Limnol. Oceanogr., 57(2), 2012, 554–566

doi:10.4319/lo.2012.57.2.0554

Allometric scaling and taxonomic variation in nutrient utilization traits and maximum growth rate of phytoplankton

Kyle F. Edwards,[a,*] Mridul K. Thomas,[a,b] Christopher A. Klausmeier,[a,c] and Elena Litchman [a,b]

[a] Kellogg Biological Station, Michigan State University, Hickory Corners, Michigan
[b] Department of Zoology, Michigan State University, Hickory Corners, Michigan
[c] Department of Plant Biology, Michigan State University, Hickory Corners, Michigan

Abstract

Nutrient utilization traits can be used to link the ecophysiology of phytoplankton to population dynamic models and the structure of communities across environmental gradients. Here we analyze a comprehensive literature compilation of four traits: maximum nutrient uptake rate; the half-saturation constant for nutrient uptake; the minimum subsistence quota, measured for nitrate and phosphate; and maximum growth rate. We also use these traits to analyze two composite traits, uptake affinity and scaled uptake affinity. All traits tend to increase with cell volume, except for scaled uptake affinity and maximum growth rate, which tend to decline with cell volume. Most scaling relationships are the same for freshwater and marine species, although important differences exist. Most traits differ on average between major taxa, but between-taxon variation is nearly always due to between-taxon variation in volume. There is some evidence for between-trait correlations that could constrain trait evolution, but these correlations are difficult to disentangle from correlation driven by cell volume. These results should enhance the parameterization of models that use size or taxonomic group to structure physiological variation in phytoplankton communities.

Nutrient utilization traits have long been used to link the ecophysiology of phytoplankton to competitive interactions and the structure of communities across environmental gradients (Eppley et al. 1969; Tilman 1982). Such traits can be used to parameterize models that predict population dynamics and competitive outcomes in the laboratory (Grover 1991), implying that trait-based models may allow a mechanistic understanding of natural dynamics and distributions (Litchman and Klausmeier 2008). Many traits have been shown to scale allometrically with cell volume, such as maximum nutrient uptake rate, the half-saturation constant for uptake, subsistence quotas, and uptake affinity (Shuter 1978; Grover 1989; Litchman et al. 2007; Tambi et al. 2009). Such scaling relationships allow the parameterization of models that predict how optimal size, or community size structure, should vary with the nutrient supply regime. For example, the relative abundance of large cells is predicted to increase with increasing nitrogen supply (Irwin et al. 2006), and nitrogen supplied in pulses may allow large cells to outcompete small cells (Stolte and Riegman 1996; Litchman et al. 2009). Likewise, major phytoplankton taxa often differ in average trait values (Smayda 1997; Litchman et al. 2007), and this can permit a more mechanistic understanding of patterns in taxonomic structure and the biogeochemical effects of taxonomic variation. For example, annual fluctuation in the relative abundance of marine diatoms, coccolithophores, and prasinophytes can be predicted from a model that incorporates taxonomic differences in nutrient and light utilization traits (Litchman et al. 2006); and predicted seasonal patterns of oceanic CO_2 uptake are altered by the inclusion of a separate functional group representing coccolithophores (Signorini et al. unpubl.).

* Corresponding author: edwar466@msu.edu

In order to better quantify interspecific variation in nutrient utilization traits, we have comprehensively compiled trait data from published studies. Our compilation includes nitrate and phosphate utilization traits of both freshwater and marine species, as nitrogen and phosphorus are the main limiting nutrients in both environments (Hecky and Kilham 1988; Elser et al. 2007). We have also compiled maximum growth rates for a large number of freshwater and marine species. We use this compilation to ask a series of questions: (1) what are the power-law exponents that describe how each trait scales with volume; (2) do the exponents differ between marine and freshwater species; (3) do average trait values differ between taxa; (4) do between-taxon differences exist when controlling for between-taxon variation in cell volume; (5) how do empirical power-law exponents compare to theoretical predictions; and (6) is there evidence for trade-offs among traits that would constrain the evolution of competitive ability. Many of these questions have been addressed previously, in separate analyses using smaller datasets or a subset of traits (Banse 1976; Shuter 1978; Grover 1989; Tang 1995; Smayda 1997; Litchman et al. 2007). In this study we synoptically address these questions using a thorough collection of currently available data.

Methods

Trait compilation—We comprehensively searched the literature for traits related to nutrient uptake and nutrient-limited growth. Specifically, we collected parameters of the Michaelis-Menten model of nutrient uptake,

$$\text{Uptake} = V_{\max}\frac{R}{K+R} \quad (1)$$

and the Droop model of phytoplankton growth,

$$\text{Net growth} = \mu_\infty \left(1 - \frac{Q_{\min}}{Q}\right) - m \quad (2)$$

where $V_{\max}$ is the maximum cell-specific nutrient uptake rate (μmol nutrient cell^{-1} d^{-1}), K is the half-saturation constant for nutrient uptake (μmol nutrient L^{-1}), R is the external nutrient concentration (μmol nutrient L^{-1}), μ_∞ is the specific growth rate at infinite quota (d^{-1}), Q is the internal nutrient concentration or quota (μmol nutrient cell^{-1}), $Q_{\min}$ is the minimum quota at which growth rate equals zero, and m is the specific mortality rate (d^{-1}).

Here we focused on the parameters $V_{\max}$, K, and $Q_{\min}$, measured for nitrate and phosphate. We denoted the nitrate parameters as $V^{\mathrm{N}}_{\max}$, K^{N}, and $Q^{\mathrm{N}}_{\min}$, and the phosphate parameters as $V^{\mathrm{P}}_{\max}$, K^{P}, and $Q^{\mathrm{P}}_{\min}$. We compared nitrate but not ammonium parameters because ammonium parameters have been measured for many fewer species. We used studies in which temperature was at or near 20°C, and light was not severely limiting. It is possible that allometric relationships and taxonomic differences involving these parameters could vary with temperature, but data on the temperature dependence of these parameters are limited, and using studies performed close to 20°C allowed us to make consistent interspecific comparisons. The maximum rate of uptake ($V_{\max}$) often declines as cellular nutrient content increases (Morel 1987), and therefore we included only estimates of $V_{\max}$ measured under conditions of intracellular nutrient depletion. We included estimates of uptake kinetics both from studies using isotopic methods, and studies using the rate of depletion of filtrate nutrient concentration. Cell volumes were determined from the literature, if not measured in the focal studies. Species and traits present in the dataset are listed in the Web Appendix (Table A1, www.aslo.org/lo/toc/vol/vol_57/issue_2/0554a.html); the dataset included 64 marine species and 59 freshwater species. If the same trait was measured on multiple occasions for a species, we used the mean trait value in our analysis.

Uptake affinity, or $V_{\max}/K$, is often used as an indicator of nutrient uptake ability at limiting concentrations, because it quantifies the clearance rate of nutrients as the external nutrient concentration becomes close to zero (Healey 1980). In order to predict species' relative abilities to grow under nutrient limitation, it may be more useful to measure the uptake affinity scaled by the minimum nutrient quota, or $V_{\max}/(K \times Q_{\min})$ (Tambi et al. 2009). This quantity measures the clearance rate of nutrients, relative to the amount of nutrient needed to grow, when external nutrient concentration becomes close to zero. Furthermore, it can be shown that under Eqs. 1, 2, as $m \to 0$, for species with equal mortality rates, the winner in competition at equilibrium is the species with the greater scaled uptake affinity (Litchman et al. 2007; Edwards et al. 2011). Therefore, we quantified allometric relationships and between-taxon differences for both uptake affinity and scaled uptake affinity, for nitrate and phosphate; we will refer to these as N affinity, P affinity, scaled N affinity, and scaled P affinity. We also tested whether scaled uptake affinity is an effective predictor of the outcome of competition by comparing the outcomes of published P-limited chemostat experiments to the outcomes predicted by those species' scaled uptake affinities. We found that scaled P affinity correctly predicted the winner in competition in 10 out of 13 pairwise comparisons, in spite of the fact that dilution rates varied from 0.07 to 0.5 d^{-1} (*see* Web Appendix, Table A1). We therefore consider scaled uptake affinity to be a useful proxy for equilibrium competitive ability under nutrient limitation. We refer to uptake affinity and scaled uptake affinity as "composite" nutrient traits, similar to R^*, defined as the break-even nutrient concentration at which growth rate equals mortality rate (Tilman 1982). We refer to $V_{\max}$, K, and $Q_{\min}$ as "primary" nutrient traits.

We also compiled estimates of maximum growth rate, $\mu_{\max}$, for 105 marine species and 124 freshwater species (*see* Web Appendix, Table A1). These estimates were compiled from studies of nutrient-limited growth, light-limited growth, and maximum growth rate as a function of temperature. We compiled maximum achievable growth rates, rather than μ_∞ from the Droop model (Eq. 2), because measurements of $\mu_{\max}$ were available for more species. If only μ_∞ was available, when possible we converted to $\mu_{\max}$ using the equation $\mu_{\max} = \mu_\infty \times (Q_{\max} - Q_{\min})/Q_{\max}$ where $Q_{\max}$ is the maximum possible nutrient quota, which is obtained when $\mu = \mu_{\max}$ (Morel 1987). All estimates of $\mu_{\max}$ were measured at or near 20°C. Marine species for which we have maximum growth rate data range in volume from 4.6×10^{-1} to 1.6×10^{7} μm^3, and the freshwater species range in volume from 1.8 to 3.8×10^{4} μm^3.

