

# Dominance of small-sized phytoplankton in a Mediterranean eutrophic coastal lagoon

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## ABSTRACT

The predator-prey relationship is generally size-specific in the pelagic food webs. Phytoplankton cell size structure can provide information on the successive levels of consumers and therefore on the energy that can flow towards the top consumers. This work focuses on phytoplankton cell size structure in a coastal lagoon (Cabras Lagoon, Italy) considered one of the most important for fishing productivity in the Mediterranean. The inter-annual and seasonal dynamics of picophytoplankton (Pico, cell size <3 µm) and Utermöhl Fraction of Phytoplankton (UFP, cell size >3 µm) were considered during almost three years in relation to the temporal dynamics of selected

environmental variables and zooplankton. Small-sized cells with a mean linear cell size <10 µm and a mean cell volume <10<sup>3</sup> µm<sup>3</sup> mainly represented UFP along the entire study period. This size class contributed the most to total phytoplankton biomass (up to 86%) and density (up to 99%) during the first part of the investigation period. A compositional change was detected: smaller species of Chlorophyceae, Bacillariophyceae, filamentous Cyanophyceae, and autotrophic nanoflagellates thrived in the second part of the study, replacing larger Mediophyceae that dominated UFP at the beginning. Picocyanobacteria rich in phycocyanin were the dominant taxa of Pico along the entire investigation period and this size class contributed the most to total phytoplankton biomass (up to 30%) and density (up to 96%) at the end of the study. The observed shift towards different and even smaller UFP and Pico in the second part of the study was most probably due to complex interactions between top-down and bottom-up effects. Indeed, an increased temperature, a decreased salinity and decreased concentrations of nutrients (mainly ammonium and orthophosphate), as well as an increased grazing pressure of rotifers on the larger Mediophyceae were simultaneous with the changes detected in phytoplankton. The obtained results highlight a longer planktonic trophic web in Cabras Lagoon that includes small phytoplankton at the base, ciliates, rotifers, and copepods. This suggests low energy availability for planktivorous fish, with possible future relevant consequences for fishing activities in this coastal lagoon.

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## Introduction

Phytoplankton are microscopic algae and cyanobacteria, but despite being tiny, their intra- and interspecific range in size is huge. Interspecific linear cell sizes span 5 orders of magnitude, and cell volumes more than 8 orders of magnitude (Finkel *et al.*, 2010; Ryabov *et al.*, 2021).

The amazing morphological phytoplankton diversity in terms of cell size and shape represents a successful adaptive strategy addressed at facing pelagic environment variability (Naselli-Flores, 2021). Specifically, cell size is the dominant trait affecting the physiology and ecological function of phytoplankton, including metabolic rate (growth, photosynthesis, respiration), light acquisition, nutrient uptake, sinking rate from the illuminated

surface layer, and predatory grazing by zooplankton (Litchman *et al.*, 2008). For these reasons, Litchman *et al.* (2010) have suggested considering cell size a master trait helpful in the analysis of phytoplankton responses to environmental conditions and their change. For example, being small is a particularly good strategy to deal with nutrient depletion because of the high surface-to-volume ratio that makes phytoplankton cells less diffusion-limited (Litchmann *et al.*, 2007). Consequently, smaller-sized phytoplankton generally dominate phytoplankton in the open ocean where nutrients are depleted and primary production is low, while larger-sized phytoplankton are generally more dominant in nutrient richer coastal waters where primary production is higher (Cabré *et al.*, 2016; Marañón *et al.*, 2007).

As primary producers at the base of aquatic food-webs, phytoplankton size structure can impact on grazers and, consequently, on the functioning of aquatic ecosystems and their relative ecosystem goods and services (Como *et al.*, 2012, 2018). However, the functioning of plankton communities is complex, and in addition to trophic interactions, various kinds of less studied biological links, such as mutualistic symbiosis, commensalism, allelopathy and competitions, may be relevant (Kéfi *et al.*, 2015; Pilosop *et al.*, 2017; Russo *et al.*, 2022). A size-based predation has been reported for plankton food chains and it is generally assumed that larger prey is eaten by larger predators: heterotrophic nanoflagellates (cell size <20 µm) feed on picoplankton (cell size <2 µm), microzooplankton (20–200 µm) feed on heterotrophic nanoflagellates and nanoplanktonic phytoplankton, and mesozooplankton (200–2000 µm) feed on microplankton (Peter and Sommer, 2012; Sommer *et al.*, 2017a). This means that the energy flow in marine pelagic food webs is largely based on size specific predator–prey relationships (Boyce *et al.*, 2015): a shift to smaller size phytoplankton at the base of the food web results in a lower quality food for mesozooplankton, making the carbon flow between primary producers and mesozooplankton longer, through heterotrophic nanoflagellates and microzooplankton, with relative losses of energy, nutrients and carbon which can reduce productivity of higher trophic levels as described by Berglund *et al.* (2007). Actually, the trophic diversity of plankton is huge, the functioning of marine pelagic food webs is complex and the efficiency of energy transfer is more variable than previously thought (D’Alelio *et al.*, 2016a; Eddy *et al.*, 2021). For example, there are pelagic tunicates centimeters in length that filter particles, including phytoplankton, four to five orders of magnitude smaller than themselves (Sutherland *et al.*, 2010). Both planktonic metazoan and protozoan consumers may change their feeding behaviors based on the available resources, modifying the overall functioning of the plankton food web in different seasons (D’Alelio *et al.*, 2016b). Seven-fold decrease in phytoplankton biomass translated into only a two-fold decrease in potential planktivorous fish biomass in a plankton community in the Gulf of Naples (D’Alelio *et al.*, 2016a). Large temporal fluctuations in the dominance of primary producers (*i.e.* macroalgae vs. phytoplankton) and, thus, in the available food items, resulted in major changes in the diet and stable isotopic signatures of several primary consumers in the hypertrophic Yundang Lagoon (Xiamen, China; Zheng *et al.*, 2020).

The economic consequences of plankton composition changes (and food web alterations) are especially important in ecosystems that are strongly exploited for fishing, such as Mediterranean coastal lagoons. In general, coastal lagoons are

transitional shallow ecosystems highly sensitive to natural and anthropogenic environmental changes, such as climate crises, whose impacts occur at global and local scales (Barbosa *et al.*, 2010; Newton *et al.*, 2014). Coastal lagoons function as tightly linked benthic-pelagic systems and, in addition to the environmental stressors and the pelagic biological interactions, phytoplankton structure and dynamics can be strongly influenced by the benthic environment in these ecosystems. Benthos can be a strong sink for phytoplankton biomass (for example, grazing by benthic size-specific filter feeders), or a source for meroplanktonic species from germination of resting stages in sediments (Cloern and Jassby, 2010) and/or for tychoplanktonic species removed from substrates by turbulence (Cahoon, 2016). Therefore, decoding phytoplankton spatial and seasonal dynamics is challenging in heterogeneous and dynamic ecosystems. In this regard, the analyses of multiannual data turned out to be a useful instrument for disentangling the intrinsic complexity of phytoplankton dynamics, at least at a local level (Morabito *et al.*, 2018; Zingone *et al.*, 2010). The scarcity of detailed observations, and the high variability within lagoons, seriously limited the establishment of general models of planktonic succession in lagoons as has been done for other marine and freshwater environments (Gilbert, 2001). To date, the environmental and biological factors affecting the entire plankton community composition and size structure in coastal lagoons have been little studied, preventing a deep understanding of plankton food web functioning in these ecosystems (Trombetta *et al.*, 2021).

