

ARTICLE

Freshwater Ecology

Phytoplankton functional composition determines limitation by nutrients and grazers across a lake productivity gradient

Marika A. Schulhof^{1,2}  | Dedmer B. Van de Waal²  | Steven A. J. Declerck^{2,3}  | Jonathan B. Shurin¹ 

¹Division of Biological Sciences, Section of Ecology, Behavior & Evolution, University of California San Diego, La Jolla, California, USA

²Department of Aquatic Ecology, Netherlands Institute of Ecology (NIOO-KNAW), Wageningen, The Netherlands

³Laboratory of Aquatic Ecology, Evolution and Conservation, KU Leuven, Leuven, Belgium

Correspondence

Marika A. Schulhof
Email: marika.schulhof@gmail.com

Funding information

National Science Foundation, Grant/Award Number: DEB 1457737; Royal Netherlands Academy of Arts and Sciences Ecology Fund; NSF-GROW Fellowship; NSF Graduate Research Fellowship

Handling Editor: Grace Wilkinson

Abstract

Functional trade-offs among ecologically important traits govern the diversity of communities and changes in species composition along environmental gradients. A trade-off between predator defense and resource competitive ability has been invoked as a mechanism that may maintain diversity in lake phytoplankton. Trade-offs may promote diversity in communities where grazing- and resource-limited taxa coexist, which determines the extent to which communities are resource- or consumer-controlled. In addition, changes in temperature may alter nutrient demands and grazing pressure, changing the balance between the two regulating factors. Our study aims to understand whether a trade-off between grazer vulnerability and nutrient limitation promotes coexistence of phytoplankton functional groups in communities that differ in trophic status, and how this trade-off may shift with warming. We conducted multifactorial experiments manipulating grazing, nutrients, and temperature in phytoplankton communities from three Dutch lakes varying in trophic status, and used a trait-based approach to classify functional groups based on grazing susceptibility. We found no associations between susceptibility to grazing and response to nutrient additions in any of the communities or temperature regimes, indicating that a competition–defense trade-off is unlikely to explain diversity within the tested communities. Instead, we observed a tendency toward both a higher grazing resistance and weaker nutrient limitation along with a shift in the functional composition of phytoplankton in communities across a gradient from low to high productivity.

KEYWORDS

competition–defense trade-off, functional traits, lakes, phytoplankton, productivity, warming

This is an open access article under the terms of the Creative Commons Attribution License, which permits use, distribution and reproduction in any medium, provided the original work is properly cited.

© 2022 The Authors. *Ecosphere* published by Wiley Periodicals LLC on behalf of The Ecological Society of America.

INTRODUCTION

An interspecific trade-off between predator defense and resource competitive ability, whereby strong resource competitors are more susceptible to predation and poor resource competitors are more defended against predators, has been invoked as a mechanism that may maintain coexistence and diversity in ecological communities (Leibold, 1996; Lubchenco, 1978; McCauley & Briand, 1979; Paine, 1966). Such trade-offs may arise from constraints on allocation of resources to different organismal functions such as resource acquisition versus morphological or chemical defenses (Kneitel & Chase, 2004). Although this competition–defense trade-off has been invoked as a mechanism explaining the coexistence of species in communities, empirical evidence for such trade-offs in natural communities is still limited. Competition–defense trade-offs can be detected based on the response of co-occurring species to manipulation of different potentially limiting factors. For example, the species most limited by resources (poor competitors) should respond most positively to resource addition and remain relatively unaffected by increased predation pressure, while species that are limited by their consumers should increase the most in response to predator removal but be less sensitive to changes in nutrient availability (Viola et al., 2010). An interspecific negative correlation in response to manipulation of these limiting factors indicates the presence of a trade-off (Figure 1).

Functional trade-offs among ecologically important traits govern the diversity of algae within communities and changes in species composition among communities along environmental gradients (Kruk et al., 2010; Litchman et al., 2007; Litchman & Klausmeier, 2008; Mandal et al., 2018; Reynolds, 1984). However, the association between resource competition and grazer resistance in phytoplankton communities, and its role as regulator of diversity, remains uncertain. Cell size and morphology of phytoplankton are important traits that influence key metabolic processes such as nutrient uptake and utilization strategies (Reynolds, 1984). Small cells are able to acquire and assimilate limiting nutrients more efficiently due to their high surface area-to-volume ratio (Chisholm, 1992; Edwards et al., 2011; Litchman et al., 2007; Marañón et al., 2013, but see Aksnes & Cao, 2011; Fiksen et al., 2013; Smith et al., 2014). Furthermore, the size of phytoplankton is known to strongly affect susceptibility to grazing by zooplankton. Larger cell size results in greater resistance to gape-limited grazers, creating a potential trade-off between nutrient competitive ability and grazing susceptibility (reviewed in Litchman et al., 2007, 2010).

Understanding functional trade-offs among algal taxonomic groups is critical in order to predict community

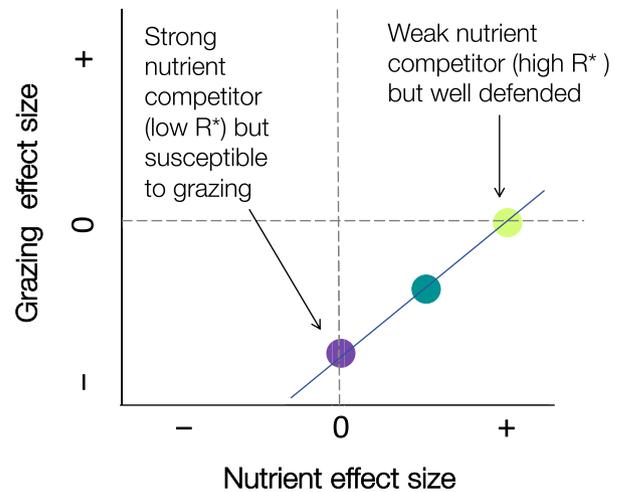


FIGURE 1 Hypothesized competition–defense trade-off. The purple symbol indicates a functional group that is susceptible to grazing but a strong nutrient competitor. Thus, it is expected to show high losses to grazing and a weak growth response to nutrient addition. In comparison, the light green functional group is less edible but a weaker nutrient competitor, and thus should have smaller losses to grazing but a stronger positive response to nutrient addition. The teal functional group is intermediate in both traits. The effect of warming may increase the effect of grazing on functional groups due to stronger top-down control in warmer environments, but decrease the effects of nutrient addition due to increased nutrient use efficiencies at higher temperatures (not represented in scheme)

responses to elevated temperatures, nutrient addition, and changes in grazing pressure (Litchman et al., 2012). Trait-based approaches to phytoplankton ecology hold promise for better understanding and predicting responses of phytoplankton communities to simultaneous global changes (Finkel et al., 2010; Litchman & Klausmeier, 2008; Reynolds, 2002; Van de Waal & Litchman, 2020). Increased temperatures are expected to alter the structure and functioning of ecological communities, including trophic interactions and size structure, and therefore may alter the sensitivity of phytoplankton species to nutrients and grazers. Higher temperatures result in increased metabolic rates and resource requirements for organisms and therefore may affect population dynamics and interspecific interactions (Brown et al., 2004). Empirical studies have demonstrated that warming alters trophic interaction strengths by enhancing top-down, consumer-driven control, causing increased grazing and thus reduced primary producer biomass (Kratina et al., 2012; O'Connor et al., 2009; Shurin et al., 2012). Moreover, warming may increase nutrient use efficiencies and requirements of some species (Baker et al., 2016; De Senerpont Domis et al., 2014; Thomas et al., 2017), while it may lower trophic transfer efficiencies (Barneche et al., 2021).

