistical significance of the maximized correlation \( r^*(Y, X) \) between diversity and the number of limiting resources, we apply randomization methods. The diversity data \( Y \) are permuted using an algorithm and pseudo-random number generator based on recommendations of Manly (1991). For each permutation, the exhaustive search algorithm is applied to find the combination of limitation thresholds producing the highest correlation between diversity and the number of limiting resources. The highest correlation for the permuted data is compared to the observed maximized correlation \( r^* \), and from the proportion of exceedances among all permutations, a one-tailed \( P \) value is calculated for the probability of obtaining a correlation equal to or higher than the observed. A one-tailed test is used because theory predicts a positive correlation between diversity and the number of limiting resources. To estimate relatively low \( P \) values, 5000 permutations were run for each data set analyzed here (Edgington 1980).

Evaluating uncertainty in the estimated values of the limitation thresholds that maximize the correlation with diversity is the most difficult problem, because a number of complications preclude computing conventional confidence intervals. We suggest that judgments about how well the data constrain the values of limitation thresholds be made using goodness-of-fit profiles similar to the “sensitivity analysis” introduced by Interlandi and Kilham (2001). After obtaining the set of thresholds maximizing the correlation between diversity and the number of limiting resources, this correlation is plotted for all possible values of the threshold for a single resource, while keeping the other thresholds at the optimized values.

This procedure produces a profile of goodness-of-fit in relation to threshold values for each resource. Four idealized profiles illustrate the typical properties of such graphs and their interpretation (Fig. 1). First, any profile has the same correlation at the highest and lowest possible values of the threshold. Biologically, setting a given threshold to its highest or lowest value implies that limitation status does not vary: the resource in question is either always limiting or never limiting. Thus the correlation between diversity and the number of limiting resources takes the value determined by the other resources, with their limitation thresholds set to optimized values. In some cases, a clear peak occurs in the correlation associated with an intermediate threshold value for the given resource (e.g., curve A, Fig. 1). When the height of the peak greatly exceeds the correlation pertaining to the highest and lowest threshold values (dashed line in Fig. 1), then there is a threshold value for the given resource that distinguishes between limiting and nonlimiting cases and in doing so improves the correlation between diversity and the number of limiting resources. When the peak is very narrow, the range of threshold values producing a strong correlation is constrained, and we interpret this as indicating low uncertainty in the estimated best-fit threshold for that resource.

Other possible profiles signify less confidence in the estimated thresholds. Comparing curve B to curve A in Fig. 1, the former has a lower and broader peak, showing that less improvement in the correlation of diversity with the number of limiting resources results from setting the threshold to its optimized value. For curve B, a wide range of threshold values produces a similar improvement in the correlation, implying greater uncertainty in the estimated threshold than for curve A. For curve C, the correlation between diversity and the number of limiting resources exhibits a local maximum for an intermediate value of the limitation threshold, but this peak barely exceeds the correlation associated with the highest and lowest possible thresholds. This indicates that very little improvement in the correlation results from setting the threshold to its optimized value, and it is about as reasonable to assume that the resource in question is either always limiting or never limiting. Such a case provides weak evidence that the limitation status of the given resource contributes to the relationship with diversity, with a highly uncertain estimate of the limitation threshold. Finally, curve D illustrates a worst-case scenario, in which the strongest correlation between diversity and the number of limiting resources occurs for the highest and lowest possible threshold values. The best correlations result from assuming that the resource is either always or never limiting, and in this case there is no evidence that changes in limitation by this resource affect diversity.

![Threshold](image-url)
Fig. 2. Physical variables in Eagle Mountain Lake and Joe Pool Lake, north Texas, USA. In panels (D) and (I), lines with filled circles show mixing depths at each sampling event (left scale); plain lines show daily wind speeds (right scale). In panels (E) and (J) boxes with roman numerals indicate the quarters of each year of the study. For panels (A) and (F), "m asl" indicates meters above sea level.

Although such profiles of correlation vs. threshold value permit some judgments about estimated limitation thresholds and their biological implications, we are unable to go further in constructing inferences about these estimates. Therefore, evaluating estimated thresholds for biological reasonableness, in light of published studies of algal growth, remains important (Interlandi and Kilham 2001).

The methods described above were used to analyze the relationship between diversity and the number of limiting resources. Regression analysis was used to further examine the relationship between diversity, total algal biomass, selected disturbance measures, and the number of limiting resources. In all analyses, randomization methods were used to determine statistical significance, and further details are presented with the results.

RESULTS

Lake physics

Large seasonal and interannual variations in lake physics were observed over the course of this study in both reservoirs (Fig. 2). Although rainfall and inflow tended to be higher in spring and autumn than in winter and summer, a regional drought from the last quarter of 1999 to the end of the study drove variations in lake level that were not strictly seasonal. Lake level at EML dropped continually throughout the study and fell especially rapidly during the dry third quarter of 2000, due to heavy withdrawals for municipal water supplies (Fig. 2A). Despite dry weather during much of the latter part of the study, lake level in JPL did not decline continually. It was managed to accumulate water during the relatively wet second quarter of 2000 (Fig. 2F) and maintain downstream flows.