Mediterranean lagoons are considered particularly vulnerable environments that deserve attention as the Mediterranean region is deemed as one of the most sensitive areas regarding on-going global warming and increased extreme climate events (Ferrarin *et al.*, 2014; IPCC, 2021). Temperature rise is expected to favour the selection of smaller-sized phytoplankton with profound consequences for the aquatic food web structure and efficiency from the very basis to the upper trophic levels (Boyce and Worm, 2015; Polovina *et al.*, 2012; Sommer *et al.*, 2017b). However, local dynamics are strongly site-specific, and processes within each region can modulate the overall patterns observed at a global level (Chust *et al.*, 2014; Lomas *et al.*, 2022).

A significant temporal variation in the trophic levels of dominant fish strongly related to fish size has been demonstrated in Cabras Lagoon, the largest coastal lagoon in Sardinia (Italy, Mediterranean Sea) and one of the most important for fishing productivity in the Mediterranean basin (Como *et al.*, 2018). Cabras Lagoon is known to be a highly complex eutrophic ecosystem (Magni *et al.*, 2022). Consistently, very high  $\delta^{15}\text{N}$  values in the small size seston (<55 µm), likely related to a high input of anthropogenic nitrogen from a large catchment area, resulted in a  $^{15}\text{N}$  enrichment maintained from primary producers up to the predators at higher trophic levels (Como *et al.*, 2012). In addition, studies on ecological data collected since 1999 showed the affirmation of very small-sized phytoplankton (linear cell size <10 µm) in Cabras Lagoon (Padedda *et al.*, 2012; Pulina *et al.*, 2011, 2012). To verify if it was only an occasional feature of phytoplankton in this ecosystem, the present work focused on the size structure of phytoplankton from Cabras Lagoon. Two different phytoplankton size classes, the picophytoplankton (cell size <3 µm; Pico) and the Utermöhl

fraction of phytoplankton (cell size  $>3 \mu\text{m}$ ; Utermöhl Fraction of Phytoplankton, UFP), were studied over three additional years (2017, 2018, 2019) not analyzed in previous studies. Inter-annual and seasonal phytoplankton dynamics were related to the temporal variation of environmental conditions in the lagoon and, for the first time in this ecosystem, to the temporal dynamics of zooplankton. We aimed: i) to confirm the dominance of small-sized phytoplankton in the lagoon also during the latest investigation period, suggesting that it is an intrinsic aspect characterizing phytoplankton of this ecosystem that reveals a low energy-efficient trophic web; ii) to detect which environmental variables affected significantly the temporal dynamics of the two phytoplankton size classes (Pico and UFP); iii) to assess if and how zooplankton grazing activity may have affected the temporal dynamics of the two size classes in the considered years.

## Materials and Methods

### Study area

Cabras Lagoon (western Mediterranean Sea;  $39^{\circ}56'37''$  N,  $08^{\circ}28'43''$  E; *Figure S1*) is a eutrophic lagoon with a mean depth of 1.5 m and about 23.8 km<sup>2</sup> large, where phytoplankton are the main primary producers. Freshwater originates from a large catchment of about 430 km<sup>2</sup> and mostly arrives in the northern part of Cabras Lagoon, carried by the river Rio Mare'e Foghe. The communication with the adjacent Gulf of Oristano is on the southern opposite side of the lagoon and it happens mainly via narrow creeks that flow into a southernmost canal. Because of an ecological dataset available since 1999 (Padedda *et al.*, 2010, 2012; Pulina *et al.*, 2011, 2012, 2016, 2020; Satta *et al.*, 2014), Cabras Lagoon has been included in the "Marine ecosystems of Sardinia" site of the Italian Long Term Ecological Research network since 2006 (<https://deims.org/d5071d21-9c8f-47ccb534-1b1162a5e09c>; Morabito *et al.*, 2018; Pugnetti *et al.*, 2013).

### Samplings and chemical-physical analyses

Data on phytoplankton and environmental variables derive from monthly samplings carried out over three years, from April 2017 to October 2019. Water samples were collected at three sampling stations, located along the natural gradient of salinity: station 1, near the lagoon's connection with the sea; station 2, in the center; station 3, near the main input of freshwater (*Figure S1*).

*In situ*, water transparency (Tra) was measured with a Secchi disk, and water temperature (Tem) and salinity (Sal) were detected using a multiparameter probe (YSI 6600V2). Water samples for phytoplankton and nutrients analyses were collected at about 20 cm depth: 250 mL samples for studying UFP (cell size  $>3 \mu\text{m}$ ) were immediately fixed in 2% acid Lugol's solution, and 100 mL samples for studying Pico (cell size  $<3 \mu\text{m}$ ) and autotrophic nanoflagellates (cell size  $>3 \mu\text{m}$ ) were immediately fixed with formaldehyde (2%). In the laboratory, orthophosphate (P-PO<sub>4</sub>), ammonium (N-NH<sub>4</sub>), nitrate (N-NO<sub>3</sub>), nitrite (N-NO<sub>2</sub>), silicate (Si-SiO<sub>4</sub>), total phosphorus (TP) and total nitrogen (TN) concentrations were obtained according to the methods of Strickland and Parsons (1972).

Zooplankton samples were collected seasonally (in summer

and autumn 2017, in winter, spring and summer 2018, in winter 2019) at station 2, with a hand-held conic net (mouth diameter 31 cm, mesh size 10  $\mu\text{m}$ ), towed for 30 m at 50 cm depth with speed of 0.8 - 1 m s<sup>-1</sup> and immediately fixed in 70% ethanol.

### Phytoplankton analyses

Five mL of fixed water samples were analyzed to estimate UFP cell density using Utermöhl's technique (Utermöhl, 1958), with an inverted microscope (Axiovert 25, Zeiss, Oberkochen, Germany). Cell counts were made at magnifications of 200 X and 400 X for more easily identifiable species, and at magnifications of 1000 X for smaller cells from at least 10% of the total bottom area of the settling chamber. Non-fixed samples were also observed to facilitate the identification of species. The species were identified according to the taxonomic literature listed in Pulina *et al.* (2012) and grouped at the class level for data analysis. Cell sizes of 20 randomly selected individuals of each taxon were measured in each sample. Cell volumes were calculated approximating the shape of each taxon to a geometric shape following Hillebrand *et al.* (1999), Vadrucchi *et al.* (2013) and "Atlas of Shapes" powered by LifeWatch Italy ([http://phytobioimaging.unisalento.it/Products/AtlasOfShapes.aspx?ID\\_Tipo=0](http://phytobioimaging.unisalento.it/Products/AtlasOfShapes.aspx?ID_Tipo=0)). For each taxon, the cell carbon content was obtained by applying the conversion formulas suggested by Menden-Deuer and Lessard (2000) to the mean cell volume. The biomass of each taxon, in terms of carbon content, was calculated by multiplying cell carbon content by the corresponding cell density.

