Eco-evolutionary differences in light utilization traits and distributions of freshwater phytoplankton

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Abstract

We compiled light utilization traits for 56 species of freshwater phytoplankton to analyze group differences, trait trade-offs, and allometric scaling relationships. We also used these traits to explain differences in major group distributions along the light availability gradient in 527 lakes in the continental United States. Major taxonomic groups differed significantly in their light utilization traits. Cyanobacteria had the highest initial slope of the growth-irradiance curve (α) and low irradiance at the onset of photoinhibition, indicating adaptation to low light environments. Green algae had the highest maximal growth rates and low α, indicating adaptation to higher light environments. Groups capable of mixotrophy had traits indicative of poor light competitive abilities and high light requirements. Key light utilization traits scaled allometrically with cell size and exhibited trade-offs leading to contrasting ecological strategies; α and cell size were conserved at the highest taxonomic level (domain), indicating a fundamental trait divergence between prokaryotic and eukaryotic phytoplankton. In line with these trait differences, major groups showed different responses to light availability in natural conditions. The relative abundances of low light–adapted groups declined with increasing light availability and vice versa. The genera mean values of the initial slopes of the growth-irradiance curves were significantly negatively correlated with the slopes of the relationships between the genus's relative abundance and light availability characterized by Secchi depth in 527 lakes. This indicates that light utilization traits can be used to explain phytoplankton distributions in nature.

Because phytoplankton are globally important primary producers, accounting for half of the Earth's primary productivity (Field et al. 1998), we need to understand how phytoplankton communities are organized. Light is a major resource for phytoplankton, one that significantly influences its total biomass and community composition, because different phytoplankton groups and individual species respond differently to light (Richardson et al. 1983). Understanding how light structures phytoplankton communities requires knowledge of the phytoplankton traits defining light utilization. Trait-based approaches are being increasingly used to explain community organization along various environmental gradients in both terrestrial and aquatic ecology (McGill et al. 2006; Litchman and Klausmeier 2008). Resource utilization traits are among the key functional traits that define the ecological niche of an organism (Chase and Leibold 2003; Litchman and Klausmeier 2008). Different trait values are associated with particular levels of resources and often correspond to distinct ecological strategies (Margalef 1978; Sommer 1984; Litchman et al. 2007). For light, there are typical relationships between light intensity and growth rates that characterize phytoplankton responses to this major resource. As is the case with photosynthesis, growth rate can be either a saturating or, if there is photoinhibition, a saturating and then declining function of irradiance (Kirk

1994; Litchman and Klausmeier 2008). Major parameters that characterize these growth-irradiance relationships are the maximum growth rate (μ_{max}); the initial slope of the growth-irradiance curve (α), which reflects the efficiency of light harvesting; and the irradiance at the onset of photoinhibition (I_{opt}) (Eilers and Peeters 1988). Note that we focus on growth-irradiance curves, not photosynthesis-irradiance curves.

Contrasting values of these light utilization traits are often interpreted as adaptations to different light environments (Richardson et al. 1983; Langdon 1987). High α generally indicates adaptation or acclimation to low light conditions through a high efficiency of utilizing light for growth (Langdon 1987), while low α would be characteristic of high light adaptation. Similarly, low I_{opt} indicates high sensitivity to photoinhibition (Eilers and Peeters 1988). High maximum growth rates are often advantageous under high resource (light) conditions, following the gleaner–opportunist trade-off (Grover 1991; Litchman and Klausmeier 2001).

Different taxonomic groups may have different strategies for light utilization: for example, cyanobacteria are thought to be adapted to low light environments and green algae to high light environments (Richardson et al. 1983; Reynolds 1984). Despite this general notion of significant group differences and the wealth of data on light-dependent growth for many species of phytoplankton, there is a paucity of meta-analyses that would quantitatively compare growth responses of major taxonomic groups to light.

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A comprehensive synthesis of existing data would allow stronger inferences related to the group differences with respect to their light utilization strategies. The knowledge of the mean trait values and their variation based on the analysis of multiple species would be useful for explaining and predicting the distributions of major groups along the light gradient in natural systems, as well as for parameterizing aquatic ecosystem models that include those taxonomic groups. Using light utilization traits to explain phytoplankton distributions in nature would improve our mechanistic understanding of community assembly in general. It is also of interest to determine whether the major parameters characterizing light-growth responses depend on cell size, because cell size is a master trait that influences most physiological and ecological characteristics (Chisholm 1992; Brown et al. 2004). The knowledge of allometric scaling of light utilization traits would provide information on the role of light levels in determining cell size distributions in natural environments (Key et al. 2010).

Here we have compiled data from published studies on growth-irradiance relationships of freshwater phytoplankton species representing major taxonomic groups. We also experimentally determined growth-irradiance relationships for several freshwater species representing phylogenetically and ecophysiologically diverse organisms. We determined key parameters for each growth-irradiance curve using commonly used models and compared major taxonomic groups with respect to their characteristic trait values. We also characterized allometric (power) relationships among light utilization traits and cell size, which improves our mechanistic understanding of size scaling in phytoplankton communities. We determined whether there are correlations between major parameters of growth responses to light. Such correlations may represent eco-physiological trade-offs and may define contrasting ecological strategies (Litchman et al. 2007). We also looked at whether major light traits are phylogenetically conserved or labile. Finally, we related mean light trait values of major taxonomic groups with their distributions along the light availability gradient in natural systems and showed that general distribution patterns can be explained by key light utilization traits.

