

The effect of salinity and water density on phytoplankton size*

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Abstract Phytoplankton size and morphology are key traits influencing their ecological roles, buoyancy, and susceptibility to grazing. We investigated how water density, driven by salinity and temperature, affects phytoplankton size structure in two systems: (1) a observational study in a saline (~14) meromictic Lake Shira (Russia), characterized by seasonal water column stratification; and (2) the gradient experiment at the METU Mesocosm System II (Türkiye) with a large salinity gradient (0.5–50). In Lake Shira, while the overall phytoplankton community showed no consistent size variations across seasons or depth zones, colonies of the cyanobacterium *Microcystis* and filaments of cyanobacterium *Planktolyngbya contorta* exhibited larger area based diameters in denser, more saline deep waters compared to the less dense epilimnion in summer and fall and a positive response of the area-based diameter to the increase in water viscosity. In the system of 5 000-L mesocosms, phytoplankton average diameter increased with salinity, likely compensating for reduced sinking velocity in denser water. These findings suggest that water density influences phytoplankton size, particularly in species with colonial forms; however, ecological implications for food web dynamics remain uncertain. Our study highlights the complex interplay of physical and biological factors shaping phytoplankton size structure in saline ecosystems.

Keyword: colonial phytoplankton; cyanobacteria; meromictic lake; mesocosm experiment; buoyancy

1 INTRODUCTION

The basis of food webs in the pelagic zone of lake ecosystems is comprised of diverse phytoplankton varying in shape and species composition. These organisms, suspended in the water column and capable of photosynthesis, play a crucial role in producing oxygen and converting

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dissolved minerals into organic matter, which is then transferred through the food web to zooplankton and fish. Since phytoplankton requires light for photosynthesis, one of its primary challenges is to remain suspended within the photic zone (Zohary et al., 2017). Additionally, they must develop strategies to protect themselves from predation, which directly shapes their size and morphology through processes like zooplankton grazing (Lüring, 2021; Eyring et al., 2025). Consequently, the size and morphology of phytoplankton cells and colonies exhibit significant diversity, influenced by seasonal changes, lake mixing regimes, water chemistry, nutrient availability, the presence and size of predators, and other environmental factors.

The size and morphology of phytoplankton are critical characteristics elucidating its ecological functions, patterns associated with growth, abundance, reproduction, resource consumption (including nutrients and light), predator avoidance, and even species diversity (Litchman and Klausmeier, 2008). While phytoplankton size responses to temperature and nutrients are well-documented (e.g., Reynolds, 2006; Mara  n, 2015; Hillebrand et al., 2022), the direct role of salinity-mediated water density remains poorly understood, particularly in inland systems. Furthermore, the interactive effects of salinity and zooplankton grazing pressure on phytoplankton size structure require further investigation (e.g., Christensen et al., 2023). A critical and unresolved challenge is to disentangle the direct, physical effects of salinity (via changes in water density and viscosity) from the co-varying and often dominant effects of temperature on phytoplankton size, both in natural and experimental settings.

Numerous studies have explored the effects of various environmental factors on the size characteristics of phytoplankton. Water density and viscosity, which are significantly affected by both temperature and salinity, directly influence buoyancy and settling rates. Larger particles are more likely to remain suspended in denser water, as demonstrated by observations from Lake Kinneret (Israel), where specific phytoplankton species exhibited larger sizes at lower temperatures (indicating denser water) than at higher temperatures (Zohary et al., 2017). According to Bergmann's rule, the size of organisms tends to decrease with increasing temperature (Blackburn et al., 1999; Watt et al., 2010). Generally, freshwater phytoplankton exhibits a trend toward smaller sizes with warming, consistent with this rule (Zohary et al., 2021).

However, there are exceptions, often attributable to indirect effects of temperature on phytoplankton size through mechanisms such as zooplankton grazing and nutrient availability (e.g., Pomati et al., 2020).

Phytoplankton size is also influenced by water turbulence (Estrada and Berdalet, 1998), which regulates access to resources. The morphological variation observed in phytoplankton reflects evolutionary adaptation to variability in turbulence (Naselli-Flores et al., 2021). Critically, density and viscosity are significantly affected by salinity. It can be hypothesized that increasing salinity leads to greater water density, thereby reducing sinking losses and potentially favoring larger phytoplankton sizes. The impact of rising salinity on aquatic food webs has emerged as a critical concern due to alarming trends in freshwater salinization (Cunillera-Montcus   et al., 2022). However, there is a notable lack of studies focusing on freshwater lakes undergoing salinization, and limited data are available to isolate the direct effects of salinity-mediated changes in viscosity and density on phytoplankton size from the pervasive and often stronger effects of temperature, particularly in complex, stratified natural systems (Naselli-Flores et al., 2021).

In this study, we performed a secondary analysis on data from two independent sources to test the hypothesis linking salinity-mediated density to phytoplankton size structure. First, we utilized long-term monitoring data from a natural system: the deep, stratified saline meromictic Lake Shira. In such lakes, the water column is permanently stratified into layers of varying density due to salinity and temperature differences, creating a natural gradient where phytoplankton communities are exposed to distinct salinity/density conditions. This provides a real-world scenario but one where salinity and temperature effects are inherently confounded, making isolation of the single physical factor of salinity challenging. Second, we analyzed data from a pre-existing controlled gradient experiment using outdoor mesocosms (0–50 salinity) at the METU Mesocosm System II (Ankara, T  rkiye), which was originally designed for other research purposes (e.g.,   zkan et al., 2023). This experiment, with minimal temperature variation across the gradient, offers a stronger test of the isolated effect of salinity. Neither the lake monitoring nor the mesocosm experiment was originally performed to test the specific hypothesis

of this study. However, by analyzing data from both—a complex natural gradient and a controlled salinity manipulation—we can robustly assess the relationship between salinity, water density, and phytoplankton size across different scales and system complexities.

We hypothesized that in both systems, the smallest phytoplankton sizes would be found in water with the lowest salinity and water density, and that this effect would be observable alongside the structuring role of zooplankton.

2 MATERIAL AND METHOD

2.1 Phytoplankton size structure in saline Lake Shira

Data on the size structure of phytoplankton in saline meromictic Lake Shira (southern Siberia, Russia) were collected through seasonal water sampling in winter (early March, under ice), spring (mid-May, two weeks after ice-off), summer (late July, during thermal stratification), and autumn (mid-October, during mixolimnion mixing) of the 2009 monitoring period. Sampling was conducted at a single, long-term reference site at the lake's center (approximately 54°30'N, 90°11'E), over the deepest point (24 m) (Rogozin et al., 2017). Sampling frequency was once per season, with water collected at 1-m intervals from the surface to the bottom using a Ruttner sampler.

