MICROALGAL FUNCTIONAL TRAITS

Is salinity a driving factor for the phytoplankton community structure of a brackish shallow Mediterranean lake?

Igor Stanković · Marija Gligora Udovič · Petar Žutinić [ORCID] **· Nikola Hanžek · Anđelka Plenković-Moraj**

Abstract Phytoplankton is a well-studied group of organisms that can change rapidly with environmental conditions, providing a reliable response to these changes. We analysed phytoplankton biomass, diversity, and its response to environmental conditions in the shallow brackish Mediterranean Lake Vransko. Although protected as Nature Park, the Lake's succession is enhanced by intensive agricultural activity and an artificial connection to the sea. Analysis of phytoplankton reveals a specific community composition strongly influenced not only by nutrients but also by salinity gradient, with species composition shifting from freshwater to brackish. Conditions of higher salinity support the dominance of brackish species, often with low biomass, while periods of low salinity are characterised by dominance of cyanobacteria or other freshwater species capable of rapidly taking up nutrients and forming algal blooms. Changes in water transparency caused by phytoplankton dynamics strongly influence the overall lake system through the availability of macrophyte growth and sediment fixation. These findings are critical for the future lake management, particularly its hydrological regime and maintenance of natural oligohaline and mesotrophic conditions. Understanding the response of phytoplankton to environmental conditions, exacerbated by anthropogenic influence and recent climate change, contributes to the protection of Mediterranean shallow lakes at local and global scales.

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Introduction

Phytoplankton play a critical role in aquatic ecosystems, contributing to carbon fixation from the atmosphere and producing the organic matter needed for food webs to function (Basu & Mackey, 2018). They

are characterized by high diversity and rapid successional shifts in species composition that occur in response to changes in environmental factors. Identifying these factors and main processes that determine the seasonal succession of species in phytoplankton and community structure is one of the central aims in ecology (Masmoudi et al., 2015).

Shallow lakes represent unique ecosystems where hydrological and environmental factors (such as wind, precipitation or water inflow) have a strong influence on plankton development (Adrian et al., 2009; Rühland et al., 2015). Species composition and phytoplankton biomass in shallow lakes are mainly influenced by recurrent mixing of the water column (Weithoff et al., 2000). Shallow brackish lakes are unique in that they tend to have a variable species community that includes representatives from both freshwater and coastal marine environments. In such ecosystems, salinity acts as a switching mechanism that can shift the phytoplankton community from freshwater to more salt-tolerant during intermediate states of nutrient loading. Fluctuating salinity levels can induce changes in light intensity (Rijstenbil, 1987; Lionard et al., 2005) and nutrient availability, such as phosphorus release from sediments (Mohleji & Verhoff, 1980; Jordan et al., 2008; Hintz & Relyea, 2019) or presence of sulfur compounds (Cole et al., 1986; Liu et al., 2019) and ammonia (Rijstenbil, 1988; Seitzinger et al., 1991), which promote proliferation of selected species and may result in decrease in overall biodiversity (Moss, 1994; Flöder & Burns, 2004; Larson & Belovsky, 2013; Velthuis et al., 2023).

Phytoplankton covers a wide range of sizes, shapes, and taxonomic affiliations with precise ecological functions and roles in aquatic ecosystem processes. The morphological adaptations together with the functionality of the species gathered in the community lead to a specific set of traits. The assignment of functional traits of phytoplankton species allowed some classifications (Salmaso et al., 2015) that can be used in the interpretation of ecological processes. One of them is the highly acclaimed Reynolds' classification (Reynolds et al., 2002), which classifies species/taxa into robustly constructed groups based on their ecological characteristics and habitat properties. It has proven to be a powerful descriptor of phytoplankton succession in a wide range of freshwater lentic (Caroni et al., 2012; Žutinić et al., 2014;

Allende et al., 2019) and lotic systems (Stanković et al., 2012; Wang et al., 2021), as well as a practical tool in bioassessment and application of phytoplankton as a biological quality element in ecological water quality assessment (Salmaso et al., 2015; Kruk et al., 2017).

In this study, we hypothesized that the phytoplankton community structure of a shallow brackish Mediterranean lake is primarily conditioned by changes in salinity. Our aim was to determine the effects of environmental variables on the phytoplankton community through a detailed analysis of phytoplankton composition and its relationship with different conditions of nutrients and salinity in a variable habitat, such as a shallow Mediterranean lake.

Materials and methods

Study area

Lake Vransko (syn. Lake Vrana) is located in the eastern Adriatic karst coastal area (Fig. 1). It is the largest natural freshwater lake in Croatia with an area of 30.02 km^2. The lake is located on the territory of Vransko jezero Nature Park, where it occupies more than half of the area. It is a polymictic,
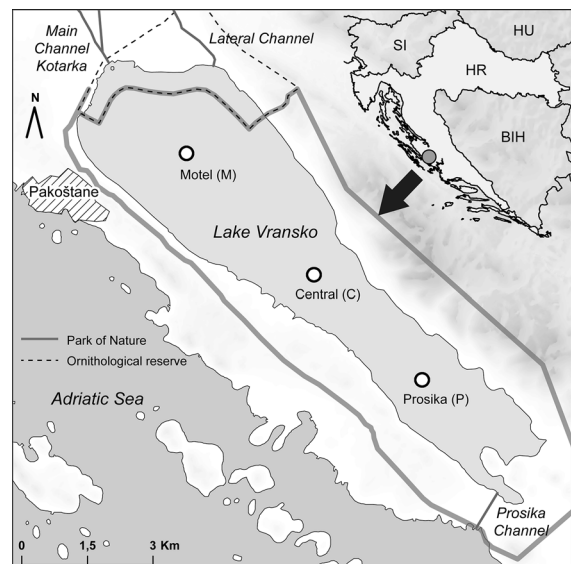
Fig. 1 Location of Lake Vransko in Croatia with main topographic features and designated sampling sites Motel (M), Prosika (P) and Central (C)

