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Moderate and extreme warming under a varied resource supply alter the microzooplankton–phytoplankton coupling in North Sea coastal communities

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Abstract

Rising temperature is one of the most visible effects of global change on Earth; however, it is barely known how moderate or extreme warming events impact the trophic interactions and the energy transfer in food webs. Combining a mesocosm approach and two-point dilution incubations, we quantified how natural plankton assemblages respond to moderate and extreme warming (+6°C vs. +12°C above ambient temperature), covering a nitrogen-to-phosphorus gradient from nutrient-saturated to limited conditions. We addressed how both drivers altered the community structure and mediated the phytoplankton growth (μ) and microzooplankton grazing (m) rates. Moderate and extreme warming effects on the microzooplankton–phytoplankton relationship differed and were mediated by time. This trophic interaction was weakened due to μ outpacing m regardless of the warming treatment at the middle of the experiment. By contrast, after the acclimation period, the trophic interaction was strengthened by increased grazing under extreme warming. The variable grazing pressure found at different temporal scales only under extreme warming could be due to a decreased microzooplankton grazing pressure with increasing temperature when prey biomass is low, and vice versa. Also, it could be a consequence of a switch toward mixotrophy or that the temperatures experienced by grazers were suboptimal compared to their prey. Finally, we found that temperature was the main driver whereas resource availability played a minor role in this trophic interaction. As climate change will intensify in the future, food webs could be less productive but more efficient, and thus, potentially support a higher secondary production.

Plankton are sentinel organisms used to track the impacts of global change on aquatic ecosystems due to their short generation times and high turnover rates allowing them to respond to changes more quickly than the rest of the food web. Within food webs, phytoplankton play a key role in regulating organic matter and nutrient cycling, trophic interactions and atmosphere-water

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gas exchange (Iversen 2023), hence any alteration on this trophic compartment may be propagated (and amplified) at higher trophic levels. The ecological relevance of microzooplankton for carbon cycling is well studied as it consumes about 60% of the global primary production (Schmoker et al. 2013). Despite the pivotal role of the phytoplankton–microzooplankton interaction, most studies evaluating its sensitivity to temperature and resource availability have considered the spatial variability (Calbet and Landry 2004; Landry et al. 2022) and microzooplanton grazing (m) and phytoplankton growth (μ) rates separately (Liu et al. 2019); however, changes over time in both rates and in the trophic coupling ($m:\mu$), in particular under global change scenarios, have received scarce attention.

Temperature is a major driver governing all biochemical reactions on Earth (Gillooly et al. 2001), and one of the most pervasive changes that the biosphere is facing (Rockström et al. 2023). According to predictions by Metabolic Theory of Ecology (Brown et al. 2004) and observational and experimental evidences (López-Urrutia et al. 2006; O'Connor et al. 2009), heterotrophic processes (e.g., m) increase faster than autotrophic ones (e.g., μ) under increasing temperatures due to their higher thermal sensitivity. However, Chen and Laws (2017) and Wang et al. (2019) showed that autotrophic processes can be as sensitive or more sensitive to temperature as heterotrophic ones, even over seasonal scales (Liu et al. 2019). Additionally, the thermal dependence of metabolism is dependent on resource availability, being stimulated as resources availability increases (Hayashida et al. 2020; Cabrerizo and Marañón 2021a), and weaker (Liu et al. 2021), or even suppressed when nutrients are limiting (Marañón et al. 2018). In this sense, Courboulès et al. (2022) found that warming reduced phytoplankton biomass due to enhanced grazing rates and increased the bacteria to phytoplankton ratios, triggering a strengthening of the microbial loop compared to the grazing chain. More recently, Vad et al. (2023) found that a short experimental extreme warming resulted in decrease of herbivorous ciliates while bacterivorous taxa dominated, which in turn resulted in a weakened top-down control of phytoplankton mediated by that short heat pulse. Finally, under high-temperature and resource conditions, Franzè et al. (2023) proposed that most of the primary production is likely being exported to deep waters due to a weakened grazer-prey coupling, but the opposite could occur, that is, an enhanced trophic transfer under high-temperature and low-nutrient availability. Therefore, the temperature–resources interplay and the potential differential thermal sensitivity of autotrophic and heterotrophic processes may determine the fate of primary production in marine ecosystems due to alterations in the microzooplankton–phytoplankton coupling.

In the present study, we address three interlinked key questions to better understand the effects of moderate and extreme warming on marine microbial food webs:

a. Does the trophic coupling between phytoplankton and microzooplankton favor the carbon export vs. trophic transfer efficiency to higher trophic levels, or vice versa?
b. What are the effects of moderate and extreme warming under a varied resources supply on the microzooplankton–phytoplankton coupling?
c. Are such effects on microzooplankton grazing consistent once the communities reached the final temperature level and after an acclimation period?

We predict a higher energy transfer efficiency over the food web under extreme compared to moderate and control temperature since microzooplankton grazing rates will increase more than phytoplankton growth due to their higher (heterotrophic vs. autotrophic processes) thermal sensitivity. To address these research gaps, we performed a mesocosms experiment in which a natural marine plankton community from the North Sea was exposed to moderate (+ 6°C) and extreme (+ 12°C) warming in respect to in situ conditions (6°C) in two consecutive phases: a ramping phase in which temperature increased by 1°C per day until it reached the target temperature treatments (moderate and extreme warming in 6 and 12 d, respectively), and a constant temperature phase (from days 12 to 27) in which communities were exposed to warming treatments mentioned above. We examined these warming effects on different biological organization levels: total biomass, stoichiometry (particulate organic carbon [POC], particulate organic nitrogen, and particulate organic phosphorus ratios), community size (microplankton, nanoplankton, and picoplankton) and trophic (autotrophs, mixotrophs, and heterotrophs) structure, as well as the microzooplankton–phytoplankton trophic interaction by quantifying the phytoplankton growth (μ) and mortality (m) rates using the two-point modification dilution method.