Lake Shira is a brackish (salinity ~14), meromictic lake with elevated sulfate and chloride concentrations. Its phytoplankton community is characterized by deep water chlorophyll maxima during summer and low diversity, with the filamentous cyanobacterium *Planktolyngbya contorta* (Lemmermann) Anagnostidis & Komárek, the green alga *Hindakia tetrachotoma* (Printz) C. Bock, Pröschold & Krienitz, and the colonial cyanobacterium *Microcystis* sp. being dominant (Zotina et al., 1999).

Samples were pre-filtered through an 80- μ m mesh to prevent clogging of the FlowCam flow cell. This size cutoff was a technical constraint of the FlowCam system and the chosen flow cell, and it assumes that colonial or filamentous particles larger than 80 μ m were either rare in Lake Shira's pelagic zone or that their contribution to total particle count and biovolume in the analyzed size range (2–50 μ m) was minimal. Subsequent analysis supported this, as particles >15 μ m were found to be rare in the processed samples. Non-fixed samples were stored

in a dark, cold place for not more than 2 d. After that, they were gently shaken to resuspend the sample, and the phytoplankton particles were subsequently analyzed using a FlowCam flow cytometer (FluidImaging Inc., USA) at 20 $\times$ magnification with a 50- μ m flow cell. For each sample, a volume of 0.2–0.5 mL (adjusted to capture at least 100 particles) was processed. The lower detection limit was set at 2 μ m of the area-based diameter (ABD) (the practical resolution limit at 20 $\times$), and the upper limit was set at 50 μ m (ABD). To capture images of entire colonies rather than individual cells, we used 10 μ m as the “distance to nearest neighbor” in the FlowCam particle capture algorithm. This means that if the distance between two particles is less than 10 μ m, they will be captured and analyzed as a single particle (see Supplementary Fig.S1 for examples of detected images). All captured images were visually inspected by a technician to remove obvious non-phytoplankton debris (e.g., mineral particles, large detritus). For the key genus *Microcystis* and *Planktolyngbya*, image libraries were manually reviewed by a phytoplankton taxonomist to identify and isolate colonies and filaments from other particles, ensuring accurate classification (see Supplementary Fig.S2 for examples of *Microcystis* and *Planktolyngbya* images separated from the entire phytoplankton dataset). Due to the non-standardized nature of manual image sorting, this procedure was limited to removing clear debris and performing targeted taxonomic isolation (*Microcystis* and *Planktolyngbya*); it was not a complete taxonomic verification of all images.

The obtained phytoplankton image libraries were analyzed using the built-in VisualSpreadsheet Color 20x software (2008) to determine the average size of the phytoplankton. Even with the 50- μ m upper limit, particles with an ABD >15 μ m were quantitatively rare in the dataset. To avoid having the mean particle size metrics be disproportionately influenced by a few, potentially stochastic, large particles, we excluded all particles with an ABD >15 μ m from the final calculation of community mean size. ABD is based on the diameter of the circle formed by all dark pixels that are segmented from the image for a single particle and crunched into a compact circle. ABD is fundamentally different from equivalent spherical diameter (ESD). The software first finds the particle's geometric center, then measures its width in 10-degree increments, and finally averages these values to

compute the ESD. ESD is closer to the diameter determined visually under the microscope, while ABD is a much better proxy of biovolume (and biomass) of plankton particles (Hrycik et al., 2019) (see Supplementary Table S1 with the example images of *P. contorta* and *Microcystis* sp. captured by FlowCam with the values of ABD and ESD). We acknowledge that this exclusion could underrepresent the response of larger taxa (e.g., large filaments or colonies) to salinity, which is a limitation of the method. The error and uncertainty associated with FlowCam size measurements are recognized; the instrument's automated size calculations (based on pixel area) can differ from true geometric dimensions. However, traditional manual microscopy for deriving mean community particle size is prohibitively labor-intensive and also subject to human error. Samples were analyzed once without replicate subsamples due to sample volume constraints, which adds to the uncertainty of the estimates. Sensitivity analyses on the effect of the 15- μm cutoff were not performed.

To identify hydrodynamically homogeneous zones, we analyzed vertical profiles of temperature, salinity, and redox potential measured with a YSI 6600 multiparameter probe (YSI Inc., USA). The chemocline (boundary between mixolimnion and monimolimnion) was defined as the depth where redox potential shifted from positive to negative. The thermocline was defined as the depth where the temperature gradient (Δt) exceeded 1- $^{\circ}\text{C}/\text{m}$, separating the epilimnion ($\Delta t < 1^{\circ}\text{C}/\text{m}$) from the hypolimnion (Zadereev and Tolomeyev, 2007).

2.2 Size structure of phytoplankton along a salinity gradient

Sampling was conducted under the EU AQUACOSM-plus project at the METU Mesocosm System II facility on November 6–7, 2021. The experiment used 16 cylindrical polyethylene mesocosms (volume: 5 000 L, depth: 1.8 m) with established plankton communities exposed to a salinity gradient (0, 2, 4, 6, 8, 10, 12, 14, 17, 20, 23, 26, 30, 35, 40, and 50). To create different salinity levels a NaCl- Na_2SO_4 mixture in a seawater Cl: SO_4 ratio was used. This experiment took place in a larger experiment performed between 9 September 2021 and 13 May 2022 (Özkan et al., 2023). Water was mixed using a submerged pump. We collected one integrated sample per mesocosm, which was pre-filtered (80- μm mesh) and fixed with Lugol's solution for later analysis.

Phytoplankton in these fixed samples was analyzed using a FlowCam8000 at 20 $\times$ magnification (50- μm flow cell) in AutoImage mode. Detection limits, sample volume processing, and the procedure for image review and exclusion of particles >15 μm were identical to the methods described for Lake Shira (see Supplementary Fig.S3 for example images of phytoplankton from METU mesocosms).