Methods

Growth-irradiance experiments—Fourteen experiments were conducted using freshwater phytoplankton species either obtained from culture collections or isolated from local lakes. Batch cultures were grown at 20°C in full-strength freshwater WC medium (Guillard 1975). Irradiance was provided by 'cool white' fluorescence lamps (Philips or General Electric). Light intensities were determined using a quantum scalar (4π) sensor (QSL-100, Biospherical Instruments) immersed in distilled water and were adjusted as needed with neutral density screens. Cultures were acclimated for 1 week to the experimental irradiance and were then diluted to 100–500 cells or filaments per milliliter before the experiment to prevent self-shading and nutrient limitation during the experiment. Growth-irradiance experiments were run for 5 d with daily sampling. Samples were preserved in Lugol's solution

(Wetzel and Likens 1995) and were counted either with a CASY counter (Schärfe-System GmbH) or with a compound microscope (in hemocytometers or Palmer cells) or under an inverted microscope using settling chambers (Lund et al. 1958). Growth rates at each irradiance were calculated by fitting a linear least-squares regression to the natural logarithm-transformed cell abundances plotted against time for each irradiance (Litchman 2000). Only the linear parts of the curves with the highest slope, corresponding to the exponential growth phase, were used (three to five data points, on average). Light traits were determined using best-fit models (*see* Curve fitting).

Fourteen growth-irradiance curves were obtained for the following 10 species: *Anabaena flos-aquae, Ankistrodesmus* sp., *Chlamydomonas reinhardtii, Cryptomonas erosa, Fragilaria crotonensis, Microcystis aeruginosa, Oocystis* sp., *Planktothrix rubescens, Rhodomonas* sp., and *Scenedesmus quadricauda*. Cultures were grown in 250-mL or 500-mL Erlenmeyer flasks with 180 mL or 400 mL of medium, respectively, at a light : dark cycle of 14 : 10 h. Twelve or more irradiance levels (single replicates), ranging from limiting to sub-saturating or saturating (0 and 350 or 500 μmol quanta m^{-2} s^{-1}), were used, ensuring a comprehensive coverage of the curve and significant model fits.

Data acquisition from literature—Using a wide literature search, we acquired data on growth at different irradiances for 83 more strains from major freshwater phytoplankton taxonomic groups (Web Appendix, www.aslo.org/lo/toc/vol_56/issue_2/589a.html), for a total of 63 unique species. Diatoms, green algae, and cyanobacteria and, to a lesser degree, cryptophytes were well represented; data on dinoflagellates and desmids were scarce. Only the data from studies conducted at or close to 20°C and grown in nutrient-rich media were used in order to exclude temperature and nutrient effects. Specific growth rate μ (d^{-1}) and μmol quanta m^{-2} s^{-1} units for irradiance were used as a common basis for the analyses; the original data were converted to these units where necessary, using the conversion factors for given light sources (most experiments were conducted under fluorescent light). We collected the growth-irradiance data from the original studies using screenshots or scans and then used 'DataThief III' software (http://www.datathief.org) to digitize the data.

Curve fitting—To obtain the main traits characterizing growth dependence on light (maximum growth rate [μ_{max}, d^{-1}], the initial slope of the growth-irradiance curve [α, μmol quanta^{-1} m^2 s d^{-1}], and the optimum irradiance after which photoinhibition ensues [I_{opt}, μmol quanta m^{-2} s^{-1}]), each of the literature ($n = 83$) and experimental ($n = 14$) curves were fitted to two growth-irradiance models, thus:

$$\text{Model 1}: \mu(I) = \frac{\mu_{max} I}{I + \frac{\mu_{max}}{\alpha}}$$

$$\text{Model 2}: \mu(I) = \frac{\mu_{max} I}{\frac{\mu_{max}}{\alpha I_{opt}^2} I^2 + \left(1 - 2\frac{\mu_{max}}{\alpha I_{opt}}\right) I + \frac{\mu_{max}}{\alpha}}$$

where μ (d^{-1}) is growth rate as a function of irradiance (I, μmol quanta m^{-2} s^{-1}). Model 1 is a Monod equation in which the growth rate saturates at high irradiance. Saturating hyperbolic relationships such as the Monod equation have been frequently used to describe algal growth as a function of irradiance (Kiefer and Mitchell 1983; Laws and Chalup 1990) and were shown to produce parameter estimates very similar to those of another commonly used model, an exponential model by Platt et al. (1980; Eilers and Peeters 1988; Macedo et al. 1998). The photoinhibition Model 2 was taken from Eilers and Peeters (1988), with the changes of parameters made by Macedo and Duarte (2006). We used a nonlinear fitting function in Mathematica 7.0 (Wolfram Research 2008) to obtain values of μ_{max}, α, and I_{opt} (in Model 2). For each data set, the best-fitting model was the one with the smallest Akaike Information Criterion corrected for small sample size (AICc) (Akaike 1974). If multiple data sets were available for individual species, we averaged major parameters obtained from each curve.