Temperature is therefore expected to alter the balance between resource limitation and grazing pressure among taxa.

Our study aims to determine whether a competition–defense trade-off is apparent within phytoplankton communities, and whether the interspecific correlation between grazer sensitivity and nutrient limitation is affected by temperature. We conducted multifactorial microcosm experiments on phytoplankton communities from three Dutch lakes and manipulated temperature, grazing pressure, and nutrient load. To test the generality of our results, we chose to work on lakes differing widely in productivity. In earlier work, we demonstrated how these factors alter growth and stoichiometric responses of entire phytoplankton communities (Schulhof et al., 2019). Here, we tested the effects of nutrient addition and grazing at ambient and elevated temperatures on distinct phytoplankton functional groups using a trait-based framework (from Kruk et al., 2010). Additionally, to test for the existence of trade-offs between competitive strength and grazer resistance, we tested for negative relationships between the effect sizes of grazing and nutrient addition across phytoplankton functional groups in each of the lakes separately at ambient and warmed temperatures.

We hypothesized that smaller, more edible functional groups would show strong negative effects of grazer addition but weak positive responses to nutrient addition, whereas larger inedible functional groups would show stronger positive responses to nutrient addition and weak negative responses to grazer addition in communities from all three lakes (Figure 1). Additionally, we hypothesized that warming would enhance top-down control due to increased grazing pressure and thus increase the negative grazing response of smaller functional groups. In contrast, we expected that warming would enhance nutrient use efficiencies, thereby reducing the positive nutrient response of larger functional groups.

MATERIALS AND METHODS

Experimental setup

Spring phytoplankton communities were collected from three lakes differing in trophic status, sampled 1 month apart: Maarsseveen (52.144402N, 5.080691E; March 2017), Tjeukemeer (52.890225N, 5.802871E; April 2017), and Loosdrecht (52.196582N, 5.080495E; May 2017). At each lake, 340 L of water from 0.5- to 1.0-m depth was collected in 10-L containers and brought to the laboratory to inoculate experiments. Plankton inocula were stored in the laboratory in the dark overnight, and experiments were inoculated the next morning. All inocula were pre-

screened through a 200- μm mesh to remove large zooplankton grazers, and gently mixed in a large cattle tank before filling equal 10 L volumes into transparent Nalgene containers that were loosely capped to allow air-flow in and out of bottles.

In earlier work (Schulhof et al., 2019), we showed that mean chl *a* (in micrograms per liter) fraction of chl *a* <30 μm , total nitrogen (TN) (in micromoles) and total phosphorus (TP) (in micromoles) were significantly different among the three sampled lakes ($p < 0.01$). Highest chl *a* concentrations occurred in Lake Tjeukemeer ($35.8 \pm 0.2 \mu\text{g L}^{-1}$), followed by Lake Loosdrecht ($19.5 \pm 0.2 \mu\text{g L}^{-1}$) and Lake Maarsseveen ($2.37 \pm 0.01 \mu\text{g L}^{-1}$). The fraction of chl *a* <30 μm showed the opposite pattern: The lowest fraction occurred in Tjeukemeer (0.60 ± 0.02), followed by Loosdrecht (0.71 ± 0.02) and Lake Maarsseveen (0.86 ± 0.001). Similar to mean chl *a*, TN and TP were highest in Lake Tjeukemeer ($79.1 \pm 1.4 \mu\text{M N}$ and $3.15 \pm 0.03 \mu\text{M P}$), followed by Loosdrecht ($46.2 \pm 3.3 \mu\text{M N}$ and $1.15 \pm 0.03 \mu\text{M P}$), while lowest concentrations were observed in Lake Maarsseveen ($25.5 \pm 1.2 \mu\text{M N}$ and $0.34 \pm 0.03 \mu\text{M P}$). Therefore, phytoplankton communities from lakes Maarsseveen, Loosdrecht, and Tjeukemeer will be referred to as the communities of “low,” “medium,” and “high” productivity, respectively. For further details, see Schulhof et al. (2019).

Using a fully factorial design, the culture containers were subjected to two temperature, nutrient, and grazing treatments, for a total of eight factorial treatment combinations. Each of the eight treatments was replicated four times, resulting in 32 experimental units for each of three experiments. The temperature treatments consisted of an ambient treatment set to the lake water temperature at the time of sampling, and a +4°C warming treatment based on plausible global change scenarios (IPCC scenario RCP8.5; IPCC, 2014). However, due to technical problems with temperature control in the incubation system, there were differences between the magnitudes of warming for each experiment. The resulting mean ambient and elevated temperatures, respectively, for each experiment were as follows: $9.6 \pm 0.5^\circ\text{C}$ and $11.0 \pm 0.2^\circ\text{C}$ for Maarsseveen, $12.0 \pm 0.4^\circ\text{C}$ and $15.0 \pm 0.5^\circ\text{C}$ for Tjeukemeer, and $15.8 \pm 0.3^\circ\text{C}$ and $20.0 \pm 0.2^\circ\text{C}$ for Loosdrecht.

For the nutrient addition treatment, nitrogen and phosphorus were added to concentrations of 1 mM NO_3^- and 0.0625 mM PO_4^{3-} (N:P molar ratio of 16:1). For the grazing treatment, *Daphnia magna* were added to a final population density of 50 individuals per culture vessel (equivalent to five individuals per liter). *Daphnia* were acquired commercially (Ruinemans Aquarium B.V., Montfoort, The Netherlands), and individuals were selected, cleaned, and subsequently cultured in the laboratory fed with *Chlamydomonas reinhardtii* cultures in

the quantity of about $0.5 \text{ mg C L}^{-1} \text{ day}^{-1}$. For each experiment, nongravid *Daphnia* subadults of similar size were selected and thoroughly washed in deionized water before being added to culture vessels. *Daphnia* biomass was determined at the end of the experiment by sieving water, drying, and weighing the remaining *Daphnia* individuals in each culture vessel.

