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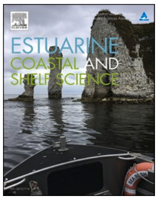

Effects of an experimental summer heatwave on a small-sized plankton community from a Mediterranean coastal lagoon

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ABSTRACT

Coastal lagoons are vulnerable ecosystems subjected to increasing anthropogenic pressures and the effects of climate change, notably in the Mediterranean region. Extreme events, such as heatwaves are predicted to become more intense and frequent, and to date their effects on planktonic food webs in transitional ecosystems have been poorly studied.

We simulated a 14-days lasting summer heatwave in laboratory to investigate its experimental effects on the whole natural plankton community collected from Cabras Lagoon (Western Mediterranean). We exposed the natural community to +5 °C increase in temperature compared to a control (environmental temperature) in presence and in absence of planktonic apical predators.

A long planktonic trophic web was detected, composed of several trophic levels dominated by very small-sized taxa: *Mnemiopsis leidyi* – rotifers – ciliates – Chlorophyceae as phytoplankton – heterotrophic nanoflagellates – picocyanobacteria and bacteria. This revealed low energy available for planktivorous fish in the lagoon which has a great economic value in relation to fishery's activities. The simulated summer heatwave strongly affected the heterotrophic community, triggering defense mechanisms in rotifers, altering ciliate abundance (with short-term increases and long-term declines), and reshaping their taxonomic composition. Warming also indirectly influenced heterotrophic nanoflagellates through intensified ciliate grazing. In contrast, bacterial and autotrophic components remained largely stable, sustaining heterotrophic changes without significant decline.

This study provides new insights on how heatwaves can affect the structure of a small-sized planktonic trophic web in coastal lagoons, contributing to a broader understanding of heatwave impacts on coastal lagoon functioning and productivity under future warmer scenarios.

1. Introduction

Marine heatwaves are prolonged periods lasting at least 5 days of anomalously warm sea surface temperatures that exceed typical conditions (Hobday et al., 2016). They are categorized from moderate to extreme events based on the degree to which daily temperature exceeds the 90th percentile of the local climatology calculated on at least 30-years daily data (Hobday et al., 2018). The Mediterranean Sea has experienced many strong heatwaves over the last decades which have triggered unprecedented climate-driven mass mortality events (Garrabou et al., 2022). The strongest marine heatwaves detected in the Mediterranean during the last 40 years took place in summer (Martínez et al., 2023), consistent with recent findings attributing this increasing trend to an intensification of the Mediterranean Sea surface temperature in summer (Ciappa, 2022). The Mediterranean region is one of the most responsive to global climate change (Lionello and Scarascia, 2018). The observed warming in the Mediterranean is expected to continue at a greater rate than the global average along with a stronger increase in the frequency and intensity of extreme weather events, such as heatwaves, intense rainfall, and droughts (Lazoglou et al., 2024).

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Coastal lagoons are among the Mediterranean aquatic ecosystems considered most vulnerable to climate change (Hassoun et al., 2025). Shallowness and restricted connection with the open sea make these transitional environments particularly sensitive to atmospheric forcing, tidal activity, and river runoff, as well as pollution, eutrophication, and modifications caused by human activities (Marinov et al., 2008; Kennish, 2016; Soria et al., 2022). Therefore, changes in environmental conditions may have a severe impact on these fragile ecosystems, which are also among the most biodiverse and productive in the world, and are crucial for the provision of important ecosystem goods and services, specifically those related to fishery and aquaculture (Pérez-Ruzafa et al., 2024).

Plankton communities have a relevant role in sustaining the functioning and productivity of coastal lagoons, serving as feeding for many species of fish and birds, and supporting a wide range of human activities, such as fisheries, shellfish farming and tourism (Cruz et al., 2020). Phytoplankton cell size is used as a proxy for determining how palatable these organisms are to their grazers (Hillebrand et al., 2022), and even if it is not a rule, energy flow in aquatic pelagic food webs is largely based on size specific predator–prey relationships (Boyce et al., 2015). A shift to smaller sized plankton has been extensively reported as a response to warming, with severe consequences for the ecosystem functioning (Albini et al., 2025). Smaller size phytoplankton at the base of the food web represents lower quality food for mesozooplankton (200–2000 μm) and indicates a low energy-efficient trophic web (e.g., microbial loop), because the carbon flow between primary producers and mesozooplankton is long, with several trophic levels, including heterotrophic nanoflagellates (cell size <20 μm) and microzooplankton (20–200 μm) (Di Pane et al., 2024). In this case, the relative losses of energy, nutrients and carbon can reduce fish productivity as described by Berglund et al. (2007).

To date, literature supports both direct and indirect effects of warming on plankton. Direct effects solicit physiological and metabolic responses of organisms, generally resulting in a reduced body size of both autotrophs and heterotrophs, an increased grazing rate (functional effects) and density of grazers (numerical effects) of micro- and mesozooplankton (Calbet et al., 2023; de Juan et al., 2023). However, these direct effects overlap with indirect effects of warming on plankton, for example those mediated by changes in size selective grazing, or by variations in water physical-chemical property, such as nutrient limitation and viscosity which alter selection on phytoplankton's morpho-functional related traits (Sommer et al., 2017; Di Pane et al., 2024).

The effects of heatwaves on a natural plankton community considering several trophic levels have been investigated through numerous experimental activities in the Baltic Sea (Müren et al., 2005; Aberle et al., 2007; Sommer et al., 2007; Lewandowska and Sommer, 2010; Lewandowska et al., 2014). Major findings indicate strong responses of heterotrophic grazers to warming, highlighting that heterotrophic processes are more sensitive to temperature changes than autotrophic ones. In all these case studies, warming affected phytoplankton indirectly, through enhanced grazing, with ciliates playing an important role when copepod abundance declined.

Despite the urgent need for such knowledge as a basis for forecasting future alterations and implementing sustainable management practices, to date this topic has been studied very little in the Mediterranean basin. Only a few experimental studies have investigated entire plankton communities from Mediterranean coastal lagoons (Vidussi et al., 2011; Pulina et al., 2020; Courboulès et al., 2021, 2022; Soulié et al., 2022, 2023; Eiglane et al., 2025).

In the present work, we simulated a 14-days lasting summer heatwave in laboratory to investigate its experimental effects on the whole plankton community collected from Cabras Lagoon, the largest coastal lagoon in Sardinia (Italy, Western Mediterranean), which has a great economic value in relation to fishery activities (11 fishing cooperatives with about 130 fishermen in total). This lagoon is part of the Italian network for Long-Term Ecological Research LTER (https://deims.org/d5071d21-9c8f-47cc-b534-1b1162a5e09c), and its trophic state, metabolism and phytoplankton ecology have been studied since 1999 (Padedda et al., 2010, 2012; Pulina et al., 2011). Cabras Lagoon is a shallow (mean depth of 1.6 m) and brackish coastal lagoon, with salinity values ranging from 6.82 PSU in March 2024 to 16.35 in October 2024, and with temperature values ranging from 11.52 °C in January 2024 to 27.78 °C in August 2024 according to monitoring field (unpublished). Previous investigations have reported the presence of very small-sized phytoplankton (linear cell size <10 μm and mean cell volume < 10^3 μm^3) in this ecosystem, and Pulina et al. (2023) hypothesized a longer planktonic trophic web that includes small phytoplankton at the base, nanoflagellates, ciliates, rotifers, and copepods. In addition, the small-sized fishes *Atherina boyeri* and *Engraulis encrasicolus* have been reported feeding on zooplankton in Cabras Lagoon (Como et al., 2018). Further, the massive occurrence of the invasive ctenophore *Mnemiopsis leidyi* is likely to exert negative impacts on Sardinian coastal lagoons over the last 10 years, not only through intensive grazing on zooplankton but also by reducing fish catches and the efficiency of fishing gears (Diciotti et al., 2016, 2017).