Statistical analyses—In order to quantify scaling relationships between nutrient traits and cell volume, we used standardized major axis (SMA) regression (also referred to as reduced major axis regression). This method is more appropriate than least-squares regression for estimating the line of best fit for the relationship between two variables (Warton et al. 2006). SMA slopes were estimated only if the two variables had a significant (at $p < 0.05$) Kendall rank-correlation coefficient. Analysis was performed with the "smatr" package in R version 2.11.0 (Warton and Ormerod 2007). Relationships between nutrient utilization traits and cell volume are typically linear when both variables are log-transformed, resulting in a power-law relationship on the linear scale (Litchman et al. 2007); we therefore performed all analyses on $\log_{10}$-transformed values.

We tested for between-taxon trait differences using analysis of variance; we tested for between-taxon trait differences while controlling for the effect of volume by performing analysis of variance and post hoc Tukey pairwise comparisons with the residuals from the SMA regressions. For taxonomic comparisons, species were classified into the following groups: diatoms, dinoflagellates, cyanobacteria, desmids, non-desmid chlorophytes, haptophytes, and raphidophytes. Freshwater and marine species were analyzed separately, because of differences in taxon representation; also, for each trait we excluded from the analysis those taxa for which the trait was measured on

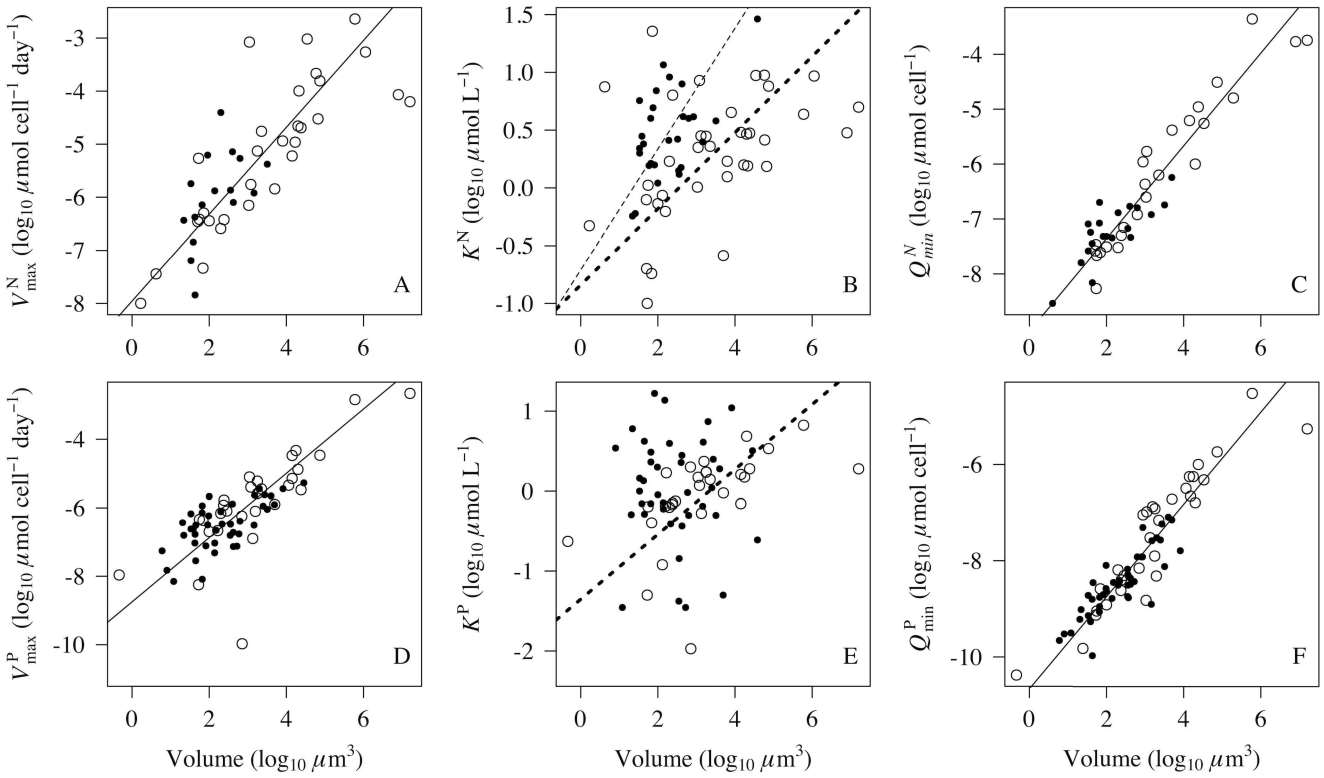

Fig. 1. Scaling relationships between primary nutrient utilization traits and cell volume. All variables are $\log_{10}$-transformed. Marine species are larger open circles, freshwater species are smaller filled circles. Lines plotted are from the SMA regressions in Table 1. If there is no significant difference in slopes between marine and freshwater species, as reported in Table 1, a single solid line is plotted. If the slopes differ significantly, a dotted line is plotted for the marine species and a dashed line is plotted for the freshwater species. If there is no significant correlation between the variables, no line is plotted. (A) V^{N}_{max}, (B) K^{N}, (C) Q^{N}_{min}, (D) V^{P}_{max}, (E) K^{P}, (F) Q^{P}_{min}.

fewer than two species. Therefore, the following groups were compared for freshwater species: cyanobacteria, diatoms, desmids, and non-desmid chlorophytes (desmids were only present for the phosphate traits). For the marine species, the following groups were compared: diatoms, dinoflagellates, chlorophytes, haptophytes, and raphidophytes. We also used *t*-tests to compare mean trait values for freshwater and marine diatoms, because diatoms from both environments were relatively well represented.

We tested for correlations between the primary nutrient utilization traits, which could indicate physiological trait constraints or correlated selection pressures. Because most traits are correlated with volume, we also tested for partial correlations that controlled for the combined effect of volume on each pair of traits. Correlations between the composite traits are the subject of a separate manuscript, in which we use techniques for multivariate analysis in the presence of missing data to better estimate the correlations between cell volume, uptake affinity, and scaled uptake affinity for N and P (Edwards et al. 2011).

Results

Trait–volume scaling—All six of the primary nutrient traits tend to increase with cell volume (Fig. 1). Four of these traits have no significant difference in slope between freshwater and marine species: V^{N}_{max}, Q^{N}_{min}, V^{P}_{max}, and Q^{P}_{min} (Table 1; Fig. 1). In contrast, K^{N} and K^{P} both have significantly different slopes for freshwater vs. marine species, with freshwater species exhibiting a steeper slope for K^{N}, but exhibiting no significant relationship with volume for K^{P} (Fig. 1B,E). Of the four composite nutrient traits, P affinity, N affinity, and scaled N affinity all have slopes that are indistinguishable for freshwater and marine species. Both P affinity and N affinity increase with cell volume, whereas scaled N affinity decreases with cell volume (Fig. 2A,B,D). Scaled P affinity exhibits significantly different slopes for freshwater and marine species, with marine species showing a negative relationship with volume, and freshwater species showing no significant relationship (Fig. 2C). Maximum growth rate tends to decline with cell volume (Fig. 3A), and marine species show a significantly shallower slope than freshwater species (Table 1).

Between-taxon trait differences—μ_{max} differs significantly between taxa for both freshwater and marine species, with and without controlling for volume (Table 2; Fig. 3C,D). Because of the greater amount of data for μ_{max}, we also tested for differences in the allometric exponent between taxa; SMA slopes did not differ by taxon for freshwater (likelihood ratio = 6.46, $p = 0.09$) or marine species (likelihood ratio = 0.58, $p = 0.96$). For marine species, after controlling for volume, mean maximum

Table 1. Coefficients for SMA regression of nutrient utilization traits and μ_{max} with cell volume. Intercepts (int.) and slopes are listed for separate regressions of freshwater and marine species, and the common intercepts and slopes are listed for regressions that combine freshwater and marine species. 95% confidence intervals for each parameter are in parentheses. ns is listed for traits not significantly correlated with volume. The final column lists the p value for a test of whether the freshwater and marine species have different slopes. p values < 0.05 are marked with an asterisk.