Materials and methods

Experimental setup of the mesocosm experiment

On March 6, 2022, an 8000-liter surface water sample was taken at Helgoland Roads station (54°11′17.88″N, 7°54′E), filtered through a 200-μm mesh to exclude mesozooplankton and maintained at in situ temperature and darkness until the following day (12 h) when it was placed into 12 indoor mesocosms (Planktotrons, 600-liter stainless steel indoor mesocosms). On March 8, the plankton communities were exposed (in quadruplicate) to 3 temperature treatments: 6°C (control temperature), 12°C (moderate), and 18°C (extreme) over 27 d. We chose 6°C as the ambient temperature as it resembles the temperature registered during the sampling day (5.4°C) and is representative of the sea surface temperature for the North Sea in March over the last two decades (Wiltshire et al. 2013). The 12°C treatment represents the temperature experienced by spring bloom communities at the end of the bloom period (i.e., May/June; Wiltshire et al. 2013) (hereafter; moderate), and the 18°C treatment simulates a worst-case scenario (e.g., an extreme heatwave event; Smale et al. 2019) and the upper limit experienced by plankton communities in the sampling site during summer (August; Wiltshire et al. 2013)

(hereafter, extreme). The temperature increase was set to 1°C per day until target temperatures were reached for the two warming treatments, and then maintained constant over the experimental period (Supporting Information Fig. S1A). Built-in rotors with silicon lips at the side, top, and bottom, gently rotate (0.14 rpm) in the Planktotrons, to prevent wall growth and to ensure homogeneous phytoplankton distribution (and that they received homogeneous irradiances) in the water column during the experiment. Light conditions were maintained constant during the experiment using two light-emitting diode units (IT2040, Evergrow, Shenzhen, China) above each Planktotron, with mean surface irradiances of $181.80 \pm 1.76\ \mu$mol photons $m^{-2}\ s^{-1}$ over a 12 h : 12 h light–dark cycle. Translucent float glass panels (Pilkington Optiwhite) were placed on top of the Planktotrons to prevent evaporation, outgassing, and cross-contamination. Daily salinity measurements (WTW IDS TetraCon 925 + Multi 3630 IDS, Xylem Analytics) indicated no differences among temperature treatments (*see* more details in Ahme et al. 2023a, 2024).

Despite excluding large grazers with a 200-μm mesh in the original sample, we observed mesozooplankton in all mesocosms; however, no significant differences among temperature treatments existed in terms of abundance and composition (Ahme et al. 2024). Thus, we assume the mesozooplankton effects on our results to be negligible.

Sampling and analysis

Water temperature was logged continuously in each Planktotron using built-in PT100 sensors (Temperature Control) whereas light intensity was monitored manually each other day using a Photosynthetically Active Radiation light meter (LI-COR LI-250A, LI-COR Biosciences). All other response variables, except the micro-grazing incubations (*see* below) were measured every 3 d (10 times in total) over the experimental period. Early in the morning (9:00 a.m.), integrated water column samples were taken from each Planktotron once the mixing process was completed using a customized polyvinyl chloride cylinder.

Inorganic nutrients, chlorophyll *a*, and stoichiometry in the mesocosms

Nitrate + nitrite and phosphate concentrations (0.2-μm pre-filtered water samples) were determined by colorimetric measurements on a continuous flow analyzer (Euro EA 3000, HEKAtech GmbH), whereas silicate was quantified by the molybdate reaction following standard protocols (Wetzel 2001). Samples for POC, particulate organic nitrogen, and particulate organic phosphorus as well as chlorophyll *a* (Chl *a*) concentrations were filtered on pre-combusted and acid-washed glass-fiber GF/C filters (Whatman) and stored at −80°C until analyzed. POC and particulate organic nitrogen filters were measured with a CHN elemental analyzer (Thermo Fisher Scientific), and particulate organic phosphorus by molybdate reaction after sulfuric acid digestion (Wetzel 2001). Chl *a* extraction was done by adding 90% ethanol to the samples, which were sonicated on ice in darkness for 30 min, and then extracted at 4°C for 24 h in darkness. Samples were measured in a fluorescence multiplate reader (SYNERGY H1, BioTek) following the protocol by Thrane et al. (2015).

Taxonomic composition in the mesocosms

Plankton community composition was assessed via 18S rRNA metabarcoding, as described in Ahme et al. (2024) as well as in the supplementary material. Annotated species were grouped based on two different categories: cell size and trophic mode. Cell size was differentiated between picoplankton (0.2–2 μm), nanoplankton (2–20 μm), and microplankton (20–200 μm), and the trophic mode between autotrophs, mixotrophs, and heterotrophs. To assign the cell size and the trophic mode to the identified species, we used the Encyclopedia of Life (http://eol.ogr), World Register of Marine Species (http://marinespecies.ogr), Nordic Microalgae (http://nordicmicroalgae.org), and PlanktonNet (https://planktonnet.awi.net) databases, and Olenina et al. (2006). For species where the required information was not available, we did specific literature searches (until September 12, 2023) using SCOPUS (http://www.scopus.com) as search engine (Supporting Information Table S1). Apicomplexa, Foraminifera, Fungi, Metazoa, Pseudofungi, Rhodophyta (multicellular species), Sagenista, and Streptophyta species identified in our samples through meta-barcoding were excluded from the analysis as we were interested in unicellular plankton organisms.