To date it has not been possible to directly assess whether Cabras Lagoon has experienced aquatic heatwaves as defined by the metrics of Hobday et al. (2016, 2018) due to the insufficient availability of long-term daily water temperature data for this site. However, it can be at least stated that Cabras Lagoon has faced several atmospheric heatwaves, mainly in summer, with peaks of air temperature anomalies exceeding +8 °C (Pulina et al., 2026).

In the present experimental study, we aimed to distinguish direct warming effects from those indirectly mediated by grazing. We exposed the summer plankton community to a +5 °C increase in temperature with respect to recorded *in situ* one, both in absence and in presence of mesozooplankton. This second condition also included the ctenophore *M. leidyi*, plentiful in the lagoon. Our approach focused on the size-structure of the incubated plankton community, with the objective of assessing which planktonic size classes, among picoplankton, nanoflagellates, phytoplankton, microzooplankton and mesozooplankton, benefited by warming (Fig. 1).

2. Material and methods

An indoor experiment was conducted following the protocol (a specifically designed framework) by Pulina et al. (2026). Water samples were collected at a sampling station located in the centre of Cabras Lagoon on July 23rd' 2024. The selected sampling site is the central one of three stations located along a salinity gradient of the lagoon and used for traditional multiannual field ecological monitoring programs carried out in this ecosystem. Salinity was intermediate (14.24 PSU) in this sampling site compared to the value detected in the southern station close to the sea (15.0 PSU), and to the northern station near to the main freshwater input (10.5 PSU). The central station was selected following the previous experimental activity performed by Pulina et al. (2020). In addition, in relation to a condition of less confinement, the presence of *Mnemiopsis leidyi* was lower in the central station compared to its abundance observed in the other sampling sites, facilitating field sample collection activities.

Water samples were collected from the superficial water layer (0.30 m depth), considered representative of the entire water column due to the shallowness and the well mixed regime of the lagoon (Padedda et al., 2012). Collected samples were immediately transferred to the laboratory. Further, the entire plankton community (unfiltered water) was incubated in semi-transparent plastic buckets and exposed in triplicate for 14 days to +5 °C increase in water temperature compared to the control units at environmental temperature corresponding in the control units to the *in situ* average water temperature of Cabras Lagoon in July, based on long-term data. A +5 °C increased temperature has been chosen because represented the maximum sea surface temperature

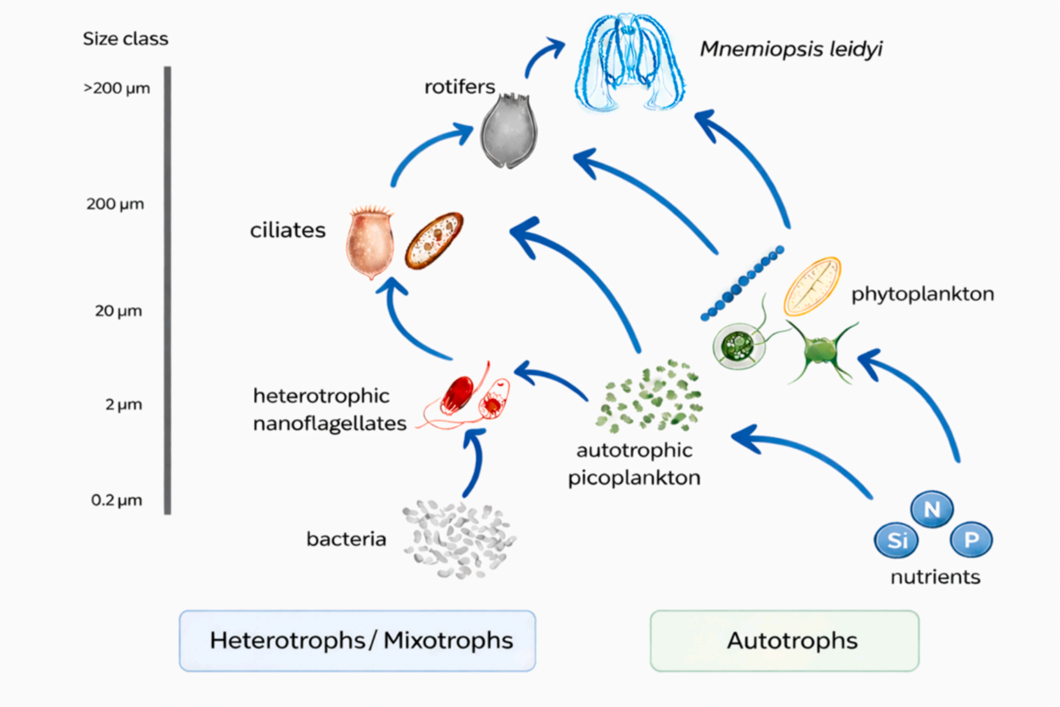

Fig. 1. Conceptual model of the planktonic trophic web investigated during the heatwave experiment in Cabras Lagoon, modified and adapted from Weisse and Montagnes (2022).

anomaly value recorded in the Mediterranean (Marullo et al., 2023) and the maximum intensity value detected during the marine heatwaves observed in the last decade in Mediterranean (Martínez et al., 2023). In addition, +5 °C treatment has been used also in one of the few previous studies on the heatwave effects on a Mediterranean coastal lagoon plankton community (Soulié et al., 2023).

To investigate the effects of the simulated heatwave on plankton community even in the absence of the larger zooplankton, 200 µm mesh-filtered water collected from the lagoon was inserted in other semi-transparent plastic buckets in laboratory and exposed in triplicate for 14 days to +5 °C increase in water temperature compared to the control triplicate at environmental temperature.

In total, 12 incubation units of 10 L were used, corresponding to four experimental conditions, each performed in triplicate:

- natural plankton community at *in situ* environmental temperature with mesozooplankton and *Mnemiopsis leidyi* as a top predator (Env M);
- natural plankton community at +5 °C above *in situ* environmental temperature with mesozooplankton and *Mnemiopsis leidyi* as a top predator (HW M);
- natural plankton community at *in situ* environmental temperature with microzooplankton as top predator (Env);
- natural plankton community at +5 °C above *in situ* environmental temperature with microzooplankton as top predator (HW).

A water heater connected to a thermostat was inserted in each unit. The thermostats permitted to regulate, control and keep constant the temperature in all buckets throughout the experiment. Light conditions simulated the ambient summer season (July): total irradiance of 2000 µmol photons m^{-2} s^{-1} (air light intensity) in a 15:9-h light/dark cycle. In each unit, water was continuously and gently mixed using an aquarium stirrer throughout the entire experiment.

2.1. Physical-chemical and plankton analyses

All the methods applied for laboratory measurements and sample analyses were described in detail in Pulina et al. (2026).

Water temperature, salinity, pH and dissolved oxygen were measured in each incubation unit with a multi-parameter probe (HL7, OTT-Hydrolab) every day at the same time throughout the experiment. At the beginning of the incubation (day 0) and at specific time (day 2, 6, 9, 14), water samples were collected from each unit for the analyses of algal nutrients (nitrate N-NO_3, nitrite N-NO_2, ammonium N-NH_4, orthophosphate P-PO_4 and silicate Si-SiO_4) carried out spectrophotometrically following Strickland and Parsons (1969). Dissolved Inorganic Nitrogen (DIN) was obtained summing up N-NO_3 + N-NO_2 + N-NH_4.

Water for the analysis of autotrophic (APP) and heterotrophic (bacteria) picoplankton (cell size 0.2 - 2 µm), autotrophic and heterotrophic (HNF) nanoflagellates (cell size 2 - 20 µm), Utermöhl fraction of phytoplankton (cell size >2 µm) and microzooplankton (body size 20 - 200 µm) was collected according to the above-mentioned time scheme (days 0, 2, 6, 9, 14) from each incubation unit.

To characterize the initial community of larger zooplankton (body size >200 µm, mesozooplankton and *M. leidyi*), an additional 10 L of unfiltered lagoon water was sampled at day 0. Final samples of the larger zooplankton community were collected from each unit on day 14.