Culture vessels were randomly positioned and submerged in temperature-controlled aquaria using the Farex SR minisystem (RKC Instruments, Tokyo, Japan) and subjected to controlled light conditions ($120 \mu\text{mol photons m}^{-2} \text{ s}^{-1}$) with a day–night cycle of 14:10 that simulated the spring light conditions in the Netherlands. Each experiment ran for a duration of 6 days, when phytoplankton communities started to enter the stationary phase of growth (see Schulhof et al., 2019 for further details). The experiments were harvested on Day 6. Samples from each culture vessel were collected, and three of four replicates for each treatment were analyzed using microscopy. Samples for microscopy were randomly chosen and fixed in Lugol's iodine until enumeration.

Microscopy and image analysis

Phytoplankton communities were counted via microscopy using the Utermöhl method (Utermöhl, 1958). Depending on the density of samples, phytoplankton in 0.5–6 ml of water were allowed to settle overnight in Utermöhl chambers and counted using an inverted microscope. For small taxa ($<30 \mu\text{m}$), fields of view were counted at $400\times$ magnification until approximately 400 units were counted. Large cells ($>30 \mu\text{m}$) were counted at $100\times$ magnification: For samples from Lake Maarsseveen, 100 transects were counted by microscopy, while for lakes Loosdrecht and Tjeukemeer, cells were counted from images taken at $100\times$ magnification because these communities were dominated by dense filamentous cyanobacteria that could more accurately be counted from images. For these lakes, 10 images equally spaced along a transect were taken at $100\times$ magnification and 2–4 images chosen from the edges and center of the transect were counted until at least 100 filaments were counted.

Cell dimensions were measured from microscope images using ImageJ (version 1.50i; <https://imagej.nih.gov/ij/>), and biovolumes were calculated based on cell geometry according to Hillebrand et al. (1999). For taxa that exhibited a constant size range across all treatments, mean measurements taken across all treatments were used to calculate biovolumes. However, for cells that varied in their length (i.e., chain-forming diatoms), treatment-specific means were calculated from length measurements taken in each

treatment. Additionally, for filamentous cyanobacterial cells that were counted via images for lakes Loosdrecht and Tjeukemeer, cell dimensions were measured on all counted cells in each image such that the total biovolume was calculated for each image. Biovolumes were then normalized by sample volume (in cubic micrometers per milliliter).

Functional groupings

After all cell biovolumes were calculated and normalized by sample volume (in cubic micrometer per milliliter), they were binned into seven morphology-based functional groups developed by Kruk et al. (2010) in order to assess the competition–defense trade-off within each of the three lakes. Kruk et al. (2010) developed a phytoplankton morphology-based functional classification scheme containing seven functional groups that correlate with functional properties of species and vary predictably in abundance with environmental conditions (Kruk et al., 2011; Kruk & Segura, 2012) and in response to zooplankton grazing (Colina et al., 2016). The seven morphology-based functional groups (FG I–VII) and their expected grazing susceptibility (low, medium, and high; Colina et al., 2016) are as follows: small organisms with high surface area-to-volume ratio (FG I; high grazing susceptibility), small flagellated organisms with siliceous exoskeletons (FG II; low grazing susceptibility), large filaments with aerotopes (FG III; low grazing susceptibility), organisms of medium size lacking specialized traits (FG IV; high grazing susceptibility), unicellular flagellates of medium to large size (FG V; medium grazing susceptibility), nonflagellated organisms with siliceous exoskeletons (FG VI; medium grazing susceptibility), and large mucilaginous colonies (FG VII; low grazing susceptibility) (Colina et al., 2016; Kruk et al., 2010).

For all three communities, FG I consisted of cells with maximum linear dimensions (MLD) up to $5 \mu\text{m}$, including picocyanobacteria, and small round flagellated and nonflagellated cells. FG II was found only in the medium-productivity community and consisted of *Dinobryon* sp. FG III, prevalent in the medium- and high-productivity lakes, consisted of filamentous cyanobacteria including *Planktothrix* sp., *Limnothrix* sp., *Dolichospermum* sp., and *Pseudanabaena* sp. FG IV consisted primarily of unicellular spherical cells with MLD of $9 \mu\text{m}$ in the low-productivity lake, and larger colonial forms in the medium- and high-productivity lakes such as *Scenedesmus* sp., *Cosmocladium* sp., *Pediastrum* sp., *Actinastrum* sp., *Tetrastrum* sp., *Tetraedron* sp., and *Ankistrodesmus* sp. FG V was present in the low-productivity lake and consisted of *Plagioselmis* sp. and dinoflagellates such as *Gymnodinium* sp. and *Ceratium* sp. The medium-

productivity lake also contained dinoflagellates, but they were rare and found in small quantities in only four samples, so they were not counted as a separate functional group and were instead included in FG IV. FG VI consisted entirely of diatoms, which in the low-productivity lake included pennate colony-forming genera such as *Asterionella* sp. and *Fragilaria* sp., and centric unicells and colonies of *Aulacoseira* sp. In the medium- and high-productivity lakes, FG VI was composed primarily of colonial (*Tabellaria* sp.) and single-celled pennate diatoms such as *Synedra* sp. and *Ceratoneis* sp. FG VII for all three communities was composed of mucilaginous cyanobacterial colonies, including *Microcystis* sp., *Aphanocapsa* sp., and *Aphanothece* sp.

Statistical methods

All statistical analyses were performed using the statistical program R version 3.4.2 (R Core Team, 2017). The mean percentages of each functional group biovolume and SE were calculated from the control treatments to characterize the functional composition of each community. We fitted generalized linear models (“glm” function in lme4 package) assuming Gaussian distributions to determine the main and interactive effects of experimental treatments on response variables (functional group biovolume) in each experiment and assessed statistical significance using an *F* test. Prior to running models, all response variables were log (or $\log[x + 1]$)-transformed to normalize variance.

Effect sizes of grazing and nutrient addition on functional group biovolumes were calculated using the ln-transformed response ratio (Hedges et al., 1999). These effect sizes were separately calculated for ambient and warmed temperatures as $RR = \ln(\bar{X}_E/\bar{X}_C)$, where \bar{X}_E is the mean value in enriched treatments (nutrient addition or grazing), and \bar{X}_C is the mean of the analogous control treatment lacking enrichment. Here, we were interested in the main effects of nutrients and grazing; thus, effect sizes were calculated using means for all treatments combined that had the same condition for nutrients and grazing (enriched vs. ambient) at each temperature, rather than for each unique multifactorial treatment combination. The variance of each effect size was calculated using the following equation:

$$\frac{(SD_E)^2}{n_E \bar{X}_E^2} + \frac{(SD_C)^2}{n_C \bar{X}_C^2}$$

where SD is the standard deviation, and *n* is the sample size (Hedges et al., 1999). Correlation tests using

Pearson’s product–moment correlation coefficient (“cor.test” function) were used to analyze whether there was a statistically significant correlation between the effect size of grazing and nutrient addition on functional group biovolumes within each community, at both ambient and warmed temperatures. Additionally, a one-way ANOVA was used to assess: (1) differences in final *Daphnia* biomass (in grams) remaining in grazing treatments between each of the three phytoplankton communities when experiments were harvested and (2) differences in *Daphnia* biomass between treatments with added grazers within each community.