	Freshwater int.	Freshwater slope	Marine int.	Marine slope	Common int.	Common slope	Slope p
V^N_{max}	−8.8(−10, −7.4)	1.3(0.82, 2.1)	−8.1(−8.8, −7.3)	0.82(0.65, 1)	−8.0(−8.5, −7.4)	0.82(0.68, 0.98)	0.069
K^N	−0.71(−1.1, −0.28)	0.52(0.37, 0.73)	−0.84(−1.2, −0.44)	0.33(0.24, 0.45)	−0.61(−0.88, −0.34)	0.33(0.26, 0.42)	0.044*
Q^N_{min}	−8.7(−9.2, −8.2)	0.68(0.51, 0.93)	−9.2(−9.6, −8.7)	0.88(0.77, 1)	−9.0(−9.3, −8.8)	0.84(0.76, 0.94)	0.13
V^P_{max}	−8.4(−8.9, −7.9)	0.81(0.64, 1)	−9.1(−10, −8.2)	1.0(0.8, 1.3)	−8.7(−9.2, −8.3)	0.94(0.81, 1.1)	0.17
K^P	ns	ns	−1.4(−1.8, −0.88)	0.41(0.29, 0.56)	−1.5(−1.9, −1.1)	0.53(0.42, 0.67)	0.0097*
Q^P_{min}	−10.5(−10.8, −10.2)	0.86(0.74, 1)	−10.6(−11.1, −10.1)	0.97(0.84, 1.1)	−10.7(−10.9, −10.4)	0.96(0.87, 1.1)	0.3
P affinity	−9(−9.7, −8.2)	1 .0(0.76, 1.3)	−8.1(−8.8, −7.5)	0.73(0.58, 0.94)	−8.5(−9, −8.1)	0.85(0.72, 1)	0.089
N affinity	−8.6(−9.8, −7.3)	1 .0(0.6, 1.7)	−8.2(−9, −7.4)	0.75(0.58, 0.98)	−8.1(−8.7, −7.6)	0.75(0.61, 0.92)	0.33
P scaled affinity	ns	ns	3.6(2.8, 4.3)	−0.55(−0.38, −0.8)	3.6(3.1, 4.1)	−0.65(−0.5, −0.83)	0.046*
N scaled affinity	−0.96(−2.1, 0.2)	0.78(0.43, 1.4)	2.9(1.9, 3.9)	−0.63(−0.44, −0.91)	2.4(1.8, 3.0)	−0.57(−0.43, −0.78)	0.54
μ_{max}	0.69(0.52, 0.86)	−0.36(−0.30, −0.43)	0.70(0.54, 0.85)	−0.24(−0.20, −0.29)	0.65(0.53, 0.76)	−0.28(−0.25, −0.32)	0.0014*

growth rates for diatoms are greater than those of chlorophytes, dinoflagellates, and cyanobacteria (Tukey pairwise comparison $p < 0.001$ for all tests). This means that at a given cell size, diatoms grow faster than other taxonomic groups. In addition, dinoflagellates and haptophytes have greater growth rates than cyanobacteria when controlling for volume ($p = 0.0081$ and $p = 0.0078$, respectively). For freshwater species, cyanobacteria have lower growth rates than diatoms, desmids, non-desmid chlorophytes, and cryptomonads when controlling for volume ($p < 0.001$ for all tests).

Most nutrient traits differ significantly between taxa when cell volume is not included as a covariate (Table 2; Figs. 4–7). However, few traits differ between taxa when the effect of volume is accounted for (Table 2; Figs. 4–7). For freshwater species, values of primary nutrient traits tend to be greatest for the desmids and smallest for the cyanobacteria, with diatoms and non-desmid chlorophytes having intermediate values (Fig. 4). For marine species, dinoflagellates and raphidophytes tend to have the largest trait values, whereas diatoms have intermediate values and haptophytes and chlorophytes have the smallest values (Fig. 6). Because few between-taxon differences remain when cell volume is controlled for, these differences are evidently driven by differences between taxa in typical cell size. For freshwater species, the exceptions are K^P, P affinity, and scaled N affinity, all of which differ between taxa while controlling for volume (Table 2; Figs. 4, 5). Tukey tests show that for K^P, desmids have significantly greater values than the other three taxa, and non-desmid chlorophytes have significant greater values than cyanobacteria ($p < 0.01$ for all tests). For P affinity, desmids have significantly greater values than cyanobacteria ($p = 0.034$). For scaled N affinity, diatoms and non-desmid chlorophytes both have significantly greater values than cyanobacteria ($p = 0.036$ and 0.026, respectively). For marine species, the traits with significant between-taxon variation when controlling for volume are K^N and scaled N affinity (Table 2; Figs. 6, 7). For K^N, chlorophytes have greater values than diatoms ($p < 0.001$), dinoflagellates ($p < 0.001$), haptophytes ($p < 0.001$), and raphidophytes ($p < 0.037$). For scaled N affinity, although there is significant between-taxon heterogeneity, no pairwise comparisons are significantly different at $p < 0.05$.

Diatoms—We compared log-transformed mean trait values for freshwater and marine diatoms using those traits measured on at least five species from each environment (μ_{max}, K^N, Q^N_{min}, V^P_{max}, K^P, Q^P_{min}, P affinity, and scaled P affinity). Marine diatoms have higher μ_{max} than freshwater diatoms (back-transformed means of 1.0 vs. 0.28, respectively; $p = 0.001$). Marine diatoms have lower K^N (back-transformed means of 1.6 vs. 3.5; $p = 0.018$), higher V^P_{max} (back-transformed means of 2.8×10^{-6} vs. 2.5×10^{-7}; $p = 0.029$), and higher K^P (back-transformed means of 1.1 vs. 0.23; $p = 0.035$). Marine diatoms are also larger on average (means of 2090 vs. 436 m^3; $p = 0.009$), but the other significant trait differences were unchanged in analyses of covariance that included volume as a covariate.

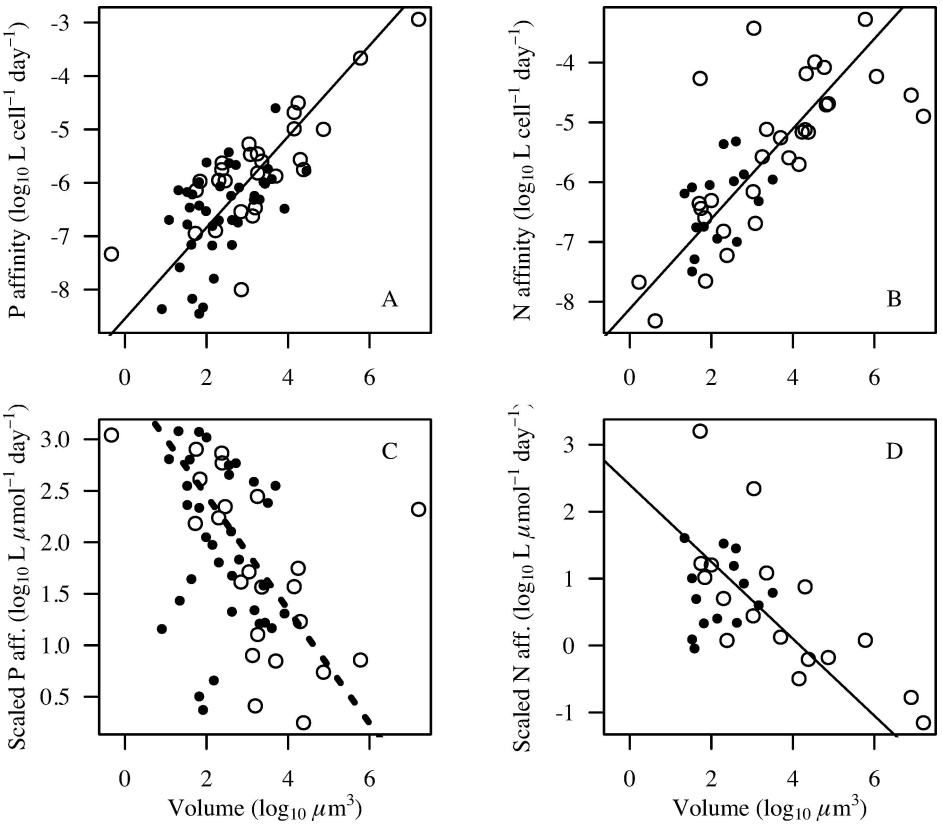

Fig. 2. Scaling relationships between composite nutrient traits and cell volume. All variables are $\log_{10}$-transformed. Lines plotted are from the SMA regressions in Table 1. If there is no significant difference in slopes between marine and freshwater species, as reported in Table 1, a single solid line is plotted. Symbols as in Fig. 1. (A) P affinity (aff.), (B) N aff., (C) scaled P aff., (D) scaled N aff.

Correlations between traits—Raw correlations between primary nutrient utilization traits are dominated by strong positive correlations (Table 3). For the marine species, all pairs of traits are significantly positively correlated. For the freshwater species, most pairs of traits either are significantly positively correlated or show no significant correlation. The single exception to this pattern is a significant negative correlation between K^{P} and K^{N} among the freshwater species (Table 3). The dominance of positive correlations among traits is likely driven by the fact that all traits tend to increase with cell volume (Fig. 1). A similar argument applies to the fact that all of the raw correlations of nutrient traits with $\mu_{\max}$ are negative (Table 3). We therefore tested for partial correlations, which control for the simultaneous effect of cell volume on each pair of traits. In this analysis, positive correlations between nutrient traits still dominate, as do negative correlations between nutrient traits and $\mu_{\max}$ (Table 3). Among marine species, five pairs of traits still show significant positive correlations between nutrient traits; among freshwater species, four pairs of traits show significant positive correlations, whereas K^{P} and K^{N} again show a negative correlation. As discussed below, the preponderance of positive correlations that remain while controlling for volume may indicate that the effect of volume is not fully removed.