shallow cryptodepression with an average water depth between 2 and 2.5 m (Šiljeg et al., 2015). The catchment consists mainly of Cretaceous and Eocene limestones. A 0.8 to 2.5 km wide limestone ridge extends between the lake and the Adriatic Sea (Fajković et al., 2012). The lake is supplied with freshwater by several permanent freshwater springs and two artificial channels, Main (Kotarka) and Lateral channel which were built at the beginning of the twentieth century. The 800 m long Prosika artificial channel on the southwestern part of the ridge connects the lake with Adriatic Sea. Due to its location in the permeable karst area, Lake Vransko is usually slightly brackish, but in years with low freshwater inflow, higher salinity values can be measured (Rubinić & Katalinić, 2014). According to the EU Water Framework Directive (WFD 2000), Lake Vransko can be classified as a brackish surface water. Ornithological Reserve in the northwestern part and especially increased agricultural activity in the catchment contribute to external nutrient loads. In the warm summer months, macrophyte vegetation consisting predominantly of *Chara papillosa* Kützing with *Chara contraria* A.Braun ex Kützing and *Stuckenia* (*Potamogeton*) *pectinata* (L.) Börner dominate the bottom of Lake uniformly with a coverage of more than 50% (Gligora et al., 2007), presumably being responsible for high nitrogen uptake and triggering nitrogen limitation for phytoplankton (van Donk & Hessen, 1993; Jeppesen et al., 1997).

Sampling and analysis

Composite samples for phytoplankton and chemical analyses of water were collected monthly from April to September during 6 years (2004, 2009, 2014, 2016, 2017 and 2019) at two stations: Vransko Motel (M) and Vransko Prosika (P), apart from 2009 when the middle of the lake (C) was sampled, and 2019 when only station Vransko Motel was sampled. Each composite sample consisted of a mixture of equal volume discrete sample aliquots taken from subsurface (0.5 m), 1 m and 2 m depths.

Phytoplankton was counted using an Olympus BX51 inverted microscope equipped with DIC (differential interference contrast), following the Utermöhl (1958) method as described in (CEN - EN 15204, 2006). A minimum of 400 settling units were counted in a transect at 400× magnification, yielding a counting error of < 10% (Lund et al., 1958; CEN

- EN 15204, 2006). Biovolumes were calculated by determining an average individual size from up to 30 randomly selected cells of each taxon and then multiplying by the observed species abundance (Rott, 1981). Biomass (fresh weight) was derived from biovolumes and used for further analyses, where 1 mm^3 l^{-1} = 1 mg l^{-1} (CEN - EN 16695, 2015). Diatoms were identified from the permanent slides made by cleaning the net samples with warm hydrochloric acid and hydrogen peroxide and mounted in the Naphrax solution (CEN-EN 15708, 2009). They were examined under Olympus BX51 microscope at ×1000 magnification equipped with DIC. Taxa were identified to the lowest possible taxonomic level (species, genus) using relevant identification keys and names were assigned according to Algaebase (Guiry & Guiry, 2021). Phytoplankton species were classified into Reynolds' functional groups (Reynolds et al., 2002; Padisák et al., 2009).

Water transparency was estimated using a Secchi disc. Temperature, pH, conductivity, salinity, and dissolved oxygen were measured on site using a WTW Multiline P4 or HACH HQ40D Portable Multi Meter by measuring vertical profiles (0.5 m, 1 m, 2 m) which were then averaged. In years when salinity was not measured directly, it was calculated from conductivity values based on the linear equation obtained from samples when both parameters were measured in years 2014, 2017, and 2019 ($y = 0.0006x - 0.1291$; $R^2 = 0.9956$). Alkalinity was measured according to APHA (2005). Ammonia (NH_4^+-N), nitrites (NO_2^--N) and nitrates (NO_3^--N) were analysed using a Dionex 3000 ion chromatograph. Total nitrogen was analysed using a Shimadzu TOC-VCPH equipped with an analyser for TN (EN 12260:2003). Soluble reactive phosphorus (SRP) and total phosphorus (TP) were determined spectrophotometrically using a Perkin Elmer Lambda UV–VIS spectrometer (EN ISO 6878:2004; EN ISO 17294-2:2016).

Data analysis

To check for significance of changes in salinity on all stations sampled, PERMANOVA (Permutational multivariate analysis of variance) in Primer-E statistical package software (Anderson et al., 2015) was utilized with sampling site as a factor. The Shannon index (*H′*) characterising community species diversity and the list of taxa that contributed more than 1% to

the total phytoplankton biomass in Lake Vransko during the study period were calculated based on species biomass in Primer v6 (Clarke & Gorley, 2006). To test the response of phytoplankton functional groups, total biomass, species number, and Shannon diversity to environmental conditions, Spearman's rho correlation coefficient was used in IBM SPSS Statistics 22 (IBM Corp. Released 2013). Canonical correspondence analysis (CCA) was performed in Canoco 5 (ter Braak & Šmilauer, 2012) to explore the relationships between Reynolds' FG assemblages and all environmental variables. Monte Carlo permutation test was applied to test the statistical significance of all axes, and forward selection was used to assess the importance of each variable. Environmental variables were normalised, and phytoplankton functional group biomass was transformed using the logarithm function prior to statistical analyses. Graphical plots were created using Grapher 15 (Grapher[TM] 2019).

Results

Physical and chemical variables of water

The description of the physical and chemical variables of the water ($n = 60$) with ranges, average values and standard deviation during the study period is given in Table 1. The salinity, total phosphorus (TP) and total nitrogen (TN) concentrations are presented in detail on Fig. 2a, b. Following the EU WFD categorization of transitional waters (WFD 2000), the 0.5‰ salinity level was set as a threshold between freshwater and brackish water (Fig. 2b). According to the physical and chemical properties of the water, Lake Vransko is a brackish lake with mesohaline, oxygen and nutrient rich water with meso- to eutrophic character.

During the study period salinity ranged from 0.7‰ (in 2016) to 3.8‰ (in 2009). Periods with low recorded salinity included years 2004, 2014, 2016, and 2019. These years were characterized by a low fluctuation of salt water spanning between 0.6 and 1.5‰, apart from the September sample of 2016 when a salinity pulse from the Prosika channel was recorded on station P (a threefold increase, from 0.65‰ up to 2‰). The year 2009 was distinguished with the highest salinity (over 3‰) measured in the middle of the Lake (station C) which lasted throughout the sampled months. For the year 2017, a very slow gradual increase of salinity levels was documented during spring on both stations (from 0.7‰ to 0.9‰, respectively), followed by a strong salinity pulse in summer with twofold and threefold salinity increase recorded on stations M and P, respectively.

