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ORIGINAL ARTICLE

Determinants of phytoplankton size structure in warm, shallow lakes

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Body size is an important trait of any organism, including phytoplankton, because it affects physiological and morphological performance, reproduction, population growth rate and competitive interactions. Understanding how interacting top-down and bottom-up factors influence phytoplankton cell size in different aquatic environments is still a challenge. Structural equation modeling (SEM) is a comprehensive multivariate statistical tool for detecting cause–effect relationship among different variables and their hierarchical structure in complex networks (e.g. trophic interactions in ecosystems). Here, several SEM models were employed to investigate the direct and indirect interaction pathways affecting the phytoplankton size structure in 44 mostly eutrophic and hypereutrophic permanent lakes in western Turkey. Among the 15 environmental variables tested, only rotifers and Carlson's Trophic Index (TSI) had significant direct positive effect on the mean phytoplankton size and size variance, respectively. The results indicate that both bottom-up and top-down factors significantly affect phytoplankton community size structure in eutrophic and hypereutrophic lakes in warm climates. Rotifer grazing increased the abundance of large-sized phytoplankton species, such as filamentous and colonial cyanobacteria and TSI affected phytoplankton size variance, with a higher size variance in hypereutrophic lakes.

KEYWORDS: phytoplankton mean size; phytoplankton size variance; structural equation modeling; rotifers; trophic state

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INTRODUCTION

Phytoplankton are the main primary producers in most aquatic ecosystems and responsible for nearly half of primary production on Earth (Field *et al*., 1998). Since they form the base of most aquatic food webs, phytoplankton productivity affects all levels of the food web (Graham and Wilcox, 2000). Moreover, they are a highly diverse group and their size ranges from picoplankton, with cell dimensions around 1–5 μm, to some colonial or filamentous species that can be visible to the naked eye (Reynolds, 2006). Cell size is a key trait for phytoplankton, because it affects fundamental survival functions, like nutrient uptake (Aksnes and Egge, 2006; Litchman and Klausmeier, 2008), sinking rate (Padisák *et al*., 2003) and grazer resistance (Pančić and Kiørboe, 2018). Both mean cell size and the variance in cell size are important characteristics of phytoplankton communities. They influence the structure of planktonic food webs, cycling of energy and materials and affect multiple ecosystem functions. Variance in cell size is positively correlated with functional diversity (Acevedo-Trejos *et al*., 2015).

Both abiotic (bottom-up) and biotic (top-down) factors affect cell size. Availability of nutrients is generally considered to be one of the main abiotic drivers of phytoplankton size (Edwards *et al*., 2012). For instance, small-celled (10–20 μm) species have an advantage under nutrient-deficient conditions due to high surface-to-volume ratio (Chisholm, 1992; Clark *et al*., 2013). In contrast, large cells perform better at high nutrient conditions, and increase nutrient uptake with strategies such as distorting the diffusive boundary layer, swimming, sinking or cell elongation (Karp-Boss *et al*., 1996). Larger cells can also contain bigger vacuoles to store more nutrients, allowing for luxury consumption (Litchman *et al*., 2009).

Eutrophication is one of the main causes of cyanobacterial blooms in recent decades (Paerl *et al*., 2011; Chirico *et al*., 2020), but temperature increases due to climate change also plays a role in increasing blooms. Temperature affects phytoplankton seasonality, community structure and size distribution (Sheridan and Bickford, 2011; Havens *et al*., 2019). Because the optimum growth temperature of cyanobacteria is usually higher than in other groups (25–35°C), they may be favored by higher temperatures (Thomas *et al*., 2016; Maliaka *et al*., 2020). Temperature increase also enhances stratification, which many cyanobacteria with gas vesicles are able to exploit (Paerl and Huisman, 2009). Cyanobacterial blooms in surface waters also may locally increase water temperature due to high light absorption by photosynthetic pigments, which in turn can enhance their competitive advantage over other phytoplankton groups (Hense and Beckmann, 2006; Mantzouki *et al*., 2018).

Grazing pressure, which is related to zooplankton community structure, has a direct effect on phytoplankton size structure in lakes (Edwards *et al*., 2011; Hulot *et al*., 2014; Frau *et al*., 2017). Although small-sized zooplankton groups, like rotifers, small-sized cladocerans and nauplii are capable of grazing on small phytoplankton species, large cladocerans and calanoid copepods usually prefer intermediate- and large-sized phytoplankton (Lampert and Sommer, 2007; Colina *et al*., 2016). In eutrophic, warm temperate and subtropical lakes (hereafter referred as warm lakes), the zooplankton community is usually dominated by small-sized zooplankton species due to predation pressure on the zooplankton by fish (Jeppesen *et al*., 1997; Vadadi-Fülöp *et al*., 2012; Tavşanoğlu *et al*., 2015), which are predominately small, omnivorous and found in high density (Meerhoff *et al*., 2012; Frau *et al*., 2015; Boll *et al*., 2016). Consequently, grazing pressure by small-sized grazers on small phytoplankton is expected to be more intense in warm or eutrophic lakes (Matsuzaki *et al*., 2018). Similarly, Ger *et al*. (2016) and Mao *et al*. (2020) suggested that top-down control on large phytoplankton species in eutrophic waters is relatively weak because of the small size of zooplankton grazers and dominance by grazer-resistant large filamentous or colonial phytoplankton.

Understanding underlying ecological mechanisms in these systems is difficult because interactions between abiotic and biotic parameters are complex and often nonlinear. To overcome these difficulties, we employ structural equation modeling (SEM)—a powerful multivariate statistical tool to evaluate two or more structural cause–effect relations to model multivariate relationships based on correlations (Grace *et al*., 2015). SEM is a classic approach (Wright, 1920, 1921) whose implementation in ecology has increased in recent years (Grace, 2006), though its application in freshwater ecosystems is still limited (Stomp *et al*., 2011; Fan *et al*., 2016; Cao *et al*., 2017; Laughlin and Grace, 2019). SEM uses confirmatory factor analysis and path analysis to infer causal relationships from complex ecological interactions, disentangling direct and indirect effects of different drivers (Anderson and Gerbing, 1988; Grace, 2006; Fan *et al*., 2016). SEM also allows flexibility in defining the directionality of trophic interactions, for example the interaction can be fixed as zooplankton → phytoplankton or phytoplankton → zooplankton.

In this study, we aimed to understand the main controlling factors on mean phytoplankton size and size variance by SEM using multi-trophic-level data from 44 Turkish lakes, allowing us to explore the effects of both top-down (fish and zooplankton) and bottom-up (temperature, TP, TN, etc.) drivers and their interactions. We hypothesized

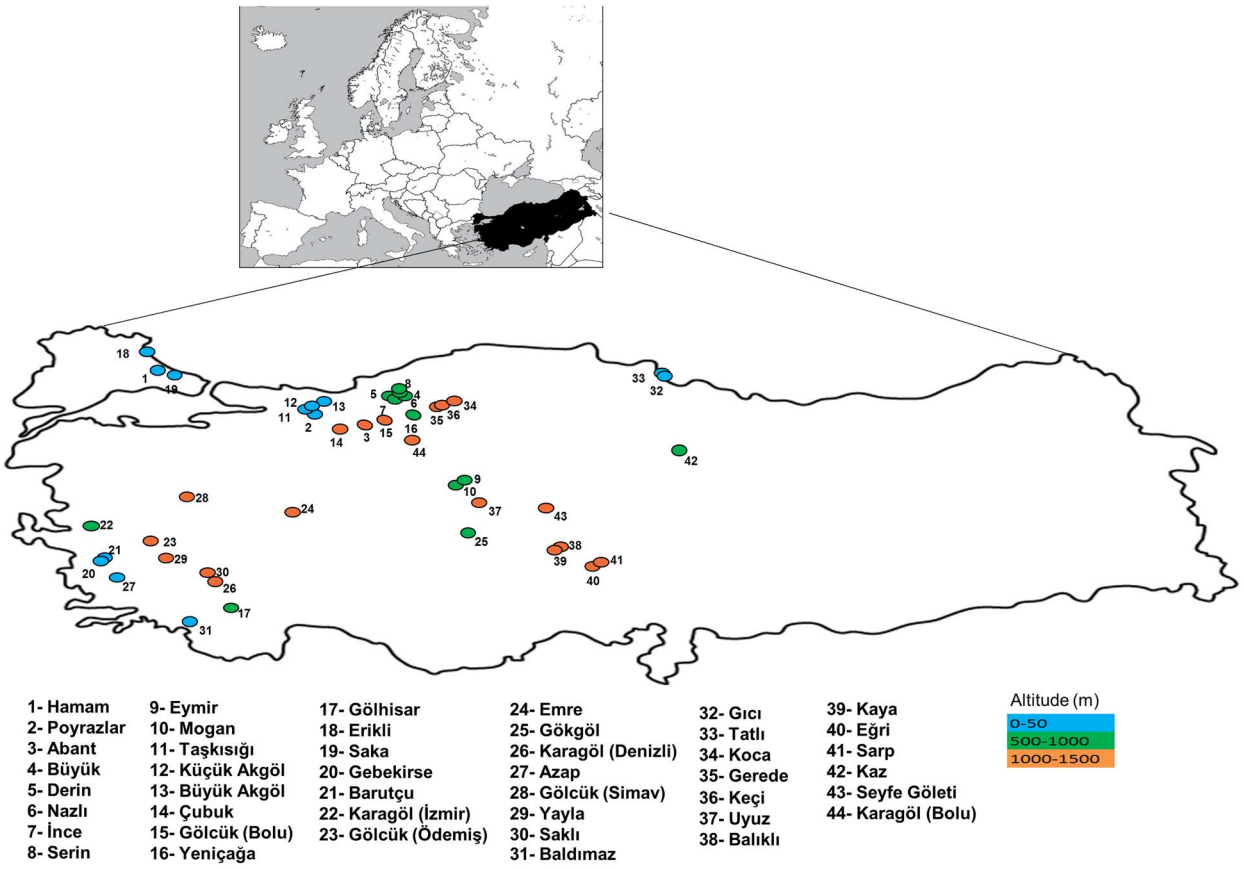

Fig. 1. Study lakes on the map of Turkey given each with a number. The color coding indicates the altitude of the lake; blue: 0–50 m.a.s.l., green:500–1000 m.a.s.l. and orange:1000–1500 m.a.s.l.