$Q^{\mathrm{N}}_{\min}$: $Q^{\mathrm{P}}_{\min}$ *ratios*—We tested whether the ratio $Q^{\mathrm{N}}_{\min}$: $Q^{\mathrm{P}}_{\min}$ tended to differ between marine and freshwater habitats. Both habitats showed broad variation in this ratio, but freshwater species had a significantly higher ratio on average (Fig. 8; freshwater median molar ratio, 24.4; marine median molar ratio, 13.6; two-sample Wilcoxon test, $p = 0.032$).

Discussion

Scaling relationships and marine–freshwater differences—We find that $V_{\max}$, K, and $Q_{\min}$ all tend to increase as cell volume increases, although this is not the case for K^{P} among freshwater species (Fig. 1; Table 1). It is noteworthy that marine and freshwater slopes are indistinguishable for those traits that appear to be the most strongly constrained by volume, $V_{\max}$ and $Q_{\min}$ (Fig. 1). In contrast, the half-saturation constants for uptake appear to be less strongly constrained by volume, and have significantly different slopes for freshwater and marine species (Fig. 1B,E), possibly reflecting different selective pressures from N or P limitation. If P limitation is more prevalent in freshwater environments, it may select for low K^{P} and thus decrease this trait's dependence on cell volume. The generally positive effect of volume on all of these traits is consistent

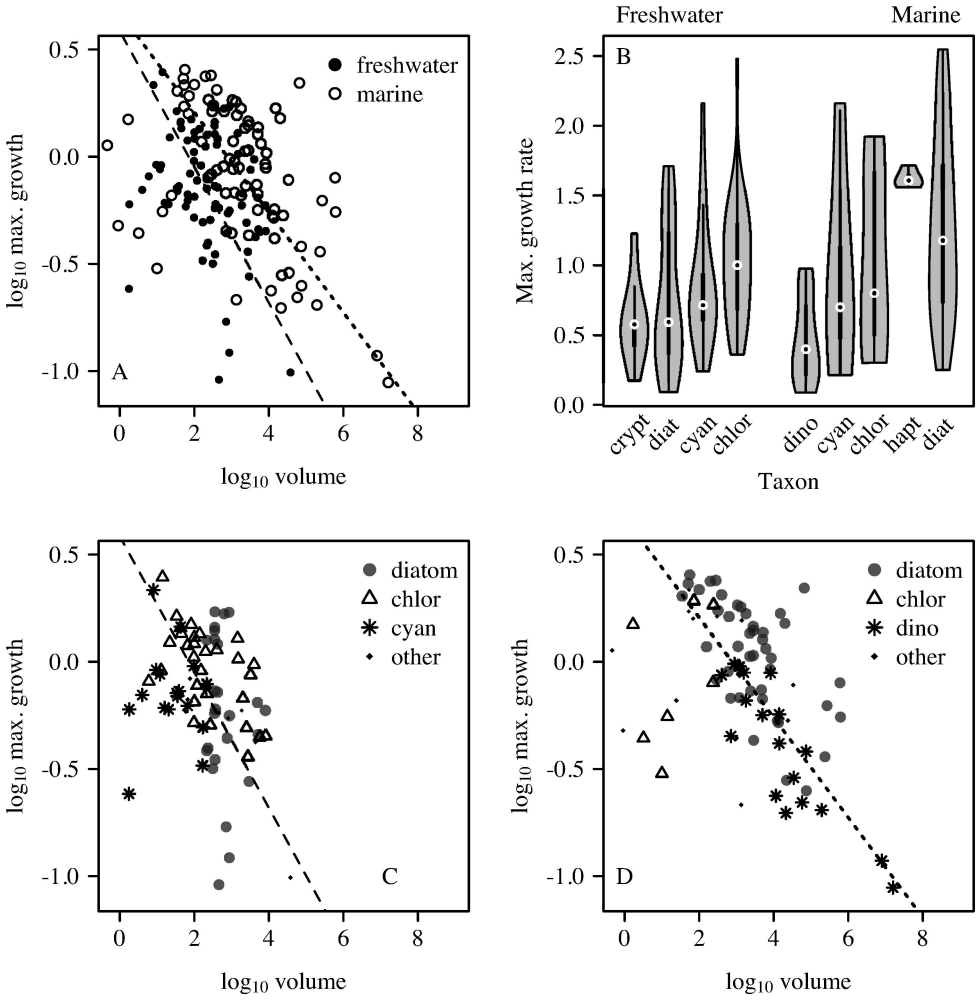

Fig. 3. Allometric scaling and between-taxon differences in μ_{max}. (A) μ_{max} vs. cell volume for freshwater and marine species. The fitted SMA regressions a plotted for marine (dashed line) and freshwater (dotted line) species. (B) Violin plots of μ_{max} (on a linear scale). The violin plots combine a box plot (showing 10th, 25th, 50th, 75th, and 90th quantiles) with a density plot. (C) μ_{max} vs. volume for freshwater species, coded by taxon. (D) μ_{max} vs. volume for marine species, coded by taxon. Chrys, chrysophytes, crypt, cryptophytes; diat, diatoms; cyan, cyanobacteria; chlor, chlorophytes; dino, dinoflagellates; hapt, haptophytes.

with prior analyses (Shuter 1978; Grover 1989; Litchman et al. 2007). These results are also largely consistent with the idea that marine and freshwater phytoplankton have similar physiological ecology (Kilham and Hecky 1988), although the different environments may modify trait patterns to some extent. For example, diatoms in marine and freshwater systems appear to occupy somewhat different ecological niches and exhibit different strategies. Marine diatoms are a fast-growing group compared to other marine taxa and appear to be adapted to high nutrient conditions. In contrast, freshwater diatoms have intermediate growth rates and low K^P compared to other freshwater taxa. They also have a relatively high P affinity among freshwater groups, likely being adapted to P-limited conditions. When marine and freshwater diatoms are compared, marine diatoms have significantly higher growth rates and K^P than freshwater diatoms; conversely, marine diatoms also tend to have lower K^N than freshwater diatoms. These differences possibly reflect different selective pressures in marine and freshwater environments. Because of the heavy silica frustules of diatoms, in marine environments diatoms benefit from mixing conditions associated with high nutrients that select for high growth rates. In freshwaters, diatoms also are associated with mixing conditions, but in shallow lakes mixed layer depths are too small and strongly select against diatoms (Ptacnik et al. 2003). Therefore, freshwater diatoms may be more prevalent in deeper lakes that also tend to be lower in P (Dillon and Rigler 1974), causing freshwater diatoms to be good P competitors.

Uptake affinity is often used as a proxy of the ability to grow or compete under nutrient limitation; therefore, it is important to note that uptake affinity on a per-cell basis increases with cell volume (Fig. 2A,B), because V_{max} scales

Table 2. Tests of between-taxon trait differences. For each trait, the F statistic and p value are listed for a one-way analysis of variance testing the effect of taxon in isolation ("Taxon only" rows). Numerator and denominator degrees of freedom are in parentheses. In addition, the Wald (W) statistic and p value are listed for a test of common intercepts across taxa in an SMA regression with cell volume ("Taxon with volume [w/vol.]" rows). P values < 0.05 are marked with an asterisk.

	V^{N}_{max}	K^{N}	Q^{N}_{min}	V^{P}_{max}	K^{P}	Q^{P}_{min}	P affinity	N affinity	P scaled affinity	N scaled affinity	μ_{max}
Marine species											
Taxon only F	4.5(4,25)	5.3(4,32)	6.3(3,19)	0.9(4,22)	0.6(4,22)	5.2(4,23)	0.81(4,20)	6.5(4,25)	3.5(4,16)	5.8(3,12)	12.2(4, 91)
Taxon only p	0.0075*	0.0022*	0.0038*	0.48	0.67	0.0038*	0.53	0.001*	0.031*	0.011*	5.6×10^{-8}*
Taxon w/vol. W	1.5	8.8	0.83	1.2	1.6	1.2	1.6	3.3	0.43	0.51	30
Taxon w/vol. p	0.24	7.10×10^{-5}*	0.5	0.35	0.21	0.35	0.22	0.029*	0.78	0.68	4.5×10^{-6}*
Freshwater species											
Taxon only F	9.2(2,13)	0.77(2,24)	3.1(2,17)	5.5(3,34)	5.1(3,31)	14(3,40)	1.7(3,31)	4.2(2,12)	2.8(3,28)	3.3(2,11)	2.3(4, 112)
Taxon only p	0.0032*	0.48	0.074*	0.0034*	0.0056*	2.80×10^{-6}*	0.19	0.042*	0.06	0.077	0.061
Taxon w/vol. W	3.2	1.6	2	2.4	13	0.57	3.1	1.1	1.2	5.2	35
Taxon w/vol. p	0.075	0.22	0.17	0.088	1.50×10^{-5}*	0.64	0.043*	0.38	0.35	0.028*	5.0×10^{-6}*

more steeply with volume than K. In contrast, scaled uptake affinity tends to decrease with cell volume, although strong support for this relationship is present only for marine species. Among freshwater species, scaled P affinity shows no relationship with cell volume (Fig. 2C). Furthermore, although the slopes for scaled N affinity cannot be distinguished for freshwater and marine species, the freshwater species occupy a limited range of volumes, and by themselves show no correlation with volume (Kendall rank correlation 0.03, $p = 0.91$; Fig. 2D). Scaled uptake affinity should be a more appropriate proxy for competitive ability under nutrient limitation, because it standardizes uptake ability by the nutrient requirement for growth. This is supported by our comparison of scaled uptake affinities with the outcome of P-limited chemostat experiments (Web Appendix, Table A2). Our results therefore support the expectation that the ability to compete for limiting nutrients tends to decline with increasing cell volume (Chisholm 1992), although this relationship may be weaker or nonexistent in freshwater habitats. These results are also consistent with a study of marine species that measured scaled P affinity directly, as the biomass-specific P turnover rate, and found that scaled P affinity declines with increasing equivalent spherical radius (Tambi et al. 2009).