Micro-grazing incubation experiments

Phytoplankton growth and protist herbivorous-induced mortality rates were measured with the dilution method (Landry and Hassett 1982) in a two-point modification (Menden-Deuer et al. 2018; Anderson and Harvey 2019; Landry et al. 2022) using undiluted (100%) and diluted (30%) seawater. The validity of this approach, compared with the traditional multipoint dilution approach, has been demonstrated in several studies which have evidenced indistinguishable growth and grazing rates with both methodologies (Worden and Binder 2003; Chen 2015; Morison and Menden-Deuer 2017). The dilution factor used was based on previous results by Chen (2015) which showed that setting up a highly diluted bottle and treating the net μ of this bottle as the instantaneous μ yields more accurate estimates. In our case, we used 30% as diluted seawater treatment to ensure enough sensitivity to detect changes in μ through Chl *a* performed measurements done during the incubation period. This dilution treatment has been proven successful in recent works with natural plankton communities (Landry et al. 2022, 2023).

To do that, integrated water samples were taken from each mesocosm, filtered again through a 200-μm mesh and pooled

together for each temperature treatment. From this water, we prepared 500-mL undiluted (100%) and diluted (30%) culture flasks (Sarstedt) (two technical replicates per temperature and dilution treatment at the start [t_0] without nutrients enrichment and only one [with nutrients enrichment] after 24 h of incubation [t_f]). t_f samples were exposed to a full-crossed combination of 25 N : P ratios, resulting in 54 experimental units, 4 for t_0 (2 × 100% and 2 × 30%), and 50 for t_f (25 × 100% and 25 × 30%) in total for the experimental day 0, and 168 (54 per temperature treatment) per day for the days 15 and 27 (Fig. 1). N concentrations added, as $NaNO_3$, ranged between 0 and 52.70 μM, and those of P, added as hydrated NaH_2PO_4, between 0 and 3.3 μM. The total dissolved nutrient additions, added as a unique pulse before starting the incubations, were based on Gerhard et al. (2019), excepting the highest concentration treatment, which was replaced by a control treatment without any nutrient addition treatment. Ultimately, the final nutrient supply consisted of the nutrients added plus the background concentrations existing in the seawater at the start of each incubation (Supporting Information Table S2), hence the final N : P ratios slightly differed between the incubation days 0 (5–228), 15 (5–353), and 27 (4–360). Thus, the generated N : P ratios were categorized into N-limited (N : P < 11), balanced (N : P = 12–39), and P-limited (N : P > 40). This categorization is based on previous work by our group in which we showed that the optimum N : P supply

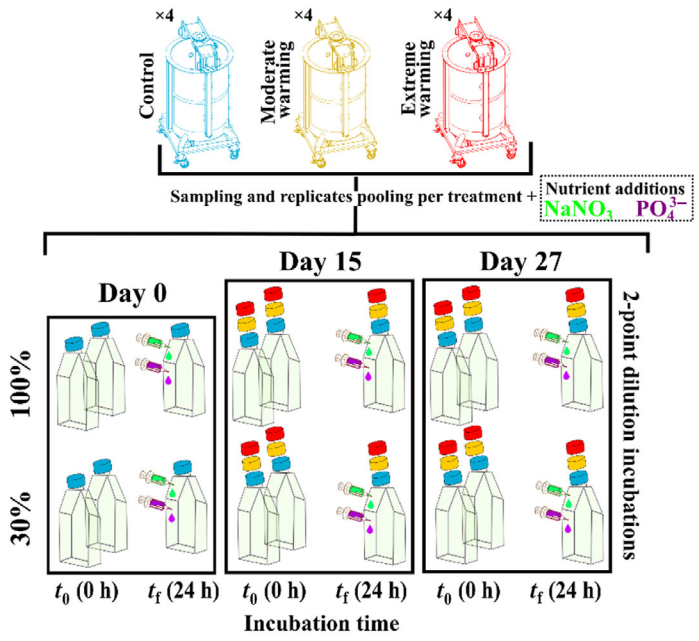

Fig. 1. Graphical scheme of the experimental design in which plankton communities were exposed to control temperature and moderate and extreme warming, and subsequent 2-point dilution incubations (100% vs. 30%) to determine the phytoplankton growth and microzooplankton grazing rates to the temperature treatments tested over a 25th nitrogen-to-phosphorus (N : P) ratios gradient (*see* "Materials and Methods" for a detailed description).

for a phytoplankton community can range from 13 to 40 (Gerhard et al. 2019). This does not imply that all ratios in the assigned category were indeed limiting. We selected an unreplicated procedure for these incubations following previous works that proposed it as an alternative in experimental studies where multiple levels (> 5) of a given driver are tested (Ellison and Gotelli 2018; Garzke et al. 2019), given the statistical power of it comes from the wide range of ×-levels used (25 N : P ratios in our case).

To generate the necessary seawater to be mixed with the samples for the diluted treatment (150 mL per bottle), additional 75 liters of seawater from a nearby coastal site at the ICBM were filtered through 0.2-μm polycarbonate filters (Millipore), sterilized for 15 min at 121°C, and stored in darkness at 4°C. The stored dilution water was subsequently acclimated to the target temperatures before being used in the dilution experiments. Once prepared, all samples were placed in 600-mL sterilized cell culture flasks (Greiner Bio-One GmbH), and incubated for 24 h in temperature-controlled rooms under the same temperature and light conditions as experienced in the Planktotrons. Samples for Chl *a* determination were collected when flasks were filled initially (t_0) and after 24 h (t_f). The procedure followed to analyze all the samples obtained was the same as explained above for Chl *a* determination.

Following Landry and Hassett (1982) and Chen (2015), the net phytoplankton growth rate (k) was calculated as:

$$k = \ln\left(\mathrm{Chl}\,a_{t_f}/\mathrm{Chl}\,a_{t_0}\right)/t$$

where $\mathrm{Chl}\,a_{t_f}$ and $\mathrm{Chl}\,a_{t_0}$ are the Chl *a* concentrations measured at the end (t_f) and the beginning (t_0) of the incubation period, respectively, and t is the duration of the incubation period (24 h).