that in warm nutrient-rich lakes, high selective grazing on small phytoplankton species is likely to lead to an increase in mean phytoplankton size, due to high abundance of small-sized grazers such as rotifers and small cladocerans. In addition, the increase of grazing resistant large-bodied species, like filamentous and colonial species of cyanobacteria, would lead to an increase in phytoplankton size variance within a lake.

METHODS

Study lakes

Turkey is located between 36–42°N latitude and 26–45°E longitude, with highly mountainous topography and with multiple climatic zones, ranging from arid, cold steppes to a warm temperate region to the hot and dry Mediterranean (Peel *et al*., 2007). We sampled 44 permanent lakes between years 2006 and 2012. The lakes are located in Western Anatolian Plateau, distributed from north to the south, ranging from warm temperate to hot Mediterranean climates. Elevation of sampling sites ranges from sea-level to 1423 m.a.s.l. and latitudinal gradient is between 37°N and 42°N (Fig. 1).

Sampling and analyses

Samples were collected during the peak-growing season (July–August) along both latitudinal and elevational gradients. All the lakes were sampled once, and depth-integrated water samples for all physico-chemical and biological variables were collected using a snap-shot sampling protocol that is widely used for sampling of lakes in different continents (Kruk *et al*., 2009; Kosten *et al*., 2012; Levi *et al*., 2014). The details of the study lakes can be found in Beklioğlu *et al*. (2020).

Abiotic variables

The depth profile of each lake was determined in parallel transects at even intervals by using a Portable Sounder (Speechtech SM-5), the number of transects in each lake was based on lake area. Temperature was measured *in situ*

with a YSI 556 MPS multi-probe (YSI, Yellow Springs, OH, USA), and Secchi disc transparency was measured with a 20-cm diameter disc at the deepest point of each lake. Depth-integrated samples including the entire water column (surface to bottom) were taken at the deepest point for each lake with a KC Denmark Ruttner sampler (3.5-L capacity with a length of 50 cm). We collected a total volume of 40 L. If a lake was too shallow to yield that volume from a single sampling point, we took several depth-integrated water samples from several points at the deepest area to collect the required volume. The water sample was mixed in a barrel and sub-samples were taken for chemical analyses and for phytoplankton and zooplankton investigation. Samples for water chemistry analyses were stored frozen until analyzed for total phosphorus (TP; Mackereth *et al*., 1978), chlorophyll-*a* (Chl-*a*; Jespersen and Christoffersen, 1987) and total nitrogen (TN; using a Scalar Auto-analyzer, San++ Automated Wet Chemistry Analyzer, Skalar Analytical, B.V. Breda, The Netherlands).

In order to determine the trophic status of the lakes, Carlson's Trophic Index (TSI; Carlson, 1977, 1996) was calculated based on TP, Chl-*a* and Secchi disc depth (SD) measurements by employing the following equations;

$$\mathrm{TSI\ (Chl}a\mathrm{)} = 9.81 * \ln(\mathrm{Chl}a) + 30.6$$
$$\mathrm{TSI\ (SD)} = 60 - 14.41 * \ln(\mathrm{SD})$$
$$\mathrm{TSI\ (TP)} = 14.42 * \ln(\mathrm{TP}) + 4.15$$

The average of these three equations was calculated as the final TSI for each lake's trophic status. TSI index ranges from 0 to 100 that indicates the most oligotropic and most eutrophic water trophic states, respectively.

Biotic variables

Fifty milliliter of water from the 40 L of composite water sample from each lake was fixed using a 2% Lugol's solution, and they were stored in 50-mL dark glass bottles for phytoplankton enumeration. Phytoplankton samples were counted according to the Utermöhl technique (1958). Samples were shaken at least 100 times, then, depending on the sample volume, were settled in Utermöhl chambers for 16–24 h. Subsequently, samples were counted in horizontal transects under an inverted microscope, until reaching 400 natural units of the most abundant species. For small species ×400 and ×630 magnifications and for large species ×20 magnification were used (Leica DMI, 4000B).

Filamentous and colonial species were counted as one unit, and, where possible, organisms smaller than 2 μm were also counted. Identification of phytoplankton species was carried out by the same person, using reference taxonomy books (Prescott, 1973; Komarek and Fott, 1983; Popovski and Pfiester, 1990; Cox, 1996; Komarek and Anagnostidis, 1999; John *et al*., 2002). Whenever possible, the dimensions of 10 individuals per phytoplankton species were measured in each lake and the same species means were applied in all lakes to calculate phytoplankton community mean size and size variances. Measurements were done with Leica image analysis program, and biovolume was calculated according to Hillebrand *et al.* (1999).

To sample for zooplankton, we filtered 20 L through a 2-μm mesh (see Beklioğlu *et al*., 2020; Çakıroğlu *et al*., 2016 for details). Zooplankton samples were stored in 50-mL dark glass bottles and preserved in 4% Lugol's iodine solution. Zooplankton counts were carried out at the genus or species level, where possible. Samples were counted until 50–100 individuals of the most abundant taxa were recorded and, when possible, body sizes of about 25 individuals of each taxon were measured and body weight was calculated from length–weight allometric relationships (Dumont *et al*., 1975; Bottrell *et al*., 1976; McCauley, 1984; Michaloudi, 2005). The biomass of each zooplankton species or genus was calculated and converted to dry weight according to Dumont et al. (1975), Ruttner-Kolisko (1977) and Malley *et al*. (1989).

Fish community structure and abundance (catch per numbers unit effort, CPUE, number net^{-1}) were determined using Lundgren multi-mesh gillnets, covering 12 mesh sizes (5, 6.5, 8, 10, 12.5, 15.5, 19.5, 24.5, 29, 35, 43 and 55 mm; see Boll *et al*., 2016 for details). The number of gillnets for each lake was determined based on the lake area (0–2 ha: 2 sets of nets, 2–20 ha: 4 sets nets, 20–100 ha: 6 sets nets and > 100 ha: 8 sets nets). The gillnets were deployed parallel to the shore, to both littoral and pelagic zones for 12 h. Detailed information can be found in Boll *et al*. (2016). Zooplanktivorous fish density (number of fish net^{-1} night^{-1}), and the total fish to zooplanktivorous fish ratio were calculated.

The macrophyte survey was conducted in transects, with a rake. Plant height, plant coverage, water depth and GPS coordinates were noted at each sampling point along each transect. Percent plant volume inhabited (PVI) data of the each of the study lakes were taken from Levi *et al*. (2014) and it was calculated based on the formula of plant coverage × average plant height/water depth (Canfield *et al*., 1984).

Data analysis

Mean size-based biovolume for each phytoplankton species and mean size-based biomass of each zooplankton species were calculated as follows:

$$\text{Mean phytoplankton size } \left(\mu\mathrm{m}^3\right) = \frac{\sum(\text{mean volume} * \text{abundance})}{\sum \text{abundance}}$$

Based on calculated size data, size variance (s^2) for phytoplankton in each lake was calculated as follows:

$$s^2\ \left(\mu m^3/L^{-1}\right) = \frac{\sum (X-\overline{X})^2}{n-1}$$
$$X = \sum (\text{Mean volume} * \text{abundance})$$

Consequently, we obtained two size-related variables for each lake: (i) mean phytoplankton size and (ii) phytoplankton size variance. The same mean size formula was also used to calculate zooplankton mean size.

SEM enabled us to determine how much variation in mean phytoplankton size and phytoplankton size variance could be explained by abiotic or biotic variables. A convincing SEM model should have the following acceptable fit measures: non-significant chi-square, low root-mean-square error of approximation (RMSEA < 0.05), high goodness-of-fit statistic (GFI > 0.09), high comparative fit index (CFI > 0.09) and low standardized root-mean-square residual (SRMR < 0.08; Browne *et al*., 1993; Kline, 2005; Hooper *et al*., 2008). If the fit measures were not satisfactory, the initial model was modified according to the reasonable biological assumptions. Analyses were repeated until the best-fit measures and significant interactions among all the remaining variables were obtained. Possible interaction pathways were tried among significant parameters and the best result was chosen according to the SEM fit parameters and significance of explanation.