Maximum growth rate tends to decline with increasing cell volume, consistent with prior results (Banse 1976; Chisholm 1992). To our knowledge, this is the first study comprehensively comparing marine and freshwater species, and we find that the allometric slope is shallower for marine species (Table 1; Fig. 3). The consequence of this difference in slopes is that, on average, marine phytoplankton of a certain size grow faster than freshwater species of the same size, and this difference is greater as cell size increases (Fig. 3A). The allometric slopes of μ_{max} for freshwater and marine species are -0.36 and -0.24 respectively, and the slope when all species are combined is -0.28 (Table 1). These slopes are steeper than some previously reported slopes (Banse 1976; Tang 1995), but this may be due to our use of SMA regression. Lines fit using ordinary least squares (OLS) have slopes biased towards zero (Warton et al. 2006), and if we fit our data using OLS the slopes are -0.10 and -0.096 for freshwater and marine species, respectively. Because allometric exponents are used to parameterize ecosystem models (Irwin et al. 2006), and to test hypothesized mechanisms for scaling relationships (DeLong et al. 2010), we recommend the use of SMA regression in comparative analyses, as discussed in detail by Warton et al. (2006).

In general, there are a number of limitations to our comparisons of freshwater and marine species. The freshwater species in our dataset cover a smaller range of cell volumes, which may limit our ability to detect scaling relationships and therefore obscure potential effects of volume on scaled uptake affinity. However, freshwater phytoplankton may have a smaller cell size range compared to marine phytoplankton in general (Litchman et al. 2009; Kamenir et al. 2010). In addition, the species in our compilation are those that phytoplankton ecologists have chosen to study in the lab, rather than a random or size-structured sample from natural communities. Future work on trait–volume scaling relationships would benefit from a

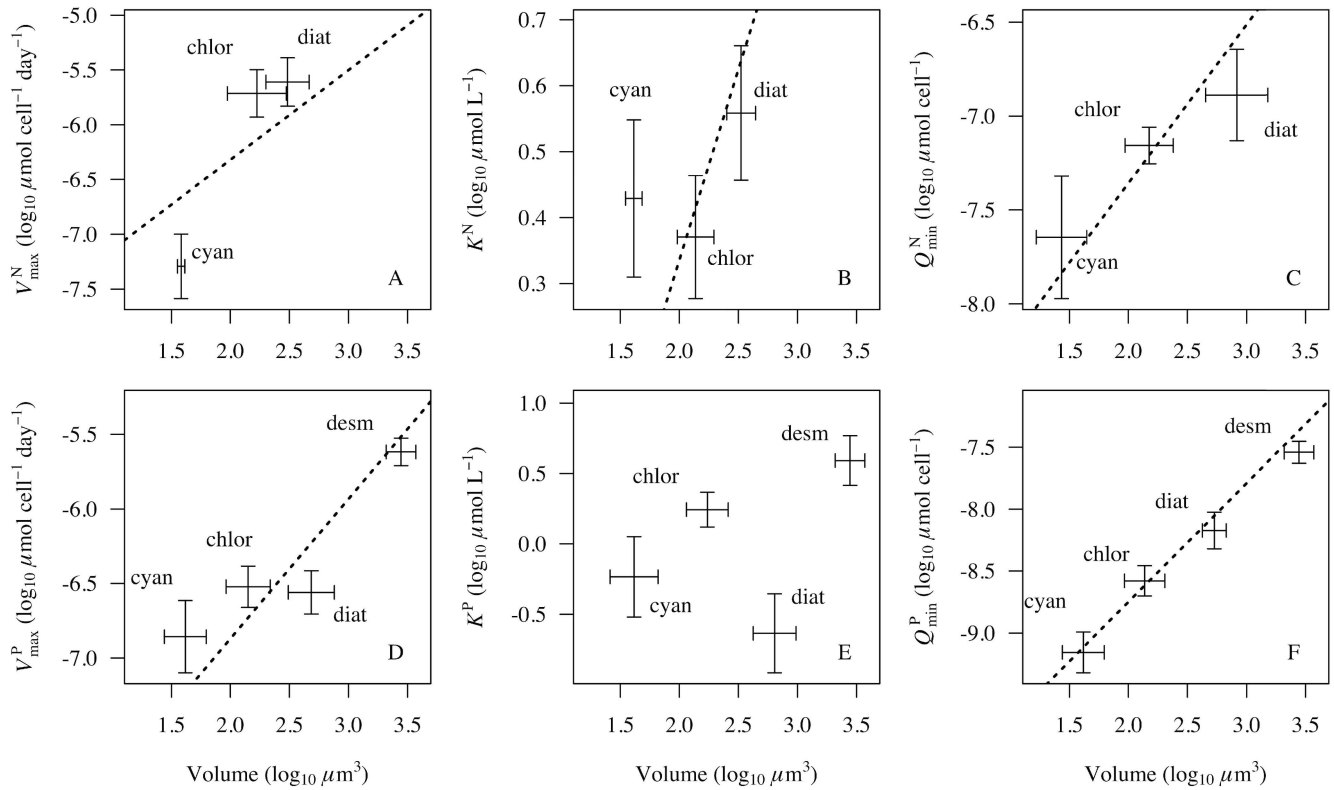

Fig. 4. Between-taxon variation in primary nutrient traits for freshwater species. All variables are $\log_{10}$-transformed. For each taxon, mean trait value and mean volume are plotted, ± 1 SE. The SMA fits from Fig. 1 are plotted as dotted lines. cyan, cyanobacteria; diat, diatoms; chlor, non-desmid chlorophytes; desm, desmids. (A) V^{N}_{max}, (B) K^{N}, (C) Q^{N}_{min}, (D) V^{P}_{max}, (E) K^{P}, (F) Q^{P}_{min}.

more comprehensive sampling of natural communities, including endpoints of the size spectrum, from picoplankton to giant diatoms.

Between-taxon differences—We find strong differences between taxa in average nutrient trait values (Figs. 3–6; Table 2). At the same time, few between-taxon differences remain when controlling for cell volume (Table 2), indicating that taxonomic variation in nutrient utilization traits is largely driven by size variation. Considering raw trait values (not corrected for volume), among marine species differences in scaled uptake affinities suggest that the relatively small haptophytes and prymnesiophytes should be better competitors under P limitation, whereas the relatively large dinoflagellates and raphidophytes should be poorer competitors (Fig. 7C). The same trends occur for scaled N affinity, although there are no data for chlorophytes (Fig. 7D). Among freshwater species, the data for scaled P affinity suggest that cyanobacteria tend to be better competitors, whereas the desmids are poor competitors, with diatoms and non-desmid chlorophytes in between (Fig. 5C). There are fewer data for scaled N affinity among freshwater species, but the present patterns suggest that cyanobacteria may be poorer competitors for nitrate (Fig. 5D); however, only two cyanobacteria are present in this analysis, and one of these species can fix nitrogen, which may compensate for low nitrate affinity.

There is significant variation in mean μ_{max} between taxa (Table 2), with most variation due to the fact that among marine species, diatoms have high growth rates relative to their cell volume, whereas among freshwater species, cyanobacteria have low growth rates relative to their cell volume (Fig. 3C,D). However, because of the relatively small size of cyanobacteria cells, their raw growth rates are still relatively high among freshwater species, on average (Fig. 3B).

$Q^{N}_{min}:Q^{P}_{min}$ *ratios*—Our analysis of the structural N:P ratio supports prior work showing broad variation in this ratio across phytoplankton (Rhee and Gotham 1980; Klausmeier et al. 2004). We also find that $Q^{N}_{min}:Q^{P}_{min}$ is greater on average for freshwater species. If the evolution of this ratio is driven by the relative availability of N and P, the difference between habitats may be due to greater prevalence of P limitation in freshwater systems (Elser et al. 2007). Alternatively, if the evolution of this ratio is driven by allocation to resource acquisition proteins vs. ribosomes (Klausmeier et al. 2004), higher ratios among freshwater species may indicate a greater tendency for nutrient or light limitation in freshwater systems, and a greater tendency for near-maximal growth rates in marine systems (Goldman et al. 1979).

