

The experimental implications of the rate of temperature change and timing of nutrient availability on growth and stoichiometry of a natural marine phytoplankton community

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Abstract

Climate change increases the need to understand the effect of predicted future temperature and nutrient scenarios on marine phytoplankton. However, experimental studies addressing the effects of both drivers use a variety of design approaches regarding their temperature change rate and nutrient supply regimes. This study combines a systematic literature map to identify the existing bias in the experimental design of studies evaluating the phytoplankton response to temperature change, with a laboratory experiment. The experiment was designed to quantify how different temperature levels (6°C, 12°C, and 18°C), temperature regimes (abrupt vs. gradual increase), timings of nutrient addition (before or after the temperature change) and nutrient regimes (limiting vs. balanced) alter the growth and stoichiometry of a natural marine phytoplankton community. The systematic map revealed three key biases in marine global change experiments: (1) 66% of the studies do not explicitly describe the experimental temperature change or nutrient regime, (2) 84% applied an abrupt temperature exposure, and (3) only 15% experimentally manipulated the nutrient regime. Our experiment demonstrated that the identified biases in experimental design toward abrupt temperature exposure induced a short-term growth overshoot compared to gradually increasing temperatures. Additionally, the timing of nutrient availability strongly modulated the direction of the temperature effect and strength of growth enhancement along balanced N : P supply ratios. Our study stresses that the rate of temperature change, the timing of nutrient addition and the N : P supply ratio should be considered in experimental planning to produce ecologically relevant results as different setups lead to contrasting directions of outcome.

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Increasing temperature and changes in nutrient regimes are among the most prevalent abiotic pressures of the last decades (Malone and Newton 2020; IPCC 2023). Both drivers exert a strong impact on phytoplankton growth (Thomas et al. 2017; Anderson et al. 2022) and stoichiometry (De Senerpont-Domis et al. 2014; Yvon-Durocher et al. 2017), which subsequently alter the nutritional quality and quantity for higher trophic levels (Sterner and Elser 2002; Hessen et al. 2013) and the carbon export out of the pelagic zone (Kwiatkowski et al. 2018). Future scenarios predict various possible combinations of temperature and nutrient availability, for example, that rising water temperatures increase stratification and thus reduce nutrient transport to surface waters (Steinacher et al. 2010), or that terrestrial run-off increases the nutrient input in coastal waters (Rabalais et al. 2009). These different scenarios stress the need to cover an extensive range of possible combinations

and underline the importance of gradient experiments, including extreme treatment levels (Collins et al. 2022). Moreover, the effects of temperature and nutrients on phytoplankton are often investigated independently (e.g., Pálffy et al. 2021; Soulié et al. 2022) or by using single species in laboratory experiments (e.g., Boyd et al. 2015; Bestion et al. 2018). However, to gain a comprehensive understanding of direct and indirect effects via species interactions (Boyd et al. 2018) and to draw conclusions on the ecosystem level, we need studies quantifying such responses at the community level.

Experimental studies have shown that the thermal dependence of phytoplankton metabolism accentuates with increasing nutrient concentration (and vice versa) (Thrane et al. 2017; Marañón et al. 2018), whereby nutrient availability changes the height and curvature of the thermal performance curve (Thomas et al. 2017). The combined effects of temperature and nutrients on the community level are expected to be more complex than patterns on single species level as phytoplankton taxa exhibit trade-offs in their ability to use resources and to outperform other taxa along their species-specific performance curves (Litchman and Klausmeier 2008). For a marine spring bloom community exposed to three temperatures and two different nutrient concentrations, Anderson et al. (2022) found higher temperatures (+3.4°C compared to ambient) to be beneficial for community growth rates under nutrient-replete conditions, but antagonistic under nutrient limitation. Applying a wide range of nutrient concentrations and ratios, Gerhard et al. (2019) found the temperature \times nutrient interaction effect on the growth rate of a freshwater community to be strongest under balanced N : P supply ratios (i.e., around the Redfield ratio) compared to extremely sub-optimal N : P supply ratios (N or P limitation). Additionally, under a balanced N : P supply ratio, nutrient concentration only slightly affected the sensitivity to temperature fluctuations (Gerhard et al. 2019). A recent analysis of long-term data showed that the North Sea is experiencing rising N : P supply ratios, potentially entailing an increasingly prevalent phosphorus limitation (Burson et al. 2016; Rönn et al. 2023) making the investigation of the interactive effects of nutrient conditions and temperature changes even more relevant in this system.

Considering phytoplankton stoichiometry (i.e., particulate N : P ratio), the *temperature-dependent physiology* hypothesis implies increasing particulate N : P ratios with higher temperatures due to a lower requirement for phosphorus-rich ribosomes relative to nitrogen-rich proteins to maintain an organism's performance (Woods et al. 2003). However, as phytoplankton taxa differ in their macronutrient requirements (Edwards et al. 2012) altering relative N and P supply may also reshape the phytoplankton community (Tilman et al. 1982). Although the phytoplankton community response to temperature increase (Striebel et al. 2016) and levels of nitrogen and phosphorus (Frost et al. 2023) was shown to be highly context-dependent, temperature change studies comprise very

heterogeneous approaches regarding their choice of experimental design.

To identify how temperature experiments with marine phytoplankton communities are designed, a systematic literature search has been conducted (see Methods; Supporting Information S1; Figs. S2.1, S2.2). It generally showed that an increase in temperature is performed either gradually (9 of 86 studies) with an applied rate of temperature change between 0.75°C d⁻¹ (Paul et al. 2021) and 2.5°C d⁻¹ (Soulié et al. 2023), but more often as an abrupt temperature exposure (72/86 studies), that is, directly placing the community on the experimental temperature below or above ambient conditions (e.g., Sommer and Lewandowska 2011; Moreau et al. 2014; Menden-Deuer et al. 2018). Even among the studies applying an abrupt temperature exposure, only half of the studies explicitly address this in the methods section (36/72), often it is not clearly stated but to be assumed from the experimental design (36/72). The abruptly applied temperature increases which were not defined as heat shock experiments were most often set to +3°C, +4°C, or +6°C, but also up to a temperature of +11.8°C compared to ambient conditions (Supporting Information Fig. S2.2). Furthermore, the literature search did not identify any study that tested the effect of different rates of temperature increase for a natural marine phytoplankton community. To our knowledge, this has only been tested for single species. In these studies, it was shown that populations abruptly exposed to temperatures above their acclimated condition achieved significantly higher growth rates than the population acclimated to this respective temperature (Kremer et al. 2018; Fey et al. 2021). This is referred to as gradual plasticity and describes phenotypic changes happening at a slower pace than the initiating environmental changes (Kremer et al. 2018). However, thermal acclimation can re-adjust the physiological processes that lead to the growth overshoot in monocultures in response to abrupt temperature exposure (Rehder et al. 2023).

Regarding the nutrient conditions during temperature change, the systematic literature map revealed that most studies use the ambient nutrient regime (46/86), but nutrient-enriched conditions are also common (19/86) to stimulate phytoplankton growth (Supporting Information Fig. S2.1). Few studies applied ambient-adapted nutrient conditions (6/86) which compensate for unusually low ambient concentrations of phosphorus or nitrogen at sampling time (Engel et al. 2011) or to achieve better comparability to a reference year or experiment (Sommer et al. 2007). Some studies (13/86) include at least two nutrient levels (also including studies using enriched treatments but with an ambient control), and only one of these also manipulated N : P supply ratios based on extended Representative Concentration Pathways scenarios (Moreno et al. 2022).

Overall, we lack studies testing if the species level response to different temperature change rates translates into natural communities or whether compensatory community dynamics may balance or outweigh the growth overshoot. Recently, it has also been shown that the temporal pattern of multiple

abiotic stressor occurrences (e.g., whether they are applied sequentially or simultaneously) defines the magnitude and direction of the combined effect, highlighting the importance but lack of consideration of timing in multi-stressor experiments (Gunderson et al. 2016; Brooks and Crowe 2019). More information is needed to compare temperature effects and their trade-offs between experimental designs in global change research and point toward the implications of choosing a certain rate of experimental temperature change, the nutrient regime, and timing of nutrient addition.