2.3 Calculation of water density and particle sedimentation velocity

For each zone/season in Lake Shira and for each mesocosm, the sinking velocity (V_s) of an average phytoplankton particle was estimated using Stokes' law:

$$V_s = \frac{2}{9} g r^2 \frac{(\rho' - \rho)}{\mu}, \quad (1)$$

where V_s is the sinking velocity of the particle, g is the acceleration of the falling surface (value), r is the radius of the particle, $\rho' = 1.15 \text{ g}/\text{cm}^3$ is the density of the sinking phytoplankton particle (Naselli-Flores et al., 2021), ρ is the density of water, μ is the viscosity coefficient of water.

We also calculated sinking velocity for a 10- μm particle for each zone/season in Lake Shira and for each mesocosm. The choice of a 10- μm particle as a baseline is a standardized reference to compare the physical effect of water density/viscosity changes across conditions, isolating it from concurrent changes in biological size.

Water density (ρ) and viscosity (μ) were calculated from measured temperature and electrical conductivity (Table 1) using the online Hamza's Reef Water Properties Calculator.

2.4 Statistical analysis

Differences in calculated sinking velocities and mean particle diameters between lake zones and seasons were tested using factorial ANOVA (factors: season, zone) in STATISTICA 8.0. To analyze the effects of environmental drivers (temperature, salinity, water density, viscosity) on particle size across both datasets, we used Generalized Additive Models (GAMs). GAMs were chosen over linear models to accommodate potential non-linear responses, which were anticipated given the complex physiological and physical interactions involved. The GAMs were fitted using the "mgcv" package in R, with Gaussian or Gamma families and identity or log link

Table 1 Seasonal average values ($\pm$ standard deviation) of temperature (T) and electrical conductivity (Cond) in different hydrodynamically isolated zones of Lake Shira and in the METU mesocosms

Lake Shira sampling						
Season \ Zone	Epilimnion		Hypolimnion		Monimolimnion	
	T ($^{\circ}\text{C}$)	Cond (mS/cm)	T ($^{\circ}\text{C}$)	Cond (mS/cm)	T ($^{\circ}\text{C}$)	Cond (mS/cm)
Winter	1.4 $\pm$ 0.8	20.7 $\pm$ 0.1			0.8 $\pm$ 1.5	20.8 $\pm$ 0.2
Spring	9.6 $\pm$ 0.6	17.9 $\pm$ 0.2	2.1 $\pm$ 0.5	20.1 $\pm$ 0.2	0.8 $\pm$ 0.8	20.9 $\pm$ 0.2
Summer	19.7 $\pm$ 0.7	17.7 $\pm$ 0.2	5.8 $\pm$ 0.5	20.1 $\pm$ 0.2	1.4 $\pm$ 0.7	20.9 $\pm$ 0.2
Autumn	7.6 $\pm$ 0.6	18.7 $\pm$ 0.2			1.4 $\pm$ 0.7	21.1 $\pm$ 0.2

Mesocosm sampling			Mesocosm sampling		
Salinity	T ($^{\circ}\text{C}$)	Cond (mS/cm)	Salinity	T ($^{\circ}\text{C}$)	Cond (mS/cm)
0	11.6	1.2	17	11.6	27.9
2	11.6	3.9	20	11.8	32.3
4	11.7	7.4	23	12.0	36.6
6	11.8	10.7	26	11.8	41.1
8	11.9	13.6	30	11.4	47.1
10	11.8	17.0	35	11.9	53.4
12	11.7	20.1	40	11.9	60.7
14	11.8	23.5	50	11.8	73.5

Conductivity units are milliSiemens per centimeter (mS/cm).

functions. Model selection was based on the lowest Akaike Information Criterion (AIC) values (R package “mgcv”; Wood, 2017).

3 RESULT

3.1 Seasonal stratification of the water column in Lake Shira and associated differences in phytoplankton particle sinking velocities

To describe the structure of meromictic Lake Shira, we define two overlapping stratification schemes: thermal (epilimnion/hypolimnion) and chemical/density-based (mixolimnion/monimolimnion). The mixolimnion is the upper, periodically mixed layer, which can be thermally subdivided into an epilimnion (warm, well-mixed surface layer) and, when present, a hypolimnion (cooler, deeper layer below the thermocline but still above the chemocline). The monimolimnion is the deep, denser, permanently stratified layer isolated by a strong chemocline (salinity/density gradient).

In winter, under ice cover, the water column was divided into a mixolimnion and a more saline monimolimnion (no distinct thermocline). As spring arrived, thermal stratification developed within the mixolimnion, creating three distinct hydrodynamically stable zones: (1) a warm, freshened epilimnion; (2) a

cooler, more saline hypolimnion below the seasonal thermocline; and (3) the high-salinity monimolimnion near the lake bottom. During summer, thermal stratification intensified, narrowing the hypolimnion and increasing density contrasts. In fall, with surface cooling, the mixolimnion became thermally mixed again, simplifying the structure back to two zones: a mixolimnion (equivalent to the epilimnion) and the monimolimnion (Fig.1).

The calculated sinking velocity for a standardized 10- μm particle was highest in the epilimnion across all seasons except winter, showing significant differences between zones (Fig.2). In winter, sinking velocities were similar in the mixolimnion and monimolimnion. The highest sinking velocity occurred in the summer epilimnion, corresponding to the period of lowest surface water density (warm, lower salinity).

3.2 Size structure of phytoplankton in Lake Shira

The seasonal dynamics of the phytoplankton community in Lake Shira were typical for this lake (Fig.3). In all seasons, the phytoplankton was dominated both in numbers (50% out of the total phytoplankton particle numbers) and biovolume (60% out of the total phytoplankton biovolume) by two cyanobacteria: filamentous *P. contorta*. and colonial

