Temperature and resources interact to shape phytoplankton size-abundance relationships at a continental scale

Vojsava Gjoni 1,2*, Douglas S. Glazier3, Bastiaan W. Ibelings1, and Mridul K. Thomas1

1 Department F.-A. Forel for Environmental and Aquatic Sciences, University of Geneva, Geneva, Switzerland
2 Department of Biological and Environmental Sciences and Technologies, University of Salento, Lecce, Italy
3 Department of Biology, Juniata College, Huntingdon, PA 16652, USA

*Correspondance: e-mail: vojsava.gjoni@unige.ch

Abstract

Communities contain more individuals of small species and fewer individuals of large species. The observed -3/4 slope relating mean size and mean abundance across communities (the ‘cross-community scaling relationship’ or CCSR) is thought to arise from a 3/4-power scaling of metabolic rate with body size. Assuming that 3/4-power metabolic scaling is universal, as claimed by the metabolic theory of ecology (MTE), size-abundance relationships should also be invariant with a scaling exponent of -3/4 or nearly so, across environmental conditions. However, we find that the CCSR slope is actually -1 overall (perhaps due to isometric metabolic scaling), and varies substantially across temperature and nutrient gradients in 1048 freshwater lake phytoplankton communities in the USA. The size-abundance relationship is most shallow at low temperatures and high nutrients, and steeper and relatively insensitive to nutrients at high temperatures. Phytoplankton communities have orders of magnitude more small or large cells depending on environmental conditions than would be predicted from the MTE. Although based on observational data, these results suggest that the environment alters either metabolic scaling or other poorly understood processes shaping community size distributions.

Keywords: Phytoplankton, Size-abundance relationship, Body size, Temperature, Resources.
Introduction

The body mass (M) of a species is strongly associated with many physiological, ecological and behavioral traits (Brown et al. 2004, Woodward et al. 2005, White et al. 2007). It is also negatively related to population abundance (N), often approximating the power function \( N \propto M^{-3/4} \) (Damuth 1981, 1991, 1998). Assuming that M is related to metabolic rate (R) as \( R \propto M^{3/4} \) (Brown et al. 2004), the total energy (E) used by each species should be \( E \propto (N \times R) \propto M^{3/4} \times M^{-3/4} \propto M^0 \) (Energetic Equivalence Rule – EER, Nee 1991, Brown et al. 2004). Furthermore, M is negatively related with the total community N either through time or across space. Such cross-community scaling relationships (CCSR, following White et al. 2007) have received increasing attention in recent years (Enquist et al. 1998, White et al. 2004, 2007, Meehan et al. 2004, Li 2002, Long and Morin 2005, Arim et al. 2011, Gjoni et al. 2018, 2019, Gjoni and Glazier 2020). A power function \( N \propto M^{-3/4} \) for the CCSR implies that at the community level an equivalent amount of energy is used by species with different body sizes.

Although the -3/4 scaling exponent of size-abundance relationships has been claimed to be universal, considerable deviations have been reported for plankton communities (Cavender-Bares et al. 2001, Huete-Ortega et al. 2012, Marañón 2015). Thus far, this variation has largely been assumed to be idiosyncratic, with little investigation of whether it is systematically related to environmental factors or ecological processes. The existence of any such systematic variation would undermine the claim that the relationships between body size, energetic requirements and population abundance described above are universal. This universality is assumed by the influential metabolic theory of ecology (MTE), which attempts to explain a wide range of ecological phenomena based on underlying physiological processes (Brown et al. 2004, West et al. 1997). Therefore, investigating variation in size-abundance relationships may help us identify weaknesses in existing theoretical models such as the MTE, as well as possible additional mechanisms that should be incorporated into them. Recent studies examining metabolic scaling have also highlighted variation in these relationships, pointing towards the need for a careful examination of the assumptions and predictions of the MTE, and a consideration of alternative models (e.g., Glazier 2014a, 2018, Harrison 2017, White et al. 2019, Kozłowski et al. 2020).

Temperature is one of the most important environmental factors affecting physiological and ecological processes (Brown et al. 2004). The MTE assumes that the rates of biochemical reactions comprising metabolism increase exponentially with increasing temperature, independently of body size (Fig. 1A; Gillooly et al. 2001, Brown et al. 2004). This implies that the size-abundance relationship should also not change across environmental temperature gradients (Fig. 1B). However,
recent studies have shown that temperature can alter size-abundance relationships (e.g., Morán et al. 2010, Gjon and Glazier 2020). Temperature may affect the size-abundance relationship in two hypothetical ways. First, the body-mass scaling of metabolic rate may become less steep at higher temperatures (Glazier 2005, 2014b, 2020, Killen et al. 2010, Glazier 2020), thereby altering the size-abundance relationship. Second, temperature may affect body size directly (‘temperature-size rules’ – TSRs, Daufresne et al. 2009, Atkinson 1994, Bergmann 1847), thereby affecting the size-abundance relationship. In short, the mechanistic connections between temperature, metabolism and body size may be more complex than assumed by MTE, thus causing size-abundance relationships to vary, rather than conform to a single theoretical slope.