Table 1 Range, average (Avg) and standard deviation (SD) values of physical and chemical variables of water in Lake Vransko over the study period from 2004 to 2019 for sampling stations Motel, Central and Prosika

Variable	Motel			Central			Prosika		
	Range	Avg	SD	Range	Avg	SD	Range	Avg	SD
Secchi depth (m)	0.6–2.5	1.5	0.5	1.0–1.5	1.2	0.5	0.6–3.1	1.8	0.5
Temperature (°C)	14.0–28.3	22.1	4.3	16.0–28.0	24.1	4.3	13.5–27.5	21.4	4.3
pH	7.1–9.3	8.4	0.5	7.6–8.7	8.2	0.5	7.0–9.3	8.3	0.5
Conductivity ($\mu S\ cm^{-1}$)	1336–3480	2091	473	5400–6460	5873	473	1407–5300	2443	473
Salinity (‰)	0.7–1.8	1.1	0.2	3.1–3.7	3.4	0.2	0.7–2.9	1.3	0.2
Alkalinity (mg $CaCO_3\ l^{-1}$)	40.0–233.0	110.1	51.6	81.0–235.5	139.1	51.6	40.0–211.0	110.3	51.6
Dissolved oxygen (mg $O_2\ l^{-1}$)	8.2–14.8	10.4	1.6	8.7–12.0	10.1	1.6	7.4–12.4	9.7	1.6
Saturation (%)	95.0–179.0	119.5	20.7	92.4–149.7	120.6	20.7	89.5–143.7	110.3	20.7
Ammonia ($\mu g\ N\ l^{-1}$)	0.004–0.394	0.068	0.106	0.020–0.240	0.093	0.106	0.004–0.574	0.113	0.106
Nitrites ($\mu g\ N\ l^{-1}$)	0.001–1.070	0.120	0.258	0.002–0.032	0.015	0.258	0.001–1.290	0.144	0.258
Nitrates ($\mu g\ N\ l^{-1}$)	0.010–2.31	0.375	0.591	0.55–1.913	0.950	0.591	0.010–2.040	0.390	0.591
Total nitrogen (TN) ($\mu g\ N\ l^{-1}$)	0.510–2.360	1.064	0.462	0.646–2.158	1.204	0.462	0.570–2.320	1.076	0.462
Soluble reactive phosphorus (SRP) ($\mu g\ P\ l^{-1}$)	0.002–0.024	0.007	0.006	0.003–0.020	0.006	0.006	0.001–0.024	0.006	0.006
Total phosphorus (TP) ($\mu g\ P\ l^{-1}$)	0.007–0.055	0.021	0.011	0.008–0.030	0.021	0.011	0.003–0.059	0.023	0.011
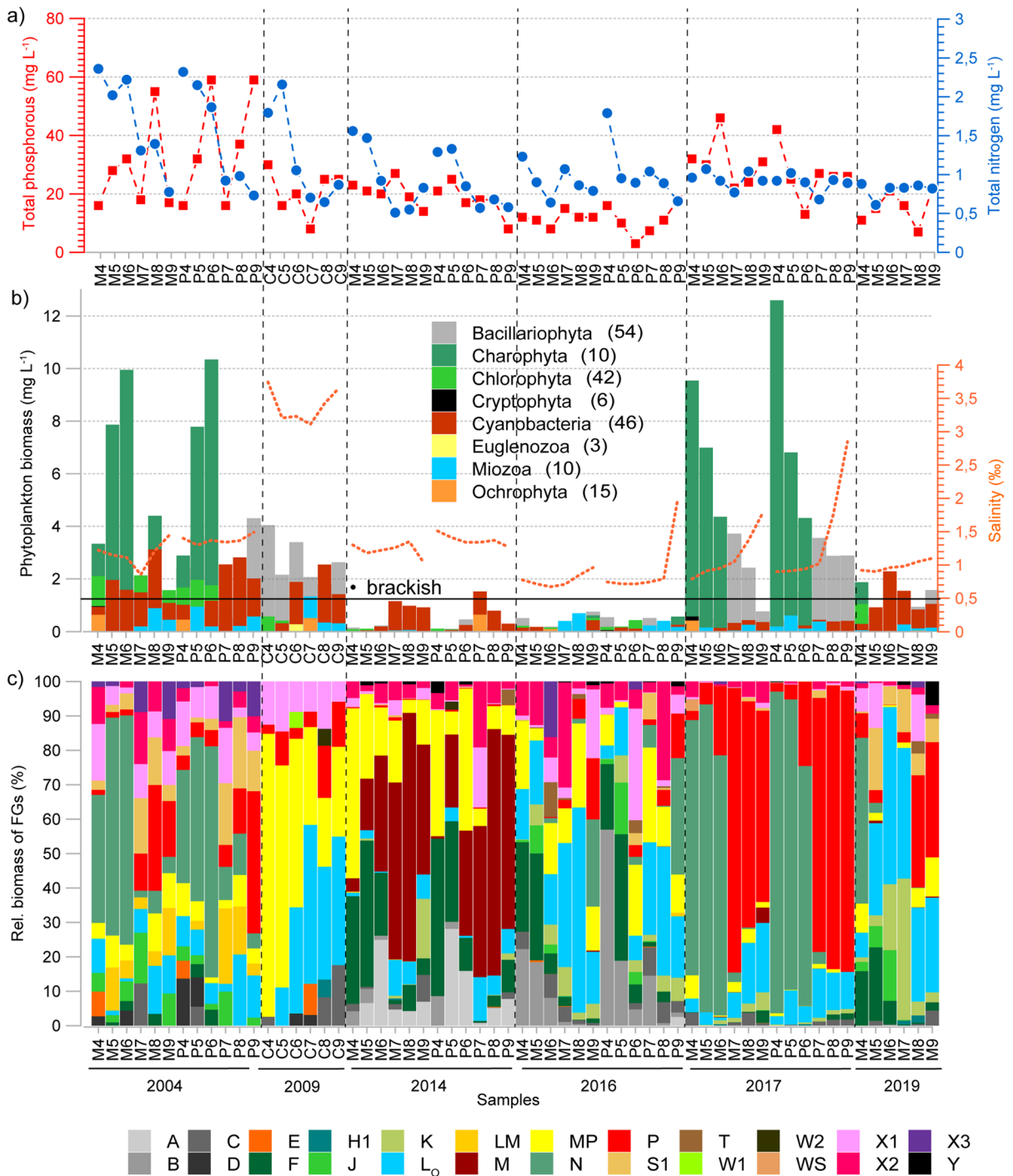
Fig. 2 Relative abundance of functional groups, biomass of phytoplankton taxonomic groups, salinity, total phosphorus, and total nitrogen at sampling sites Motel (M), Prosika (P) and Central (C) in Lake Vransko throughout the study period. The number of taxa in each taxonomic group is given in parentheses. Samples are labelled with the sampling site code and a month