From both net phytoplankton growth rates (that is, k_{30} and k_{100}), we calculated the phytoplankton mortality rates induced by grazing due to protist herbivorous as $m = (k_{30} - k_{100})/(1 - x)$, x being the dilution factor used.

We calculated the intrinsic phytoplankton growth rates (μ) as the sum of $k_{100} + m$.

Data and statistical analysis

We used resource use efficiency (RUE) as a proxy to track the functional change in relation to species change (Hodapp et al. 2019). Resource use efficiency was defined as the biomass production in POC (in μmol C L^{-1}) per unit total nitrogen (nitrate plus nitrite) or phosphorus (in μM). We used N to calculate the RUE because it is well known that Chl *a* (a proxy of phytoplankton biomass and the variable used in our dilution experiments) varies mostly as a function of N (rather than P) availability (*see* Supporting Information Fig. S1B–D; Palomares-García et al. 2006). The relative contribution (%) of each size fraction (microplankton, nanoplankton, and picoplankton) and trophic mode (autotroph, heterotroph, and mixotroph) to the

total community was calculated as the quotient between the total number of amplicon sequence variants (ASVs) belonging to plankton species identified of a given size fraction or trophic mode fraction respect to the total number of amplicon sequence variants (i.e., microplankton + nanoplankton + picoplankton for size structure, and autotroph + heterotroph + mixotroph for trophic mode). The predator : prey availability ratio over the experimental period was calculated as the quotient between the amplicon sequence variants of autotrophs vs. those of heterotroph plus mixotroph (*see* Ahme et al. 2023b and Supporting Information Materials and Methods for more details). This ratio constitutes a rough estimate of prey availability and was used to relate if a higher (or lower) grazing pressure was accompanied by predator : prey availability ratios > 1 (or < 1) (predator dominates over prey, or vice versa). Ratios between different functional groups of plankton are also a useful indicator of environmental change (Wasmund 2017). We grouped heterotroph and mixotroph as several experimental findings indicate that mixotrophs behave as photoheterotrophs (i.e., photosynthetic apparatus mainly provides energy but not fixed carbon), and heterotrophy increases with warming (Wilken et al. 2013; Cabrerizo et al. 2019; Lepori-Bui et al. 2022). The appropriateness of the procedure followed with the amplicon sequence variant data to estimate the predator : prey ratio is based on recent findings by Andersson et al. (2023), who showed a good agreement between the relative gene copy number and carbon biomass in coastal phytoplankton communities.

To assess to what degree the balance between μ and m determined the dynamics of phytoplankton biomass, we calculated the accumulation rates as the difference between μ and m, and the proportion of the primary production available (in percentage) for higher trophic levels by dividing m over μ (Calbet and Landry 2004; Anderson and Harvey 2019; Cabrerizo and Marañón 2021b). A trophic coupling between microzooplankton and phytoplankton occurs when m equals μ (relationship m vs. μ, on the 1 : 1 line), a strengthening of this trophic interaction occurs when m outpaces μ (below the line 1 : 1) and a weakening when the opposite situation takes place (above the line 1 : 1).

A repeated measures one-way ANOVA was used to test significant differences between temperature treatments on Chl *a*, RUE_N, N : P and C : P ratios, inorganic nutrients, C : Chl *a* ratio, species richness from the Planktotrons, predator : prey availability ratio, and accumulation rates from micro-grazing incubations. A repeated measures two-way ANOVA was used to test significant differences between temperature treatments and the different cell size (or trophic mode) fractions. Linear (or a power) regression analyses were used to assess the relationship between μ and m and the $m : \mu$ ratio over the experimental N : P ratio gradient considered and for each temperature treatment, and Chl *a* vs. POC (as a proxy of phytoplankton biomass). Assumptions of normality (by Shapiro Wilk's test and error's distribution analysis), homogeneity of variances (by Levene's test), sphericity (by Mauchly's test), and independence were checked to be satisfied before ANOVA and regression analysis were performed. When a significant temperature effect was detected, a least significant differences (LSD) post hoc test was used to evaluate significant differences within temperature levels. All statistical analyses were performed in R v.4.3.1 (R Core Team 2022) with RStudio v. 2023.09.0.

Results

Community biomass, RUE, and stoichiometry in the mesocosms

Warming had a significant effect on Chl *a*, RUE_N, N : P, and C : P ratios (Fig. 2; Supporting Information Table S3). Regardless of the temperature treatment, Chl *a* (and POC, between 400 and 1600 μmol C L^{-1}; Supporting Information Fig. S2) concentrations increased up to day 12. By contrast, from here to the end of the experiment, their concentrations significantly increased only under extreme warming (Supporting Information Fig. S1A). The RUE_N was almost constant until day 15 with no significant differences among temperature treatments (LSD post hoc test, $p > 0.05$; Fig. 2b). However, later during the experiment, it increased to values ~ 12, 6, and 4 under extreme, moderate, and control conditions, respectively, due to a significant temperature × time interaction (Supporting Information Table S3). No significant differences were found for RUE_P among temperature treatments over the experimental period (F-test = 0.34, df = 2, $p = 0.73$; Supporting Information Fig. S3). Particulate N : P and C : P ratios remained relatively stable until day 15, whereas during the 2nd half of the experiment, the particulate N : P ratio was significantly higher under extreme than moderate and control temperature, and the treatments also reached the maximum values at different time points (first the extreme and moderate, then the control; LSD post hoc, $p < 0.01$; Fig. 2c). No significant differences between treatments existed for the particulate C : P ratio although their values increased from day 18 (Fig. 2d).