In both lakes, temperature varied seasonally from 7°–10°C in winter to ~30°C in summer (Fig. 2E, J). These temperature variations were not accompanied by seasonal thermal stratification. In both lakes, deep mixing prevailed during periods of low temperature (Fig. 2D, I). Wind speed tended to be high during the second quarter of each year when lakes were warming, and deep mixing frequently interrupted any stratification that developed.

Algal community composition

In both lakes, phytoplankton communities were broadly similar (Table 1): diatoms, chlorophytes, and
cyanobacteria usually had higher biomass than other algal divisions. In EML, common taxa included centric diatoms (Cyclotella and Stephanodiscus), Cryptomonas, Tetraedron, and unidentified nanoflagellates with greatest linear dimension of 10–20 μm (the larger of two size classes enumerated). In JPL, common taxa again included Cryptomonas, Cyclotella, Ceratium, Tetraedron, and smaller nanoflagellates (greatest linear dimension <10 μm).

**Relationships between diversity and biomass**

Because theories about diversity predict a curvilinear, concave-down relationship with biomass (Grime 1979, Tilman and Pacala 1993, Huston 1994), quadratic multiple regression was applied to genus richness in relation to total algal biomass (Table 2, Fig. 3). In EML, the linear term of the regression was significant (randomization test, \( P = 0.0044 \)), while the quadratic term was marginal (randomization test, \( P = 0.051 \)). The regression explained 23% of the variation in genus richness and was strongly influenced by the two data points with highest total algal biomass. In JPL, both linear and quadratic terms were significant (randomization tests, \( P < 0.01 \)), the regression model explained 35% of the variation in genus richness, and it was robust to removal of one or two points with high biomass. In both lakes, the quadratic regression models portray curvilinear, concave-down relationships in which diversity rises for most of the observed range of biomass and falls only for the highest observed biomasses (Fig. 3).

### Table 2. Quadratic regression models relating genus richness to total algal biomass.

<table>
<thead>
<tr>
<th>Term</th>
<th>Eagle Mountain Lake</th>
<th>Joe Pool Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Coefficient</td>
<td>SE</td>
</tr>
<tr>
<td>Linear</td>
<td>8.42</td>
<td>2.76</td>
</tr>
<tr>
<td>Quadratic</td>
<td>-1.67</td>
<td>0.78</td>
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*Note: \( P \) values are from randomization tests with 5000 permutations, with linear terms entered in the models before quadratic terms.*
in JPL ($r^* = 0.772, P = 0.0002$, randomization test). Goodness-of-fit profiles (Fig. 4) indicate varying degrees of confidence in the estimated limitation threshold for the four resources (Table 3). In EML, the profile for nitrogen shows that the best-fit threshold is associated with a peak correlation (0.570) well above the correlations associated with the highest and lowest thresholds (each 0.341), indicating that variations in nitrogen limitation contribute strongly to the relationship with diversity (Fig. 4A). This peak is broad, with two additional local maxima, and shows that a range of nitrogen thresholds from -3.5 to 8.9 μmol/L would produce a relationship about as strong as that of the optimized threshold of 3.67 μmol/L. The best-fit threshold for phosphorus has a peak correlation (0.570) that modestly exceeds the correlations associated with the highest and lowest thresholds (each 0.502), indicating that variations in phosphorus limitation might not contribute strongly to the relationship with diversity (Fig. 4B). The local peak at the estimated threshold of 0.161 μmol/L is very narrow, but additional local peaks imply that a range of phosphorus thresholds from -0.28 to 0.60 μmol/L would also produce a relationship about as strong as that of the optimized threshold. The goodness-of-fit profile for silicon shows great uncertainty, with no local maximum above the correlations associated with the highest and lowest thresholds (each 0.570, Fig. 4C). Thus the relationship between diversity and number of limiting resources is best if silicon is regarded either as always or never limiting. Interlandi and Kilham (2001) found the same result for silicon. Like them, we assign the highest admissible threshold of 129 μmol/L as the best-fit estimate, adopting the assumption that silicon is always limiting, based on growth-kinetic literature (reviewed by Interlandi and Kilham [2001]) suggesting that some diatoms are silicon-limited even at relatively high silicon concentrations. The best-fit threshold for light has a peak correlation (0.570) exceeding the correlations associated with the highest and lowest thresholds (each 0.436), indicating that variations in light limitation contribute to the relationship with diversity (Fig. 4D). The breadth of this peak shows that a range of light thresholds from -10 to 14 mol/m² would produce a relationship about as strong as that of the optimized threshold of 11.7 mol/m².