Resource supply may also have a key role in determining the slope of size-abundance relationships. EER predicts that resources are equally partitioned between species in a community regardless of their body size (i.e., without changing the slope). Furthermore, increased resource availability increases the energy available for, not only maintenance metabolism, but also growth and reproduction, thereby increasing population abundance, as observed for birds (Meehan et al. 2004) and plankton (Abrams 1993, Carmeno et al. 2008). However, if the increases in growth and reproduction occur independently of body size, then greater resource availability may increase the intercept of size-metabolism and size-abundance relationships without changing their slopes (~3/4, Fig. 1C and 1D).

Deviation from the predictions of the EER may be driven by size asymmetries in resource acquisition (Nee et al. 1991, Cotgreave 1994, Russo et al. 2003) or access (Holling 1992, Ernest 2005). Furthermore, according to the ‘resource rule’ (RR), changes in resource availability cause changes in body maintenance, growth and reproduction that modify the adult body size of species (McNab 2010, Huston and Wolverton 2011). Large species are able to allocate more energy to growth under high-resource supply conditions because of their higher resource uptake rates but are extirpated under resource-limited conditions. Small species require fewer resources and therefore do relatively better when resources are limited (Litchman and Klausmeier 2008, Qu et al. 2019, Yang et al. 2021). Low resource supply levels should therefore decrease the size-abundance relationship slope (i.e., make it more negative), whereas high levels of resource supply should increase the slope (i.e., make it more positive).

Temperature and resources may interact to shape the size-abundance distribution, though presently we do not have clear expectations of how this would occur (see e.g., Sommer et al. 2017). There is extensive evidence from a wide variety of organisms that temperature and resource availability interact to shape organismal and population growth rates (Brett 1971, Thomas et al. 2017, Huey and Kingsolver 2019), feeding rates (Rall et al. 2012), body stoichiometry (Kendrick and
Benstead 2013), resource requirements (Thomas et al. 2017, Lewington-Pearce et al. 2019), body sizes (Kimmance et al. 2006) and possibly assimilation rates and resource-use efficiencies. At the community and ecosystem level, they alter food web structure, ecosystem metabolism (O’Connor et al. 2009), community composition and trait distributions (Roselli and Basset 2015, Ryan et al. 2017), and trophic interactions (Dell et al. 2014, Bideault et al. 2019). Any one of these mechanisms could plausibly lead to temperature-resource interactions shaping size-abundance relationships, and future modelling work would be valuable in exploring these.

We, therefore, investigated how temperature and resource availability shape the size-abundance relationship of phytoplankton communities in 1048 lakes in the USA. To do so, we compared the mean body size and total abundance of whole communities. Our study takes advantage of continental-scale observational data on community properties to (i) shed light on the possible need for improvements in an important theoretical framework, the metabolic theory of ecology, and (ii) highlight how environmental change may have more complex effects on aggregate community and ecosystem properties than is presently assumed.

Our study has potentially important implications for, not only an improved understanding of community structure, a central topic of ecology, but also of how communities and ecosystems respond to climate warming and nutrient enrichment, the two key drivers of ecosystem change in lakes and oceans (e.g., Moss 2012). Phytoplankton communities, in particular, are important to study because they are vital components of aquatic food webs and biogeochemical cycles, and are highly responsive to environmental change (Van de Waal and Litchman 2020). They are also especially useful for undertaking CCSR studies because they exhibit a broad range of cell sizes (Finkel et al. 2010), and extensive data exists for the cell or colony size and abundance of species in phytoplankton communities under different environmental conditions, thus allowing for rigorous statistical tests to be carried out.

Material and Methods

We used observational data from >1000 US lakes to model the effects of temperature, nutrients, and zooplankton on the CCSR (White et al. 2007).

NLA dataset

We used data from continental US lakes collected as part of the 2012 National Lakes Assessment survey conducted by the US Environmental Protection Agency (Fig. 2). NLA is a synoptic sampling program of lakes, reservoirs, and ponds implemented across the continental US (Pollard et al. 2018).
The lakes were sampled once in the summer (June–September) and were selected from the National Hydrography Database (https://nhd.usgs.gov/). At each lake, an extensive set of environmental variables was measured, but we provide sampling details only for variables used in our analysis. Field crews used standardized sampling methods across all sites, with collections made during the morning to early afternoon. All data are freely available online (https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys).

Variables examined in this study

To examine the CCSR slopes across lakes of the NLA dataset, we used the following variables (biological, chemical, and physical measurements and samples were taken at a deep, open water location (i.e., <50 m) in each lake ecosystems:

- **Cell volume**: for each sample (corresponding to a different lake) all cell biovolumes of all identified taxa were quantified (μm$^3$ mL$^{-1}$). Formulae for solid geometric shapes that most closely match the cell shape were used to estimate biovolume (Hillebrand et al. 1999). Cell biovolumes of each taxon were then divided by the population density (cells mL$^{-1}$) to estimate the cell volume (S).