Plankton community size structure and trophic modes in the mesocosms

The community size structure was dominated by nanoplanktonic species regardless of the temperature treatment and over the entire experimental period (Fig. 3a; Supporting Information Table S3). This group contributed between ~ 70% (6°C) on day 0 and 61% and 82% (12°C and 18°C vs. 6°C) on day 27, whereas microplankton accounted for ~ 36% (day 0) and 17–30% (day 27, 6–12°C and 18°C, respectively). The contribution of picoplankton to the community was minor in all treatments and at all timepoints (< 3% of the total; Fig. 3a). When we grouped the species into trophic modes, autotrophs had the highest contribution to the community at the beginning (> 50%; days 3, 6, and 9) and at

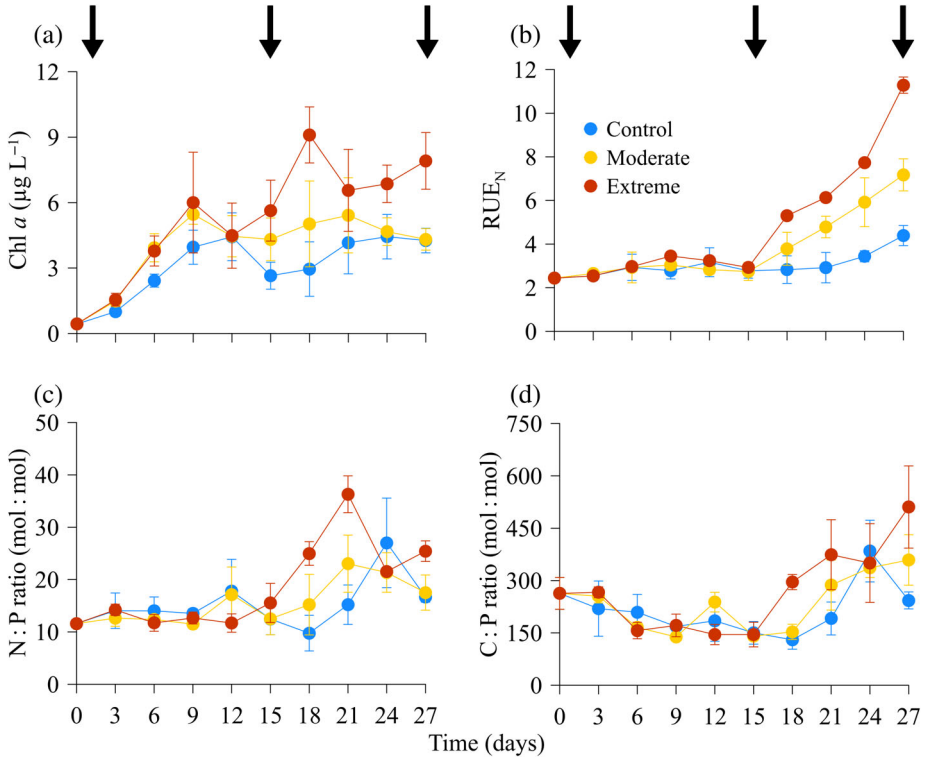

Fig. 2. Mean (± SD) chlorophyll *a* (Chl *a*) (**a**), nitrogen-specific resource use efficiency (RUE_N) (**b**), particulate organic nitrogen : phosphorus (N : P) (**c**), and particulate organic carbon : phosphorus (C : P) (**d**) ratios in plankton communities exposed to three temperature treatments (control, 6°C; moderate, 12°C; and extreme, 18°C) over the experimental period. Black arrows represent the micro-grazing incubation days.

the end of the experimental period (up to 60%; days 24 and 27) under control temperature (Fig. 3b). Under moderate and extreme warming, their contribution maintained constant at short-term but decreased below 40%, in particular under moderate warming, at the end of the experimental period. Heterotrophs exhibited an opposite response pattern to autotrophs, with a maximum contribution in the middle of the experiment, in particular under control, and the lowest values at the start and at the end of the experimental period. Mixotrophs contribution increased over time, with values being significantly higher under moderate than control and extreme temperature conditions (LSD post hoc test, $p < 0.05$). These variations in taxonomic composition matched with reductions in the C : Chl *a* ratio (Supporting Information Fig. S4A) and the total species richness (Supporting Information Fig. S4B), although without significant differences between temperature treatments (C : Chl *a*: $F = 0.58$, $p = 0.59$; richness: $F = 0.18$, $p = 0.84$).

Microzooplankton–phytoplankton coupling in dilution experiments: Prey availability, accumulation rates, and interaction strength

The predator : prey ratio showed values around 1 at the beginning of the experimental period. From here, they increased above 1 (i.e., predators > prey availability), in particular under moderate warming at day 12, where maximum values of ca. 3 were measured (Fig. 4a). From mid-experiment (day 15), the predator : prey ratios decreased in all treatments but lowest values occurred under control temperature, followed by moderate and extreme temperature treatments. The changing ratios translated into marked variations in the accumulation rates over time (Fig. 4b). These rates significantly increased under moderate and extreme warming treatments at day 15 compared to initial conditions (Supporting Information Table S3), reaching values between 0.5 and 1 d^{-1}, but decreased under control temperature. By contrast, at day 27, all rates were positive; however, we found that they were significantly higher (LSD post hoc test, $p < 0.01$) under moderate and control temperatures compared to extreme temperature conditions (Fig. 4b).

The relationship between μ and m provides further insights into how microzooplankton grazing controls phytoplankton biomass. On day 0, all μ rates fell close the 1 : 1 line ($R = 0.74$, $F_{23} = 9.08$, $p < 0.01$; Fig. 5a), suggesting that μ and m were closely balanced due to a strong coupling between both groups. Additionally, the coupling was not significantly influenced by the N : P supply ratio (a similar response pattern was observed at days 15 and 27; Fig. 6a; Supporting Information Table S4). On day 15 and under control conditions, μ rates were below the line 1 : 1 reflecting the inability of phytoplankton to avoid microzooplankton grazing control. By contrast, under moderate and extreme warming, these rates were above the line 1 : 1 (Fig. 5b). On day 27, we observed that the

weakened trophic coupling, that is, values above the 1 : 1 line were only maintained under moderate warming ($R = 0.90$, $F_{23} = 88.35$, $p < 0.0001$; Fig. 5c).