For Pico cell counts, from 2 to 5 mL (depending on cell density) of fixed water samples were filtered onto 0.2- $\mu\text{m}$  black-stained polycarbonate membranes (Nucleopore). Duplicate slides were prepared and observed using a microscope (Axiovert 100, Zeiss) equipped with green (BP520–560 nm/FT580 nm/LP590 nm) and blue (BP450–490 nm/FT510 nm/LP520 nm) filter sets, at 1000 X magnification. At least 20 random fields of view and a minimum of 100 randomly selected cells of each taxon (picocyanobacteria rich in phycoerythrin, P-PE; picocyanobacteria rich in phycocyanin, P-PC; picoeukaryotes, P-Euk) were counted for each slide (MacIsaac and Stockner, 1993). When it was not possible to recognize a specific taxon, cells were counted as picophytoplankton undetermined (Pico-und). Total picophytoplankton (Pico) counts were the sum of P-PE, P-PC, P-Euk, and Pico-und. Cell sizes of about 200 randomly selected individuals from each Pico group were measured on each slide. The cell volume of each group was calculated assuming that the shape of the cell was spherical or cylindrical with hemispheric ends and using the Bratbak formulas (1985). To calculate cell carbon content, the conversion factors of 250 fg C  $\mu\text{m}^{-3}$  and of 220 fg C  $\mu\text{m}^{-3}$  were applied to the mean volume of picocyanobacteria and P-Euk, respectively (Tamigneaux *et al.*, 1995). The biomass of each Pico group was obtained by multiplying the cell carbon content by the corresponding cell density.

Autotrophic nanoflagellates (cell size 3-20  $\mu\text{m}$ ) were also counted during Pico cell counts. At least 200 randomly selected flagellate cells were counted and measured for each slide. Their cell volume and biomass were calculated as described above for UFP.

For each sample, total UFP and Pico density and biomass

were calculated as the sum of the cell density and biomass, respectively, of all taxa and groups observed, including autotrophic nanoflagellates into UFP.

Since the arithmetic average of individual morphological traits does not properly represent the community structure because common and rare taxa receive the same weight when calculating the average, weighted average of volume was also estimated for the whole UFP community according to Kruk *et al.* (2015).

## Zooplankton analyses

In the laboratory, fixed zooplankton samples were fractionated into mesozooplankton (Meso) and microzooplankton (Micro), using sieves of mesh size 200  $\mu\text{m}$  and 20  $\mu\text{m}$ , respectively, and preserved in fresh 70% ethanol. Each subsample was brought to the volume of 10 mL or 5 mL (depending on the richness of the subsample) by removal of the supernatant and analysed under a stereoscope (Leica M80, Wetzlar, Germany) at 20-50 X magnification to perform a qualitative-quantitative analysis by direct enumeration of the organisms in 1 mL aliquots.

For each subsample, counted specimens were identified according to available literature (Avancini *et al.*, 2006; Cicero *et al.*, 2016). The individuals were grouped at different levels: phylum (Nematoda, Rotifera), subphylum (Naupli), class (Copepoda, Ostracoda, Bivalvia, Gastropoda, Polychaeta, Appendicularia), subclass (Teleostei), order (Decapoda). Taxa that never contributed at least 2% to total zooplankton density were not included into the data analyses. Metazoan eggs were not considered either, as they are not primary consumers.

## Data analyses

Monthly data from the three sampling stations of the entire study period were included in all statistical analyses. In addition, seasons were considered as: winter = January – March, spring = April – June, summer = July – September, autumn = October – December. Autumn 2019 was only represented by the month October.

To ascertain annual, seasonal, and spatial differences in Pico density and biomass, in UFP density and biomass, and selected environmental variables (Tra, Tem, Sal, N-NH<sub>4</sub>, N-NO<sub>2</sub>, N-NO<sub>3</sub>, Si-SiO<sub>4</sub>, P-PO<sub>4</sub>), three-way analysis of variance (ANOVA) was performed considering three fixed factors (years, seasons and

sampling stations), individually and combined. Since autumn 2019 included only the month “October”, one-way ANOVA was also performed to assess significant differences in the same aforementioned biological and environmental variables among October 2017, October 2018, and October 2019, to confirm (or not) what we detected for the season “autumn”. Prior to ANOVA analyses, the two assumptions were confirmed for all pooled data: normal distribution (Kolmogorov-Smirnov test) and homogeneity of variance (Bartlett test). All data required logarithmic [ln(x)] transformation to meet ANOVA assumptions.

When significant differences in the dependent variables based on factors were observed, post hoc Tukey’s pairwise comparisons test was performed.

The Generalized Linear Mixed Model (GLMM) was used (R package MASS) to investigate the influence of selected environmental variables related to the total biomass and total density of Pico and UFP. This kind of model is an extension of the Generalized Linear Model (GLM) and provides a more flexible approach to analyse non-normal data when random effects are present (Bolker *et al.*, 2008). Two GLMM were applied: one model included Tem, Tra, Sal, P-PO<sub>4</sub>, N-NO<sub>3</sub>, N-NO<sub>2</sub>, N-NH<sub>4</sub> and P-PO<sub>4</sub> as fixed terms (predictor variables) and the seasonality as the random variable (random term; Pinheiro *et al.*, 2012); the other model included the same selected environmental variables and the months as the random term.

All the statistical analyses were performed using R 2.15.2 software (R Core Team, 2020), and an *a priori* level of significance was established at  $p < 0.05$ .

## Results

### Pico and UFP inter-annual and seasonal dynamics

Monthly values of Pico and UFP biomass and density along the study period were reported in *Table S1*.

Considering Pico inter-annual dynamics, after an initial peak in spring 2017 and another peak in spring 2018, maximum values of both biomass and density were detected at the end of the study period, from spring to autumn 2019 (*Figures S2, S3*). Pico biomass and density observed in 2019 resulted significantly different from the values observed in 2017 and 2018 (*Figures S2, S3*; *Table 1*; Tukey’s test:  $p < 0.001$ ). Specifically, spring density in 2019 was higher than spring density values in 2018

**Table 1.** Results of the three-way ANOVA (*F*-test and *P*-value) to assess significant differences in cell biomass and density of picophytoplankton (Pico) and Utermöhl fraction of phytoplankton (UFP) among years, seasons and sampling stations. Interaction between factors (years x seasons, years x stations, years x seasons x stations) are also shown. Significant results ( $p < 0.05$ ) are in bold.

	Years		Seasons		Stations		Years x seasons		Years x stations		Seasons x stations		Years x seasons x stations	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
Cell biomass														
Pico	<b>10.30</b>	<b>&lt;0.001</b>	<b>4.64</b>	<b>0.01</b>	0.01	0.99	<b>3.47</b>	<b>0.01</b>	0.03	0.99	0.11	0.99	0.33	0.97
UFP	2.21	0.12	<b>3.90</b>	<b>0.01</b>	0.18	0.83	<b>3.44</b>	<b>0.01</b>	0.61	0.65	0.64	0.70	0.36	0.96
Cell density														
Pico	<b>21.31</b>	<b>&lt;0.001</b>	<b>6.84</b>	<b>&lt;0.001</b>	0.07	0.93	<b>6.66</b>	<b>&lt;0.001</b>	0.15	0.96	0.22	0.97	0.41	0.93
UFP	<b>16.80</b>	<b>&lt;0.001</b>	1.98	0.13	0.02	0.98	<b>2.40</b>	<b>0.04</b>	0.68	0.61	0.42	0.86	0.35	0.96

(Tukey's test:  $p=0.040$ ), and summer and autumn values of both biomass and density observed in 2019 were higher than the values observed in summer and autumn in 2017 and 2018 (Tukey's test:  $p<0.001$ ). Even Pico biomass (ANOVA:  $F=26.21$ ,  $p=0.002$ ) and density (ANOVA:  $F=33.48$ ,  $p=0.001$ ) detected in October 2019 were significantly higher than the biomass (Tukey's test:  $p=0.025$ ) and density (Tukey's test:  $p=0.019$ ) observed in October 2018 (Tukey's test:  $p=0.020$ ) and higher than the biomass (Tukey's test:  $p<0.001$ ) and density (Tukey's test:  $p<0.001$ ) observed in October 2017.