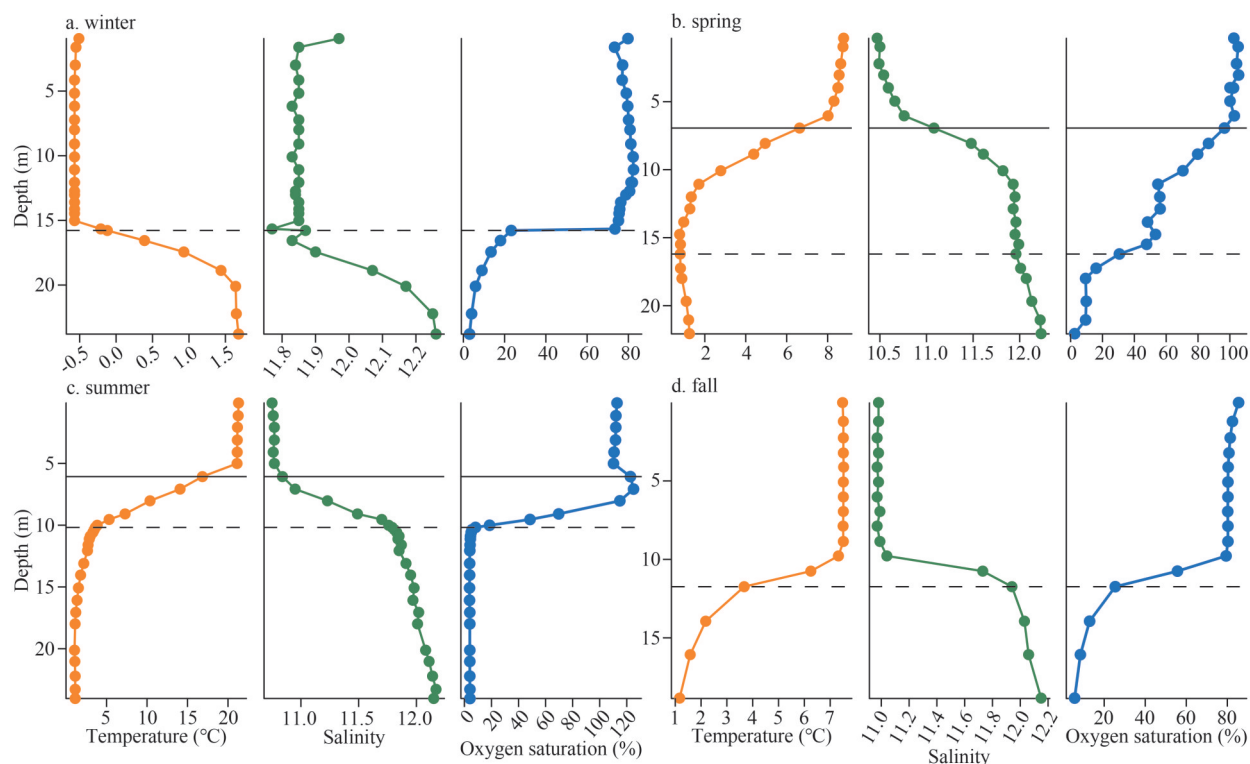


Fig.1 Typical vertical distributions of temperature (orange line, °C), oxygen saturation (blue line, %), and salinity (green line) and the depths of the thermocline (horizontal gray line) and chemocline (horizontal dashed gray line) in the pelagic zone of Lake Shira in different seasons

In winter and fall, the chemocline depth is shown; a distinct thermocline was absent as the mixolimnion was thermally homogeneous. Note that x-axes for temperature are season-specific to show details.

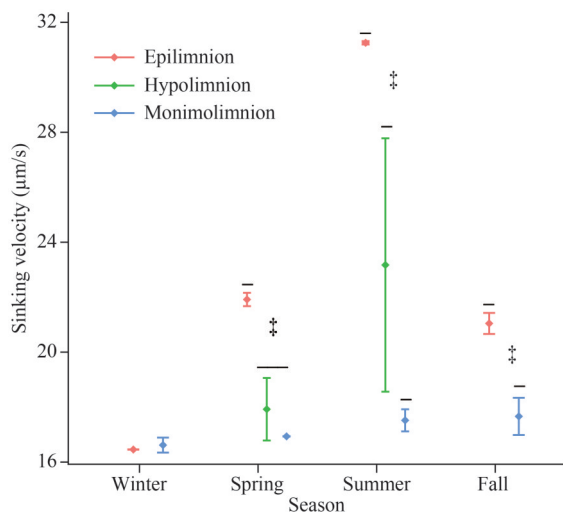


Fig.2 Sinking velocity of 10- μm diameter particles in different seasons within the hydrodynamically stable zones of Lake Shira

‡: seasonal sinking velocities in zones connected by horizontal black lines differ significantly ($P < 0.01$, post hoc Tukey HSD test).

Microcystis sp. The abundance and bio volume of phytoplankton in the epilimnion were relatively

stable across seasons (7 000–11 000 particles/mL and $(5\text{--}8) \times 10^5 \mu\text{m}^3/\text{mL}$, respectively). The maximal numbers and biovolume of phytoplankton were observed in July (summer) with the deep water maximum located in the hypolimnion (up to 21 000 particles/mL and $16 \times 10^5 \mu\text{m}^3/\text{mL}$, respectively). High numbers (19 000 particles/mL) and biovolume ($24 \times 10^5 \mu\text{m}^3/\text{mL}$) of phytoplankton were observed in deep monimolimnion in October (autumn) that is settled biomass accumulated over the growing season (Fig.3).

Analysis of the entire phytoplankton community (2–15- μm ABD) revealed no significant differences in average ABD of particles across zones in spring and summer (Fig.4a). In winter the deep monimolimnetic phytoplankton had smaller ABD than the epilimnetic phytoplankton, while in fall the ABD of monimolimnetic phytoplankton was larger than that of the epilimnetic phytoplankton (Fig.4a). At the same time water viscosity showed no relationship with this community-wide average ABD (Fig.4b).