Mesozooplankton and *M. leidyi* were analyzed using water samples collected at the start (T0) and at the end (T4) of the experiment as reported in detail in Pulina et al. (2026). At the end of the experiment (T4), the entire volume of each experimental unit was filtered through a 30-µm mesh, in order to collect all retained organisms and life stages, including small zooplankton and potential resting eggs, and the retained material was subsequently preserved in 70% ethanol for analysis. All organisms were taxonomically identified, counted and measured using light microscopy from a subsample corresponding to at least 10% of the total sample volume.

Microzooplankton and Utermöhl fraction of phytoplankton were analyzed according to the Utermöhl technique (1958), autotrophic nanoflagellates and HNF were analyzed using epifluorescence microscopy (Zeiss, Axiovert 100), and picoplankton samples (APP and bacteria) were processed by CytoFlex flow cytometer (Beckman Coulter), as reported in detail in Pulina et al. (2026).

2.2. Data and statistical analyses

Autotrophic nanoflagellates and Utermöhl fraction of phytoplankton data were merged into a single autotrophic group of cell size >2 μm, hereafter called phytoplankton.

Data visualization was conducted using Jupyter Notebook with Python version 3.13.5 and statistical analyses were performed using software R version 4.5.0. Taxonomic icons were obtained from PhyloPic (https://www.phylopic.org) under a Creative Commons license. A linear mixed-effects model (LME) was applied to evaluate the effect of temperature (fixed effect) on biological variables (cell density of APP and bacteria; cell biomass of HNF, phytoplankton and microzooplankton) and algal nutrients (DIN, P-PO_4, Si-SiO_4), while accounting for temporal variability (random effect). A generalized least squares (GLS) model with temperature as a fixed effect was first used to assess the effect of temperature without considering temporal variability. Subsequently, the LME model was applied with temperature as a fixed effect and experimental days as a random effect, to account for repeated measures over time. With the aim to intercept crucial experimental times, the analyses have been made considering different temporal ranges: from day 0 to day 6 (day 0 – day 6), from day 0 to day 9 (day 0 – day 9) and from day 0 to day 14 (day 0 – day 14). The significance of the random effect was tested by means of a likelihood ratio test (LRT) comparing the LME model with the GLS model. To assess significant differences among treatments, an ANOVA was performed on the LME model, followed by Tukey's post hoc test to identify which treatment pairs differed significantly, including on specific experimental days. Data were log- or square root-transformed before the analysis to meet the assumptions of linear models (normality and homoscedasticity of residuals).

For the larger zooplankton, differences in total zooplankton density among treatments at the end of the experiment (day 14) were tested using a Kruskal–Wallis non-parametric test followed by Dunn's post hoc pairwise comparisons with Bonferroni correction. Because the initial sampling time (start) was not replicated, no inferential analyses across sampling times or repeated-measures models were applied to zooplankton data.

Kruskall-Wallis test has been also used to assess significant differences among water temperature treatments during the experimental activities.

3. Results

3.1. Physical-chemical conditions

In the control treatments (Env, Env M), temperature values were maintained at 27.01 ± 0.25 °C, whereas in the heatwave treatments (HW, HW M) the temperature was kept at 31.84 ± 0.22 °C (Kruskall–Wallis test: HW – Env $p < 0.001$, HW M – Env M $p < 0.001$; Fig. 2a).

Regarding nutrients, after an initial increase, DIN decreased gradually in all treatments, with a stronger decrease in the heatwave treatments (HW and HW M) from day 2 to day 9 (Fig. 2b–Table 1). From day 9 to day 14, an abrupt DIN decline was recorded in the HW, HW M, and Env M treatments, whereas in the control treatment without the larger zooplankton (Env) the decrease was more attenuated. P-PO_4 fluctuated in all treatments throughout the experiment, while Si-SiO_4 increased progressively from day 0 to day 14 in all treatments, with a stronger increase under heatwave (HW and HW M) (Fig. 2c and d, Table 1).

Among the other physical-chemical parameters, salinity increased

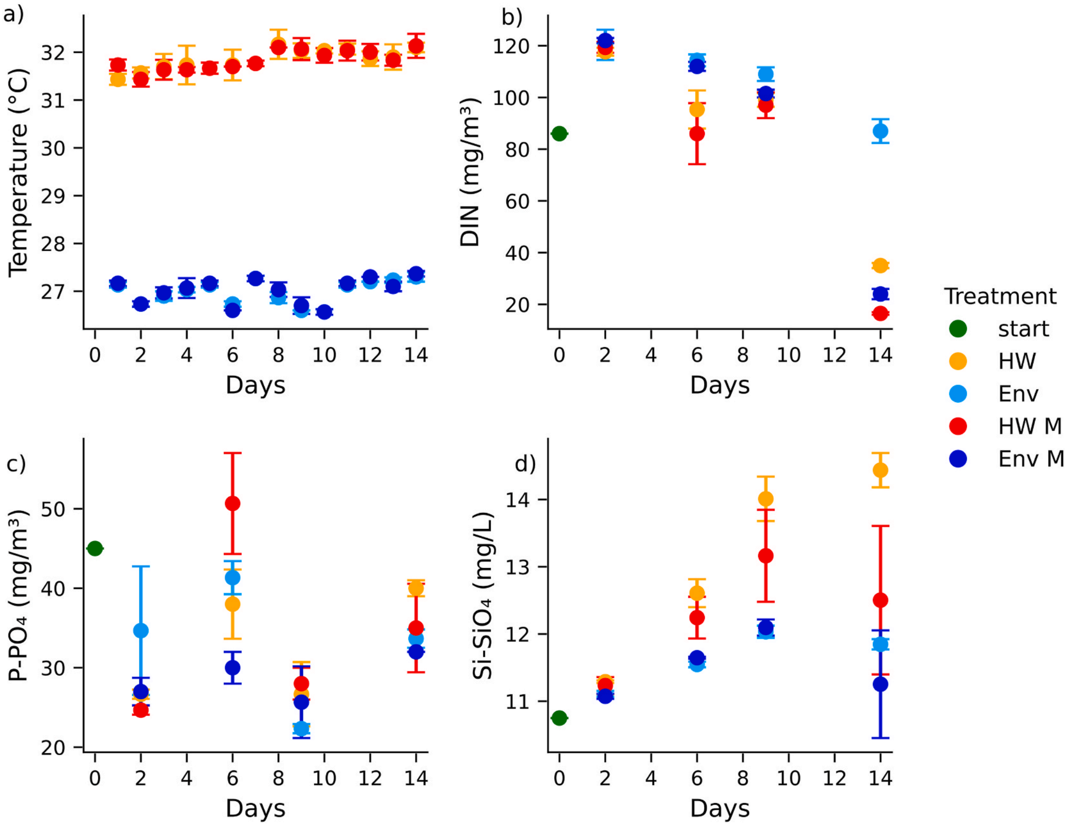

Fig. 2. Temporal trends of key environmental variables measured during the experiment in the different treatments. Panels show: **(a)** water temperature (°C); **(b)** dissolved inorganic nitrogen, DIN (mg m^{-3}); **(c)** dissolved phosphorus P-PO_4 (mg m^{-3}); and **(d)** dissolved silica Si-SiO_4 (mg L^{-1}). Points represent mean values ± standard deviation (SD) across replicates (n = 3) and sampling days.

Table 1

Results of linear mixed-effects (LME) model for each response variable (DIN = dissolved inorganic nitrogen, P-PO$_4$ = orthophosphate, Si-SiO$_4$ = silicate), with temperature (explanatory variable) in presence (HW M) and in absence (HW) of larger zooplankton as a fixed factor and time (day) as a random effect. Not significant results ($p > 0.05$) are in italics.

		day 0 - 6		day 0 - 9		day 0 - 14	
Response variable	**Explanatory variable**	F	P	F	P	F	P
DIN	HW	17.15	<0.001	13.07	0.002	17.15	<0.001
	Time x HW		<0.001		<0.001		<0.001
	HW M	6.33	0.025	*3.41*	*0.080*	5.03	0.034
	Time x HW M		<0.001		0.004		<0.001
P-PO$_4$	HW	*0.01*	*0.931*	*1.05*	*0.319*	*0.01*	*0.931*
	Time x HW		<0.001		<0.001		<0.001
	HW M	*4.08*	*0.063*	4.59	0.045	*2.56*	*0.122*
	Time x HW M		0.001		0.046		0.090
Si-SiO$_4$	HW	30.29	<0.001	18.23	<0.001	30.30	<0.001
	Time x HW		<0.001		<0.001		<0.001
	HW M	7.806	0.014	10.65	0.004	10.15	0.004
	Time x HW M		<0.001		<0.001		<0.001

consistently in the heatwave treatments from 14.24 ± 0.00 PSU at day 0 to 20.94 ± 0.23 PSU in HW and to 20.87 ± 0.54 PSU in HWM at day 14 (Table S1).