- **Population density**: phytoplankton population size per unit area (cells mL$^{-1}$) of all taxa was summed to estimate total in each lake population density (D).

- **Temperature**: multi-parameter water quality meters (or sonde) were used to measure temperature (T - °C) at the open water location (i.e., <50 m) in each lake ecosystems.

- **Nutrients**: water samples were inspected and stored at 4°C in darkness. The samples were then filtered (0.4μm) and nitrate concentration (N - mg L$^{-1}$) of each sample from each lake was measured with flow injection automated colorimetric analysis (e.g., ion chromatography).

- **Zooplankton predation**: mean biomass of zooplankton (μg L$^{-1}$) in each lake was used as a proxy for zooplankton predation pressure (Z), see Appendix S1: Fig. S1.

The NLA sampling of both biotic and abiotic components and sample processing procedures are described in detail and discussed elsewhere (https://www.epa.gov/national-aquatic-resource-surveys/manuals-used-national-aquatic-resource-surveys).

Data exclusion rules

1. A small number of lakes were visited and measured more than once. To avoid complications associated with non-independence of data points, we included data from only the first visit to each lake.
2. For three lakes in situ temperatures >60 °C were recorded. We treated these as errors and excluded these temperature values. For reference, the next highest temperature recorded was 35.5 °C.

3. We also excluded zooplankton species that were recorded as being parasitic or carnivorous from our analyses, with the goal of capturing the herbivorous zooplankton. Not all species had an ecological descriptor, so this step only excluded species for which information was available in the dataset.

4. Data from 85 lakes were excluded from the model fitting process because of the absence of measurements of at least one of our study variables.

After these exclusion steps, we were left with data from 1048 lakes.

**Data processing steps**

1. For some lakes the mean nitrate concentration was below the detection limit of 0.001 (mg L⁻¹), and thus recorded as a zero. We replaced all zeroes with values that were half the detection limit i.e., 0.0005.

2. For each lake, we calculated the following quantities:
   - total population density of phytoplankton cells in each lake
   - mean volume of all cells in each lake
   - mean zooplankton biomass in each lake

**Data analysis**

We quantified the CCSR and its dependence on environmental variation by using linear models. As a first step, we developed a priori hypotheses about how environmental variation should affect the CCSR.

**Hypotheses evaluated**

First, we tested whether the slope for the phytoplankton CCSR for all lakes sampled was equal to -3/4, as predicted by the MTE. Second, we tested whether the CCSR scaling slope was invariant, regardless of variation in water temperature and nutrient availability, again as predicted by the MTE (Fig. 1). These environmental variables were selected because they are both known to affect metabolic scaling relationships (see Introduction). Third, we tested whether increased zooplankton biomass (a proxy for predation pressure) reduces the total density of phytoplankton (and thus the
CCSR intercept), especially at higher temperatures because prey consumption per unit predator biomass should increase exponentially with temperature.

Model fitting

We translated these hypotheses into the following linear model, which we fitted to the data:

\[ A = \alpha + \beta_1(S) + \beta_2(T) + \beta_3(N) + \beta_4(Z) + \beta_5(S \times T) + \beta_6(S \times Z) + \beta_7(S \times N) + \beta_8(T \times Z) + \beta_9(T \times N) + \beta_{10}(S \times T \times N) + \epsilon \]

where \( A \) (or abundance) is the mean cell density of all phytoplankton species, \( S \) (or size) is the mean volume of all phytoplankton cells, \( T \) is the mean temperature, \( N \) is the mean nitrate concentration, and \( Z \) is the biomass concentration of herbivorous zooplankton. We calculated means per lake (e.g., mean temperature) when multiple measurements were taken during one sampling effort (no vertical structure in these variables was recorded and so this averaging does not bias measurements). All variables except temperature \( T \) were log_{10}-transformed before model fitting.

Justification of modelling choices and exploration of alternatives.

1) This model does not include all possible interactions. We intentionally excluded one possible 2-way interaction (\( Z \times N \)), three possible 3-way interactions (\( Z \times N \times T \), \( Z \times S \times T \), \( Z \times N \times S \)) and the 4-way interaction (\( S \times T \times N \times Z \)). We restricted the terms in our model based on the hypotheses described earlier, in part to meaningfully evaluate them with a relatively small dataset of ~1000 points. All results presented are based on this restricted model.

2) Because some phytoplankton species are colonial, our quantification of abundance and volume at the cell level arguably misses important aspects of phytoplankton ecology. We therefore also analyzed our data at the colony level and found similar patterns (Appendix S1: Fig. S2 and S3).