We collected species cell volumes (μm^3) either from the original sources or from available databases (Finnish Lakes phytoplankton data and the North American Water Quality Assessment [NAWQA]: http://diatom.acnatsci.org/nawqa/2001biovol.asp). If there was no cell volume information for a given species in those sources, we collected species sizes (μm) from the taxonomic literature (Komárek and Anagnostidis 1999, 2005; Komárek and Zapomělová 2007) and calculated cell volumes (μm^3) following the method of Hillebrand et al. (1999). The species cell volumes and literature source are given in the Web Appendix.

Group comparison—In most cases, we used classes as major taxonomic groups. However, within Chlorophyceae (green algae), we separated the Desmidiaceae (Order Zygnematales) from other orders (Chlorococcales, Tetrasporales, and Volvocales). The Zygnematales are distinguished by their type of reproduction and lack of flagella (Bold and Wynne 1984); they have been suggested to belong to a separate class (Zygnemaphyceae; Round 1971) and often occupy ecological niches that are different from those of other green algae (Bold and Wynne 1984). Within groups, data were tested for normal distribution (JMP 8, SAS Institute) and log-transformed to achieve normal distribution, where needed. Groups were compared using one-way ANOVA, and pairwise comparisons were done using *t*-tests (JMP 8, SAS Institute).

Allometric relationships—To determine whether there is an allometric dependence of main light traits on cell size (volume, μm^3), we regressed log-transformed α, μ_{max} and I_{opt} against log cell volume and fitted two regression models: the ordinary least squares (OLS) regression to compare allometric exponents with other studies exploring cell size relationship (Banse 1982) and the reduced major axis (RMA) regression recommended for allometric relationships (Warton et al. 2006).

We also explored whether there are relationships among the light utilization traits by regressing one of the traits against another. The values were log-transformed to achieve normal distribution. To look for multivariate relationships among the traits, we performed a principal component analysis (PCA; Joliffe 2002) on log cell volume, log μ_{max}, and log α based on the correlation matrix.

Trait conservatism—We also assessed whether major light utilization traits in phytoplankton were evolutionarily conserved and, if so, at what taxonomic levels. We characterized each species in the database taxonomically, specifying the domain, kingdom, phylum, order, class, family, genus, and species. We used a combination of major taxonomic schemes, the Integrated Taxonomic Information System (itis.gov) and the Tree of Life database (tolweb.org), to classify each species. We ran nested ANOVA models on each trait (log-transformed) to determine how much trait variance was explained at each taxonomic level within the nested taxonomy (Kerkhoff et al. 2006). Significance of the variance components was assessed using a likelihood ratio test, in which lower taxonomic levels were added sequentially to see if the addition improved the model (Kerkhoff et al. 2006). These analyses were run using the *R* statistical platform.

Data on taxonomic group distributions in lakes—We explored whether the compiled light utilization traits could be used to explain the distribution of major taxonomic groups along the light availability gradients in nature. We used the Environmental Protection Agency data set from the National Eutrophication Survey (EPA NES) conducted in 1973–1975 to determine the relationship between the relative abundance of major taxonomic groups and light availability. In this survey, more than 500 lakes were sampled for physical, chemical, and biological parameters, including light availability and phytoplankton community composition (USEPA 1975*a*,*b*). Each lake was sampled at its deepest point; integrated samples were taken from the surface up to 4.6 m deep or from the surface to the lower limit of the photic zone (1% of incident light). Most lakes were sampled three times a year to provide information on spring, summer, and fall conditions (total number of samples: $n = 1553$). Phytoplankton samples were preserved with acid Lugol's solution and counted at the Environmental Monitoring and Support Laboratory in Las Vegas, Nevada. Prior to counting, samples were concentrated using the settling method. Counting was done at 400X until a minimum of 100 fields was viewed or until the dominant form was observed a minimum of 100 times (USEPA 1975*b*).