3.2. Larger zooplankton

Total abundance of larger zooplankton increased from the beginning (day 0) to the end (day 14) of the experiment (Fig. 3a), although this increase was less pronounced under heatwave conditions (HW M). At day 0, the larger zooplankton community was strongly dominated by small-sized individuals of *M. leidyi* (Fig. 4a) with total density approximately 5.3 ind. L^{-1}. Most individuals were larval and juvenile stages with a maximum total length of about 2 cm. In contrast, only a few rotifer individuals were detected at the start (approximately 0.01 ind. L^{-1}). At the end of the experiment (day 14), total zooplankton density differed significantly among treatments (Kruskal–Wallis test, $p = 0.047$) and a pronounced increase in rotifers was observed. Small rotifers, belonging to the genus *Brachionus* spp. Became the dominant taxon under both experimental conditions (Env M and HW M), accounting for more than 90% of the mesozooplankton community and reaching densities exceeding 10^7 ind. L^{-1} (Fig. 3a and 4a). *Brachionus* spp. with a low abundance but with resting eggs were observed under warming.

In contrast, *M. leidyi* was completely absent from all final samples. Post hoc Dunn comparisons with Bonferroni correction revealed no significant pairwise differences among treatments. In particular, no significant difference in total zooplankton density was detected between environmental and heatwave conditions when mesozooplankton was present (ENV M vs HW M; $p = 1.0$). Meroplanktonic larvae of annelida, bivalvia, cirripedia contributed only marginally and only at the final sampling point, confirming their minor role in the pelagic component despite detectable absolute abundances (Fig. 4a). Throughout the experiment, Cyclopoida and Harpacticoida were detected only with occasional observations of their exuviae at very low relative abundances, mainly under heatwave conditions (≤10% in HW M; Fig. 4a).

3.3. Microzooplankton

Significant effects of heatwave on microzooplankton and ciliates were observed from day 0 to day 6, when biomass of microzooplankton increased at the heatwave treatments (HW and HW M) in relation to a strong increase of ciliate abundance (Fig. 3b and 4b, Table 2 and S2). After day 6, microzooplankton and ciliate abundance decreased strongly under warming conditions (HW and HW M). Statistically significant effects of the heatwave on ciliates, in the presence of the larger zooplankton, were also detected from day 0 to day 9 and from day 0 to day 14 (Fig. 3b and 4b, Table S2).

Heterotrophic phytoplankton, mainly the dinoflagellate *Oxyrrhis marina*, dominated the starting community of microzooplankton at day 0 (Fig. 4b). Ciliate contribution increased in all treatments on day 2, but more strongly in the heatwave treatments (HW and HW M) contributing with values > 75% to total microzooplankton biomass. Ciliates were the main component of the microzooplankton community in all the treatments on day 6 and day 9, while their contribution was lower in presence of the larger zooplankton (HW M and Env M) on day 14. Heterotrophic phytoplankton, mainly the Chrysophyceae *Rhizochrysis* sp., contributed with a value of 80% in the environmental treatment in absence of the larger zooplankton (Env) on day 14 (Fig. 4b).

Regarding ciliate taxonomic composition, small Oligotrichia (linear cell size 14.06 μm, mean cell volume 5992.76 μm^3) dominated the starting ciliate community on day 0 (Fig. 4c). Significant effects of heatwave were detected on the biomass of Oligotrichia, Euplotia and Scuticociliatia, which increased strongly from day 0 to day 6 (Fig. S1 and Table S2). Indeed, more subclasses contributed most to total ciliate biomass under warming (HW and HW M) on day 6, mainly Choreotrichia, Euplotia and Oligotrichia. In contrast, under environmental conditions (Env and Env M), Euplotia alone accounted for more than 90% of the total ciliate biomass. Euplotia (linear cell size 24.25 μm, mean cell volume 36013.93 μm^3) dominated the total biomass of ciliates in all treatments on day 9, and only under warming (HW and HW M) on day 14 when Scuticociliatia (linear cell size 12.30 μm, mean cell volume 334.35 μm^3) contributed to total ciliate biomass with values > 20% under environmental conditions (Env and Env M, Fig. 4c).

3.4. Heterotrophic nanoflagellates

Total biomass of HNF decreased in all treatments, with a stronger decline under heatwave conditions (HW and HW M) from day 2 onwards (Fig. 3c). A significant effect of heatwave in the presence of larger zooplankton (HW M) was detected from day 0 to day 6 and of both heatwave conditions (HW and HW M) from day 0 to day 9 and from day 0 to day 14 (Table 2).

3.5. Phytoplankton

The starting phytoplankton community on day 0 was dominated by species of mean cell volume < 10^3 μm^3, with the three most abundant species represented by the chlorophyte *Monoraphidium* cf. *minutum* (Chlorophyceae), mean cell volume 1.62 μm^3; the *Merismopedia* sp. (Cyanophyceae), mean cell volume 0.63 μm^3; the undetermined pennate Bacillariophyceae, mean cell volume 6.48 μm^3 (Fig. 5).

Significant effects of heatwave on total phytoplankton biomass were not detected during the experiment, neither in the presence nor in the absence of larger zooplankton (Fig. 3d–Table 3). Chlorophyceae was the class contributing most to the total phytoplankton biomass throughout the experiment, but Bacillariophyceae contribution increased significantly from day 9 under both heatwave conditions (HW and HW M),