The results of PERMANOVA confirmed a significant influence of spatial changes in salinity when sampling sites were used as a factor ($P = 0.0001$; $P < 0.05$), showing that salinity was significantly different on all three sampling sites. Regarding nutrient concentrations, both TP and TN have shown annual and interannual variability (Fig. 2a). The concentration of TP ranged from 2 μg P l^{-1} (in 2016) up to 60 μg P l^{-1} (in 2004), with years 2009, 2014, 2016, and 2019 characterized by lower values (> 30 μg P l^{-1}). In the year 2004 TP concentration on both stations gradually increased towards late summer (September), while during 2017 the highest TP concentrations were recorded at the beginning of summer (June). The highest concentration of TN was measured in 2004 (2.3 mg N l^{-1}) and the lowest in 2014 (0.4 mg N l^{-1}). Years with a higher variation of TN were 2004 and 2009, in which a spring concentration of TN decreased by a threefold in summer (from 2.3 mg N l^{-1} to 0.7 mg N l^{-1}, respectively). Similarly, years 2014 and 2016 were characterized by a twofold decrease of TN concentration. Finally, the years 2017 and 2019 were marked by a nearly steady concentration of TN (around 0.8 mg N l^{-1} in both years).

Phytoplankton composition and diversity

Phytoplankton dynamics expressed as biomass of taxonomic groups, relative abundance of functional groups together with salinity, TP and TN are shown in Fig. 2. Among the 186 taxa identified throughout the study period, taxonomic groups with the highest number of taxa were Bacillariophyta (54), Cyanobacteria (46) and Chlorophyta (42). A high number of taxa (118) appeared in at least one sample, accounting for > 1% of the total biomass (Supplementary Table 1). Nevertheless, only 18 taxa were dominant or subdominant and contributed > 20% biomass (mg of wet weight) in individual samples.

Total phytoplankton biomass varied from 0.25 mg l^{-1} to 15.09 mg l^{-1} in 2017 and 2004, respectively (Fig. 2b). In 2004, which was characterized by low salinity and high TP, Charophyta contributed the most to the total biomass, with a bloom of *Cosmarium tenue*, which belongs to the functional group **N** (Fig. 2a–c). Several cyanobacterial representatives were also present with high biomass, especially *Komvophoron pallidum* and *Planktolyngbya contorta* from functional group **S1**, and *Gomphosphaeria aponina* from functional group $\mathbf{L_M}$.

The bloom of *C. tenue* also occurred in 2017 in similar conditions of low salinity and high TP, but once salinity began to increase and TP decreased (Fig. 2a, b), diatom *Synedropsis roundii* became dominant as the representative of functional group **P** (Fig. 2c). In 2009, when the highest salinity values were present at moderate to low TP values, Bacillariophyta and Cyanobacteria alternated in dominance. The first group was represented by the species *Tetramphora croatica* and *Navicula trivialis*, which belong to the functional group **MP**, while functional group $\mathbf{L_O}$ included mainly the cyanobacterial representatives *Chroococcus minutus*, *Chroococcus turgidus*, *Merismopedia tranquilla* and *Snowella lacustris*.

The years 2014, 2016 and 2019 were characterized by low salinity, low nutrient concentration and low phytoplankton biomass. In 2014, Cyanobacteria were dominant or co-dominant with Chlorophyta. Functional group **M** included most cyanobacterial species with *Microcystis aeruginosa* and *Microcystis novacekii* as typical representatives, while Chlorophyta were represented with functional group **F** including *Dictyosphaerium subsolitarium* and *Raphidocelis danubiana* as the most dominant. The year 2016 was the most diverse in terms of changes in the dominance of different taxonomic and functional groups. Nevertheless, it was dominated by benthic diatoms from functional group **MP**, *Peridiniopsis borgei* representing Miozoa (dinoflagellates) and functional group $\mathbf{L_O}$, chlorophytes represented by *Crucigenia tetrapedia* and *R. danubiana* from functional group **F**, and cryptophyte species *Plagioselmis nannoplanctica* belonging to functional group **X2**. The year 2019 was characterized by a brief spring dominance of *C. tenue*, which was displaced by cyanobacterial species *M. punctata*, *Rhabdoderma lineare*, *Snowella atomus* and *Woronichinia compacta* from functional group $\mathbf{L_O}$ and *Anathece smithii* from functional group **K**. Towards the end of the sampling season with a slow increase in salinity, they were replaced by diatom *S. roundii* from codon **P**.