RESULTS

Percentage of biovolume by functional type

Mean percentages of each functional group by biovolume (\pm SE) differed in control treatments for each community. For the low-productivity community (sampled in March 2017), 39% were functional groups with high grazing susceptibility (small cells with high S:V [FG I]: $5.8 \pm 0.70\%$; medium cells with no specializations [FG IV]: $33.5 \pm 11.6\%$), 24% were groups with intermediate grazing susceptibility (medium-to-large flagellates [FG V]: $0.73 \pm 0.48\%$; nonflagellated with siliceous exoskeleton [FG VI]: $23.3 \pm 5.3\%$), and 37% were groups with low susceptibility to grazers (large mucilaginous colonies [FG VII]: $36.6 \pm 8.1\%$) (Figure 2).

In the medium-productivity community (sampled in May 2017), 93% were groups with low grazing susceptibility (large filaments with aerotopes [FG III]: $93.1 \pm 1.5\%$; small flagellated organisms with siliceous exoskeletons [FG II]: $0.033 \pm 0.016\%$; FG VII: $0.055 \pm 0.043\%$), while groups with medium (FG VI: $4.7 \pm 2.3\%$) and high grazing susceptibility (FG I: $0.49 \pm 0.19\%$; FG IV: $1.6 \pm 0.83\%$) accounted for 7% of the remaining biovolume (Figure 2).

The high-productivity community (sampled in April 2017) was also dominated (83%) by groups with low grazing susceptibility (FG III: $71.6 \pm 3.0\%$; FG VII: $11.8\% \pm 3.2\%$), while groups of intermediate (FG VI: $6.7 \pm 1.0\%$) and high grazing susceptibility (FG I: $2.2 \pm 0.48\%$; FG IV: $7.8 \pm 0.70\%$) collectively comprised 17% (Figure 2).

Treatment effects on functional group biovolumes

In the low-productivity community, all functional groups with high and medium grazing susceptibility (I, IV, V, VI) increased in response to nutrient addition and showed losses due to grazing ($p < 0.05$ – 0.001 ; Table 1,

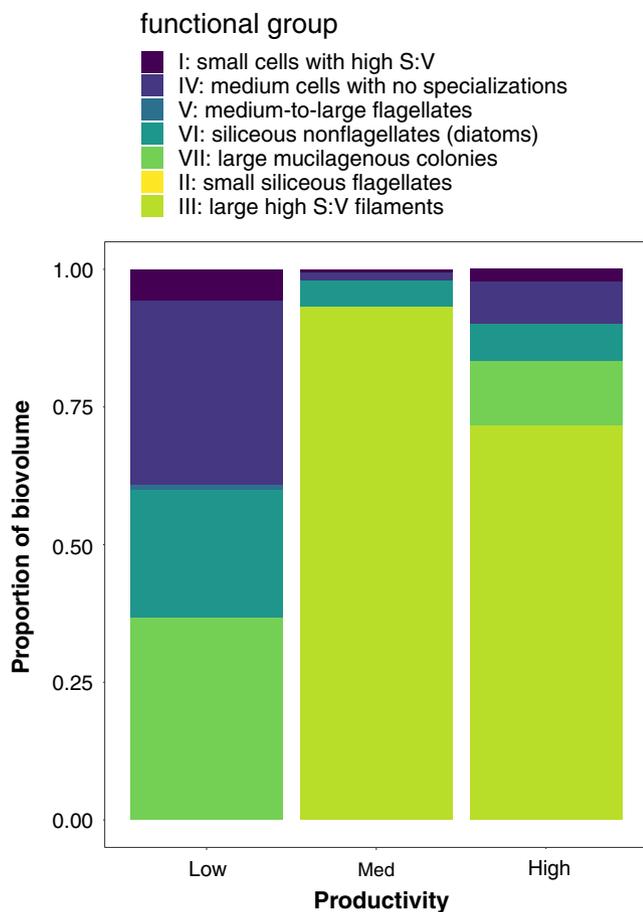


FIGURE 2 Mean relative abundances of each functional group in the control treatment for each community. Colors correspond to functional groups, where shades of purple indicate high grazing susceptibility, shades of blue/teal indicate medium grazing susceptibility, and shades of green/yellow indicate low grazing susceptibility. Grazing susceptibility categories for each functional group are from Colina et al. (2016)

Appendix S1: Figure S1). There were no significant main effects of warming or interactions with nutrients or grazing on any group.

In the medium-productivity community, nutrient addition significantly increased biovolumes of functional groups highly susceptible to grazers (I and IV; $p < 0.001$ and $p < 0.01$, respectively) but decreased biovolume of group III (low grazer susceptibility; $p < 0.01$), especially at warm temperatures (temperature \times nutrients, $p < 0.05$; Table 1, Appendix S1: Figure S2). Additionally, there was a significant effect of nutrient addition on group II (low grazer susceptibility), resulting in lower biovolumes when combined with grazing treatments ($p < 0.05$; Table 1, Appendix S1: Figure S2), although there was no significant interaction with grazing.

In the high-productivity community, biovolumes of only functional groups IV and VI showed differences among treatments. For group IV (high grazing susceptibility),

grazing had a positive effect on biovolume ($p < 0.05$), and nutrient addition had a positive effect when warmed (temperature \times nutrients, $p < 0.05$) (Table 1, Appendix S1: Figure S3). For group VI (medium grazing susceptibility), temperature had a negative effect on biovolume ($p < 0.01$) and nutrients had a positive effect ($p < 0.05$) (Table 1, Appendix S1: Figure S3).

Effect sizes of grazer and nutrient addition within lakes

The effect size of grazing and nutrient addition on functional groups show distinct patterns within each community (Figure 3). Functional groups in the low-productivity community generally show a positive response to nutrient addition and losses to grazing at both temperatures. In the medium-productivity community, functional groups of all grazing susceptibilities show variable responses to nutrient addition and grazing ranging from positive to negative. However, in the high-productivity community, all functional groups show little-to-no effects of grazing nor nutrient addition at both temperatures (Figure 3). We found no significant correlations between the effect size of grazer and nutrient addition on functional groups within any community at ambient temperatures or warmed temperatures.

Daphnia biomass across communities

Mean *Daphnia* biomass (in grams) remaining at the end of experiments in grazing treatments differed significantly across the three phytoplankton communities ($p < 0.01$). The lowest productivity community had the highest *Daphnia* biomass remaining (0.0052 g) followed by the high-productivity (0.0025 g) and medium-productivity (0.0015 g) communities. However, there was no difference in *Daphnia* biomass between treatments with added grazers within each community.