To fill the knowledge gaps outlined above, we experimentally addressed how the growth and stoichiometric responses were not only altered by the temperature level, but also their rate of temperature increase and the timing of nutrient addition. A microcosm study was conducted by exposing a natural phytoplankton spring community off the German coast at the Helgoland roads permanent sampling site to a nitrogen to phosphorus ratio gradient (from severe limitation to balanced ratios) across three temperature levels applied with either a gradual or abrupt temperature increase, and with nutrient addition during or after the temperature change (Fig. 1). Two consecutive microcosm experiments allowed for explicitly testing the following hypotheses:

(H1) The phytoplankton community growth rate and particulate N:P ratio depend on the rate of temperature change (abrupt vs. gradual) in interaction with nutrient supply ratios: precisely, (H1a) the growth performance of the abrupt exposure treatments is expected to show an overshoot compared to the gradually increasing temperature treatments (based on Anderson et al. 2022), with larger differences at higher temperatures (until the thermal optimum) under balanced nutrient conditions. (H1b) Limiting nutrient conditions lead to reduced growth rates which is strengthened at higher temperature levels (Thomas et al. 2017), and further decreased by abrupt temperature exposure.

By comparing the performance of communities that received the nutrient addition before vs. after the temperature increase, it is possible to disentangle whether (H2) the phytoplankton community growth rate and particulate N:P ratio depend on the timing of nutrient addition in interaction with the supplied nutrient ratios: Specifically, (H2a) when previously acclimated to an elevated temperature under ambient nutrient conditions, a nutrient addition after the temperature increase is expected to result in lower community growth rates and particulate N:P ratios compared to a community receiving the same nutrient additions before temperature increase. (H2b) This effect may also be strengthened under unbalanced or limiting nutrient conditions, as the community already used all remaining nutrients during thermal acclimation and drives into complete limitation.

Methods

Systematic literature map

A systematic literature search was performed, using the ISI Web of Knowledge as a search engine, to identify how experimental studies that investigate natural marine phytoplankton communities apply experimental temperature change treatments. The search and analysis followed the guideline of Preferred Reporting Item for Systematic Reviews and Meta-analysis in Ecology and Evolutionary biology (O'Dea et al. 2021) and matched 486 studies from which 83 papers and thus, 86 experimental designs remained after screening the full-texts. See Supporting Information S1 for details on the search string, inclusion criteria, categorization, the flow-chart of report screening, and a PRISMA-EcoEvo checklist. For extracting the information from the full-texts, only the method section and referred Supporting Information of each paper were considered.

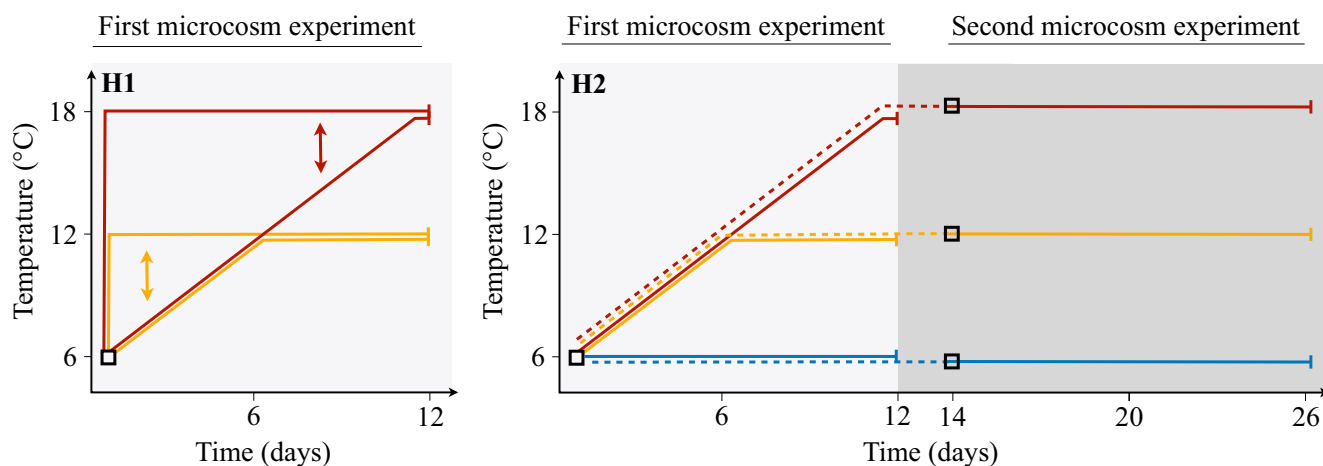


Fig. 1. Conceptual overview of experimental temperature treatments applied for testing the hypotheses (H1, H2). The line colors represent the final temperatures at 6 (blue), 12 (orange), and 18°C (red). For H1, an abrupt temperature exposure and a gradual temperature increase were applied. The black square represents the time point of nutrient addition to the microcosms. For H2, the dashed line indicates the thermal acclimation phase (under ambient nutrients) in indoor mesocosms before starting the microcosm experiment.

Experimental design

The initial plankton community originated from surface seawater at a depth of 5 m collected off the coast of Helgoland Roads long-term time series site in the German part of the North Sea (54°11, 3'N, 7°54, 0'E) on 06 March 2022 at 05:00 h (UTC) using a diaphragm pump and filtered through a 200- μm mesh to reduce mesozooplankton. The water was transported using eight 1000-liter polyethylene Intermediate Bulk Containers (IBC, AUER Packaging GmbH) onboard the German RV *Heincke*. A temperature of 5.4°C and a salinity of 30.7 PSU were recorded for the collection time and location. The phytoplankton community showed an initial concentration of $0.44 \pm 0.13 \mu\text{g}$ chlorophyll *a* L^{-1} .

The collected seawater was used to set up a mesocosm experiment in 600-liter stainless-steel tanks (analyzed in Ahme et al. 2024) and simultaneously run bottle incubations (microcosms) on the March 8, 2022. The effect of two gradual temperature increase scenarios (12°C and 18°C in steps of 1°C d^{-1}) and an ambient temperature control (6°C) on phytoplankton functional responses was tested in the Planktotrons indoor mesocosm facility (Gall et al. 2017). In addition, two consecutive microcosm experiments using 160 mL cell culture bottles (SARSTEDT AG & Co. KG) with ventilated caps were conducted. The mesocosms and microcosms experienced identical light conditions set to 175 μmol photons $\text{s}^{-1} \text{m}^{-2}$ from LED units (IT2040, Evergrow) and a day–night cycle of 12 h : 12 h chosen according to field conditions during that time of the year.

The first microcosm experiment started at the beginning of the mesocosm experiment using the initial phytoplankton community. In addition to the control (6°C), these microcosms were exposed to two temperature levels (12°C and 18°C) either as an abrupt exposure or as a gradual increase (1°C d^{-1}) and supplied with a wide gradient of N : P supply ratios (Table 1) as a unique pulse at the start of the incubation.

The communities used in the second microcosm experiment acclimated to their experimental temperature under ambient nutrient conditions in the mesocosms. The water for setting up the microcosm experiments was pooled across the four replicated mesocosms after the 18°C temperature ramp was completed (Fig. 1). The acclimated phytoplankton communities were placed at the respective constant experimental temperatures which they originated from (6°C, 12°C, and 18°C). The communities were supplied with the same nutrient matrix as a unique pulse at the start of the microcosm incubation. Accordingly, these microcosms started the incubation with different community compositions due to temperature-dependent species sorting during the acclimation phase, while the community dynamics in the first experiment were simultaneously temperature- and nutrient-dependent. In total, both microcosm experiments ran in duplicated and summed up to 400 units (8 temperature change scenarios \times 5 N levels \times 5 P levels \times 2 replicates). Both microcosm experiments were terminated after 12 d.

Table 1. Matrix of phosphorus (P) and nitrogen (N) concentrations and the resulting dissolved N : P ratios including the background concentration and the applied nutrient additions at the start of the first microcosm experiment. The ambient concentration (background concentration) refers to the lowest experimental level and is displayed in bold.