Comparison of scaling exponents to theoretical predictions—The observed scaling exponents of nutrient utilization

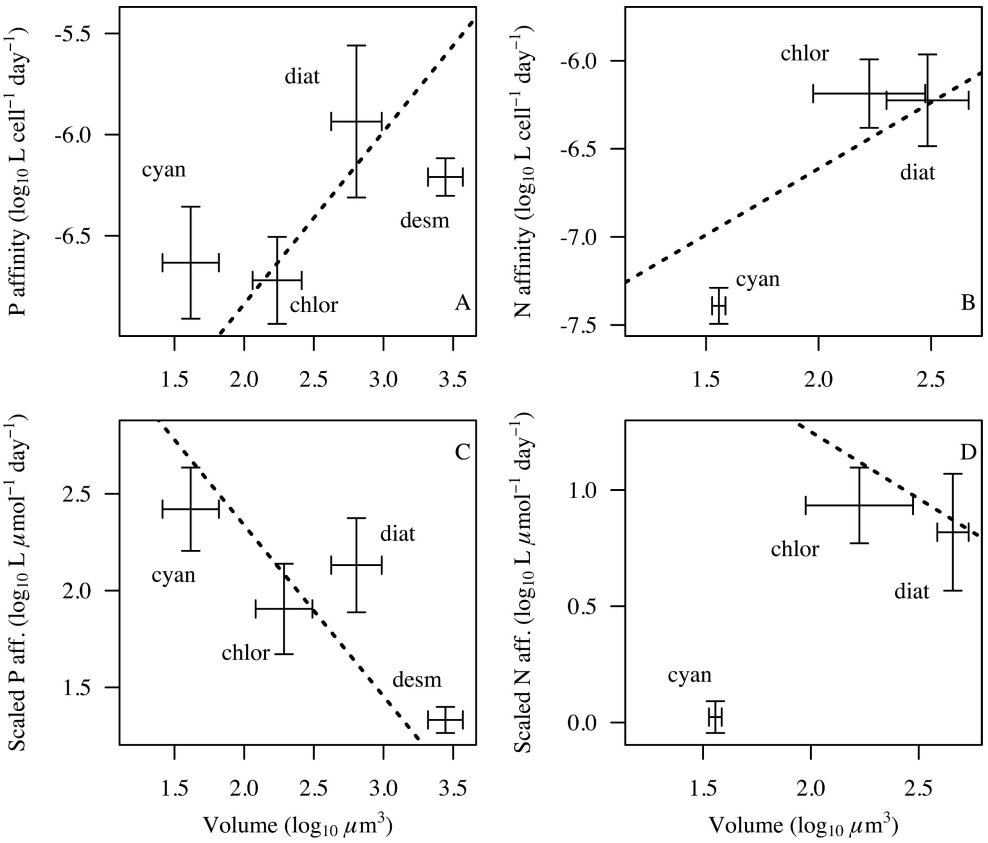

Fig. 5. Between-taxon variation in composite traits for freshwater species. All variables are $\log_{10}$-transformed. For each taxon, mean trait value and mean volume are plotted, ± 1 SE. The SMA fits from Fig. 2 are plotted as dotted lines. cyan, cyanobacteria; diat, diatoms; chlor, non-desmid chlorophytes; desm, desmids. (A) P affinity, (B) N affinity, (C) scaled P affinity, (D) scaled N affinity.

traits can be compared to those expected under a mechanistic model of nutrient uptake (Aksnes and Egge 1991). If V_{max} is determined by the number of uptake sites on the cell surface, and if the density of uptake sites does not vary with cell volume, then cell-specific maximum uptake rate should be proportional to surface area. For spherical cells, this will result in a scaling exponent of 2/3 for V_{max} relative to cell volume. Although a prior analysis of nitrate traits in marine species found a scaling exponent consistent with two-thirds scaling (Litchman et al. 2007), with the current, larger dataset, both V^{N}_{max} and V^{P}_{max} scale more steeply than 2/3 (95% confidence intervals are [0.68, 0.98] and [0.81, 1.1], respectively; Table 1). However, an interspecific comparison has found that species with greater cell volume tend to show greater deviation from a spherical shape, and as a result the surface area : volume ratio declines with increasing cell volume, but it declines more slowly than expected for spherical cells (Reynolds 2006). Therefore, our results for the scaling of V_{max} are not inconsistent with a scaling relationship driven by surface area.

K appears to be less constrained by cell volume than either V_{max} or Q_{min}, and unlike these traits, K exhibits different scaling patterns for freshwater and marine species, with K^{P} showing no correlation with volume for freshwater species (Table 1; Fig. 1). Therefore, although cell volume may affect K by altering the mass transfer coefficient (Aksnes and Egge 1991), our results suggest that other factors beyond cell volume strongly affect K.

We hypothesized earlier (Litchman et al. 2007) that allometric exponents for Q_{min} may range from 2/3 to 1, if we assume that the minimum nutrient quota is contained in the cytoplasm that occupies most of the cell volume in small cells (isometric scaling), or is distributed along the cell wall in cells with large vacuoles (thus scales with cell surface, 2/3 of volume). We are, however, unaware of mechanistic predictions for whether Q_{min} scaling exponents should be different for N and P. Intriguingly, Q^{N}_{min} scales allometrically with volume (95% confidence interval [0.76, 0.94]; Table 1), whereas the scaling of Q^{P}_{min} is indistinguishable from isometric (95% confidence interval [0.87, 1.1]; Table 1). This also implies that the ratio $Q^{N}_{min} : Q^{P}_{min}$ may decline as volume increases. In our dataset, this is true when marine and freshwater species are combined (Kendall rank correlation: −0.28, $p = 0.013$), although not when marine and freshwater species are analyzed separately (marine correlation −0.059, $p = 0.77$; freshwater correlation −0.25, $p = 0.14$).

The allometric scaling of uptake affinities and scaled uptake affinities are consistent with the scaling of the

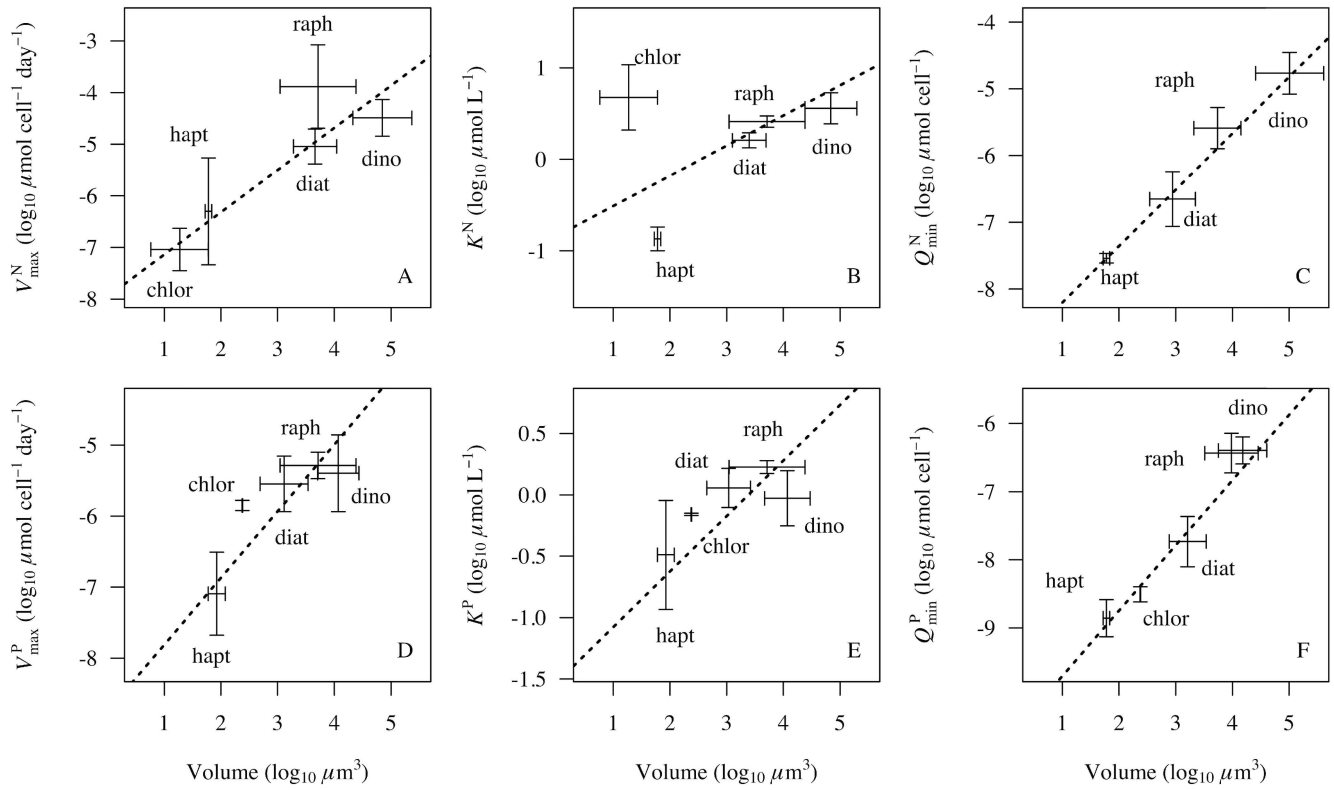

Fig. 6. Between-taxon variation in primary nutrient traits for marine species. All variables are $\log_{10}$-transformed. For each taxon, mean trait value and mean volume are plotted, ± 1 SE. The SMA fits from Fig. 1 are plotted as dotted lines. hapt, haptophytes; diat, diatoms; chlor, chlorophytes; dino, dinoflagellates; raph. raphidophytes. (A) V^{N}_{max}, (B) K^{N}, (C) Q^{N}_{min}, (D) V^{P}_{max}, (E) K^{P}, (F) Q^{P}_{min}.

component parameters V_{max}, K, and Q_{min}. V_{max} tends to increase more steeply with volume than K, resulting in an increase in cell-specific uptake affinity with volume (Fig. 2). When uptake affinity is scaled by Q_{min}, this results in a decrease in scaled uptake affinity with increasing volume, because the scaling exponents of V_{max} and Q_{min} tend to be similar. The exception to this trend is scaled P affinity for freshwater species, which does not decline with increasing volume; this may be due to the fact that K^{P} does not decline with volume.