Taken together, growth and grazing patterns indicated that the microzooplankton consumption ($m : \mu$ ratio) accounted, on average throughout temperatures, for 87% of

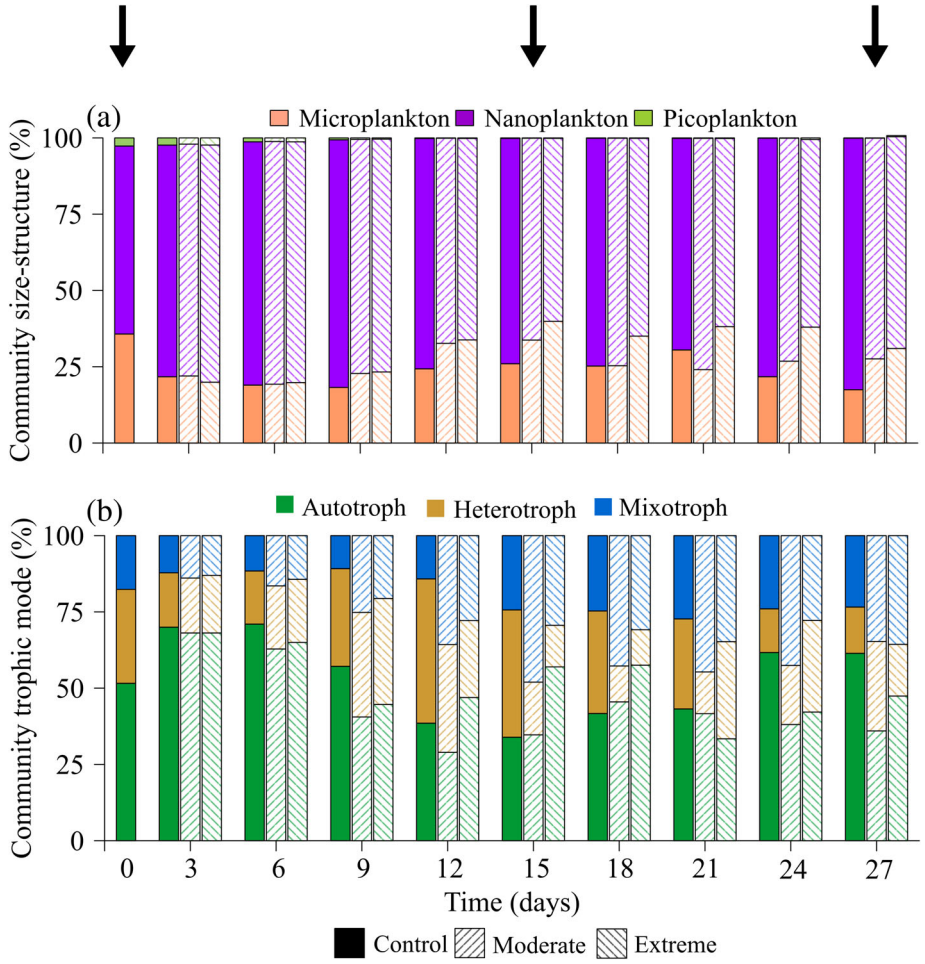

Fig. 3. Mean relative contribution (%) of microplankton, nanoplankton, and picoplankton (**a**), and autotrophs, mixotrophs, and heterotrophs (**b**) to the total community in plankton communities exposed to three temperature treatments (control, 6°C; moderate, 12°C; and extreme, 18°C) over the experimental period. Black arrows represent the micro-grazing incubation days.

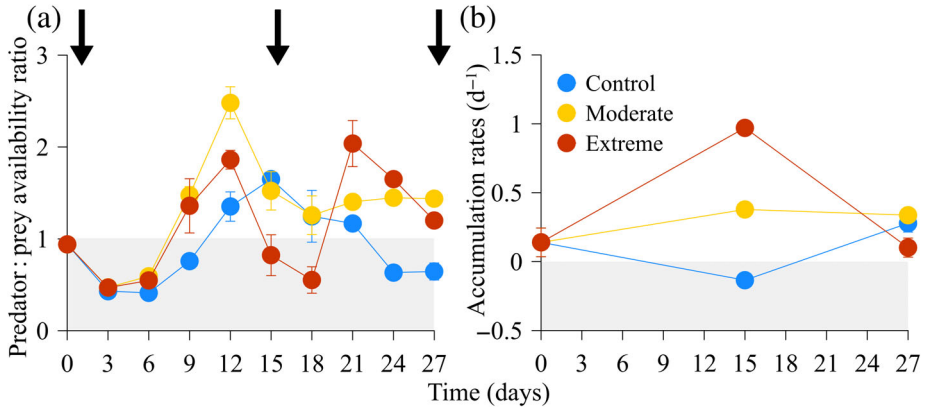

Fig. 4. Mean (± SD) predator (heterotrophs plus mixotrophs) and prey (autotrophs) availability ratios (**a**), and accumulation rates (**b**) in plankton communities exposed to three temperature treatments (control, 6°C; moderate, 12°C; and extreme, 18°C) over the experimental period. Black arrows represent the micro-grazing incubation days. Standard deviation bars do not appear when they are smaller than the symbol size.