Number of taxa (S) and Shannon diversity index (H') are displayed on Fig. 3. The years 2004 and 2009 were characterized by high Shannon diversity index and low number of taxa. High values of both indices were recorded in 2014 and 2016, after which the number of taxa and diversity index dropped in 2017.
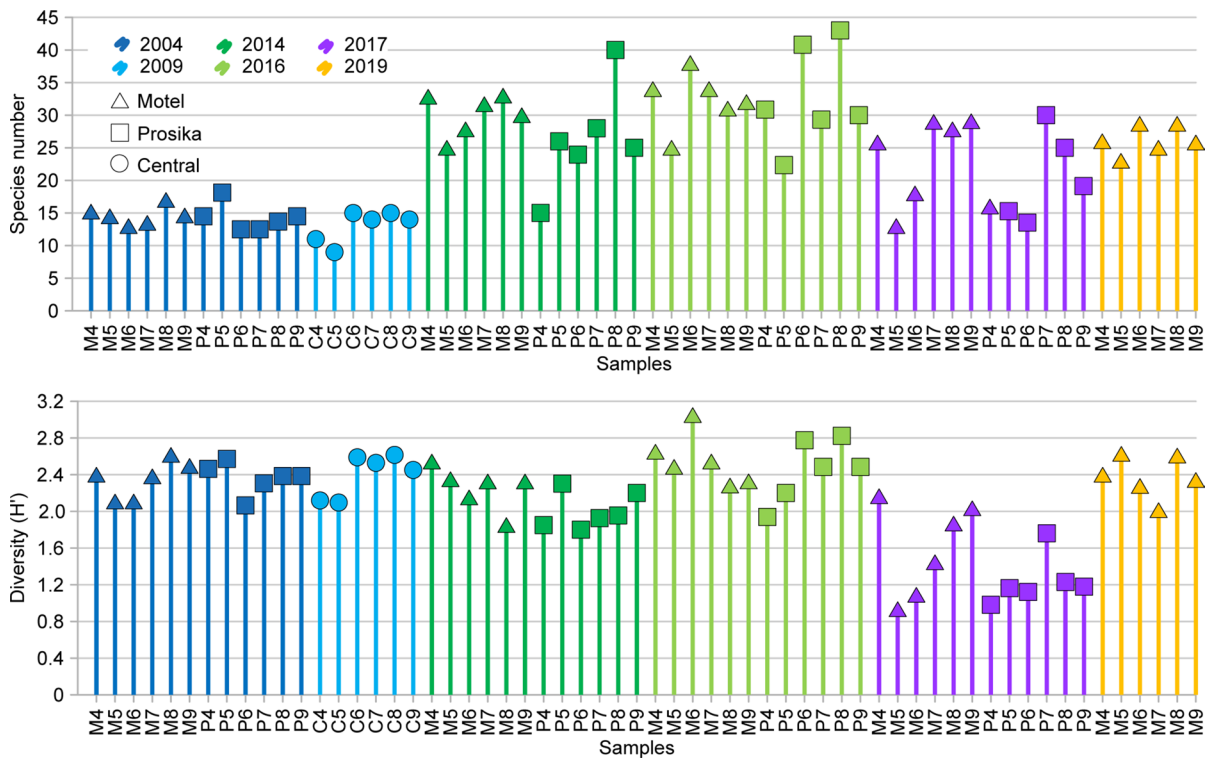
Fig. 3 Number of taxa (S) and Shannon diversity index (H') of phytoplankton at sampling sites Motel (M), Prosika (P) and Central (C) in Lake Vransko throughout the study period. Samples are labelled with the sampling site code and a month. Years are marked with different colours, and sampling sites with different symbols

This was followed by a slow increase in both indices again in 2019.

Response of phytoplankton to environmental conditions

Biological descriptors such as functional groups, total biomass, species number and Shannon diversity index showed significant Spearman's rho correlations with environmental variables (tested levels of significance: $P \leq 0.05$ and $P \leq 0.01$; Table 2). Total phytoplankton biomass correlated positively with SRP, TP and nitrates, and negatively with Secchi depth and ammonium, but had no significant correlation with salinity. The relationship between phytoplankton functional groups and environmental variables indicated several main determining factors of the phytoplankton community dynamics. Higher biomass of the benthic functional group **MP** was measured during the period of high salinity and showed a positive correlation, while functional groups with typical freshwater representatives (**B**, **F**, **J**, **K**, **T**) showed a negative correlation with salinity. Functional group **N,** characterized by the highest measured biomass in 2017, showed a negative correlation with salinity and a positive correlation with TN, SRP and TP, while functional group **P**, represented mainly by a typical brackish water species *S. roundii*, correlated positively with salinity and TP. Phytoplankton richness, presented as species number, significantly decreased with higher salinity and higher TP, while phytoplankton diversity increased with high concentration of nitrogen ions, decreased with higher concentration of TP and showed no significant correlations with salinity.

The relationship between phytoplankton composition based on functional groups and environmental variables is summarized on the CCA ordination, reflecting the importance of salinity, water transparency and nutrients in shaping the phytoplankton community in Lake Vransko (Fig. 4; Table 3). The eigenvalues of the first two axes (0.147 and 0.103, respectively) explained 59.0% in constrained

Table 2 Spearman's rho correlations (2-tailed) between selected biological descriptors and environmental variables

	Secchi depth	Temperature	pH	Conductivity	Salinity	Alkalinity	Oxygen concentration	Saturation	Ammonia	Nitrites	Nitrates	Total nitrogen	Soluble reactive phosphorus	Total phosphorus
A	**0.338****	n.s	n.s	0.281*	n.s	0.288*	− **0.367****	− 0.308*	**0.413****	n.s	n.s	− **0.344****	n.s	n.s
B	0.288*	n.s	− 0.285*	− **0.409****	− **0.433****	0.309*	n.s	n.s	**0.505****	**0.711****	n.s	n.s	n.s	− **0.431****
C	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
D	n.s	n.s	n.s	n.s	0.276*	n.s	n.s	n.s	n.s	n.s	**0.372****	0.315*	n.s	n.s
E	n.s	n.s	n.s	n.s	n.s	n.s	0.266*	n.s	n.s	0.262*	n.s	n.s	n.s	− 0.317*
F	n.s	n.s	n.s	− 0.256*	− 0.290*	**0.364****	n.s	− 0.311*	n.s	n.s	n.s	n.s	n.s	n.s
H1	n.s	**0.435****	**0.376****	n.s	n.s	− 0.297*	− 0.272*	n.s	n.s	− **0.362****	− **0.396****	− **0.355****	n.s	n.s
J	n.s	n.s	n.s	− **0.365****	− 0.284*	n.s	n.s	n.s	n.s	**0.376****	0.272*	n.s	0.278*	n.s
K	n.s	n.s	n.s	− **0.435****	− **0.474****	− **0.443****	n.s	n.s	− **0.349****	n.s	− **0.682****	− **0.410****	n.s	− 0.296*
L$_O$	− 0.258*	n.s	n.s	n.s	n.s	− **0.406****	n.s	0.320*	− **0.428****	n.s	n.s	n.s	n.s	n.s
LM	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	0.322*	n.s	n.s	**0.355****
M	0.283*	n.s	n.s	n.s	n.s	n.s	− **0.394****	− 0.275*	n.s	− **0.394****	− 0.281*	− **0.425****	n.s	n.s
MP	n.s	n.s	n.s	0.312*	**0.360****	n.s	n.s	n.s	n.s	n.s	**0.554****	n.s	n.s	n.s
N	− **0.405****	n.s	n.s	− **0.358****	− 0.268*	− 0.303*	0.320*	n.s	− **0.391****	n.s	n.s	0.277*	**0.506****	0.279*
P	− 0.327*	n.s	**0.352****	n.s	0.293*	− **0.640****	n.s	0.271*	− **0.646****	− **0.441****	n.s	n.s	n.s	**0.475****
S1	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	− **0.434****	n.s	n.s	n.s	0.257*	n.s
T	n.s	n.s	n.s	− 0.318*	− **0.340****	n.s	n.s	n.s	n.s	n.s	− **0.408****	− 0.321*	n.s	− **0.464****
W1	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
W2	n.s	0.256*	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
WS	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
X1	n.s	n.s	n.s	n.s	0.258*	n.s	n.s	n.s	n.s	n.s	**0.548****	n.s	n.s	n.s
X2	0.291*	n.s	n.s	− 0.255*	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
X3	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	0.308*	n.s	n.s	n.s
Y	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s	n.s
TB	− **0.478****	n.s	0.311*	n.s	n.s	− 0.311*	n.s	n.s	− **0.522****	− 0.308*	0.282*	n.s	**0.413****	**0.528****
Spec. Num	0.271*	n.s	n.s	− **0.337****	− **0.412****	n.s	n.s	n.s	n.s	n.s	− **0.526****	− **0.407****	− 0.313*	− **0.379****
Shannon	n.s	n.s	− 0.294*	n.s	n.s	n.s	n.s	n.s	0.309*	**0.497****	0.313*	n.s	n.s	− **0.403****