The average ABD of colonial cyanobacterium

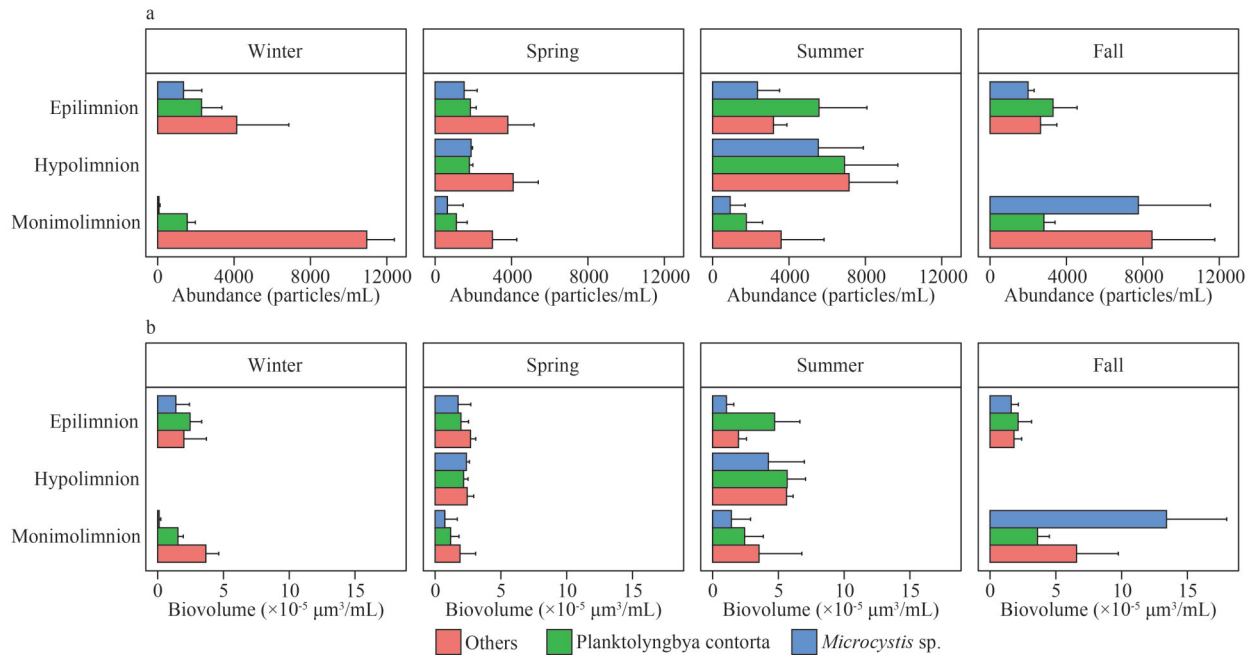


Fig.3 Seasonal dynamics and depth distribution of phytoplankton in Lake Shira

Microcystis and filamentous cyanobacterium *Planktolyngbya* also showed no differences across hydrodynamically stable zones in winter and spring (Fig.4c, e). The average colony ABD of the cyanobacterium *Microcystis* and filamentous cyanobacterium *Planktolyngbya* showed systematic differences, being significantly larger in the denser monimolimnion compared to the epilimnion during summer and fall (Fig.4c, e). Water viscosity showed clear relationship with average ABD of colonial cyanobacterium *Microcystis* and filamentous cyanobacterium *Planktolyngbya* (Fig.4d, f).

The size distribution of *Microcystis* colonies shifted significantly between these zones across all seasons except spring, with a higher proportion of colonies $>8\ \mu\text{m}$ in the monimolimnion (Fig.5a). Seasonally, the summer epilimnion had the highest proportion of very small colonies ($<4\ \mu\text{m}$) (Fig.5b).

3.3 Size structure of phytoplankton in the METU Mesocosm System II salinity gradient experiment

Phytoplankton abundance and biovolume were highest at the salinity extremes (0.5, 2, and 50) (Fig.6a, b). Salinity had a significant positive effect on the average diameter of all phytoplankton particles (2–15 μm), with a ~ 1.5 -fold increase from ~ 4 to $\sim 6\ \mu\text{m}$ across the gradient (Fig.6c). This shift was driven by a significant decrease in the proportion of 2–6- μm particles ($r=-0.60$, $P=0.014$) and an increase in 7–10- μm particles ($r=0.61$, $P=$

0.011) with rising salinity (Fig.6d, e).

The calculated sinking velocity for a standardized 10- μm particle decreased strongly with increasing salinity ($r=-0.99$, $P<0.001$, Fig.7a), demonstrating the physical effect of increased water density and viscosity. However, when sinking velocity was calculated for particles of the actual mean diameter observed in each mesocosm, there was no significant correlation with salinity ($r=0.29$, $P=0.27$, Fig.7b). This suggests the biological response (size increase) may offset the physical effect on sinking for the dominant phytoplankton.

4 DISCUSSION

4.1 Integrating lake and mesocosm results on phytoplankton size and salinity

Our study employed complementary approaches—a natural saline meromictic lake (Lake Shira) and a controlled mesocosm salinity gradient (the METU Mesocosm System II)—to investigate how salinity-mediated water density affects phytoplankton size structure. In the natural system (Lake Shira), we observed a significant, viscosity-dependent size response in the colonial cyanobacterium *Microcystis* and filamentous cyanobacterium *P. contorta*, but no community-wide signal. In the controlled mesocosm salinity gradient, we documented a clear, positive relationship between salinity and the average size of the entire phytoplankton community. This integration