N ($\mu\text{mol L}^{-1}$)	P ($\mu\text{mol L}^{-1}$)				
	0.31	1.68	2.30	3.00	3.64
18.07	58	11	8	6	5
40.77	131	24	18	14	11
51.17	165	30	22	17	14
61.37	198	36	26	20	17
70.77	228	42	31	24	19

The nutrient treatments of both microcosm experiments were achieved by using five N and five P levels (similar to Gerhard et al. 2019) creating a wide gradient of N : P molar supply ratios (Table 1). The addition of N (NaNO_3) and P (NaH_2PO_4) to the seawater was conducted as a unique pulse at the start of the respective microcosm experiment. Ultimately, the final nutrient supply (total dissolved nutrients) consisted of the concentration in seawater plus the added nutrients and ranged from 18.07 to 70.77 $\mu\text{mol N L}^{-1}$ and 0.31 to 3.64 $\mu\text{mol P L}^{-1}$. The background concentration of dissolved nutrients was measured from the water samples before filling the bottles at the beginning of each microcosm experiment using a continuous flow auto-analyzer (Euro EA 3000; HEKATEch GmbH). The ambient nutrient conditions were 0.31 $\mu\text{mol P L}^{-1}$ and 18.07 $\mu\text{mol N L}^{-1}$ for the first microcosm experiment (Table 1), but differed between the temperature levels at 6°C (0.21 $\mu\text{mol P L}^{-1}$, 16.78 $\mu\text{mol N L}^{-1}$), 12°C (0.20 $\mu\text{mol P L}^{-1}$, 11.48 $\mu\text{mol N L}^{-1}$) and 18°C (0.20 $\mu\text{mol P L}^{-1}$, 18.58 $\mu\text{mol N L}^{-1}$) at the start of the second microcosm run. In the following, a balanced nutrient supply refers to both N and P being equally abundant or equally rare (Cardinale et al. 2009) corresponding to an N : P supply ratio of $\sim 16 : 1$ (Redfield 1958). Continuous data loggers (HOBO Pendant, Onset) monitored the temperature conditions during the experiment.

Every other day, 1 mL sample from each homogenized experimental unit was pipetted into a 48-well microplate (SARSTEDT AG & Co. KG) to measure in vivo autofluorescence of chlorophyll *a* (395/680 Ex./Em.) as a proxy for biomass using a SYNERGY H1 microplate reader (BioTek®). After 12 d, the experiments were terminated and one replicate was filtered onto precombusted acid-washed glass microfiber filters (Whatman® GF/C) to quantify their respective particulate carbon, nitrogen, and phosphorus content. This has also been done for the respective starting communities.

Filters for particulate organic carbon (POC) and nitrogen (PON) were dried at 60°C and measured using an elemental

auto-analyzer (Flash EA 1112, Thermo Scientific). The filters for particulate organic phosphorus (POP) were precombusted and analyzed by molybdate reaction after peroxydisulfate digestion (Wetzel and Likens 2003). The N : P ratio was calculated as the ratio between the molar masses of PON and POP.

The phytoplankton community composition throughout the mesocosm experiment and thus, the respective starting communities of the microcosm experiment (Supporting Information Fig. S2.3), were analyzed via V4 region of the 18S rRNA gene metabarcoding and is discussed in detail in Ahme et al. (2024). The thermal performance curve (TPC) of the start community showed a thermal optimum at 18°C (corresponding to the highest experimental temperature) and positive effect sizes of temperature on community growth were found between 7°C and 29°C (for details, see Ahme et al. 2024; Supporting Information Fig. S2.4).

Statistical analyses

Linear growth rates μ (d^{-1}) were calculated manually as the slope of a linear regression based as $(\ln(N_{t1}) - \ln(N_{t0}) / (t1 - t0))$, with N as the autofluorescence at the chosen start ($t0$) and endpoint ($t1$) of the first experiment. The two points have been chosen as the exponential growth phase, that is, the time interval between the end of the lag phase and before the biomass of the first samples within a temperature treatment reached the decay phase (see times series; Supporting Information Figs. S2.5–S2.10). The majority of units that were gradually increased to 18°C went into their decay phase before reaching their final temperature (Supporting Information Fig. S2.6). This resulted in a calculation between days 2 and 8 for the abrupt temperature exposure treatments and control in the first experiment, between days 4 and 10 for the gradual temperature increase in the first experiment, and days 2–6 for the second experiment.

To test for the effect of the rate of temperature change on the response of phytoplankton growth and particulate N : P ratios to temperature and nutrient supply, log-response ratios (LRRt) were calculated as $\log_{10}(\mu_1/\mu_2)$, with μ_1 as the mean growth rate of the abrupt temperature exposure treatment, and μ_2 as the mean growth of the gradually increasing temperature treatments for each temperature. To test for the effect of timing of nutrient addition relative to temperature change, LRRn were calculated as $\log_{10}(\mu_1/\mu_2)$, with μ_1 as the mean community growth rate when nutrients were added during the gradual temperature change (experiment 1) and μ_2 as the mean community growth rate when nutrients were added after the gradual acclimation (experiment 2) to test for the effect of nutrient availability during temperature change.

For all following analyses, the applied nutrient ratios were categorized into nitrogen-limited (final N : P ratio ≤ 11), balanced (12–39), or phosphorus-limited (> 40) nutrient conditions. This is based on Gerhard et al. (2019) who showed that the optimum N : P supply for a phytoplankton community

ranges between 13 and 40. This does not imply that all ratios in the assigned category were indeed limiting. For the statistical analyses of H1, generalized linear models (GLM) on the gradual and abrupt temperature increase treatments (12°C and 18°C) of the first experiment have been performed (μ , particulate N : P ratio \sim temperature level * N : P supply ratio * rate of temperature change; and LRRt \sim N : P supply ratio * temperature level). For the statistical analyses of H2, generalized linear models (μ , particulate N : P ratio \sim temperature level * N : P supply ratio * nutrient availability during temperature change; and LRRn \sim temperature level * N : P supply ratio) were conducted. Due to a right-shifted distribution of the growth data, a box-cox transformation with an exponent of three was used. The GLM for the particulate N : P ratio was run with log-transformed data. All GLMs were post-evaluated with a Tukey High Significant Differences post hoc test (Supporting Information Tables S2.1–S2.5).

All statistical results were interpreted as significant for a significance level of $\alpha = 0.05$ and were performed using the R statistical environmental version 4.2.3 (R Core Team 2023). All plots were created using the “ggplot2” package (Wickham 2016).

Results

The type of temperature increase

Whether the temperature change has been experienced as an abrupt exposure or a gradual increase showed a significant main effect on community μ (Table 2). An abrupt temperature exposure significantly increased overall μ at 12°C (by 9%) and 18°C (by 11%) compared to a gradual temperature change (Fig. 2; Supporting Information Fig. S2.11). Additionally, phosphorus-limited growth conditions significantly decreased community μ compared to both other nutrient conditions (Fig. 3; Supporting Information Table S2.1). When nutrients are limiting, especially in the gradual temperature increase treatments, community μ is less dependent on temperature compared to balanced nutrient conditions (i.e., similar μ over a 12°C thermal breadth) (Fig. 3). Although, no significant effect of the rate of temperature change on particulate N : P ratios has been found, significant differences between the three nutrient supply scenarios (N- or P-limited and balanced) were observed in which the N-limited nutrient conditions led to the lowest particulate N : P ratios, whereas P-limited conditions generated the highest particulate N : P ratios, mirroring the supplied ratios (Supporting Information Fig. S2.12; Table 2; Supporting Information Table S2.2). The LRRt was not significantly affected by temperature or nutrient conditions. Therefore, the general growth performance was affected by the rate of temperature change regardless of the final temperature level and nutrient conditions. Furthermore, no interactive effects of the rate of temperature change with the nutrient supply ratio or temperature level have been found for any response variable.

Table 2. GLMs of the rate of temperature change (rate), nitrogen to phosphorus (N : P) supply ratios (N-limited, P-limited, balanced), and temperature (T) on phytoplankton community growth rate (μ), particulate N : P ratios and log-response ratio between the abrupt and gradual temperature change treatment (LRRt).

