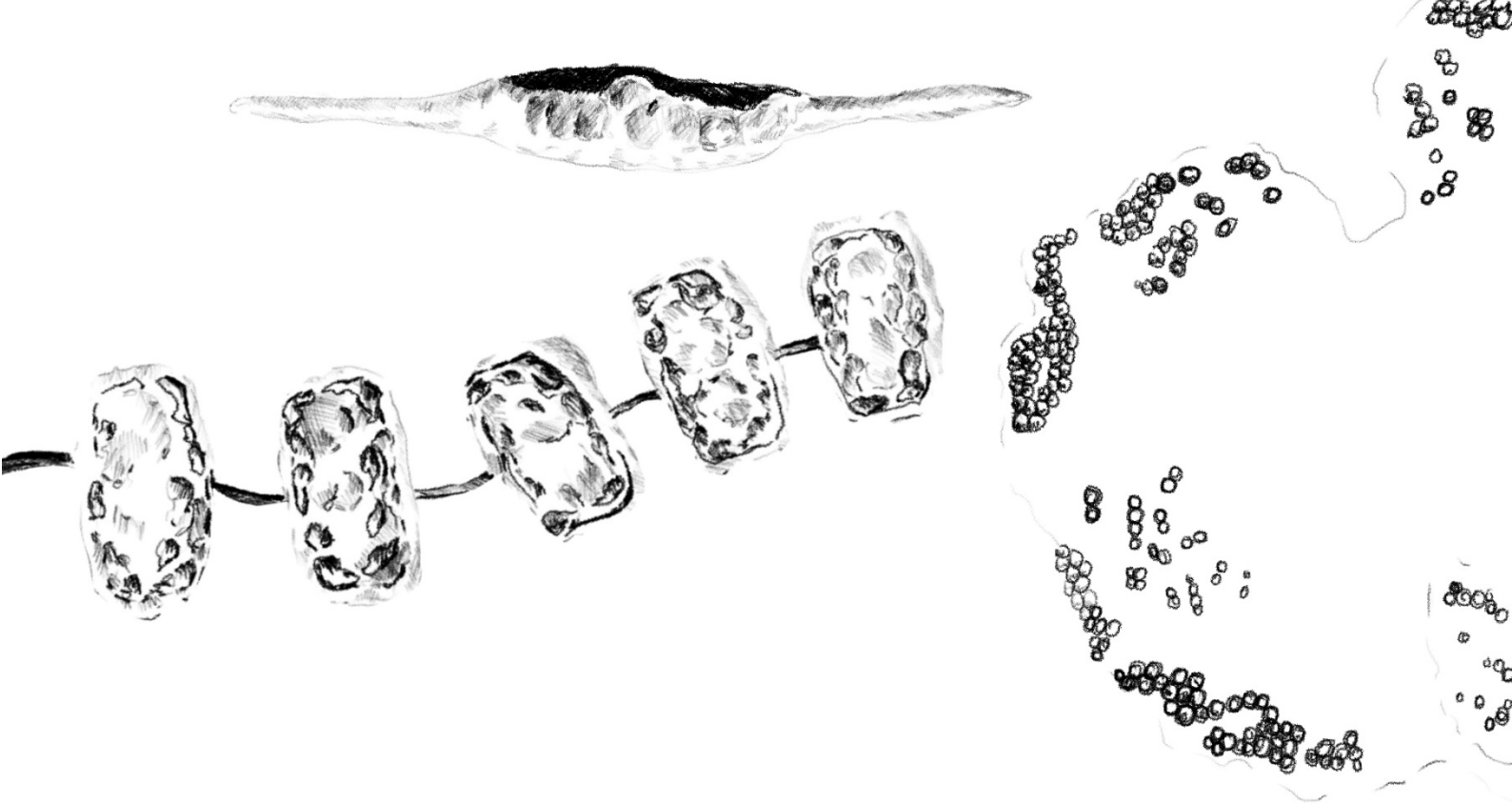
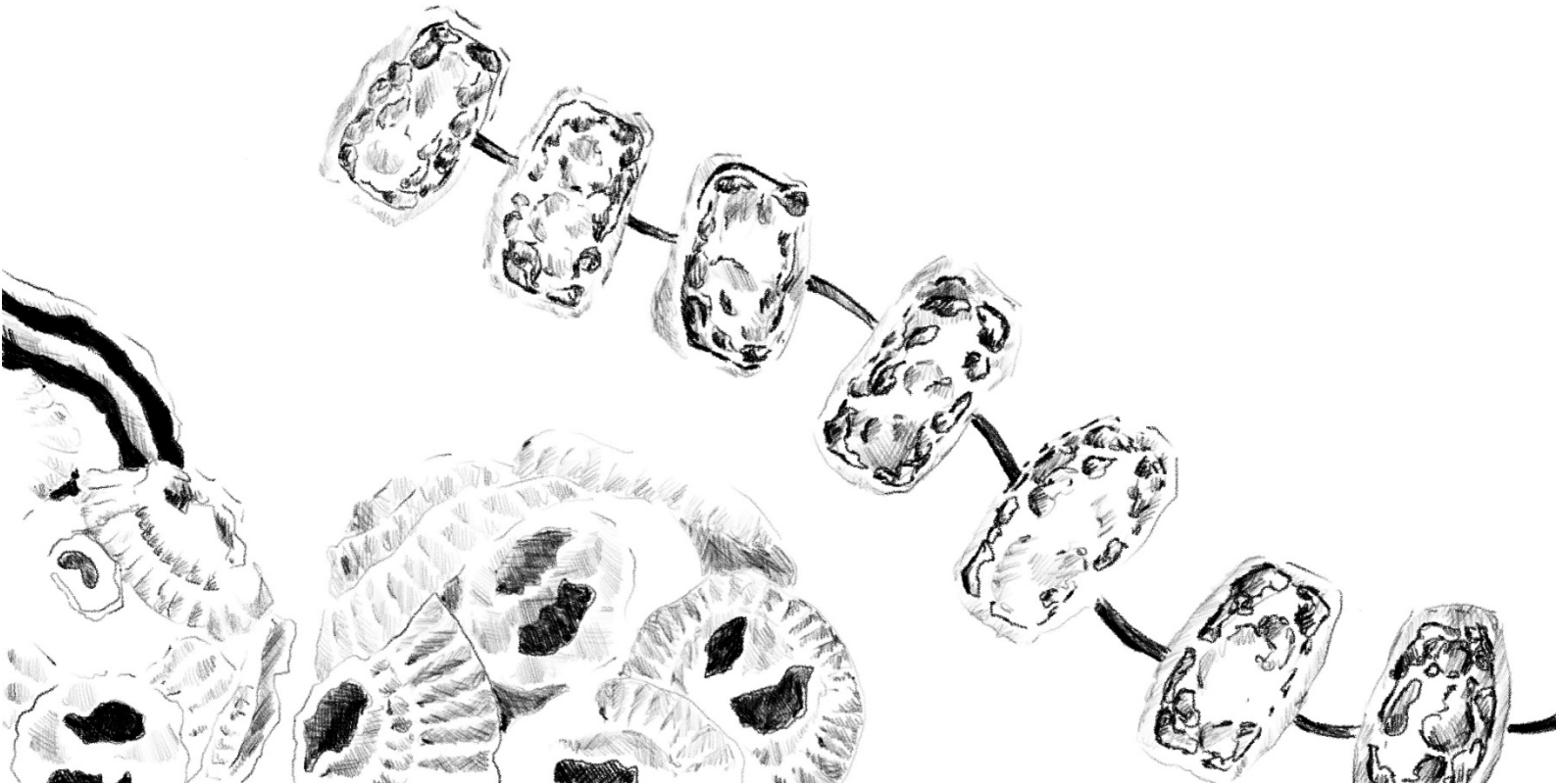


How Temperature Shapes Marine Protist Communities

Mechanistic assessment across seasonal and spatial scales

Antonia Ahme



Cover design and artwork by Hannah Stowe

How temperature shapes marine protist communities

Mechanistic assessment across seasonal and spatial scales

Dissertation zur Erlangung des akademischen Grades einer

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Antonia Ahme: How temperature shapes marine protist communities – Mechanistic assessment across seasonal and spatial scales, Bremen, March 2024. This cumulative dissertation summarises the work conducted at the Alfred-Wegener-Institute, Helmholtz Centre for Polar and Marine Research, and the University of Bremen between September 2020 and March 2024.

"Chaos: When the present determines the future, but the approximate present does not approximately determine the future."

Edward Lorenz, 2005

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Summary

Protist communities play a crucial role in marine ecosystems as they sustain the food web, contribute substantially to the atmosphere's oxygen and drive major biogeochemical cycles. Anthropogenic drivers, including global warming, can significantly alter the marine environment and induce changes in community composition. Given the high functional diversity among marine protists, shifts in community composition can have significant consequences for the ecosystem. To predict future ecosystem dynamics, it is therefore essential to identify the patterns that govern community reorganisation under environmental change. Although microbial ecologists have focused on ocean warming for some time, a mechanistic analysis of temperature-induced community changes across different systems is still lacking. To fill this gap, this thesis aims to systematically assess the temperature responses of marine protist communities and associated ecosystem functions by identifying overarching patterns as well as system-specific attributes across different seasonal and spatial scales. The study focuses on two regions that are oceanographically connected and particularly affected by global warming: The Arctic Ocean and the North Sea.

Due to the 'Arctic amplification', the Arctic Ocean is warming almost four times faster than the global average. Additionally, Arctic protist communities may face increased competition from temperate organisms that are being transported from lower latitudes by saltier and warmer currents, a process known as 'Atlantification'. Chapter 2 therefore addresses the impact of warming on the invasion potential of temperate species by incubating a community from a mixing zone of Atlantic and Arctic waters at different temperatures. While the results show that temperate species profited from warming, there was no relative increase in heterotrophic or picoplanktonic species. This is likely because nutrients were kept replete, denying them an advantage under warming. Moreover, the results reveal a thermal limit for many Arctic species between 6 °C and 9 °C, which underscores the importance of thermal histories in community reorganisation under warming.

The species' thermal histories were also identified as the primary determinants of protist reorganisation in a North Sea spring community, for both phototrophs (chapter 3.1) and heterotrophs (chapter 3.3). Temperate species were found to tolerate significantly higher temperatures than their Arctic counterparts in chapter 2, maintaining a stable diversity even 12 °C above ambient conditions. Although warming did not affect the Shannon diversity of phototrophic protists overall, it did change the composition and resulting ecosystem functions. Warming also weakened trophic coupling (chapter 3.3) and increased variability among replicate mesocosms. Furthermore, altering the N:P ratio along with temperature and varying

the rate of warming led to partially reversed temperature responses concerning the communities' growth rate (chapter 3.2). This highlights nutrients as potential modulators of temperature responses and emphasises the significance of considering an appropriate experimental design.

To investigate whether the same patterns apply to a summer community and to additionally assess the effect of short-term fluctuations on top of mean warming, a North Sea summer community was exposed to a marine heatwave under ambient conditions and an integrated future scenario (+ 3 °C, N:P ratio of 25, pCO2 of 1000 ppm). In the summer community, the susceptibility towards abiotic change was determined by the trophic mode (chapter 4.1). The study discovered that the diversity of heterotrophic protists was resistant to heatwaves and largely resilient to global change. However, phototrophic diversity was affected by both, which had consequences for the entire planktonic food web (chapter 4.2). Particularly the temperature drop at the end of the heatwave imposed a strong selective pressure on phototrophs, consistent with the community responses to the ambient control in the other experiments (chapter 2 & 3). Despite the diverging effects of temperature on heterotrophic and phototrophic diversity, it did not alter the relative proportion of heterotrophs to phototrophs across all three experiments, contradicting predictions of the metabolic theory of ecology.

In conclusion, this thesis provides systematic insights into the temperature responses of marine protist communities from different habitats. Integrating the results of three different experiments, I showed the importance of the species' thermal histories for community reorganisation. The globally important genus *Phaeocystis* mainly thrives under intermediate warming, while diatoms occupy diverse thermal niches but are less competitive when nutrients become limiting. The relative contribution of these two groups determined the functional output across experiments, making the degree of warming a crucial factor for future ecosystems. Ocean warming is likely to have a particularly negative impact on Arctic spring and temperate summer communities, possibly due to the thermal proximity of these habitats to the species' upper thermal limits. Overall, this work emphasises the importance of integrating results from different spatial and seasonal scales and thereby highlights the potential benefits that arise from conducting community-wide studies.

Zusammenfassung

Gemeinschaften von Protisten spielen eine entscheidende Rolle in marinen Ökosystemen, da sie die Grundlage des marinen Nahrungsnetzes bilden, wesentlich zum Sauerstoffgehalt der Atmosphäre beitragen und einen wichtigen Teil biogeochemischer Kreisläufe darsellen. Anthropogene Einflüsse, einschließlich der globalen Erwärmung, können die Meeresumwelt erheblich beeinflussen und dadurch zu Veränderungen in der Zusammensetzung dieser Gemeinschaften führen. Angesichts der großen funktionellen Vielfalt innerhalb der Gruppe der Protisten kann dies erhebliche Auswirkungen auf das Ökosystem haben. Für die Vorhersage künftiger Ökosystemdynamiken ist es daher wichtig, zu identifizieren nach welchen Prinzipien sich diese Gemeinschaften verändern und reorganisieren. Obwohl sich Ökolog*innen seit einiger Zeit auf die Erwärmung der Ozeane konzentrieren, fehlt eine mechanistische Analyse der temperaturbedingten Veränderungen von Protistengemeinschaften in verschiedenen Systemen. Um diese Lücke zu schließen, untersucht die vorliegende Doktorarbeit die Reaktionen mariner Protistengemeinschaften auf Temperaturveränderungen und die damit verbundenen Ökosystemfunktionen systematisch, indem übergreifende Muster und systemspezifische Merkmale über verschiedene saisonale und räumliche Skalen hinweg ermittelt werden. Die Studie konzentriert sich auf zwei Regionen, die ozeanographisch miteinander verbunden und von der globalen Erwärmung besonders betroffen sind: den arktischen Ozean und die Nordsee.

Aufgrund der 'arktischen Verstärkung' erwärmt sich der arktische Ozean fast viermal so schnell wie der globale Durchschnitt. Darüber hinaus sehen sich die arktischen Protistengemeinschaften einer verstärkten Konkurrenz durch Organismen aus gemäßigten Breiten ausgesetzt, die mit den salzigeren und wärmeren Strömungen zunehmend aus niedrigeren Breiten in die Arktis eindringen – die sogenannte 'Atlantifizierung'. Nach einer übergreifenden Einleitung befasst sich Kapitel 2 daher mit den Auswirkungen der Erwärmung auf das Invasionspotenzial gemäßigter Arten. Dafür wurde eine Gemeinschaft aus einer Mischzone von atlantischem und arktischem Wasser bei unterschiedlichen Temperaturen inkubiert. Die Ergebnisse zeigen eine drastische Zunahme temperater Arten erst bei einer Erwärmung von 9 °C. Darüber hinaus zeigte sich für viele arktische Arten eine thermische Grenze zwischen 6 °C und 9 °C, was die Bedeutung der thermischen Historie für die Umstrukturierung von Gemeinschaften bei Erwärmung unterstreicht.

Die thermische Vergangenheit der Arten wurde auch als Hauptfaktor für die Umstrukturierung der Protistengemeinschaft in einer Frühlingsinkubation in der Nordsee identifiziert, sowohl für phototrophe (Kapitel 3.1) als auch für heterotrophe Organismen

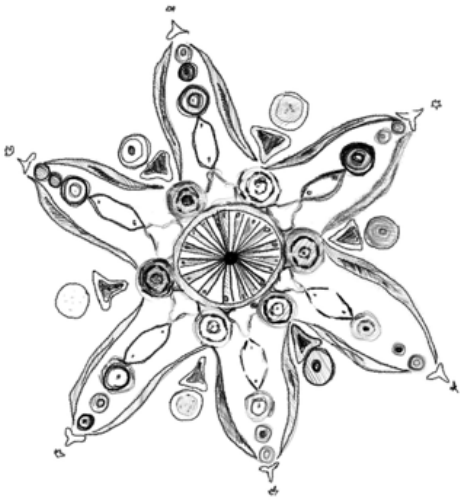
(Kapitel 3.3). Es zeigt sich, dass die Arten der gemäßigten Breiten deutlich höhere Temperaturen tolerieren, als ihre arktischen Artgenossen und ihre Diversität sogar bei Temperaturen von + 12 °C stabil halten. Obwohl die Shannon-Diversität der phototrophen Protisten durch Erwärmung insgesamt nicht beeinflusst wurde, veränderte sich ihre Zusammensetzung und die daraus resultierenden Ökosystemfunktionen. Erwärmung schwächte auch die trophische Kopplung (Kapitel 3.3) und erhöhte die Variabilität zwischen den Replikaten. Darüber hinaus führte die gleichzeitige Veränderung des N:P-Verhältnisses und der Erwärmungsrate zu teilweise umgekehrten Reaktionen hinsichtlich der Wachstumsrate der Gemeinschaft (Kapitel 3.2). Dies identifiziert Nährstoffe als potenzielle Modulatoren von Temperaturreaktionen und unterstreicht die Bedeutung eines geeigneten experimentellen Designs.

Um zu untersuchen, ob die identifizierten Mechanismen saisonal spezifisch sind oder ob die gleichen Muster im Sommer gelten und um die Auswirkungen kurzfristiger Schwankungen zusätzlich zur mittleren Erwärmung zu bewerten, wurde eine Sommergemeinschaft in der Nordsee einer marinen Hitzewelle ausgesetzt, sowohl unter Umgebungsbedingungen als auch unter einem potentiellen Zukunftsszenario (+ 3 °C, N:P-Verhältnis von 25, pCO2 von 1000 ppm). In der Sommergemeinschaft wurde die Anfälligkeit gegenüber abiotischen Veränderungen durch den trophischen Modus bestimmt (Kapitel 4.1). Die Studie ergab, dass die Vielfalt der heterotrophen Protisten gegenüber Hitzewellen resistent und gegenüber globalen Veränderungen weitgehend resilient war. Die phototrophe Vielfalt wurde jedoch von beiden Szenarien beeinflusst, was sich auf das gesamte planktonische Nahrungsnetz auswirkte (Kapitel 4.2). Insbesondere der Temperaturabfall am Ende der Hitzewelle übte einen starken Selektionsdruck auf die phototrophen Protisten aus, übereinstimmend mit den Reaktionen der Gemeinschaft auf die niedrigsten Temperaturen in den anderen Inkubationen (Kapitel 2 und 3). Trotz der unterschiedlichen Auswirkungen der Temperatur auf die heterotrophe und phototrophe Diversität änderte sich ihr relatives Verhältnis in allen drei Experimenten nicht, was den Vorhersagen der metabolischen Theorie der Ökologie widerspricht.

Zusammenfassend lässt sich sagen, dass diese Arbeit systematische Einblicke in die Temperaturreaktionen von marinen Protistengemeinschaften aus verschiedenen Lebensräumen bietet. Anhand der Ergebnisse von drei verschiedenen Experimenten konnte ich zeigen, wie wichtig die thermische Vergangenheit der Arten für die Umstrukturierung von Gemeinschaften ist. Die weltweit verbreitete Gattung *Phaeocystis* profitiert hauptsächlich bei mittlerer Erwärmung, während Kieselalgen diverse thermische Nischen besetzen, aber weniger konkurrenzfähig sind, wenn die Nährstoffe knapp werden. Der relative Beitrag dieser beiden

Gruppen bestimmt die funktionelle Leistung in allen Experimenten, so dass der Grad der Erwärmung ein entscheidender Faktor für künftige Ökosysteme ist. Die Erwärmung des Ozeans wird sich wahrscheinlich besonders negativ auf die arktischen Frühlings- und gemäßigten Sommergemeinschaften auswirken, was an der Nähe dieser Lebensräume zur oberen thermischen Grenze der Arten liegt. Insgesamt unterstreicht diese Arbeit, wie wichtig es ist, Ergebnisse aus verschiedenen räumlichen und saisonalen Maßstäben zu integrieren, und verdeutlicht damit die Vorteile, die sich aus der Durchführung von gemeinschaftsweiten Studien ergeben könnten.