In JPL, the best-fit threshold for nitrogen has a peak correlation (0.772) that greatly exceeds the correlations associated with the highest and lowest thresholds (each 0.496), indicating that variations in nitrogen limitation contribute strongly to the relationship with diversity (Fig. 4E). The breadth of this peak shows that a range of nitrogen thresholds from -6.6 to 11 μmol/L would produce a relationship about as strong as that of the optimized threshold of 10.1 μmol/L. The goodness-of-fit profile for phosphorus shows that the best-fit threshold has a peak correlation (0.772) that slightly exceeds the correlations associated with the highest and lowest thresholds (each 0.736), indicating that variations in phosphorus limitation contribute weakly to the relationship with diversity (Fig. 4F). The peak around the optimized threshold of 0.255 μmol/L suggests that a narrow range of phosphorus thresholds from -0.24 to 0.29 μmol/L would produce a relationship about as strong that of the optimized threshold. The goodness-of-fit profile for silicon again reveals high uncertainty with no local maximum above the correlations associated with the highest and lowest thresholds (each 0.772, Fig. 4G). As in EML, we assign the highest admissible threshold of 109 μmol/L as the best-fit estimate, adopting the assumption that silicon is always limiting. The goodness-of-fit profile for light shows similar uncertainty, with no local maximum above the correlations associated with the highest and lowest thresholds (each 0.772, Fig. 4D). Thus the relationship between diversity and number of limiting resources is best if light is regarded either as always or never limiting. We adopt the former assumption that light is always limiting in assigning the highest threshold of 32.5 mol/m² as the best-fit estimate. Deep mixing is common in JPL (Fig. 21) and the water is turbid (attenuation coefficient, K, ranges 0.5-1.6 m⁻¹); thus it seems bi-
Fig. 4. Goodness-of-fit profiles based on the correlation (r) between diversity and number of limiting resources. For each resource in each lake, the limitation threshold is varied through the full range of admissible values while limitation thresholds for other resources are assigned their optimized values. Key to abbreviations: DIN, dissolved inorganic nitrogen; SRP, soluble reactive phosphorus; SRSi, soluble reactive silicon; DLD, daily light dose.
TABLE 3. Estimated limitation thresholds of resource availability in each lake.

<table>
<thead>
<tr>
<th>Resource</th>
<th>Eagle Mountain Lake (N = 50)</th>
<th>Joe Pool Lake (N = 51)</th>
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<tbody>
<tr>
<td>DIN (µmol/L)</td>
<td>3.67 20</td>
<td>10.1 29</td>
</tr>
<tr>
<td>SRP (µmol/L)</td>
<td>0.16 9</td>
<td>0.255 44</td>
</tr>
<tr>
<td>SRSi (µmol/L)</td>
<td>129* 50</td>
<td>109* 51</td>
</tr>
<tr>
<td>DLD (mol/m²)</td>
<td>11.7 33</td>
<td>32.5* 51</td>
</tr>
</tbody>
</table>

Notes: An asterisk indicates cases where the highest and lowest admissible thresholds both optimized the correlation between diversity and the number of limiting resources, and the highest admissible threshold is reported. Resource abbreviations are: DIN, dissolved inorganic nitrogen; SRP, soluble reactive phosphorus; SRSi, soluble reactive silicon; DLD, daily light dose.

Nitrogen showed strongly seasonal dynamics, with low concentration during warm periods and higher concentration during cold periods (Fig. 5A, F). In both lakes, phosphorus displayed similar but weaker tendencies; in particular the increase in concentration appeared to be suppressed during the dry winter of 1999–2000 (Fig. 5A, F). For both nitrogen and phosphorus in both lakes, the estimated limitation thresholds (Table 3) depict limitation by these nutrients predominantly during warm weather (Fig. 5).

In contrast, silicon exhibited a continual increase in both lakes with less hint of seasonal variation (Fig. 5C, H). In EML, during 1998 silicon concentration followed temperature, rising from spring through autumn and declining in the winter of 1998–1999. A similar rise in the spring through autumn of 1999, was not followed by a decline in the dry winter of 1999–2000, and concentration remained high through the end of the study (Fig. 5C). In JPL, silicon concentration was also elevated in the dry winter of 1999–2000 and especially during the very dry third quarter of 2000 (Fig. 5H).

Dynamics of resource availabilities were broadly similar in the two lakes (Fig. 5). In both EML and JPL, it is logically plausible to assume that light is usually limiting.

Dynamics of resource availabilities were broadly similar in the two lakes (Fig. 5). In both EML and JPL, it is logically plausible to assume that light is usually limiting.

![Fig. 5](image-url)
Light availability was low in both lakes during cold periods and high but variable during warm periods (Fig. 5D, I). The limitation threshold for light was reasonably well determined in EML and depicts light limitation as occurring continually during winter, but only occasionally in summer. Given the similarities of resource dynamics in these lakes, the differing strengths of their relationships between diversity and the number of limiting resources resulted primarily from differences in the dynamics of diversity. Diversity in EML did not appear to be strongly seasonal in the first year of the study, though increases did occur in the warm periods of 1999 and 2000 (Fig. 5E). The modest correlation between diversity and number of limiting resources results from the increased number of limiting resources indicated during these two summers. In contrast, diversity in JPL was more strongly seasonal: in every year it was low during winter and spring, rose in summer, and fell in autumn (Fig. 5J). The strong correlation of diversity with the number of limiting resources results from limitation by nitrogen and phosphorus during the period of elevated diversity in warm weather in every study year in JPL.