Effect	df	μ		LRRt		N : P ratio	
		F	P	F	P	F	P
T	1	0.22	0.638	0.18	0.676	1.78	0.186
Ratio	1	50.85	<0.001*	1.36	0.268	74.00	<0.001*
Rate	1	28.49	<0.001*	—	—	2.53	0.115
T * Ratio	1	0.69	0.501	—	—	1.05	0.355
T * Rate	1	0.01	0.937	1.08	0.348	2.09	0.152
Ratio * Rate	1	0.55	0.577	—	—	0.59	0.585
T * Ratio * Rate	1	0.30	0.742	—	—	0.41	0.663

The timing of nutrient addition

The timing of nutrient availability showed significant main effects on community μ and particulate N : P ratios as well as complex interactive patterns (Table 3). Adding nutrients before temperature change led to an overall positive effect on community μ at 12°C and 18°C compared to 6°C, while adding nutrients after the temperature change reversed this effect (Fig. 2; Supporting Information Fig. S.2.11). This reversal was displayed in highest overall μ at 6°C when P was limiting after the temperature acclimation (Fig. 2). The reversed temperature effect was accentuated at balanced N : P supply ratios in the lowest temperature treatment reflecting the significant three-way interaction between the timing of nutrient

addition, temperature level, and nutrient supply ratio (Table 2). Moreover, the LRRn showed that the effect size was significantly shaped by the interaction of temperature level and nutrient supply ratio as well as both main effects (Table 2), with positive overall effects of the availability of nutrients during temperature change in the warming treatments compared to ambient temperature, and a pronounced negative effect under balanced nutrient supply under ambient temperature. Furthermore, it is evident from the measured background concentrations of dissolved phosphorus at the respective start conditions ($0.31 \mu\text{mol L}^{-1}$ in the first experiment and $0.21 \mu\text{mol L}^{-1}$ in the second experiment) and the growth response of the treatments without nutrient

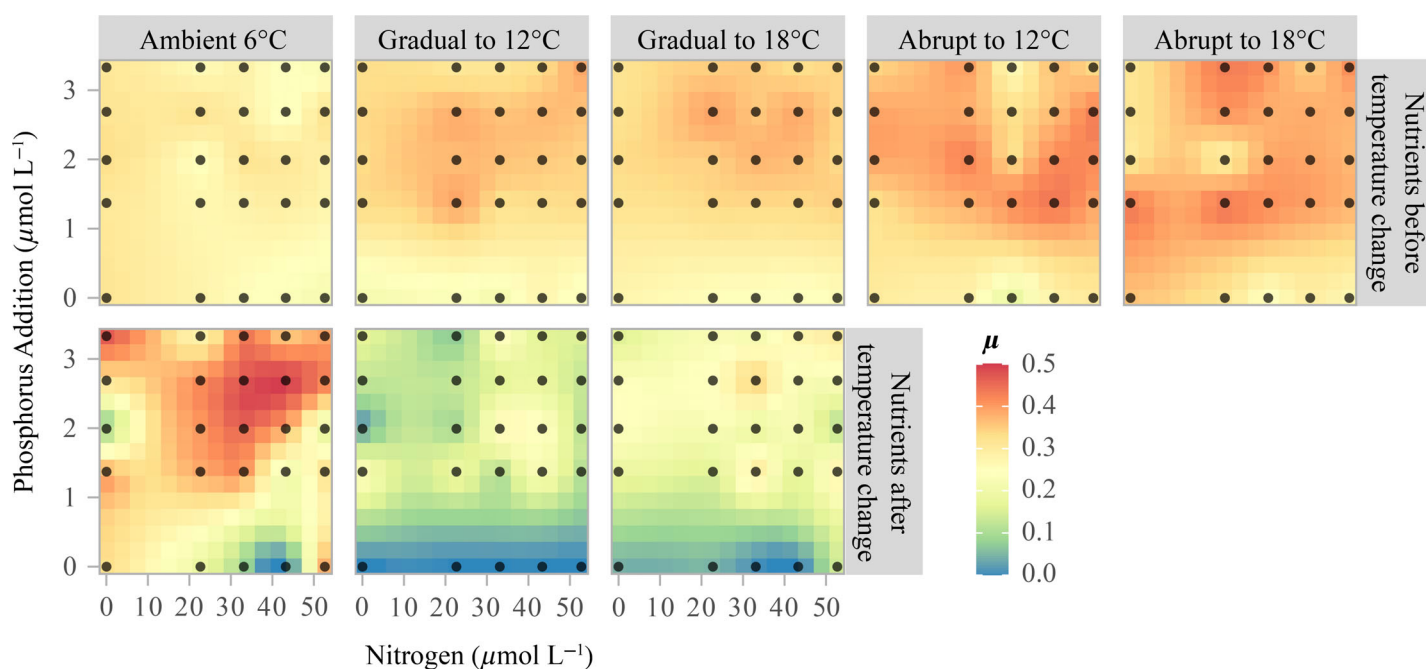


Fig. 2. Interpolated response surfaces of the growth rate (μ) over nitrogen and phosphorus supply ($\mu\text{mol L}^{-1}$). All values below 0 have been set equal to 0. The points mark the tested experimental conditions. The rows represent the first experiment with nutrients added during the temperature change (upper) or the second experimental phase with nutrient additions after the temperature change (lower).

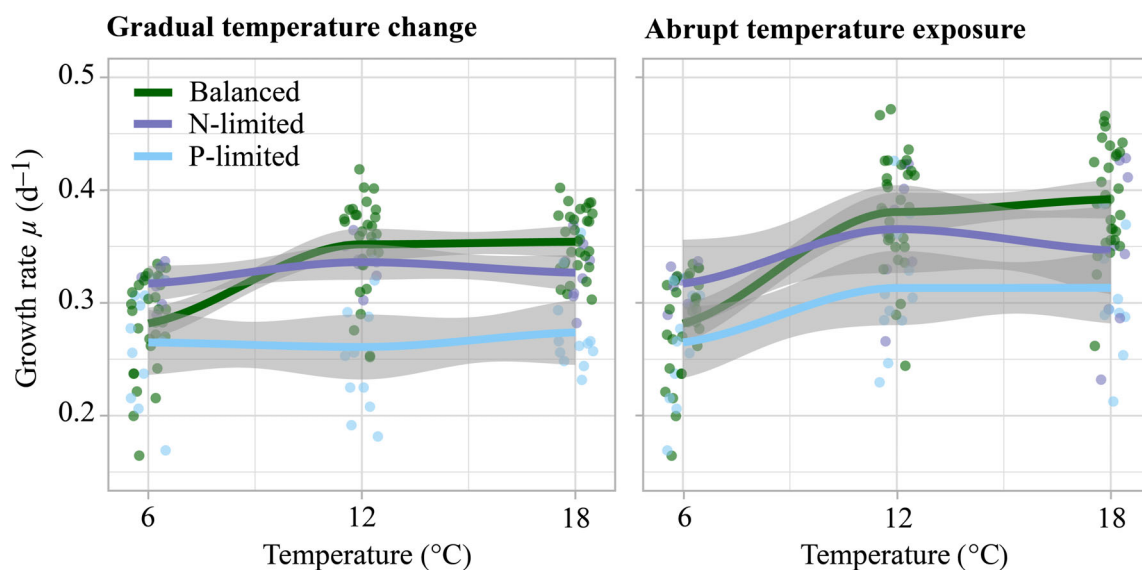


Fig. 3. Growth rate (d^{-1}) of the phytoplankton community across experimental temperatures. Colors indicate the applied nutrient conditions. Each point represents an individual observation. The gray areas show smoothed conditional means with a sensitivity of 0.8 and a GAM fit.

addition within the nutrient supply matrix that the P-limitation strengthened during the course of the thermal acclimation.