1
Introduction



1.1 Marine microbial communities: the hidden diversity

Although they are not visible to the bare eye (Sanz 2011), marine microbes can be considered major players in the global ocean, as they account for approximately 70% of the total marine biomass (Bar-On et al. 2018). Unlike most other organisms, they are not only passively affected by global change but also influence its dynamics as they regulate global biogeochemical cycles and thereby affect the marine carbonate system, deoxygenation and the stoichiometry of the ocean (Wolf-Gladrow et al. 2007, van de Waal et al. 2010, Richardson and Bendtsen 2017). Additionally, their community composition is a determinant of the amount of carbon that is exported to the deep sea or fueled into marine food webs (Tréguer et al. 2018, Duret et al. 2020, Li et al. 2023). Therefore, it is essential to comprehend and forecast microbial community dynamics, despite the high complexity involved. Marine microbes span a wide phylogenetic spectrum (De Vargas et al. 2015) and comprise multiple different functional groups (Sunagawa et al. 2015). They exhibit various forms throughout the year (Alvain et al. 2008) and in different locations (Carradec et al. 2018). However, considering their high applicability as model systems to answer ecological questions (Altermatt et al. 2015) and their critical relevance to marine ecosystems (Wetzel 2001, Naselli-Flores and Padisák 2023), unravelling marine microbial community dynamics is a task worthy of being tackled.

1.1.1 Taxonomic diversity

While marine microbes span all three domains of life including archaea and bacteria, this thesis focuses on protists, i.e. unicellular eukaryotes (O'Malley et al. 2013). It further focuses on the planktonic fraction, which refers to the attribute of being passively transported through the ocean with currents (Tappan 1979). Eukaryotic microbes can have various taxonomic identities spanning several supergroups (Figure 1; Burki et al. 2020). Although their exact phylogeny in its deep branches and origin is still under debate (Adl et al. 2019, Keeling and Burki 2019, Burki et al. 2020), it is increasingly unravelled thanks to high-throughput-sequencing technologies and large-scale international sampling campaigns (De Vargas et al. 2015, Burki et al. 2020). Some of the most important marine genera belong to closely related groups. For example, diatoms and chrysophytes belong to the stramenopiles, dinoflagellates and ciliates are grouped to the alveolates, and coccolithophores can be found within haptista. Sequencing technologies enabled a higher genetic resolution; however, a vast number of marine eukaryotes remain devoid of morphological identification and largely uncultured. These groups are named depending on the higher taxonomic rank they belong to, i.e. marine ochrophytes (MOCH), marine stramenopiles (MAST) and marine alveolates (MALV).

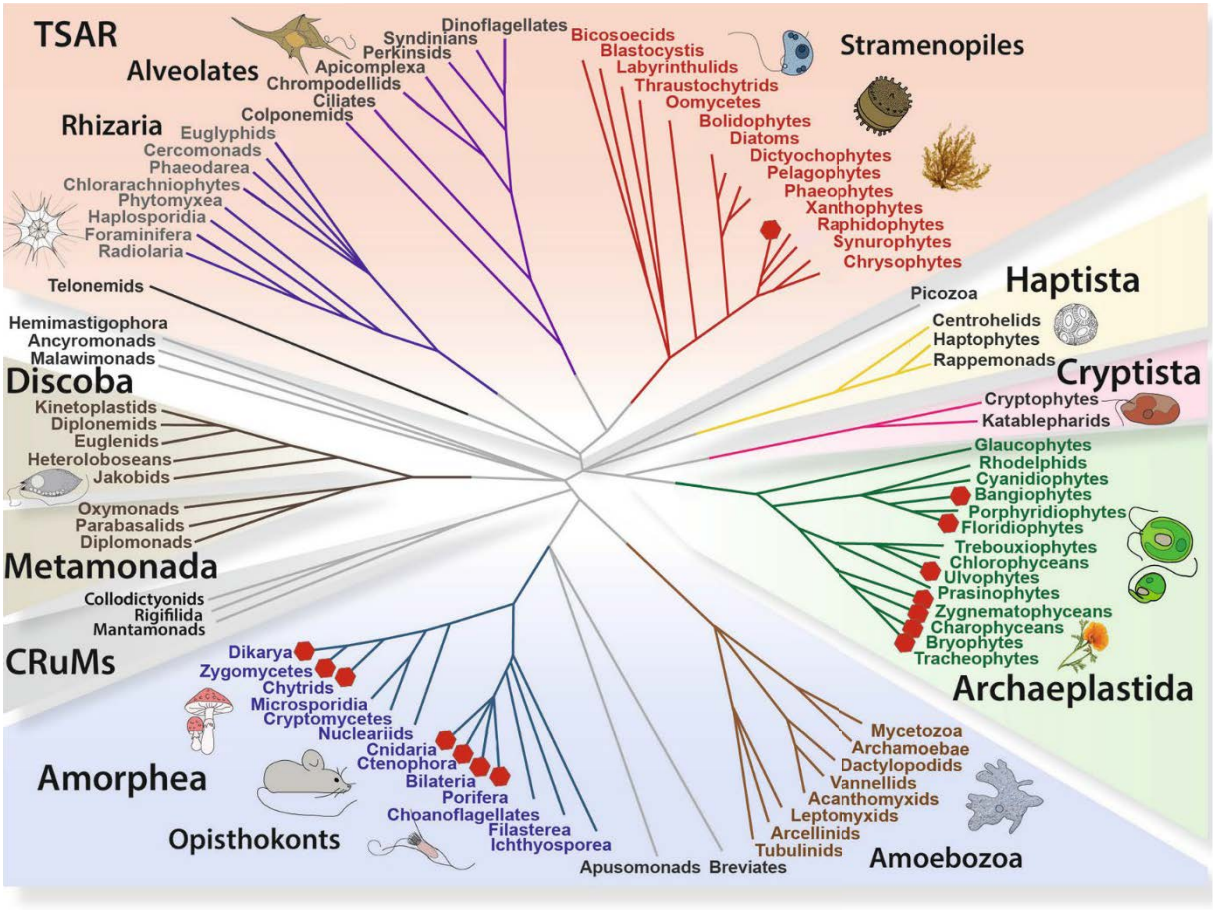


Figure 1: One potential phylogenetic tree of eukaryotes including several supergroups (coloured areas) of which most organisms are unicellular (red hexagons indicate lineages containing at least one multicellular taxon). Grey lines indicate preliminary assumptions. Modified after Bachy et al. (2022).

Apparent from the eukaryotic tree of life, marine microbes comprise a vast taxonomic diversity, with an expected number of 150.000 different species (De Vargas et al. 2015). Generally, taxonomic diversity can be differentiated into several components and described by various indices. Diversity components within a given community or area include species richness, i.e. the total number of unique species, and species evenness, a measure of the relative abundance of each species (Pielou 1966). The most commonly used diversity metric is the Shannon index (abbreviated as H'; Ortiz-Burgos 2016), which integrates both species richness and evenness and is defined by

$$H' = -\sum_{n=1}^{n} (p_i * \ln p_i)$$

with p_i representing the proportion of each species in the sample. It is generally assumed that a high diversity can buffer an ecosystem against abiotic perturbations (Vallina et al. 2017, Bestion et al. 2020, Bestion et al. 2021). However, Hillebrand et al. (2008; 2012) argue that for accurately assessing the effect of biodiversity on ecosystem functioning, not only the taxonomic but also the functional composition of the community needs to be considered.

1.1.2 Functional biodiversity

In addition to their phylogenetic position, planktonic microbes can be differentiated regarding their trait diversity which is defined by Petchey and Gaston (2006) as "understanding communities based on what organisms do, rather than on their evolutionary history". From these traits, functional biodiversity emerges as an overarching framework that combines taxonomic diversity with the trait spaces of the species within a community. A high functional biodiversity is considered beneficial for ecosystem productivity (Vallina et al. 2017, Fontana et al. 2018) and assumed to be a better predictor for community dynamics than taxonomic identity alone (Fontana et al. 2018, Henson et al. 2021). Therefore, the concept has received great attention in the scientific literature (Petchey and Gaston 2006 and references therein). Recently, trait variability has even been offered as an elegant explanation to the 'paradox of the plankton', which describes the puzzling observation that a limited range of resources can support a wide range of microbial species and enable coexistence (Menden-Deuer et al. 2021). For eukaryotic microbes, functional biodiversity can be considered from different perspectives. At their core, these revolve around functional traits exhibited by individual organisms, which define the functional role of these individuals within the ecosystem but also determine their functional impact on the ecosystem (Glibert 2016).

Marine microbes harbour an indefinite amount of functional traits that can be described along the major ecological axes of their niche and are in detail summarised by Litchman and Klausmeier (2008) as well as Litchman et al. (2010). The most important ones for this thesis are thermal and nutrient-related traits, which are usually described by growth rate as a measure of fitness. For example, the thermal traits of an organism can be visualised by its thermal performance curve (TPC; sometimes also referred to as thermal reaction norm) which is a unimodal, left-skewed function of an organism's growth rate over temperature (Figure 2a; Kingsolver 2009). TPCs provide several parameters that define the competitive ability of a given species at a certain temperature or under thermal changes: the optimum temperature for growth, the slopes of the ascending and descending parts, the lower and upper thermal limits and the thermal breadth (Figure 2a; Huey and Stevenson 1979, Angilletta and Michael 2009). Traits related to nutrients can be described in a similar way for organisms that need dissolved inorganic nutrients. In this case, the growth rate of the population is described by a saturating function of the external concentration of a specific nutrient (Figure 2b; Monod 1949, Eppley et al. 1969). Relevant traits for organisms that rely on the uptake of particulate nutrients include feeding efficiency, motility and the food particle size range (Litchman et al. 2013). Species also

differ in their requirement for various nutrients as described by their intracellular stoichiometry (Quigg et al. 2003, Finkel et al. 2009).

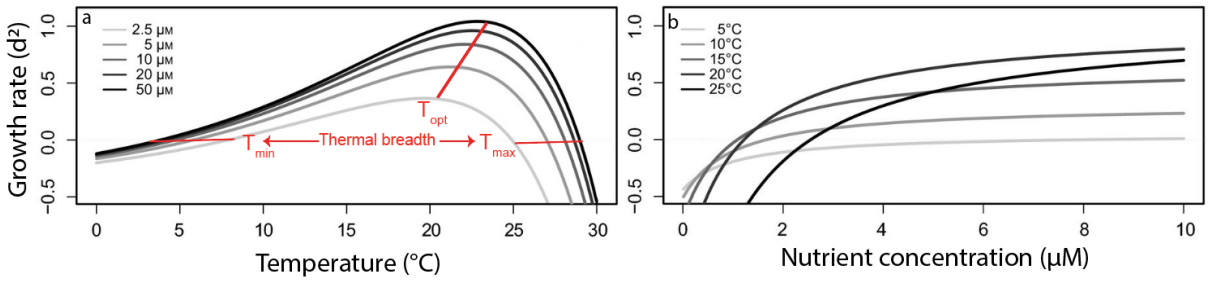


Figure 2: Joint effects of differing temperature and nutrient levels on an organism's growth rate based on a model of Thomas et al. (2017) showing both, (a) changes in the thermal performance curve with varying nutrient concentrations and (b) changes in the Monod curve with varying temperatures. Red colour indicates different thermal traits. Modified after Thomas et al. (2017).

Both thermal and nutrient-related traits have been observed to correlate with cell size and seem to give smaller organisms a competitive advantage in low-nutrient and high-temperature conditions (Peter and Sommer 2012, 2013, Hillebrand et al. 2022). Potential reasons for their competitiveness is a higher surface-to-volume ratio, a smaller diffusion boundary layer and a lower nutrient demand (Finkel et al. 2009). Furthermore, their growth rates can increase faster with warming compared to larger cells (Kremer et al. 2017). Beyond that, cell size can affect an organism's sinking velocity, grazing defence and light absorption, rendering it a master trait of unicellular eukaryotes (Finkel et al. 2009, Marañón 2015, Hillebrand et al. 2021).

1.1.3 Trade-offs and functional groups

Physiologically restricted trade-offs and trait correlations have led to the evolution of contrasting ecological strategies and universal interdependencies among functional traits. (Litchman et al. 2007, Litchman and Klausmeier 2008). Especially thermal and nutrient-related traits can change along the environmental axes of each other (Figure 2; Thomas et al. 2017). For instance, Lewington-Pearce et al. (2019) have found that higher temperatures raise the minimum nutrient requirements of several phototrophic protists, likely due to higher metabolic investment in chaperones that assist in protein folding under heat stress (Verghese et al. 2012). Vice versa, nutrient limitation seems to inhibit the adaptive potential of microbes to cope with high temperatures (Aranguren-Gassis et al. 2019, Wu et al. 2021) and can even suppress the general temperature-dependence of cellular metabolic rates (Marañón et al. 2018). As these trade-offs may vary among sub-processes of microbial population dynamics (Bieg and Vasseur 2024) and between species (Sunday et al. 2023), they will ultimately determine how a microbial

community restructures in response to environmental changes (De Senerpont Domis et al. 2014, Verbeek et al. 2018, Serra-Pompei et al. 2019).

Based on specific trait sets, several classifications into functional groups within the ecosystem have been established. One of them focuses on how energy and carbon are assimilated. Eukaryotic microbes can be phototroph, i.e. produce organic matter and energy via photosynthesis, heterotroph, i.e. take up other organisms and organic material via phago- or osmotrophy, or mixotroph defined as a combination of the two (Chakraborty et al. 2017). Another classification differentiates microbes into producers, bacterivores, detritivores, micrograzers, intraguild predators and parasites, based on the trophic position and the food type (Wieczynski et al. 2021). However, both classifications are not mutually exclusive and all functional groups are interactively affecting each other. Phototrophic protists make up a large fraction of global primary producers (Field et al. 1998) while their nutritional quality has an important impact on the growth of heterotrophic protists (John and Davidson 2001). These, in turn, contribute substantially to the recycling of nutrients for producers and the termination of blooms, either via micrograzing (Schmoker et al. 2013) or by infecting them as parasites (Suter et al. 2022).

Lastly, eukaryotic microbes can be defined according to their biogeochemical impact on the ecosystem (Hood et al. 2006, Litchman et al. 2015), so whether they can silicify (e.g. diatoms and dictyochophytes), calcify (e.g. coccolithophores & foraminifera) or produce volatile sulphur compounds (e.g. some haptophytes). Since all different organisms play roles in the cycling of the major elements (carbon, nitrogen, phosphorus) and are important for other ecosystem functions (Naselli-Flores and Padisák 2023), the best classification will vary depending on the research question and the focus of interest (Petchey and Gaston 2006).

1.1.4 Variations across spatial and seasonal scales

Functional biodiversity together with environmental filtering can induce differences in community composition between seasons (Lewandowska et al. 2015) and across biogeographic gradients (Louthan et al. 2021, Sommeria-Klein et al. 2021, Dutkiewicz et al. 2024). This thesis focuses on communities from two regions that are majorly contributing to global primary production and are connected via the Norwegian Current: The North Sea and the European part of the Arctic Ocean (Field et al. 1998). Both regions suffer from more pronounced warming compared to other oceanic basins – the North Sea mainly based on its shallowness (Schrum et al. 2016, IPCC 2021, De Amorim et al. 2023) and the Arctic due to 'Arctic amplification' (Previdi et al. 2021, Rantanen et al. 2022). Another ongoing process is the so-called

'borealization' of Arctic phytoplankton phenology, including the development of a secondary phototrophic bloom in autumn similar to that of temperate oceans (Figure 3; Ardyna et al. 2014, Fujiwara et al. 2018, Ardyna and Arrigo 2020). Furthermore, the 'atlantification' of the Arctic Ocean (Polyakov et al. 2017, Asbjørnsen et al. 2020, Noh et al. 2024) requires an evaluation of the invasion potential of temperate organisms under environmental change (Carter-Gates et al. 2020, Zhang et al. 2023). Thus, studying temperate and Arctic communities in concordance is crucial to projecting future ecosystem states of both habitats (Lovell et al. 2023).

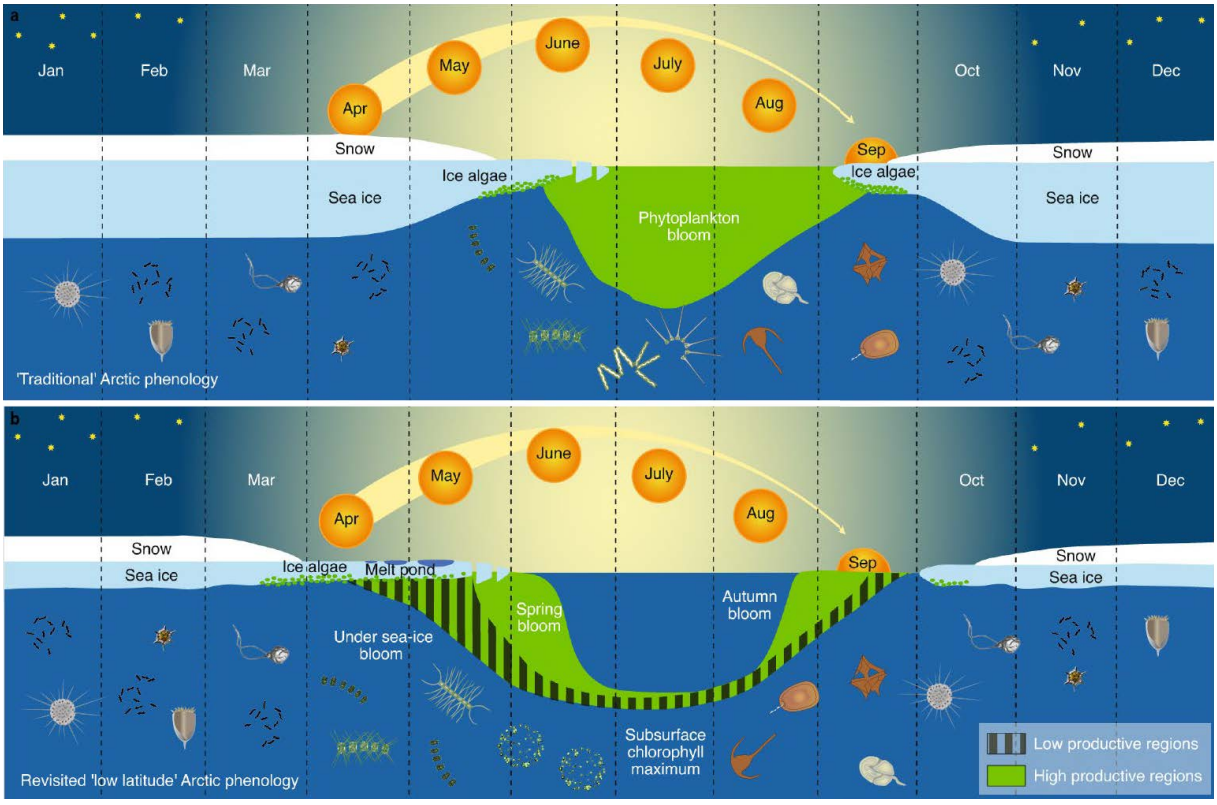


Figure 3: Schematic of the bloom phenology and composition of (a) Arctic communities before climate change and (b) communities under climate-change-induced shifts in abiotic conditions with a secondary autumn bloom similar to that in temperate oceans. Modified after Ardyna and Arrigo (2020).