DISCUSSION

We found that functional groups of phytoplankton in the community from the most eutrophic lake were less sensitive to *both* nutrient addition and grazing as compared to the communities from lakes with lower trophic status. Moreover, the majority of functional groups were not affected by the warming treatments in any of the communities. We found no support for the hypothesized trade-off between competitive ability for nutrients and resistance to grazing across phytoplankton functional categories within these communities.

TABLE 1 *p* values from generalized linear models for functional groups

Functional group (grazing susceptibility)	Temperature (tmp)	Nutrients (nut)	Grazing (grz)	tmp × nut	tmp × grz	nut × grz	tmp × nut × grz
Low-productivity community							
I (high)	0.59	<0.001	<0.01	0.43	0.57	0.43	0.20
IV (high)	0.24	<0.01	<0.01	0.11	0.23	0.75	0.10
V (medium)	0.23	<0.05	<0.01	0.77	0.85	0.86	0.49
VI (medium)	0.60	<0.001	<0.05	0.81	0.20	0.58	0.60
VII (low)	0.12	0.49	0.44	0.96	0.23	0.64	0.67
Medium-productivity community							
I (high)	0.83	<0.001	0.051	0.84	0.31	0.59	0.71
IV (high)	0.12	<0.01	0.93	0.46	0.91	0.94	0.73
VI (medium)	0.98	0.99	0.77	0.18	0.13	0.12	0.99
II (low)	0.37	<0.05	0.91	0.45	0.92	0.094	0.91
III (low)	<0.05	<0.01	0.57	<0.05	0.56	0.96	0.76
VII (low)	0.14	0.32	0.68	0.25	0.54	0.70	0.20
High-productivity community							
I (high)	0.50	0.13	0.84	0.31	0.83	0.82	0.44
IV (high)	0.58	0.059	<0.05	<0.05	0.95	0.39	0.95
VI (medium)	<0.01	<0.05	0.27	0.66	0.74	0.23	0.65
III (low)	0.70	0.64	0.92	0.99	0.65	0.94	0.22
VII (low)	0.39	0.89	0.73	0.71	0.59	0.91	0.24

Note: Significant *p* values are shown in boldface.

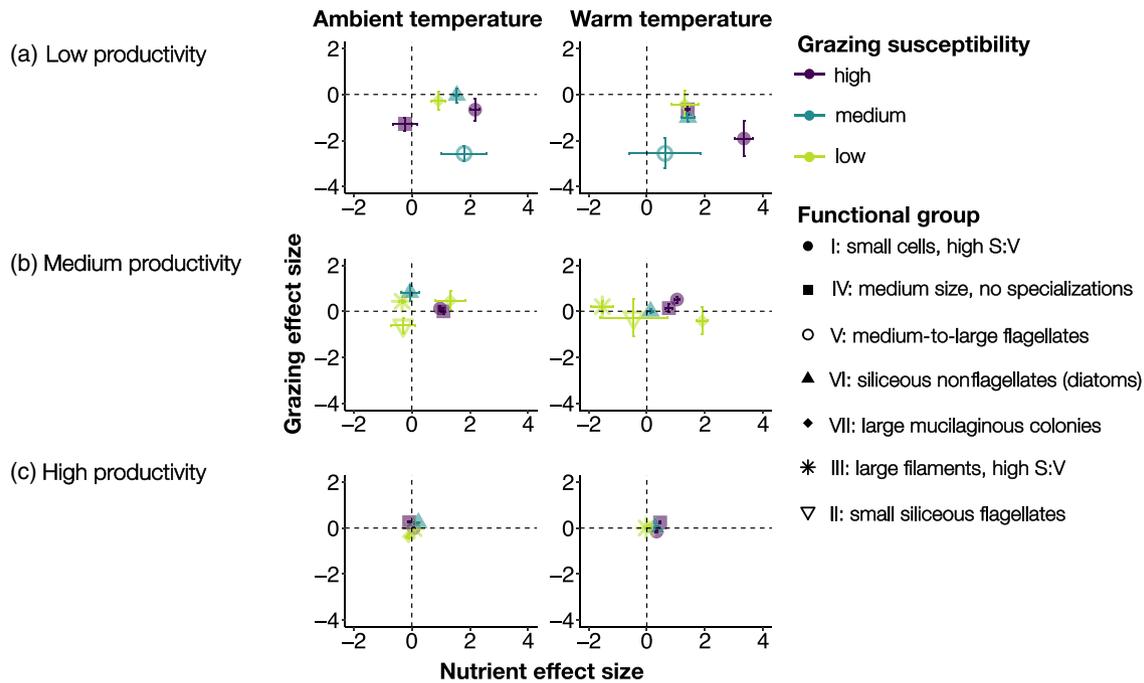


FIGURE 3 Effect sizes of grazing and nutrient addition (\pm variance) on mean functional group biovolumes are shown at ambient and warmed temperatures for each community (low (a), medium (b), and high productivity (c)). Shapes indicate functional groups, and colors denote grazing susceptibility. Grazing susceptibility categories for each functional group are from Colina et al. (2016)

While we found notable differences in functional composition and the main effect sizes of nutrient and grazer addition treatments in communities of differing trophic status, these differences were not consistent with the competition–defense trade-off. Functional groups in the least productive community showed strong biomass reductions upon grazer addition and strongly enhanced growth when nutrients were added. In contrast, functional groups in the two more productive lake communities showed relatively weaker responses to nutrient and grazer treatments. Our findings indicate that trade-offs between grazing resistance and competitive ability did not promote coexistence of functional groups within the tested lake phytoplankton communities.

The relatively small effect of grazing on functional groups within the most productive communities is likely a result of higher abundance of inedible functional groups (Figure 2) in nutrient-rich environments. Small-celled species are better nutrient competitors in low-nutrient environments, while large-celled species thrive in eutrophic or fluctuating nutrient environments (Chisholm, 1992; Cloern, 2018; Edwards et al., 2011; Irwin et al., 2006; Kiørboe, 1993; Litchman et al., 2007; Litchman et al., 2010). Thus, increasing abundance of large-celled, colonial, and filamentous species in higher nutrient environments is consistent with expectations, and these large and colonial groups are likely excluded from more oligotrophic communities due to nutrient limitation. In the low-productivity community, every functional group except VII (large mucilaginous colonies) responded strongly to grazers and nutrients. It is possible that different taxa within functional groups were limited by grazers or nutrients, but at the functional group level, coexistence was not constrained by alternate limiting factors. In the high-productivity community, the positive effect of grazing on functional group IV was likely a result of consumer-driven nutrient recycling (Elser & Urabe, 1999; Schulhof et al., 2019).