3) We used dissolved nitrate as a proxy for resource availability, even though phosphorus is more commonly the growth-limiting nutrient in lakes. Unfortunately, dissolved phosphate concentrations were not measured as part of the NLA. We believe our approach is reasonable because dissolved phosphorus concentrations are highly correlated with nitrate concentrations in nature (Ma et al. 2021). The NLA did measure total phosphorus, but this is arguably a better measure of biomass rather than nutrient availability. We replaced nitrate concentrations of 0 with values half the detection limit. In the absence of good ways to impute these values, we explored simpler approaches such as excluding them (replacing with NA). Doing so did not alter our conclusions.

4) There is some uncertainty associated with estimates of the mean phytoplankton volume in each lake. We have ignored this uncertainty in the models presented, but we subsequently explored
weighting points by 1/SE of the mean volume estimate. This weighting did not alter any conclusions and so we present the simpler models here.

**Tools used**

We used the R statistical environment 4.1.2 (R Core Team, 2018) and the function `lm()` to fit linear models. To visualise output from the fitted models, we used the following packages: `interactions`, `modelsummary`, `sjPlot`, `sjmisc`, `ggplot2`, `ggeffects`, `ggthemes` and `janitor`, for data analysis and interactions plots.

**Results**

The slope of the size-abundance relationship for all phytoplankton communities in our dataset was substantially lower than the MTE expected value of -3/4, but not different from -1 (Fig. 3, Table 1, p < 0.001).

The inclusion of environmental predictors in the model revealed strong (and likely causal) relationships that alter the observed CCSR slope of -1 that is presented in Fig. 3. In particular, temperature (T) and total nitrogen concentration (a proxy for resource availability, N) interacted strongly to alter the slope. At low N, there was a small increase in abundance with increasing T that was similar across all size classes (Figure 4A). However, at high N, the abundance of small phytoplankton increased relative to that of large phytoplankton with increasing T, making the slope more negative (Fig. 4B). Viewing the same relationship from a different perspective, at low T, increasing N increased the abundance of large phytoplankton more than that of small phytoplankton, thus making the slope more positive (Fig. 5A). However, at high T, N had relatively little effect on the abundance of both small and large phytoplankton, thus resulting in no strong changes in the slope or intercept (Fig. 5B). Specifically, the CCSR slope increases with increasing N at low T and decreases with increasing N at high T (Fig. 6).

**Discussion**

Our findings are inconsistent with MTE predictions in two ways. First, the slope for the size-abundance relationship we observed was not -3/4 (Brown et al. 2004), but -1. This isometric size-abundance relationship has also been reported for phytoplankton assemblages by Cavender-Bares et al. (2001), Huete-Ortega et al. (2012), and Marañón (2015). Second, although the MTE predicts invariant size-abundance relationship regardless of taxon and environment, we found that temperature and resource supply interact to shape the slope of lake phytoplankton communities. These results
suggest possible weaknesses in the MTE framework and point towards the importance of
environmental effects on either metabolism, body size, or the physiological and ecological processes
connecting metabolism with size-abundance relationships.

However, the isometric size-abundance relationship that we found is still consistent with the
EER relating metabolism to size-abundance relationships. For reasons that are not well-understood,
the metabolic scaling slope has been found to be ~1 for phytoplankton (Huete-Ortega et al. 2012,
Marañón 2015) instead of 3/4, implying that the slope of the size-abundance relationship should be
the reciprocal of 1, i.e., -1 as we observed. Proportional (1 on 1) relationships of cell size with
metabolic rate and abundance result in equal energy expenditure for all cell size classes, as predicted
by the EER. Therefore, although we did not find the canonical -3/4 scaling of the MTE, the -1 slope
that we did find is, in fact, the reciprocal of the metabolic scaling exponent in phytoplankton and
therefore consistent with the EER.

The interactive effects of temperature and nutrient availability on the size-abundance
relationship slope are inconsistent with a simple link between metabolism and size-abundance
relationships. To summarise our findings: small organisms are favored (more abundant than expected
based on -3/4 scaling) under either high temperatures or low resource conditions. Large organisms
are favored (more abundant than expected) only when there is a combination of low temperatures and
high resource conditions. The benefits of small size under low resource conditions are well
understood in phytoplankton: small cells are more “effective” at assimilating resources (light and
nutrients) and require fewer resources per cell, thus giving them a competitive advantage over larger
cells under low resource conditions (i.e., R*, Tilman 1982). However, why small individuals are
favored under high temperature and high resource conditions is less clear. Small phytoplankton cells
are commonly observed to be more abundant at high temperatures, but this observation is usually
attributed to low nutrient conditions, because of the earlier nutrient-use effectiveness argument and
because temperature and nutrients covary negatively in lakes (Vucic-Pestic et al. 2020). This does
not explain our results here, because our model implies that small individuals are more abundant even
when high temperatures and high nutrient concentrations co-occur. The apparent conundrum can be
resolved if small individuals’ competitive advantage increases with temperature independent of
resource concentration. This appears to be the case in phytoplankton: small cells’ competitive
advantage for nutrient uptake and usage increases with increasing temperature, prior theoretical work
based on empirical measurements of phytoplankton physiology supports this claim (Reumann et al.
2014). If true, this would explain why large individuals are advantaged only in cold, resource-rich
conditions. However, why small individuals are favored under high temperature and high resource
conditions is less clear.
Two explanations for the patterns in our dataset that we can rule out are the intraspecific patterns of the temperature-size rule (TSR) and the resource rule (RR) as strictly defined (Daufresne et al. 2009; McNab 2010). The TSR is a plastic response of individuals to being grown at colder temperatures. The RR states that individuals tend to be larger in areas with high resource availability (McNab 2010). These may not apply to our study because there is no any direct intraspecific variation in any of our estimates. Individual body sizes were not measured separately for each lake; instead, a single size estimate for each species was applied. Because there is no intraspecific variation in our estimates, intraspecific variation cannot explain directly any of the patterns that we observe.