The North Sea is a temperate shelf sea with a depth of mostly less than 50 m that is characterised by a high thermal seasonality spanning from below 0 °C in winter up to peak temperatures of 20 °C in summer (Wiltshire and Manly 2004). Temperate protists have adapted to these annual fluctuations and exhibit a wide thermal breadth and comparably high optimum temperatures for growth (Thomas et al. 2012, Boyd et al. 2013). Seasonal succession patterns are therefore assumed to be mainly driven by light and nutrients rather than temperature (Wiltshire et al. 2015, González-Gil et al. 2022) although the exact determinants are complex and multi-faceted (Glibert 2016). Generally, these patterns consist of a shift from nutrient-affine phototrophic groups like diatoms in spring to mixotrophic flagellates and microzooplankton that profit from

their heterotrophic lifestyle when nutrients are depleted in summer (Käse et al. 2020). Additionally, wind-driven mixing can cause a resupply of nutrients and foster a second bloom in autumn in coastal temperate oceans like the North Sea. In winter, insufficient light and nutrient supply result in biomass decay and a remineralisation of nutrients which are then available again to primary producers in the following spring (Brockmann and Kattner 1997). Due to substantially different community compositions, the results of global change studies are highly dependent on the season in which they took place (Staehr and Sand-Jensen 2006, Yvon-Durocher et al. 2017). The diversity of the protist community is usually higher in summer compared to spring (Sommer et al. 2015) which may give them a higher resilience towards environmental change (Tilman et al. 2014, Bestion et al. 2021). However, as summer communities live closer to their thermal limits, they could also be more susceptible depending on the abiotic stressor of interest (Chen 2015). Clarification can only be achieved by comparing the responses of spring and summer communities in a similar experimental setup.

In contrast to their temperate counterparts, many Arctic species are considered endemic, i.e. unique to this ecosystem (Darling et al. 2007, Šupraha et al. 2022), due to the strong selective pressures of their habitat. The low temperatures, which remain around 0 – 2 °C throughout the year, result in a narrower thermal breadth (Stock et al. 2019) and can even restrict heterotrophic growth (Rose and Caron 2007). Furthermore, depending on the latitude, light can be absent for up to six months in winter (Ljungström et al. 2021), which has led phototrophic protists to evolve various overwintering strategies including the formation of dormant resting stages (McQuoid et al. 2002) and an extremely high efficiency to use low light intensities (Hoppe 2021). Still, the development of the Arctic bloom at the onset of the polar dawn mirrors that of the temperate spring bloom and is also assumed to be mostly driven by light (Leu et al. 2015). This is reflected in the succession of similar taxonomic groups albeit different species, like for example representatives of the diatom genus *Thalassiosira* or the haptophyte *Phaeocystis* (Wang et al. 2010, Šupraha et al. 2022). While the Arctic season for phototrophic growth may be prolonged due to global change (Ardyna and Arrigo 2020), increasing temperatures could push many polar protists beyond their thermal limits similar to temperate summer species. It is currently unclear what degree of warming would give species from lower latitudes a competitive advantage at higher latitudes and change the community composition (Giesler et al. 2023).

1.2 Small but mighty: how marine microbes sustain the ecosystem

Planktonic protists are crucial for the ecosystems they inhabit due to their ability to perform a wide range of ecosystem functions (Bachy et al. 2022) which they can partly stabilise under environmental pressures through their functional biodiversity (Tilman et al. 2014).

1.2.1 Ecosystem functions

Marine microbes produce, transform and recycle organic matter and thereby drive all major biogeochemical cycles (Katz et al. 2004, Worden et al. 2015). Phototrophic protists usually form the starting point of the marine food web and trophic energy flux by producing oxygen, building up organic carbon compounds via photosynthesis (Falkowski et al. 1998, Field et al. 1998) and thereby sequestering carbon dioxide from the atmosphere. Through cellular metabolic processes, other elemental nutrients are attached to these carbon compounds in variable ratios (van de Waal and Boersma 2012) which define the nutritional quality for higher trophic levels including heterotrophic protists (Thomas et al. 2022). These, in turn, recycle and control the biomass of primary producers either through grazing or parasitic consumption (Bachy et al. 2022) and thereby release parts of the organic carbon again as inorganic carbon dioxide via respiration. The interplay of photo- and heterotrophic processes can aid the export of organic nutrients out of the photic zone as it affects the aggregation of organic particles, their sinking speed, remineralisation rates and the efficiency of the bacterially mediated microbial loop (Laurenceau-Cornec et al. 2015, Bach et al. 2019, Bachy et al. 2022). Depending on the exact community composition and the abiotic environment, marine protists export more or less of various elements such as nitrogen, phosphorus, silica, sulphur, calcium and most importantly carbon (Guidi et al. 2009, Dutkiewicz et al. 2013, Le Moigne et al. 2015). Lastly, under certain environmental conditions, some protists are capable of producing toxins and can form harmful algae blooms, which negatively affect fish, birds, marine mammals and even humans (Grattan et al. 2016) and may increase in the future (Wells et al. 2020).

1.2.2 Functional similarity as buffer against perturbations

An important component of the perturbation stability of all ecosystem functions outlined in the previous chapter is the functional similarity or niche overlap between different species within a community (Biggs et al. 2020, Eisenhauer et al. 2023). This concept is often termed 'functional redundancy' and refers to the difference between taxonomic and functional diversity concerning a specific ecosystem function. It describes whether different taxonomic groups with the same functional traits can perform an ecosystem function, such as primary productivity or carbon

export, to the same extent under a given range of environmental conditions (Figure 4; Louca et al. 2018). There are different ways to describe functional redundancy within a community: either through the effect trait similarity of species along an environmental gradient (Figure 4a, c) or through the response of functional richness to species loss (Figure 4b, d). In each case, a high functional redundancy resembles when species can be lost from the community without any consequences for the functional output, either as a single effect trait (Figure 4a) or as the general functional richness (Figure 4b). A potential mechanism underlying the development of functionally similar species from taxonomically disparate groups is environmental filtering (Schaum et al. 2013, Fontana et al. 2016, Chen et al. 2021).

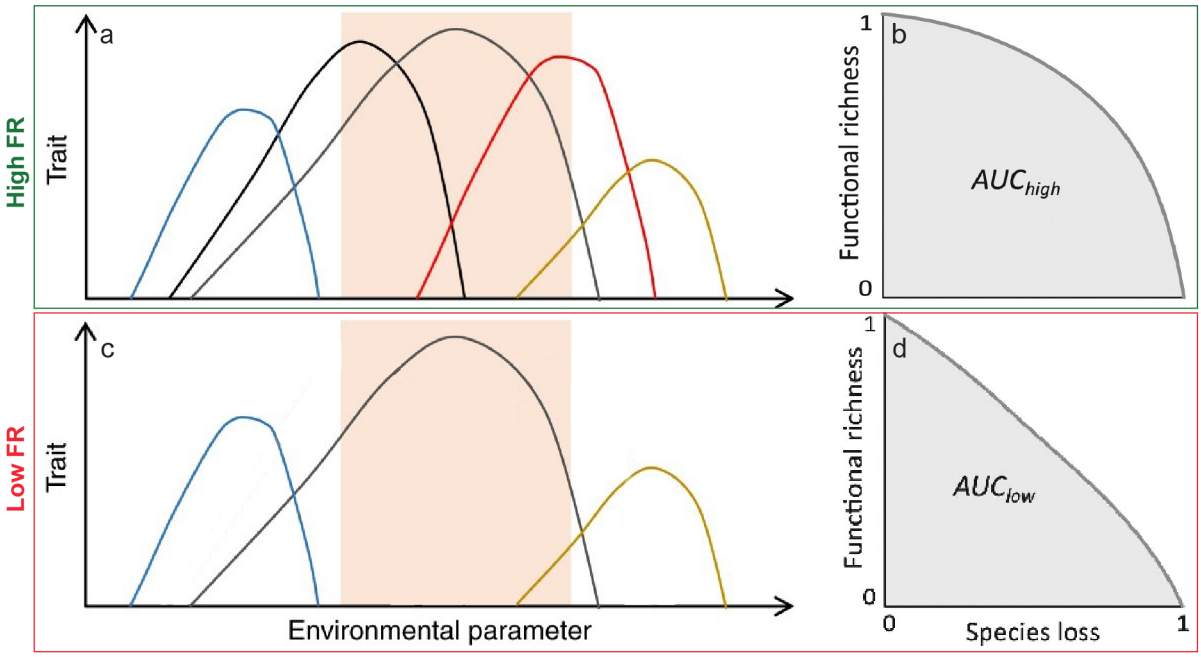


Figure 4: A high (green rectangle; a & b) and low (red rectangle; c & d) functional redundancy (FR) either described by the traits of species (represented by different colours) as functions of an environmental parameter (a & c) or as the functional richness as a function of species loss (b & d). The red-shaded area is the range in which traits are unaltered and the area under the curve (AUC) indicates the degree of functional redundancy. Modified after Hoppe et al. (2018) and Teichert et al. (2017)

While many experimental studies suggested that taxonomic and functional diversity are two independent axes of variation (Louca et al. 2016, Goldford et al. 2018, Gerhard et al. 2021, Ramond et al. 2023) others propose that functions vary along with the phylogeny (Galand et al. 2018, Ramond et al. 2019, Isobe et al. 2020, McCain et al. 2021). However, the degree of functional redundancy depends on the type of perturbation and habitat (Fetzer et al. 2015), the environmental history of a community (Zhong et al. 2020) as well as on the investigated ecosystem functions (Meyer et al. 2018), which may explain these variations among experimental studies.

1.3 Drivers of microbial community composition

Considering the differential impact of various species on the ecosystem, it is crucial to identify the patterns that govern the structural composition of microbial communities to understand and predict future ecosystem functioning. Overall, ecological dynamics can be assessed on different levels (Guimarães 2020). The first one is the trait set of individual organisms and their phenotypic plasticity (Fontana et al. 2016, Kremer et al. 2018), determining the physiological optima and limits of a given organism within its environment. The next level are trait variations between individuals of the same species (i.e. intraspecific diversity) which can cause genotype sorting and determine a population's demographic rates like overall mortality, growth and reproduction (Bolnick et al. 2011, Wolf et al. 2018, Wolf et al. 2019, Listmann et al. 2020). The third level is the community context, which describes how trait-driven variations in growth and loss rates between species and their interactive dynamics shape the taxonomic and functional composition of a community (Wieczynski et al. 2021). Lastly, the environmental context and biogeographic connectivity are important components of community dynamics, especially when the abiotic conditions change (Walther 2010, Garcia et al. 2018, Ward et al. 2021). Most studies that investigate environmental change focus on single species or functional groups (Boyd et al. 2018). However, bearing in mind the four ecological levels (individual trait sets, intra-specific variations, community and environmental contexts), the outcome of single-species experiments might not reflect the actual situation in the field (Hall et al. 2018, McClean et al. 2019). Furthermore, relatively small changes within some functional groups could have large knock-on effects on other groups (Camarena-Gómez et al. 2018). Next to the detailed physiological insight gained from controlled single-species experiments, this highlights the importance of investigating communities as a whole including their complexity (Walther 2010, Russell et al. 2011).

Microbial communities are often considered chaotic systems (Box 1) due to their high sensitivity to initial conditions. Even slight variations in these conditions, combined with non-linear dynamics such as exponential growth and additive driver effects, can lead to unpredictable patterns over time (Roy and Majumdar 2022). The complexity of microbial systems arises from the intricate interactions between the organisms and their environment, as well as feedback loops within the system (Benincà et al. 2008, Telesh et al. 2019, Rogers et al. 2022). These interactions can cause significant variations over time, which can be quantified using the Lyapunov exponent (Box 1; Nazarimehr et al. 2017). As a result, microbial communities display irregular oscillations or bifurcations, sometimes leading them to evolve into different states, i.e. alternative attractors (Box 1; van Nes and Scheffer 2004, Feng et al.

2006, Säterberg and McCann 2021). They can also switch between various dynamic or chaotic behaviors within short timeframes (Becks and Arndt 2008).

1.3.1 Components of community dynamics

Stochasticity (Huisman and Weissing 1999) and determinism (Menden-Deuer et al. 2021) have each been used to explain the 'paradox of the plankton', although chaotic systems counterintuitively combine both components (Zhou and Ning 2017, Ning et al. 2019). While deterministic processes set the overall structure and trends, stochastic processes introduce variability and uncertainty through random and probabilistic events. Examples of stochastic processes include random mutations, ecological drift, probabilistic dispersal, or chance encounters between microorganisms (Caruso et al. 2011, Stegen et al. 2012, Zhou et al. 2014, Evans et al. 2017). Studying stochastic elements in the development of microbial systems is especially important considering environmental change, as external forcing can increase the stochasticity and unpredictability of a given system by introducing variability-frequencies that are not characteristic for either the internal or external variability (Pálffy et al. 2021, Mayersohn et al. 2022). Paradoxically, these elements exist even in mechanisms considered deterministic, such as competition (Huisman and Weissing 1999, 2001), predation (Vandermeer 1993, Becks et al. 2005, Northfield et al. 2021), symbiosis (Graham et al. 2007) and infection (Agnihotri and Kaur 2019). Due to the complex interplay of stochastic and deterministic components within chaotic systems, it is important to consider both when studying microbial community dynamics.

BOX 1: GLOSSARY OF TERMS FROM CHAOS THEORY

Chaos: To classify a system as chaotic, it must be sensitive to initial conditions, deterministic and non-linear (Smith 2007).

(Ecological) stochasticity: Stochasticity in general refers to the property that future states of a system cannot be determined from previous ones. Ecological stochasticity in particular is defined as ecological processes that generate community diversity patterns indistinguishable from those generated by random chance alone (Zhou and Ning 2017).

Determinism describes the property that later states of a system are determined by, or inevitably follow from, its earlier states (Kent 2007).

Lyapunov exponent is a quantity that shows the intrinsic instability of trajectories in a system and are computed as the average rate of exponential convergence or divergence of trajectories that are nearby in the phase space (Nazarimehr et al. 2017).

Alternative attractors refer to alternative stable states, cycles or equilibria towards which a system can evolve under certain conditions (Säterberg and McCann 2021).

1.3.2 Deterministic processes

Contrary to stochasticity, deterministic processes are defined as predictable and rule-based interactions within microbial systems (Box 1) and can be modelled using specific functions. Within ecological research, they can either be grouped into biotic vs. abiotic factors or into bottom-up vs. top-down control (Figure 5). Biotic factors cover all processes mediated by living organisms and are essential in structuring microbial communities (Dutkiewicz et al. 2024). They can be considered within or between different trophic levels. When a higher trophic level affects lower ones, for example through predation or infection, they exert top-down control on them. This usually leads to a lowered population size of the most abundant species (Winter et al. 2010, Flynn et al. 2022) and sometimes even prevents blooms from forming (Tillmann 2004). Through this frequency-dependent selection, grazers and parasites can increase the species evenness and diversity of their prey communities (Hillebrand et al. 2007), although there are also cases of selective feeding or infection of rare species (Gaul and Antia 2001, Liu et al. 2014). Vice versa, when a lower trophic level affects a higher one, for example via its nutritional content or abundance, this is termed bottom-up control.

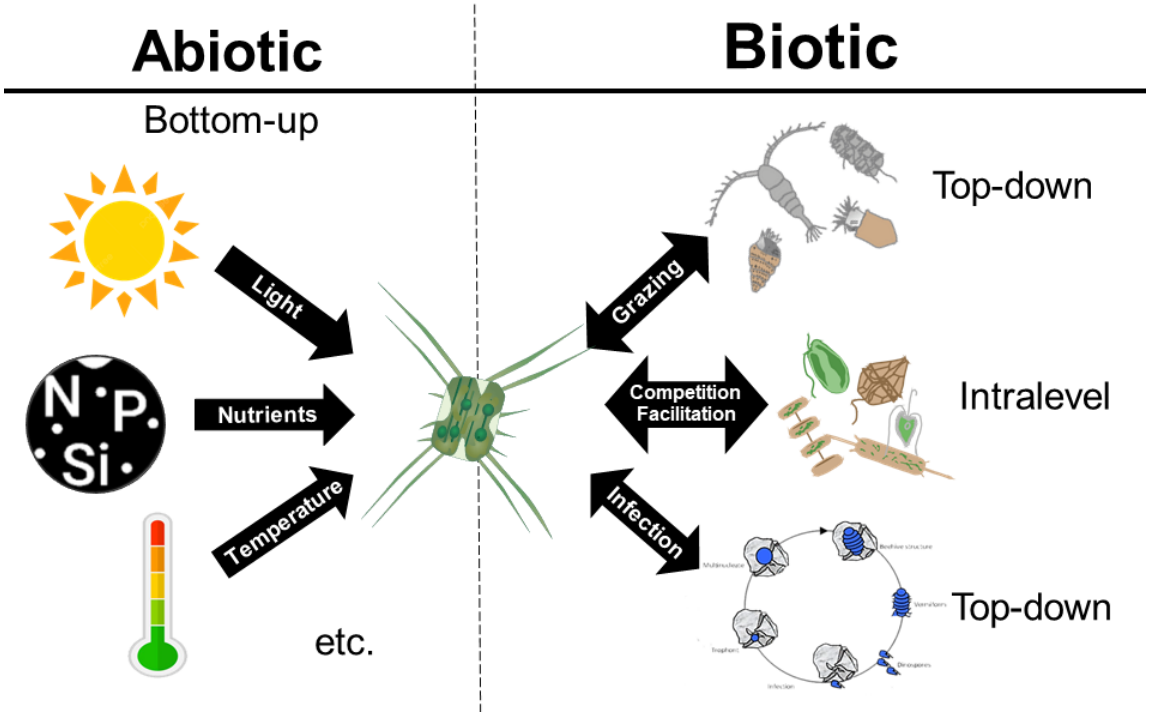


Figure 5: Collection of deterministic processes for the example of phototrophic protists within a microbial community. For phototrophs, abiotic drivers are synonymous with bottom-up drivers and include light, nutrients and temperature. Biotic drivers include top-down control by higher trophic levels, such as grazing by micro- or mesozooplankton, and infection by viruses, bacteria, or eukaryotic parasites, as well as intra-level interactions in the form of competition and facilitation. Modified after Uwe John.

Biotic interactions among organisms on the same level can be beneficial in the case of facilitation and mutualistic symbioses (Zélé et al. 2018) or antagonistic in the case of competition (Sommer 2002). Especially in a marine planktonic setting confronted with environmental change, these biotic interactions gain significance (Bi et al. 2021, Giesler et al. 2023) as organisms are transported along currents and can easily invade new ecosystems if the conditions become favourable. While these concepts can be helpful in explaining a status quo, they are not definitive. For example, a predatory relationship can evolve into endosymbiosis (Horas et al. 2022), and mutualistic symbioses can turn to parasitism under changing environmental conditions (Drew et al. 2021). In fact, most biotic factors depend on and interact with abiotic factors which are physical or chemical aspects like temperature, nutrient concentrations, carbon dioxide partial pressure, light intensity, salinity etc. (Hillebrand et al. 2007, Flynn et al. 2022, Giesler et al. 2023). For phototrophs, these are also considered as bottom-up control. While many abiotic factors are important in shaping planktonic communities, the most prominent aspect of current environmental change that directly affects all organisms is temperature (Litchman and Thomas 2023).

1.3.3 Temperature: the master driver of ecological processes

Compared to the shifts of most abiotic factors whose overall direction is less predictable and depends on the region (Laufkötter et al. 2015, Tuerena et al. 2022, Röthig et al. 2023), changes in temperature are more straightforward and uncertainties or regional differences only concern the degree of change (IPCC 2021, Rantanen et al. 2022). Mean ocean temperatures are gradually rising (IPCC 2021), posing a constant press disturbance for marine organisms, and in addition, the intensity, frequency and duration of heatwaves (i.e. sudden pulse disturbances) are increasing (Oliver et al. 2019, Laufkötter et al. 2020, Barkhordarian et al. 2024). As mentioned above (chapter 1.3), coastal areas like the North Sea and polar regions like the Arctic Ocean are especially prone to these changes (Rantanen et al. 2022, De Amorim et al. 2023). In the North Sea, sea surface warming projections for 2100 span from + 1 °C to + 4.5 °C (Schrum et al. 2016) and in the Arctic even from + 3 °C to + 7 °C (Meredith 2019, Rantanen et al. 2022). Simultaneously, potentially permanent heatwaves of up to + 5 °C are projected for the end of the century in a worst-case scenario (Oliver et al. 2019). Considering that metabolic rates scale with temperature (Brown et al. 2004), and that there are high intra- and interspecific differences in thermal dependencies (Zhang et al. 2014), the projected changes will likely affect the future composition and dynamics of marine microbes.