Potential constraints among nutrient utilization traits—A raw correlation analysis reveals a preponderance of positive correlations among nutrient traits (Table 3). These positive correlations may partially constrain the evolution of competitive ability, because for a given nutrient, a positive correlation between V_{max} and K, or between V_{max} and Q_{min}, constrains the evolution of equilibrium competitive ability, whereas a positive correlation between K and Q_{min} has the opposite effect (Litchman et al. 2007). However, if these trait correlations are driven solely by cell size, then the only relevant constraints for trait evolution are those that will constrain size evolution, i.e., the trait–volume scaling parameters (Litchman et al. 2009). Our partial correlation analysis, which tested for trait correlations after controlling for the effect of cell volume, yielded equivocal results. This analysis yielded only positive significant correlations between nutrient traits, with the exception of K^{P} vs. K^{N} for freshwater species, which is also negative in the raw correlation analysis (Table 3). Furthermore, the significant correlations occurred among pairs of traits that were strongly correlated in the raw correlation analysis (Table 3). It therefore seems possible that the partial correlation analysis did not succeed in fully removing the correlated effect of volume on nutrient traits. Measurement error for either nutrient traits or cell volume, along with plasticity in cell volume, will tend to add noise to the trait–volume relationship. This noise will prevent the partial correlation from fully removing the true relationship between a trait and volume. The same considerations apply to the negative correlations between μ_{max} and nutrient traits (Table 3).

However, some of the partial correlations may indeed represent constraints, or correlated selection pressures. The negative correlation between K^{P} and K^{N} among freshwater species is unlikely to be driven by volume, and may contribute to a trade-off in competitive ability for nitrate vs. phosphate among freshwater species. Among the positive partial correlations, nearly all would constrain the evolution of high scaled uptake affinity for a single nutrient (for marine species, V^{N}_{max} vs. Q^{N}_{min}, V^{P}_{max} vs. K^{P}; for freshwater species, V^{N}_{max} vs. Q^{N}_{min}), or would constrain the simultaneous evolution of high scaled uptake affinity for both nutrients (for marine species, V^{N}_{max} vs. K^{P}, V^{P}_{max} vs. K^{N}; for freshwater species, V^{N}_{max} vs. V^{P}_{max}, V^{P}_{max} vs. Q^{N}_{min}). Therefore, these relationships may represent eco-evolutionary

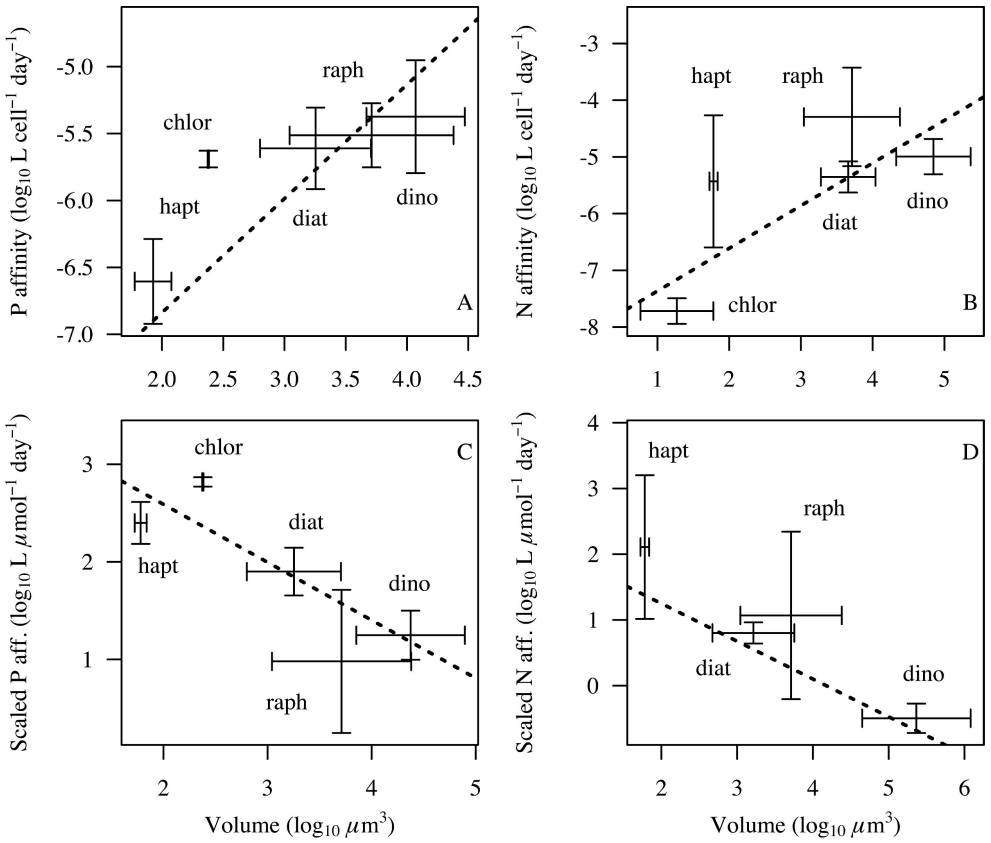

Fig. 7. Between-taxon variation in composite traits for marine species. All variables are $\log_{10}$-transformed. For each taxon, mean trait value and mean volume are plotted, ± 1 SE. The SMA fits from Fig. 2 are plotted as dotted lines. hapt, haptophytes; diat, diatoms; chlor, chlorophytes; dino, dinoflagellates; raph, raphidophytes. (A) P affinity, (B) N affinity, (C) scaled P affinity, (D) scaled N affinity.

Table 3. Correlation matrices for between-trait relationships. Entries in the upper right are for marine species, entries in the lower left are for freshwater species.

	V^{N}_{max}	K^{N}	Q^{N}_{min}	V^{P}_{max}	K^{P}	Q^{P}_{min}	μ_{max}
Raw Kendall rank correlations							
V^{N}_{max}		0.28*	0.69**	0.6**	0.71**	0.54*	−0.37*
K^{N}	0.27		0.53*	0.6**	0.52*	0.56*	−0.27*
Q^{N}_{min}	0.66**	0.13		0.66**	0.69**	0.84**	−0.61**
V^{P}_{max}	0.43*	−0.066	0.52*		0.59**	0.66**	−0.22
K^{P}	−0.14	−0.45*	−0.3	0.19		0.64**	−0.14
Q^{P}_{min}	0.7**	−0.037	0.55**	0.42**	−0.0034		−0.35
μ_{max}	−0.029	−0.16	−0.22	−0.2	−0.032	−0.2	
Partial Kendall rank correlations that control for cell volume							
V^{N}_{max}		0.086	0.45*	0.34	0.53*	0.072	−0.12
K^{N}	0.18		0.032	0.37*	0.19	0.078	−0.091
Q^{N}_{min}	0.57*	0.065		0.087	0.13	0.53*	−0.35*
V^{P}_{max}	0.39*	−0.1	0.62**		0.31*	0.13	0.06
K^{P}	0.051	−0.38*	−0.0095	0.19		0.20	0.12
Q^{P}_{min}	0.62*	−0.18	0.3	0.21	0.059		−0.23
μ_{max}	0.024	−0.12	−0.25	−0.2	−0.027	−0.042	

* $p < 0.05$, ** $p < 0.001$.

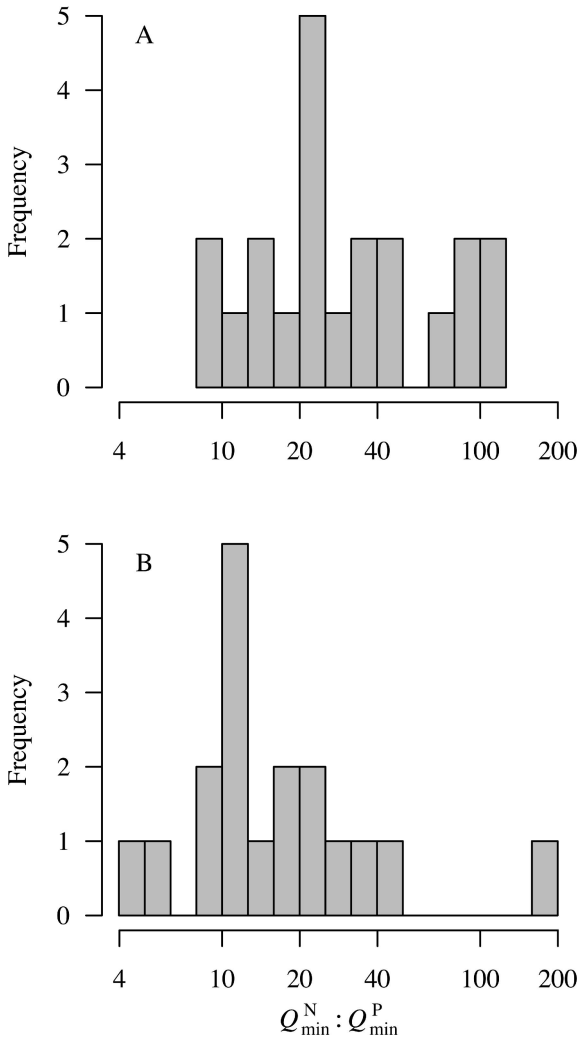

Fig. 8. Distribution of Q^{N}_{min}:Q^{P}_{min} molar ratios. X-axis is on a log scale. (A) Freshwater species, (B) marine species.

trade-offs among nutrient utilization traits. Edwards et al. (2011) used the same dataset to test for trade-offs among scaled N affinity, scaled P affinity, and cell volume. They found evidence for a general three-way trade-off among these three traits, with the relationship between scaled N affinity and scaled P affinity most evident in freshwater species, and the relationship between cell volume and the two nutrient traits most evident in marine species.