n.s.: not significant; total number of samples for all variables is 60

*Correlation is significant at the $P \leq 0.05$ level (in bold)

**Correlation is significant at the $P \leq 0.01$ level (in bold)
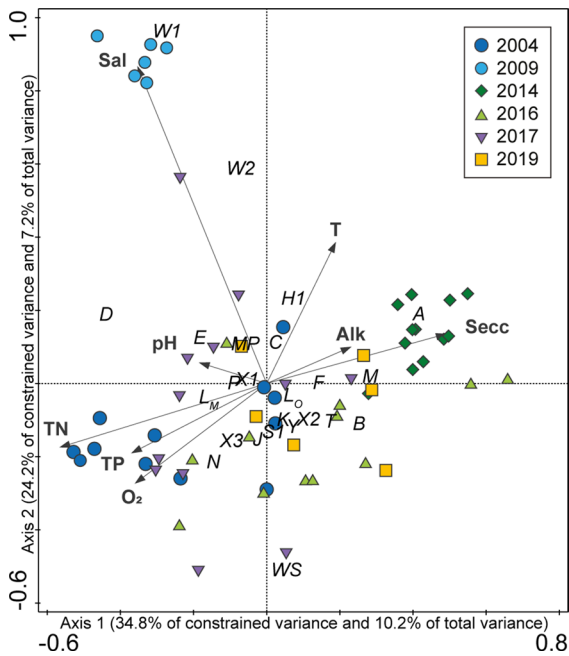
Fig. 4 Canonical correspondence analysis (CCA) triplot of phytoplankton functional groups, environmental variables and samples from all years studied. Environmental variables are abbreviated as: *Secc* Secchi depth, *T* temperature, *O₂* Dissolved oxygen, *Sal* Salinity, *Alk* Alkalinity, *TP* Total phosphorus, *TN* Total nitrogen

Table 3 Results of the canonical correspondence analysis (CCA) between FGs and environmental variables

	Axis 1	Axis 2
Axis summary statistics and variance in species data[a]		
Eigenvalues	0.147	0.103
FGs-environment correlations	0.779	0.791
Cumulative percentage variance		
Of FGs data	10.2	17.4
Of FGs-environment relation	34.8	59.0
Correlations of environmental variables and redundancy axes[b]		
Variable		
Secchi depth	0.3818	0.1071
Temperature	0.1467	0.3047
pH	− 0.143	0.0449
Salinity	− 0.2748	0.6847
Alkalinity	0.1792	0.0789
Dissolved oxygen	− 0.2798	− 0.2156
Total nitrogen	− 0.4399	− 0.1377
Total phosphorus	− 0.2873	− 0.1501

[a]Axis summary statistics of the two canonical axes extracted and the percentage of variance explained by CCA ordination

[b]Correlation of environmental variables with ordination axes

variance of the functional group composition and environmental data. Pearson correlation analysis between functional groups and environmental variables (0.779 and 0.791, respectively) calculated for the first 2 axes indicated significant positive correlation between abiotic values and the phytoplankton functional group variables. According to the Monte Carlo permutation test, the ordination of the two axes was statistically significant ($P < 0.05$). Axis 1 correlated strongly with TN ($R = −0.440$), Secchi depth ($R = 0.382$), TP ($R = −0.287$) and O₂ ($R = −0.280$), while axis 2 had a high correlation with salinity ($R = 0.685$) and temperature ($R = 0.305$). The highest positive correlation with salinity and temperature were shown by euglenophytes from the coda **W1** and **W2,** together with pennate diatoms belonging to group **MP,** *Cyclotella meneghiniana* from group **C** and heterocytous cyanobacterial taxon *Anabaena* from association **H1**. Colonial chrysophyte genus *Dinobryon* from codon **E** correlated positively with salinity and

pH. Coda **P** (*Synedropsis roundii*) and **X1** (mainly chlorococcales from the genera *Ankistrodesmus* and *Monoraphidium*) were positioned close to the centroid with equal influence of all variables plotted in the biplot, while functional groups **N** (*Cosmarium tenue*), **L_M** (*Gomphosphaeria aponina*), **X3** (*Koliella longiseta* f. *tenuis*) correlated positively with nutrients (TP, TN) and dissolved oxygen concentration, and negatively with salinity. Centric diatoms from functional groups **A** (*Thalassiosira* spp.) and **B** (*Pantocsekiella ocellata*) colonial cyanobacteria from codon **M** (*Microcystis* spp.), and chlorophytes belonging to groups **F** and **T** were negatively associated with pH, salinity and nutrient concentration and positively associated with alkalinity and water transparency. Typical freshwater representatives, such as filamentous cyanobacteria from group **K** (*Aphanocapsa* spp.), colonial small-celled cyanoprokaryotes from group **S1** (*Planktolyngbya* spp.), flagellated cryptophytes from coda **X2** and **Y** (mainly *Plagioselmis nannoplanctica* and *Cryptomonas* spp.), with codon **J** coenobial chlorophytes (*Lemmermania tetrapedia*), correlated negatively with salinity.