Our sample size was relatively small (44 lakes), thus we could only use four environmental variables as explanatory variables in SEM to achieve adequate statistical power. We used a pearson correlation matrix as a first step to eliminate variables with high correlation coefficients and prevent multicollinearity (correlation coefficient > 0.8; Maruyama, 1998; Fig. 2 and Supplementary Fig. S1). Among the possible variables, nutrients, temperature and grazing are known to be among the main drivers of phytoplankton community structure (Edwards *et al*., 2012; Hulot *et al*., 2014; Thomas *et al*., 2017), and zooplanktivorous fish may have indirect effect on phytoplankton via grazing on zooplankton (Meerhoff *et al*., 2012). Consequently, TSI, temperature, total zooplankton biomass, biomasses of different zooplankton taxa, zooplanktivorous fish and total fish zooplanktivorous fish ratio were chosen as the main parameters to include to the SEM.

Only four explanatory variables may not be enough to explain complex ecological pathways. Therefore, different SEMs were used to find the best model that explains the most interactions and main regulation patterns. The different SEMs were constructed by replacing TSI with TP and TN, separately, and by replacing total zooplankton biomass with different zooplankton groups i.e. Cladocera, Rotifera and Copepoda and zooplankton mean size. In addition, a model using the ratio of zooplanktivorous fish to total fish biomass was compared with one using zooplanktivorous fish biomass. Subsequently, the best model among all different SEMs was chosen.

To meet the normality assumption, we log transformed phytoplankton size, phytoplankton size variance, TSI, surface temperature, TP, TN, total zooplankton, Chl-*a*, latitude, Secchi disc depth, air temperature and zooplankton size. For measurements that included zero values (total fish/zooplanktivorous fish ratio, zooplanktivorous fish, cladocera, copepod, rotifer and PVI), we $\log(x+z)$ transformed the data with z set to 50% of detection limit for the biotic variables and 1 for elevation.

We calculated Mardia's coefficient of multivariate kurtosis and skewness values for each variable to check multivariate and univariate normality assumptions (Kline, 2005; Wang *et al*., 2011). Acceptable Mardia's coefficient of multivariate kurtosis should be smaller than 1.96 (Wang *et al*., 2011) and it was calculated as −1.59. Skewness and kurtosis values for each variable were also calculated to check univariate normality assumption. All of our variables fell within the acceptable range for SEM of −3 to +3 for skewness, −10 to +10 for kurtosis (Kline, 2005; Weston and Gore, 2006).

Change in biomasses of total zooplankton, Cladocera, Copepoda, Rotifera and cyanobacteria biovolume among different trophic states were tested using Kruskall–Wallis test with Bonferroni correction ($P > 0.05$).

R software (lavaan package) version 3.1.3 (R Development Core Team, 2015) was used to conduct all statistical analyses.

RESULTS

Water temperature of the lakes ranged from 16 to 32°C (Table I). Elevation ranged from 0 to 1423 m.a.s.l. The lowest Secchi disc transparency was recorded in Lake Küçük Akgöl (0.2 m) and the highest was recorded in Lake Abant (9 m), consistent with the TP concentrations, as the highest TP concentration was recorded in Lake Küçük Akgöl (632 μg L^{-1}) and the lowest TP was measured in Lake Abant (15 μg L^{-1}). The highest TN concentration was 2340 μg L^{-1} (Lake Balıklı) and the lowest TN value was 238 μg L^{-1} (Lake Poyrazlar). According to the TSI classification, there were 6 mesotrophic, 30 eutrophic and 8 hypereutrophic lakes. Mean submerged plant PVI was 19% across all lakes, however no macrophytes were recorded in 9 of the study sites

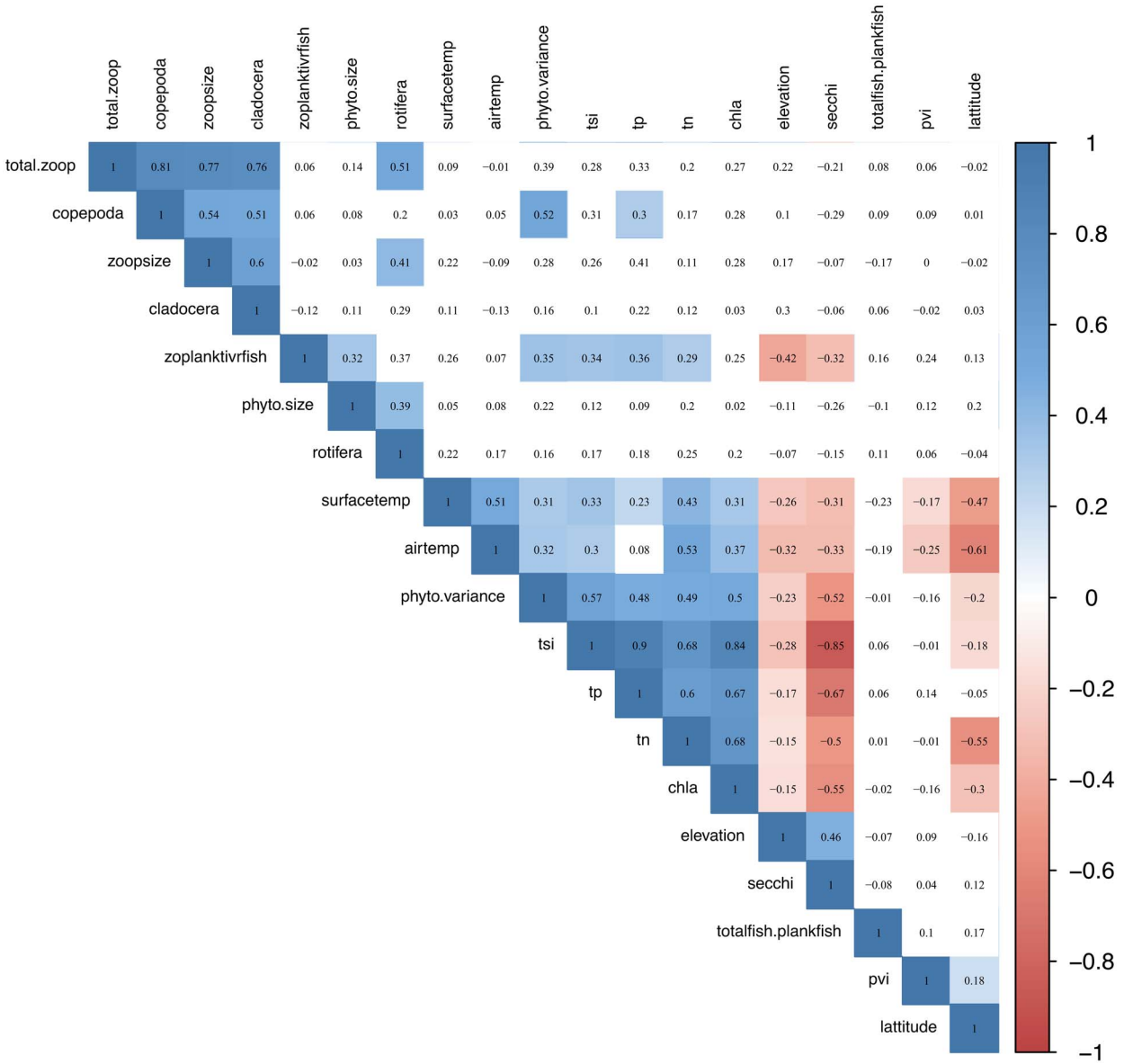

Fig. 2. Pearson correlation matrix and correlation coefficients for PVI, zooplanktivorous fish, total fish/zooplanktivorous fish, latitude, altitude, Copepoda, zooplankton size (zoopsize), total zooplankton biomass (total zoop), Cladocera, phytoplankton size variance (phyto variance), TSI, TP, Chl-*a*, TN, air temperature, phytoplankton mean size (phyto size) and rotifera.

(Lakes Baldımaz, Derin, Buyuk, Ince, Eymir, Taşkısığı, Karagöl, Seyfe and Barutçu). The most prevalent macrophyte taxa were *Ceratophyllum* sp., *Myriophyllum* spp., *Potamogeton* spp. and *Najas marina*. Cyprinidae were the dominant fish group in study lakes and mostly juvenile *Cyprinus* and *Carassius* spp. were observed in all lakes (Boll *et al*., 2016). Abundance of total zooplanktivorous fish for each lake is presented in supplementary material (Supplementary Fig. S2), and the details of fish taxonomy can be found in Boll *et al*. (2016).