Functional groups in the lowest productivity community showed strongest positive responses to nutrient addition likely because they were most nutrient-limited in their ambient environment. In contrast, the weaker response to nutrient addition in the highest productivity community for all functional groups may have occurred because nutrient limitation was alleviated by higher ambient nutrient availability. Therefore, the nutrient treatment may have had a smaller relative impact on the most productive community than on the least productive community. Increasing nutrient supply alleviated both grazer susceptibility and nutrient limitation as more grazer-resistant forms dominated communities. The filamentous cyanobacteria, which dominated the intermediate and high-productivity communities in our experiments, are likely very unpalatable for *Daphnia* and presumably

caused the reduced *Daphnia* biomass we observed in these communities. Filamentous cyanobacterial species can mechanically obstruct the *Daphnia* filtering apparatus, even for larger bodied species such as *D. magna* used in our experiments (DeMott et al., 2001; Gliwicz, 1990; Gliwicz & Lampert, 1990). Additionally, filamentous cyanobacteria produce toxic and possibly allelopathic chemical compounds (Fulton, 1988; Lampert, 1981) and are of low nutritional quality (Brett & Müller-Navarra, 1997). As a result, large-bodied *Daphnia* often cannot maintain growth in the presence of high densities of toxic filamentous cyanobacteria (DeMott et al., 2001). Therefore, large *Daphnia* may be rare in eutrophic lakes dominated by filamentous cyanobacteria (Hansson et al., 2007). Inhibition of grazing and therefore growth of large *Daphnia* species by filamentous cyanobacteria has been shown even when supplemented with edible algae (DeMott et al., 2001), indicating that a dominance by filamentous forms may provide an associational defense for more edible phytoplankton species by suppressing grazer abundance. Our results suggest the existence of associational defense for edible taxa in the presence of defended cells, as the edible functional groups showed no significant losses to grazing in the two lakes dominated by filamentous cyanobacteria, but were reduced by *Daphnia* in the least productive lake. Facilitation by inedible functional groups may thus allow grazing-resistant and grazing-susceptible groups to coexist in highly productive environments.

Our results do not provide empirical evidence that variation among functional groups in limitation by nutrients versus grazers promotes local coexistence within lakes. That is, the groups that were reduced in abundance by grazer introduction often also responded positively to fertilization. Our results indicate that the most grazing-tolerant species were likely excluded from the low-productivity lake due to their inability to persist at low-nutrient conditions, as predicted in a modeling study (Cadier et al., 2019). Thus, the best nutrient competitors and most grazing-susceptible phytoplankton dominated the phytoplankton community from the least productive lake, while defended species that are poor nutrient competitors were most prevalent in the communities from the more eutrophic lakes. The variable effect of warming and its interactions with nutrient addition on functional groups in each community is consistent with the literature, indicating that the effect of warming depends on trophic state and species composition of phytoplankton in lakes (Huber et al., 2008; Rigosi et al., 2014). Warming effects on phytoplankton productivity can range from positive to negative depending on phosphorus availability, with constrained or negative effects under phosphorus scarcity and positive effects under eutrophic conditions (De Senerpont Domis et al., 2014; Tadonl  k  , 2010).

The near absence of taxa with both low grazer resistance and high nutrient efficiency in these lakes likely precluded us from finding trade-offs, given that species sorting was constrained due to our experimental setup (i.e., the short-term duration and the absence of immigration from the regional species pool). Furthermore, trade-offs might have been detected if we had used grazers typically found in these lakes, such as copepods and rotifers that are more selective in their grazing and possibly also more gape-limited than *Daphnia*. It is possible that small grazers <200 μm , such as copepod nauplii, rotifers, and ostracods, were not filtered out of inocula and might have grazed upon small phytoplankton taxa. However, given that small cells represented the smallest fraction of biomass in all three phytoplankton communities (<6%), these small grazers are unlikely to have impacted the overarching patterns we observed, especially in the medium- and high-productivity communities that were dominated by filamentous cyanobacteria. Our findings may indicate that this trade-off is not the most important factor in determining the functional diversity of phytoplankton communities and may also suggest that the grazer susceptibility classifications for the functional groupings did not reliably predict the responses of the functional groups, especially in the least productive lake where taxa with strategies along the size axis were represented more equally.

We note some factors that may have potentially confounded our findings. First, the communities from the different lakes were sampled at different times in spring due to logistical constraints and therefore differed in ambient temperature at the time of sampling. The lowest productive community was sampled early in spring (March 2017) and was followed by sampling of the highest (April 2017) and intermediate (May 2017) productivity communities. These ambient temperature differences possibly affected the sensitivity of the communities to the treatments. Second, due to technical issues, the warming treatments differed between the communities, resulting in a 1.4°C increase for the lowest productivity, 4°C for the intermediate-productivity, and 3°C for the highest productivity community. Despite the potential consequences of differences in ambient temperature and temperature treatments, warming had a minimal impact on functional groups in all three communities. Thus, the observed patterns within the different communities under ambient treatments may have been primarily driven by responses of the phytoplankton functional groups to the nutrient and grazing treatments (Table 1, Figure 3).

Trade-offs have the potential to promote diversity and coexistence when competing taxa experience limitation by different biotic or abiotic factors, preventing competitive exclusion. In our experiments, the competition–defense trade-off was not apparent in lake phytoplankton communities of varying trophic status, at both ambient

and warmed temperatures. However, there was a robust pattern in which both top-down and bottom-up control decreased in more productive communities that were dominated by inedible functional groups, namely filamentous cyanobacteria. We found that functional groups subject to different limiting factors (nutrients or grazers) tended to occur in different lakes rather than the same lake (Figure 2), supporting the idea that covariation in traits related to nutrient competitive ability and tolerance of grazers may drive turnover of functional groups among lakes (Kneitel & Chase, 2004; Ptacnik et al., 2010).

ACKNOWLEDGMENTS

Thank you to Ellen van Donk for supporting this research collaboration. Additionally, we are grateful to Nico Helmsing for providing general technical assistance; Erik Reichman for extensive assistance in field sampling; Dennis Waasdorp for assistance identifying, analyzing, and counting microscope data; and Guus van den Heuvel and Justin Jansen for their help with running and harvesting the experiments. We also would like to thank Thijs Frenken and Alena Gsell for sharing their expertise and for their help harvesting the experiments. This project was funded by an NSF Graduate Research Fellowship, NSF-GROW Fellowship, Royal Netherlands Academy of Arts and Sciences Ecology Fund, and NSF DEB award 1457737.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

Marika A. Schulhof was involved in designing and executing experiments, processing samples, analyzing data, and writing and editing the manuscript; Dedmer B. Van de Waal was involved in designing and executing experiments, processing samples, and carefully editing the manuscript; Steven A. J. Declerck helped with experimental design and carefully edited the manuscript; and Jonathan B. Shurin was involved in the design of the study, advised data analysis, and carefully edited the manuscript.