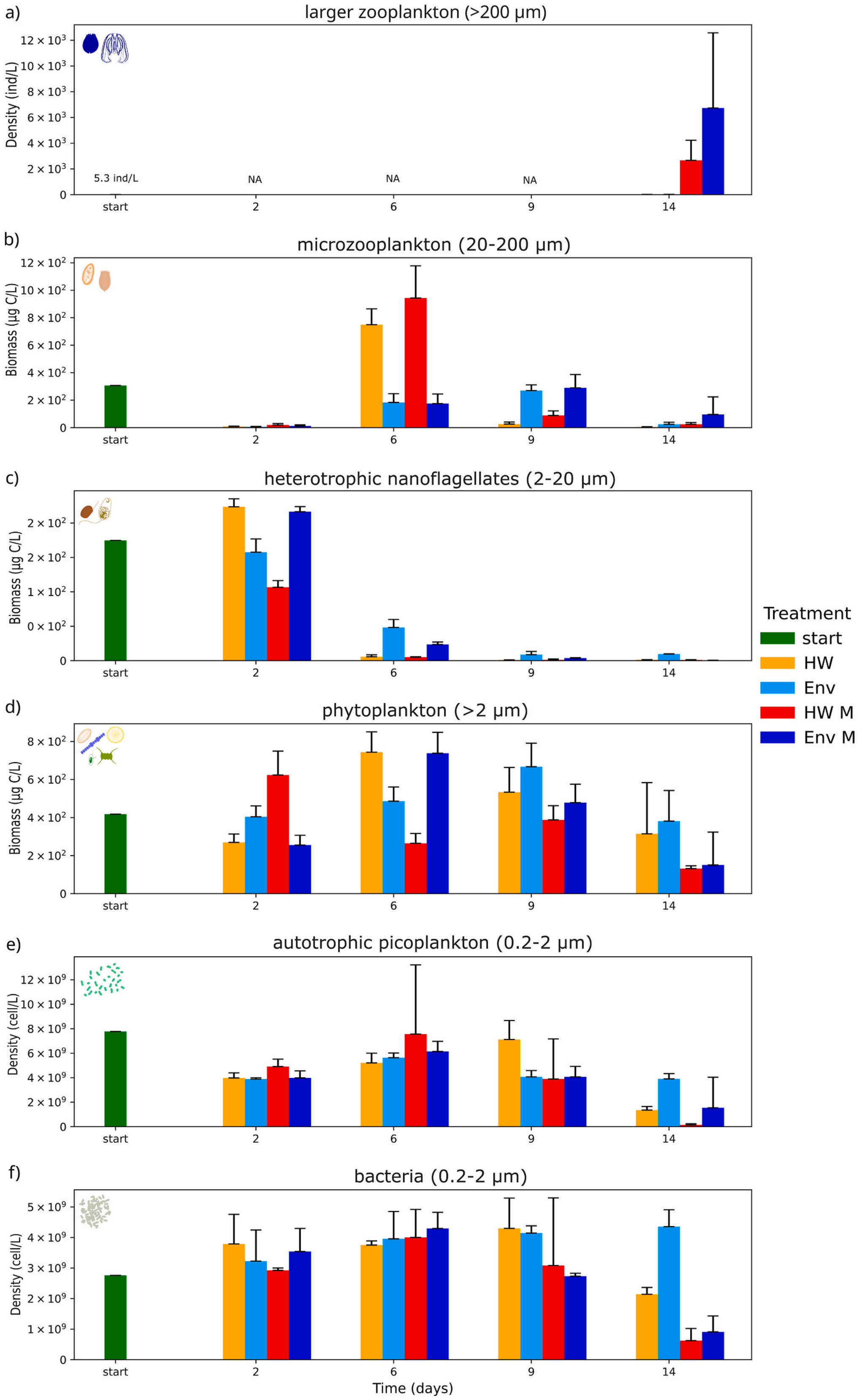

Fig. 3. Panel plot showing the temporal variation of all planktonic groups investigated under different experimental treatments: **(a)** mesozooplankton density (ind L^{-1}), **(b)** microzooplankton biomass (µg C L^{-1}), **(c)** heterotrophic nanoflagellate biomass (µg C L^{-1}), **(d)** phytoplankton biomass (µg C L^{-1}), **(e)** autotrophic picoplankton density (cells L^{-1}) and **(f)** bacterial density (cells L^{-1}). Bars represent mean values for each treatment, with error bars indicating variability among replicates (±SD). Treatments are indicated by colors: start (green), HW (orange), Env (light blue), HW M (red) and Env M (dark blue). *NA* indicates time points where measurements were not available. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

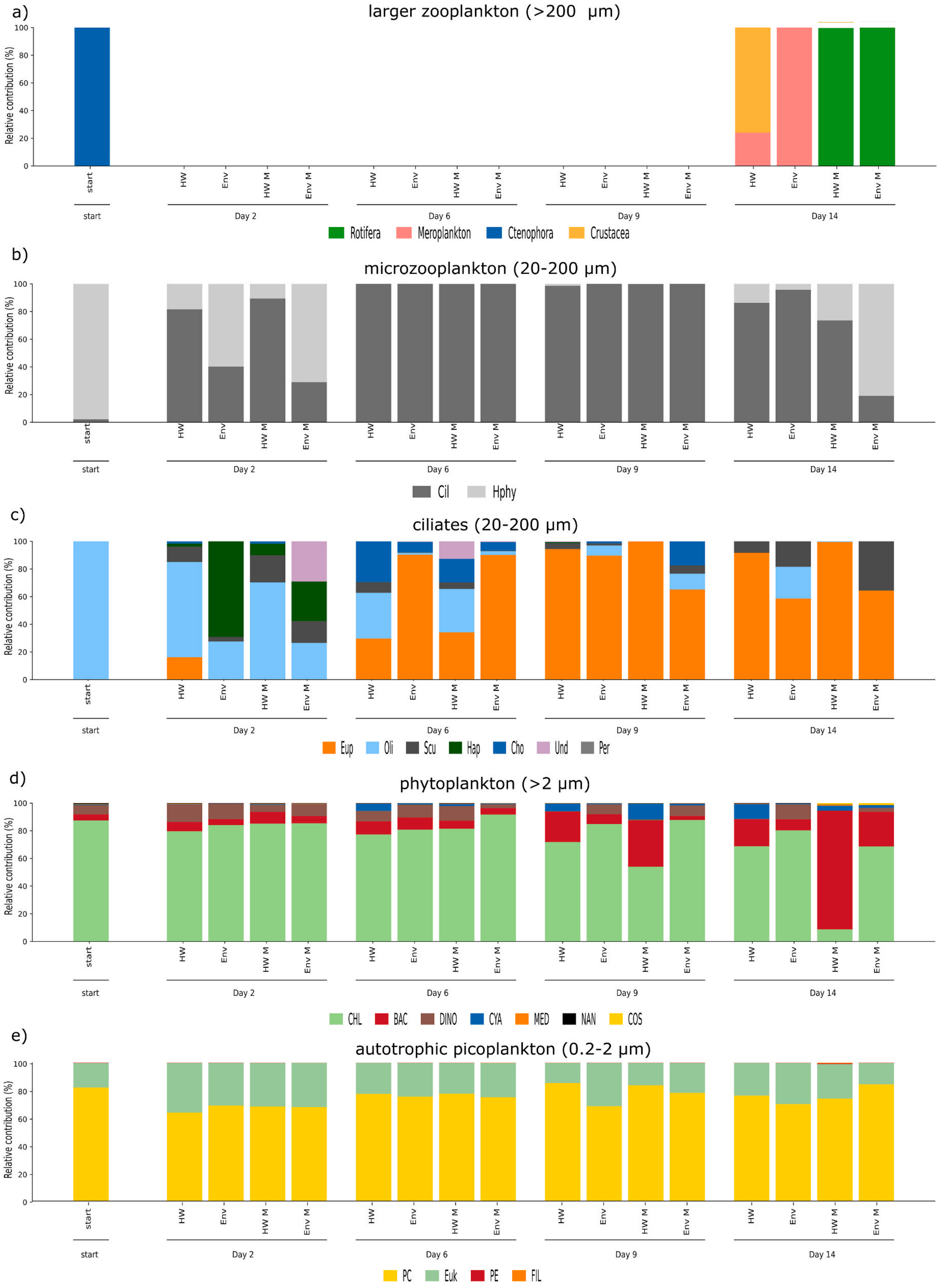

Fig. 4. Panel plot showing the relative contribution (%) of the main planktonic groups over time under different experimental treatments. Panels show changes in community composition for **(a)** mesozooplankton, **(b)** microzooplankton (CIL = ciliates, Hphy = heterotrophic phytoplankton), **(c)** ciliates (Cho = Choreotrichia, Eup = Euplotia, Hap = Haptoria, Oli = Oligotrichia, Per = Peritrichia, Scu = Scuticociliatia, Und = undetermined), **(d)** phytoplankton (BAC = Bacillariophyceae, CHL = Chlorophyceae, COS = Coscinodiscophyceae, CYA = Cyanophyceae; DINO = Dinophyta, MED = Mediophyceae, NAN = autotrophic nanoflagellates) and **(e)** autotrophic picoplankton (PE = picocyanobacteria rich in phycoerythrin, FIL = filaments, EUK = picoeukariotes, PC = picocyanobacteria rich in phycocyanin) at the start of the experiment (start) and after 2, 6, 9 and 14 days. Bars represent the proportional contribution of each taxonomic group within each treatment. Treatments are indicated along the x-axis (HW, Env, HW M, Env M).