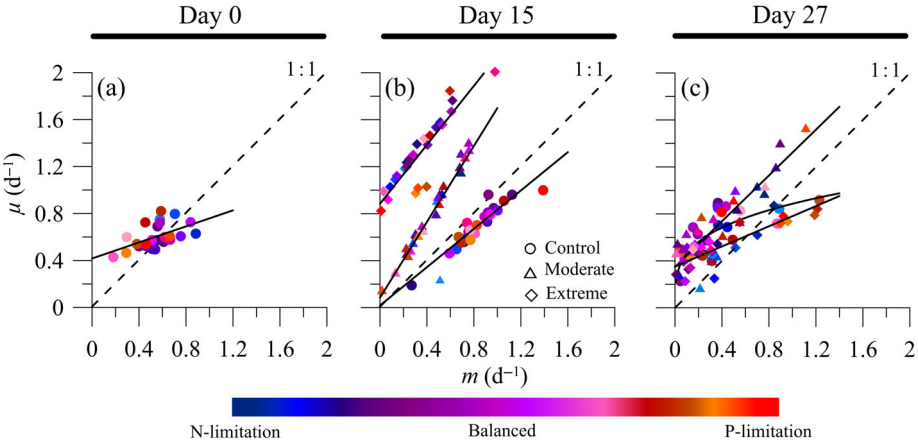

Fig. 5. Mean instantaneous phytoplankton growth (μ) vs. microzooplankton grazing (m) rates at days 0, 15, and 27 in plankton communities exposed to three temperature treatments (control, 6°C; moderate, 12°C; and extreme, 18°C) over a dissolved nitrogen : phosphorus (N : P) ratios gradient (*see* Supporting Information Fig. S3 for specific ratios). The dashed line represents the 1 : 1 relationship and the solid lines are the linear or nonlinear fits for each temperature treatment.

phytoplankton growth, that is, grazing exerted a strong top-down control on primary production available at day 0 (Fig. 6a). Once the communities were exposed to the target temperature conditions, we observed that the $m : \mu$ ratio increased under control conditions ($R = 0.94$, $F_{23} = 168.89$, $p < 0.0001$), reaching values > 120% (Fig. 6b), whereas it decreased under extreme warming conditions ($R = 0.91$, $F_{23} = 105.70$, $p < 0.0001$; Fig. 6b). In the warming conditions, the $m : \mu$ ratios ranged, on average for N : P ratios, between 24% (extreme warming) and 51% (moderate warming). Finally, on day 27, the mean $m : \mu$ ratios over the dissolved N : P gradient were at ~ 50% under control temperature and moderate warming but increased up to 72% under the extreme warming (Fig. 6c).

Discussion

Our work shows a contrasting effect of moderate and extreme warming on the microzooplankton–phytoplankton relationship mediated by time. Moreover, the effects of temperature on this trophic interaction exceeded those exerted by the resources supply. This dominant effect of temperature can be explained by a too short incubation period to observe a detectable change due to the resources supply in the biomass of the phytoplankton community, and subsequently in grazers (Cáceres et al. 2013; Landry et al. 2022). Thus, our findings with North Sea plankton communities suggest that the variability in phytoplankton growth and microzooplankton grazing rates is mainly driven by temperature, whereas the resource supply played a minor role.

Transient responses of microzooplankton and phytoplankton to moderate and extreme warming

A continuous temperature increase (1°C d^{-1}) prompted a decoupling in the trophic coupling due to a weakening in the trophic interaction strength ($\mu > m$; Fig. 5b). Our $m : \mu$ estimates (24–51%) are lower compared to prior (> 60–90%) observational (Steinberg and Landry 2017) and experimental (Rose et al. 2009; Menden-Deuer et al. 2018; Horn et al. 2020) findings, but higher than recently reported by Franzé et al. (2023) in a coastal ecosystem. The decoupling between m and μ rates denotes a "weak" role of microzooplankton in the marine food web. A weakening in the trophic interaction entails a decrease in the energy transfer efficiency toward higher trophic levels but an enhanced carbon export out of the euphotic zone due to increased accumulation rates (Fig. 4b).

Two responsible processes were proposed by Franzè et al. (2023) to explain the low grazing pressure measured under warming and nutrient-enrichment conditions: changes in composition toward less palatable species (Anderson et al. 2022) and that μ were maximal. We found support for the explanation as the proportion of these species (mainly nanoplanktonic and microplanktonic chain-forming diatoms) was significantly higher under extreme compared to the control and moderate warming (~ 26 vs. ≤ 18; Anderson et al. 2024). By contrast, we did not observe any change in the contribution of picoplankton to the total community over time. The low contribution reported (3%) is consistent with previous findings in the study area (values < 2%; Wollschläger et al. 2015) and in other temperate coastal and upwelling ecosystems (Marañón 2015). Our results are also in line with the 2nd proposed argument, as μ_{max} (1.8 d^{-1}) exceeded the m_{max} (< 0.8 d^{-1}) (Fig. 3). A higher μ_{max} is consistent with the enhanced accumulation rates observed under warming conditions (Fig. 4b) and agrees with previous results by Mojica et al. (2021) who found that increased accumulation is based on faster rates of change in phytoplankton division rates.

Two additional plausible explanations for the reduced grazing pressure found could be a switch toward mixotrophy and that the temperatures experienced by grazers were more suboptimal