The most robust community response to warming is lower compositional stability and a higher turnover rate, as several experimental and modelling studies have observed (Hillebrand et al. 2012, Dutkiewicz et al. 2013, Henson et al. 2021). In particular, the combination of mean warming and short-term fluctuations like marine heatwaves can push some species beyond their upper thermal limits and thereby induce a compositional reorganisation (Stefanidou et al. 2018, Kling et al. 2020, Samuels et al. 2021). Several studies have contrarily observed compositional stability towards temperature (Stefanidou et al. 2019, Filiz et al. 2020, Briddon et al. 2023), highlighting the need to determine the degree of warming that induces shifts within a given system.

Although temperature has the inherent capacity to increase stochasticity by speeding up the divergence rate of different trajectories (see chapter 1.3.1; Striebel et al. 2016, Pálffy et al. 2021), some dynamics can be explained deterministically. For example, thermal trait variations (see chapter 1.1.2) can govern compositional shifts and explain the patterns that result from warming between functional groups (Anderson et al. 2021, Katkov and Fussmann 2023, Anderson et al. 2024), within the same functional group (Boyd et al. 2013, Bestion et al. 2018) and even intra-specifically (Anderson and Rynearson 2020, Kling et al. 2023). Furthermore, the metabolic theory of ecology suggests that temperature has a greater stimulatory effect on community respiration than on photosynthesis. As a consequence, heterotrophs may exhibit higher growth rates under warming than phototrophs, altering the metabolic balance of ecosystems. Many studies support this assumption (López-Urrutia 2008, Boscolo-Galazzo et al. 2018, Barton et al. 2020), but there is evidence that the thermal dependence of metabolic rates varies among ecosystems (Yvon-Durocher et al. 2012), community compositions (Chen and Laws 2017) and nutrient regimes (Chen et al. 2012). Therefore, it is important to consider not only temperature alone but also other drivers that may modulate its effects (Litchman and Thomas 2023, Seifert et al. 2023).

1.3.3 Temperature modulations by multiple drivers

In natural ecosystems, variations of abiotic factors rarely occur in isolation (Figure 6). Warming itself can, for example, cause thermal stratification, leading to fewer nutrients in the upper water layers and a higher mean irradiance experienced by pelagic organisms in the euphotic zone (Cermeño et al. 2008, Strom and Fredrickson 2008, van de Poll et al. 2018, Li et al. 2020). In addition, warming leads to the melting of sea ice and glaciers in polar regions, which results in freshwater input to the ocean, causing a lower salinity and sometimes increasing the nutrient concentrations (Pan et al. 2019, Møller et al. 2023, Röthig et al. 2023). The rising levels of

carbon dioxide not only contribute to global warming but also lead to a decrease in the pH levels of the ocean (Caldeira and Wickett 2003, IPCC 2014). Simultaneously, humans cause terrestrial runoffs of nutrients and chemical pollutants into coastal oceans originating from urban, agricultural and industrial activities (Grizzetti et al. 2012).

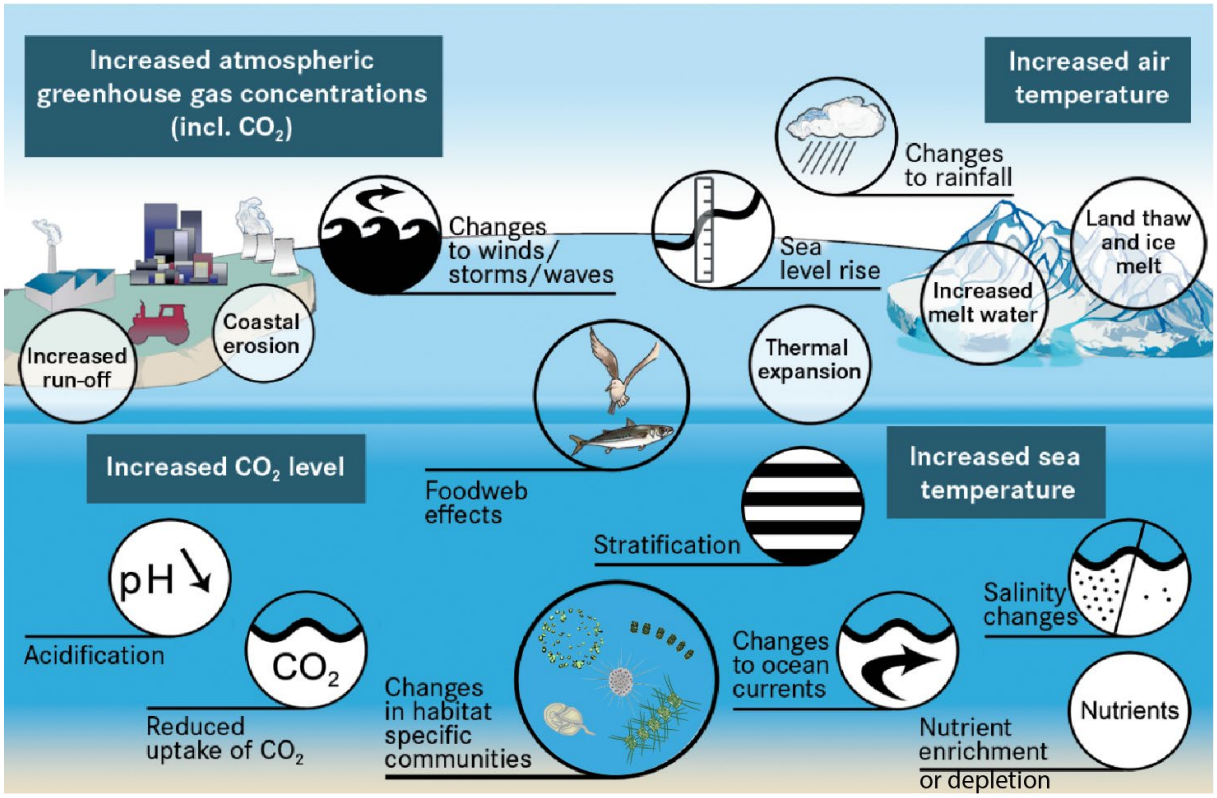


Figure 6: A selection of multiple drivers caused by anthropogenic pressures that can affect marine protist communities. Modified after OSPAR (2010).

The effect of temperature can be modulated by these multiple drivers to varying degrees. For example, factors such as salinity and the carbon dioxide partial pressure are likely only minor modulators. Although interactions between these factors and temperature have been observed for specific species (Bozzato et al. 2019, Seifert et al. 2020) and in other ecosystems such as peatlands (Kilner et al. 2024), the effects may not be strong enough to manifest themselves on the community level in marine systems (Sommer et al. 2015, Hoppe et al. 2018, Stefanidou et al. 2019, Briddon et al. 2023). Contrarily, concurrent changes in macro- (e.g. nitrate, phosphate and silicate) and micronutrients (e.g. iron) can drastically alter the community response to temperature changes (Rose et al. 2009, Verbeek et al. 2018, Gerhard et al. 2019, Anderson et al. 2022). This can be intensified by the indirect effect of warming on nutrient uptake, leading to faster nutrient depletion, which alters the competition environment (Lewandowska et al. 2014, Serra-Pompei et al. 2019). However, all mentioned drivers can change the functional output of protist communities under warming independently of their effect on the composition

(Dutkiewicz et al. 2013, De Senerpont Domis et al. 2014, Cabrerizo et al. 2021, Moreno et al. 2022). Thus, we need a holistic approach to disentangle single and interactive driver effects on community composition and functions to be able to predict future changes.

1.4 Studying complex community structures: approaches and limitations

Considering the high complexity of marine protist communities regarding their trait diversity and abiotic modulators, precise and appropriate methods are imperative to unravel microbial processes. Next to studying communities in their natural habitat via field sampling, potential future dynamics can be assessed through controlled and adjustable laboratory setups. These comprise a broad variety of experimental designs, technical systems and analytical methods. While there is no single ideal way to perform experiments, knowing the benefits and limitations of each approach can help to choose the most suitable complementary combination for a given research question.

1.4.1 Experimental designs

Environmental factors can be applied alone or together, at different speeds or intervals, and using various levels. An advantage of single-driver experiments is the high explanatory power, which can be helpful if one specific factor dominates in a certain setting to predict community responses (Boyd et al. 2018). However, in some environments, several drivers act concurrently, and single-driver studies could under- or overestimate the actual outcome due to additive, synergistic or antagonistic effects of the interactive response (Koussoroplis and Wacker 2016). Therefore, multiple driver experiments often allow a more realistic assessment of future community changes. These can be full-factorial, i.e. spanning several drivers alone and their interaction along different levels, or integrated scenario-based designs combining projections for multiple drivers into a few treatments (Boyd et al. 2018, Moreno et al. 2022). Furthermore, drivers can either be applied suddenly, gradually or as fluctuations, depending on the ecosystem and its specific projections (Gerhard et al. 2023). While some authors advocate the need for integrating drivers to project future changes more realistically (Moreno et al. 2022), especially model parametrization requires full-factorial or gradient designs at the cost of replication (Collins et al. 2022, Seifert et al. 2023, Thomas and Ranjan 2024). However, the chosen design does not only depend on the research objective but also the logistical and financial feasibility of the given experimental system.

1.4.2 Experimental systems

Manipulative experiments can be performed in various systems that differ in terms of their volume and can contain single or multiple functional groups. Nanocosms contain the smallest possible volume of less than a millilitre (Volpe et al. 2021). Therefore, for comparably larger microbes such as protists, any inoculum will rarely contain the full diversity and they are highly prone to changes in the physical or chemical conditions. Consequently, their use for studies on community dynamics is restricted to short-term incubations of bacteria or the trait-characterisation of single strains isolated from community experiments (Garcia et al. 2018, Argyle et al. 2021, Bishop et al. 2022). Systems with a volume of a hundred millilitres up to a few litres (i.e. microcosms) are mostly used for studies with lower complexity, such as artificial communities or single groups (De Senerpont Domis et al. 2007, Boyd et al. 2018, Bestion et al. 2020). The higher replication that can be achieved comes at the cost of lower realism and bottle effects, i.e. sampling and handling biases that selectively reduce the amount of rare and more fragile individuals (Venrick et al. 1977, Calvo-Díaz et al. 2011, Grattepanche et al. 2019). Still, microcosms are especially valuable in settings in which larger volumes are not logistically possible, such as ship-based research expeditions (Altermatt et al. 2015). More realistic ecological studies, which necessitate the inclusion of a higher complexity, are usually conducted in mesocosms, containing several hundred litres (Stewart et al. 2013, Gall et al. 2017). For these, a high workforce and specific facilities with access to the water body of interest are needed and the logistical and financial challenges often entail an integrated or single driver rather than a factorial design (Boyd et al. 2018, Moreno et al. 2022).

The incubation time of any system may span from days to years and needs to be thoroughly chosen as it can impact the outcome of experiments (Barton et al. 2020). For the assessment of short-term phenomena such as heatwaves or environmental shifts along currents (e.g. to test invasion potential), incubations of several days sometimes suffice. Although these may not fully resolve all knock-on effects, they can still provide valuable insights into initial survival rates and help identify the baseline diversity upon which prolonged processes can act. Longer incubation times are preferable to investigate phenomena that occur on larger timescales such as climate change, especially if an assessment of the evolutionary potential is part of the research question. However, due to the complexity of marine protist communities, longer-term experiments are particularly prone to diverge into different trajectories and therefore strongly depend on the initial conditions (see chapter 1.3; Drake et al. 1996, Benincà et al. 2008). Thus, they should rather be performed in large volumes to ensure capturing a representative inoculum (Rasconi et al. 2017) and if possible have a higher replication (Goldford et al. 2018). Overall,

most analytical methods require certain amounts of biomass, which is why the scale of the experimental system is also an essential determinant of the number of parameters that can be assessed.

1.4.3 Making the invisible visible: analytical methods

Marine microbial communities cannot be assessed without the help of proxies to track their composition. Several methods have been developed to identify the species present within a community: flow cytometry, microscopy and metabarcoding (for a detailed description see Altermatt et al. 2015). All three methods have their use cases and disadvantages. Flow cytometers often provide automatic high-throughput measurements, allowing for the processing of many samples in a short amount of time (Props et al. 2016). Additionally, they measure total counts and thereby enable the calculation of growth rates and other abundance-based metrics (Utermöhl 1958). Flow cytometry can contingently be used to determine taxonomic identities based on differences in cell size (forward scatter), granularity (side scatter) and fluorescence. However, many instruments are only capable of measuring organisms smaller than 20 µm. Microscopy also enables an assessment of abundances with the advantage of being cheaper than flow cytometry. Furthermore, it is theoretically possible to identify organisms larger than 2 µm at the species level. The downside is its labour intensity and that the precision highly depends on the expertise of the investigator. Although not being quantitative, sequencing technologies such as metabarcoding offer a solution as they enable the rapid processing of many samples at once and can determine all present species, including rare or cryptic ones, as long as there is an available reference sequence in databases (Hoerstmann et al. 2022, Clark et al. 2023). While these come with the advantage of not being biased by the experience of the user, they do have some technical biases (Figure 7) and can therefore only inform on relative abundances (Mäki et al. 2017, van der Loos and Nijland 2021). Still, thanks to ongoing methodological advancements (Yeh et al. 2018, Jurburg et al. 2022), metabarcoding remains the most promising tool to track community changes in an experimental setup (Lopes dos Santos et al. 2022) and in ecosystems like the Arctic Ocean (Mock et al. 2022, Clark et al. 2023).

Altogether, an awareness of the complexity of marine microbial processes and the challenges that come with studying them is essential to advance our understanding of their dynamics under environmental change. Knowing the benefits and disadvantages of available approaches can guide the development of specific research questions as well as the interpretation of experimental results.

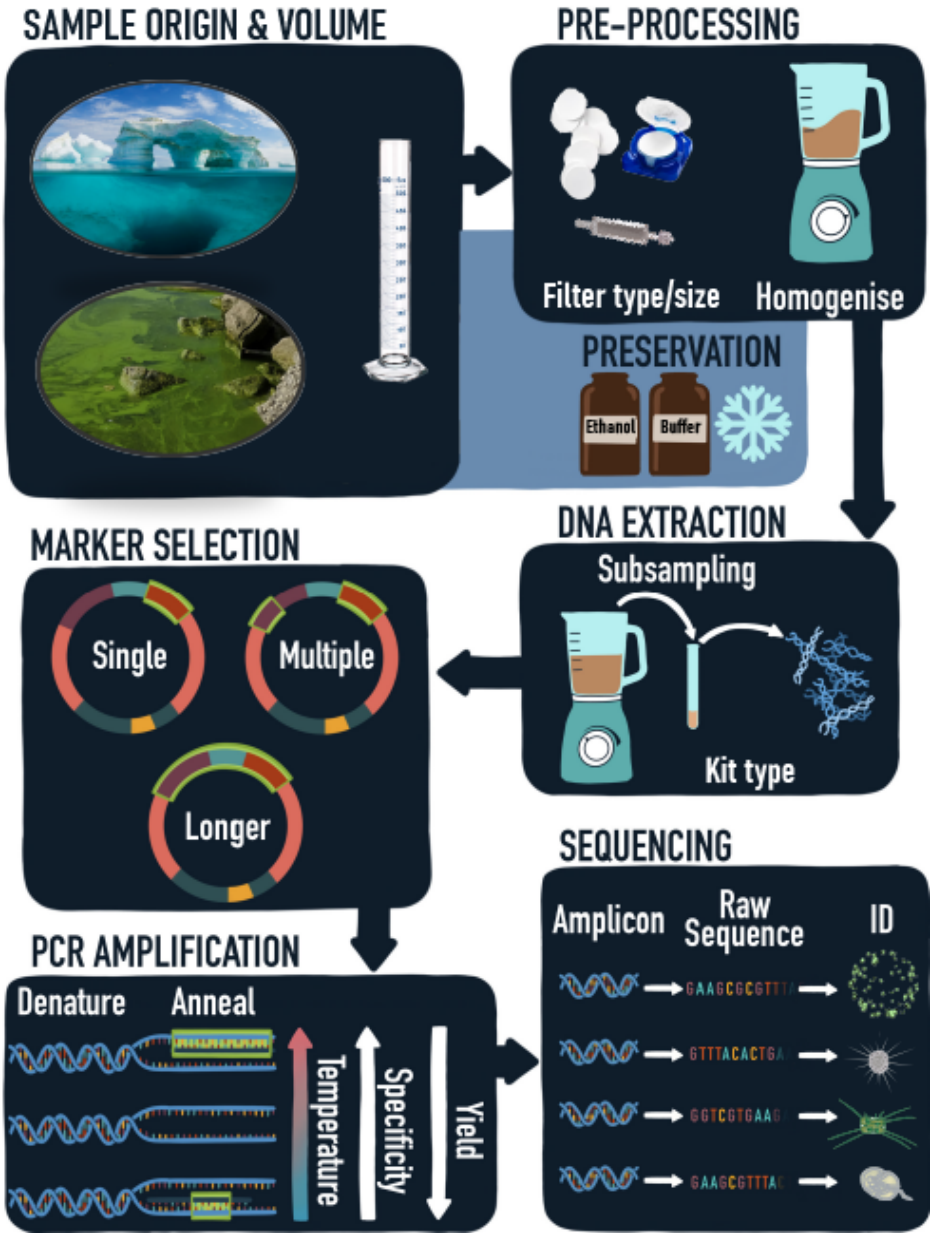


Figure 7: Overview of steps involved in metabarcoding in which technical biases can be introduced. Modified after van der Loos and Nijland (2021).

1.5 Aims and outline of the thesis

This thesis aims to expand our knowledge of the temperature responses of marine protist communities and assess the related functional changes. The main goals are to unravel principles that govern community reorganisation (objective 1), identify prevailing species (objective 2), assess potential consequences for the ecosystem (objective 3) and evaluate how temperature responses may be modulated by other drivers (objective 4). Accounting for the complexity and diversity of marine microbes, communities from different environments were incubated in three consecutive experiments that form the core chapters of this thesis (chapters 2–4) and resulted in a total of six publications (Figure 8). The responses of all communities are compared to determine critical temperatures (objective 5) and assess which marine microbial communities may be particularly vulnerable to environmental change (objective 6).