Table 2
Results of linear mixed-effects (LME) model for each response variable (total biomass of microzooplankton, heterotrophic phytoplankton – Hphy, heterotrophic nanoflagellates – HNF, total density of bacteria), with temperature (explanatory variable) in presence (HW M) and in absence (HW) of larger zooplankton as a fixed factor and time (day) as a random effect. Not significant results ($p > 0.05$) are in italics.

		day 0 - 6		day 0 - 9		day 0 - 14	
Response variable	**Explanatory variable**	F	P	F	P	F	P
Microzooplankton	HW	8.15	0.013	*0.27*	*0.606*	*2.09*	*0.162*
	Time x HW		<0.001		<0.001		<0.001
	HW M	7.50	0.016	*3.77*	*0.067*	*0.68*	*0.417*
	Time x HW M		<0.001		<0.001		<0.001
	HW	*3.26*	*0.093*	*0.07*	*0.797*	*0.23*	*0.633*
Hphy	Time x HW		<0.001		<0.001		<0.001
	HW M	*0.16*	*0.699*	*0.01*	*0.910*	*0.02*	*0.881*
	Time x HW M		<0.001		<0.001		<0.001
HNF	HW	*3.52*	*0.081*	9.01	0.007	17.28	<0.001
	Time x HW		<0.001		<0.001		<0.001
	HW M	20.26	<0.001	27.39	<0.001	19.20	0.000
	Time x HW M		<0.001		<0.001		<0.001
Bacteria	HW	*0.20*	*0.658*	*0.26*	*0.617*	*1.53*	*0.228*
	Time x HW		*0.086*		0.014		*0.098*
	HW M	*1.76*	*0.205*	*0.33*	*0.572*	*0.35*	*0.560*
	Time x HW M		0.002		*0.254*		<0.001

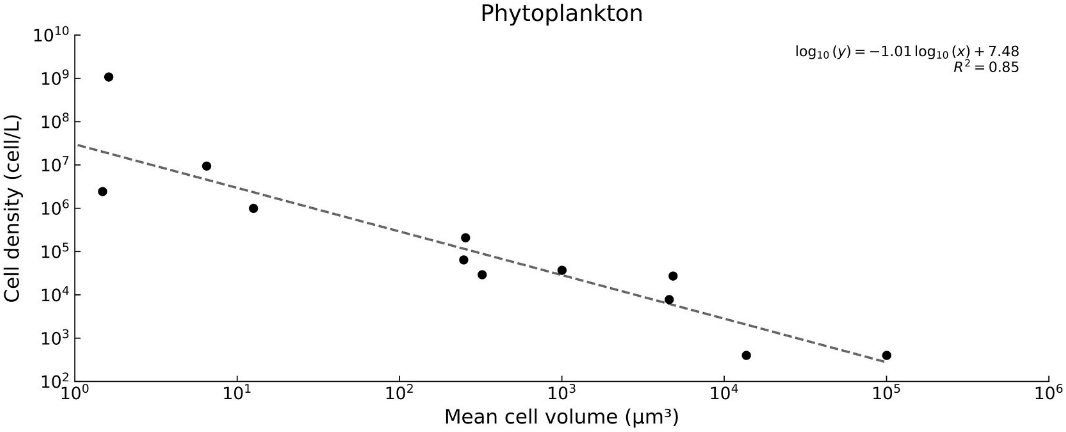

Fig. 5. Cell density *vs* mean cell volume of starting phytoplankton community. Each black dot represents a phytoplankton species detected at day 0.

Table 3
Results of linear mixed-effects (LME) model for each response variable (total biomass of phytoplankton, Bacillariophyceae and Chlorophyceae; total density of autotrophic picoplankton – APP), with temperature (explanatory variable) in presence (HW M) and in absence (HW) of larger zooplankton as a fixed factor and time (day) as a random effect. Not significant results ($p > 0.05$) are in italics.

		day 0 - 6		day 0 - 9		day 0 - 14	
Response variable	**Explanatory variable**	F	P	F	P	F	P
Phytoplankton	HW	*0.00*	*0.950*	*0.338*	*0.568*	*0.90*	*0.352*
	Time x HW		0.004		<0.001		<0.001
	HW	*0.05*	*0.828*	*0.30*	*0.591*	*0.47*	*0.497*
	Time x HW M		*1.000*		*1.000*		0.031
Bacillariophyceae	HW	*2.63*	*0.127*	10.82	0.004	16.16	<0.001
	Time x HW		<0.001		<0.001		<0.001
	HW M	*0.03*	*0.857*	*4.19*	*0.055*	8.92	0.006
	Time x HW M		*1.000*		*0.617*		0.026
Chlorophyceae	HW	*0.04*	*0.851*	*1.42*	*0.248*	*2.43*	*0.132*
	Time x HW		0.011		0.011		0.044
	HW M	*2.35*	*0.148*	5.40	0.031	5.26	0.031
	Time x HW M		*0.546*		*0.592*		<0.001
APP	HW	*0.35*	*0.565*	*2.84*	*0.108*	*1.02*	*0.323*
	Time x HW		<0.001		<0.001		<0.001
	HW M	*0.48*	*0.499*	*0.01*	*0.919*	*0.62*	*0.438*
	Time x HW M		*0.075*		*0.073*		<0.001

accounting for more than 80% under heatwave conditions in presence of larger zooplankton at day 14 (Fig. 4d). Indeed, absolute values of total Bacillariophyceae biomass also increased significantly from day 9 onwards under both heatwave conditions (HW and HW M), meanwhile total Chlorophyceae biomass decreased from day 6 to day 14 in the presence of larger zooplankton, and especially at the heatwave condition (HW M) at day 14 (Fig. 6; Table 3).

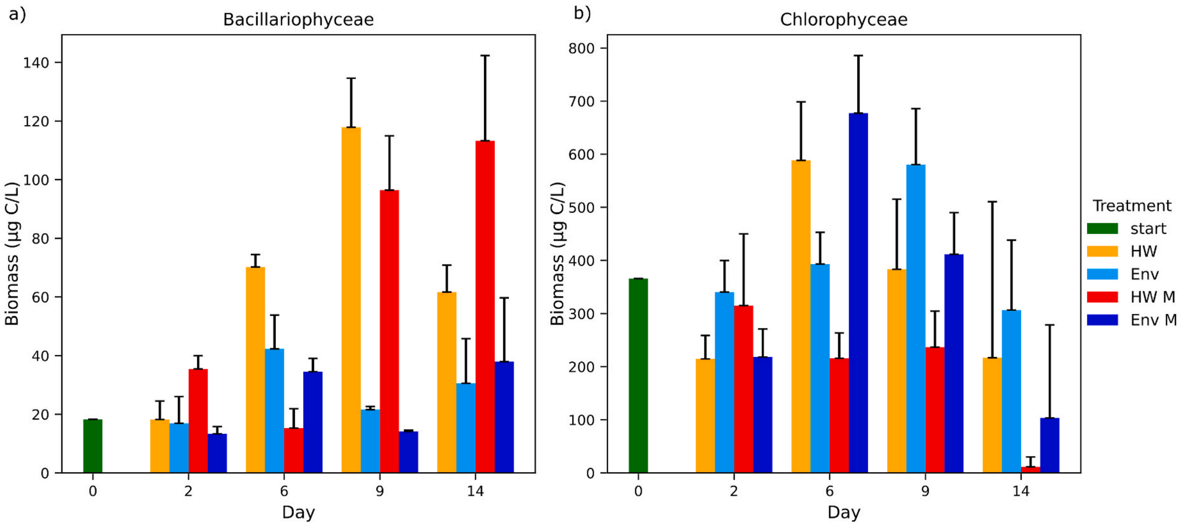

Fig. 6. Panel plot showing the temporal variation of the phytoplankton groups that contributed the most to total phytoplankton abundance under different experimental treatments: **(a)** total biomass of Bacillariophyceae and **(b)** total biomass of Chlorophyceae. Bars represent mean values for each treatment, with error bars indicating variability among replicates (±SD). Treatments are indicated by colors: start (green), HW (orange), Env (light blue), HW M (red) and Env M (dark blue). *NA* indicates time points where measurements were not available. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

3.6. *Picoplankton (APP and bacteria)*

Cell densities of APP and bacteria were both of 10^9 magnitude order, with picocyanobacteria contributing more than 60% to total APP density in all treatments throughout the experiment (Fig. 3e, f, 4e). Significant effects of the heatwave were not observed either on the total bacterial abundance or on the total APP abundance (Tables 2 and 3). A strong decrease of total APP density and of total bacteria density were detected at the end of the experiment: APP abundance was lower under warming conditions (HW and HW M) and bacteria abundance was lower in the presence of larger zooplankton (HW M and Env M) at day 14.