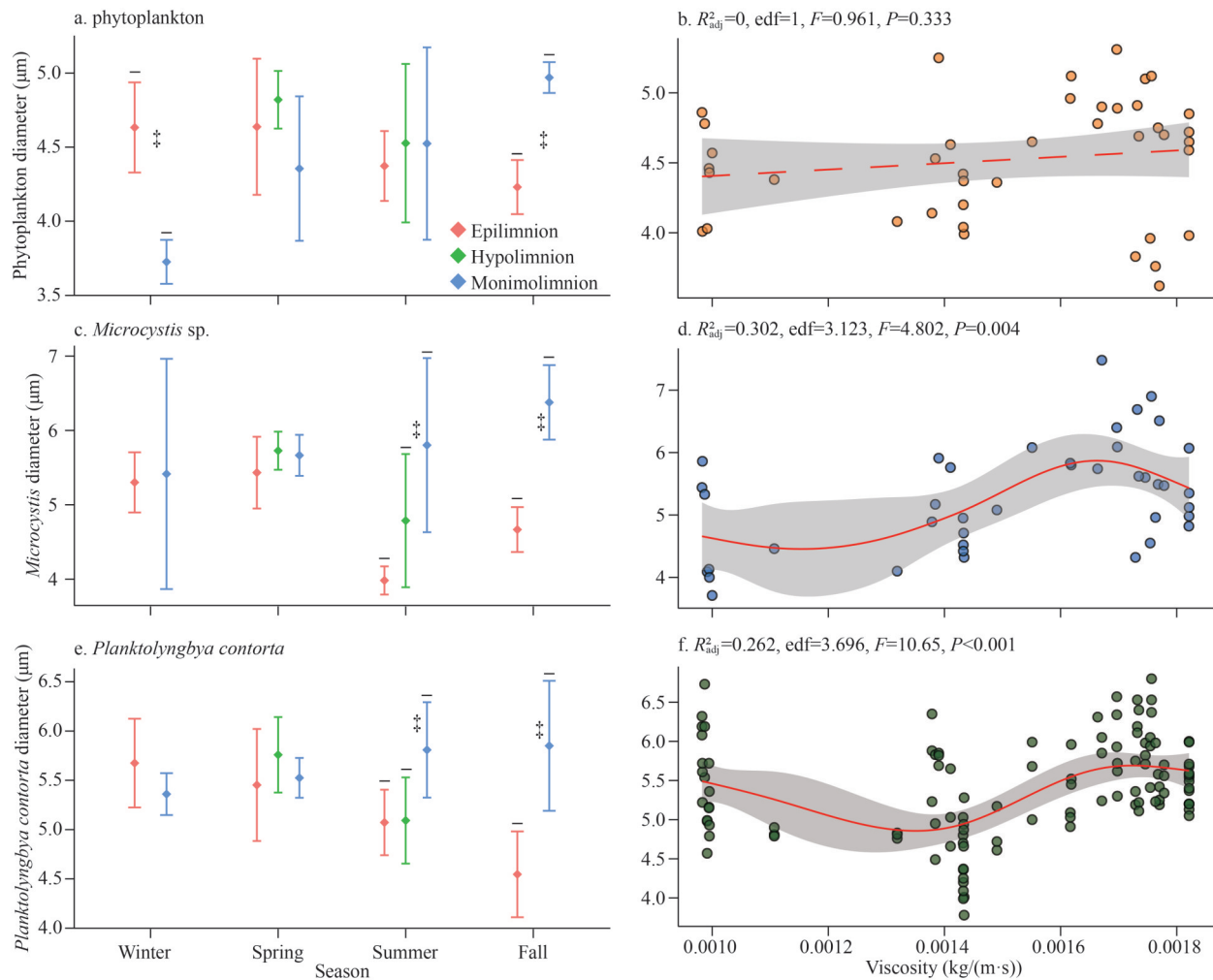


Fig.4 Average ABD of all phytoplankton particles (2–15 μm) (a), *Microcystis* colonies (c), and *P. contorta* filaments (e) in different seasons within hydrodynamically stable zones of Lake Shira; relationship between water viscosity and the ABD of all phytoplankton particles (b), *Microcystis* colonies (d), and *P. contorta* filaments (f)

†: ABD in zones connected by horizontal black lines differ significantly ($P<0.05$, Post hoc Tukey HSD test). The GAM family gaussian (identity link function) was used for (c–d). edf : effective degrees of freedom.

reveals that while a direct physiological or biophysical response to water density exists, its expression at the ecosystem level is often moderated or obscured in natural settings by concurrent factors like thermal stratification, species interactions, and grazing.

4.1.1 Phytoplankton size in a stratified saline lake

Analysis of Lake Shira demonstrated that the size of *Microcystis* colonies was inversely related to the calculated sinking velocity in different water layers. The smallest colonies were found in the summer epilimnion, the zone with the lowest water density and highest potential sinking loss. This supports the hypothesis that buoyancy regulation is a key driver for this genus, with larger colony size potentially conferring an advantage in denser, more viscous

waters by reducing sinking rates. The mechanistic link may involve osmotic regulation and cell turgor pressure, as noted in some studies (e.g., Farkas et al., 2023). The size of *P. contorta* filaments was also positively affected by the increased water viscosity but the effect of water viscosity on the ABD was less pronounced than for the *Microcystis*.

At the same time, we found no consistent differences in the average size of the entire phytoplankton community (2–15 μm) across seasons or density gradients. These findings contradict those of a previous theoretical study by Prokopkin et al. (2010), who demonstrated that sedimentation (directly related to the viscosity of the water) is one of the main factors shaping the vertical distribution of phytoplankton in Lake Shira. This divergent

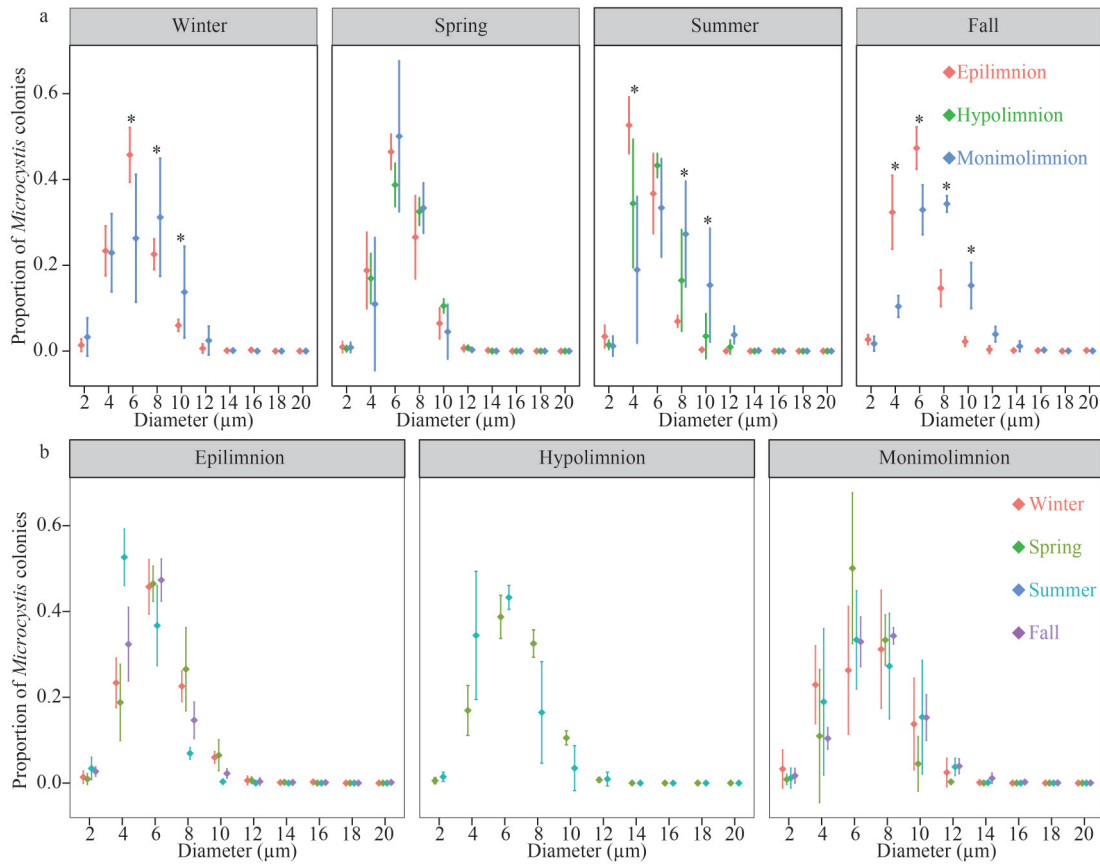


Fig.5 Size distribution of *Microcystis* colonies in Lake Shira between the epilimnion and monimolimnion across seasons (a), and across different seasons (b)