Pico biomass and density varied significantly among seasons of the same year as well (Figures S2, S3; Table 1). Autumn Pico biomass and density were higher than winter biomass (Tukey's test:  $p=0.020$ ) and density (Tukey's test:  $p<0.001$ ) in 2019, and summer Pico density was also higher than winter density in the same year (Tukey's test:  $p=0.002$ ). Spring Pico biomass and density were higher than summer biomass (Tukey's test:  $p=0.030$ ) and density (Tukey's test:  $p=0.020$ ) in 2017.

Considering UFP inter-annual dynamics, maximum values of both biomass and density were detected at the beginning of the study period, from summer 2017 to winter 2018 (Figures S2, S3). UFP density in summer 2017 was significantly higher than the values observed in summer 2019, and UFP density in autumn 2017 was higher than the values observed in autumn 2018 (Figures S2, S3; Table 1; Tukey's test:  $p=0.004$  and Tukey's test:  $p=0.031$ , respectively). Even UFP density in October 2017 was higher than in October 2018 and 2019 with a significant difference only with October 2018 (ANOVA:  $F=26.39$ ,  $p=0.002$ ; Tukey's test:  $p=0.003$ ). UFP biomass detected in winter 2018 was significantly higher than the values of biomass observed in winter 2019 (Figures S2, S3; Table 1; Tukey's test:  $p=0.022$ ). In addition, while no significant differences in UFP biomass among autumns of the different years were observed, UFP biomass in October 2017 was significantly higher than the biomass detected in October 2018 and in October 2019 (ANOVA:  $F=18.39$ ,  $p=0.005$ ; Tukey's test:  $p=0.004$  and Tukey's test:  $p=0.03$ , respectively).

UFP biomass and density also varied among seasons of the same year (Figures S2, S3; Table 1): autumn UFP biomass and density were higher than spring biomass (Tukey's test:  $p=0.029$ ) and density (Tukey's test:  $p=0.031$ ) in 2017, and winter UFP biomass was higher than spring biomass in 2018 (Tukey's test:  $p=0.022$ ).

Excluding spring 2017, Pico contribution to total phytoplankton biomass and density increased throughout the study period, while UFP contribution decreased (Figure S4). The highest Pico contribution to total phytoplankton biomass (up to 30%) and density (up to 96%) was observed in spring, summer, and autumn 2019. On the contrary, the highest UFP contribution to total phytoplankton biomass (up to 86%) and density (up to 99%) was observed from summer 2017 to winter 2018.

Significant differences in Pico and UFP biomass and density were not detected among sampling stations in Cabras Lagoon during the investigated years (Table 1).

### Pico and UFP composition

Considering Pico composition, P-PE (mean linear cell size  $1.16\pm 0.27 \mu\text{m}$  and mean volume  $0.86\pm 0.43 \mu\text{m}^3$ ) and P-PC (mean linear cell size  $1.23\pm 0.40 \mu\text{m}$  and mean volume

$0.96\pm 0.55 \mu\text{m}^3$ ) were observed throughout the investigated period with similar contributions to total biomass and density (Figures S5, S6). P-PC dominated in all seasons in 2017 and 2019 with contributions always  $>60\%$  to Pico total biomass and density. P-PE maximum contributions to Pico total biomass and density were observed in autumn 2017 and in winter 2018 ( $>20\%$ ). P-Euk (mean linear cell size  $1.50\pm 0.01 \mu\text{m}$  and mean volume  $1.77\pm 0.01 \mu\text{m}^3$ ) were observed only in spring 2018, when dominated Pico total biomass and density (85% and 71%, respectively).

Considering UFP composition (Figures S5, S6), Trebouxiophyceae (mainly *Chlorella* sp., mean linear cell size  $3.84\pm 0.69 \mu\text{m}$  and mean volume  $8.05\pm 0.30 \mu\text{m}^3$ ) contributed the most to total UFP biomass in spring 2017 (43%). This class was even more important in terms of density, with a contribution of at least 13% up to a maximum of 41% from spring 2017 to winter 2018. Mediophyceae (mainly *Cyclotella/Thalassiosira* sp., mean linear cell size  $9.74\pm 6.73 \mu\text{m}$  and mean volume  $869.01\pm 1680.44 \mu\text{m}^3$ ) dominated the total UFP biomass, especially at the beginning of the study period, from summer 2017 to spring 2018 and in autumn 2018 with a contribution  $>60\%$ . The contribution of Mediophyceae to total phytoplankton biomass and density was lower in the second part of the investigation period, especially from winter 2019 onwards, when a significant presence affirmation of other groups was also detected. Autotrophic nanoflagellates (mainly Pyramimonadophyceae and Cryptophyceae undetermined, mean linear cell size  $7.33\pm 0.25 \mu\text{m}$  and  $9.39\pm 3.54 \mu\text{m}$ , respectively, and mean volume  $37.57\pm 8.50 \mu\text{m}^3$  and  $130.68\pm 115.65 \mu\text{m}^3$ , respectively) contributed the most from spring 2018 to the end of the study period, dominating the total UFP biomass in winter (61%) and autumn 2019 (57%). The contribution of Bacillariophyceae (mainly Pennales undetermined, mean linear cell size  $9.01\pm 2.12 \mu\text{m}$  and mean volume  $15.31\pm 2.21 \mu\text{m}^3$ ) and of Cyanobacteria (mainly *Pseudanabaena catenata* Lauterborn, mean linear cell size  $4.09\pm 1.00 \mu\text{m}$  and mean volume  $6.82\pm 2.30 \mu\text{m}^3$ ) to total phytoplankton biomass was major from summer 2018 onwards (up to 23% in autumn 2019 and up to 18% in summer 2018, respectively), and the contribution of Chrysophyceae (mainly *Keophysiron spirale* (Lackey) Conrad, mean linear cell size  $4.33\pm 0.81 \mu\text{m}$  and mean volume  $27.16\pm 12.68 \mu\text{m}^3$ ) to total phytoplankton biomass was up to 18% in summer 2019. Chlorophyceae (mainly *Monoraphidium minutum* (Nägeli) Komárková-Legnerová, mean linear cell size  $3.42\pm 0.66 \mu\text{m}$  and mean volume  $1.82\pm 1.49 \mu\text{m}^3$ ) contributed especially in terms of density. Contribution was always  $>20\%$  from autumn 2017 to spring 2019 and this class dominated the total UFP density in spring 2017 (57%), in autumn 2018 (62%), and in autumn 2019 (78%).

As described above, the most abundant UFP taxa detected in the Cabras Lagoon throughout the investigated period were small in linear cell size ( $<10 \mu\text{m}$ ) and in mean cell volume ( $<10^3 \mu\text{m}^3$ ; Figure S7). Considering all UFP taxa observed during the entire study period, those proliferating with cell densities  $>10^7 \text{ cell L}^{-1}$  were those with a mean cell volume  $<1000 \mu\text{m}^3$  and taxa proliferating with cell densities  $>10^8 \text{ cell L}^{-1}$  were those with a mean cell volume  $<100 \mu\text{m}^3$  (Figure S8). In addition, the observed change in UFP composition resulted in a reduction in the UFP community volume in the second part of the investigation period (Figure S9).

## Inter-annual and seasonal environmental conditions

Monthly values of the selected environmental variables along the study period were reported in *Table S2*.