Relationships between diversity and disturbance

To explore the association between diversity and disturbance and compare it to the relationship with number of limiting resources, we used regression analysis. We sought first to identify disturbance indices having strong relationships with diversity. Because the intermediate disturbance hypothesis proposes that such relationships are curvilinear, quadratic regressions of genus richness against each disturbance index were conducted: quadratic terms were entered in regression models after linear terms, and significance of these terms was assessed in this order with randomization tests (following the algorithm of Manly [1991]).

In EML, three disturbance indices had moderately strong relationships with diversity: the standard deviation of lake level, inflow, and wind speed (Table 4). Regression modeling suggested a quadratic, concave-down relationship of diversity with the standard deviation of lake level (Fig. 6A), but significance of the quadratic term was sensitive to deletion of a single data point with an exceptionally high value of the standard deviation. With this datum deleted, the linear term remained significant and $R^2$ dropped only slightly from 0.292 to 0.288. Regression modeling suggested a nearly linear decrease of diversity with inflow in EML, but this relationship was sensitive to deletion of a single data point with an exceptionally high inflow. With this datum deleted, neither the linear nor the quadratic term remained significant, and $R^2$ dropped from 0.180 to 0.064. Regression modeling also suggested a nearly linear decrease of diversity with wind speed in EML, a relationship without isolated, extreme observations (Fig. 6B). Despite the presence of influential, extreme observations in some regressions, we report analyses based on all data here (and below) because the underlying meteorological data have no discernable errors and corroborate one another.

In JPL, two disturbance indices had moderately strong relationships with diversity: mixing depth and wind speed (Table 4). Regression modeling suggested nearly linear decreases of diversity with both mixing depth and wind speed in JPL (Fig. 6A, B). Neither relationship had isolated, extreme observations.
FIG. 6. Diversity in relation to number of limiting resources and disturbance in (A–C) Eagle Mountain Lake (EML) and (D–F) Joe Pool Lake (JPL). Observations are coded by the number of limiting resources as determined from best-fit estimates of limitation thresholds (Table 3): solid circles, one limiting resource; open circles, two limiting resources; solid triangles, three limiting resources; open triangles, four limiting resources. (A) Diversity and the standard deviation of lake level in EML. Lines show predictions of the best regression model (Table 5) for the mean value of wind speed and different numbers of limiting resources: solid line, one limiting resource; long dashed line, two limiting resources; medium dashed line, three limiting resources; short dashed line, four limiting resources. (B) Diversity and mean wind speed in EML. Lines show predictions of the best regression model (Table 5) for the mean value of the standard deviation of lake level and different numbers of limiting resources as in panel (A). (C) Diversity and number of limiting resources in EML. The line shows predictions of the best regression model (Table 5) for mean values of the standard deviation of lake level and mean wind speed. (D) Diversity and mixing depth in JPL. Lines show predictions of the best regression model (Table 6) for different numbers of limiting resources: long dashed line, two limiting resources; medium dashed line, three limiting resources; short dashed line, four limiting resources. (E) Diversity and mean wind speed in JPL. (F) Diversity and number of limiting resources in JPL. The line shows predictions of the best regression model (Table 6) for the overall mean of wind speed.
TABLE 5. Best predictive regression model for diversity in Eagle Mountain Lake.

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
<th>SE</th>
<th>P</th>
<th>R²</th>
<th>C_p</th>
<th>Overall P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. limiting resources</td>
<td>3.23</td>
<td>1.00</td>
<td>0.019</td>
<td>0.518</td>
<td>2.685</td>
<td>0.0002</td>
</tr>
<tr>
<td>SD of lake level</td>
<td>1.08</td>
<td>0.39</td>
<td>0.038</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(SD of lake level)²</td>
<td>-1.63</td>
<td>0.98</td>
<td>0.23</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Wind speed</td>
<td>-0.086</td>
<td>0.036</td>
<td>0.082</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>(Wind speed)²</td>
<td>-2.69</td>
<td>1.24</td>
<td>0.11</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: P values from randomization tests with 5000 permutations are given for each term when added last to the model; the overall P value for explained variation is also based on a randomization test with 5000 permutations.

† For an explanation of C_p, see Kleinbaum et al. (1998).

 Relationships between diversity, number of limiting resources, and disturbance

After identifying disturbance indices associated with diversity in each lake, we sought to compare resources and disturbance as predictors of diversity with the following regression modeling strategy. For each lake, we first selected the two disturbance indices having the strongest relationships with diversity as revealed by the quadratic regressions summarized above. For EML, these were the standard deviation of lake level and wind speed. For JPL, these were mixing depth and wind speed. Then we constructed a “maximal” multiple regression model with the following terms: one linear term for the number of limiting resources as determined above from the best-fit estimates of limitation thresholds (Table 3); two linear terms for the chosen disturbance indices; two quadratic terms for the chosen disturbance indices; two second-order interaction terms for the number of limiting resources with each of the disturbance indices; and two third-order interaction terms for the number of limiting resources with the square of each of the disturbance indices. Quadratic terms for disturbance indices were included because of their theoretical basis in the intermediate disturbance hypothesis, and interactions with the number of limiting resources were included because mechanistic relationships could occur among resources and disturbance indices (e.g., when inflow or deep mixing supplies nutrients to the surface waters). To reduce collinearity, number of limiting resources and disturbance indices were centered by subtracting the mean before calculating the higher order terms.