We regressed the relative abundance (arsine square root–transformed; Sokal and Rohlf 1981), including the null occurrences, of each taxonomic group (at both class and genus levels) for which we have the light trait data against Secchi depth, used as a proxy for light availability (Megard et al. 1980). The slope of this regression line characterizes the dependence of a group's (class or genus) relative abundance on light availability: for example, a negative slope indicates that a given group decreases in its relative abundance with increasing light availability. Next, we explored whether the light-related traits could explain the distribution of individual groups along the light availability
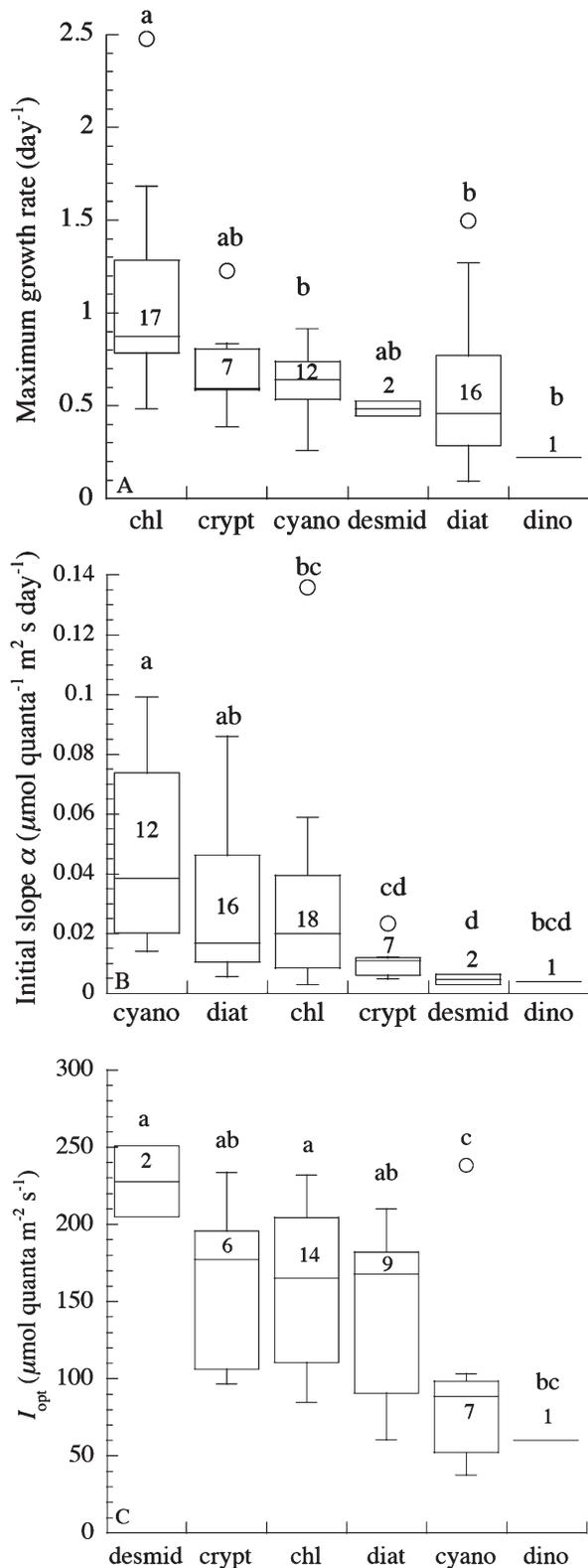
gradient by regressing the slope of the relative group abundance vs. Secchi depth onto the given light-related trait to determine the nature of the relationship, if any.

Results

Curve fitting—Ten literature curves that gave poor fits to both models were discarded. Based on AICc, 38 data sets were fit best by Model 1 and 49 by Model 2. We forced two curves to use Model 2 based on visual inspection of the fits. The obtained parameters and references are given in the Web Appendix. Parameter estimates of μ_{max} and α were typically negatively correlated; other parameter estimate pairs in Model 2 showed no consistent correlation.

Taxonomic group differences—Major taxonomic groups of phytoplankton differed in their light utilization traits (e.g., μ_{max}, α, and I_{opt}; Fig. 1). On average, green algae as a group had the highest maximum growth rates (μ_{max}) and dinoflagellates the lowest (Fig. 1A). Other major groups, such as cryptophytes, cyanobacteria, and diatoms, had intermediate maximum growth rates. Cyanobacteria as a group had significantly higher α values than did many other taxonomic groups, followed by diatoms and green algae (Fig. 1B). Diatoms were similar to cyanobacteria in having relatively low maximum growth rates and high α values. They were, however, more resistant to photoinhibition than were cyanobacteria, with higher I_{opt}. Based on a single species for which data were available, dinoflagellates had the lowest α values; desmids and cryptophytes also had low α values. Except for desmids, all groups with low α values were mixotrophs. I_{opt}, the irradiance at the onset of inhibition, also differed across groups, with cyanobacteria having the lowest and green algae (Chlorophyceae and desmids) the highest values, on average (Fig. 1C).

Allometric relationships—Major parameters of growth-irradiance curves depended significantly on cell size. Cell volumes spanned ca. five orders of magnitude. Green algae covered the entire cell volume range (10^0–10^4), whereas diatoms and cryptophytes had mostly large ($10^{2.5}$–10^4) and cyanobacteria mostly small (10^0–$10^{2.5}$) cell volumes. The maximum growth rates (μ_{max}) were negatively related to cell size, and the exponents of the allometric relationship were -0.08 ± 0.04 for the OLS regression ($R^2 = 0.07$, $p = 0.046$; Fig. 2A) and -0.30 ± 0.06 for the RMA regression (same R^2 and p-value) of the log–log-transformed data. The initial slope α also decreased significantly with cell size, with the exponent of -0.16 ± 0.06 (OLS regression of log–log-transformed data, $R^2 = 0.11$, $p = 0.01$; Fig. 2B) and -0.47 ± 0.11 (RMA regression). The I_{opt} did not exhibit a significant relationship with cell size (data not shown).

Fig. 1.   Box plots of light utilization trait distributions for major taxonomic groups. Groups are arranged in order of decreasing means. Pairwise comparisons were run on log-transformed data; significantly different groups ($p < 0.05$) do not have letters in common. Numbers by each group indicate number of species included. Circles are outliers. Group names