Discussion

Phytoplankton biomass in shallow brackish lakes is determined by the strong interaction of nutrients and salinity (Meerhoff et al., 2012). Such systems are usually characterized by periodic alternations between freshwater and brackish phases, depending on the environmental stresses exerted on them (Obolewski et al., 2018). Although the salinity threshold below 0.5‰ (WFD, 2000) was not noted in Lake Vransko during the study period, the years with very low intrusion of seawater were regarded as the lake's freshwater phase, whilst the years with pulses of salinity were characterized as brackish phase (Fig. 2b). During the freshwater phase, the phytoplankton biomass of a subsequent brackish Lake Vransko is mainly controlled by the availability of nutrients (TP and TN) and water transparency. The years 2004 and 2017, when the highest biomass was reached, were both characterized by the spring bloom of *Cosmarium tenue*. This small-sized unicellular charophyte species is an indicator of mesotrophic conditions (Coesel & Meesters, 2007) included in codon **N**, which is characteristic of spring and early summer periods in temperate, shallow lakes with a mixed layer. The combination of both R- and SR-strategies allows this desmid to exploit its high affinity for phosphorus at low concentrations (Reynolds, 2006) and successfully dominate the phytoplankton community. Gligora Udovič and Plenković-Moraj (2003) highlighted species of the genus *Cosmarium* as important members of the spring–summer phytoplankton community of Njivice and Ponikve lakes, two shallow Mediterranean lakes in the northern region of Adriatic Sea (Croatia). Gligora et al. (2007) reported that the phytoplankton community of Lake Vransko in 2004 was clearly determined by the winter-spring prevalence of *Cosmarium tenue*, which was controlled by the availability of nutrients, mainly nitrogen compounds. In the summer and autumn of 2004, *C. tenue* was replaced by *Synedropsis roundii* (described as *Synedra* sp.) after the concentration of nutrients decreased (Gligora et al., 2007). This coincides with two summer-related events, the occurrence of high macrophyte cover and the drop in freshwater levels with consequent saltwater intrusion through the Prosika channel. The high cover of aquatic macrophytes in Lake Vransko in summer is probably responsible for high nitrogen uptake, triggering nitrogen limitation

for phytoplankton (van Donk & Hessen, 1993; Jeppesen et al., 1997; Gligora et al., 2007). This influence on phytoplankton biomass and recorded changes in species dominance (Gligora et al., 2007) was also confirmed here, with high (Shannon) species diversity and low numbers of taxa present in 2004, 2009 and 2017, both showing a negative correlation with nitrate, TN, SRP and TP. On the other hand, long-term saltwater intrusion can cause fundamental changes in natural biogeochemical cycles and ecosystem structure and function (Widney et al., 2019), which include soil salinization, increased ammonium release, decreased plant productivity, declining species richness, and decreased N retention (Herbert et al., 2015; White Jr. & Kaplan, 2017). Increased ammonium release can lead to further eutrophication (Howarth & Paerl, 2008; Widney et al., 2019), which is more prevalent in oligotrophic systems, as the nitrification process usually starts earlier in lakes of higher trophic status and has a faster ammonium turnover (Leoni et al., 2018).

As shown by statistical analyses, the salinity below 2‰ and moderate availability of both TP and TN still support development of *Cosmarium tenue*. The summer dominance of the codon **P** representative *Synedropsis roundii* is associated with the depletion of nitrogen from the water column (Gligora et al., 2007), since phosphorus limitation during the summer period only affects *C. tenue* (Sommer, 1987). Indeed, *Synedropsis roundii* becomes competitive for phosphorus even in environments with low TP and TN concentrations and therefore displaces *Cosmarium* during summer periods when nitrogen is depleted from the water column. Lake Vransko as a shallow, temperate, continuously mixed lake with an average depth of 2–3 m represents an optimal habitat for groups **N** and **P** (Padisák et al., 2009), both of which indicate meso- to eutrophic conditions. Both coda **N** and **P** generally respond poorly to increased salinity, indicating that in years when these functional groups dominated the community (2004 and 2017), salinity was in the lower range.

In addition to *C. tenue*, several cyanobacterial representatives with high biomass were also present, especially the solitary, filamentous, non-nitrogen-fixing species *Komvophoron pallidum* and *Planktolyngbya contorta* from the **S1** functional group, and *Gomphosphaeria aponina* from the **L$_M$** functional group. Both **S1** species are shade-adapted cyanoprokaryotes

characteristic of turbid mixing environments and sensitive to flushing (Reynolds et al., 2002; Padisák et al., 2009), which may be common in the mixing period. On the other hand, the species of codon L_M are typical of small- to medium-sized lakes of higher trophic levels and usually occur in a highly irradiated part of the water column. Their representative, the small cyanobacterium *G. aponina*, is a freshwater species (Komárek & Hindák, 1988; Komárek & Anagnostidis, 1999) that achieves the favoured conditions of high light availability by forming spherical colonies in which each cell is connected to mucilaginous stalks and by buoyancy-regulating gas vesicles (Reynolds, 2006). In a series of mesocosm bioassays with varying N and P concentrations, Ma et al. (2015) showed that *Cosmarium* growth is strongly promoted under the influence of moderate N and P supply. These findings are congruent with our results, as the biomass of *C. tenue* significantly exceeded that of colonial and filamentous cyanobacteria.