We then sought to simplify the maximal model and find the best predictive model based on the statistics R² and C_p, following the criteria that R² should be close to that of the maximal model and C_p close to \(2p - k + 1\), where \(p\) is the number of terms in a simplified model and \(k\) the number of terms in the maximal model (Kleinbaum et al. 1998). All possible regression models derived by simplifying the maximal model were examined, subject to the restrictions (1) that models with higher order terms contain all component lower order terms and (2) that linear terms for the number of limiting resources and at least one disturbance index be retained. The first restriction is necessary to avoid bias, and the second, was imposed so that the final model would serve to compare resources and disturbance as predictors of diversity.

Once the best predictive model was chosen for each lake, we tested significance with a randomization test (Manly 1991). We also decomposed the explained proportion of variance in diversity into three components associated uniquely with number of limiting resources, uniquely with disturbance, and with shared variations in resources and disturbance, following the approach of Whittaker (1984), which is the univariate version of the decomposition undertaken for multivariate ecological data by Borcard et al. (1992).

The best predictive model for diversity in EML contained five terms: number of limiting resources and linear and quadratic terms for both the standard deviation of lake level and wind speed (Table 5). Interaction terms were never significant in any of the candidate models examined. The best model explained 51.8% of the variation in diversity, compared to 52.6% explained by the maximal model. Three terms are not significant, but removing them reduces explained variation considerably, so we chose to retain them. There is also one highly influential datum involving an exceptionally high standard deviation of lake level (Fig. 6A). However, this observation is well supported in the other data and is due to a rapid rise in lake level caused by heavy inflow and rainfall. Therefore we retained this datum in fitting the best regression model. This regression model portrays curvilinear, concave-down relationships between diversity and the disturbance indices, standard deviation of lake level and wind speed (Fig. 6A, B), and an increasing relationship between diversity and number of limiting resources (Fig. 6C). However, the quadratic relationship with the standard deviation of lake level is heavily influenced by the extreme datum noted above, and through the range of typical observations, diversity increases with the standard deviation of lake level.

The best predictive model for diversity in JPL contained two terms: number of limiting resources and a linear term for mixing depth (Table 6). Interaction terms were never significant in any of the candidate models examined. The best model explained 63.9% of the variation in diversity, compared to 66.6% explained...

<table>
<thead>
<tr>
<th>Term</th>
<th>Coefficient</th>
<th>SE</th>
<th>P</th>
<th>$R^2$</th>
<th>Cp†</th>
<th>Overall P</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. limiting resources</td>
<td>6.89</td>
<td>0.84</td>
<td>0.0002</td>
<td>0.639</td>
<td>-0.793</td>
<td>0.0002</td>
</tr>
<tr>
<td>Mixing depth</td>
<td>-0.40</td>
<td>0.17</td>
<td>0.15</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Notes: *P* values from randomization tests with 5000 permutations are given for each term when added last to the model; the overall *P* value for explained variation is also based on a randomization test with 5000 permutations.

† For an explanation of $C_p$, see Kleinbaum et al. (1998).

The predictive regression models reproduced much of the temporal pattern of diversity in both lakes (Fig. 7), including the relatively low diversity in EML during the first year of the study and the highly seasonal patterns in JPL. We sought to estimate the components of explained variance uniquely associated with number of limiting resources and disturbance, respectively. Following Whittaker (1984), the proportion of variance in diversity uniquely associated with number of limiting resources is the change in $R^2$ that results from adding the number of limiting resources to a regression model already containing diversity indices. Likewise, the proportion of variance in diversity uniquely associated with disturbance is the change in $R^2$ that results from adding disturbance indices to a regression model already containing the number of limiting resources. Given covariation between the number of limiting resources and disturbance, a regression model with both sets of independent variables explains a higher proportion of variance in diversity than the sum of these unique components, with this difference representing the proportion of variance associated with covariation in the number of limiting resources and disturbance.

Based on the estimated components of explained variance, the two lakes differ in how strongly diversity is associated with the number of limiting resources and disturbance (Table 7). In EML, 11.5% of the variance in diversity is uniquely associated with the number of limiting resources, while 19.3% is uniquely associated with disturbance and 21.0% with shared variations in resources and disturbance. Thus the influence of the number of limiting resources is relatively weak, and much of this influence results from covariation with disturbance. Most of this covariation is due to a cor-

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**Fig. 7.** Observed diversity (line with dots) and predictions (plain solid line) from the best regression models (Tables 5 and 6) for each lake. Boxes with roman numerals indicate the quarters of each year of the study.
TABLE 7. Decomposition of explained variance based on the predictive regression models for diversity (Tables 5 and 6).