According to Pearson's correlation results, phytoplankton mean size was positively correlated with Rotifera biomass and zooplanktivorous fish abundance (Fig. 2 and Supplementary Fig. S1), whereas variance in phytoplankton size was positively correlated with Copepoda, zooplanktivorous fish abundance, temperature, TSI, TP, TN, Chl-*a* and negatively correlated with elevation, secchi and latitude. The highest positive correlation was observed between TSI and secchi (−0.85). No significant correlation was observed for total fish/zooplanktivorous fish ratio

Table I: Main physical, chemical and biological characteristics of the study lakes (n = 44)

Variables	Range	Mean	Median
Elevation (m)	0–1423	749.4	972.5
Latitude (°N)	36.7–41.9	39.7	38.9
Summer mean air temperature (°C)	19.6–29.4	23.7	23.2
Surface water temperature (°C)	16–32.4	24.5	25
TP (μg L^{-1})	15–632.6	121.2	81.1
TN (μg L^{-1})	238.8–2340	1084.6	972.9
TSI	38.9–83.7	61.9	63.2
Chlorophyll-*a* (μg L^{-1})	1.8–181.1	31	14.4
Total phytoplankton biovolume (mm^3 L^{-1})	0.1–76.7	14.5	6.5
Total zooplankton biomass (μg L^{-1})	0.1–678.3	62.5	13.4
Cladoceran biomass (μg L^{-1})	0–62.7	7.4	1.8
Copepod biomass (μg L^{-1})	0–623.7	23.5	1.9
Rotifer biomass (μg L^{-1})	0–133.3	6.3	1.4
Zooplanktivorous fish (number net^{-1} $night^{-1}$)	0–1210	100	1.3
Total fish/Zooplanktivorous fish (number net^{-1} $night^{-1}$)	0–168	8.8	27.5
Submerged plants PVI	0–79.9	19.3	6.3

(see Fig. 2 and Supplementary Fig. S1 details for other variables).

Phytoplankton and Zooplankton Taxonomic Composition

Total phytoplankton biovolumes were generally high in nutrient-rich lakes (Figs 3a and 4). The percentage of Cyanobacteria was higher in eutrophic and hypereutrophic lakes compared with mesotrophic ones and significantly increased with TSI value ($R^2 = 0.11$, $P < 0.05$). Cyanobacterial species were mostly from the genera *Microcystis*, *Merismopedia* and *Anabaena* (some species from this genus were recently renamed *Dolichospermum* and *Sphaerospermopsis*). Detailed phytoplankton taxonomy and biovolume data for each lake are presented in supplementary material (Supplementary Tables S1 and S2). Although cyanobacteria biovolume differences among mesotrophic-eutrophic ($P < 0.05$) and mesotrophic-hypereutrophic ($P < 0.05$) lake groups were statistically significant, the difference between eutrophic and hypereutrophic lakes (as determined by TSI) was not significant ($P > 0.05$ by Kruskal–Wallis/Bonferroni; Fig. 4c). Bacillariophyta, Dinophyta and Chlorophyta contributions were high in mesotrophic lakes, while in eutrophic and hypereutrophic lakes, cyanobacteria, Chlorophyta and Cryptophyta groups contributed the most to biovolume (Fig. 4b). The lowest cyanobacteria biovolume was observed in mesotrophic lakes and the highest cyanobacteria biovolume was observed in hypereutrophic lakes (Fig. 4c).

Total zooplankton biomass did not show a consistent increase along the TP gradient, although a few lakes that had very high biomasses had the highest TP (Fig. 3c and Supplementary Fig. S5). Mean zooplankton size differed among lakes (Fig. 3d) and the highest mean zooplankton size was observed in Copepoda group (Supplementary Fig. S6). *Bosmina* and *Ceriodaphnia* were the most frequent Cladoceran taxa and in eutrophic and mesotrophic lakes, small-bodied (0.3–0.5 mm) cladoceran species such as *Bosmina*, *Chydorus* and *Alona* were dominant. *Brachionus*, *Trichocerca*, *Poliarthra*, *Keratella* and *Filina* were the most commonly observed rotifer taxa. Both calanoid and cyclopoid copepods were observed across all lakes and dominant in eutrophic and hypereutropic lakes. Detailed zooplankton taxonomy and biomass data for each lake are given in supplementary material (Supplementary Tables S3 and S4). The difference among total biomasses of Cladocera, Copepoda and Rotifera and among different trophic states were not statistically significant ($P > 0.05$ by Kruskal–Wallis/Bonferroni; Fig. 5).

SEM of phytoplankton mean size

Mean phytoplankton size did not show clear pattern with increasing TP (Fig. 3b). However, mean size was generally high at high TP concentration. Moreover, the highest mean unit size was observed in cyanobacteria group (Supplementary Fig. S5). Among different models that tested different explanatory variables (Fig. 6a), phytoplankton mean size was best explained by the biomass of rotifers, zooplanktivorous fish and TP (Fig. 6b). This SEM model did not reveal direct effects of TP and zooplanktivorous fish on mean phytoplankton size, but they acted indirectly through rotifer biomass, which had a significant, direct and positive effect on phytoplankton mean size ($R^2 = 0.15$, $P < 0.01$). Overall SEM results explained 15% of total variance in mean size (RMSEA = 0, $X^2 = 0.524$, $df = 3$, GFI = 0.98, CFI = 1 and SRMR = 0.061; Fig. 6b).

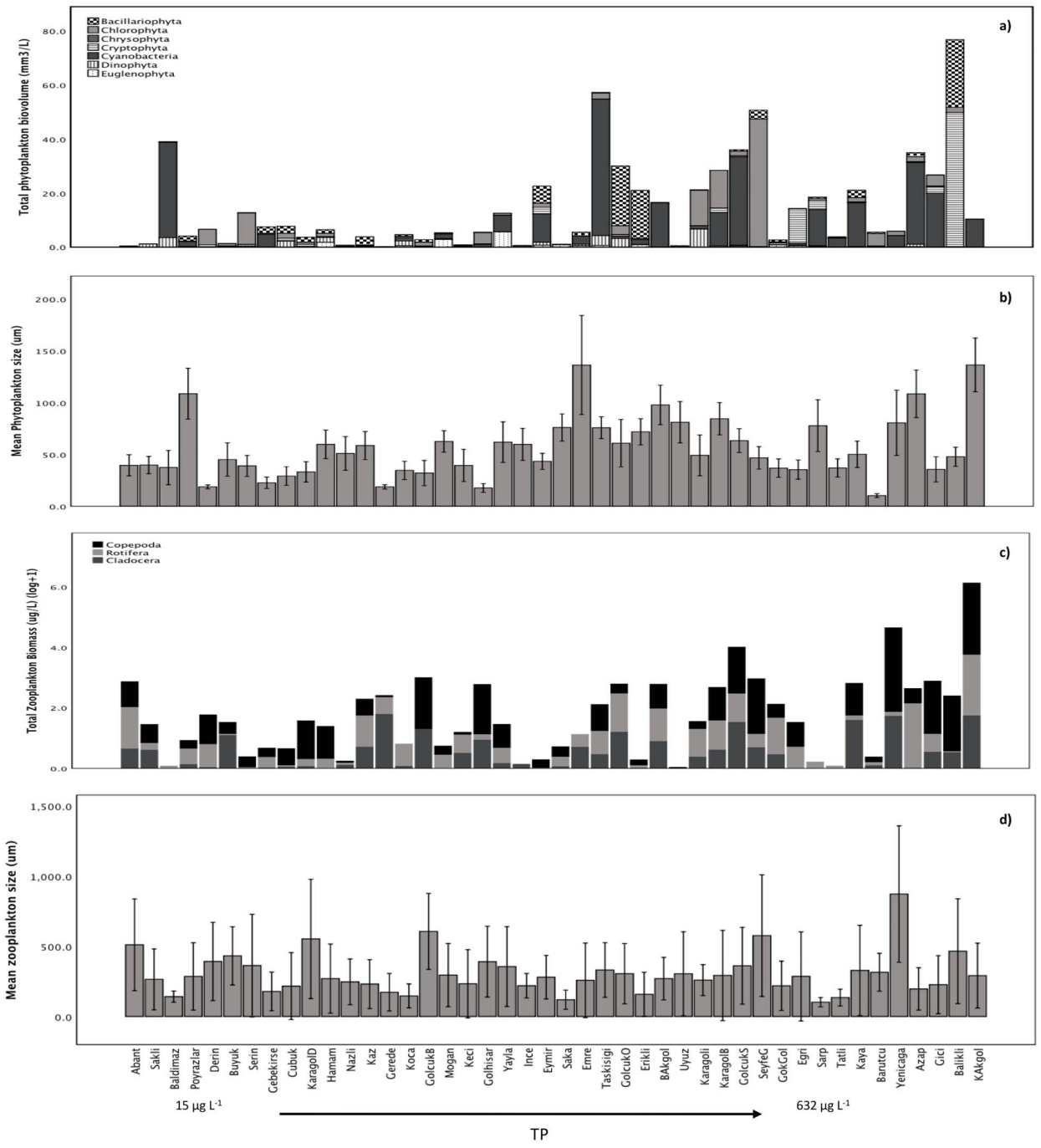

Fig. 3. (**a**) Total phytoplankton biovolume ($mm^3\ L^{-1}$) for each study site along the TP concentration gradient, increasing from left to right, (**b**) Mean phytoplankton size (µm) for each study site, (**c**) Total zooplankton biomass ($\mu g\ L^{-1}$) for each study site, (**d**) Mean zooplankton size (µm) for each study site.