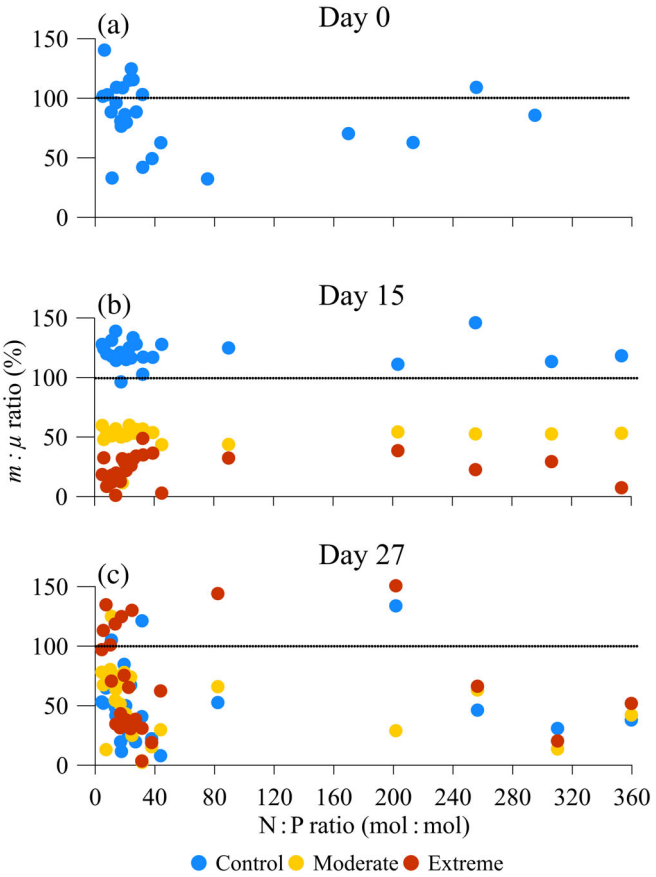

Fig. 6. Mean instantaneous phytoplankton growth (μ) and microzooplankton grazing (m) ratios (i.e., grazing pressure, %) at the initial time (day 0), once the communities reached the target temperature treatments (day 15), and after the acclimation period (day 27) in plankton communities exposed to three temperature treatments (control, 6°C; moderate, 12°C; and extreme, 18°C) over a dissolved nitrogen : phosphorus (N : P) ratio gradient between 0 and 360. The dashed line ($m : \mu = 100$) denotes that all primary production generated was consumed by microzooplankton grazing.

for them than for their prey. Although mixotrophy was not measured here, potentially mixotrophic species (dinophyta, haptophyta, and unidentified MAST clade species) represented between 30% and 50% of the total community under warming conditions (Fig. 4a). A potentially higher phototrophic activity subsequently would explain why despite the increase in mixotrophic grazers mediated by increased availability of prey biomass and RUE_N (Fig. 2b), the grazing pressure was lower. Additionally, our communities grew under optimal light conditions which favor phototrophy in mixotrophs.

Concerning the 2nd explanation, there is evidence in coastal ecosystems showing that microzooplankton has optimal temperatures > 3°C higher than that of phytoplankton (Liu et al. 2019). By considering this explanation and that our phytoplankton community had their growth optimum at 18°C (Anderson et al. 2024), we assume that grazers were growing under less optimal temperatures than their prey.

Acclimation of microzooplankton and phytoplankton to moderate and extreme warming

Once communities were acclimated for more than 2 weeks to warming, grazing pressure was only accentuated under extreme warming. The contrasting response pattern between days 15 and 27 entails a shift toward a strengthening of the trophic coupling, and potentially, a higher energy transfer efficiency to higher trophic levels but lower C export. This increased grazing pressure under warmer conditions agrees with the idea by Rose and Caron (2007) that acclimated microzooplankton growth rates increase faster than do those of phototrophs at temperatures above 15°C. The discrepancy between grazing pressure observed during transient and acclimated communities only under extreme warming could be based on the different Chl *a* concentrations, with a decrease in microzooplankton grazing pressure with increasing temperature when Chl *a* concentrations are low (i.e., values ~ 3 μg L^{-1}) but increases in such pressure when Chl *a* concentrations are high (Chen et al. 2012). Franzè and Menden-Deuer (2020) have also suggested that discrepancies in the grazing pressure can be due to microzooplankton needing a longer time than phytoplankton to acclimate to the experimental thermal environment. Thus, we cannot discard that the different acclimation time to the target temperature elapsed on day 15 for plankton communities under moderate (9 d) and extreme (3 d) warming, may have influenced the responses observed at day 27. Finally, we can discard that the increased grazing pressure observed on day 27 was due to a higher energetic demand mediated by a lowered prey nutritional quality. We did not find differences between temperature treatments for phytoplankton stoichiometric (C : N : P) ratios (Fig. 2).

Conclusions

Our findings for a temperate coastal community suggest, partially in contrast to what we predicted, that temperature changes can differentially affect microzooplankton and phytoplankton. For phytoplankton, higher growth rates and reductions in micro-grazing pressure could trigger sudden phytoplankton blooms, and potentially increase the carbon sequestration and its exportation to deep waters. This argumentation matches with the prediction that phytoplankton blooms will expand and intensify in a warmer 21st century (Dai et al. 2023), although we are aware that mesocosm experiments cannot fully replicate natural environmental complexity, hence the effects shown do not necessarily represent what may occur in natural ecosystems.

Once phytoplankton and microzooplankton are acclimated to temperature for a similar period, grazing pressure accentuated, in particular under the extreme warming, and the trophic transfer efficiency boosted. This evidence shows that no universal assumption can be made about the role that micrograzers play in marine food webs. Although ocean-scale models and climate change scenarios consider a constant grazing pressure, our findings support the idea that grazing

remains as the largest uncertainty source for marine carbon cycling (Rohr et al. 2023). Moreover, assuming a constant grazing implies considering a fixed thermal sensitivity. This argumentation has been recently questioned for phytoplankton by Anderson et al. (2024), meaning that the use of a constant thermal sensitivity for different phytoplankton groups in models leads to an unrealistic outcome in terms of community composition and significantly alters their competitive ability. Since this aspect remains unevaluated for grazers, it ultimately impacts our current estimates of biogeochemical processes (e.g., carbon export).

Therefore, if evolutionary time scales do not compensate for the differential and variable thermal sensitivity of phytoplankton growth and microzooplankton grazing (reported here, we predict that warming could lead to less productive but more efficient food webs, and thus could potentially support a higher secondary production in the future).

Data availability statement

The data supporting the findings of this study are openly available in PANGAEA (https://doi.org/10.1594/PANGAEA.961155).

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Conflict of Interest

None declared.

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