<table>
<thead>
<tr>
<th>Component</th>
<th>Eagle Mountain Lake</th>
<th>Joe Pool Lake</th>
</tr>
</thead>
<tbody>
<tr>
<td>Explained variance</td>
<td></td>
<td></td>
</tr>
<tr>
<td>No. limiting resources</td>
<td>0.115</td>
<td>0.505</td>
</tr>
<tr>
<td>Disturbance</td>
<td>0.193</td>
<td>0.043</td>
</tr>
<tr>
<td>Covariation in resources and disturbance</td>
<td>0.210</td>
<td>0.092</td>
</tr>
<tr>
<td>Unexplained variance</td>
<td>0.482</td>
<td>0.361</td>
</tr>
</tbody>
</table>

Note: The sum of the components of explained variance is $R^2$ for the regression model.

relation between the number of limiting resources and wind speed ($r = -0.375$), while the correlation between number of limiting resources and the standard deviation of lake level is weak ($r = 0.006$). In contrast, the relative influence of limiting resources on diversity is much stronger in IPL: 50.5% of the variance in diversity is uniquely associated with the number of limiting resources, while only 4.3% is uniquely associated with disturbance and 9.2% with shared variations in resources and disturbance. These shared variations result from a correlation between number of limiting resources and mixing depth ($r = -0.214$).

**DISCUSSION**

We sought to assess the influence of limiting resources on phytoplankton diversity in the lakes we studied, to compare this influence to that of disturbance, and to assess whether the role of disturbance was consistent with the Intermediate Disturbance Hypothesis. We found that the influences of both limiting resources and disturbance on phytoplankton diversity can be strong, but that they also vary between lakes and also cannot be disentangled. We found mixed support for the Intermediate Disturbance Hypothesis. Before discussing these findings we note a caveat.

For this study, we analyzed in detail genus richness as a measure of phytoplankton diversity. In exploratory analyses we computed other measures of diversity: “species” richness as the count of taxa identified at greatest resolution in our data (67% of taxa identified to species level, 32% to genus level) and Simpson’s diversity index based on biomass, as used by Interlandi and Kilham (2001), at genus and species levels of taxonomic resolution. In IPL, all diversity measures were strongly correlated with the number of limiting resources: the lowest optimized correlation ($r^*$) was that for genus richness (0.772) and the highest for species richness (0.795). In EML, genus richness had the highest correlation with the number of limiting resources ($r^* = 0.570$) and Simpson’s index for species the lowest (0.516). In both lakes genus and species richness had very similar relationships to disturbance indices, and in IPL this also held for measures based on Simpson’s index. However, measures based on Simpson’s index in EML were unrelated to disturbance indices. A strong bloom of the dinoflagellate *Ceratium hirundinella* (up to 50% of biomass) in EML during summer 2000 greatly decreased Simpson’s index, but not richness, leading these classes of diversity measures to disagree. Apart from relationships with disturbance in EML, our results appear robust to alternative measures of diversity.

As a prelude to the central themes of this study, we examined relationships between phytoplankton diversity and biomass. Theory that considers the influence of limiting resources or disturbance on diversity often leads to a relationship with biomass: either concave-down (Grime 1979, Tilman and Pacala 1993, Huston 1994) or monotonically decreasing (Siegel 1998). Other studies of phytoplankton diversity have found the latter (Sommer 1993, Interlandi and Kilham 2001), in contrast to the concave-down relationships found here, although high variability complicates this comparison. Relationships between diversity and biomass suggest that limiting resources or disturbance might be important controls of phytoplankton diversity in the lakes studied. In theory many mechanisms are consistent with diversity–biomass relationships, so such relationships alone do little to diagnose important processes.

With a correlation coefficient of 0.77, the association between phytoplankton diversity and the number of limiting resources in IPL is statistically significant ($P = 0.0002$, randomization test) and stronger than that in EML and in three lakes in the Yellowstone ecosystem (Interlandi and Kilham 2001). The corresponding rank-correlation coefficient was 0.35 in an analysis pooling data from all three Yellowstone lakes. The correlation between diversity and the number of limiting resources was 0.57 in EML, which is also larger than that found in the Yellowstone lakes, but the correlation in EML was not statistically significant ($P = 0.083$, randomization test). Though weaker, the correlation in Yellowstone lakes was based on a larger sample size ($N > 200$) than the correlations in Texas reservoirs ($N = 50$ or 51). Thus it is possible that the correlation in Yellowstone lakes is statistically significant. Our experience leads us to recommend randomization methods to assess such significance. Random permutations of our data sets often produce apparent correlations in a range of 0.3–0.5 that would be deemed statistically significant by conventional methods with large sample sizes ($N >$...
50). Randomization tests appear much more conservative for the analyses conducted here.

In JPL, the relationship between diversity and number of limiting resources displayed consistent seasonal dynamics, while such dynamics were less apparent in EML (compare Fig. 5E, J). According to our analyses, nitrogen and phosphorus regularly limited phytoplankton in summer, increasing the number of limiting resources relative to winter. The longer mean water residence time in JPL (2.5 yr) than in EML (0.5 yr) could allow stronger internal nutrient dynamics relative to external forcing by inflows, leading to seasonal depletion of multiple nutrients and promoting phytoplankton diversity as predicted by resource competition theory.