←

are diat (Bacillariophyceae), chl (Chlorophyceae), crypt (Cryptophyceae), cyano (Cyanophyceae), desmid (Desmidiales), and dino (Dinophyceae). (A) Maximum growth rates (μ_{max}). (B) Initial slopes of the growth-irradiance curves (α). (C) Irradiance at the onset of inhibition (I_{opt}). Note that not all species were photoinhibited; hence, there are fewer values for each group.
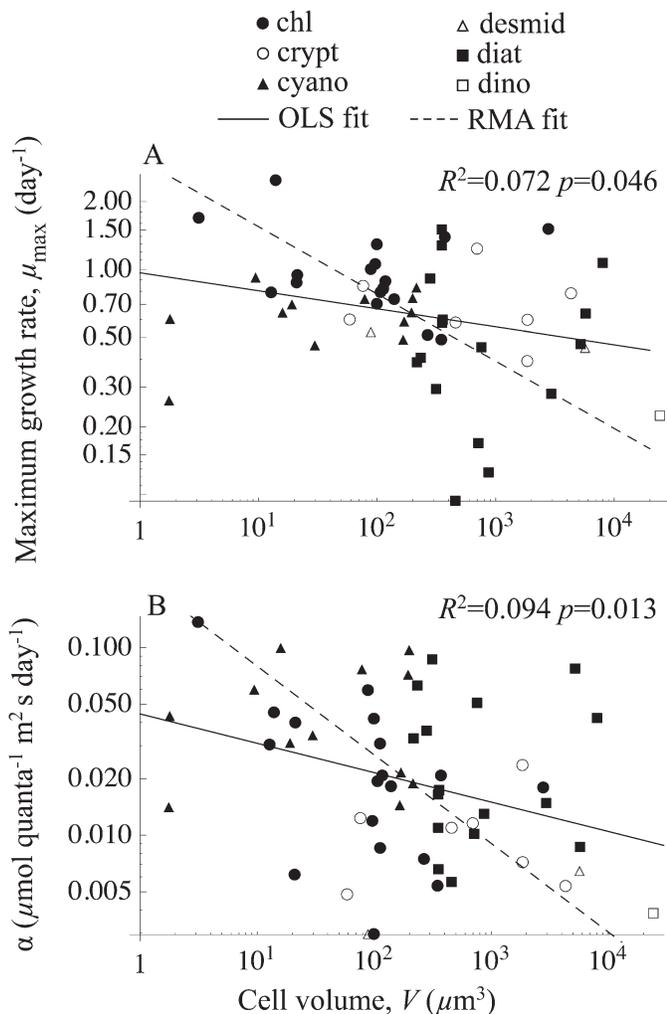
Fig. 2. Light utilization traits vs. cell size. (A) Maximum growth rate (μ_{max}). (B) Initial slope of the growth-irradiance curve (α). The fit is the power relationship of all trait values as a function of size on a log–log scale.

Fig. 3. Relationship between the maximum growth rate μ_{max} vs. initial slope of the growth-irradiance curve, α.

Relationships among traits—There was a weakly significant positive relationship between the maximum growth rate μ_{max} and the initial slope of the growth-irradiance curve α ($p = 0.05$, log–log-transformed data; Fig. 3). I_{opt} and α were weakly negatively correlated ($p = 0.056$, log–log-transformed data). μ_{max} and I_{opt} were not significantly correlated.

In the PCA, the largest principal component (PC) explained the majority of the variation, with an eigenvalue of 1.58, and the other two PCs had similar eigenvalues, so we consider only the largest PC. Its eigenvector was (log cell volume, log μ_{max} log α) = (1, −0.917, −0.994), indicating that the largest amount of variation in traits reflects that larger cells grow slower, both at low and high irradiance.

Trait conservatism—Of the major traits analyzed, α and cell size were conserved at the highest taxonomic level (domain), as inferred from the relatively large percentage of trait variance explained at this level (Table 1). In contrast, μ_{max} and I_{opt} were more labile at higher taxonomic levels,
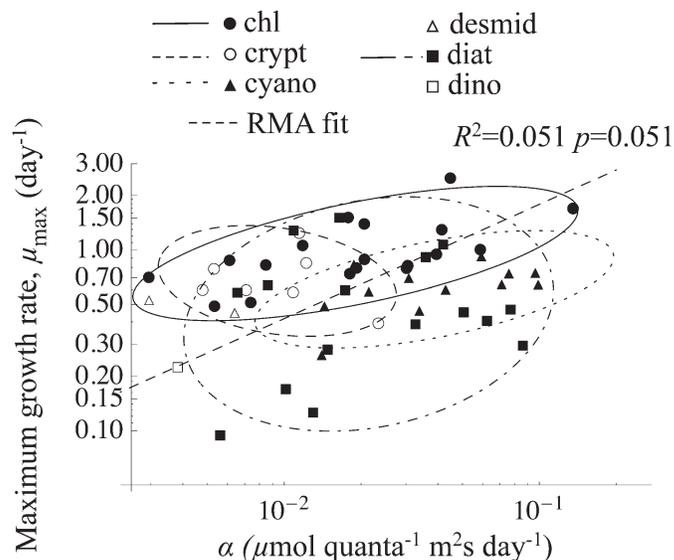
having the highest percentage of variance explained at the order level (Table 1). The residual variance at the species or genus level was substantial for all traits except I_{opt} (Table 1), indicating high adaptability at these lower taxonomic levels.