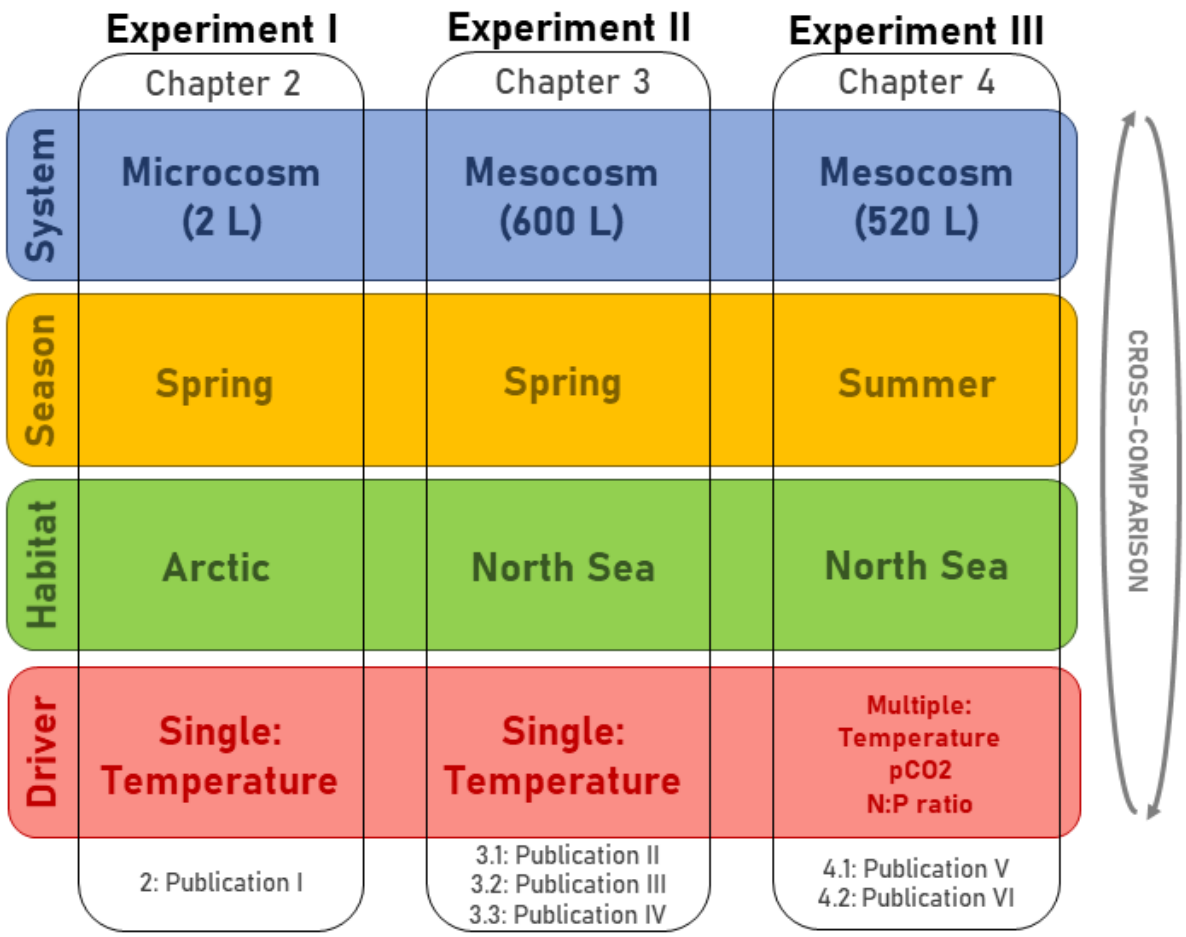


Figure 8: Overview of the chapters of this thesis and how they are related to each other.

In addition to the main objectives of this thesis, which will be achieved by combining the results of all three experiments, each publication has specific sub-objectives. These aim to fill current knowledge gaps in the literature and cover particular aspects of the protist communities' warming responses.

Chapter 2 (publication I) focuses on a microbial community sampled from one of the fastest-warming oceans in the world – the Arctic Ocean. At the same time, organisms here may be particularly vulnerable due to their comparably stable environmental history. While several studies on warming effects have been conducted in strictly polar waters (Coello-Camba 2015, Hoppe et al. 2018), so far, none have been performed with communities in which Arctic species compete with organisms advected from lower latitudes. In publication I, I therefore incubated a microbial community from the Fram Strait at different temperatures. In this area, Atlantic water flows into the Arctic Ocean and carries along temperate plankton that are potentially better adapted to cope with stronger warming compared to their Arctic counterparts. Investigating the competition among species with varying environmental histories enables a better understanding of the sorting mechanisms of a warming environment.

Contrarily, in chapter 3, I want to assess the thermal limits of temperate communities that lack the potential of diversity-stabilising invasions from lower latitudes, which is why I chose to investigate a community from the North Sea. Surprisingly, only a few studies have conducted temperature incubations of North Sea communities (e.g. Lassen et al. 2010, Moreno et al. 2022) and to our knowledge, none focussed on future temperature increases along the spring-summer transition. Therefore, a community was sampled at the start of the spring bloom and exposed to temperatures that they may experience along the development of the bloom by the end of the century. During the incubation, the responses of the replicates partly diverged, which allowed for an additional assessment of how the degree of warming affects the compositional and functional variability in chapter 3.1 (publication II). Chapter 3.2 (publication III) covers particular aspects of the experimental design in ocean warming studies and chapter 3.3 (publication IV) focuses on the role of protist grazers. Furthermore, the publications of chapter 3 act as an anchor for the overall cross-comparison to the other two publications, as the investigated community was either taken from the same habitat or the same season (Figure 8).

In the first chapters, a potential modulating role of nutrients on the communities' temperature response could be identified. Therefore, building on the knowledge obtained from applying temperature as a single driver in chapters 2 and 3.1 (publications I & II), and the insights on varying nutrient ratios from chapters 3.2 and 3.3 (publications III & IV), chapter 4 goes one step further towards a more realistic assessment by combining temperature, nutrients,

and the carbon dioxide partial pressure into an integrated future scenario. Selecting a North Sea summer community, the focus was again on species that may be particularly susceptible to temperature increases, as they already live near their optimal temperature. Especially under short-term temperature fluctuations, thermal limits of temperate summer species may be reached. Therefore, a marine heatwave treatment was added to investigate whether its influence on the protist community composition (chapter 4.1; publication V) and the ecosystem functions (chapter 4.2; publication VI) may differ under ambient vs. potential future conditions.

In chapter 5, the major findings from all experiments are synthesised to answer the objectives of this thesis and discussed within the context of current research. The results from different experimental systems, seasons and habitats are compared to differentiate overarching patterns from system-specific attributes. Finally, I provide an overview of potential future research directions that could follow and expand the insights obtained in this thesis.

1.6 List of publications of the thesis

Publication I
Experiment I
Chapter 2

Ahme A., Von Jackowski A., McPherson R. A., Wolf K., Hoppman M., Neuhaus S., John U. (2023): Winners and losers of atlantification: The degree of ocean warming affects the structure of arctic microbial communities. *Genes* (14) 3, 623. doi: 10.3390/genes14030623.

The experiment was planned together with KW and UJ. The experiment was conducted by myself and UJ. The samples were processed by myself. The data analysis was performed by myself, AVJ, RM, MH and SN. Results were interpreted by myself with the help of the co-authors. The manuscript was written by myself, and revised with the help of the co-authors.

Publication II
Experiment II
Chapter 3.1

Ahme A., Happe A., Striebel M., Cabrerizo M. J., Olsson M., Giesler J., Schulte-Hillen R., Sentimenti A., Kühne N., John U. (2024): Warming increases the compositional and functional variability of a temperate protist community. *Science of the Total Environment* 926, 171971. doi: 10.1016/j.scitotenv.2024.171971.

The experiment was planned by myself with the help of UJ and MS. The experiment was conducted by myself with the help of the co-authors. The samples were processed by myself and AH. The data analysis was performed by myself, MC and JG. Results were interpreted by myself with the help of the co-authors. The manuscript was written by myself, and revised with the help of the co-authors.

Publication III
Experiment II
Chapter 3.2

Happe A., Ahme A., Cabrerizo M. J., Gerhard M., John U., Striebel M. (2024): The experimental implications of the rate of temperature change and timing of nutrient availability on growth and stoichiometry of a natural marine phytoplankton community. Under review in *Limnology & Oceanography*.

The experiment was planned by AH and MS with the help of myself. The experiment was conducted by AH with the help of myself. The samples were processed by AH. The data analysis was performed by AH, MG and MS. Results were interpreted by AH with the help of myself and all co-authors. The manuscript was written by AH, and revised by myself and the co-authors.

Publication IV
Experiment II
Chapter 3.3

Cabrerizo M. J., Happe A., Ahme A., John U., Olsson M., Striebel M. (2024): Moderate and extreme warming under a varied resource supply alter the microzooplankton-phytoplankton coupling in marine communities. Submitted to *Limnology & Oceanography*.

The experiment was planned by MC and MS with the help of myself. The experiment was conducted by MC with the help of myself. The samples were processed and the data analysis was performed by MC. Results were interpreted by MC with the help of myself and all co-authors. The manuscript was written by MC, and revised by myself and the co-authors.

Publication V
Experiment III
Chapter 4.1

Ahme A., Kirstein I. V., Meunier C., Wohlrab S., John U. (2024): Microbial Meltdown: Concurrent global change and heatwaves disturb phototrophic more than heterotrophic protist diversity. To be submitted.

The experiment was planned by IVK and CM. The experiment was conducted by myself and the co-authors. The samples were processed by myself. The data analysis was performed by myself and SW. Results were interpreted by myself with the help of the co-authors. The manuscript was written by myself, and revised with the help of the co-authors.

Publication VI
Experiment III
Chapter 4.2

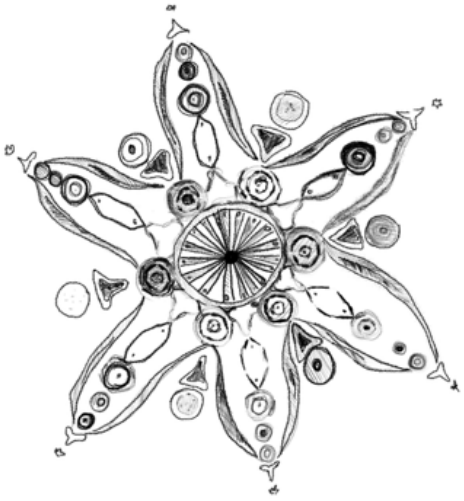
Meunier C. L., Schmidt J., Ahme A., Balkoni A., Berg K., Blum L., Boersma M., Brüwer J. D., Fuchs B. M., Gimenez L., Guignard M., Schulte-Hillen R., Krock B., Rick J., Stibor H., Stockenreiter M., Tulatz S., Weber F., Wichels A., Wiltshire K. H., Wohlrab S., Kirstein I. V. (2024): Plankton communities today and tomorrow – impacts of global change and marine heatwaves in a multiple-driver mesocosm experiment. Submitted to *Science of the Total Environment*.

The experiment was planned by CM and IVK. The experiment was conducted by all co-authors. The samples were processed by myself and all co-authors. The data analysis was performed by CM and IVK. Results were interpreted by CM with the help of myself and all co-authors. The manuscript was written by CM and IVK, and revised by myself and the co-authors.

The declaration of own contribution to manuscripts can be found at the end of the thesis.

2

Arctic spring community



PUBLICATION I

Winners and losers of atlantification: The degree of ocean warming affects the structure of arctic microbial communities

Published in Genes (2023)

genes

MDPI

Article

Winners and Losers of Atlantification: The Degree of Ocean Warming Affects the Structure of Arctic Microbial Communities

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Abstract: Arctic microbial communities (i.e., protists and bacteria) are increasingly subjected to an intrusion of new species via Atlantification and an uncertain degree of ocean warming. As species differ in adaptive traits, these oceanic conditions may lead to compositional changes with functional implications for the ecosystem. In June 2021, we incubated water from the western Fram Strait at three temperatures (2 °C, 6 °C, and 9 °C), mimicking the current and potential future properties of the Arctic Ocean. Our results show that increasing the temperature to 6 °C only minorly affects the community, while an increase to 9 °C significantly lowers the diversity and shifts the composition. A higher relative abundance of large hetero- and mixotrophic protists was observed at 2 °C and 6 °C compared to a higher abundance of intermediate-sized temperate diatoms at 9 °C. The compositional differences at 9 °C led to a higher chlorophyll a:POC ratio, but the C:N ratio remained similar. Our results contradict the common assumption that smaller organisms and heterotrophs are favored under warming and strongly indicate a thermal limit between 6 °C and 9 °C for many Arctic species. Consequently, the magnitude of temperature increase is a crucial factor for microbial community reorganization and the ensuing ecological consequences in the future Arctic Ocean.

Keywords: Fram Strait; West Spitsbergen Current; incubation experiment; species composition; traits; thermal limits; cell size; trophic mode; pelagic microorganisms; microplankton

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1. Introduction

The Arctic ecosystem is dramatically changing and increasingly influenced by the Atlantic Ocean due to a weakening of the Atlantic Meridional Overturning Circulation [1]. This so-called "Atlantification" implies a northward expansion of Atlantic water into the Arctic Basin, resulting in an increase in temperature and salinity, rapid sea ice decline, as well as an intrusion of temperate species [2]. In particular, the West Spitsbergen Current is the largest driver of Atlantification by transporting Atlantic water northwards [2]. Over the last decades, the Atlantic water in the Fram Strait and the West Spitsbergen Current has been steadily warming [3]. Approximately half of Atlantic water transport carried northwards by the West Spitsbergen Current recirculates in the Fram Strait [4] and eventually becomes part of the southward outflow of polar water, namely the East Greenland Current [5]. These dynamic and mixed properties make the Fram Strait and West Spitsbergen Current an ideal place to study water that is representative of an Arctic Ocean increasingly exhibiting Atlantic characteristics.

In addition to Atlantification, the Arctic is generally warming faster than the global average—a phenomenon referred to as Arctic amplification [6,7]. The co-occurrence of Atlantification and Arctic amplification is expected to affect the microbial community composition, as advected individuals may cope better with the new conditions than local

species [8]. Anticipating shifts in community structure under abiotic change is complex and depends on various factors. Recently, trait differences among competing species have been identified as a good predictor for planktonic reorganization [9]. With regard to warming, traits such as the cell size [10] and the trophic mode [11] are known to affect the fitness and performance of a species, as they influence the thermal reaction norm for maximum growth [12].

Smaller cells were long believed to have an advantage under increasing temperatures as their supposedly higher mass-specific growth rates should enable them to outcompete larger cells [13]. Therefore, warming was expected to result in a community shift towards smaller species [14,15]. This assumption has been challenged by the repeated observation of growth rates peaking at intermediate cell sizes, even under higher temperatures [10,16,17]. However, in accordance with both theories, it is generally expected that comparably larger species suffer a competitive disadvantage when temperatures increase.

Another group that is assumed to experience a competitive disadvantage under warming is photoautotrophs. While the metabolism of phytoplankton is limited by their photosynthetic rate, heterotrophic plankton depends on food uptake and the rate at which it respires it [11]. Although all metabolic processes increase with temperature up to a point, the rate of increase is slower for photosynthesis compared to respiration. This is due to different temperature dependencies of the central chemical reactions—the production of ATP from glycolysis and the tricarboxylic acid cycle for respiration being more sensitive than Rubisco carboxylation for photosynthesis [18,19]. Therefore, several authors have suggested that while warming may also enhance phytoplankton growth, it disproportionally favors heterotrophic organisms [20–22]. Consequently, grazing pressure may increase [11,23].

Liu et al. [24] also found micro-grazers to be disproportionally advantaged by high temperatures but identified thermal optima as potential vectors for community response. This is in line with another set of studies that suggests most shifts in planktonic composition can be explained by the thermal niche of the respective species [12,25,26]. As thermal reaction norms are usually the result of adaptation and often reflect the biogeography of species [27–29], temperate organisms should have higher optimum and maximum temperatures for growth than polar organisms. This assumption is confirmed by the ongoing expansion of temperate species into the Arctic realm [30]. However, whether temperate or polar species will prevail may depend on their thermal optima and limits relative to the actual temperature increase occurring in the Arctic.

Currently, the degree of warming in Arctic waters remains unclear [6] but may be crucial in determining planktonic reorganization. This is particularly important considering the presence of temperate species with other metabolic traits, which are advected via Atlantification. Still, studies on the consequences of concurrent warming and Atlantification of the Arctic Ocean for local plankton communities are scarce. The aim of this study was to experimentally simulate the effect of different temperature scenarios on the composition and characteristics of microbial communities (i.e., protists and bacteria) from Atlantic water inflow to the Arctic Ocean. We hypothesized that small temperate heterotrophs increase in relative abundance with rising temperatures. Furthermore, we expected the diversity to decrease with increasing temperatures due to the dominance of a few well-adapted species.

2. Materials and Methods

2.1. Study Site and Seawater Physical Properties

Our experiment was performed on board the German research icebreaker RV Polarstern during the PS126 expedition to the long-term ecological research observatory Hausgarten in the Fram Strait in May/June 2021 [31]. To capture a plankton community with both Atlantic and Arctic characteristics, we chose a sampling site in the central Fram Strait (Figure 1a), where warm and salty Atlantic water recirculates and subducts under colder and fresher Polar water. The hydrographic properties of the water at this depth suggest that it is predominately mixed polar surface water (Figure S1), which is cold (<1 °C) and relatively fresh (34), overlaying an Atlantic water layer. However, the sample location

shows high near-surface variability where warm (>0 °C) Atlantic waters often dominate (Figure 1b,c). Thus, it is anticipated that at least some of the species at the sample site are those found in Atlantic water and represent a background Atlantic community. A total of 30 L of seawater was collected from the chlorophyll maximum at 15 m at station HG-IV on 1 June 2021 (12:00 UTC, Figure S1). We used a ship-based SBE911 + CTD/rosette system (Sea-Bird Scientific, Washington, DC, USA) equipped with a standard suite of oceanographic sensors and 24 × 12 L OTE water sampling bottles. The sea-surface temperature and salinity were measured by two SBE21 thermosalinographs and two auxiliary SBE38 temperature sensors (Sea-Bird Scientific, Washington, DC, USA) installed in an underway seawater flow-through system on board the *Polarstern* [32]. The seawater inlet is located 11 m under the water's surface. If not otherwise noted, salinity is always expressed after PSS-78 and is, therefore, unitless.

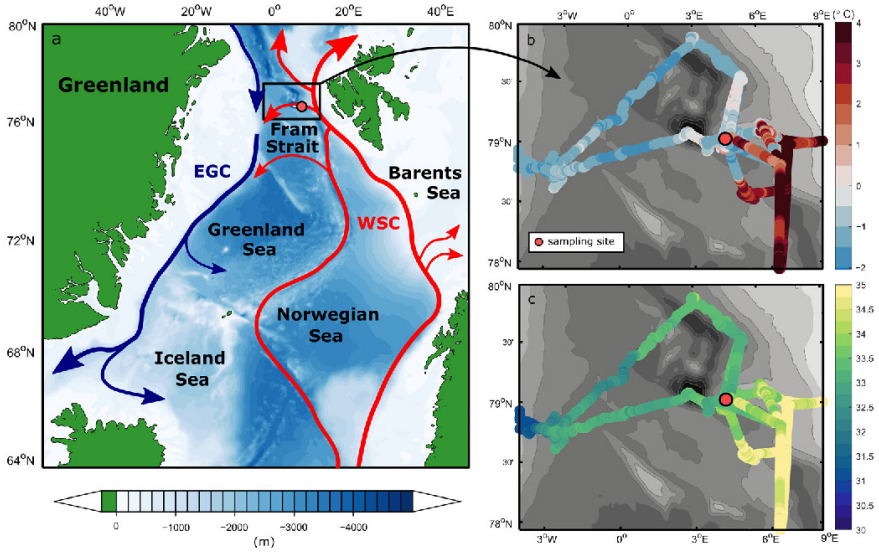

Figure 1. (**a**) Schematic of the Atlantic water circulation in the Nordic Seas and the near-surface (**b**) temperature and (**c**) salinity in the Fram Strait during PS126. The location of the sampling site HG-IV is highlighted as the red circle (4° 22.23′ W, 79° 4.86′ N). On the map, the red arrows represent the northwards flow of warm Atlantic water into the Arctic Ocean as the West Spitsbergen Current, with a large fraction of this water recirculating in the Fram Strait. The blue arrows denote the flow of modified Atlantic water southward within the East Greenland Current along the continental shelf break. The sketched currents are adapted from Beszczynska-Möller et al. [3].

2.2. *Experimental Set-Up*

To remove metazoan plankton and ensure the same community composition within all treatments, we filtered the water through an ethanol-cleaned 150 µm mesh into an acid-cleaned 25 L carboy and gently mixed it. Water from the carboy was distributed evenly into nine autoclaved 2.3 L glass bottles (three temperature treatments in triplicates), which were closed with gas-tight lids. The remaining water was used to sample parameters of the starting community (t0) in triplicate. We took care not to introduce any bubbles during the filling procedure to prevent more fragile organisms, such as ciliates, from dying [33].