*: proportions differ significantly ($P < 0.05$, ANOVA post hoc test).

outcome likely stems from several factors. First, and most critically, Lake Shira's stratification tightly couples changes in salinity with strong thermal gradients. The warmer, fresher epilimnion and the colder, saltier monimolimnion present a scenario where the opposing physical effects of temperature (decreasing density) and salinity (increasing density) on water viscosity are in tension, and the biological response to temperature (often smaller size) may counteract or mask any response to salinity alone. This confounding is a fundamental limitation for isolating salinity effects in stratified natural systems. Future work using in vitro density manipulations might resolve this limitation. Second, the observed *Microcystis* and *P. contorta* signal may be diluted in the community average by other taxa responding differently or not at all. Third, we cannot rule out that the observed size patterns, particularly the larger *Microcystis* colonies and *P. contorta* filaments in the deep, saline monimolimnion, are influenced by reduced zooplankton grazing pressure

in that isolated layer, allowing colonies to persist longer (e.g., Butts et al., 2022).

Factors such as zooplankton grazing and nutrient content may have obscured the relationship (Eyring et al., 2025). The size of phytoplankton particles consumed by zooplankton depends on species composition. Cladocerans and microzooplankton (ciliates, rotifers) primarily consume phytoplankton within the size range 5–15 μm, whereas copepods mainly consume phytoplankton objects larger than 10 μm (Sommer and Sommer, 2006). The Lake Shira zooplankton community includes both calanoid copepods and microzooplankton (rotifers, ciliates) (Zadereev and Tolomeyev, 2007). The impact of such a zooplankton community on the size characteristics of phytoplankton is difficult to predict. Moreover, the zooplankton in Lake Shira is unevenly distributed with depth (Zadereev and Tolomeyev, 2007), making it even more challenging to assess its effect on the size of phytoplankton community without conducting excessive grazing experiments.

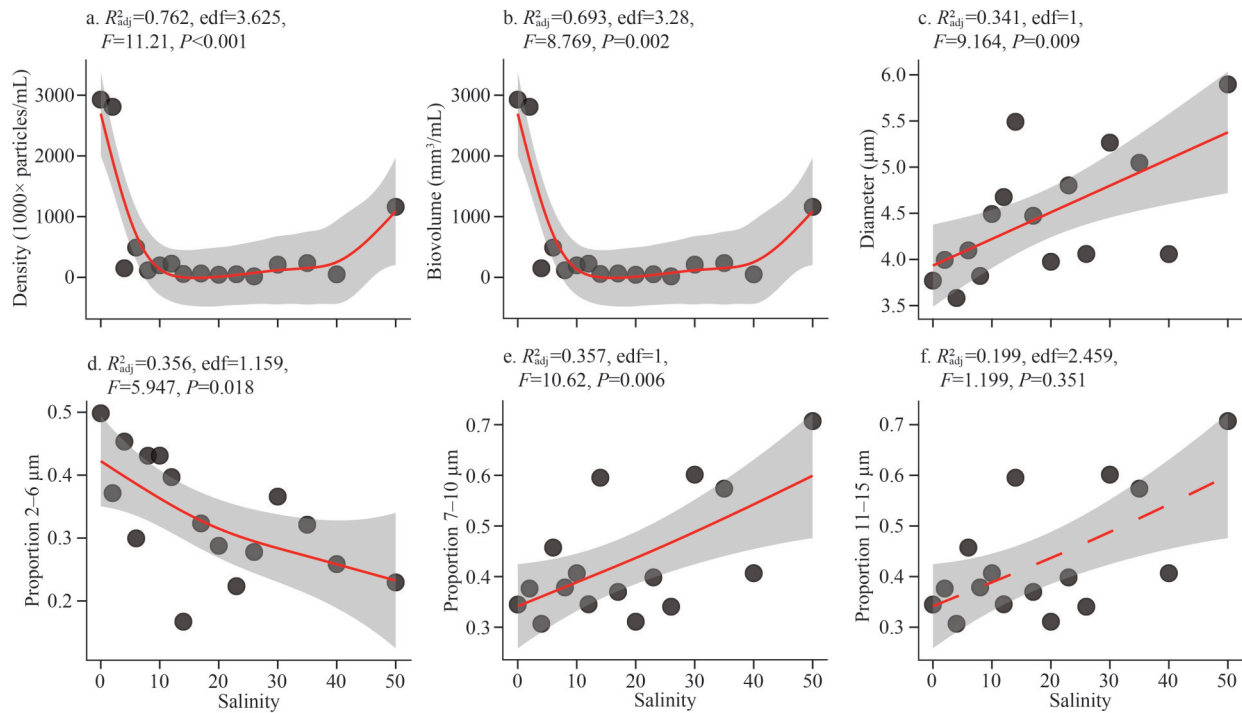


Fig.6 Total abundance (a), biovolume (b), and average particle diameter (2–15- μ m range) (c) of phytoplankton across the salinity gradient; proportion of particles in the size ranges 2–6 μ m (d), 7–10 μ m (e), and 11–15 μ m (f)

The GAM family Gamma (log link function) was used for (a–b), and the family gaussian (log link function) for (c–f).