However, the TSR and RR are sometimes applied to community-level patterns as well, despite the fact that the ecophysiological mechanisms underpinning the interspecific patterns may be considerably different and more complex than the physiological mechanisms governing the intraspecific patterns. Setting aside these caveats about mechanisms for the moment, we see in our data that there is an increase in the relative abundance of small species at high temperatures, consistent with the ‘species shift hypothesis’ of Daufresne et al. (2009). There is growing evidence for this hypothesis where warming favors an increase in the relative proportion of small species in communities (Daufresne et al. 2009; Morán et al. 2010; Gjoni and Glazier 2020; Saito et al. 2021; Perkins 2021; Pomeranz et al. 2021; Zohary et al. 2021), which could be considered a community-level TSR (Perkins 2021). If the RR is extrapolated to the interspecific level, it would be consistent with our findings: the relative density of large species increases (causing a less negative CCSR slope) as resource availability increases. Size-abundance relationships in planktonic communities often show this pattern (Gaedke et al. 2004, Morán et al. 2010, Barton et al. 2013, Marañón 2015, Guiet et al. 2016, Sprules and Barth 2016). Limited resource supply is associated with a more negative slope (small organisms acquire more resources), whereas a rich supply of resources is associated with a less negative slope (large organisms acquire more resources). But despite their general agreement, the lack of precise, agreed-upon mechanisms (and scale) in the TSR and RR means that there is no simple way to link them from individual- to community-level to explain a temperature-resource interaction mechanistically, as we have attempted.

Another factor that may affect phytoplankton CCSRs and their relation to temperature and resource availability is size-selective predation by zooplankton. A possible hypothesis requiring testing is that predation by zooplankton on relatively large phytoplankton cells may be higher at high vs. low temperatures (cf. Pomati et al. 2020). Size-selective predation by zooplankton can alter the size distribution of phytoplankton communities (Wollrab and Diehl 2015, Sommer et al. 2016). Predation may also cause temperature-sensitive changes in the metabolic scaling of prey organisms (Glazier et al. 2011, Glazier et al. 2020a,b), but whether such effects can alter size-abundance
relationships is unknown. In fact, we have found that zooplankton biomass (a proxy for predation) does not appear to affect the size-abundance relationships of lake phytoplankton communities under various temperature and nutrient conditions (see Appendix S1 - Fig. S1). However, there are also other important predators that feed on phytoplankton cells, such as mussels (Kagami and Urabe 2001), that we have not considered in this study. Unfortunately, very little is known about the interactive effects of ecological factors on size-abundance relationships and even less about the mechanisms underlying them.

In this regard, Pomati et al. (2021) reported that temperature, resource levels and zooplankton predation interacted to affect on the slope of phytoplankton size-abundance relationships in lake ecosystems. Specifically, increasing temperature positively affected the size-abundance relationship slope, whereas increasing nutrient availability negatively affected the slope, which implies that low temperature and high resource availability favor small vs. large individuals (the opposite of that predicted by a community-level TSR or RR). In contrast to the patterns that we have reported, Pomati et al. (2020) also found that the effect of temperature on the size-abundance relationship slope was greatest at low nutrient levels, and the effect of nutrient supply was greatest at high temperature. Why these anomalous patterns occur is not known, but perhaps they are related to size-selective predation by zooplankton, which interacted with the effects of temperature and resource supply (Pomati et al. 2020), unlike what we found in our study (Figure S1).

In any case, our results provide no support for any of the predictions of the MTE regarding the effects of temperature and resource supply. Contrary to the MTE and inferences from it, the size-abundance relationship slope varies across environmental conditions, the intercept of the size-abundance relationship does not increase with increasing resource availability, and increases rather than decreases with increasing temperature. Although various hypotheses, including the metabolic-level boundaries hypothesis (Glazier 2010, 2014b, Killen et al. 2010), the viscosity hypothesis (Verberk and Atkinson 2013), the thermal acclimation hypothesis (Fossen et al. 2019) and the size-dependent oxygen-uptake hypothesis (Rubalcaba et al. 2020) all predict that the slope for the rate of maintenance metabolism should decrease with increasing temperature, temperature effects on the size-abundance patterns that we observed across phytoplankton communities apparently cannot be explained simply in terms of these hypothetical physiological effects.