Group distribution patterns—Major taxonomic groups displayed different responses to light availability in lakes across the United States. Cyanobacterial relative abundance strongly declined with increasing Secchi depth (Fig. 4; Table 2). The relative abundance of chrysophytes, dinoflagellates, and cryptophytes was significantly positively correlated with Secchi depth (Table 2). Interestingly, all groups with positive slopes are mixotrophs. The slope for diatoms was positive but not significant (Table 2). Groups with high average α values tended to decline in relative abundance with increasing Secchi depth, having a negative slope (cyanobacteria and chlorophytes and desmids). Conversely, groups with low α values tended to increase in relative abundance with increasing Secchi depth. The slopes of the regressions of classes' ($n = 6$) relative abundances vs. Secchi depth were weakly negatively related

Table 1. Nested partitioning of variance (percent) for major light utilization traits across all taxonomic levels. Bold numbers are statistically significant, as determined by the likelihood ratio test ($p < 0.05$).

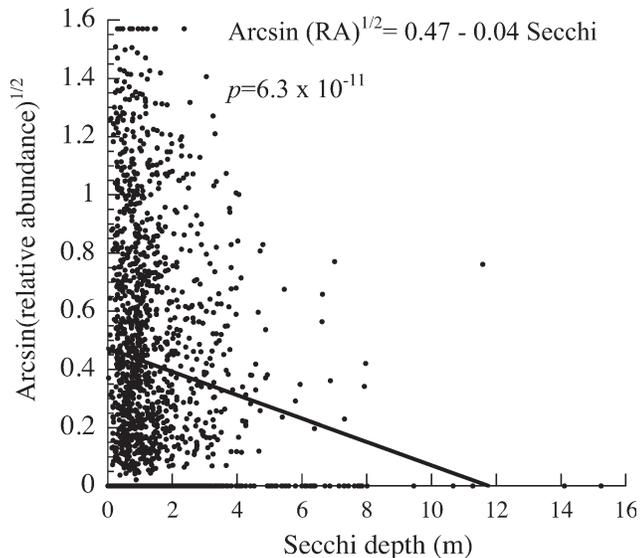| Level	μ_{max}	α	I_{opt}	Cell size
Domain	<0.1	**21.2**	0.90	**26.7**
Kingdom	**15.4**	<0.1	<0.1	**21.8**
Phylum	<0.1	<0.1	**14.9**	<0.1
Class	<0.1	<0.1	<0.1	<0.1
Order	**57.1**	**21.3**	**76.9**	**22.6**
Family	<0.1	<0.1	0.32	6.5×10^{-13}
Genus	<0.1	<0.1	<0.1	**21.9**
Species	**27.5**	**57.5**	6.9	6.9

Fig. 4.   Relative abundances (RA) of cyanobacteria (arcsine square root–transformed) against Secchi depth (Secchi) in 527 lakes across the continental United States ($n = 1553$, most lakes were sampled three times a year) sampled in 1973–1975 within the EPA NES.

to the class's average α, the initial slope of the growth-irradiance curve ($R^2 = 0.57$, $p = 0.08$). When individual genera were considered ($n = 28$), the relationship between the slope of the relative abundance vs. Secchi depth and the genus's average α values was even more significant ($R^2 = 0.16$, $p = 0.03$; Fig. 5). α was the only physiological trait (not the μ_{max} or I_{opt}) that was significantly related to the slope of the group's relative abundance vs. Secchi depth.

Discussion

Group differences—Our extensive meta-analysis of growth responses of major taxonomic groups to irradiance demonstrates that despite considerable within-group variation, these groups differ significantly in their light utilization traits, corresponding strategies, and distribution patterns along natural light gradients. Cyanobacteria as a group appear to be adapted to low light environments by having significantly higher initial slopes, α, of the growth-irradiance curves and low I_{opt}. Therefore, our results strongly support other studies indicating that cyanobacteria efficiently utilize low light and, thus, should thrive in low light environments (Van Liere and Walsby 1982). They often dominate eutrophic lakes characterized by high nutrients and low light (Reynolds 1987). The efficient utilization of low light is an adaptive strategy in such environments. The high efficiency of light utilization in cyanobacteria may have evolved as a result of low solar luminosity (30% lower than at present; Milo 2009) at the time of cyanobacteria's origin ca. 3.8 billion yr ago. A high susceptibility to photoinhibition (low I_{opt}), possibly arising from the structure of the photosynthetic apparatus and small cell size (*see* below), would be less detrimental under lower light conditions at that time. Adaptation of cyanobacteria to low light is associated with efficient

Table 2.   Slopes of the OLS regressions of the relative abundances of various taxonomic groups (arcsine square root–transformed) against Secchi depth in 527 lakes across the continental United States sampled in 1973–1975 within the EPA NES.

Group	Slope	SE	Slope *p*-value
Cyanobacteria	−0.04	0.006	<0.001
Diatoms	0.0061	0.005	0.27
Green algae (Chlorococcales, Tetrasporales, and Volvocales only)	−0.010	0.0037	0.01
Cryptophytes	0.028	0.0047	<0.001
Dinoflagellates	0.0068	0.0015	<0.001
Chrysophytes	0.017	0.002	<0.001
Desmids	−0.0028	0.0015	0.06

light-harvesting capability (chlorophyll *a* [Chl *a*]–specific extinction coefficient) and chlorophyll-to-carbon (Chl : C) ratios (Langdon 1988). High values of the Chl *a*–specific extinction coefficient, compared to other groups (e.g., green algae and diatoms), are in part due to the presence of accessory pigments such as phycocyanins (Kirk 1994). In addition, cyanobacteria often show physiological responses to light, such as regulation of the amount of accessory pigments (chromatic adaptation), and buoyancy control that increase their light competitive abilities even further (Klemer 1991; Litchman 2003; Huisman et al. 2004).