According to the GLMM with seasonality as a random term, among the selected environmental variables, Temp, Sal, N-NH<sub>4</sub> and P-PO<sub>4</sub> significantly influenced the seasonal dynamic of both Pico and UFP throughout the multiannual period in Cabras Lagoon, but exactly in an opposite way: the environmental variables that positively influenced one phytoplankton size class, negatively influenced the other, and *vice-versa* (Table 2). The same findings came out from the GLMM with monthly as a random term, but in this case, N-NH<sub>4</sub> affected significantly only UFP dynamics (Table S3).

Considering only the environmental variables affecting significantly phytoplankton throughout the multiannual period in Cabras Lagoon, the values of Sal and P-PO<sub>4</sub> differed significantly among sampling stations (Table 3). These

differences never occurred in the same season of the same year, according to Tukey's test. Looking at the inter-annual and seasonal dynamics, summer and autumn Temp values increased during the years, and autumn 2019 was statistically warmer than autumn 2017 (Figure S10; Table 3; Tukey's test:  $p=0.002$ ). October 2019 was significantly warmer than October 2017 and October 2018 (ANOVA:  $F=303.3$ ,  $p<0.001$ ; Tukey's test:  $p<0.001$ ). Spring and summer Temp were significantly higher than autumn in 2017 (Tukey's test:  $p<0.001$ ; winter 2017 data are not available) and winter in 2018 (Tukey's test:  $p<0.001$ ). Instead, summer and autumn were significantly warmer than winter in 2019 (Tukey's test:  $p<0.001$ ).

Sal in 2017 was significantly higher than Sal detected in 2018 and 2019 (Figure S10; Table 3; Tukey's test:  $p<0.001$ ). Specifically, Sal observed in spring, summer and autumn 2017 was higher than the values observed in the same seasons of 2018 (Tukey's test:  $p<0.001$ ). Sal in October 2017 was significantly higher than Sal in October 2018 and in October 2019 (ANOVA:  $F=31.5$ ,  $p=0.001$ ; Tukey's test:  $p=0.001$  and Tukey's test:

**Table 2.** Results (*t*-value and *p*-value) of the generalized linear mixed-effect model (GLMM) applied on the Picophytoplankton (Pico) and Utermöhl fraction of phytoplankton (UFP) total cell density and on the Pico and UFP total biomass along the study period (2017, 2018, 2019) in Cabras Lagoon. The selected environmental variables (Secchi disk transparency, Tra; water temperature, Temp; salinity, Sal; ammonium, N-NH<sub>4</sub>; nitrate, N-NO<sub>3</sub>; nitrite, N-NO<sub>2</sub>; orthophosphate, P-PO<sub>4</sub>; reactive silica, Si-SiO<sub>4</sub>) were used as fixed terms and the seasonality was used as random term. Significant effects ( $p<0.05$ ) are in bold.

	Cell biomass				Cell density			
	Pico		UFP		Pico		UFP	
	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>	<i>t</i>	<i>p</i>
Tra	-0.365	0.716	0.911	0.366	1.003	0.320	-0.825	0.412
Temp	0.849	0.399	<b>-3.692</b>	<b>0.000</b>	<b>1.820</b>	<b>0.044</b>	<b>-3.971</b>	<b>0.000</b>
Sal	<b>-2.452</b>	<b>0.017</b>	<b>6.842</b>	<b>0.000</b>	-1.159	0.251	<b>6.677</b>	<b>0.000</b>
N-NH <sub>4</sub>	<b>2.418</b>	<b>0.019</b>	<b>-2.995</b>	<b>0.004</b>	1.045	0.300	-1.589	0.117
N-NO <sub>3</sub>	0.906	0.368	-1.027	0.308	-0.038	0.970	-0.883	0.381
N-NO <sub>2</sub>	-1.820	0.074	1.942	0.057	-1.161	0.250	1.510	0.136
P-PO <sub>4</sub>	<b>-2.845</b>	<b>0.006</b>	1.425	0.159	<b>-2.852</b>	<b>0.006</b>	<b>2.024</b>	<b>0.047</b>
Si-SiO <sub>4</sub>	0.182	0.856	0.841	0.404	1.237	0.221	-0.700	0.487

**Table 3.** Results of the three-way ANOVA (*F*-test and *P*-value) to assess significant differences in the selected environmental variables (Secchi disk transparency, Tra; water temperature, Temp; salinity, Sal; ammonium, N-NH<sub>4</sub>; nitrite, N-NO<sub>2</sub>; nitrate, N-NO<sub>3</sub>; orthophosphate, P-PO<sub>4</sub>; reactive silica, Si-SiO<sub>4</sub>) among years, seasons and sampling stations. Interaction between factors (years x seasons, years x stations, seasons x stations, years x seasons x stations) are also shown. Significant results ( $p<0.05$ ) are in bold.

	Years		Seasons		Stations		Years x seasons		Years x stations		Seasons x stations		Years x seasons x stations	
	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>	<i>F</i>	<i>p</i>
	Tra	<b>6.03</b>	<b>0.00</b>	<b>9.41</b>	<b>&lt;0.001</b>	0.18	0.83	<b>3.42</b>	<b>0.01</b>	1.73	0.16	1.46	0.22	1.12
Temp	1.16	0.32	<b>54.03</b>	<b>&lt;0.001</b>	0.19	0.83	<b>4.52</b>	<b>0.00</b>	0.05	0.99	0.09	0.99	0.13	0.99
Sal	<b>67.83</b>	<b>&lt;0.001</b>	<b>6.09</b>	<b>&lt;0.001</b>	<b>7.14</b>	<b>0.00</b>	<b>10.14</b>	<b>&lt;0.001</b>	0.73	0.58	0.25	0.96	0.36	0.96
N-NH <sub>4</sub>	<b>23.09</b>	<b>&lt;0.001</b>	<b>3.12</b>	<b>0.03</b>	1.99	0.15	2.32	0.06	0.30	0.87	0.31	0.93	0.23	0.99
N-NO <sub>2</sub>	<b>4.91</b>	<b>0.01</b>	<b>6.04</b>	<b>0.00</b>	1.68	0.20	<b>5.69</b>	<b>&lt;0.001</b>	0.26	0.90	0.87	0.52	0.37	0.95
N-NO <sub>3</sub>	<b>4.51</b>	<b>0.02</b>	<b>16.97</b>	<b>&lt;0.001</b>	<b>6.65</b>	<b>0.00</b>	<b>4.84</b>	<b>0.00</b>	1.16	0.34	<b>6.08</b>	<b>&lt;0.001</b>	1.06	0.41
P-PO <sub>4</sub>	2.64	0.06	0.92	0.40	<b>7.48</b>	<b>0.00</b>	<b>0.15</b>	<b>0.99</b>	0.05	0.99	2.40	0.05	0.05	0.99
Si-SiO <sub>4</sub>	<b>12.45</b>	<b>&lt;0.001</b>	<b>19.85</b>	<b>&lt;0.001</b>	<b>10.56</b>	<b>&lt;0.001</b>	0.74	0.62	<b>4.86</b>	<b>0.00</b>	0.11	0.98	0.54	0.85

$p=0.007$ , respectively). Sal in spring 2017 was also higher than Sal in spring 2019 (Tukey's test:  $p=0.002$ ). Sal in winter 2018 was higher than Sal in winter 2019 (Tukey's test:  $p<0.001$ ). Considering seasonal intra-annual differences, Sal in spring and summer was higher than Sal in winter in 2019 (Tukey's test:  $p<0.001$ ); Sal detected in autumn was significantly higher than in winter (Tukey's test:  $p<0.001$ ) and spring (Tukey's test:  $p=0.03$ ) in 2019.