DATA AVAILABILITY STATEMENT

Data (Schulhof et al. 2021) are available from Dryad: <https://doi.org/10.5061/dryad.9w0vt4bh4>.

ORCID

Marika A. Schulhof  <https://orcid.org/0000-0003-0412-3625>

Dedmer B. Van de Waal  <https://orcid.org/0000-0001-8803-1247>

Steven A. J. Declerck  <https://orcid.org/0000-0001-6179-667X>

Jonathan B. Shurin  <https://orcid.org/0000-0001-7870-1972>

REFERENCES

- Aksnes, D. L., and F. J. Cao. 2011. "Inherent and Apparent Traits in Microbial Nutrient Uptake." *Marine Ecology Progress Series* 440: 41–51.
- Baker, K. G., C. M. Robinson, D. T. Radford, A. S. McInnes, C. Evenhuis, and M. A. Doblin. 2016. "Thermal Performance Curves of Functional Traits Aid Understanding of Thermally Induced Changes in Diatom-Mediated Biogeochemical Fluxes." *Frontiers in Marine Science* 3: 44.
- Barneche, D. R., C. J. Hulatt, M. Dossena, D. Padfield, G. Woodward, M. Trimmer, and G. Yvon-Durocher. 2021. "Warming Impairs Trophic Transfer Efficiency in a Long-Term Field Experiment." *Nature* 592: 76–9.
- Brett, M. T., and D. Müller-Navarra. 1997. "The Role of Highly Unsaturated Fatty Acids in Aquatic Foodweb Processes." *Freshwater Biology* 38(3), 483–99.
- Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. "Toward a Metabolic Theory of Ecology." *Ecology* 85(7): 1771–89.
- Cadier, M., K. H. Andersen, A. W. Visser, and T. Kiørboe. 2019. "Competition–Defense Tradeoff Increases the Diversity of Microbial Plankton Communities and Dampens Trophic Cascades." *Oikos* 128: 1027–40.
- Chisholm, S. W. 1992. "Phytoplankton Size." In *Primary Productivity and Biogeochemical Cycles in the Sea*, edited by P. G. Falkowski and A. D. Woodhead, 213–37. New York: Plenum Press.
- Cloern, J. E. 2018. "Why Large Cells Dominate Estuarine Phytoplankton." *Limnology and Oceanography* 63: S392–409.
- Colina, M., D. Calliari, C. Carballo, and C. Kruk. 2016. "A Trait-Based Approach to Summarize Zooplankton–Phytoplankton Interactions in Freshwaters." *Hydrobiologia* 767(1): 221–33.
- De Senerpont Domis, L., D. B. Van de Waal, N. R. Helmsing, E. Van Donk, and W. M. Mooij. 2014. "Community Stoichiometry in a Changing World: Combined Effects of Warming and Eutrophication on Phytoplankton Dynamics." *Ecology* 95(6): 1485–95.
- DeMott, W. R., R. D. Gulati, and E. Van Donk. 2001. "Daphnia Food Limitation in Three Hypereutrophic Dutch Lakes: Evidence for Exclusion of Large-Bodied Species by Interfering Filaments of Cyanobacteria." *Limnology and Oceanography* 46(8): 2054–60.
- Edwards, K. F., C. A. Klausmeier, and E. Litchman. 2011. "Evidence for a Three-Way Trade-Off between Nitrogen and Phosphorus Competitive Abilities and Cell Size in Phytoplankton." *Ecology* 92(11): 2085–95.
- Elser, J. J., and J. Urabe. 1999. "The Stoichiometry of Consumer-Driven Nutrient Recycling: Theory, Observations, and Consequences." *Ecology* 80(3): 735–51.
- Fiksen, Ø., M. J. Follows, and D. L. Aksnes. 2013. "Trait-Based Models of Nutrient Uptake in Microbes Extend the Michaelis-Menten Framework." *Limnology and Oceanography* 58: 193–202.
- Finkel, Z. V., J. Beardall, K. J. Flynn, A. Quigg, T. A. V. Rees, and J. A. Raven. 2010. "Phytoplankton in a Changing World: Cell Size and Elemental Stoichiometry." *Journal of Plankton Research* 32(1): 119–37.
- Fulton, R. S. 1988. "Grazing on Filamentous Algae by Herbivorous Zooplankton." *Freshwater Biology* 20(2): 263–71.
- Gliwicz, Z. M. 1990. "Daphnia Growth at Different Concentrations of Blue-Green Filaments." *Archiv für Hydrobiologie* 120: 51–65.
- Gliwicz, Z. M., and W. Lampert. 1990. "Food Thresholds in *Daphnia* Species in the Absence and Presence of Blue-Green Filaments." *Ecology* 71(2): 691–702.
- Hansson, L. A., S. Gustafsson, K. Rengefors, and L. Bomark. 2007. "Cyanobacterial Chemical Warfare Affects Zooplankton Community Composition." *Freshwater Biology* 52(7): 1290–301.
- Hedges, L. V., J. Gurevitch, and P. S. Curtis. 1999. "The Meta-Analysis of Response Ratios in Experimental Ecology." *Ecology* 80(4): 1150–6.
- Hillebrand, H., C.-D. Dürselen, D. Kirschtel, U. Pollinger, and T. Zohary. 1999. "Biovolume Calculation for Pelagic and Benthic Microalgae." *Journal of Phycology* 35(2): 403–24.
- Huber, V., R. Adrian, and D. Gerten. 2008. "Phytoplankton Response to Climate Warming Modified by Trophic State." *Limnology and Oceanography* 53(1): 1–13.
- IPCC, 2014. Climate Change 2014: Synthesis Report. In *Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (p. 151), edited by R.K. Pachauri and L.A. Meyer. Geneva, Switzerland: IPCC.
- 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." *Journal of Plankton Research* 28(5): 459–71.
- Kiørboe, T. 1993. "Turbulence, Phytoplankton Cell Size, and the Structure of Pelagic Food Webs." *Advances in Marine Biology* 29: 1–72.
- Kneitel, J. M., and J. M. Chase. 2004. "Trade-Offs in Community Ecology: Linking Spatial Scales and Species Coexistence." *Ecology Letters* 7(1): 69–80.
- Kratina, P., H. S. Greig, P. L. Thompson, T. S. A. Carvalho-Pereira, and J. B. Shurin. 2012. "Warning Modifies Trophic Cascades and Eutrophication in Experimental Freshwater Communities." *Ecology* 93(6): 1421–30.
- Kruk, C., V. L. M. Huszar, E. T. H. M. Peeters, S. Bonilla, L. Costa, M. Lüring, C. S. Reynolds, and M. Scheffer. 2010. "A Morphological Classification Capturing Functional Variation in Phytoplankton." *Freshwater Biology* 55(3): 614–27.
- Kruk, C., E. T. H. M. Peeters, E. H. Van Nes, V. L. M. Huszar, L. S. Costa, and M. Scheffer. 2011. "Phytoplankton Community Composition Can Be Predicted Best in Terms of Morphological Groups." *Limnology and Oceanography* 56(1): 110–8.
- Kruk, C., and A. M. Segura. 2012. "The Habitat Template of Phytoplankton Morphology-Based Functional Groups." *Hydrobiologia* 698(1): 191–202.
- Lampert, W. 1981. "Inhibitory and Toxic Effects of Blue-Green Algae on *Daphnia*." *Internationale Revue der gesamten Hydrobiologie und Hydrographie* 66(1): 285–98.
- Leibold, M. A. 1996. "A Graphical Model of Keystone Predators in Food Webs: Trophic Regulation of Abundance, Incidence, and Diversity Patterns in Communities." *The American Naturalist* 147: 784–812.
- Litchman, E., P. de Tezanos Pinto, C. A. Klausmeier, M. K. Thomas, and K. Yoshiyama. 2010. "Linking Traits to Species Diversity and Community Structure in Phytoplankton." *Hydrobiologia* 653(1): 15–28.