Green algae have the highest maximum growth rates, relatively low α values, and high I_{opt}. Therefore, our results confirm the notion that green algae are a relatively high–light adapted group, compared to cyanobacteria (Richard-
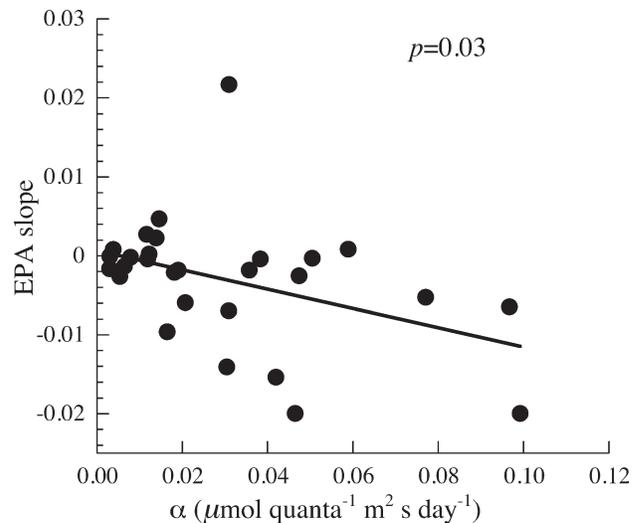

Fig. 5.   Slope of the regression of a genus's relative abundance (arcsine square root–transformed) in 1553 samples (527 lakes, most sampled three times a year) against Secchi depth plotted vs. that genus's average α. Slopes were determined for each genus by fitting OLS regression to the data, as in Fig. 4 (1553 samples), and α values for each genus are averages across species. The line is the OLS fit ($R^2 = 0.17$, $p = 0.03$).

son et al. 1983). Desmids appear to be even more high light adapted than other green algae, as shown by their lower light utilization efficiencies (α) but higher irradiances for photoinhibition (I_{opt}). Diatoms had low maximum growth rates but high α values. This suite of traits indicates good low light competitive abilities. They, however, have a higher photoinhibition tolerance (higher I_{opt}) than cyanobacteria, possibly indicating adaptation to well-mixed conditions with exposure to both low and high light. Mixotrophs (dinoflagellates and cryptophytes) had low α values. In addition, dinoflagellates (based on a single species but supported by other studies [Smayda 1997]) had low maximum growth rates. Such a combination of light utilization traits makes these groups poor light competitors. However, their motility and mixotrophy likely offset their poor light utilization abilities.

The majority of species and strains for which the data were available occur in temperate lakes; it would be interesting to see whether including representatives from other geographic regions, such as the tropics, would alter the observed relationships. Moreover, including the marine species in such analyses would help compare the role of evolutionary history vs. the environment-specific selection pressures.

Relationships between traits—We found that major light utilization traits are significantly correlated: μ_{max} is positively related to the initial slope of the growth-irradiance curve, α. This indicates that species with high maximum growth rates tend to have high initial slopes of the growth-irradiance curves and, thus, a high efficiency of utilizing low light, which makes them better adapted to low light environments. This result is not driven by the correlation between parameter estimates in curve fitting, because those were negatively correlated. A negative correlation of I_{opt} with α indicates that species adapted to low light (high α) may be more susceptible to photoinhibition. This indicates a trade-off in adaptation to low vs. high irradiances, reflecting physiological constraints on light traits that preclude the existence of a 'superspecies' with respect to light utilization capabilities.

Trait conservatism and the role of phylogeny—We were able to estimate variances at all levels except within species. Ideally taxonomic sampling would be more comprehensive. However, previous simulation work (Ackerly 2000) has indicated that sparse taxonomic sampling often recovers a strong estimate of the 'true' phylogenetic signal in continuous trait data. This is particularly true in cases with random sampling and is less true when the samples are taken from single communities that are highly structured as a result of convergent adaptations. Given the nature of the present data set, we suggest that the taxonomic sampling is dense enough to provide a reliable estimate of the phylogenetic signal in the traits studied.

High conservatism of the major light utilization trait, α, as well as the cell size (high percent of variance explained at the domain level) may be indicative of a fundamental evolutionary divergence of these traits between eukaryotes and prokaryotes, likely related to differences in cellular organization. High values of α and, hence, a greater light utilization efficiency in prokaryotes (cyanobacteria) may arise as a result of the particular structure of their photosynthetic apparatus (i.e., absence of chloroplasts). In cyanobacteria, thylakoids (photosynthetic membranes) are located in the cytoplasm. Consequently, light has to cross only the cell wall to reach thylakoids, thus resulting in a high α. In eukaryotic phytoplankton, light has to cross both the cell wall and the chloroplast membranes, two or more, depending on the evolutionary origin of the group (Yoon et al. 2004; Hackett et al. 2007). A greater light attenuation by multiple membranes may lower light utilization efficiency, thereby resulting in lower α.