Additionally, the acclimation under ambient nutrients (i.e., nutrients added after warming) led to lower particulate N : P ratios compared to communities with access to nutrients during temperature change, and thus an increasing divergence occurred between the treatments until an N : P supply ratio of ~ 40 (Fig. 4). Beyond this threshold which also marks the P-limited scenario, the P-limitation led to a temperature-dependent increase in particulate N : P ratios. This increase was strongest at 18°C, whereby the communities that acclimated to temperature under nutrient depletion reached particulate N : P ratios 1.5-fold higher than communities with nutrients available during temperature change (Fig. 4). This reflects the highly significant three-way interactive effect of

timing of nutrient availability, temperature level, and ratio of supply nutrients (Table 3).

Discussion

With the type of temperature change and the timing of nutrient availability relative to warming, this study covers two key aspects not considered before when we evaluate the interplay between temperature and nutrient supply in experimental approaches, and how it modulates the growth response and stoichiometry in marine phytoplankton. First, the rate of temperature change influences how phytoplankton respond to warming, that is, abrupt temperature exposure overestimates the phytoplankton growth rates when compared with those obtained under a gradual temperature increase. Second, the timing of nutrient availability (under a balanced N : P

Table 3. GLMs of the timing of nutrient availability (NutAv), N : P supply ratios as a categorical variable (N-limited, P-limited, balanced), and temperature (T) on phytoplankton community growth rate (μ), particulate N : P ratios and the log-response ratio between treatments with nutrients added during versus after the temperature change (LRRn).

Effect	df	μ		LRRn		N : P ratio	
		F	P	F	P	F	P
T	2	36.30	<0.001*	115.92	<0.001*	2.34	0.101
Ratio	1	65.95	<0.001*	25.03	<0.001*	137.16	<0.001*
NutAv	1	93.54	<0.001*	—	—	38.27	<0.001*
T * Ratio	4	1.60	0.175	5.84	0.212	6.54	<0.001*
T * NutAv	2	90.25	<0.001*	—	—	0.01	0.988
Ratio * NutAv	1	13.81	0.005*	—	—	7.75	<0.001*
T * Ratio * NutAv	2	2.58	0.037*	—	—	6.75	<0.001*

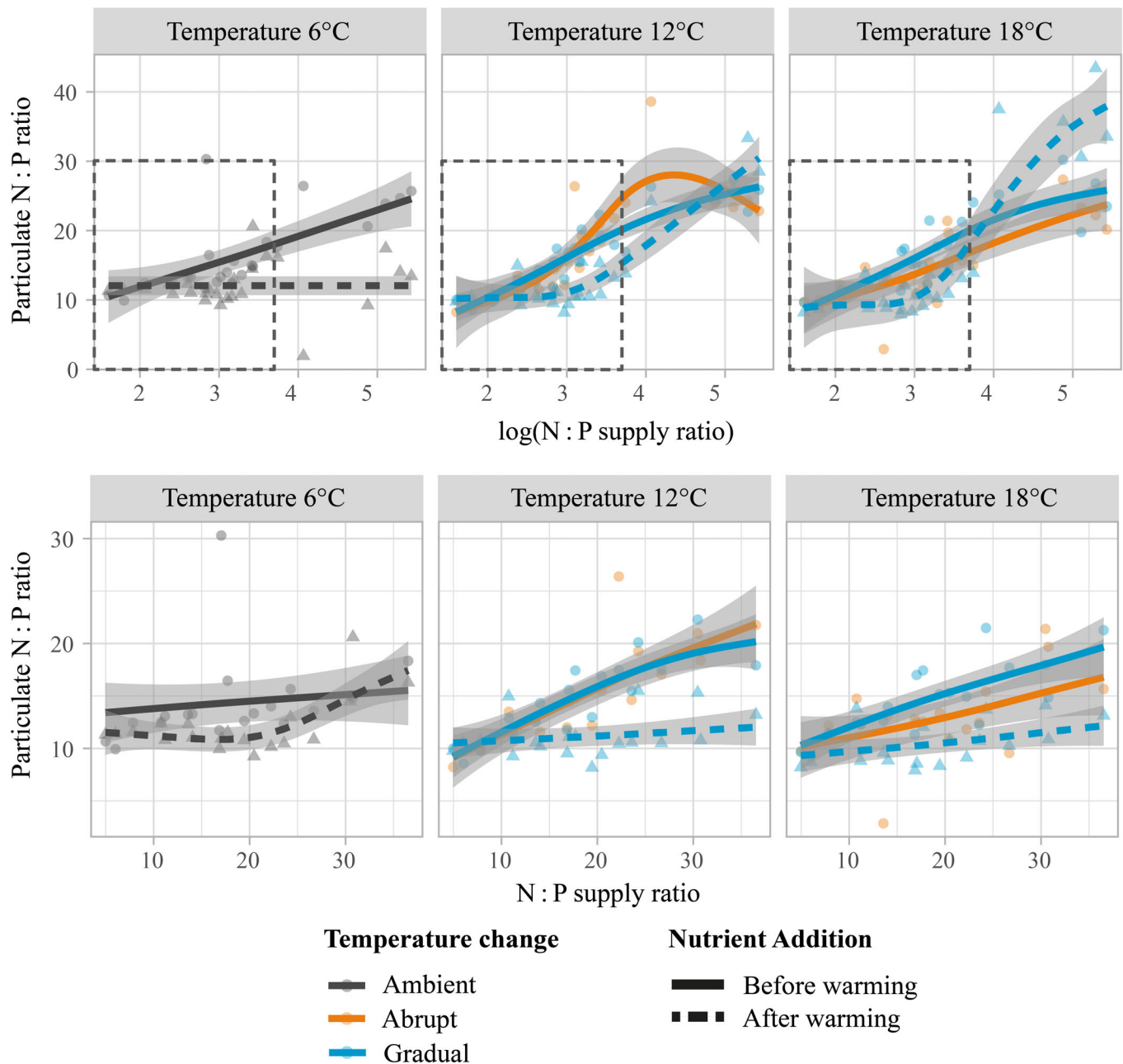


Fig. 4. Phytoplankton final particulate N : P ratios across N : P supply ratios (including background concentration) and experimental temperatures. The upper panels include all treatments on a logarithmic scale (to visualize the effects of very high N : P supply ratios), the lower panels focus on the low to intermediate N : P supply ratios (≤ 42) by excluding the lowest phosphorus level. The rectangle in the upper panels represents the area shown in the lower panels. A GAM smoothing has been applied. The color indicates an abrupt (red) or gradual (blue) temperature change. The line type and shape of points represent ambient nutrient conditions during temperature change (dashed line and triangles) or nutrient additions before temperature change (solid line and circles).

supply) determines the magnitude and direction of the effects of temperature change on phytoplankton. On the one hand, some of the found patterns (e.g., the growth overshoot under abrupt temperature exposure) are in accordance with findings in monoculture studies (e.g., Kremer et al. 2018; Fey et al. 2021). Still, on the other hand, natural communities show more complex patterns and interactive effects with the

rate of temperature change and timing of nutrient availability driving their biological adjustments.

Abrupt vs. gradual temperature increase

Phytoplankton community growth rates generally increased with warming although depending on the rate of temperature change by overshooting in the abruptly exposed

temperature treatments compared to the gradual temperature increase treatments. The natural phytoplankton spring community used in our experiments was sampled at 5.4°C ambient temperature which is close to the identified thermal minimum in the community TPC. This suggests a community at the initiation of its spring bloom as thermal limitation was slowly alleviated in the field. With the thermal optimum of the community TPC at 18°C and being exposed to high temperature variability in the North Sea (Wiltshire and Manly 2004), the studied spring community naturally held a high potential for a positive response to higher temperatures. The broad thermal breadth displayed by the community TPC can potentially be explained by species in the community living below their temperature optimum to avoid detrimental effects of supra-optimal temperatures (Thomas et al. 2012) and/or (summer) species that were already present in low abundance ready to thrive at higher temperatures.