In order to keep cells in suspension, bottles containing the natural communities were mounted in triplicates onto three plankton wheels that turned at a speed of one round per minute. The plankton wheels stood in temperature-controlled incubation containers, which were kept at three different temperatures. We chose 2 °C as the lowest level because it was close to the mean temperature of 0.84 °C for the upper 100 m (Figure S1), and similar mean temperatures have also been recorded at this station previously [34]. The other two

temperature levels of 6 °C and 9 °C were chosen to represent different scenarios of Arctic amplification (+4 °C and +7 °C respectively, [6]). All communities were exposed to 24 h artificial daylight with an irradiance of 30 µmol photons m^{-2} s^{-1} (SunStrip 35W fresh, ECONLUX GmbH, Köln, Germany) for ten days.

The nutrient concentrations in the field were low (0.06 ± 1.23 µM NO_3^-, 0.21 ± 0.08 µM PO_4^{3-} , 0.08 ± 0.39 µM $Si(OH)_4$); therefore, we added macro- and micronutrients to enable an investigation of the otherwise growth-limited photoautotrophic summer community. Nutrient pulses can also naturally occur through mixing events of short-term frontal systems and commonly enhance production in the surface waters of the eastern Fram Strait during summer [35,36]. Based on recommendations by Calbet and Saiz [37], we added 50 µM NO_3^-, 4.7 µM PO_4^{3-}, and 25 µM $Si(OH)_4$, as well as trace metals and vitamins in accordance with the F/2 R medium concentrations. During the first days of the experiment, six bottles per temperature were incubated for a micrograzing experiment (not included in this dataset), with half of them undiluted and the other half diluted 1:5 with 0.22 µm filtered seawater taken from near the sampling location. After three days, we pooled the diluted and undiluted communities from t0 at each temperature. These pooled communities were then once again diluted (1:5), and nutrients were added as at t0 before they were incubated in triplicates for the last seven days of the experiment. Importantly, this did not result in any differences among our treatments, as community composition stayed stable at all temperatures during the first three days (Figure S2).

pH was measured at tfin using a pH meter (EcoScan pH 5, ThermoFisher Scientific, Waltham, MA, USA) with a glass electrode (Sentix 62, Mettler Toledo, Columbus, OH, USA) one-point calibrated with a technical buffer solution (pH 7, Mettler Toledo, Columbus, OH, USA). At t0 and tfin, samples for total alkalinity (TA) and dissolved nutrients were filtered through a 0.22 µm cellulose-acetate syringe filter (Nalgene, Rochester, NY, USA) and stored at 4 °C in 125 mL borosilicate bottles and 15 mL polycarbonate tubes, respectively. TA was measured by duplicate potentiometric titration using a TitroLine alphaplus autosampler (Schott Instruments, Mainz, Germany) and corrected with certified reference materials from A. Dickson (Scripps Institution of Oceanography, San Diego, CA, USA). The full carbonate system was calculated for tfin using the software CO2sys [38] with dissociation constants of carbonic acid by Mehrbach et al. [39], refitted by Dickson and Millero [40]. Dissolved nutrients were measured colorimetrically at t0 and tfin on a continuous-flow autoanalyzer (Evolution III, Alliance Instruments, Freilassing, Germany) following standard seawater analytical methods for nitrate and nitrite [41], phosphate [42], silicate [43], and ammonium [44].

2.3. Biomass Parameters

Biomass parameters were sampled in triplicate from t0 and from each replicate bottle at tfin. After thoroughly inverting the bottles, we vacuum-filtered (<−200 mbar) 300 mL for chlorophyll *a* (chl*a*), 200 mL for particulate organic carbon and nitrogen (POC/PON), and the same volume of sterile water for blanks onto pre-combusted glass-fiber filters (GF/F Whatman, Maidstone, UK). These were put into 2 mL cryovials (Sarstedt, Nümbrecht, Germany) and kept at −80 °C until processing. Filters for chl*a* were manually shredded in 6 mL of 90% acetone and extracted for 20 h at 8 °C according to the EPA method 445.0 [45]. The extract was centrifuged to remove residual filter snips, and chl*a* was determined on a Trilogy fluorometer (Turner Designs, San Jose, CA, USA) after correcting for phaeopigments via acidification (1 M HCl). Filters for POC/PON were also acidified (0.5 M HCl) and dried for 12 h at 60 °C. Analysis was performed using a gas chromatograph CHNS-O elemental analyzer (EURO EA 3000, HEKAtech, Wegberg, Germany). The chl*a*:POC ratio was calculated by dividing the chl*a* concentration by the POC concentration, and the C:N ratio was calculated by dividing the molar mass of POC by PON.

2.4. Community Composition and Diversity Analyses

Eukaryotic and bacterial community compositions were assessed by means of metabarcoding. A total of 500 mL of sample water was carefully vacuum-filtrated onto polycarbonate filters (0.8 µm nominal pore size, Nucleopore, Whatman, Maidstone, UK). Sequential filtration onto 0.2 µm pore size filters was not possible, which undoubtedly biased the analysis toward particle-associated heterotrophic bacteria. Filters were put into 2 mL cryovials containing 650 µL of warm extraction buffer and stored at −80 °C. After cell disruption with a MagNa Lyser (Roche Diagnostics, Basel, Switzerland), DNA extraction was performed according to the manufacturer's protocol using the NucleoSpin Soil extraction kit (Macherey-Nagel GmbH, Düren, Germany). DNA concentration was quantified using a NanoDrop 8000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and normalized to 5 ng μL^{-1}. Amplicons of the variable region 4 (V4) of the 18S rRNA and 16S rRNA gene for eukaryotes and bacteria, respectively, were generated according to the standard protocol of amplicon library preparation (16S Metagenomic Sequencing Library Preparation, Part #15044223 Rev. B. Illumina, San Diego, CA, USA) using the forward primer CCAGCASCYGCGGTAATTCC and reverse primer ACTTTCGTTCTTGAT for 18S rRNA gene sequencing [46] and the forward primer GTGCCAGCMGCCGCGGTAA and reverse primer GGACTACHVGGGTWTCTAAT for 16S rRNA gene sequencing [47], all including an Illumina tail. Single samples were indexed using the Nextera XT Index Kit v2 Set A primers (Illumina, San Diego, CA, USA), and the resulting libraries were pooled, one pool each for 18S and 16S rRNA gene sequencing. Sequencing was performed on a MiSeq sequencer (Illumina, San Diego, CA, USA), producing 300 base pair paired-end gene amplicon reads. Demultiplexing and FASTQ sequence file generation were carried out using the Generate FASTQ workflow of the MiSeq sequencer software. Primers were removed with v2.8 cutadapt [48], and further processing of the sequence data was performed using the v1.18 DADA2 R package [49]. In consideration of the read quality, which usually drops towards the 3′-end, the forward reads were trimmed after 240 to 260 base pairs, and the reverse reads were trimmed after 200 to 210 base pairs. For each pool, error rates were learned independently, and sequences were denoised. Paired-end reads were merged with a minimum overlap of 50 base pairs allowing 0 mismatches, and chimeras were predicted and removed. Taxonomic assignment of the resulting amplicon sequence variants (ASVs) was performed using the reference databases PR2 (v4.12.0) for eukaryotes and SILVA (v138) for bacteria [50,51].

For downstream analyses, ASVs with a count of less than ten reads in replicate sample means were removed, as well as potential contaminations, metazoans, and fungi (Table S1). After rarefaction to confirm a sufficient sequencing depth, all samples were scaled to the lowest depth as described by Beule and Karlovsky [52]. Lastly, ASVs were normalized by centered log ratio (CLR) transformation [53] after removing the zeros with multiplicative simple replacement [54]. Processing of the data was performed using R v4.21 [55] with RStudio v2022.07.2 [56] and the packages dplyr (v1.0.10), vegan (v2.6-2), SRS (v0.2.3), zCompositions (v1.4.0.1), propr (v4.2.6), easyCODA (v0.34.3), and BiodiversityR (v2.14.4).

Annotated species were grouped according to three different categories: cell size, trophic mode, and thermal niche (Tables S2 and S3). Cell size was differentiated into picoplankton (here defined as <2 µm), nanoplankton (here defined as 2–20 µm), and microplankton (>20 µm), and trophic mode was differentiated into heterotrophs, autotrophs, mixotrophs, and parasitic. The data of both categories were assembled through an unstructured literature search via WoRMS [57] based on previous categorizations by Hörstmann et al. [58] and Schneider et al. [59]. The classification of thermal niches into Arctic, Arctic-temperate and cosmopolitan was performed as described by Šupraha et al. [60]. Whenever categories were unclear, species were marked as uncategorized.

To obtain a measure for phenotypic diversity [61–63], 3.5 mL of the sample were preserved with hexamine-buffered formalin (0.5% final concentration) and stored at −80 °C after dark incubation for 15 min. For analysis, samples were thawed at room temperature, vortexed, and measured at a fast speed for three minutes using an Accuri C6 flow cytometer

(BD Sciences, Franklin Lakes, NJ, USA) after setting the threshold of the FL-3 channel to 900. Phenotypic diversity (D2) was calculated for each sample based on the flow cytometric fingerprint according to Props et al. [61], using the values of FSC-H, SSC-H, FL-2, FL-3, and FL-4. To generate Figure 2, replicate C of the 6 °C treatment had to be removed due to an erroneous measurement of the flow cytometer and, thus, a missing D2 value. However, excluding D2 as a constraint, 6 °C replicate C clustered together with the other replicates, and the overall pattern did not change (Figure S4). Species richness and evenness were calculated from the sequencing data before transformation (Table S3).

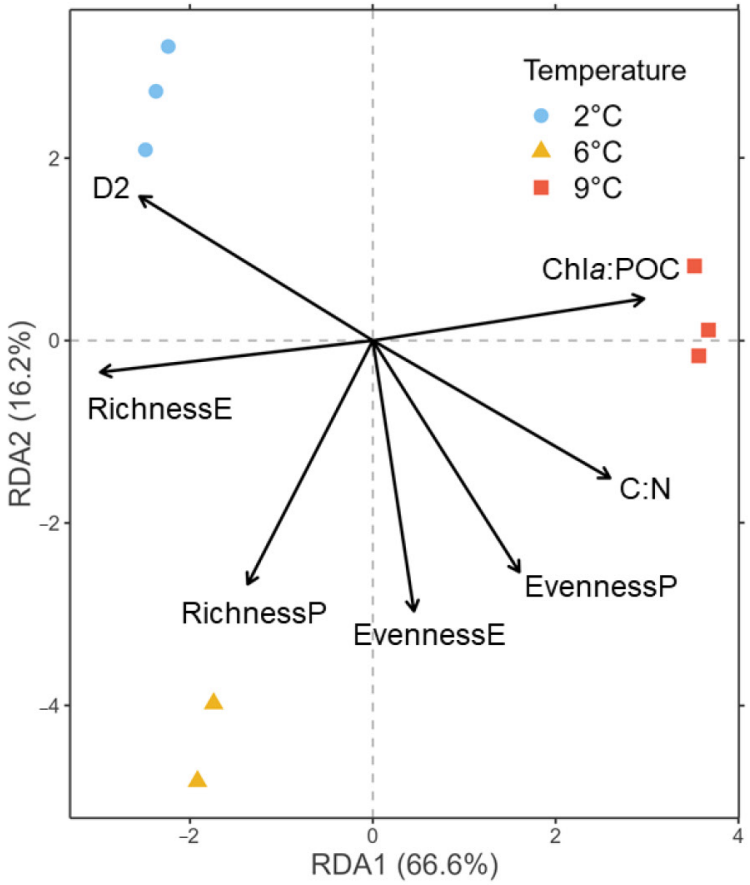

Figure 2. RDA of the CLR-transformed ASV counts of the 18S rRNA gene library color-coded to temperatures at tfin using biomass and diversity parameters as constraints represented by arrows. P = prokaryotes, E = eukaryotes, D2 = phenotypic diversity.

2.5. *Data Handling & Analyses*

To examine the relative community composition, we took the replicate mean of the read abundance after normalization. ASV data grouped according to cell size, trophic mode, and thermal niche were analyzed after normalization and boxcox transformation by means of correspondence analysis (CA), according to Greenacre [64]. Furthermore, the parameters chl*a*:POC, C:N, D2, prokaryotic/eukaryotic richness, and evenness were used as explanatory matrices for a redundancy analysis (RDA) with the CLR-transformed ASV tables as response matrices at tfin. Before conducting any statistical tests, all parameters were checked for homogeneity of variance with Levene's test and met the assumption. As the data of at least one temperature per parameter were not normally distributed, we log-transformed them and performed pairwise t-tests with Bonferroni correction to detect

differences in biomass and diversity parameters between temperatures (Table S4). The choice of this test was based on a priori assumptions on existing differences between the temperatures based on our hypothesis. We checked whether the results would differ using one-way ANOVAs and post hoc Tukey's tests (R Script on GitHub), and they did not. The significance level of all statistical tests was set to 0.05, and all data in the tables and text are shown as mean ± one standard deviation of the three replicates.

3. Results

3.1. Physical Ocean Properties and Water Masses

In the CTD profile taken at the time of sampling (Figure S1), a 20 m deep cold and fresh polar surface water layer overlaid a sharp pycnocline, with a warm Atlantic water layer at ~70 to 450 m. Near-surface temperature and salinity measured by the thermosalinograph throughout the experiment suggest strong variability in this part of the Fram Strait. During repeated visits to the sampling site, temperatures and salinities in the upper water column ranged between −1.6 °C and 4 °C and 32.5 and 35, respectively, in a period of only a few weeks (Figure 1b,c). The sampling site was located close to the Svalbard continental shelf, in a transition zone characterized by an Atlantic water recirculation regime (indicated in Figure 1a). There, conditions alternate between the warm Atlantic-influenced West Spitsbergen Current and the colder and fresher Polar water towards the central and western Fram Strait at a relatively high rate (see also von Appen et al. [65]). These findings suggest the presence of a highly variable and dynamic mixed polar surface water layer in the upper ~70 m (Figure S1), exhibiting properties of both Atlantic and Arctic waters over time.

3.2. Biomass and Diversity Parameters

During the ten days of incubation, the communities were neither nutrient- nor carbon-limited (Table S5). Chl*a*:POC was significantly higher and eukaryotic species richness was significantly lower at 9 °C than at 2 °C and 6 °C (Tables S3 and S4). The phenotypic diversity (D2) was significantly lower at 9 °C only compared to 2 °C. All other pairwise t-tests were not significant. The directions of change in all parameters in relation to temperature treatments are visualized in the RDA plot (Figure 2). Here, 66.6% of the variation is constrained by the first RDA axis, with 9 °C associated with a high chl*a*:POC ratio, while eukaryotic species richness increases in the direction of 2 °C and 6 °C. The two lower temperatures spread across the second RDA axis (16.2%), of which 2 °C was associated with high D2. Considering the t-tests, the effects of the C:N ratio, eukaryotic species evenness, and prokaryotic species richness can be considered negligible for the interpretation of the RDA (Table S4).

3.3. Community Composition—Eukaryotes

The size classes as well as trophic and thermal groups contributed to the variance among temperature treatments, as is evident from the CA plots in Figure 3. Generally, all replicates of the temperature treatments clustered together. Regarding size differences (Figure 3a), nanoplanktonic individuals (2–20 µm) mainly led to the clustering of 9 °C away from the two other temperatures on CA dimension one, with 93.7% of the variance constrained. On the second CA dimension (5.6%), 2 °C and 6 °C clustered away from each other based on differences in pico- and microplankton read abundances. In terms of trophic mode (Figure 3b), the three temperature treatments mainly spread out along the first axis (90.8%), with 6 °C and 2 °C on one side being gradually dominated more by hetero- and mixotrophy and 9 °C on the other side comprising more phototrophic and parasitic organisms. The replicates at 9 °C also spread out on the second axis (8.1%), along with the explanatory variables phototrophy and parasitism pointing in opposite directions. In the thermal niche CA (Figure 3c), 9 °C samples clustered away from 6 °C and 2 °C along the explanatory variable cosmopolitan on the first axis, which constrained 89.1% of the variance. The variables Arctic and Arctic-temperate pointed towards samples of the two colder temperatures and spread out slightly along the second axis (7.2%).

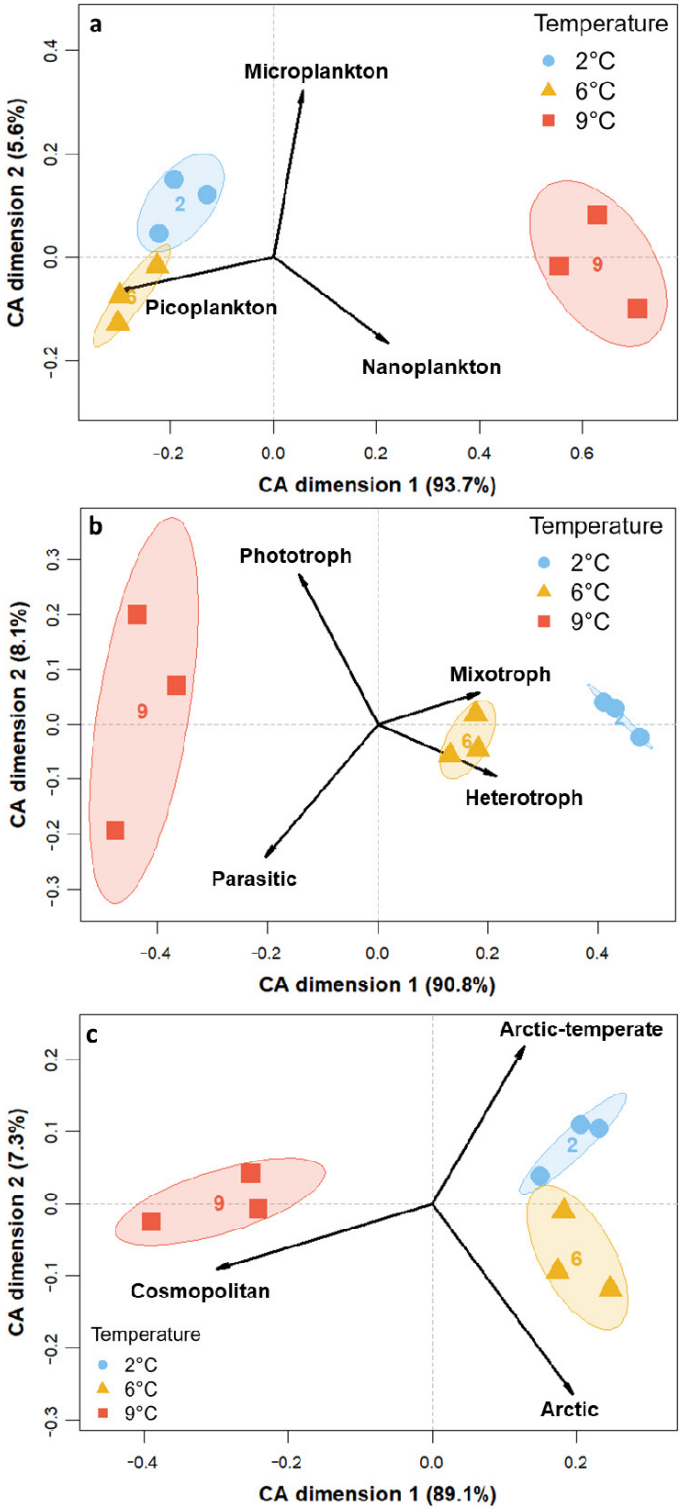

Figure 3. CA for the transformed read counts of the 18S rRNA gene library color-coded to temperatures at tfin constrained by (**a**) size classes, (**b**) trophic mode, and (**c**) thermal niche.