Regarding algal nutrients,  $N-NH_4$  values in 2017 were significantly higher than values observed in 2018 (Figure S10; Table 3; Tukey's test:  $p<0.001$ ) and 2019 (Tukey's test:  $p<0.001$ ). Specifically, values observed in spring 2017 were significantly higher than values observed in spring 2018 (Tukey's test:  $p<0.001$ ) and 2019 (Tukey's test:  $p<0.001$ ); values observed in autumn 2017 were significantly higher than values observed in autumn 2018 (Tukey's test:  $p=0.01$ ). Even the concentrations detected in October 2017 were only significantly higher than the concentrations detected in October 2018 (ANOVA:  $F=29.02$ ,  $p=0.002$ ; Tukey's test:  $p=0.001$ ).  $N-NH_4$  values varied significantly among seasons only in 2017, with spring values higher than summer ones (Tukey's test:  $p=0.04$ ).  $P-PO_4$  values detected in 2017 were significantly higher than the values observed in 2018 and 2019 (Figure S10; Table 3; Tukey's test:  $p<0.001$ ). Specifically,  $P-PO_4$  values observed in summer and spring 2017 were significantly higher than values observed in summer (Tukey's test:  $p<0.001$ ) and spring 2019 (Tukey's test:  $p=0.04$ ). Also,  $P-PO_4$  in summer 2018 was significantly higher than  $P-PO_4$  in summer 2019 (Tukey's test:  $p<0.001$ ).  $P-PO_4$  values varied significantly among seasons only in 2018, with winter values lower than summer values (Tukey's test:  $p<0.001$ ).

## Zooplankton temporal dynamics and composition

Zooplankton density was  $<1$  ind  $L^{-1}$  until winter 2018 and increased strongly in spring 2018 (maximum peak of 2.59 ind  $L^{-1}$ ) and in summer 2018 (Figure S11). The Meso fraction dominated the total zooplankton density from autumn 2017 (60%) to spring 2018 (100%), while the Micro fraction dominated in summer 2017 (70%), in summer 2018 (90%), and in winter 2019 (60%) (Figure S11).

Regarding zooplankton composition, Polychaeta larvae represented zooplankton in both Meso and Micro fractions in summer 2017 (100% to the total zooplankton density) and contributed 63% to the total Micro density in autumn 2017 as well (Figure S12; Table S4). Copepods (mainly adults and copepodite stages) dominated total Meso density from autumn 2017 (91%) to spring 2018 (85%) and contributed up to 50% (mainly as copepodites and naupli) to the total Micro density in winter 2018, showing the same contribution of Crustacea naupli. Rotifera contributed 44% to the total Meso density in winter 2018 and dominated both Meso and Micro in summer 2018 (88% and 98%, respectively) and in winter 2019 (56% and 89%, respectively). Nematoda contributed 4% to the total Micro density in autumn 2017.

## Discussion

In this work, the temporal dynamics of two phytoplankton size classes, Pico (cell size  $<3 \mu m$ ) and UFP (cell size  $>3 \mu m$ ), were investigated throughout a multiannual period of almost

three study years, from April 2017 to October 2019, in a eutrophic Mediterranean lagoon. Results showed that the size structure of phytoplankton changed strongly, alternating periods of prevalence of UFP on Pico and *vice versa*: UFP dominated at the beginning of the study, especially from summer 2017 to winter 2018; Pico dominated the total phytoplankton density in spring 2017 and from summer 2018 to the end of the study period. Pico maximum cell density peak of  $1.87 \times 10^9$  cell  $L^{-1}$  observed in summer 2019 doubled the UFP maximum peak of  $0.95 \times 10^9$  cells  $L^{-1}$  observed in autumn 2017.

The contribution of Pico to total phytoplankton biomass was clearly lower, up to 30% in spring 2017 and in autumn 2019 when maximum peaks of about  $300 \mu g C L^{-1}$  were detected. The Pico community was mainly composed of picocyanobacteria, with a higher contribution of P-PC for a longer period compared to the contribution of P-PE. This is in line with the known preference of P-PC for salinity  $<25$  (Bec *et al.*, 2011; Xia *et al.*, 2017), normally measured in Cabras Lagoon (Pulina *et al.*, 2012). Such a relevant presence of Pico in Cabras Lagoon agrees with other studies that have indicated increased Pico presence in other Mediterranean eutrophic coastal lagoons (Bec *et al.*, 2011; Collos *et al.*, 2009; Pulina *et al.*, 2018; Sorokin *et al.*, 2004). Pico are well known to be the main contributors of biomass and primary productivity in oligotrophic regions, such as oceanic ecosystems (Wang *et al.*, 2022), whereas they have been little investigated in coastal lagoons so far. For this reason, our findings add new ecological information on this component, underlining the necessity of considering them even in studies on phytoplankton from eutrophic transitional environments. The Pico cell density detected in Cabras Lagoon was higher than the values detected previously in other eutrophic Sardinian Lagoons (up to  $10^8$  cells  $L^{-1}$ ; Pulina *et al.*, 2017 and 2018), and closer to values detected in other Mediterranean coastal lagoons (Venice Lagoon, Italy; Sorokin *et al.*, 2004; Bagnas Lagoon, France; Bec *et al.*, 2011), in an Australian coastal lagoon (Coorong Lagoon, Shapira *et al.*, 2010) and other brackish waters worldwide (Baltic Sea; Mazur-Marzec *et al.*, 2013; Neuse River Estuary, USA, Paerl *et al.*, 2020). Comparison of biomass values is harder since Pico biomass data are scarce in the literature. Pico biomass values observed in Cabras Lagoon in the current work were much higher than the values detected previously in the other Sardinian lagoons (up to  $60 \mu g C L^{-1}$ ; Pulina *et al.*, 2017 and 2018) and much higher than the few values available for coastal waters (about  $10 \mu g C L^{-1}$  in Chesapeake Bay, Atlantic Ocean; Cai *et al.*, 2010, and in Ionian Sea; Caroppo *et al.*, 2014) and for brackish waters (up to  $180 \mu g C L^{-1}$  in Baltic Sea; Mazur-Marzec *et al.*, 2013).

In the present study, small-sized cells mainly represented UFP along the entire study period: they were  $<10 \mu m$  in mean linear cell size and  $<10^3 \mu m^3$  in mean cell volume. An UFP compositional change was also observed from spring 2018 onwards: the increased abundance of Pico at the end of the study was accompanied by the increased abundance of smaller species of Chlorophyceae (*Monoraphidium minutum*) and Bacillariophyceae (Pennales undetermined), smaller autotrophic nanoflagellates, and a smaller filamentous species of cyanobacteria (*Pseudanabaena catenata*), which replaced the larger Mediophyceae (*Cyclotella/Thalassiosira* sp.) which dominated at the beginning of the study when the maximum UFP abundances were observed. Already published results on

ecological data collected previously reported small-sized phytoplankton in Cabras Lagoon (Padedda *et al.*, 2012; Pulina *et al.*, 2011, 2012, 2016). Specifically, cyanobacteria of picoplanktonic cell size (about 3  $\mu\text{m}$ ) were the most abundant during the ecological studies performed in the periods 2000-2002 and 2007-2009 in this ecosystem, together with other small species (cell size 3-10  $\mu\text{m}$ ) of *Cyclotella*, *Fragilaria*, *Thalassiosira*, *Chlorella* and with *Monoraphidium minutum* among the most abundant taxa. The size and taxonomic structure of phytoplankton detected in previous studies were confirmed by data reported in the present work obtained with a more appropriate method of analysis applied to investigate Pico, the smallest fraction of phytoplankton.