High conservatism of cell size may be due to the different degrees of cellular complexity and the presence or absence of nucleus determining how large a cell can get. On average, prokaryotic cells are much smaller than eukaryotic cells (Gregory 2001). This finding was supported by our analysis, with cyanobacterial cells being smaller than the eukaryotic phytoplankton. Cell size also likely affects the low light utilization efficiency (*see* Allometric scaling of light traits, below). To overcome some of the negative consequences of small cell size (e.g., high grazer susceptibility), cyanobacteria are often colonial or filamentous. Two other traits, μ_{max} and I_{opt}, appear to be more labile, with the greatest percent of variance explained at the order level. The data also indicate that even for more conserved traits, α and cell size, there is a significant diversity at the species or genus level, potentially indicating local adaptation and diversification.

Phytoplankton taxonomy is fluid, and the taxonomic affiliations of organisms change periodically. These changes in taxonomic classification may affect the results regarding trait conservatism; however, the results at the highest taxonomic levels (domain and kingdom) should be reasonably robust, as these divisions are more stable.

Allometric scaling of light traits—Light utilization traits were significantly correlated with cell size. The negative correlation of the initial slope α, obtained from the growth-irradiance curves, with cell size indicates that smaller cells may be more efficient at utilizing low light, as was suggested previously (Geider et al. 1986; Fujiki and Taguchi 2002). Small cells tend to have higher light absorption efficiency, because with increasing cell size and, consequently, a greater path length for light, self-shading decreases the pigment absorption efficiency, resulting in the packaging effect (Falkowski and Raven 1997; Finkel 2001; Fujiki and Taguchi 2002). In line with our findings, previous experimental data showed that cell size decreases under low light (Claustre and Gostan 1987).

The allometric scaling of maximum growth rates with cell size has been reported before, and the exponent (linear regression slope of the log–log-transformed data) derived from our data compilation is similar to the previously obtained exponents (Banse 1982; Tang 1995; Finkel 2001). The exponents for α are greater than for μ_{max}, indicating a stronger control of light utilization efficiency by cell size compared to the maximum growth rates. Because small cells tend to have both high α and μ_{max}, they may be well

adapted to both low and high light, as was noted before for marine phytoplankton (Geider et al. 1986).

The dependence of major light utilization traits on cell size indicates that different light levels may result in different size distributions of phytoplankton communities. It would be interesting to test if there is a higher relative abundance of small cells under low light, because of their higher efficiency of low light utilization. Low nutrient levels often result in a preponderance of small cells because of their efficient nutrient acquisition due to a high surface area–to-volume ratio (Chisholm 1992; Raven 1998) and are often associated with high light conditions, as observed in oligotrophic systems. Therefore, the pattern in phytoplankton size distributions due to light may be obscured by the opposing influence of low nutrients, which selects for smaller sizes (Chisholm 1992; Raven 1998). The use of multivariate statistical methods should help in distinguishing the effects of light (vs. nutrients) on cell size distributions.

Different size distributions driven by differences in light (and nutrient) levels may lead to contrasting zooplankton size distributions and abundance, with resultant effects that can percolate to higher trophic levels. Therefore, different levels of available irradiance—through the mediation of the size distribution of phytoplankton—may have a profound effect on the community structure and energy transfer in aquatic ecosystems. Knowledge related to the light utilization traits in major phytoplankton groups and their allometric scaling should help us predict phytoplankton community organization along light gradients in natural systems.

Relating traits to group distributions along the light gradient—Our analysis of the distributions of major taxonomic groups along the light availability gradient among lakes showed that groups differ in their responses to light in accordance with their light utilization traits. In particular, the initial slope of the growth-irradiance curve (α) was significantly negatively correlated with the slope of the relationship between the group's relative abundance and Secchi depth (Fig. 5). Cyanobacteria's relative abundance decreased the most with increasing light availability (the most negative slope of their relative abundance vs. Secchi depth; Table 2). This pattern agreed well with the low light–adapted strategy of this group inferred from the light utilization traits (high α, low I_{opt}) and may be associated with their preference for high nutrient conditions (Huisman et al. 2005) that result in high biomass and, consequently, low light availability. Groups whose traits indicate lack of low light adaptation (dinoflagellates and cryptophytes, capable of mixotrophic nutrition) tended to increase in relative abundance with increasing light availability, probably as a result of their association with low nutrient conditions (Pollinger 1988) that are often characterized by high light availability. Our preliminary analysis of the relative abundance of major groups as a function of the total phosphorus (TP) concentration in 527 lakes across the United States strongly supports this notion: cyanobacteria and green algae show a highly significant positive response to TP, while dinoflagellates, cryptophytes, chrysophytes, and diatoms have negative relationships with TP. In this analysis we used Secchi depth

as a proxy for light availability to phytoplankton, and although not a perfect measure (e.g., the depth of the mixed layer significantly affects light availability), this parameter is frequently used to characterize light climate and was available for all lakes in the EPA NES database. Future refinements may include calculating the depth of the mixed layer from temperature data, where available, and using it, together with light attenuation coefficients, to estimate light availability more precisely.

In summary, we showed that major taxonomic groups of freshwater phytoplankton differ in their light utilization traits, and these differences reflect fundamental evolutionary divisions, up to the domain level. Different trait value combinations define distinct ecological strategies that correspond well to the group distributions along the light availability gradient in lakes. The obtained traits may allow stronger inferences related to the group differences with respect to their light utilization strategies and distribution patterns in nature. They can also be used to meaningfully parameterize models of phytoplankton to investigate phytoplankton community assembly along major environmental gradients.

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