An especially surprising result, and contrary to the MTE, was that increasing temperature caused an increase in the intercept of the size-abundance relationship of phytoplankton communities. According to the MTE, increasing temperature should increase the metabolic rate and thus resource demand of phytoplankton cells, thus decreasing their abundance at a given resource level. The opposite pattern that was observed may be the result of increased temperature causing increased
population growth rates made possible by increased rates of protein synthesis, as found in phytoplankton communities by Toseland et al. (2013). Temperature may also affect the mortality rates of phytoplankton cells (Baker and Geider 2021), and, in turn, population growth rates and possibly size-abundance relationships, a hypothesis requiring testing.

In summary, our results regarding temperature and resource supply effects on the size-abundance relationship of phytoplankton communities provide partial support for a community-level TSR and RR, but no support for the MTE. Specifically, increasing temperature causes an increase in the abundance of small phytoplankton cells, but a decrease in the abundance of large cells, and thus a decrease in the size-abundance relationship slope, as expected from a community-level TSR, but only at high nutrient levels. By contrast, increasing resource (nutrient) supply causes an increase in the abundance of large phytoplankton cells, but a decrease in the abundance of small cells, and thus an increase in the size-abundance relationship slope, as expected from a community-level RR, but only at low temperatures. However, contrary to the TSR, temperature has a minimal effect on the size-abundance relationship slope at low or medium resource (nutrient) levels. Also, contrary to the RR, resource availability has no significant effect on the size-abundance relationship slope at medium or high temperatures. Therefore, at the interspecific community level, TSR and RR are ecologically contingent: i.e., in phytoplankton communities they are observed only in specific ecological contexts.

Although some aspects of our results still require further explanation, they demonstrate that predictions from major ecological rules or theories are contingent on environmental conditions. Phytoplankton size-abundance relationships may in general not follow the MTE, unlike that observed for other taxa (see also Cavender-Bares et al. 2001, Huete-Ortega et al. 2012, Marañón 2015). Resource availability may change the expression of a community-level TSR (see also Tabi et al. 2020) and temperature may change the expression of a community-level RR. Understanding how size-abundance relationships integrate environmental change represent a major advance in our present of how these changes affect ecological communities (Petchey and Belgrano 2010). Thus, these conclusions have important implications for predicting the effects of human-caused climate change and nutrient enrichment on natural communities and ecosystems, such as freshwater lakes.
Acknowledgements

Vojlsa Gjoni was supported by SNF Scientific exchange grand scholarship provided by the Swiss National Foundation in Switzerland. We thank Ethan White for his insightful discussions that helped improve this paper. We also thank Uriah Daugaard for helping to make the map of this study.

References


Figure legends

Figure 1 - Effects of temperature (T) and resource supply (R) on log-log relationships of metabolism and abundance with body size, as predicted by the Metabolic Theory of Ecology (MTE): a. increasing temperature (T₁ to T₂ to T₃) should increase metabolism in a similar - proportional - way for organisms of different body size (i.e., with a constant metabolic scaling slope, bₘ = 3/4), and therefore b. decrease population abundance without the size-abundance relationship slope (bₐ = -3/4), and as predicted by the Energetic Equivalence Rule (EER): c. when resource availability increases (R₁ to R₂ to R₃), metabolism should increase in a similar - proportional - way for organisms of different body size, and when resource availability decreases (R₃ to R₂ to R₁) metabolism should decrease proportionally (i.e., with a constant metabolic scaling slope, bₘ = 3/4). Correspondingly, d. when resource availability increases (R₁ to R₂ to R₃), although metabolic demand for maintenance should increase, simultaneous increases in growth and reproduction may nevertheless increase abundance, whereas when resource availability decreases (R₃ to R₂ to R₁), abundance should decrease, both without changing the size-abundance relationship slope (bₐ = -3/4).

Figure 2 - Map of the distribution of National Lakes Assessment sampling locations. Points are color-coded based on: A. Water temperature – T (°C), and B. log (nitrate concentration) – N (mg/L).

Figure 3 - The relationship between total population density and individual cell size (volume μm³) of phytoplankton communities in 1048 lakes of the continental United States of America. Each point represents mean cell size and total population density of the species present in each lake community.

Figure 4 – Temperature (T) and nutrients (N) interact to alter size-abundance relationships across 1048 lake phytoplankton communities. This figure is a visualization of a multiple regression model predicting total log₁₀ (density) with mean log₁₀ (cell volume), temperature and log₁₀ (nitrate concentration), as well as interactions between those predictors. See methods for details of the fitted model. Although all predictors are treated as continuous in the model, we ‘slice’ the regression surface at 3 temperatures (10°, 20°, 30°C) and 2 nutrient concentrations (0.06 mg L⁻¹ and 6 mg L⁻¹) to visualize changes in slope driven by these predictors. See Fig. 5 for a different visualization of the same model.