- Litchman, E., K. F. Edwards, C. A. Klausmeier, and M. K. Thomas. 2012. "Phytoplankton Niches, Traits and Eco-Evolutionary Responses to Global Environmental Change." *Marine Ecology Progress Series* 470: 235–48.
- Litchman, E., and C. A. Klausmeier. 2008. "Trait-Based Community Ecology of Phytoplankton." *Annual Review of Ecology, Evolution, and Systematics* 39(1): 615–39.
- Litchman, E., C. A. Klausmeier, 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." *Ecology Letters* 10(12): 1170–81.
- Lubchenco, J. 1978. "Plant Species Diversity in a Marine Intertidal Community: Importance of Herbivore Food Preference and Algal Competitive Abilities." *The American Naturalist* 112: 23–39.
- Mandal, S., R. A. Wilkins, and J. B. Shurin. 2018. "Compensatory Grazing by *Daphnia* Generates a Trade-Off between Top-Down and Bottom-up Effects across Phytoplankton Taxa." *Ecosphere* 9(12): e02537.
- Marañón, E., P. Cermeño, D. C. López-Sandoval, T. Rodríguez-Ramos, C. Sobrino, M. Huete-Ortega, J. M. Blanco, and J. Rodríguez. 2013. "Unimodal Size Scaling of Phytoplankton Growth and the Size Dependence of Nutrient Uptake and Use." *Ecology Letters* 16(3): 371–9.
- McCauley, E., and F. Briand. 1979. "Zooplankton Grazing and Phytoplankton Species Richness: Field Tests of the Predation Hypothesis." *Limnology and Oceanography* 24: 243–52.
- O'Connor, M. I., M. F. Piehler, D. M. Leech, A. Anton, and J. F. Bruno. 2009. "Warming and Resource Availability Shift Food Web Structure and Metabolism." *PLoS Biology* 7(8): 3–8.
- Paine, R. T. 1966. "Food Web Complexity and Species Diversity." *The American Naturalist* 100: 65–75.
- Ptácnik, R., T. Andersen, P. Brettum, L. Lepistö, and E. Willén. 2010. "Regional Species Pools Control Community Saturation in Lake Phytoplankton." *Proceedings of the Royal Society B: Biological Sciences* 277(1701): 3755–64.
- R Core Team. 2017. *R: A Language and Environment for Statistical Computing*. Vienna, Austria: R Foundation for Statistical Computing. <https://www.R-project.org/>
- Reynolds, C. S. 1984. *The Ecology of Freshwater Phytoplankton*. Cambridge: Cambridge University Press.
- Reynolds, C. S. 2002. "Towards a Functional Classification of the Freshwater Phytoplankton." *Journal of Plankton Research* 24(5): 417–28.
- Rigosi, A., C. Carey, B. W. Ibelings, and J. D. Brookes. 2014. "The Interaction between Climate Warming and Eutrophication to Promote Cyanobacteria Is Dependent on Trophic State and Varies among Taxa." *Limnology and Oceanography* 59(1): 99–114.
- Schulhof, M. A., J. B. Shurin, S. A. J. Declerck, and D. B. Van de Waal. 2019. "Phytoplankton Growth and Stoichiometric Responses to Warming, Nutrient Addition and Grazing Depend on Lake Productivity and Cell Size." *Global Change Biology* 25: 2751–62.
- Shurin, J. B., J. L. Clasen, H. S. Greig, P. Kratina, and P. L. Thompson. 2012. "Warming Shifts Top-Down and Bottom-Up Control of Pond Food Web Structure and Function." *Philosophical Transactions of the Royal Society B: Biological Sciences* 367(1605): 3008–17.
- Smith, S. L., A. Merico, K. W. Wirtz, and M. Pahlow. 2014. "Leaving Misleading Legacies behind in Plankton Ecosystem Modelling." *Journal of Plankton Research* 36: 613–20.
- Tadonlélé, R. D. 2010. "Evidence of Warming Effects on Phytoplankton Productivity Rates and Their Dependence on Eutrophication Status." *Limnology and Oceanography* 55(3): 973–82.
- Thomas, M. K., M. Aranguren-Gassis, C. T. Kremer, M. R. Gould, K. Anderson, C. A. Klausmeier, and E. Litchman. 2017. "Temperature–Nutrient Interactions Exacerbate Sensitivity to Warming in Phytoplankton." *Global Change Biology* 23(8): 3269–80.
- Utermöhl, H. 1958. "Zur vervollkommnung der quantitativen phytoplankton-methodik: Mit 1 Tabelle und 15 abbildungen im Text und auf 1 Tafel." *Internationale Vereinigung für theoretische und angewandte Limnologie: Mitteilungen* 9(1): 1–38.
- Van de Waal, D. B., and E. Litchman. 2020. "Multiple Global Change Stressor Effects on Phytoplankton Nutrient Acquisition in a Future Ocean." *Philosophical Transactions of the Royal Society B* 375: 20190706.
- Viola, D. V., E. A. Mordecai, A. G. Jaramillo, S. A. Sistla, L. K. Albertson, J. S. Gosnell, B. J. Cardinale, and J. M. Levine. 2010. "Competition-Defense Tradeoffs and the Maintenance of Plant Diversity." *Proceedings of the National Academy of Sciences* 107(40): 17217–22.

SUPPORTING INFORMATION

Additional supporting information may be found in the online version of the article at the publisher's website.

How to cite this article: Schulhof, Marika A., Dedmer B. Van de Waal, Steven A. J. Declerck, and Jonathan B. Shurin. 2022. "Phytoplankton Functional Composition Determines Limitation by Nutrients and Grazers across a Lake Productivity Gradient." *Ecosphere* 13(3): e4008. <https://doi.org/10.1002/ecs2.4008>