SEM of phytoplankton size variance

The best SEM of variance in phytoplankton size included TSI, and altitude as predictors (Fig. 6c). Although TSI has a direct effect on size variance, altitude had an indirect effect ($R^2 = 0.32$, $P < 0.05$). Overall, our model explained 32% of variance in phytoplankton size variance (RMSEA = 0, $X^2 = 0.255$, $df = 1$, GFI = 0.996, CFI = 1 and SRMR = 0.025; Fig. 6c).

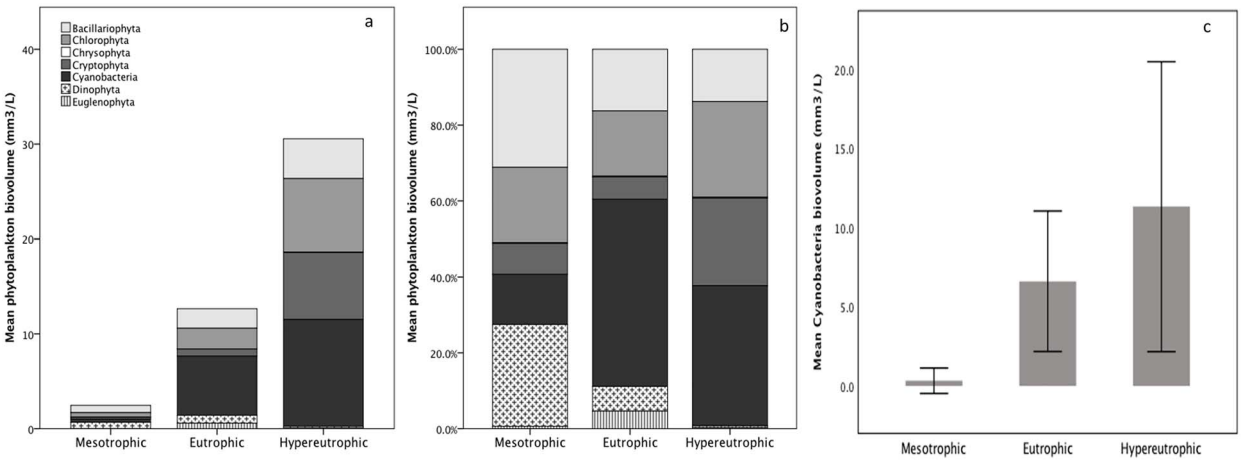

Fig. 4. Phytoplankton community composition in the study lakes grouped based on the total TSI index classification (**a**) mean phytoplankton biovolume (**b**) percent contribution of phytoplankton groups (**c**) Mean cyanobacteria biovolume in the study lakes.

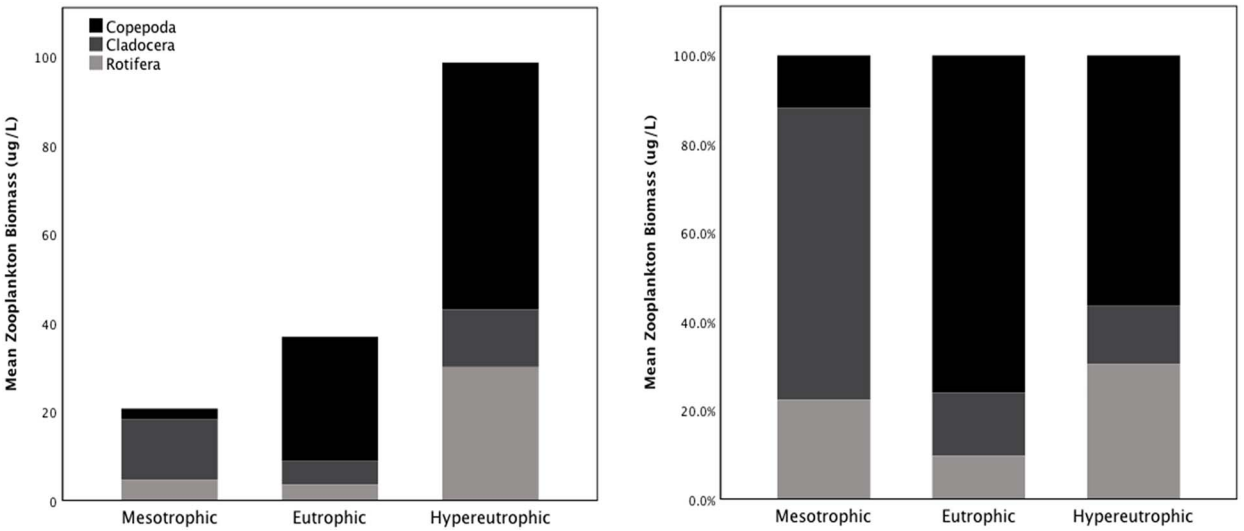

Fig. 5. Mean zooplankton biomass of major taxonomic groups in lakes based on the total TSI index classification (**a**) mean zooplankton biomass (**b**) percent contribution of zooplankton groups.

DISCUSSION

The results of SEM suggest that in warm lakes selective grazing by small-sized zooplankton, mainly rotifers, had a direct effect on phytoplankton mean size while TSI has a direct positive effect on variance in phytoplankton size. These results were in accordance with our first and second hypotheses.

The significant direct positive effect of rotifers on phytoplankton mean size (Fig. 6b) may be the result of rotifers' selective grazing pressure on small-sized phytoplankton. Owing to their small size, rotifers generally are not considered a potential phytoplankton size and biomass regulator. However, at high enough densities, rotifers may have a strong impact on phytoplankton biomass, especially for small-sized species (Lionard *et al*., 2005). A similar effect was observed by Wong *et al*. (2016). According to their results, average phytoplankton size tends to increase with eutrophication due to higher grazing pressure by micro-zooplankton (small rotifers and ciliates, smaller than 200 μm) on small phytoplankton species, eventually causing a proportional loss of 44 and 53% in small and medium phytoplankton species, respectively (Wong *et al*., 2016).

In our study, cyanobacteria species had the largest cells and their biomass was higher in eutrophic and hypereutrophic lakes (Fig. 4c and Supplementary Fig. S5). Rotifers can only graze on small organisms up to 5 μm