4. Discussion

This study confirms the presence of very small autotrophic cells at the base of the plankton food web in Cabras Lagoon, as previously reported by Pulina et al. (2012, 2023). The starting natural autotrophic plankton community exposed to an experimental summer heatwave was very small-sized, being mainly composed of pico-cyanobacteria and the chlorophyte *Monoraphidium* cf. *minutum*.

Since warming is widely reported to favor smaller phytoplankton over larger cells (Sommer et al., 2017a, 2017b), this work provides a case study to explore the response of a planktonic trophic web under heatwave, when extremely small autotrophic cells occupy the first trophic level. Moreover, as heatwaves are expected to become a key selective driver in a warmer future scenario, pushing many species to their physiological limits (Calbet and Saiz, 2023), this work provides new insights into which planktonic taxa may benefit from abrupt warming events in Mediterranean coastal lagoons, while also assessing the role of predator-prey interactions across the entire plankton community, from pico-to meso-zooplankton.

Our results reported no significant effects of the simulated heatwave on the total abundance of APP or phytoplankton. Instead, warming affected the phytoplankton taxonomic composition, benefiting small Bacillariophyceae undetermined Pennate and suppressing even smaller Chlorophyceae after nine experimental days via rotifer grazing. Therefore, our findings show that the initial very small-sized autotrophic plankton community was competitive under abrupt and intense thermal stress, such as the simulated heatwave. This supports the idea that a higher surface-area-to-volume ratio and a thinner diffusive boundary layer, which facilitate rapid resource exchange and characterize smaller cells, represent a successful biological strategy under warming conditions. This pattern is well documented in the literature, where cell size reduction has been proposed as a third universal response to climate warming (Litchman et al., 2007; Daufresne et al., 2009; Van de Waal et al., 2020; Heinrichs et al., 2024). Indirect warming effects mediated by nutrient limitation can be also ruled out in the present work, as P-PO_4 and DIN never became limiting during the experiment. Instead, an indirect heatwave effect mediated by predator–prey interactions acted only on the abundance of small Chlorophyceae.

A similar experiment was previously conducted by Pulina et al. (2020), with a natural winter plankton community from Cabras Lagoon and excluding the large zooplankton (>200 µm size). In that study, the community was exposed to a +3 °C and +6 °C heatwave for sixteen days. The starting phytoplankton community was mainly composed of Chlorophyceae species (*Chlorella* with a mean cell volume of 16.9 µm^3) and Mediophyceae species (*Cyclotella* and *Thalassiosira* with mean cell volumes of 160.1 and 3761.9 µm^3, respectively). A significant increase in phytoplankton abundance was measured under warming, along with an increased abundance of Chlorophyceae which replaced Mediophyceae. These changes were driven by a shift of ciliate taxonomic composition that resulted in a preferred feeding on the larger Mediophyceae rather than the smaller Chlorophyceae (Pulina et al., 2020).

Looking at results of other similar investigations on coastal lagoon plankton communities from the Mediterranean area, Vidussi et al. (2011) reported that experimental warming effects on spring phytoplankton from Thau Lagoon (France) were small. They suggested that predator-prey relationships between copepods and ciliates were the main drivers, ultimately benefiting the phytoplankton. In a more recent outdoor mesocosm experiment in Thau Lagoon, Soulié et al. (2022, 2023) investigated the responses of a spring plankton community to two consecutive experimental heatwaves of +5 °C compared to the controls. They detected positive warming effects on the diatoms during the first heatwave, likely related to the higher Si-SiO_4 concentrations found in the heated treatment, highlighting that nutrient availability often overrides temperature in phytoplankton responses to heatwaves. In addition, they observed a decreased abundance of Chlorophytes under the heatwave.

In our study, the observed increase in Bacillariophyceae under warming may have been favored by the concurrent rise in Si-SiO_4

concentrations and by low zooplankton grazing pressure at the highest temperature.

Temperature rise is the most often suggested key driver of changes in zooplankton phenology (Forsblom et al., 2024). In the Inter-governmental Panel on Climate Change (IPCC, 2023) Sixth Assessment Report, a systematic review of marine phenology studies suggested that zooplankton timing is responding faster compared to that observed for other marine animals (Cooley, 2022). It was found that the effect of simulated heatwave on the plankton community in the coastal north-western Mediterranean Thau lagoon during late spring/early summer was the potential shift in coastal zooplankton communities from a copepod-dominated to a meroplankton-dominated community (Zervoudaki et al., 2024). Similarly, in the present study, significant effects of the simulated heatwave on zooplankton were measured, consisting in changes in abundance and profound taxonomic compositional shifts in both mesozooplankton (>200 μm) and microzooplankton (<200 μm). A general increase in the abundance of larger zooplankton was observed at the end of the experiment, but it was significant only under *in situ* (environmental) temperature. Very small individuals of *Mnemiopsis leidyi* were completely absent on day 14, indicating a collapse of this species under laboratory treatment conditions. Our findings are consistent with those of Gambill et al. (2015), who reported that temperatures exceeding 27 °C impose metabolic stress on *M. leidyi* populations, resulting in negative growth in 1.5-mm larvae.

The rotifer *Brachionus* spp. Became the dominant species in all treatments by the end of the experiment. The presence of resting eggs under warming suggests a species strategy in response to unfavorable extreme temperatures. However, different responses to heat of heat-tolerant and heat-sensitive *Brachionus* species were observed in short-term heat exposure (Paraskevopoulou et al., 2020). Thus, the production of resting eggs by *Brachionus* species under heatwave conditions remains controversial. Although some *Brachionus* species are euryhaline, salinity is a well-recognized factor influencing rotifer population dynamics and life-history traits, including resting egg hatching and reproductive performance. In particular, changes in salinity, rather than absolute salinity levels, have been shown to negatively affect population growth and sexual reproduction (Kim and Hagiwara, 2011). Accordingly, salinity increases associated with warming and enhanced evaporation may have acted as an additional stressor under heatwave conditions.

An important role of rotifers in the summer plankton community of Cabras Lagoon was previously detected with data from the field (Pulina et al., 2023). Rotifers prey upon bacteria, heterotrophic flagellates, small ciliates and phytoplankton (Bonecker and Aoyagui, 2005; Sweeney et al., 2022). The decreased abundance of bacteria and of the small Chlorophyceae *Monoraphidium* cf. *minutum* observed at the end of the present experiment (day 14) in the presence of larger zooplankton regardless of the temperature (HW M and Env M), suggests a feeding of *Brachionus* spp. on these taxa. Moreover, the extremely low abundance of copepods, in general the dominant component of the mesozooplankton community in coastal lagoons, observed during the experiment may reflect the influence of top-down control processes, potentially associated with the presence of *Mnemiopsis leidyi*. Copepods are known to represent a major prey item for this ctenophore, which is widely recognized as an efficient predator of mesozooplankton in coastal and estuarine ecosystems (Costello et al., 1999). The relatively high densities of *M. leidyi* recorded at the beginning of the experiment may have contributed to the lack of copepods in the initial samples at T0 through sustained grazing pressure. Such predation could have promoted shifts in zooplankton community structure, favoring smaller-sized taxa, such as rotifers.