Hillebrand *et al.* (2022) reported that phytoplankton cell volume around 100  $\mu\text{m}^3$ , such as the main representative mean cell volume of the size class UFP in Cabras Lagoon, is a very interesting ecological trait: carbon fixation was, in fact, reported to be maximum at cell sizes around 100  $\mu\text{m}^3$ , leading to the lowest respiration to photosynthesis ratio at this value of cell volume.

In the present work, inter-annual and seasonal dynamics of Pico and UFP were related to the temporal dynamics of selected environmental variables, and of micro- and meso-zooplankton for the first time in Cabras Lagoon. October 2019 (autumn 2019 in this study) was significantly warmer than October 2017 and October 2018 in Cabras Lagoon and it was characterised by temperatures very close to the summer values. In addition, with the narrowest range of temperature variation, from 24.3  $^{\circ}\text{C}$  to 27.9  $^{\circ}\text{C}$ , the summer 2019 plus October 2019 was the longest warm period in the present study. Changes in species composition with a shift towards smaller-sized organisms, as well as decrements in the average cell size of individual species, have been predicted as a response to ocean warming (Sommer *et al.*, 2017b). A strong positive correlation between temperature and small phytoplankton, even among temperature and picocyanobacteria, has been extensively reported in the literature in relation to a direct effect of temperature or due to an indirect effect mediated by a lower nutrient availability in the water and/or a more intense zooplankton grazing activity (Collos *et al.*, 2009; D'Alelio *et al.*, 2020; Peter and Sommer, 2012, 2013).

In an experimental activity, a plankton community from Cabras Lagoon was incubated in a laboratory excluding mesozooplankton and it was exposed to heating (Pulina *et al.*, 2020). A decreased abundance of Pico was detected under warming together with a significant change in UFP taxonomic composition, consisting of the increase of the smaller *Chlorella* sp., which replaced the larger *Cyclotella* sp. and *Thalassiosira* sp. It was related to a direct and positive effect of heating on ciliate abundance in the absence of their predators (mesozooplankton), as well as a taxonomic composition shift of ciliates with the affirmation of ciliates Scuticociliatida that preyed upon nanoflagellates and Pico.

Indeed, studies on eutrophic areas show grazing as one of the main drivers behind picocyanobacteria seasonal dynamics, surpassing the role of temperature and nutrient availability in structuring communities (Caroppo, 2015). Interpreting phytoplankton dynamics in relation to environmental changes in field-based study is tricky, because a lot of different variables including both environmental variables (at local and global scale) and biological relationships can interact and overlap with

each other in nature. In the present work, the period summer-autumn 2019 (maximum Pico contribution) was the longest warm period, and values of salinity and of N-NH<sub>4</sub> and P-PO<sub>4</sub> were lower than the values observed in the summer 2017-winter 2018 period (maximum UFP contribution). According to statistics, smaller UFP species and Pico benefited from these conditions of warmer water and lower salinity and N-NH<sub>4</sub> and P-PO<sub>4</sub> concentrations at the end of the study period. Small-cell sized phytoplankton are expected to be favoured under lower nutrient concentration because of a higher surface area to volume ratio, a smaller diffusion boundary layer that enables rapid nutrient exchange through the cell surface, and a better competitive ability in nutrient utilisation compared to large-cell sized phytoplankton (Harris, 1986; Litchman and Klausmeier, 2008; Van de Waal *et al.*, 2020). Temperature, salinity and N-NH<sub>4</sub> concentrations, strictly related to the freshwater flows through the lagoon, were identified as the main drivers of plankton community dynamics investigated in the hypersaline Coorong Lagoon (South Australia): during high freshwater flow, phytoplankton-zooplankton interactions dominated the food web, while at low flow, bacteria, viruses, and nano/picoplankton interactions were more dominant (Hemraj *et al.*, 2017).

Mesozooplankton (mainly copepods) are well known to feed on microzooplankton and phytoplankton >10  $\mu\text{m}$  in linear cell size (especially diatoms large 20–200  $\mu\text{m}$ ) and >500  $\mu\text{m}^3$  in cell volume (Lewandowska and Sommer, 2010; Lewandowska *et al.*, 2014; Peter and Sommer, 2012). Pico, nanoflagellates, flagellates >100  $\mu\text{m}$ , and filamentous cyanobacteria are instead well-known to be inedible for copepods (Paul *et al.*, 2021). Among smaller zooplankton, rotifers are generally under-represented in zooplankton feeding studies, they are more abundant in freshwaters, and they prey upon bacteria, heterotrophic flagellates, small ciliates and phytoplankton (Bonecker and Aoyagui, 2005; Sweeney *et al.*, 2022). Rotifers may be eaten by copepods but can also compete with copepods for the same prey (Fussman, 1996). Instead, ciliate feeding habits were studied in depth: they are recognized to prey mainly upon pico- and nanoplanktonic cell size, but they are also able to eat larger phytoplankton, such as many diatoms (Johansson *et al.*, 2004; Strom *et al.*, 2001). Therefore, according to the literature, the cell size and taxa composition structure of phytoplankton observed in the present work in Cabras Lagoon was not palatable to copepods, and they may have been more likely eaten by microzooplankton. Unfortunately, apart from rotifers, naupli and small copepod stages, the available data-set does not provide deeper insights into microzooplankton, and data on ciliates and heterotrophic phytoplankton are not available in the current work. A significant role of ciliates can only be hypothesized in Cabras Lagoon also according to the results described by Pulina *et al.* (2020) which demonstrated a significant grazing activity of ciliates on phytoplankton in this ecosystem. Looking at the temporal dynamic of phytoplankton and zooplankton in the present study, the strong decrement of UFP abundance and the shift in UFP composition towards smaller UFP taxa started in spring 2018, just when the maximum peak of mesozooplankton copepods was detected in the lagoon. Copepods may have preyed upon microzooplankton since microzooplankton was not detected at this time in the lagoon.

In turn, the increased abundance of the smallest phytoplankton may have been determined by the reduced grazing activity of microzooplankton on them. Rotifers appeared in Cabras Lagoon in winter 2018, just when salinity started to decrease, confirming the strong physiological constraints of saltwater on most rotifers, and that they are particularly sensitive to salinity changes, as highlighted by Suikkanen *et al.* (2013). In a study where plankton composition was investigated in three different North African coastal lagoons, rotifers dominated the zooplankton biomass in the less salty lagoon (Lake Manzala, Egypt, mean salinity of 10.85; Ramdani *et al.*, 2009). The peaks of maximum abundance of rotifers observed in summer 2018 and in winter 2019 in Cabras Lagoon coincided with a strong decreased abundance of Mediophyceae, suggesting an intense rotifer grazing pressure on this phytoplankton group, while smaller phytoplankton thrived. This suggests a preferred feeding of rotifers on *Cyclotella/Thalassiosira* sp., but not on the smaller phytoplankton such as *Monoraphidium minutum*, small Pennales undetermined, autotrophic nanoflagellates and the filamentous *Pseudanabaena catenata*. Since the abundance of these smaller phytoplankton started to increase from spring 2018 onwards, just when the highest abundances of copepods first and rotifers then were detected, we can hypothesize that both copepods and rotifers preyed upon ciliates as well, reducing their grazing pressure on the smaller phytoplankton. In Mar Menor Lagoon (Spain), a Mediterranean coastal lagoon where the composition and temporal dynamics of both phytoplankton and zooplankton were investigated, copepods abundance decreased in late summer when a maximum peak of ciliates and of larger phytoplankton were also observed. On the contrary, the abundance of autotrophic nanoflagellates decreased in this period, suggesting a preferred feeding for copepods on large phytoplankton and ciliates, and for ciliates on the autotrophic nanoflagellates (Gilbert, 2001).