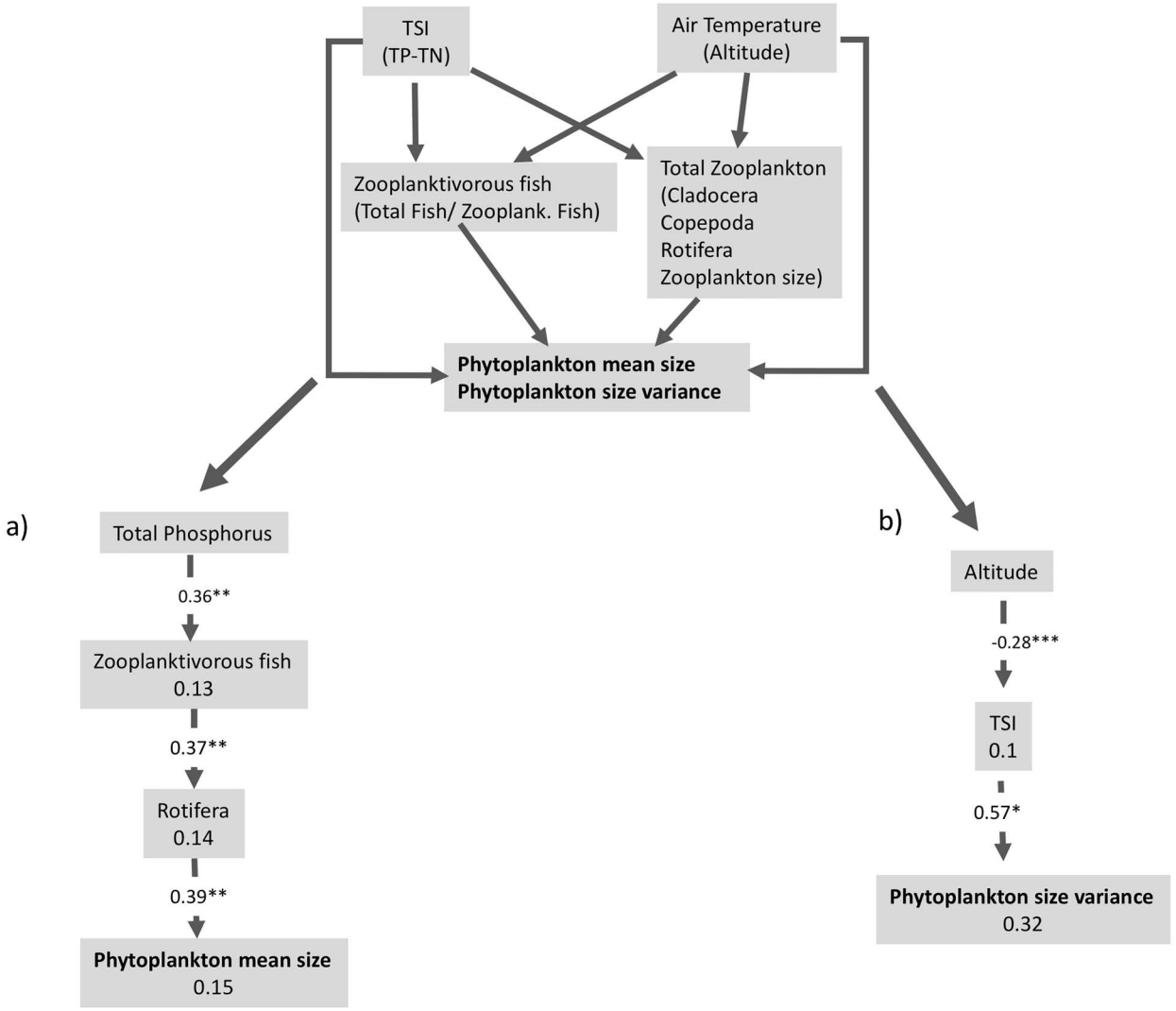

Fig. 6. Phytoplankton mean size and size variance SEM analysis results (**a**) initial SEM diagram with all variables from each group were tested individually, (**b**) Phytoplankton mean size SEM results, (**c**) phytoplankton size variance SEM results. Arrows represent casual positive relationship, coefficients and significance values are presented on arrow lines. R^2 values are given under variable names. $P < 0.05$*; 0.01**; 0.001***.

(Silvia *et al.*, 2019) and the abundance of potential rotifer prey was much lower than other phytoplankton groups in our study (Supplementary Fig. S4). The lack of small species could be a result of rotifers' grazing but we cannot rule out grazing by protozoa and other small zooplankters. Rotifers also graze on picoplankton (size class: < 2 μm), but our inverted microscope counts, though with certain limitations, indicated very few picoplankton cells (<%1 of total biovolume) (Crosbie *et al.*, 2003; Carrick *et al.*, 2017; Wei *et al.*, 2019; Supplementary Fig. S3). Rotifers could also have affected picoplankton biovolume, but our study was not designed to capture picoplankton and so a separate investigation needs to be conducted to understand picoplankton community response.

Our finding that TP and zooplanktivorous fish have indirect effects on phytoplankton mean size through increasing the small-sized grazer biomass, namely rotifers, is in accordance with other studies from warm eutrophic lakes. Other studies of warm and eutropic lakes have found zooplankton communities to be dominated by small-sized zooplankton species as a result of selective predation by small omnivorous fish on large-sized grazers (Meerhoff *et al.*, 2012; Frau *et al.*, 2015; Boll *et al.*, 2016).

TSI was the main determinant of phytoplankton size variance in our lakes (Fig. 6c). The increase in variance was likely driven by an increase in the percentage of large-sized phytoplankton species (such as filamentous or colonial cyanobacteria) that often have high temperature and nutrient requirements (Paerl and Huisman, 2008;

Kosten *et al*., 2012; Lürling *et al*., 2018). TSI values are calculated based on TP, Chl-*a* and Secchi-depth and so increased TSI is consistent with the increased nutrient needs of large-sized species. Consistent with this, we found a positive and significant correlation between TSI and cyanobacterial biovolume ($P < 0.05$). Cyanobacteria contribution was significantly higher in eutrophic and hypereutrophic lakes, compared to mesotrophic ones in our dataset (Fig. 4c).

The distribution of elevations in our study was bimodal with 13 lakes between 0 and 50 m classified as lowland and the other 31 lakes between 535 and 1423 m classified as highland (Fig. 1; see also Beklioğlu *et al.,* 2020). TSI was negatively affected by elevation according to the SEM analysis results for size variance (Fig. 6c), but of the three components of TSI, only SD depth differed significantly between highland and lowland lakes ($P < 0.01$; Supplementary Fig. S7). Temperature ($P < 0.05$) and total cyanobacteria biovolume ($P < 0.01$) also were significantly higher in lowland lakes (Supplementary Fig. S7), indicating that high temperature may promote cyanobacteria increase in lowland lakes. Furthermore, the interaction of temperature and nutrients may also promote cyanobacteria blooms more than expected (Elliot, 2012), either by a major increase in total biomass or by a proportional increase in certain taxa (Kosten *et al*., 2012).

Our results suggest that rotifers may have a significant impact on phytoplankton mean size. In addition, TSI had a positive effect on phytoplankton size variance. The explained variances were 0.15 (mean size) and 0.32 (size variance) in 2 SEM models. Two other studies using long-term data, found similar total variance explained by SEM, 0.40 (Pätynen *et al*., 2015) and 0.14 (Du *et al*., 2015) for phytoplankton and Chl-*a*, respectively.

It is important to note a few caveats. First, some of the samples were collected over a time period of 6 years which could introduce bias due to long term climate change. Second, we were constrained in our use of predictor variables by the relatively small number of lakes in our dataset (despite the tremendous sampling effort required). As a result, we may have oversimplified the actual ecological interactions. A larger dataset might explain more ecological interactions or increase explained variance percentages. Although our dataset has some limitations, our results highlight the sensitivity of cell size distributions to biotic and abiotic variables, such as nutrient levels and zooplankton grazing. Our results showed that SEM can be useful in understanding casual relationships in phytoplankton mean size and variance in mean size.

CONCLUSION

Our results imply that rotifer grazing and trophic state have significant impact on phytoplankton size and variance in size regulation respectively. The effect of rotifers on community size is likely through decreasing the prevalence of small cells susceptible to grazing. The effect of trophic state on variance is likely through increasing the abundance of filamentous and colonial species which require high nutrients. Our results highlight the sensitivity of cell size structure to environmental variables and suggest that trait-based approaches, using cell size in particular, can be a tool to assess ecological responses. Although SEM analyses are common in other disciplines (sociology, economics, etc.), their use in ecology, especially in freshwater ecology, is still relatively rare. Our study provides a new example of using SEM to detect cause–effect relationship among variables and the hierarchical effects of lake food web components on phytoplankton size and size variance. We encourage other freshwater ecologists to use this approach to improve our knowledge of complex ecological networks and interactions in freshwater systems.

SUPPLEMENTARY DATA

Supplementary data is available at *Journal of Plankton Research* online.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

REFERENCES

Acevedo-Trejos, E., Brandt, G., Bruggeman, J. and Merico, A. (2015) Mechanisms shaping size structure and functional diversity of phytoplankton communities in the ocean. *Sci. Rep.*, **5**, 8918.

Aksnes, D. and Egge, J. K. (2006) A theoretical model for nutrient uptake in phytoplankton. *Mar. Ecol. Prog. Ser.*, **70**, 65–72.

Anderson, J. C. and Gerbing, D. W. (1988) Structural equation modeling in practice: a review and recommended two-step approach. *Psychol. Bull.*, **103**, 411–423.

Pätynen, A., Kotamäki, N., Arvola, L., Tulonen, T. and Malve, O. (2015) Causal analysis of phytoplankton development in a small humic lake using structural equation modelling. *Inland Waters*, **5**, 231–239.

Beklioğlu, M., Bucak, T., Levi, E. E., Erdoğan, Ş., Özen, A., Filiz, N., Bezirci, G., Çakıroğlu, A. İ. *et al.* (2020) Influences of climate and nutrient enrichment on the multiple trophic levels of Turkish shallow lakes. *Inland Waters*, **10**, 173–185.

Boll, T., Levi, E. E., Bezirci, G., Özuluğ, M., Tavşanoğlu, Ü. N., Çakıroğlu, A. İ., Özcan, S., Brucet, S. *et al.* (2016) Fish assemblage and diversity in lakes of western and Central Turkey: role of geo-climatic and other environmental variables. *Hydrobiologia*, **771**, 31–44.

Bottrell, H., Duncan, A., Gliwicz, Z. M., Grygierek, E., Herzig, A., Hillbricht-Ilkowska, A., Kurasawa, H. and Larsson, P. (1976) A review of some problems in zooplankton production studies. *Norweg. J. Zool.*, **24**, 419–456.

Browne, M. W., Cudeck, R., Bollen, K. A. and Long, J. S. (1993) Alternative ways of assessing model fit. *Sociol. Methods Res.*, **154**, 136–136.

Çakıroğlu, A. İ., Levi, E. E., Tavşanoğlu, Ü. N., Bezirci, G., Erdoğan, Ş., Filiz, N., Andersen, T. J., Davidson, T. A. *et al.* (2016) Inferring past environmental changes in three Turkish lakes from sub-fossil Cladocera. *Hydrobiologia*, **778**, 295–312.

Canfield, D. E., Shireman, J. V., Colle, D. E., Haller, W. T., Watkins, C. E. and Maceina, M. J. (1984) Prediction of chlorophyll-a concentrations in Florida lakes: importance of aquatic macrophytes. *Can. J. Fish. Aquat.*, **41**, 497–501.