In species-specific studies, a higher performance under abrupt thermal changes in comparison with gradual changes has been attributed to gradual plasticity (Kremer et al. 2018). The growth rates of the community abruptly exposed to higher temperatures exceeded those of the gradually increasing temperature treatments, potentially due to a temporal delay in physiological acclimation such as regulations in respiration rate, photosynthetic machinery, and resource acquisition (Barton et al. 2020; Fey et al. 2021). However, in the long-term, a gradual abiotic change can lead to a higher end-point performance (Collins and de Meaux 2009). Thereby, surviving gradual warming on the species level is determined by acclimation and evolutionary processes, while surviving abruptly temperature exposure is based on resistance mechanisms (Peck et al. 2009). In natural phytoplankton assemblages, interspecific and intraspecific competition and selection can complement mechanisms based on physiological regulations (Bestion et al. 2018). For intraspecific population dynamics, sudden environmental changes may lead to the streamlining of a few well-adapted genotypes while gradual changes maintain higher genetic variability, thus buffering against additional perturbations (Hughes and Stachowicz 2004). Regarding interspecific competition, species that are more temperature-tolerant to high temperatures have a competitive advantage under abruptly temperature exposure that potentially results in an abrupt dominance shift toward more thermally resilient species. Contrarily, a gradual temperature increase provides more time for physiological adjustments within different species (Fey et al. 2021) alongside interspecific competitive interactions and with this reduces abrupt shifts in community composition and increases a potential proliferation of species with a more sustainable resource use. Overall, an abrupt temperature exposure may be predominantly driven by the species' physiological limits (Stefanidou et al. 2018) whereas, during a gradual change, competitive interactions gain importance.

Although we confirmed a short-term growth overshoot at both abruptly exposed temperature levels (in line with our

hypothesis H1), the difference in growth rate between the gradual and abrupt temperature exposures did not increase with increasing temperature, contradicting our hypothesis H1a. Furthermore, when nutrients were limiting (especially under gradual temperature increase), growth was completely independent of temperature resulting in similar growth rates over a 12°C thermal breadth. This reinforces the idea that nutrient limitation suppresses the thermal dependence of physiological processes which has been explicitly tested for single species (Marañón et al. 2018) and observed for a freshwater community (O'Connor et al. 2009).

Moreover, we found phosphorus-limited nutrient conditions to suppress community growth equally among the gradual temperature treatments (which partly rejects H1b). In line, Anderson et al. (2022) found (gradual) warming to be beneficial for community growth only under nutrient-amended conditions, whereas under nutrient limitation, warming acted as a second stressor and decreased community growth rates compared to those of the initial community. In our study, however, even with the second stressor of phosphorus limitation, abrupt temperature exposure still increased community growth for both higher temperatures compared to ambient temperature, underlining an increased phosphorus use efficiency (*temperature-dependent physiology* hypothesis). Despite lower relative phosphorus requirements with increased temperature, a phosphorus threshold concentration is likely a prerequisite for positive net community growth. Nevertheless, the results of our study suggest that the background concentration of nitrogen was not actually limiting community growth.

When applying a gradual increase in temperature, also the rate of environmental change determines which biological processes are important for the successful performance of an organism (Peck et al. 2009). Even among the studies inducing a gradual temperature increase, experimental warming applied within marine system studies is usually 10,000–100,000 times faster than predicted ocean warming (Peck et al. 2009). This has practical reasons and only this limitation makes laboratory experiments for global change research feasible. However, thermal responses determined by such relatively fast temperature change experiments should be used with care for predicting climate change effects on phytoplankton. Furthermore, it needs to be considered that the exponential growth phase during a gradual temperature increase may not cover the entire warming process and thus, affect the interpretation of calculated growth rates.

Thermal acclimation is a good way to let physiological processes adjust prior to experimental manipulation in monocultures (Rehder et al. 2023). However, acclimation such as the gradual increase in temperature conducted in this experiment changed the taxonomic composition during the acclimation period (i.e., period of gradual increase) (Ahme et al. 2024; Supporting Information Fig. S2.3). Consequently, communities arose with potentially different nutritional requirements, strategies, and limitations that may respond differently to

experimental treatments such as the later addition of nutrients.

The timing of nutrient addition: Growth

Our results further demonstrate that the community growth response depended on the timing of nutrient addition, the interaction with the nutrient supply ratio, and additionally the threefold interaction with both and the temperature level (which is in line with our hypothesis H2). The overall increase in phytoplankton community growth rate with warming (up to the optimum temperature) under nutrient-enriched conditions is an often-observed pattern in experimental studies (Bestion et al. 2018; Aranguren-Gassis et al. 2019; Fernández-González et al. 2020) and can be attributed to an increase in metabolic rates with higher temperatures under sufficiently available resources that support growth (Eppley 1972; Raven and Geider 1988). However, when the community was acclimated to its respective experimental temperature under ambient nutrient conditions and received a nutrient addition afterward, the ambient temperature treatment showed the highest growth performance (confirming our hypothesis H2a).

Although significant interactions of nutrient conditions and temperature have been demonstrated for the growth response in species-specific studies (Thomas et al. 2017; Aranguren-Gassis et al. 2019; Fernández-González et al. 2020), a temperature–nutrient interaction was not found in this experiment. This may be explained by the capability of a diverse community to buffer nutrient-dependent responses to temperature as long as minimum phosphorus requirements are covered. This potential minimum threshold was observed in the first experiment showing community growth despite phosphorus limitation, whereas in the second experiment, phosphorus was entirely depleted before the start of the experiment which led to the timing of nutrient availability to reverse the temperature effect. Therein, an increased metabolism could not be sustained under extreme phosphorus limitation and led to a collapse of the community (as predicted in hypothesis H2b). Similarly, Verbeek et al. (2018) found a relatively high phytoplankton community biomass under replete nutrients, but detrimental temperature effects under strengthening oligotrophication, highlighting that with a lack of available nutrients, the increased resource demand to maintain increased physiological processes cannot be satisfied.

The timing of nutrient addition: Stoichiometry

The type of nutrient limitation (P or N limitation) determined how the timing of nutrient addition (before vs. after temperature change) affected the particulate N:P ratios (which supports our hypothesis H2). While P-limitation exerted an interactive effect between nutrient supply, temperature level, and timing of nutrient addition, the N-limiting scenario did not show any significant differences in particulate N:P ratios compared to a balanced N:P supply.

In theory, higher temperatures increase the organismal N:P ratios due to a lower requirement in phosphorus-rich ribosomes relative to nitrogen-rich proteins to maintain growth as predicted by the *temperature-dependent physiology* hypothesis (Woods et al. 2003). Although we did not find a temperature main effect on phytoplankton N:P ratios, our study showed a divergence (i.e., increasing difference) in particulate N:P ratios in response to the timing of nutrient addition with increasing N:P supply ratios (≤ 40) which was only found for the warming treatments.

The N:P supply ratio around 40 lies within a range shown for a transition into a complete phosphorus limitation (Geider and La Roche 2002). From this transition point onwards, the communities that received nutrients during warming already started to saturate at particulate N:P ratios of ~ 25 , while only the communities that received the nutrient addition after thermal acclimation exceeded the others at 18°C with particulate N:P ratios of up to 40. The particulate ratio of 40 may reach physiological limits leading to a saturation with increasing N:P supply ratios which has also been shown for a freshwater phytoplankton community (Gerhard et al. 2019). In line, Klausmeier et al. (2004) also showed this particulate ratio to be at the upper end of structural N:P ratios of phytoplankton. The differences in phytoplankton community N:P ratios might be explained by two mechanisms: First, different phytoplankton species with specific particulate N:P ratios dominate under the respective experimental condition (Finkel et al. 2009), and second, the particulate N:P ratio of the present species change in response to the experimental condition (stoichiometric plasticity) (Yvon-Durocher et al. 2015). Due to the lack of community composition data at the end of the experiments, we are not able to determine the exact mechanism underpinning the response pattern observed here, however, it is likely that they act together in creating this complex interactive pattern as they are not mutually exclusive.

Implications for experimental design

The systematic literature map revealed an overrepresentation of abrupt temperature increase experiments and lack of clear reporting on the rate of temperature increase and experimental nutrient conditions, whereas our experimental results highlighted that an abrupt temperature exposure induces a short-term community growth overshoot compared to gradually increasing temperature, but without effects on the particulate N:P ratio. The addition of nutrients after (vs. before) thermal acclimation leads to a complex reversed temperature effect on growth and a response divergence with increasing N:P supply ratio in particulate N:P ratios.