Resource competition theory also suggests that the less eutrophic status of JPL compared to EML could produce its stronger relationship between diversity and number of limiting resources. When resource supplies are moderate, natural variations in supply produce large relative changes in resource ratios, providing opportunities for many species to coexist; when resource supplies are high, natural variations in supply produce smaller relative changes in resource ratios, favoring dominance by the small number of species best adapted to that narrow range of resource ratios (Tilman 1982). The weaker relationship between diversity and number of limiting resources in the relatively oligotrophic Yellowstone lakes (Interlandi and Kilham 2001) is consistent with the prediction that resource-ratio effects are also weaker in resource-poor habitats than in those with moderate resource supplies (Tilman 1982). The proposed relationships between resource-ratio effects, diversity, and resource supplies have been questioned, due to debatable underlying assumptions (Abrams 1997) but our study appears consistent with them.

The equilibrium theory of resource competition predicts that each additional limiting resource permits one additional coexisting species, so that the relationship between genus richness and limiting resources should have a slope of one at most (if additional species belong to different genera). In contrast, relationships between genus richness and the number of limiting resources alone have slopes of 4.3 and 7.8 in EML and JPL, respectively (with somewhat lower slopes in the predictive multiple regressions including disturbance indices; Tables 5 and 6, illustrated in Fig. 6C, F). Slopes steeper than one might indicate that with high numbers of limiting resources, competitive chaos elevates the number of coexisting competitors above that predicted by equilibrium theory (Huisman and Weissing 1999). Alternatively, high numbers of limiting resources might interact with other processes such as disturbance or spatiotemporal heterogeneity, to produce diversity above that predicted by resources alone.

Resource competition theory predicts the relationships we found between diversity and the number of limiting resources in these lakes. Previous studies of phytoplankton and nutrient dynamics in these lakes corroborate this mechanism. Grover et al. (1999) determined kinetics of nitrogen- and phosphorus-dependent growth of phytoplankton taxa from Texas reservoirs (including EML). When nitrogen or phosphorus is below the limitation thresholds estimated here for either lake, many of these taxa would suffer limited growth at 85% or less of their maximal rates (the criterion for limitation suggested by Interlandi and Kilham [2001]). During this study, enrichment bioassays and seston stoichiometry were also used as indicators of phytoplankton nutrient limitation (Chrzanowski and Grover 2001a) and corroborate limitations inferred from relationships with diversity. In both lakes, when nitrogen and phosphorus concentrations are below the estimated limitation thresholds, growth responses to enrichment are often stronger than during nonlimited periods. In both lakes, when phosphorus concentrations are below the estimated limitation thresholds, seston N:P ratios are usually higher than during nonlimited periods. Interestingly, previous bioassay studies found that limitation by trace metals was common (Sterner [1994] for JPL; Chrzanowski and Grover [2001a] for EML and JPL). The high diversity of both lakes suggests that there are more limiting resources than the four studied here, and metals such as iron and molybdenum are likely candidates.

When interpreting the relationship between diversity and the number of limiting resources, we portrayed the latter as causing the former through the mechanism of resource competition. However, recent experiments manipulating plant diversity suggest the converse: that high diversity causes depletion of available resources (Naeem et al. 1994, Tilman et al. 1996, 1997, Hooper and Vitousek 1997, Engelhardt and Ritchie 2001; but see Grime 1997, Huston 1997). If factors other than resource competition regulate diversity in these lakes, there may nevertheless be patterns resembling those expected from resource competition. We have presented evidence that disturbance affects diversity in these lakes. Trophic relationships and “top-down effects” mediated by grazers also affect phytoplankton diversity (Leibold 1996, 1999). Downing (2002) determined zooplankton densities in these lakes during the last year (only) of this study, finding that small zooplankton such as rotifers and nauplii were most numerous, and used dilution experiments to estimate phytoplankton mortality to grazing by microzooplankton (rotifers, nauplii, and protozoa). Phytoplankton diversity appeared unrelated to either zooplankton density or grazing mortality in EML. In JPL, there was a negative correlation between diversity and estimated mortality to microzooplankton grazing ($r = -0.62, P = 0.026$, randomization test) and negative correlations of similar magnitude between phytoplankton diversity and densities of several crustacean grazers (J. P. Grover and M. Downing, unpublished data).

We found that several indices of disturbance were associated with phytoplankton diversity in these lakes,
though the indices with strongest associations and the nature of their relationships differed between lakes. Diversity was related to wind speed in both lakes, to mixing depth in JPL, and to the standard deviation of lake level in EML. Relationships with mixing depth, wind speed, or other indicators of mixing events are reported in earlier observational studies (Chorus and Schlag 1993, Sommer 1993, Trifonova 1993; but see Rojo and Alvarez Cobelas 1993), and experimental mixing in mesocosms altered phytoplankton diversity (Flöder and Sommer 1999).