Cao, X., Wang, J., Liao, J., Gao, Z., Jiang, D., Sun, J., Zhao, L., Huang, Y. *et al.* (2017) Bacterioplankton community responses to key environmental variables in plateau freshwater lake ecosystems: a structural equation modeling and change point analysis. *Sci. Total Environ.*, **580**, 457–467.

Carlson, R. E. (1977) A trophic state index for lakes. *Limnol. Oceanogr.*, **22**, 361–369.

Carlson, R. E. and Simpson, J. (1996) *A Coordinator's Guide to Volunteer Lake Monitoring Methods*, North American Lake Management Society, Madison, WI, p. 96.

Carrick, H. J., Cafferty, E., Ilacqua, A., Pothoven, S. and Fahnenstiel, G. L. (2017) Seasonal abundance, biomass and morphological diversity of picoplankton in Lake superior: importance of water column mixing. *Int. J. Hydro.*, **1**, 187–197.

Chirico, N., António, D. C., Pozzoli, L., Marinov, D., Malagó, A., Sanseverino, I., Beghi, A., Genoni, P. *et al.* (2020) Cyanobacterial blooms in Lake Varese: analysis and characterization over ten years of observations. *Water*, **12**, 675.

Chisholm, S. W. (1992) Phytoplankton size. In Falkowski, P. G. and Woodhead, A. D. (eds.), *Primary Productivity and Biogeochemical Cycles in the Sea*, Plenum Press, New York, pp. 213–237.

Clark, J., Daines, S., Williams, H. and Lenton, T. (2013) Environmental selection and resource allocation determine spatial patterns in picophytoplankton cell size. *Limnol. Oceanogr.*, **58**, 1008–1022.

Colina, M., Calliari, D., Carballo, C. and Kruk, C. (2016) A trait-based approach to summarize zooplankton– phytoplankton interactions in freshwaters. *Hydrobiologia*, **767**, 221.

Cox, E. J. (1996) *Identification of Freshwater Diatoms from Live Material*, Chapman and Hall, London.

Crosbie, N. D., Teubner, K. and Weisse, T. (2003) Flow-cytometric mapping provides novel insights into the seasonal and vertical distributions of freshwater autotrophic picoplankton. *Aquat. Microb. Ecol.*, **33**, 53–66.

du, X., García-Berthou, E., Wang, Q., Liu, J., Zhang, T., Li, Z. *et al.* (2015) Analyzing the importance of top-down and bottom-up controls in food webs of Chinese lakes through structural equation modeling. *Aquat. Ecol.*, **49**, 199–210.

Dumont, H. J., DE Velde, I. and Dumont, S. (1975) The dry weight estimate of biomass in a selection of Cladocera, Copepoda and Rotifera from the plankton, periphyton and benthos of continental waters. *Oecologia*, **19**, 75–97.

Edwards, K. F., Klausmeier, C. A. and Litchman, E. (2011) Evidence for a three-way trade-off between nitrogen and phosphorus competitive abilities and cell size in phytoplankton. *Ecology*, **92**, 2085–2095.

Edwards, K. F., Thomas, M. K., Klausmeier, C. A. and Litchman, E. (2012) Allometric scaling and taxonomic variation in nutrient utilization traits and maximum growth rate of phytoplankton. *Limnol. Oceanogr.*, **57**, 554–566.

Elliot, A. (2012) Predicting the impact of changing nutrient load and temperature on the phytoplankton of England's largest lake, Windermere. *Freshw. Biol.*, **57**, 400–413.

Fan, Y., Chen, J., Shirkey, G., John, R., Wu, S. R., Park, H. and Shao, C. (2016) Applications of structural equation modeling (SEM) in ecological studies: an updated review. *Ecol. Process.*, **5**, 19.

Field, C., Behrenfeld, M., Randerson, J. and Falkowski, P. (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. *Science*, **281**, 237–240.

Frau, D., Battauz, Y. and Sinistro, R. (2017) Why predation is not a controlling factor of phytoplankton in a Neotropical shallow lake: a morpho-functional perspective. *Hydrobiologia*, **788**, 115–130.

Frau, D., Devercelli, M., José de Paggi, S., Scarabotti, P., Mayora, G., Battauz, Y. and Senn, M. (2015) Can top-down and bottom-up forces explain phytoplankton structure in a subtropical and shallow ground water connected lake? *Mar. Freshw.*, **66**, 1106–1115.

Ger, K. A., Urrutia-Cordero, P., Frost, P. C., Hansson, L.-A., Sarnelle, O., Wilson, A. E. and Lüring, M. (2016) The interaction between cyanobacteria and zooplankton in a more eutrophic world. *Harmful Algae*, **54**, 114.

Graham, L. E. and Wilcox, L. W. (2000) *Algae*, Prentice Hall, International Limited, London, UK, p. 640.

Grace, J. B. (2006) *Strucutral Equation Modeling and Natural Systems*, Cambridge University Press, New York.

Grace, J. B., Scheiner, S. M. and Jr Schoolmaster, D. R. (2015) Structural equation modeling: building and evaluating causal models. Chapter 8. In Fox, G. A., Negrete-Yankelevich, S. and Sosa, V. J. (eds.), *Ecological Statistics: from Principles to Applications*, Oxford Univ. Press, Oxford, U.K., pp. 169–200.

Havens, K. E., Ji, G., Beaver, J. R., Fulton, R. S. and Teacher, C. E. (2019) Dynamics of cyanobacteria blooms are linked to the hydrology of shallow Florida lakes and provide insight into possible impacts of climate change. *Hydrobiologia*, **829**, 43–59.

Hense, I. and Beckmann, A. (2006) Towards a model of cyanobacteria life cycle – effects of growing and resting stages on bloom formation of N2-fixing species. *Ecol. Model.*, **195**, 205–218.

Hillebrand, H., Dürselen, C., Kirschtel, D., Zohary, T. and Pollingher, U. (1999) Biovolume calculation for pelagic and benthic microalgae. *J. Phycol.*, **35**, 403–424.

Hooper, D., Coughlan, J. and Mullen, M. (2008) Structural equation modelling: guidelines for determining model fit. *Electron. J. Bus. Res. Methods*, **6**, 53–60.

Hulot, F. D., Lacroix, G. and Loreau, M. (2014) Differential responses of size-based functional groups to bottom–up and top–down perturbations in pelagic food webs: a meta-analysis. *Oikos*, **123**, 1291–1300.

Jeppesen, E., Jensen, J. P., Søndergaard, M., Lauridsen, T., Pedersen, L. J. and Jensen, L. (1997) Top-down control in freshwater lakes: the role of nutrient state, submerged macrophytes and water depth. *Hydrobiologia*, **342-343**, 151–164.

Jespersen, A. M. and Christoffersen, K. (1987) Measurements of chlorophyll-a from phytoplankton using ethanol as extraction solvent. *Archiv f ür Hydrobiologie*, **109**, 445–454.

John, D. M., Whitton, B. A. and Brook, A. J. (2002) *The Freshwater Algal Flora of the British Isles, An Identification Guide to Freshwater and Terrestrial Algae*, Cambridge University Press, Cambridge, pp. 433–468.

Karp-Boss, L., Boss, E. and Jumars, P. A. (1996) Nutrient fluxes to planktonic osmotrophs in the presence of fluid motion. *Oceanogr. Marine Biol.*, **34**, 71–107.

Kline, R. B. (2005) *Principles and Practice of Structural Equation Modeling*, 2nd edn, The Guilford Press, New York.

Komarek, J. and Anagnostidis, K. (1999) Cyanoprokaryota 1. Teil: Chroococcales. In Ettl, H., Gartner, G., Heynig, H. and Mollenhauer, D. (eds.), *Süsswasserflora von Mitteleuropa*, Spektrum Akademischer Verlag, Heidelberg, pp. 1–548.

Komarek, J. and Fott, B. (1983) Chlorococcales, 7. Teil. 1Halfte. In Elster, J. and Ohle, W. (eds.), *Das Phytoplankton des Süsswassers*, E. Schweizerbart'sche Verlagsbuchhandlung, Stuttgart, pp. 1–1043.

Kosten, S., Huszar, V. L. M., Bécares, E., Costa, L. S., VAN Donk, E., Hansson, L.-A., Jeppesen, E., Kruk, C. *et al.* (2012) Warmer climate boosts cyanobacterial dominance in lakes. *Glob. Chang. Biol.*, **18**, 118–126.

Kruk, C., Rodrıguez-Gallego, L., Meerhoff, M., Quintans, F., Lacerot, G., Mazzeo, N., Scassoö, F., Paggi, J. C. *et al.* (2009) Determinants of biodiversity in subtropical shallow lakes (Atlantic coast, Uruguay). *Freshwater Biol.*, **54**, 2628–2641.

Lampert, W. and Sommer, U. (2007) *Limnoecology*, Oxford University Press, New York, pp. 152–180.