Figure 5 - Temperature (T) and nutrients (N) interact to alter size-abundance relationships across 1048 lake phytoplankton communities. This figure is another visualization (see Fig. 4) of a multiple
regression model predicting total log$_{10}$ (density) with mean log$_{10}$ (cell volume), temperature and log$_{10}$ (nitrate concentration), as well as interactions between those predictors. See methods for details of the fitted model. Although all predictors are treated as continuous in the model, we ‘slice’ the regression surface at 2 temperatures (10°C, and 30°C) and 3 nutrient concentrations (0.06 mg L$^{-1}$, 0.6 mg L$^{-1}$, 6 mg L$^{-1}$) to visualize changes in slope driven by these predictors.

Figure 6 - An alternate visualisation of the multiple regression results presented in Fig. 4 and 5. The colour variation indicates the slope of the size-abundance relationship across temperature and nitrate gradients, estimated from the fitted regression. The green points represent individual lakes and indicate the temperature and nitrate conditions at all 1048 lakes in the dataset (note that the lowest nitrate values represent measurements below the detection limit; see methods for more detail). Regions of parameter space outside the range of the lakes in the data are shown in white (upper left and right corners).

Appendix S1

Figure S1 – Temperature (T), nutrients (N) and zooplankton biomass (Z) interact to alter size-abundance relationships across 1048 lake phytoplankton communities. This figure is another visualization of a multiple regression model predicting total log(density) with mean log$_{10}$ (cell volume), temperature, log$_{10}$ (nitrate concentration), log$_{10}$ (zooplankton biomass) as well as interactions between those predictors. See methods for details of the fitted model. Although all predictors are treated as continuous in the model, we ‘slice’ the regression surface at 3 nutrient concentrations (0.06 mg L$^{-1}$, 0.6 mg L$^{-1}$, 6 mg L$^{-1}$), 2 temperatures (10° C, and 30°C), and 2 zooplankton biomasses (10 μg L$^{-1}$ and 1.000 μg L$^{-1}$) to visualize changes in slope driven by these predictors. As can be seen, zooplankton biomass had no significant effect on phytoplankton size-abundance relationship slopes, regardless of temperature or nutrient supply.

Figure S2 - Temperature (T) and nutrients (N) interact to alter size-abundance relationships across 1048 lake phytoplankton communities. The fitted model is based on colony level size and density, rather than cell size and density, as shown in Fig. 3 and 4. This figure is a visualization of a multiple regression model predicting total log$_{10}$ (density) with mean log$_{10}$ (column volume), temperature and log$_{10}$ (nitrate concentration), as well as interactions between those predictors. Although all predictors are treated as continuous in the model, we ‘slice’ the regression surface at 3 temperatures (10° C,
20°C, 30°C) and 2 nutrient concentrations (0.06 mg L\(^{-1}\) and 6 mg L\(^{-1}\)) to visualize changes in slope driven by these predictors.

Figure S3 – Temperature (T) and nutrients (N) interact to alter size-abundance relationships across 1048 lake phytoplankton communities. The fitted model is based on colony level size and density, rather than cell size and density, as shown in Fig. 3 and 4. This figure is another visualization of a multiple regression model predicting total log\(_{10}\) (density) with mean log\(_{10}\) (column volume), temperature and log\(_{10}\) (nitrate concentration), as well as interactions between those predictors. Although all predictors are treated as continuous in the model, we ‘slice’ the regression surface at 3 nutrient concentrations (0.06 mg L\(^{-1}\), 0.6 mg L\(^{-1}\), 6 mg L\(^{-1}\)) and 2 temperatures (10°C, and 30°C).
Table legends

Table 1 - Results of least squares regression analyses of log_{10} total density of all cells in the community (individual cells/m^2) in relation to log_{10} cell volume (volume \( \mu \text{m}^3 \)).

Table 2 - Temperature (T), nutrients (N), and zooplankton (Z) all affect the CCSR at cell-based level cell volume (S). We restricted the model to these terms based on a priori hypotheses and performed no model selection steps thereafter; therefore, the p-values here are unbiased. Although the effect of zooplankton is statistically significant, it is of limited practical significance and so we focus on temperature and nutrients in the paper. However, all figures are based on this model and incorporate the effects of zooplankton. We include visualizations that show the limited effects of zooplankton in the model in the appendix (Appendix S1: Fig. S1).