With regard to microzooplankton, this study confirms the strategic role of small ciliates in the planktonic trophic web of Cabras Lagoon, as previously reported in both experimental (Pulina et al., 2020) and field studies (Pulina et al., 2023). Heatwave benefited ciliates over heterotrophic phytoplankton from day 0 to day 6, supporting findings on a lower tolerance of heterotrophic phytoplankton to abrupt exposure to high temperatures compared to ciliates (Ferreira et al., 2022; Calbet and Saiz, 2023; Calbet, 2025). Specifically, *Oxyrrhis marina,* which represented the starting heterotrophic phytoplankton community of the present experiment, is known to be negatively affected by warming due to increased respiration process (Calbet et al., 2022). Our findings highlighted a direct positive warming effect on the abundance of several groups of ciliates (Choreotrichia, Euplotia, Oligotrichia and Scuticociliatida) during the first six experimental days in relation to the absence of rotifers in the HW treatment and a low grazing pressure where rotifers were potentially present (HW M). On the other hand, warming negatively affected ciliate abundance and their taxonomical diversity after day 6, with Euplotia completely replacing the smaller Oligotrichia which dominated the starting community of ciliates. These results are consistent with those of several studies on ciliates indicating oligotrichs as generally more successful in cooler water (Calbet, 2025). Vidussi et al. (2011) also observed a significant effect of heatwaves on ciliate abundance in opposite directions over the short and long term, when the spring plankton community from Thau Lagoon was exposed to an experimental heatwave. Here, the authors related ciliate dynamics under warming to the abundance of their predators, the copepods, and to the availability of their prey, the nanoflagellates. Similarly, Pulina et al. (2020) reported a direct positive effect of warming on ciliates in absence of mesozooplankton lasting for the entire 16-days experiment, highlighting a physiological advantage at elevated temperatures (Calbet, 2025).

Our findings also indicated an increased salinity in the heatwave treatments, likely driven by enhanced water evaporation. The salinity range observed during the experiment fell within the natural variability of Cabras Lagoon, which is known to experience strong seasonal fluctuations, with seasonal differences sometimes exceeding 10 PSU, as reported in field studies (Pulina et al., 2011, 2023). We acknowledge that the faster experimental alteration of physico-chemical water conditions may have contributed, through combined effects with warming and grazing, to the observed changes in the plankton community, which primarily involved heterotrophic components. However, although salinity varied consistently over the course of the experiment, values remained within the brackish range typically tolerated by the ciliates observed, which are described in the literature as brackish and marine taxa whose abundance tends to decline only under hypersaline conditions (Nche-Fambo et al., 2016; Weisse and Montagnes, 2022; Li et al., 2024). In the present study, ciliates initially benefited from warming, high availability of HNF and low predation by the rotifer *Brachionus* spp. Until day 6. This led to a negative effect of warming on HNF, mediated by the increased abundance of ciliates feeding on them during the first days of the heatwave. After day 6, ciliates were negatively affected by warming and by the reduced availability of HNF, resulting in a compositional shift away from Oligotrichia, which mainly feed on nanoflagellates (Zöllner et al., 2009; Chen et al., 2020) toward Euplotia, which are filter feeders primarily consuming bacteria and small algae (Liu et al., 2020). In addition, we hypothesize an increased abundance of *Brachionus* spp. After day 6, which may have benefited from the absence of the ctenophore *M. leidyi* and the very high abundance of ciliates observed at the beginning of the experiment. No predator-prey relationships between HNF and bacteria were detected in the present study, unlike what was observed by Pulina et al. (2020) with the winter plankton community of Cabras Lagoon. They indicated a cascade effect ciliates-HNF-bacteria under warming in absence of the larger zooplankton, causing an increased abundance of bacteria from day 9. In the present summer plankton community, we only detected a strong decrease in bacterial abundance in the presence of larger zooplankton, regardless of temperature (HW M and Env M) on days 9 and 14, suggesting predation by heat-tolerant rotifers on bacteria. Although temperature can potentially enhance bacterial growth rates, increasing their abundance in the absence of bacterivorous protists (Vidussi et al., 2011), our results indicate that bacteria, as well as APP were strongly controlled by very small meso (rotifers) - and micro (ciliates) -zooplankton.

Dominance of very small autotrophic and heterotrophic planktonic components, together with altered grazing pathways, enhances the relative importance of microbial processes and modifies carbon and nutrient cycling, with possible consequences for trophic transfer efficiency toward higher trophic levels (Di Pane et al., 2024). A recent study, demonstrated altered nutrient uptake and carbon fixation rates due to short-term thermal stress (5 days at +2 °C and +4 °C increased temperature) in coastal microbial plankton communities, suggesting potential impacts on biogeochemical processes during heatwave events (López-Sandoval et al., 2025). Moreno et al. (2022) experimentally studied the responses of a coastal summer plankton community from the North Sea to different future ERCP scenarios. They observed the emergence of nanophytoplankton at the expense of larger diatoms, an increased abundance of microzooplankton and a decreased abundance of mesozooplankton in the ERCP 8.5 scenario (+3 °C and −0.3 pH). These dynamics were described as indicative of a microbial loop dominance in this future scenario, and of a potential diminution of energy transfer to higher trophic levels. Indeed, heatwave-driven changes in plankton size structure and predator–prey interactions may have implications beyond community composition, potentially influencing ecosystem functioning. Microzooplankton is described as key functional component of planktonic food webs, mediating rapid nutrient recycling to primary producers and forming an important trophic link between unicellular plankton and higher metazoan consumers (Calbet and Saiz, 2005; Loder et al., 2011b). According to Meunier et al. (2025), changes in microzooplankton abundance and composition, such as those detected under heatwave in the present work, significantly affect secondary consumers altering the entire pelagic food-web functioning.

5. Conclusions

This study provides strong evidence of heatwave effects on a long planktonic trophic web in the Mediterranean Cabras Lagoon, composed of multiple trophic levels with very small-sized taxa: small ctenophore *Mnemiopsis leidyi* – rotifers – small ciliates – HNF – small Chlorophyceae, picocyanobacteria and bacteria. This confirms the low energy availability for planktivorous fish in the lagoon, which is heavily exploited for fishery activities, an issue that had previously been hypothesized based on field data and experiments (Pulina et al., 2020, 2023). The simulated summer heatwave strongly affected the heterotrophic component of the plankton community: it directly and negatively impacted rotifers, while directly and positively affecting ciliates during the first six days in absence of rotifers. Warming affected indirectly and negatively HNF through ciliate feeding on them. Bacteria, as well as the autotrophic component of the plankton community was found undisturbed by the heatwave, sustaining heterotrophic changes without significant decline, at least until day 14 when bacteria and Chlorophyceae were preyed by rotifers. Overall, this study demonstrates that heatwaves events can profoundly reshape coastal lagoon planktonic food webs, both directly and by altering trophic interactions, as recently reported by Eglaine et al. (2025). The results of this study contribute to a broader understanding of heatwave impacts on coastal plankton communities and allow further predictions on how coastal lagoons may function under a warmer future. This is especially important to guide new management practices aimed at safeguarding several goods and services supplied by these highly biodiverse but fragile environments.

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CRediT authorship contribution statement

Silvia Pulina: Conceptualization, Formal analysis, Funding acquisition, Methodology, Project administration, Supervision, Validation, Writing – original draft, Writing – review & editing. **Jessica Titocci:** Data curation, Formal analysis, Methodology, Visualization, Writing – review & editing. **Lyudmila Kamburska:** Conceptualization, Formal analysis, Methodology, Validation, Writing – review & editing. **Marco Cherchi:** Formal analysis, Methodology, Validation, Writing – review & editing. **Cristina Pittalis:** Formal analysis, Methodology, Validation, Writing – review & editing. **Roberta Piscia:** Data curation, Formal analysis, Validation, Writing – review & editing. **Raffaella Sabatino:** Data curation, Formal analysis, Methodology, Writing – review & editing. **Ilaria Vaccarelli:** Formal analysis, Methodology, Writing – review & editing. **Andrea Di Cesare:** Conceptualization, Formal analysis, Methodology, Supervision, Writing – review & editing. **Ilaria Rosati:** Conceptualization, Data curation, Methodology, Writing – review & editing. **Bachisio M. Padedda:** Conceptualization, Formal analysis, Methodology, Supervision, Validation, Writing – review & editing.

Declaration of competing interest

The authors declare no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ecss.2026.109796.

Data availability

Data will be made available on request.

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