These findings evidence that the selection of a combination of temperature change rate and timing of nutrient supply in future global change biology studies may not be trivial. If the study is conducted as a batch culture with one unique pulse, the rate of temperature change or even the decision of

whether the nutrients are applied during the acclimation phase (i.e., simultaneously with the temperature change) or at the beginning of the experiment (i.e., after the temperature change) can lead to significantly different outcomes in terms of community growth and stoichiometry.

Gunderson et al. (2016) already reported on the bias in experimental design toward simultaneously applied multiple stressors, rather than a range of different and potentially more realistic temporal patterns, with the consequence of predominantly finding synergistic effects of multiple stressors. In addition, the effects of several stressors were longer-lasting when the time lag between their occurrence was increased (Brooks and Crowe 2019). Therefore, the results of our study emphasize the need for considering the timing in multiple stressor studies (i.e., temperature increase and nutrient limitation level, in our case). Additionally, the results evidence the need for multi-level driver experiments to generate response surfaces that can contribute to the improvement of predictive models (Collins et al. 2022). Often, global change studies only consider two levels for a given driver (i.e., control vs. manipulated), while the results indicate complex interactive patterns when changes in the N : P supply ratio are considered among temperature scenarios.

To summarize, when designing a laboratory or mesocosm experiment aimed at testing the effect of temperature change on natural phytoplankton communities, we propose to carefully consider the rate of temperature change, the timing of nutrient addition and the N : P supply ratio to produce ecologically relevant results. Being aware of the implications of different rates of temperature change as well as nutrient additions and its timing, and clearly stating this and the reason for the decision in the methods section improves the interpretation of results, comparability across studies, and the transfer to natural systems.

Data availability statement

The data that support the findings of this study are openly available in PANGAEA (<https://doi.org/10.1594/PANGAEA.963753>). The associated R scripts are provided in a public GitHub repository (<https://github.com/AnikaHappe/AQUACOSM2022>).

References

- Ahme, A., and others. 2024. Warming increases the compositional and functional variability of temperate protist communities. *Sci. Total Environ.* **926**: 171971. doi:10.1016/j.scitotenv.2024.171971
- Anderson, S. I., and others. 2022. The interactive effects of temperature and nutrients on a spring phytoplankton community. *Limnol. Oceanogr.* **67**: 634–645.
- Aranguren-Gassis, M., C. T. Kremer, C. A. Klausmeier, and E. Litchman. 2019. Nitrogen limitation inhibits marine diatom adaptation to high temperatures. *Ecol. Lett.* **22**: 1860–1869.
- Barton, S., and others. 2020. Evolutionary temperature compensation of carbon fixation in marine phytoplankton. *Ecol. Lett.* **23**: 722–733.
- Bestion, E., B. Garcia-Carreras, C. E. Schaum, S. Pawar, and G. Yvon-Durocher. 2018. Metabolic traits predict the effects of warming on phytoplankton competition. *Ecol. Lett.* **21**: 655–664.
- Boyd, P. W., and others. 2015. Physiological responses of a Southern Ocean diatom to complex future ocean conditions. *Nat. Clim. Change* **6**: 207–213.
- Boyd, P. W., and others. 2018. Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change—A review. *Glob. Change Biol.* **24**: 2239–2261.
- Brooks, P. R., and T. P. Crowe. 2019. Combined effects of multiple stressors: New insights into the influence of timing and sequence. *Front. Ecol. Evol.* **7**: 387.
- Burson, A., M. Stomp, L. Akil, C. P. D. Brussaard, and J. Huisman. 2016. Unbalanced reduction of nutrient loads has created an offshore gradient from phosphorus to nitrogen limitation in the North Sea. *Limnol. Oceanogr.* **61**: 869–888.
- Cardinale, B. J., H. Hillebrand, W. S. Harpole, K. Gross, and R. Ptacnik. 2009. Separating the influence of resource “availability” from resource “imbalance” on productivity–diversity relationships. *Ecol. Lett.* **12**: 475–487.
- Collins, S., and J. de Meaux. 2009. Adaptation to different rates of environmental change in *Chlamydomonas*. *Evolution* **63**: 2952–2965.
- Collins, S., H. Whittaker, and M. K. Thomas. 2022. The need for unrealistic experiments in global change biology. *Curr. Opin. Microbiol.* **68**: 102151.
- De Senerpont-Domis, L. N., D. B. Van de Waal, N. R. Helmsing, E. Van Donk, and W. M. Mooji. 2014. Community stoichiometry in a changing world: Combined effects of warming and eutrophication on phytoplankton dynamics. *Ecology* **95**: 1485–1495.
- Edwards, K. F., M. K. Thomas, C. A. Klausmeier, and E. Litchman. 2012. Allometric scaling and taxonomic variation in nutrient utilization traits and maximum growth rate of phytoplankton. *Limnol. Oceanogr.* **57**: 554–566.
- Engel, A., and others. 2011. Effects of sea surface warming on the production and composition of dissolved organic matter during phytoplankton blooms: Results from a mesocosm study. *J. Plankton Res.* **33**: 357–372.
- Eppley, R. W. 1972. Temperature and phytoplankton growth in the sea. *Fish. Bull.* **70**: 1063–1085.
- Fernández-González, C., M. Pérez-Lorenzo, N. Pratt, C. M. Moore, T. S. Bibby, and E. Marañón. 2020. Effects of temperature and nutrient supply on resource allocation, photosynthetic strategy, and metabolic rates of *Synechococcus* sp. *J. Phycol.* **56**: 818–829.