Appendix S1

Table A1 - Temperature (T), nutrients (N), and zooplankton (Z) all affect the CCSR at colony-based level cell volume (S). We restricted the model to these terms based on a priori hypotheses and therefore present valid p-values here. Although the effect of zooplankton is statistically significant, it is of limited practical significance and so we focus on temperature and nutrients in the paper. However, all figures are based on this model and incorporate the effects of zooplankton. We include visualizations that show the limited effects of zooplankton in the model in the appendix (Appendix S1: Fig. S2 and S3).
Fig. 1

A) log abundance vs. log body size for species $T_1$, $T_2$, and $T_3$ with $b = -3/4$

B) log abundance vs. log body size for species $R_1$, $R_2$, and $R_3$ with $b = 3/4$

C) log abundance vs. log body size for species $T_1$, $T_2$, and $T_3$ with $b = -3/4$

D) log abundance vs. log body size for species $R_1$, $R_2$, and $R_3$ with $b = -3/4$
Fig. 2

A

B

T ($^\circ$C)

N (mg/L)
Fig. 5

(A) Low T

(B) High T

Fig. 6
Fig. S1

(A) Low Z

Log Density

Low T  High T

Log Cell Volume

(B) High Z

Log Density

Low T  High T

Log Cell Volume

N (mg/L)

-3  -1.5  0

820
821  Fig. S1
822
Fig. S2

- (A) Low N
- (B) High N

Fig. S3

- (A) Low T
- (B) High T
<table>
<thead>
<tr>
<th>CCSR</th>
<th>Slope</th>
<th>95% CI</th>
<th>Intercept</th>
<th>95% CI</th>
<th>R²</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phytoplankton</td>
<td>-0.98</td>
<td>-1.04 to -0.92</td>
<td>6.62</td>
<td>6.48 to 6.77</td>
<td>0.44</td>
<td>&lt;0.0001</td>
</tr>
</tbody>
</table>

Table 1

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Estimates</th>
<th>CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>2.13</td>
<td>0.24 – 4.01</td>
<td>0.027</td>
</tr>
<tr>
<td>S</td>
<td>0.38</td>
<td>-0.40 – 1.16</td>
<td>0.339</td>
</tr>
<tr>
<td>T</td>
<td>0.14</td>
<td>0.06 – 0.22</td>
<td>0.001</td>
</tr>
<tr>
<td>N</td>
<td>-0.68</td>
<td>-1.39 – 0.02</td>
<td>0.058</td>
</tr>
<tr>
<td>Z</td>
<td>0.64</td>
<td>0.33 – 0.96</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>S * T</td>
<td>-0.05</td>
<td>-0.08 – -0.01</td>
<td>0.008</td>
</tr>
<tr>
<td>S * Z</td>
<td>-0.06</td>
<td>-0.14 – 0.03</td>
<td>0.188</td>
</tr>
<tr>
<td>S * N</td>
<td>0.38</td>
<td>0.08 – 0.69</td>
<td>0.013</td>
</tr>
<tr>
<td>T * Z</td>
<td>-0.01</td>
<td>-0.02 – 0.00</td>
<td>0.073</td>
</tr>
<tr>
<td>T * N</td>
<td>0.02</td>
<td>-0.01 – 0.05</td>
<td>0.160</td>
</tr>
<tr>
<td>S * T * N</td>
<td>-0.01</td>
<td>-0.03 – -0.00</td>
<td>0.032</td>
</tr>
</tbody>
</table>

Observations | 1048
R² / R² adjusted | 0.57 / 0.56
Table 2

<table>
<thead>
<tr>
<th>Predictors</th>
<th>Estimates</th>
<th>CI</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>(Intercept)</td>
<td>3.03</td>
<td>0.48 – 5.59</td>
<td>0.020</td>
</tr>
<tr>
<td>S</td>
<td>0.02</td>
<td>-0.71 – 0.75</td>
<td>0.956</td>
</tr>
<tr>
<td>T</td>
<td>0.12</td>
<td>0.01 – 0.23</td>
<td>0.038</td>
</tr>
<tr>
<td>N</td>
<td>-0.39</td>
<td>-1.37 – 0.60</td>
<td>0.440</td>
</tr>
<tr>
<td>Z</td>
<td>0.24</td>
<td>-0.53 – 1.00</td>
<td>0.544</td>
</tr>
<tr>
<td>S * T</td>
<td>-0.04</td>
<td>-0.07 – -0.01</td>
<td>0.016</td>
</tr>
<tr>
<td>S * Z</td>
<td>-0.01</td>
<td>-0.09 – 0.07</td>
<td>0.742</td>
</tr>
<tr>
<td>S * N</td>
<td>0.30</td>
<td>0.01 – 0.58</td>
<td>0.040</td>
</tr>
<tr>
<td>T * Z</td>
<td>0.00</td>
<td>-0.03 – 0.03</td>
<td>0.964</td>
</tr>
<tr>
<td>T * N</td>
<td>0.02</td>
<td>-0.03 – 0.06</td>
<td>0.405</td>
</tr>
<tr>
<td>N * Z</td>
<td>-0.13</td>
<td>-0.41 – 0.15</td>
<td>0.358</td>
</tr>
<tr>
<td>T * N * Z</td>
<td>0.00</td>
<td>-0.01 – 0.02</td>
<td>0.539</td>
</tr>
<tr>
<td>S * T * N</td>
<td>-0.01</td>
<td>-0.03 – -0.00</td>
<td>0.033</td>
</tr>
</tbody>
</table>

Observations: 1048

R^2 / R^2 adjusted: 0.54 / 0.53