Our extensive compilation of nutrient utilization traits in marine and freshwater phytoplankton shows that there are fundamental scaling relationships for most traits, and these relationships are usually shared by marine and freshwater phytoplankton. However, there are some trait differences that possibly reflect different selective pressures between marine and freshwater environments, such as the relative strength of limitation by different nutrients. Major taxa differ in their nutrient utilization traits, reflecting contrasting ecological strategies that are often driven by differences in cell volume. Our data also suggest that some taxa, such as diatoms, may have different strategies in marine vs. freshwater environments, highlighting the interaction of phylogeny and environmental controls.

Acknowledgments

This work was in part supported by National Science Foundation grant 09-28819 to E.L. and C.A.K., 08-45932 to E.L., and a James S. McDonnell Foundation grant to C.A.K. and E.L. We thank Jonathan J. Cole and two anonymous reviewers for helpful comments. This is Kellogg Biological Station contribution No. 1635.

References

Aksnes, D. L., and J. K. Egge. 1991. A theoretical model for nutrient uptake in phytoplankton. Mar. Ecol. Prog. Ser. **70:** 65–72, doi:10.3354/meps070065

Banse, K. 1976. Rates of growth, respiration and photosynthesis of unicellular algae as related to cell size—review. J. Phycol. **12:** 135–140.

Chisholm, S. W. 1992. Phytoplankton size, p. 213–237. *In* P. G. Falkowski and A. D. Woodhead [eds.], Primary productivity and biogeochemical cycles in the sea. Plenum.

DeLong, J. P., J. G. Okie, M. E. Moses, R. M. Sibly, and J. H. Brown. 2010. Shifts in metabolic scaling, production, and efficiency across major evolutionary transitions. Proc. Natl. Acad. Sci. USA **107:** 12941–12945, doi:10.1073/pnas.1007783107

Dillon, P. J., and F. H. Rigler. 1974. Test of a simple nutrient budget model predicting phosphorus concentration in lake water. J. Fish. Res. Board Can. **31:** 1771–1778, doi:10.1139/f74-225

Edwards, K. F., C. A. Klausmeier, and E. Litchman. 2011. Evidence for a three-way tradeoff between nitrogen and phosphorus competitive abilities and cell size in phytoplankton. Ecology **92:** 1085–1095.

Elser, J. J., and others. 2007. Global analysis of nitrogen and phosphorus limitation of primary producers in freshwater, marine and terrestrial ecosystems. Ecol. Lett. **10:** 1135–1142, doi:10.1111/j.1461-0248.2007.01113.x

Eppley, R. W., J. N. Rogers, and J. J. McCarthy. 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnol. Oceanogr. **14:** 912–920, doi:10.4319/lo.1969.14.6.0912

Goldman, J. C., J. J. McCarthy, and D. G. Peavey. 1979. Growth influence on the chemical composition of phytoplankton in oceanic waters. Nature **279:** 210–215, doi:10.1038/279210a0

Grover, J. P. 1989. Influence of cell shape and size on algal competitive ability. J. Phycol. **25:** 402–405, doi:10.1111/j.1529-8817.1989.tb00138.x

———. 1991. Dynamics of competition among microalgae in variable environments: Experimental tests of alternative models. Oikos **62:** 231–243, doi:10.2307/3545269

Healey, F. P. 1980. Slope of the monod equation as an indicator of advantage in nutrient competition. Microb. Ecol. **5:** 281–286, doi:10.1007/BF02020335

Hecky, R. E., and P. Kilham. 1988. Nutrient limitation of phytoplankton in freshwater and marine environments: A review of recent evidence on the effects of enrichment. Limnol. Oceanogr. **33:** 796–822, doi:10.4319/lo.1988.33.4_part_2.0796

Irwin, A. J., Z. V. Finkel, O. M. E. Schofield, and P. G. Falkowski. 2006. Scaling-up from nutrient physiology to the size-structure of phytoplankton communities. J. Plankton Res. **28:** 459–471, doi:10.1093/plankt/fbi148

Kamenir, Y., Z. Dubinsky, and R. Harris. 2010. Taxonomic size structure consistency of the English Channel phytoplankton. J. Exp. Mar. Biol. Ecol. **383:** 105–110, doi:10.1016/j.jembe.2009.12.009

Kilham, P., and R. E. Hecky. 1988. Comparative ecology of marine and freshwater phytoplankton. Limnol. Oceanogr. **33:** 776–795, doi:10.4319/lo.1988.33.4_part_2.0776

Klausmeier, C. A., E. Litchman, T. Daufresne, and S. A. Levin. 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. Nature **429:** 171–174, doi:10.1038/nature02454

Litchman, E., and C. A. Klausmeier. 2008. Trait-based community ecology of phytoplankton. Annu. Rev. Ecol. Evol. Syst. **39:** 615–639, doi:10.1146/annurev.ecolsys.39.110707.173549

———, ———, J. R. Miller, O. M. Schofield, and P. G. Falkowski. 2006. Multi-nutrient, multi-group model of present and future oceanic phytoplankton communities. Biogeosciences **3:** 585–606, doi:10.5194/bg-3-585-2006

———, ———, O. M. Schofield, and P. G. Falkowski. 2007. The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. Ecol. Lett. **10:** 1170–1181, doi:10.1111/j.1461-0248.2007.01117.x

———, ———, and K. Yoshiyama. 2009. Contrasting size evolution in marine and freshwater diatoms. Proc. Natl. Acad. Sci. USA **106:** 2665–2670, doi:10.1073/pnas.0810891106

Morel, F. M. M. 1987. Kinetics of nutrient uptake and growth in phytoplankton. J. Phycol. **23:** 137–150.

Ptacnik, R., S. Diehl, and S. Berger. 2003. Performance of sinking and nonsinking phytoplankton taxa in a gradient of mixing depths. Limnol. Oceanogr. **48:** 1903–1912, doi:10.4319/lo.2003.48.5.1903

Reynolds, C. S. 2006. Ecology of phytoplankton. Cambridge Univ. Press.

Rhee, G. Y., and I. J. Gotham. 1980. Optimum N : P ratios and coexistence of planktonic algae. J. Phycol. **16:** 486–489, doi:10.1111/j.1529-8817.1980.tb03065.x

Shuter, B. J. 1978. Size-dependence of phosphorus and nitrogen subsistence quotas in unicellular micro-organisms. Limnol. Oceanogr. **23:** 1248–1255, doi:10.4319/lo.1978.23.6.1248

Smayda, T. J. 1997. Harmful algal blooms: Their ecophysiology and general relevance to phytoplankton blooms in the sea. Limnol. Oceanogr. **42:** 1137–1153, doi:10.4319/lo.1997.42.5_part_2.1137

Sterner, R., and J. J. Elser. 2002. Ecological stoichiometry. Princeton Univ. Press.

Stolte, W., and R. Riegman. 1996. A model approach for size-selective competition of marine phytoplankton for fluctuating nitrate and ammonium. J. Phycol. **32:** 732–740, doi:10.1111/j.0022-3646.1996.00732.x

Tambi, H., G. A. F. Flaten, J. K. Egge, G. Bodtker, A. Jacobsen, and T. F. Thingstad. 2009. Relationship between phosphate affinities and cell size and shape in various bacteria and phytoplankton. Aquat. Microb. Ecol. **57:** 311–320, doi:10.3354/ame01369

Tang, E. P. Y. 1995. The allometry of algal growth rates. J. Plankton Res. **17:** 1325–1335, doi:10.1093/plankt/17.6.1325

Tilman, D. 1982. Resource competition and community structure. Princeton Univ. Press.

Warton, D. I., and J. Ormerod. 2007. smatr: (Standardised) major axis estimation and testing routines. R package version 2.1. Available from http://web.maths.unsw.edu.au/~dwarton

———, I. J. Wright, D. S. Falster, and M. Westoby. 2006. Bivariate line-fitting methods for allometry. Biol. Rev. **81:** 259–291, doi:10.1017/S1464793106007007

Associate editor: Jonathan J. Cole

Received: 04 October 2011
Accepted: 03 January 2012
Amended: 20 January 2012