The highest salinity levels (above 3‰) with moderate to low nutrients in 2009 negatively impacted total phytoplankton biomass and richness (number of species). However, these conditions were not limiting for the functional group **MP,** which is tolerant to frequent mixing and turbidity and is mainly composed of benthic diatoms (*Tetramphora croatica* and *Navicula trivialis*) and cyanobacterial representatives of the codon L_O (*Chroococcus minutus*, *Chroococcus turgidus*, *Merismopedia tranquila* and *Snowella lacustris*), which are assigned to shallow, medium to large lakes with a wide trophic range (Reynolds et al., 2002; Padisák et al., 2009). Both coda showed higher biomass and a positive correlation with salinity. *Navicula trivialis* is a benthic freshwater pennate diatom that is widely distributed in lakes and tolerates a wide range of pH, conductivity, and alkalinity (Lange-Bertalot, 2001; Rushforth & Spaulding, 2010). *Tetramphora croatica* is a benthic pennate diatom that has only recently been described and previously only detected from sediment core and diatom mat samples from Lake Vransko (Caput Mihalić et al., 2019). Nanoplanktonic species from the genus *Chroococcus* (*C. minutus*, *C. turgidus*), which occur as small spherical or compact colonies enveloped in mucus, are common in mesotrophic and eutrophic alkaline lakes with higher alkalinity (Reynolds, 2006; Komárek & Johansen, 2015). The sheet-forming colonial *M. tranquila* and the gas-vacuolate, stalk-forming colonial

S. lacustris are also common in freshwater ecosystems (Komárek & Anagnostidis, 2000; Komárek & Johansen, 2015), as indicators of eutrophic conditions (Fernández et al., 2012; Bukowska et al., 2017). All of the above cyanobacterial taxa are known to form loose associations with emergent plants in shallow lakes and are known members of planktonic and metaphytic assemblages in shallow lakes (Vincent, 2009; Komárek & Johansen, 2015). However, high correlation of codon W1 with salinity during conditions of higher salinity in 2009 (Fig. 4) was not corroborated by further statistics (Table 2), most likely as result of a sole observation. *Euglena obtusa*, a representative of this association, is a cosmopolitan, widespread species which can be found in both freshwater and brackish systems and estuaries, usually occurring in habitats rich in organic matter, and is an indicator of moderate organic pollution (Ernest & Pringsheim, 1949; Steffensen, 1974; Reynolds et al., 2002; Wolowski, 2003).

Low concentrations of TP and TN, coupled with low salinity, in 2014, 2016, and 2019 constrained total phytoplankton biomass, confirming the strong causal relationship between primary production, water transparency and nutrient availability in Lake Vransko. These years were characterized by a strongly expressed macrophyte vegetation consisting predominantly of *Chara papillosa* Kützing with *Chara contraria* A.Braun ex Kützing and *Stuckenia* (*Potamogeton*) *pectinata* (L.) Börner as subdominant species, covering 60–90% of the total lake bottom (Alegro et al., 2019; Vuković et al., 2020). Both characeans are subcosmopolitan algae that have been described from a range of meso- and eutrophic shallow habitats and regularly form associations with *Stuckenia* (Van den Berg et al., 1999; Schneider et al., 2016). Although primarily a freshwater species, *C. contraria* is reported to be sensitive to reduced light and increased salinity (Steinhardt & Selig, 2011) whereas *C. papillosa* is known for its intermediate ecophysiological characteristics and salinity-specific adaptation to different habitats (Boegle et al., 2010; Nowak & Schubert, 2019). *Stuckenia pectinata* is a rhizomatous, vascular aquatic plant characteristic of eutrophic or brackish waters where it can form dense stands in lakes, is tolerant of disturbance, and has been found in highly calcareous, nutrient-poor lakes (Hill et al., 2004).

Although conditions of low nutrients, high light availability, and low salinity favoured stable macrophyte dominance over the 3-year period (2014, 2016 and 2019, respectively), several phytoplankton taxa from the Cyanobacteria and Chlorophyta groups were nevertheless successful in exploiting the remaining gaps, possibly through allelochemical competition (Mulderij et al., 2007; Mohamed, 2017) or via seasonal dynamics (Muylaert et al., 2010; Sayer et al., 2010; They et al., 2014). In 2014 the main representatives included cyanobacteria from codon **M** (*Microcystis aeruginosa*, *M. novacekii*), which co-dominated with chlorophytes from functional group **F** (*Dictyosphaerium subsolitarium* and *Raphidocelis danubiana*). The phytoplankton assemblage of 2019 was even more clearly dominated by cyanobacteria, namely by the groups **L_O** (*Merismopedia tranquila, Rhabdoderma lineare*, *Snowella atomus* and *Woronichinia compacta*) and **K** (*Anathece smithii*). The habitat template of these associations, which includes clear, shallow, medium-sized lakes covering a wide trophic status (Reynolds et al., 2002; Padisák et al., 2009), was consistent with the ecological and morphological characteristics of Lake Vransko and was also confirmed by statistical analyses (Spearman's rho correlation and CCA, Table 2; Fig. 4).

The lowest measured salinity and TP were the defining factors for the highest recorded diversity and species richness in 2016. Despite being well adapted to these variables (Reynolds et al., 2002; Padisák et al., 2009), several functional groups, such as benthic diatoms from functional group **MP** (*Mastogloia smithii*, *Envekadea hedinii*), functional group **L_O** (*Peridiniopsis borgei*), chlorophytes from functional group **F** (*Crucigenia tetrapedia*, *Raphidocelis danubiana*) and functional group **X2** (*Plagioselmis nannoplanctica*), alternated with an overall low biomass. However, neither group was able to occupy a permanently dominant position, reflecting the conditions under which the effects of shading, nutrient limitation, resuspension of sediment particles and excretion of allelopathic substances from macrophytes severely limited phytoplankton growth (Gligora et al., 2007; Mulderij et al., 2007).

Analysis of phytoplankton over several years in the Mediterranean shallow Lake Vransko reveals a specific community composition that is strongly influenced not only by nutrients but also by the salinity gradient, with species composition shifting from freshwater to brackish water, confirming our hypothesis. Conditions of higher salinity support the dominance of brackish benthic species, often of low biomass, with the dominance of *Tetramphora croatica*. Periods of low salinity are characterised by freshwater composition, with a shift in dominance between *Cosmarium tenue/Synedropsis roundii*, cyanobacteria or colonial green algae capable of rapidly uptake nutrients and form algal blooms. The results suggest that changes in lake transparency due to changes in algal community structure strongly influence the overall lake system through the availability of macrophyte growth and sediment fixation. A highly dynamic and specific environment, such as that present in Lake Vransko, supports high levels of diversity and the discovery of new species. The results of this study are valuable for future management of the lake, particularly with respect to maintaining the hydrologic regime and natural oligohaline and mesotrophic conditions. Understanding the responses of phytoplankton to environmental conditions, exacerbated by anthropogenic influence and current climate change, contributes to the protection of Mediterranean shallow lakes at local and global scales.

Declarations

Conflict of interest All authors declare they have no financial or non-financial interests to disclose.

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