After ten days of incubation, 2 °C and 6 °C showed a more similar composition in comparison to 9 °C (Figure 4a). The 6 °C treatment had slightly higher relative abundances of Choanoflagellatea, Dictyochophyceae, Mammielophyceae, marine Stramenopile (MAST) clades, Picozoa, and Spirotrichea than the 2 °C treatment. In contrast, all these groups were absent or only present in low relative abundances at 9 °C. Furthermore, Bacillariophyta and Syndiniales showed substantially higher relative abundances at 9 °C compared to the two lower temperatures. The dominant group at 2 °C and 6 °C was Dinophyceae, whereas Bacillariophyta dominated at 9 °C. The shifts in relative species abundances of the two main phototrophic classes are shown in Figure 4b,c. The phylum of Bacillariophyta (Figure 4b) comprised the biggest compositional differences among all temperatures. *Thalassiosira nordenskoeldii* was present in all treatments, whereas *Chaetoceros gelidus* had higher relative abundances in the two warming scenarios. *Pseudo-nitzschia* sp., *Chaetoceros cinctus*, and *Thalassiosira rotula* were found in the highest relative abundances at 9 °C but were not detected at 2 °C and were only present in low relative abundances at 6 °C. *Fragilariopsis cylindrus* and *Thalassiosira antarctica* were relatively less abundant or absent at 9 °C. Within the phylum of Haptophyta (Figure 4c), mainly the species *Phaeocystis pouchetii* was present and showed the highest relative abundances at 6 °C, followed by 2 °C and then 9 °C. At 2 °C and 6 °C, *Micromonas polaris* and *commoda* of the class Mammiellophyceae were still present in low relative abundances, whereas they were absent at 9 °C (Figures S5 and S6).

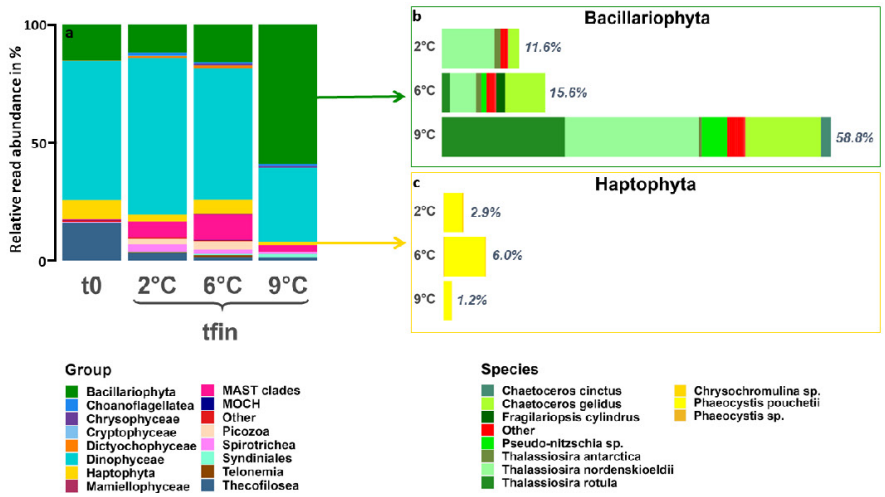

Figure 4. (**a**) ASV-based eukaryotic community composition on class level at the start (t0) and at all treatment temperatures after ten days (tfin). Windows show the relative contribution and species composition of (**b**) Bacillariophyta (green) and (**c**) Haptophyta (yellow) at tfin of all temperature treatments. ASVs with an abundance of fewer than 100 reads among all temperatures were categorized as "other".

3.4. Community Composition—Bacteria

After ten days, the α- and β-diversity differed among all temperature treatments. As part of the α-diversity analysis, sample completeness using coverage-based rarefaction and extrapolation sampling curves for species richness was greater than 99.97% with 95% confidence intervals. Further interpreting the diversity showed that bacterial richness significantly increased with temperature (ANOVA, $F_{(3,8)} = 23.41$, $p < 0.001$), while the evenness was significantly higher for the 2 °C and 9 °C treatments than for 6 °C (Table S4). Analyzing the diversity using distance matrices further confirmed significant differences between the temperature treatments, which roughly explains 64% of the total variation (PCoA using Bray–Curtis, MANOVA, $p = 0.0003$). The abundant classes included *Bacteroidia* and *Gammaproteobacteria* (Figure 5a). *Bacteroidia* abundances ranged from 5% to 47% rela-

tive abundance, while *Gammaproteobacteria* ranged from 49% to 88% relative abundance. The abundant taxa within *Bacteroidia* included *Polaribacter* (39% to 77%), *Aurantivirga* (7% to 36%), and *Ulvibacter* (8% to 14%). More abundant members within *Gammaproteobacteria* included *Colwellia* (18% to 61%), *Marinomonas* (~13%), the *SAR92* clade (26%), and *Neptuniibacter* (29%).

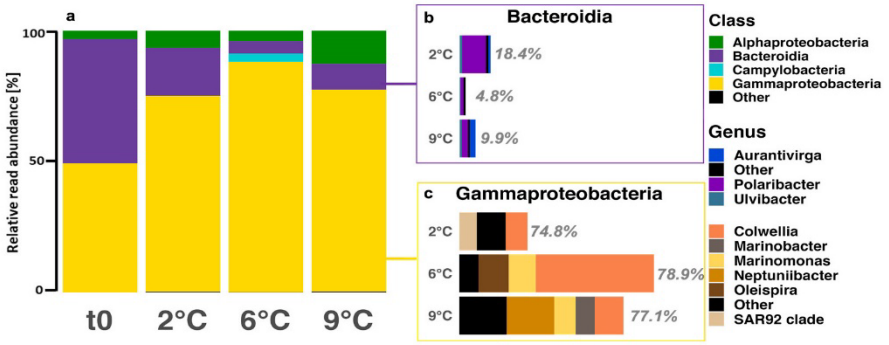

Figure 5. (**a**) ASV-based bacterial community composition on class level at the start (t0) and at all treatment temperatures after ten days (tfin). Windows show the relative contribution and genus composition of (**b**) *Bacteroidia* (purple) and (**c**) *Gammaproteobacteria* (yellow) at tfin of all temperature treatments. ASVs with an abundance of fewer than 100 reads among all temperatures were categorized as "other".

4. Discussion

4.1. Warming Induces an Increase in Photoautotrophic, Intermediate-Sized Organisms

While the C:N ratio appeared to be resistant to warming (Tables S3 and S4, see also [66]), the chl*a*:POC ratio significantly increased at 9 °C, indicating either an up-regulation of the cellular chl*a* quota of phytoplankton [67] or a higher biomass of phototrophic compared to heterotrophic organisms. Our trophic group data support the latter, as heterotrophy and mixotrophy seem to have been disadvantageous under warming (Figures 3b and S3). While this is in contrast to assumptions made by the metabolic theory of ecology (MTE; [18]), it is supported by Petchey et al. [68], who found a greater extinction frequency of higher trophic positions under warming. A reason for the deviation of our findings from the MTE could be the timespan of the experiments. On short timescales and under nutrient-replete conditions, such as during blooms and in our experiment, fast growers like diatoms may have an advantage over slower-growing heterotrophic and mixotrophic organisms. Predictions by the MTE may only manifest on longer timescales, over years to decades [22]. Additionally, our study only assessed micrograzing and did not account for mesozooplankton, which was removed by the 150 µm mesh (see methods). Increased grazing pressure under warming, as proposed by other authors [69,70], might be restricted to larger organisms and thus could not be accounted for in our experiment. Overall, the cell size and the thermal niche appeared to have been more fundamental than the trophic level for community reorganization under increasing temperatures.

In terms of cell size, warming to 9 °C resulted in a relative increase in intermediate (2–20 µm) as well as a reduction of large (20–150 µm) and small (0.8–2 µm) eukaryotes (Figures 3a and S3). Other studies have also found intermediate-sized organisms to exhibit higher growth rates compared to smaller and larger ones with increasing temperatures [10]. While this is generally in line with the theory of unimodal size scaling of planktonic growth [13], it contrasts predictions from the allometric theory of cell size decreasing with temperature [71]. The observation of this pattern on geological and biogeographical scales but not in controlled experiments could be due to other correlates, such as nutrients and grazing [17]. Nevertheless, results may differ when additional factors related to Atlantification are considered, such as decreased salinity [72]. Interestingly, the increase in intermediate phytoplankton with temperature did not confirm the predicted and modeled

growth of small temperate phytoplankton, such as *Emiliania huxleyi* [73] or *Phaeocystis globosa* [74], as these were not present in the field community that we sampled. However, some studies indicate that future Arctic temperatures may not be warm enough for them to be competitive [75,76].

4.2. Tipping Point for Arctic Key Eukaryotes Lies between 6 °C and 9 °C

The eukaryotic species' evenness was similarly high across treatments; thus, no single species dominated the communities (Figure S2). Eukaryotic species richness, however, was lower at 9 °C compared to the other two temperatures, which indicates that much fewer species were able to cope with the higher temperature (Figure S2). A decrease in Arctic phytoplankton richness under warming has also been projected by Benedetti et al. [77], who found temperature to be the main driver of changes in species diversity. Additionally, the phenotypic diversity was significantly higher at 2 °C (Tables S3 and S4), indicating that at 6 °C, the phenotypic characteristics already adapted to the higher temperatures and became more similar. Overall, the lower phenotypic and taxonomic richness found at 9 °C could make the communities more vulnerable to other drivers, as the standing diversity usually increases the communities' resilience to environmental change [78].

The eukaryotic community composition exhibited the same pattern as the species richness. It was similar between 2 °C and 6 °C, whereas clear qualitative differences could be observed at 9 °C (Figure 4a). This can be attributed to an almost 4-fold higher relative diatom sequence read abundance. Additionally, cosmopolitan species were relatively more abundant at 9 °C, while relatively fewer organisms were detected that could cope with both Arctic and temperate habitats (Figure 3c). Our results suggest an upper thermal limit between 6 °C and 9 °C, which is further supported by other studies observing the growth rates of many Arctic species to decline above 6 °C [79,80]. If future temperatures in the Arctic Ocean reach 9 °C, it may be too warm for Arctic picoplankton and too cold for temperate picoplankton to thrive [81]. Depending on the nutrient conditions, the Arctic may then become favorable for temperate diatoms with comparably high growth rates at lower temperatures [27].

4.3. Species-Specific Responses to Warming

Among diatoms, species such as *T. rotula* and *Pseudo-nitzschia* sp. already increased in relative abundance at 6 °C but only started to dominate the community at 9 °C (Figure 4b). Contrastingly, *T. antarctica* and *F. cylindrus* were more prevalent at the two colder temperatures, which is in accordance with their grouping as either Arctic-temperate or solely Arctic, respectively (Table S2). This is supported by a study on the thermal reaction norms of several marine phytoplankton groups [27], which found temperate diatoms drastically increase their growth rates at 10 °C in comparison to 5 °C. Consistently, a study on polar diatoms found that temperatures above 6 °C tend to be supra-optimal for them [82]. Similar results were found for an Arctic *Chaetoceros* strain [83]. However, the intra-specific variation approaching the thermal limits of diatoms appears to be high [82,84], and therefore polar species might adapt to warming in the longer term. Furthermore, one has to keep in mind that the sampling procedure might have excluded larger phytoplankton such as *Coscinodiscus* spp. or long chains and thereby might have skewed these results.

In the Arctic, the genus *Phaeocystis* is predicted to be a 'climate change winner' in regards to both warming and Atlantification [85–88]. Our results refine this prediction by showing that the degree of warming can be critically important in determining the future role of *Phaeocystis* in the Arctic (Figure 4c). While warming to 6 °C in our experiment and to 4.5 °C during a warm water anomaly in the eastern Fram Strait [85] led to a relative increase in the Arctic species *P. pouchettii*, their abundance decreased when temperatures rose further. Similarly, Wang et al. [75] found the upper thermal limit of *P. pouchettii* to be between 8 °C and 12 °C, while the temperate *P. globosa* did not grow below 12 °C. We conclude that if in situ temperatures in the Arctic were to rise above 8 °C but did not reach

12 °C, neither the Arctic nor the temperate *Phaeocystis* species may play a major role unless evolutionary adaptation takes place.

While the cosmopolitan chlorophyte *Micromonas* spp. [89] was only a minor contributor within our starting community, it can dominate the picophytoplankton fraction during summer [90]. At 9 °C, it was completely diminished (no sequence reads left) after the ten days of incubation (Figure S5). Even though it appears to be growing well at 6 °C [91] and has shown a high adaptation potential to warming [92], its population may crash rapidly if temperatures exceed this upper limit. Studies have shown the importance of this genus to overwintering standing stocks and deep-sea export [93–95], indicating consequences for the ecosystem if temperatures rise to 9 °C.

Notably, the observed thermal pattern also held true for other organisms, such as the Dictyochophyta, an unidentified Picozoa, as well as several groups of the MAST clade (see also [96]), which all diminished between 6 °C and 9 °C. While the role and importance of these groups are not yet clear, their thermal limits are congruent with the other species that show an Arctic distribution. On the other hand, some organisms (e.g., Syndiniales and Crysophytes) were absent at 2 °C but relatively increased at 6 °C and even more so at 9 °C, which may be indicative of a lower limit of these potentially temperate organisms. These results point towards some kind of universality of the thermal limits between 6 °C and 9 °C found in our study.

4.4. Bacterial Diversity and Composition Response to Warming

A notable outcome of our study is linking the bacterial responses to temperature, which is equally important in controlling Arctic bacteria as organic matter [97,98]. However, we must acknowledge that our study is biased towards particle-associated bacteria (>0.8 µm), excluding many smaller free-living bacteria in our analysis [99]. Despite the bias, the dataset allows us to explore details that are usually unavailable for the class *Gammaproteobacteria* compared to *Alphaproteobacteria* and *Bacteroidia* [100].

The microbial community was dominated by *Gammaproteobacteria*, particularly *Colweilla*, with minor contributions by *Bacteroidia*, particularly *Polaribacter* (Figure 5). Throughout the incubation, we observed an increase in the relative abundance of *Colweilla*, peaking at 6 °C, and a decrease in the relative abundance of *Polaribacter* (Figure 5). *Colweilla* likely thrives on the sea ice and terrestrial organic matter during the 2 °C and 6 °C incubation [101,102]. Similarly, *Polaribacter* also thrives on terrestrial organic matter [101] in addition to degrading polymeric organic compounds from phytoplankton [97]. Although *Colweilla* and *Polaribacter* are probably responsible for most of the polysaccharide-derived carbon utilization during the incubation, *Polaribacter* may prove to be more resilient to ongoing changes in the Arctic Ocean, given their ability to respond to phytoplankton-derived or terrestrial organic matter [101].

The phytoplankton community shifted towards temperate diatoms at 9 °C, which affected the microbial richness and evenness. The enhanced presence of diatoms prompted the re-appearance of *Aurantivirga* (Figure 5b), which has been linked to Arctic phytoplankton blooms as early responders to fresh organic matter input [103,104]. Furthermore, *Marinobacter* increased in relative abundance, which is reported to be associated with eukaryotes [105] and enriched in particles [106].

5. Conclusions

This study experimentally investigated the potential effect of different degrees of warming on the composition and characteristics of a microbial community in an increasingly Atlantified Arctic Ocean. We uncovered a clear thermal limit for many Arctic phytoplankton species between 6 °C and 9 °C and a concurrent gradual increase in temperate species. Additionally, the bacterial community also changed in response to warming and will, therefore, likely be altered by Atlantification. Our results highlight the importance of the thermal niche for explaining community reorganization under warming as temperate species increasingly invade the Arctic ecosystem. Predictions made by the metabolic theory

of ecology that propose heterotrophy to become more prevalent when temperatures rise could not be supported by our experimental set-up and outcome. An intermediate cell size, however, appears to be of advantage, which supports the theory of unimodal scaling of body size. The communities became less diverse in taxonomic richness as well as phenotypic characteristics, leaving them likely more vulnerable to other abiotic changes. Therefore, future studies need to integrate more and different drivers that correlate with the ongoing changes in temperatures. These should include varying nutrient, salinity, and light conditions to account for a more realistic scenario of a future Arctic ecosystem in which temperate organisms may be restricted by other abiotic factors. Overall, our experimental results imply that the future composition of Arctic microbial communities strongly depends on the intensity of warming in the Arctic Ocean.

Supplementary Materials: The supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/genes14030623/s1, Table S1: Sequencing statistics from the DADA2 pipeline for all samples after each filtering step and the ratio of final reads to raw reads. The reads containing meaningful taxa were used for downstream analyses; Table S2: Classification of the ASV-based taxonomic groups into three different size classes, four different trophic modes and three different thermal niches. Groups which could not clearly be classified are noted as "uncategorized"; Table S3: Details of biomass and diversity parameters at tfin for each temperature; Table S4: p-values of the pairwise t-tests after bonferroni correction for each temperature pair and biomass or diversity parameter; Table S5: Carbonate chemistry and dissolved nutrients of all three treatments at the end of experimental incubation ($n = 3$); Figure S1: The temperature and salinity profile at the sampling site HG-IV. The 15 m sampling depth is marked by the horizontal line. The three dominant water masses in the region (modified after [107]. are indicated by the shaded areas; Figure S2: Replicate-merged bar graphs of the ASV-based class composition after three days for the two unpooled treatments (Unpooled1 = seawater, Unpooled2: 1:5 diluted seawater) and the resulting pools of each temperature that continued the experimental incubation; Figure S3: Bar plot of the mean relative read abundances for each temperature and (a) size groups, (b) trophic modes and (c) thermal niches; Figure S4: RDA plot of the ASVs of each temperature constrained by the biomass and diversity parameters without D2; Figure S5: Relative contribution and species composition of the class of Mammiellophyceae at all treatment temperatures after ten days.

Author Contributions: Conceptualization, A.A. and U.J.; Data curation, A.A., R.A.M., M.H. and S.N.; Formal analysis, A.A., A.V.J., R.A.M. and M.H.; Funding acquisition, U.J.; Investigation, A.A., R.A.M. and K.K.E.W.; Methodology, A.A., K.K.E.W., M.H., S.N. and U.J.; Resources, U.J.; Software, S.N.; Supervision, K.K.E.W. and U.J.; Validation, A.A., K.K.E.W. and M.H.; Visualization, A.A., A.V.J., R.A.M. and M.H.; Writing—original draft, A.A., A.V.J. and R.A.M.; Writing—review & editing, A.A., A.V.J., R.A.M., K.K.E.W., M.H., S.N. and U.J. All authors have read and agreed to the published version of the manuscript.