- Fey, S. B., C. T. Kremer, T. J. Layden, and D. A. Vasseur. 2021. Resolving the consequences of gradual phenotypic plasticity for populations in variable environments. *Ecol. Monogr.* **91**: e01478.
- Finkel, Z. V., J. Beardall, K. J. Flynn, A. Quigg, T. A. V. Rees, and J. A. Raven. 2009. Phytoplankton in a changing world: Cell size and elemental stoichiometry. *J. Plankton Res.* **32**: 119–137.
- Frost, P. C., and others. 2023. Interactive effects of nitrogen and phosphorus on growth and stoichiometry of lake phytoplankton. *Limnol. Oceanogr.* **68**: 1172–1184.
- Gall, A., and others. 2017. Planktotrons: A novel indoor mesocosm facility for aquatic biodiversity and food web research. *Limnol. Oceanogr. Methods* **15**: 663–677.
- Geider, R., and J. La Roche. 2002. Redfield revisited: Variability of C:N:P in marine microalgae and its biochemical basis. *Eur. J. Phycol.* **37**: 1–17.
- Gerhard, M., A. M. Koussoroplis, H. Hillebrand, and M. Striebel. 2019. Phytoplankton community responses to temperature fluctuations under different nutrient concentrations and stoichiometry. *Ecology* **100**: e02834.
- Gunderson, A. R., E. J. Armstrong, and J. H. Stillman. 2016. Multiple stressors in a changing world: The need for an improved perspective on physiological responses to the dynamic marine environment. *Annu. Rev. Mar. Sci.* **8**: 357–378.
- Hessen, D. O., J. J. Elser, R. W. Sterner, and J. Urabe. 2013. Ecological stoichiometry: An elementary approach using basic principles. *Limnol. Oceanogr.* **58**: 2219–2236.
- Hughes, A. R., and J. J. Stachowicz. 2004. Genetic diversity enhances the resistance of a seagrass ecosystem to disturbance. *Proc. Nat. Acad. Sci. USA* **101**: 8998–9002.
- IPCC, 2023. Summary for Policymakers, p. 1–34. In Core Writing Team, H. Lee and J. Romero [eds.], *Climate Change 2023: Synthesis Report. Contribution of Working Groups I, II and III to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change*. IPCC. doi:10.59327/IPCC/AR6-9789291691647.001
- Klausmeier, C. A., E. Litchman, T. Daufrense, and S. A. Levin. 2004. Optimal nitrogen-to-phosphorus stoichiometry of phytoplankton. *Nature* **429**: 171–174.
- Kremer, C. T., S. B. Fey, A. A. Arellano, and D. A. Vasseur. 2018. Gradual plasticity alters population dynamics in variable environments: Thermal acclimation in the green alga *Chlamydomonas reinhardtii*. *Proc. R. Soc. B. Biol. Sci.* **285**: 20171942.
- Kwiatkowski, L., O. Aumont, L. Bopp, and P. Ciais. 2018. The impact of variable phytoplankton stoichiometry on projections of primary production, food quality, and carbon uptake in the global ocean. *Global Biogeochem. Cycles* **32**: 516–528.
- Litchman, E., and C. A. Klausmeier. 2008. Trait-based community ecology of phytoplankton. *Annu. Rev. Ecol. Evol. Syst.* **39**: 615–639.
- Malone, T. C., and A. Newton. 2020. The globalization of cultural eutrophication in the coastal ocean: Causes and consequences. *Front. Mar. Sci.* **7**: 670.
- Marañón, E., M. Pérez-Lorenzo, P. Cermeño, and B. Mouriño-Carballido. 2018. Nutrient limitation suppresses the temperature dependence of phytoplankton metabolic rates. *ISME J.* **12**: 1836–1845.
- Menden-Deuer, S., C. Lawrence, and G. Franze. 2018. Herbivorous protist growth and grazing rates at in situ and artificially elevated temperatures during an Arctic phytoplankton spring bloom. *PeerJ* **6**: e5264.
- Moreau, S., and others. 2014. Effects of enhanced temperature and ultraviolet B radiation on a natural plankton community of the Beagle Channel (southern Argentina): A mesocosm study. *Aquat. Microb. Ecol.* **72**: 155–173.
- Moreno, H. D., and others. 2022. An integrated multiple driver mesocosm experiment reveals the effect of global change on planktonic food web structure. *Commun. Biol.* **5**: 179.
- O'Connor, M. I., M. F. Piehler, D. M. Leech, A. Anton, and J. F. Bruno. 2009. Warming and resource availability shift food web structure and metabolism. *PLoS Biol.* **7**: e1000178.
- O'Dea, R. E., and others. 2021. Preferred reporting items for systematic reviews and meta-analyses in ecology and evolutionary biology: A PRISMA extension. *Biol. Rev. Camb. Philos. Soc.* **96**: 1695–1722.
- Pálffy, K., A. W. Kovács, V. Kardos, I. Hausz, and G. Boros. 2021. Elevated temperature results in higher compositional variability of pioneer phytoplankton communities in a mesocosm system. *J. Plankton Res.* **43**: 142–155.
- Paul, C., U. Sommer, and B. Matthiessen. 2021. Composition and dominance of edible and inedible phytoplankton predict responses of Baltic Sea summer communities to elevated temperature and CO₂. *Microorganisms* **9**: 2294.
- Peck, L. S., M. S. Clark, S. A. Morley, A. Massey, and H. Rossetti. 2009. Animal temperature limits and ecological relevance: Effects of size, activity and rates of change. *Funct. Ecol.* **23**: 248–256.
- R Core Team. 2023. R: A language and environment for statistical computing. R Foundation for Statistical Computing, Available from <https://www.R-project.org/>
- Rabalais, N. N., R. E. Turner, R. J. Díaz, and R. D. Justic. 2009. Global change and eutrophication of coastal waters. *ICES J. Mar. Sci.* **66**: 1528–1537.
- Raven, J. A., and R. J. Geider. 1988. Temperature and algal growth. *New Phytol.* **110**: 441–461.
- Redfield, A. C. 1958. The biological control of chemical factors in the environment. *Am. Sci.* **46**: 205–221.
- Rehder, L., B. Rost, and S. D. Rokitta. 2023. Abrupt and acclimation responses to changing temperature elicit divergent physiological effects in the diatom *Phaeodactylum tricorutum*. *New Phytol.* **239**: 1005–1013.

- Rönn, L., and others. 2023. Harmonisation of the phytoplankton assessment in the German and Dutch Wadden Sea. Interreg V A project “Wasserqualität—Waterkwaliteit”—Synthesis report. Report prepared on behalf of NLWKN and Rijkswaterstaat, Oldenburg/Lelystad, 2023.
- Sommer, U., and others. 2007. An indoor mesocosm system to study the effect of climate change on the late winter and spring succession of Baltic Sea phyto- and zooplankton. *Oecologia* **150**: 655–667.
- Sommer, U., and A. Lewandowska. 2011. Climate change and the phytoplankton spring bloom: Warming and overwintering zooplankton have similar effects on phytoplankton. *Glob. Change Biol.* **17**: 154–162.
- Soulié, T., F. Vidussi, S. Mas, and B. Mostajir. 2022. Functional stability of a coastal Mediterranean plankton community during an experimental marine heatwave. *Front. Mar. Sci.* **9**: 831496.
- Soulié, T., F. Vidussi, S. Mas, and B. Mostajir. 2023. Functional and structural responses of plankton communities toward consecutive experimental heatwaves in Mediterranean coastal waters. *Sci. Rep.* **13**: 8050.
- Stefanidou, N., S. Genitsaris, J. Lopez-Bautista, U. Sommer, and M. Moustaka-Gouni. 2018. Effects of heat shock and salinity changes on coastal Mediterranean phytoplankton in a mesocosm experiment. *Mar. Biol.* **165**: 145.
- Steinacher, M., and others. 2010. Projected 21st century decrease in marine productivity: A multi-model analysis. *Biogeosciences* **7**: 979–1005.
- Sterner, R. W., and J. J. Elser. 2002. *Ecological stoichiometry: The biology of elements from molecules to the biosphere.* Princeton Univ. Press.
- Striebel, M., S. Schabhtl, D. Hodapp, P. Hingsamer, and H. Hillebrand. 2016. Phytoplankton responses to temperature increases are constrained by abiotic conditions and community composition. *Oecologia* **182**: 815–827.
- Thomas, M. K., C. T. Kremer, C. A. Klausmeier, and E. Litchman. 2012. A global pattern of thermal adaptation in marine phytoplankton. *Science* **338**: 1085–1088.
- Thomas, M. K., and others. 2017. Temperature-nutrient interactions exacerbate sensitivity to warming in phytoplankton. *Glob. Change Biol.* **23**: 3269–3280.
- Thrane, J. E., D. O. Hessen, and T. Andersen. 2017. Plasticity in algal stoichiometry: Experimental evidence of a temperature-induced shift in optimal supply N:P ratio. *Limnol. Oceanogr.* **62**: 1346–1354.
- Tilman, D., S. S. Kilham, and P. Kilham. 1982. Phytoplankton community ecology: The role of limiting nutrients. *Annu. Rev. Ecol. Syst.* **13**: 349–372.
- Verbeek, L., A. Gall, H. Hillebrand, and M. Striebel. 2018. Warming and oligotrophication cause shifts in freshwater phytoplankton communities. *Glob. Change Biol.* **24**: 4532–4543.
- Wetzel, R. G., and G. E. Likens. 2003. *Limnological analyses.* Springer-Verlag.
- Wickham, H. 2016. *ggplot2: Elegant graphics for data analysis.* Springer-Verlag.
- Wiltshire, K. H., and B. F. J. Manly. 2004. The warming trend at Helgoland Roads, North Sea: phytoplankton response. *Helgol. Mar. Res.* **58**: 269–273.
- Woods, H. A., and others. 2003. Temperature and the chemical composition of poikilothermic organisms. *Funct. Ecol.* **17**: 237–245.
- Yvon-Durocher, G., M. Dossena, M. Trimmer, G. Woodward, and A. P. Allen. 2015. Temperature and the biogeography of algal stoichiometry. *Glob. Ecol. Biogeogr.* **24**: 562–570.
- Yvon-Durocher, G., C. E. Schaum, and M. Trimmer. 2017. The temperature dependence of phytoplankton stoichiometry: Investigating the roles of species sorting and local adaptation. *Front. Microbiol.* **8**: 2003.

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Conflict of Interest

None declared.

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