Laughlin, D. C. and Grace, J. B. (2019) Discoveries and novel insights in ecology using structural equation modeling. *Ideas Ecol Evol*, **12**, 28–34.

Levi, E. E., Çakıroğlu, A. İ., Bucak, T., Odgaard, B. V., Davidson, T. A., Jeppesen, E. and Beklioğlu, M. (2014) Similarity between contemporary vegetation and plant remains in the surface sediment in Mediterranean lakes. *Freshwater Biol.*, **59**, 724–736.

Lionard, M., Azemar, F., Bouletreau, S., Muylaert, K., Tackx, M. and Vyverman, W. (2005) Grazing by meso- and micro- zooplankton on phytoplankton in the upper reaches of the Schelde estuary (Belgium/the Netherlands). *Estuar. Coast. Shelf Sci.*, **64**, 764–774.

Litchman, E. and Klausmeier, C. A. (2008) Trait-based community ecology of phytoplankton. *Annu. Rev. Ecol. Evol. Syst.*, **39**, 615–639.

Litchman, E., Klausmeier, C. A. and Yoshiyama, K. (2009) Contrasting size evolution in marine and freshwater diatoms. *Proc. Natl. Acad. Sci. U. S. A.*, **106**, 2665–2670.

Lürling, M., Mello, M., VAN Oosterhout, F., DE Senerpont Domis, L. and Marinho, M. M. (2018) Response of natural cyanobacteria and algae assemblages to a nutrient pulse and elevated temperature. *Front. Microbiol.*, **9**, 1851.

Mackereth, F. I. H., Heron, J. and Talling, J. F. (1978) *Water Analysis: Some Revised Methods for Limnologists*, Freshwater Biological Association, London.

Maliaka, V., Verstijnen, Y. J. M., Faassen, E. J., Smolders, A. J. P. and Lürling, M. (2020) Effects of guanotrophication and warming on the abundance of green algae, cyanobacteria and microcystins in Lake Lesser Prespa. *Greece. Plos One*, **15**, e0229148.

Malley, D. F., Lawrence, S. G., Maclver, M. A. and Findlay, W. J. (1989) *Range and Variation in Estimates of Dry Weight for Planktonic Crustacea and Rotifera from Temperate North American Lakes*, Canadian Technical Report of Fisheries and Aquatic Sciences, pp. 1–49.

Mao, Z., Gu, X., Cao, Y., Zhang, M., Zeng, Q., Chen, H., Shen, R. and Jeppesen, E. (2020) The role of top-down and bottom-up control for phytoplankton in a subtropical Shallow Eutrophic Lake: evidence based on Long-term monitoring and modeling. *Ecosystems*, **23**, 1449–1463.

Mantzouki, E., Campbell, J., Loon, E., Visser, P., Konstantinou, I., Antoniou, M., Giuliani, G., Vieira, D. M. *et al.* (2018) A European multi Lake survey dataset of environmental variables, phytoplankton pigments and cyanotoxins. *Sci. Data.*, **5**, 180226.

Maruyama, G. (1998) *Basics of Structural Equation Modeling*, Sage, Thousand Oaks, CA.

Matsuzaki, S. S., Suzuki, K., Kadoya, T., Nakagawa, M. and Takamura, N. (2018) Bottom-up linkages between primary production, zooplankton, and fish in a shallow, hypereutrophic lake. *Ecology*, **99**, 2025–2036.

McCauley, E. (1984) The estimation of the abundance and biomass of zooplankton in samples. In Downing, J. A. and Rigler, F. H. (eds.), *A Manual on Methods for the Assessment of Secondary Productivity in Freshwaters*, pp. 228–265.

Meerhoff, M., Teixeira-de Mello, F., Kruk, C., Alonso, C., González-Bergonzoni, I., Pacheco, J. P., Lacerot, G., Arim, M. *et al.* (2012) Environmental warming in Shallow Lakes: a review of potential changes in community structure as evidenced from space-for-time substitution approaches. *Adv. Ecol. Res.*, **46**, 259–349.

Michaloudi, E. (2005) Dry weights of the zooplankton of Lake Mikri Prespa (Macedonia, Greece). *Belg. J. Zool.*, **135**, 223–227.

Padisák, J., Soróczki-Pintér, É. and Rezner, Z. (2003) Sinking properties of some phytoplankton shapes and the relation of form resistance to morphological diversity of plankton – an experimental study. *Hydrobiologia*, **500**, 243–257.

Paerl, H. W. and Huisman, J. (2009) Climate change: a catalyst for global expansion of harmful cyanobacterial blooms. *Environ. Microbiol. Rep.*, **1**, 27–37.

Paerl, H. W. and Huisman, J. (2008) Blooms like it hot. *Science*, **320**, 57–58.

Paerl, H. W., Hall, N. S. and Calandrino, E. S. (2011) Controlling harmful cyanobacterial blooms in a world experiencing anthropogenic and climatic-induced change. *Sci.Total Envrion.*, **409**, 1739–1745.

Pančić, M. and Kiørboe, T. (2018) Phytoplankton defence mechanisms: traits and trade-offs. *Biol. Rev.*, **93**, 1269–1303.

Peel, M. C., Finlayson, B. L. and McMahon, T. A. (2007) Updated world map of the Köppen-Geiger climate classification. *Hydrol Earth Syst Sci Discuss*, **4**, 439–447.

Popovski, J., and Pfiester, L. A. (1990) *Dinophyceae (Dinoflagellida), Band 6*. In: Ettl H, Gerloff J, Heynig.

Prescott, G. W. (1973) *Algae of the Western Great Lakes Area*, 5th edn, William C. Brown of Dubuque Co., Iowa.

R Core Team (2015) *R: A Language and Environment for Statistical Computing*. R Foundation for Statistical Computing. Vienna, Austria. ISBN: 3-900051-07-0. http://www.R-project.org/

Reynolds, C. (2006) General index. In *The Ecology of Phytoplankton (Ecology, Biodiversity and Conservation)*, Cambridge University Press, Cambridge, pp. 524–535.

Ruttner-Kolisko, A. (1977) Suggestions for biomass calculations of plankton rotifers. *Arch Hydrobiol*, **8**, 71–76.

Sheridan, J. A. and Bickford, D. (2011) Shrinking body size as an ecological response to climate change. *Nat Clim. Change*, **1**, 401–406.

Silvia, J. V. F., Baumgartner, M. T., Miracle, M. R., Dias, J. D., Rodrigues, L. C. and Bonecker, C. (2019) Can zooplankton grazing affect the functional features of phytoplankton in subtropical shallow lakes? – experiment in situ in the south of Brazil. *Limnetica*, **38**, 773–785.

Stomp, M., Huisman, J., Mittelbach, G. G., Litchman, E. and Klausmeier, C. A. (2011) Large-scale biodiversity patterns in freshwater phytoplankton. *Ecology*, **92**, 2096–2107.

Tavşanoğlu, U. N., Maleki, R. and Akbulut, N. (2015) Effects of salinity on the zooplankton community structure in two maar lakes and one freshwater lake in the Konya closed basin, Turkey. *Ekoloji*, **24**, 25–32.

Thomas, M. K., Kremer, C. T. and Litchman, E. (2016) Environment and evolutionary history determine the global biogeography of phytoplankton temperature traits. *Glob. Ecol. Biogeogr.*, **25**, 75–86.

Thomas, M. K., Aranguren-Gassis, M., Kremer, C. T., Gould, M. R., Anderson, K., Klausmeier, C. A. and Litchman, E. (2017) Temperature–nutrient interactions exacerbate sensitivity to warming in phytoplankton. *Glob. Chang. Biol.*, **23**, 3269–3280.

Utermöhl, H. (1958) Zur Vervollkommnung der quantitativen phytoplankton. *Method Verh. Int, Ver. Limnol.*, **9**, 1–38.

Vadadi-Fülöp, C., Sipkay, C., Mészáros, G. and Hufnagel, L. (2012) Climate change and freshwater zooplankton: what does it boil down to? *Aquat. Ecol.*, **45**, 501–519.

Wang, G., Li, H., An, M., Ni, J., Ji, S. and Wang, J. (2011) A regional-scale consideration of the effects of species richness on above-ground biomass in temperate natural grasslands of China. *J. Veg. Sci.*, **22**, 414–424.

Wei, Y., Sun, J., Zhang, X., Wang, J. and Huang, K. (2019) Picophytoplankton size and biomass around equatorial eastern Indian Ocean. *Microbiol. Open.*, **8**, e629.

Weston, R. and Gore, P. A. (2006) A brief guide to structural equation Modeling. *Couns. Psychol.*, **34**, 719–751.

Wong, W. H., Rabalais, N. N. and Turner, R. E. (2016) Size-dependent top-down control on phytoplankton growth by microzooplankton in eutrophic lakes. *Hydrobiologia*, **763**, 97–108.

Wright, S. (1920) The relative importance of heredity and environment in determining the piebald pattern of Guinea-pigs. *Proc. Natl. Acad. Sci.*, **6**, 320–332.

Wright, S. (1921) Correlation and causation. *J. Agric. Res.*, **20**, 557–585.