Although wind speed was also associated with diversity in EML, mixing depth was not. The relationship between diversity and the standard deviation of lake level in EML suggests that higher wind speed might simply be associated with hydrological drivers of variation in lake level, such as rainfall and inflow. That no convincing relationships were found between diversity and these latter two indices of disturbance might indicate that as a more integrative measure of disturbance, variation in lake level is more useful than its components. Our findings for EML agree with earlier studies linking phytoplankton to hydrological events. In a natural riverine lake (Grasmere, United Kingdom), events of hydraulic flushing strongly influenced phytoplankton species composition and population dynamics (Reynolds and Lund 1988), although diversity was not examined. In the Hartbeesport Dam reservoir (South Africa), events of hydraulic flushing reduced the summertime dominance of phytoplankton by a single species, *Microcystis aeruginosa*, thus increasing diversity (Zohary et al. 1996, Hambright and Zohary 2000).

At face value, regression modeling supports the predictions of the Intermediate Disturbance Hypothesis in EML, for both the standard deviation of lake level and wind speed. However, curvilinearity for the standard deviation of lake level rests on a single observation, out of the normal range of variation. Regression modeling suggests that phytoplankton diversity in EML peaks when the standard deviation of lake level is ~9 cm in 10 d. All but one extreme observation are below this value, where diversity is an increasing function of the standard deviation of lake level. Regression modeling also suggests that phytoplankton diversity in EML peaks when the mean wind speed is ~3 m/s over 10 d, a value roughly equal to the lower quartile for 10-d mean wind speed. Wind speed thus usually exceeds that maximizing diversity, falling on the descending limb of the relationship with diversity. In JPL, regression modeling suggests monotonically decreasing relationships of diversity with mixing depth and wind speed. These could be the descending limbs of concave-down relationships, but that would imply that mixing depths and wind speeds low enough to produce the ascending limb are rare. Therefore, in both lakes, diversity is usually a monotonic function of disturbance.

Previous studies of phytoplankton diversity and disturbance also provide mixed support for the Intermediate Disturbance Hypothesis. A field mesocosm experiment found the predicted concave-down relationships between phytoplankton diversity and both the frequency and intensity of mixing disturbances (Flöder and Sommer 1999). In some observational and comparative field studies, relationships between phytoplankton diversity and disturbance have been interpreted as supporting the Intermediate Disturbance Hypothesis (Chorus and Schlag 1993, Sommer 1993, Trifonova 1993). As each of these authors emphasizes, however, the field evidence is inconclusive for various reasons. In one field study, phytoplankton diversity was unrelated to disturbance indices (Rojo and Alvarez Cobelas 1993).

Classical definitions of disturbance emphasize processes that decrease biomasses of competitors (e.g., Grime 1979), but do not directly affect their resources. In contrast, disturbances studied by limnologists affect both competitor biomass and resource availability. Episodic mixing reduces available light by circulating algae through deeper, darker water and can increase nutrient availability when nutrient-rich deep water is mixed to the surface. Rainfall and inflow mobilize and deliver nutrients from the watershed and can also reduce light availability by delivering sediment. In our lakes, higher wind speed and deeper mixing are associated with fewer limiting resources, suggesting that wind-induced nutrient enrichment occurs. Such coupling of disturbance and resource dynamics complicates the dissection of the controls on diversity in phytoplankton. In one lake (EML), shared variation in resources and disturbance explained more variation in phytoplankton diversity than the unique effects of these factors, and in the other lake (JPL), shared variation in resources and disturbance explained more variation in phytoplankton diversity than the unique effect of disturbance.

In conclusion, this study shows that limiting resources and disturbance influence the diversity of phytoplankton in the field. These factors vary in relative importance between lakes of similar size and climatic setting. Moreover, this study shows that the coupling of limnological disturbances to resource availability can lead to large joint effects of these factors on diversity. Unique influences of disturbance or limiting resources on diversity were measured and were large for limiting resources in one lake, but we conjecture that further study of disturbance and limiting resources will often find large, shared influences on phytoplankton diversity.

Acknowledgments

This work was supported by US EPA Grant R825868 to J. P. Grover and T. H. Chrzanowski. Although the research described in this article has been funded by the U.S. Environmental Protection Agency, it has not been subjected to the Agency’s required peer and policy review and therefore does
not necessarily reflect the views of the Agency and no official endorsement should be implied. We thank F. Krangioso, J. Hardwick, M. Hurt, K. Pennebaker, B. Smith, R. Miller, M. A. Stout, and R. A. Curry for technical assistance. Hydrological data were provided by the Tarrant Regional Water District (TRWD) and the U.S. Army Corps of Engineers. We thank M. Ernst (TRWD) and G. Clingenpeel (Trinity River Authority) for discussions of reservoir dynamics and P. N. Grover for advice on wind data. We thank S. Findlay and three anonymous reviewers for comments on an earlier draft of this paper.

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APPENDIX
A description of computational procedures is available in ESA's Electronic Data Archive: Ecological Archives M074-012-A1.

SUPPLEMENT
Files containing variables analyzed in this paper and the biomasses of algal genera in each lake are available in ESA's Electronic Data Archive: Ecological Archives M074-012-S1.