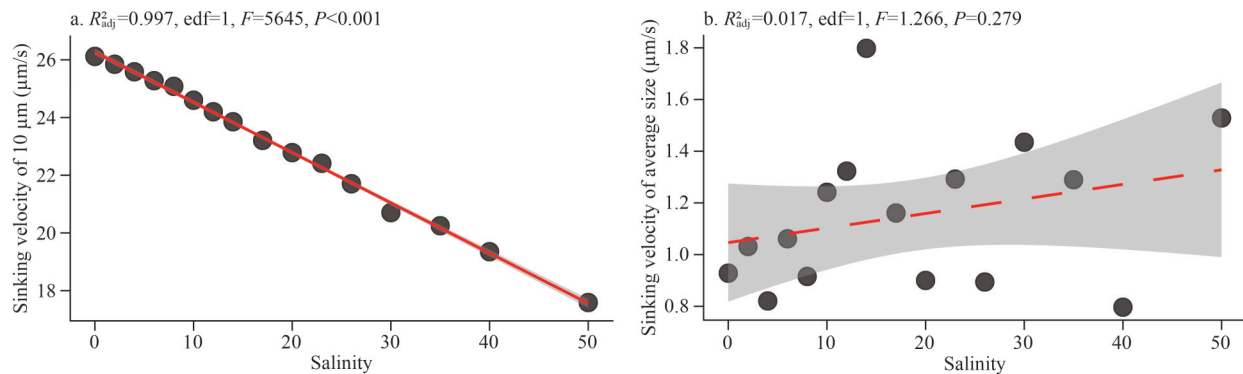


Fig.7 Calculated sinking velocity of a standardized 10 μ m diameter particle (a) and particles with the measured average diameter (b) from the phytoplankton community in each mesocosm, plotted against salinity

The GAM family gaussian (identity link function) was used for (a–b).

4.1.2 Phytoplankton size in the salinity gradient mesocosms experiment

The mesocosm experiment provided a clearer test by controlling for temperature (which was nearly constant across tanks) and minimizing grazing pressure (sampling occurred in late autumn when zooplankton abundance was low (personal communication (Arik et al., in prep))). Here, we observed a robust, 1.5-fold increase in average phytoplankton diameter ($\sim 4\text{--}6\text{ }\mu\text{m}$) across the 0–50 salinity gradient. This positive relationship

provides stronger evidence for a direct effect of increased water density and viscosity, independent of temperature, where larger cell or colony size becomes hydrodynamically favorable to maintain position in the water column (Chindia and Figueredo, 2018). The calculated sinking velocity for particles of this actual mean diameter remained constant across salinities, indicating that the biological size increase effectively compensated for the physical changes in the medium, optimizing buoyancy.

4.2 Ecological implication and the role of zooplankton

The size structure of phytoplankton in natural habitats is highly variable and affected by numerous factors. Specifically, salinity may pose a stress under which a given phytoplankton species will reduce cell size (Nagai and Imai, 1999; Aizdaicher and Markina, 2010; Farkas et al., 2023). However, with rising salinity and accompanying changes in other factors such as temperature and nutrient content, the structure of phytoplankton and zooplankton will vary, resulting in controversial outcomes in the size structure of phytoplankton. For example, in tropical lakes and reservoirs, salinity increases may favor picocyanobacteria development (Amorim et al., 2020), negatively affect large green algae colonies, and favor smaller diatoms, cyanobacteria, and phytoflagellates (Amorim and do Nascimento Moura, 2021, 2022). At the same time, in temperate zones, higher salinities are expected to increase the average cell size of primary producers (Svensson et al., 2014).

Our findings have specific implications for temperate freshwater ecosystems experiencing salinization, such as from road salt application (Dugan et al., 2017). The mesocosm results suggest that in isolation, increasing salinity could select for slightly larger phytoplankton via biophysical mechanisms. However, the Lake Shira results underscore that in real ecosystems, this signal will be modulated by seasonal temperature cycles. In winter and early spring, when road salt application is high but water temperatures are low, the combined high density from both low temperature and elevated salt might strongly favor larger or more buoyant phytoplankton morphologies. In summer, warming could offset the density effect of residual salts, potentially weakening the salinity-driven size signal. Therefore, predicting phytoplankton size responses requires considering the synchronous seasonal trajectories of both temperature and salinity.

The modest ($\sim 2 \mu\text{m}$) increase in mean size of phytoplankton observed in the mesocosms and colonial *Microcystis* in Lake Shira sits at the lower boundary of particle sizes efficiently consumed by cladocerans (Zadereev et al., 2023) and is typical prey for microzooplankton like ciliates and rotifers (Gilbert, 2022). Its direct impact on grazing may therefore be subtle. However, salinity exerts a far stronger, direct control on zooplankton community composition, causing major shifts from cladoceran-

to copepod- or rotifer-dominated assemblages (Lin et al., 2017; Gutierrez et al., 2018; Zadereev et al., 2022). Consequently, the dominant effect of salinity on trophic transfer likely operates through this restructuring of the grazer community, rather than through the relatively minor changes in phytoplankton size it induces (Jeppesen et al., 2007). Furthermore, the size differences we observed could themselves be a combined result of the direct physical effect of water density and an indirect effect mediated by a relaxation of grazing pressure from salinity-intolerant zooplankton (e.g., large cladocerans).

The ecological significance of the observed size shift may be more pronounced for biogeochemical cycles (Eyring et al., 2025). A shift from 4 to 6 μm represents a ~ 3.4 -fold increase in cell volume, which can influence nutrient uptake kinetics, growth rates, and ultimately, carbon export potential. Nutrient ratios and fluctuations can affect differences in the size distribution of marine and freshwater algae, as demonstrated for diatoms (Litchman et al., 2009). The size structure of phytoplankton may also affect the settling rate of organic matter, influencing the capacity of aquatic ecosystems to sequester carbon (Chindia and Figueredo, 2018). Future work should compare these findings to marine systems where salinity effects are better documented, to assess if convergent evolutionary responses exist across the freshwater-marine divide.

5 CONCLUSION

In conclusion, salinity exerts a direct, positive effect on phytoplankton size via increased water density, a mechanism robustly demonstrated under controlled conditions. In natural stratified lakes, this physical signal is often masked by counteracting temperature gradients and biotic interactions, becoming clearly visible primarily in taxa with strong buoyancy regulation. Future research should aim to isolate these mechanisms further, perhaps through in vitro density manipulation experiments. The ecological consequence of this size shift, while secondary to salinity's direct impact on zooplankton composition, may further modify trophic dynamics by altering the accessibility of phytoplankton resources to different grazers and influencing biogeochemical pathways. Predicting the planktonic response to freshwater salinization requires an integrated framework that considers the direct

physical effects on buoyancy, the physiological effects of ions across trophic levels, and the resulting shifts in ecological interactions.

6 DATA AVAILABILITY STATEMENT

All data generated or analyzed in this study are included in this article and are also available from the corresponding author upon reasonable request.

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Electronic supplementary material

Supplementary material (Supplementary Table S1 and Figs.S1–S3) is available in the online version of this article at <https://doi.org/10.1007/s00343-026-5133-5>.