In Cabras Lagoon, the longer warm period in 2019 may have kept high the grazing rates of copepods and/or rotifers on ciliates whose decreased grazing pressure on Pico may explain the strong affirmation of Pico from spring 2019 onwards, together with the decrement in salinity and concentrations of N-NH<sub>4</sub> and P-PO<sub>4</sub>. Indeed, the literature reported extensively a direct positive effect of experimental warming on the planktonic top-predators (mesozooplankton or microzooplankton when mesozooplankton are absent) and indirect cascade effects in the trophic web on phytoplankton (Lewandowska and Sommer, 2010; Lewandowska *et al.*, 2014; Sommer *et al.*, 2007; Vidussi *et al.*, 2011).

## Conclusions

The dominance of phytoplankton large <10 μm in mean linear cell size and <10<sup>3</sup> μm<sup>3</sup> in mean cell volume in Cabras Lagoon was highlighted in this work. According to our findings, we retain that both environmental variations and changes in zooplankton composition affected synergistically phytoplankton in Cabras Lagoon during the study period 2017-2019. Specifically, higher temperature and lower salinity and N-NH<sub>4</sub> and P-PO<sub>4</sub> values, together with a higher grazing pressure of rotifers on the larger UFP species and very likely of copepods and rotifers on ciliates determined a shift towards

the affirmation of even smaller UFP taxa and of Pico. This framework suggests a longer planktonic trophic web in Cabras Lagoon, including copepods, rotifers, ciliates, and small phytoplankton that may translate into a low availability of energy for planktivorous fish.

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Online supplementary material:

Figure S1. Study site and location of sampling stations.

Figure S2. Inter-annual and seasonal dynamics of (A) Picophytoplankton (Pico) and (B) Utermöhl Fraction of Phytoplankton (UFP) total biomass in Cabras Lagoon throughout the study period (2017, 2018, 2019). \*Autumn 2019 was represented only by October.

Figure S3. Inter-annual and seasonal dynamics of (A) Picophytoplankton (Pico) and (B) Utermöhl Fraction of Phytoplankton (UFP) total density in Cabras Lagoon throughout the study period (2017, 2018, 2019). \*Autumn 2019 was represented only by October.

Figure S4. Picophytoplankton (Pico) and Utermöhl Fraction of Phytoplankton (UFP) contribution to (A) total phytoplankton biomass and (B) density in Cabras Lagoon throughout the study period (2017, 2018, 2019). \*Autumn 2019 was represented only by October.

Figure S5. Contribution of (A) each Picophytoplankton (Pico) group to total picophytoplankton (Pico) biomass (P-PE, picocyanobacteria rich in phycoerythrin; P-PC, picocyanobacteria rich in phycocyanin; Pico-Euk, picoeukaryotes; Pico-und, picophytoplankton undetermined), and contribution of (B) each Utermöhl Fraction of Phytoplankton (UFP) group to total UFP biomass (Bac, Bacillariophyceae; Chl, Chlorophyceae; Chr, Chrysophyceae; Cya, Cyanophyceae; Dino, Dinophyceae; Fla, autotrophic nanoflagellates; Med, Mediophyceae; Tre, Trebouxiophyceae) in Cabras Lagoon along the study period (2017, 2018, 2019). \*Autumn 2019 was represented only by October.

Figure S6. Contribution of (A) each Picophytoplankton (Pico) group to total Picophytoplankton (Pico) density (P-PE, picocyanobacteria rich in phycoerythrin; P-PC, picocyanobacteria rich in phycocyanin; Pico-Euk, picoeukaryotes; Pico-und, picophytoplankton undetermined), and contribution of (B) each Utermöhl Fraction of Phytoplankton (UFP) group to total UFP density (Bac, Bacillariophyceae; Chl, Chlorophyceae; Chr, Chrysophyceae; Cya, Cyanophyceae; Dino, Dinophyceae; Fla, autotrophic nanoflagellates; Med, Mediophyceae; Tre, Trebouxiophyceae) in Cabras Lagoon along the study period (2017, 2018, 2019). \*Autumn 2019 was represented only by October.

Figure S7. Inter-annual and seasonal dynamics of mean cell volume of all UFP taxa observed in Cabras Lagoon during the study period (2017, 2018, 2019). Taxa abundance is not included. \*Autumn 2019 was represented only by October.

Figure S8. Scatterplot of mean cell volume (x axis) versus cell density (y axis) of each Utermöhl Fraction of Phytoplankton taxon recorded in Cabras Lagoon along the study period (2017, 2018, 2019).

Figure S9. Inter-annual and seasonal dynamics of UFP community volume in Cabras Lagoon during the study period (2017, 2018, 2019). Weighted-average of volume of the entire UFP community is showed. \*Autumn 2019 was represented only by October.

Figure S10. Inter-annual and seasonal dynamics of selected environmental variables (A, temperature; B, salinity; C, ammonium, N-NH<sub>4</sub>; D, orthophosphates, P-PO<sub>4</sub>) in Cabras Lagoon throughout the study period (2017, 2018, 2019). \*Autumn 2019 was represented only by October.

Figure S11. Inter-annual and seasonal dynamics of (A) Mesozooplankton (Meso) and (B) Microzooplankton (Micro) total density and their contribution to total zooplankton density in Cabras Lagoon throughout the study period (2017, 2018, 2019).

Figure S12. Contribution of (A) each taxon of Mesozooplankton (Meso) and of (B) Microzooplankton (Micro) to total zooplankton density (Biv, Bivalvia veliger; Cop, Copepoda; Nau, Crustacea Naupli; Pol, Polychaeta larvae; Rot, Rotifera; Nem, Nematoda) observed in Cabras Lagoon throughout the study period (2017, 2018, 2019).

Table S1. Monthly mean and Standard Deviation (SD) of Picophytoplankton (Pico) and Utermöhl Fraction of Phytoplankton (UFP) cell density and biomass along the study period.

Table S2. Monthly mean and Standard Deviation (SD) of the selected environmental variables (Tra, Secchi disk transparency; Temp, water temperature; Sal, salinity; N-NH<sub>4</sub>, ammonium; N-NO<sub>3</sub>, nitrate; N-NO<sub>2</sub>, nitrite; P-PO<sub>4</sub>, orthophosphate; Si-SiO<sub>4</sub>, reactive silica) along the study period.

Table S3. Results (t-value and p-value) of the Generalized Linear Mixed-Effect Model (GLMM) applied on the Picophytoplankton (Pico) and Utermöhl Fraction of Phytoplankton (UFP) total cell density and on the Pico and UFP total biomass along the study period (2017, 2018, 2019) in Cabras Lagoon. The selected environmental variables (Tra, Secchi disk transparency; Temp, water temperature; Sal, salinity; N-NH<sub>4</sub>, ammonium; N-NO<sub>3</sub>, nitrate; N-NO<sub>2</sub>, nitrite; P-PO<sub>4</sub>, orthophosphate; Si-SiO<sub>4</sub>, reactive silica) were used as fixed terms and months were used as random term. Significant effects (p<0.05) are in bold.

Table S4. Total density (ind L<sup>-1</sup>) and relative abundance (%) of main zooplankton groups detected in Cabras Lagoon.