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References

1. Tesi, T.; Muschitiello, F.; Mollenhauer, G.; Miserocchi, S.; Langone, L.; Ceccarelli, C.; Panieri, G.; Chiggiato, J.; Nogarotto, A.; Hefter, J.; et al. Rapid Atlantification along the Fram Strait at the beginning of the 20th century. *Sci. Adv.* **2021**, *7*, eabj2946. [CrossRef]
2. Polyakov, I.V.; Pnyushkov, A.V.; Alkire, M.B.; Ashik, I.M.; Baumann, T.M.; Carmack, E.C.; Goszczko, I.; Guthrie, J.; Ivanov, V.V.; Kanzow, T.; et al. Greater role for Atlantic inflows on sea-ice loss in the Eurasian Basin of the Arctic Ocean. *Science* **2017**, *356*, 285–291. [CrossRef]
3. Beszczynska-Möller, A.; Fahrbach, E.; Schauer, U.; Hansen, E. Variability in Atlantic water temperature and transport at the entrance to the Arctic Ocean, 1997–2010. *ICES J. Mar. Sci.* **2012**, *69*, 852–863. [CrossRef]
4. Marnela, M.; Rudels, B.; Houssais, M.N.; Beszczynska-Möller, A.; Eriksson, P.B. Recirculation in the Fram Strait and transports of water in and north of the Fram Strait derived from CTD data. *Ocean Sci.* **2013**, *9*, 499–519. [CrossRef]
5. de Steur, L.; Hansen, E.; Gerdes, R.; Karcher, M.; Fahrbach, E.; Holfort, J. Freshwater fluxes in the East Greenland Current: A decade of observations. *Geophys. Res. Lett.* **2009**, *36*, L23611. [CrossRef]
6. Rantanen, M.; Karpechko, A.Y.; Lipponen, A.; Nordling, K.; Hyvärinen, O.; Ruosteenoja, K.; Vihma, T.; Laaksonen, A. The Arctic has warmed nearly four times faster than the globe since 1979. *Commun. Earth Environ.* **2022**, *3*, 168. [CrossRef]
7. Previdi, M.; Smith, K.L.; Polvani, L.M. Arctic amplification of climate change: A review of underlying mechanisms. *Environ. Res. Lett.* **2021**, *16*, 093003. [CrossRef]
8. Oziel, L.; Baudena, A.; Ardyna, M.; Massicotte, P.; Randelhoff, A.; Sallee, J.B.; Ingvaldsen, R.B.; Devred, E.; Babin, M. Faster Atlantic currents drive poleward expansion of temperate phytoplankton in the Arctic Ocean. *Nat. Commun.* **2020**, *11*, 1705. [CrossRef]
9. Cadotte, M.W.; Arnillas, C.A.; Livingstone, S.W.; Yasui, S.-L.E. Predicting communities from functional traits. *Trends Ecol. Evol.* **2015**, *30*, 510–511. [CrossRef]
10. Fernandez-Gonzalez, C.; Maranon, E. Effect of temperature on the unimodal size scaling of phytoplankton growth. *Sci. Rep.* **2021**, *11*, 953. [CrossRef]
11. Boscolo-Galazzo, F.; Crichton, K.A.; Barker, S.; Pearson, P.N. Temperature dependency of metabolic rates in the upper ocean: A positive feedback to global climate change? *Glob. Planet. Chang.* **2018**, *170*, 201–212. [CrossRef]
12. Anderson, S.I.; Rynearson, T.A. Variability approaching the thermal limits can drive diatom community dynamics. *Limnol. Oceanogr.* **2020**, *65*, 1961–1973. [CrossRef]
13. Atkinson, D.; Ciotti, B.J.; Montagnes, D.J.S. Protists decrease in size linearly with temperature: Ca. 2.5% $^{\circ}C^{-1}$. *Proc. R. Soc. Lond. Ser. B Biol. Sci.* **2003**, *270*, 2605–2611. [CrossRef]
14. Morán, X.A.G.; López-Urrutia, Á.; Calvo-Díaz, A.; Li, W.K.W. Increasing importance of small phytoplankton in a warmer ocean. *Glob. Chang. Biol.* **2010**, *16*, 1137–1144. [CrossRef]
15. Daufresne, M.; Lengfellner, K.; Sommer, U. Global warming benefits the small in aquatic ecosystems. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 12788–12793. [CrossRef]
16. Marañón, E. Cell Size as a Key Determinant of Phytoplankton Metabolism and Community Structure. *Annu. Rev. Mar. Sci.* **2015**, *7*, 241–264. [CrossRef]
17. Hillebrand, H.; Acevedo-Trejos, E.; Moorthi, S.D.; Ryabov, A.; Striebel, M.; Thomas, P.K.; Schneider, M.L. Cell size as driver and sentinel of phytoplankton community structure and functioning. *Funct. Ecol.* **2021**, *36*, 276–293. [CrossRef]
18. Brown, J.H.; Gillooly, J.F.; Allen, A.P.; Savage, V.M.; West, G.B. Toward a metabolic theory of ecology. *Ecology* **2004**, *85*, 1771–1789. [CrossRef]
19. Chen, B.; Landry, M.R.; Huang, B.; Liu, H. Does warming enhance the effect of microzooplankton grazing on marine phytoplankton in the ocean? *Limnol. Oceanogr.* **2012**, *57*, 519–526. [CrossRef]
20. Hancke, K.; Glud, R.N. Temperature effects on respiration and photosynthesis in three diatom-dominated benthic communities. *Aquat. Microb. Ecol.* **2004**, *37*, 265–281. [CrossRef]
21. Regaudie-de-Gioux, A.; Duarte, C.M. Temperature dependence of planktonic metabolism in the ocean. *Glob. Biogeochem. Cycles* **2012**, *26*, GB1015. [CrossRef]
22. Yvon-Durocher, G.; Caffrey, J.M.; Cescatti, A.; Dossena, M.; Giorgio, P.D.; Gasol, J.M.; Montoya, J.M.; Pumpanen, J.; Staehr, P.A.; Trimmer, M.; et al. Reconciling the temperature dependence of respiration across timescales and ecosystem types. *Nature* **2012**, *487*, 472–476. [CrossRef]
23. Chen, B. Assessing the accuracy of the "two-point" dilution technique. *Limnol. Oceanogr. Methods* **2015**, *13*, 521–526. [CrossRef]
24. Liu, K.; Chen, B.; Zhang, S.; Sato, M.; Shi, Z.; Liu, H. Marine phytoplankton in subtropical coastal waters showing lower thermal sensitivity than microzooplankton. *Limnol. Oceanogr.* **2018**, *64*, 1103–1119. [CrossRef]

25. Anderson, S.I.; Barton, A.D.; Clayton, S.; Dutkiewicz, S.; Rynearson, T.A. Marine phytoplankton functional types exhibit diverse responses to thermal change. *Nat. Commun.* **2021**, *12*, 6413. [CrossRef]
26. Chen, B. Thermal diversity affects community responses to warming. *Ecol. Model.* **2022**, *464*, 109846. [CrossRef]
27. Boyd, P.W.; Rynearson, T.A.; Armstrong, E.A.; Fu, F.; Hayashi, K.; Hu, Z.; Hutchins, D.A.; Kudela, R.M.; Litchman, E.; Mulholland, M.R.; et al. Marine phytoplankton temperature versus growth responses from polar to tropical waters–outcome of a scientific community-wide study. *PLoS ONE* **2013**, *8*, e63091. [CrossRef]
28. Huertas, I.E.; Rouco, M.; López-Rodas, V.; Costas, E. Warming will affect phytoplankton differently: Evidence through a mechanistic approach. *Proc. R. Soc. B Biol. Sci.* **2011**, *278*, 3534–3543. [CrossRef]
29. Thomas, M.K.; Kremer, C.T.; Klausmeier, C.A.; Litchman, E. A Global Pattern of Thermal Adaptation in Marine Phytoplankton. *Science* **2012**, *338*, 1085–1088. [CrossRef]
30. Greco, M.; Werner, K.; Zamelczyk, K.; Rasmussen, T.L.; Kucera, M. Decadal trend of plankton community change and habitat shoaling in the Arctic gateway recorded by planktonic foraminifera. *Glob. Chang. Biol.* **2022**, *28*, 1798–1808. [CrossRef]
31. Soltwedel, T. *The Expedition PS126 of the Research Vessel POLARSTERN to the Fram Strait in 2021*; 1866–3192; Alfred-Wegener-Institut, Helmholtz-Zentrum für Polar-und Meeresforschung: Bremerhaven, Germany, 2021; pp. 1–131.
32. Hoppmann, M.; Tippenhauer, S.; Soltwedel, T. Continuous thermosalinograph oceanography along RV POLARSTERN cruise track PS126. Alfred Wegener Institute, Helmholtz Centre for Polar and Marine Research, Bremerhaven. *Pangaea* **2022**. [CrossRef]
33. Löder, M.G.J.; Aberle, N.; Klaas, C.; Kraberg, A.C.; Wiltshire, K.H. Conserving original in situ diversity in microzooplankton grazing set-ups. *Mar. Biodivers. Rec.* **2010**, *3*, E28. [CrossRef]
34. Soltwedel, T.; Bauerfeind, E.; Bergmann, M.; Bracher, A.; Budaeva, N.; Busch, K.; Cherkasheva, A.; Fahl, K.; Grzelak, K.; Hasemann, C.; et al. Natural variability or anthropogenically-induced variation? Insights from 15 years of multidisciplinary observations at the arctic marine LTER site HAUSGARTEN. *Ecol. Indic.* **2016**, *65*, 89–102. [CrossRef]
35. Nöthig, E.-M.; Ramondenc, S.; Haas, A.; Hehemann, L.; Walter, A.; Bracher, A.; Lalande, C.; Metfies, K.; Peeken, I.; Bauerfeind, E.; et al. Summertime Chlorophyll a and Particulate Organic Carbon Standing Stocks in Surface Waters of the Fram Strait and the Arctic Ocean (1991–2015). *Front. Mar. Sci.* **2020**, *7*, 350. [CrossRef]
36. Kaiser, P.; Hagen, W.; von Appen, W.-J.; Niehoff, B.; Hildebrandt, N.; Auel, H. Effects of a Submesoscale Oceanographic Filament on Zooplankton Dynamics in the Arctic Marginal Ice Zone. *Front. Mar. Sci.* **2021**, *8*, 625395. [CrossRef]
37. Calbet, A.; Saiz, E. How much is enough for nutrients in microzooplankton dilution grazing experiments? *J. Plankton Res.* **2018**, *40*, 109–117. [CrossRef]
38. Pierrot, D.; Wallace, D.W.R.; Lewis, E.R.; Pierrot, D.; Wallace, R.; Wallace, D.W.R.; Wallace, W.E. *MS Excel Program Developed for CO_2 System Calculations*; US Department of Energy: Oak Ridge, TN, USA, 2011.
39. Mehrbach, C.; Culberson, C.H.; Hawley, J.E.; Pytkowicx, R.M. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol. Oceanogr.* **1973**, *18*, 897–907. [CrossRef]
40. Dickson, A.G.; Millero, F.J. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep Sea Res. Part A. Oceanogr. Res. Pap.* **1987**, *34*, 1733–1743. [CrossRef]
41. Armstrong, F.A.J.; Stearns, C.R.; Strickland, J.D.H. The measurement of upwelling and subsequent biological process by means of the Technicon Autoanalyzer®and associated equipment. *Deep Sea Res. Oceanogr. Abstr.* **1967**, *14*, 381–389. [CrossRef]
42. Eberlein, K.; Kattner, G. Automatic method for the determination of ortho-phosphate and total dissolved phosphorus in the marine environment. *Fresenius' Z. Für Anal. Chem.* **1987**, *326*, 354–357. [CrossRef]
43. Grasshoff, K.; Kremling, K.; Ehrhardt, M. *Methods of Seawater Analysis*; John Wiley & Sons: Hoboken, NJ, USA, 2009.
44. Koroleff, F. Direct determination of ammonia in natural waters as indophenol blue. *Inf. Tech. Methods Seawater Anal.* **1970**, 19–22.
45. Arar, E.J.; Collins, G.B. *Method 445.0 In Vitro Determination of Chlorophyll a and Pheophytin ain Marine and Freshwater Algae by Fluorescence*; U.S. Environmental Protection Agency: Washington, DC, USA, 1997.
46. Bradley, I.M.; Pinto, A.J.; Guest, J.S. Design and evaluation of illumina MiSeq-compatible, 18S rRNA gene-specific primers for improved characterization of mixed phototrophic communities. *Appl. Environ. Microbiol.* **2016**, *82*, 5878–5891. [CrossRef] [PubMed]
47. Fadeev, E.; Salter, I.; Schourup-Kristensen, V.; Nöthig, E.-M.; Metfies, K.; Engel, A.; Piontek, J.; Boetius, A.; Bienhold, C. Microbial Communities in the East and West Fram Strait During Sea Ice Melting Season. *Front. Mar. Sci.* **2018**, *5*, 429. [CrossRef]
48. Martin, M. Cutadapt removes adapter sequences from high-throughput sequencing reads. *EMBnet J.* **2011**, *17*, 10–12. [CrossRef]
49. Callahan, B.J.; McMurdie, P.J.; Rosen, M.J.; Han, A.W.; Johnson, A.J.; Holmes, S.P. DADA2: High-resolution sample inference from Illumina amplicon data. *Nat. Methods* **2016**, *13*, 581–583. [CrossRef] [PubMed]
50. Quast, C.; Pruesse, E.; Yilmaz, P.; Gerken, J.; Schweer, T.; Yarza, P.; Peplies, J.; Glöckner, F.O. The SILVA ribosomal RNA gene database project: Improved data processing and web-based tools. *Nucleic Acids Res.* **2013**, *41*, D590–D596. [CrossRef]
51. Guillou, L.; Bachar, D.; Audic, S.; Bass, D.; Berney, C.; Bittner, L.; Boutte, C.; Burgaud, G.; de Vargas, C.; Decelle, J.; et al. The Protist Ribosomal Reference database (PR2): A catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. *Nucleic Acids Res.* **2013**, *41*, D597–D604. [CrossRef]
52. Beule, L.; Karlovsky, P. Improved normalization of species count data in ecology by scaling with ranked subsampling (SRS): Application to microbial communities. *PeerJ* **2020**, *8*, e9593. [CrossRef]
53. Sisk-Hackworth, L.; Kelley, S.T. An application of compositional data analysis to multiomic time-series data. *NAR Genom. Bioinform.* **2020**, *2*, lqaa079. [CrossRef]

54. Martín-Fernández, J.-A.; Hron, K.; Templ, M.; Filzmoser, P.; Palarea-Albaladejo, J. Bayesian-multiplicative treatment of count zeros in compositional data sets. *Stat. Model.* **2015**, *15*, 134–158. [CrossRef]
55. R Core Team. *R: A Language and Environment for Statistical Computing*; R Foundation for Statistical Computing: Vienna, Austria, 2022. Available online: https://www.R-project.org/ (accessed on 19 January 2023).
56. RStudioTeam. *RStudio: Integrated Development Environment for R. RStudio*; PBC: Boston, MA, USA, 2022. Available online: http://www.rstudio.com/ (accessed on 19 January 2023).
57. Ahyong, S.; Boyko, C.B.; Bailly, N.; Bernot, J.; Bieler, R.; Brandão, S.N.; Daly, M.; De Grave, S.; Gofas, S.; Hernandez, F.; et al. World Register of Marine Species (WoRMS). 2022. Available online: https://www.marinespecies.org/ (accessed on 25 February 2023).
58. Hörstmann, C.; Buttigieg, P.L.; John, U.; Raes, E.J.; Wolf-Gladrow, D.; Bracher, A.; Waite, A.M. Microbial diversity through an oceanographic lens: Refining the concept of ocean provinces through trophic-level analysis and productivity-specific length scales. *Environ. Microbiol.* **2022**, *24*, 404–419. [CrossRef] [PubMed]
59. Schneider, L.K.; Anestis, K.; Mansour, J.; Anschutz, A.A.; Gypens, N.; Hansen, P.J.; John, U.; Klemm, K.; Martin, J.L.; Medic, N.; et al. A dataset on trophic modes of aquatic protists. *Biodivers. Data J.* **2020**, *8*, e56648. [CrossRef] [PubMed]
60. Šupraha, L.; Klemm, K.; Gran-Stadniczeňko, S.; Hörstmann, C.; Vaulot, D.; Edvardsen, B.; John, U. Diversity and biogeography of planktonic diatoms in Svalbard fjords: The role of dispersal and Arctic endemism in phytoplankton community structuring. *Elem. Sci. Anthr.* **2022**, *10*, 00117. [CrossRef]
61. Props, R.; Monsieurs, P.; Mysara, M.; Clement, L.; Boon, N. Measuring the biodiversity of microbial communities by flow cytometry. *Methods Ecol. Evol.* **2016**, *7*, 1376–1385. [CrossRef]
62. Fontana, S.; Thomas, M.K.; Moldoveanu, M.; Spaak, P.; Pomati, F. Individual-level trait diversity predicts phytoplankton community properties better than species richness or evenness. *ISME J.* **2018**, *12*, 356–366. [CrossRef] [PubMed]
63. Zhong, D.; Listmann, L.; Santelia, M.E.; Schaum, C.E. Functional redundancy in natural pico-phytoplankton communities depends on temperature and biogeography. *Biol. Lett.* **2020**, *16*, 20200330. [CrossRef]
64. Greenacre, M. Compositional Data Analysis. *Annu. Rev. Stat. Its Appl.* **2021**, *8*, 271–299. [CrossRef]
65. von Appen, W.-J.; Wekerle, C.; Hehemann, L.; Schourup-Kristensen, V.; Konrad, C.; Iversen, M.H. Observations of a Submesoscale Cyclonic Filament in the Marginal Ice Zone. *Geophys. Res. Lett.* **2018**, *45*, 6141–6149. [CrossRef]
66. Tanioka, T.; Matsumoto, K. A meta-analysis on environmental drivers of marine phytoplankton C: N: P. *Biogeosciences* **2020**, *17*, 2939–2954. [CrossRef]
67. Thompson, P.A.; Guo, M.-x.; Harrison, P.J. Effects of variation in temperature. I. On the biochemical composition of eight species of marine phytoplankton. *J. Phycol.* **1992**, *28*, 481–488. [CrossRef]
68. Petchey, O.L.; McPhearson, P.T.; Casey, T.M.; Morin, P.J. Environmental warming alters food-web structure and ecosystem function. *Nature* **1999**, *402*, 69–72. [CrossRef]
69. Schaum, C.E.; Team, S.R.; ffrench-Constant, R.; Lowe, C.; Ólafsson, J.S.; Padfield, D.; Yvon-Durocher, G. Temperature-driven selection on metabolic traits increases the strength of an algal–grazer interaction in naturally warmed streams. *Glob. Chang. Biol.* **2018**, *24*, 1793–1803. [CrossRef] [PubMed]
70. Gibert, J.P. Temperature directly and indirectly influences food web structure. *Sci. Rep.* **2019**, *9*, 5312. [CrossRef]
71. Marañón, E.; Cermeno, P.; Lopez-Sandoval, D.C.; Rodriguez-Ramos, T.; Sobrino, C.; Huete-Ortega, M.; Blanco, J.M.; Rodriguez, J. Unimodal size scaling of phytoplankton growth and the size dependence of nutrient uptake and use. *Ecol. Lett.* **2013**, *16*, 371–379. [CrossRef] [PubMed]
72. Li, W.K.; McLaughlin, F.A.; Lovejoy, C.; Carmack, E.C. Smallest algae thrive as the Arctic Ocean freshens. *Science* **2009**, *326*, 539. [CrossRef]
73. Neukermans, G.; Oziel, L.; Babin, M. Increased intrusion of warming Atlantic water leads to rapid expansion of temperate phytoplankton in the Arctic. *Glob. Chang. Biol.* **2018**, *24*, 2545–2553. [CrossRef]
74. Vernet, M.; Richardson, T.L.; Metfies, K.; Eva-Maria, N.; Peeken, I. Models of plankton community changes during a warm water anomaly in Arctic waters show altered trophic pathways with minimal changes in carbon export. *Front. Mar. Sci.* **2017**, *4*, 160. [CrossRef]
75. Wang, X.; Tang, K.W.; Wang, Y.; Smith, W.O. Temperature effects on growth, colony development and carbon partitioning in three Phaeocystis species. *Aquat. Biol.* **2010**, *9*, 239–249. [CrossRef]
76. Zhang, Y.; Klapper, R.; Lohbeck, K.T.; Bach, L.T.; Schulz, K.G.; Reusch, T.B.H.; Riebesell, U. Between- and within-population variations in thermal reaction norms of the coccolithophore Emiliania huxleyi. *Limnol. Oceanogr.* **2014**, *59*, 1570–1580. [CrossRef]
77. Benedetti, F.; Vogt, M.; Elizondo, U.H.; Righetti, D.; Zimmermann, N.E.; Gruber, N. Major restructuring of marine plankton assemblages under global warming. *Nat. Commun.* **2021**, *12*, 5226. [CrossRef]
78. Bestion, E.; Barton, S.; Garcia, F.C.; Warfield, R.; Yvon-Durocher, G. Abrupt declines in marine phytoplankton production driven by warming and biodiversity loss in a microcosm experiment. *Ecol. Lett.* **2020**, *23*, 457–466. [CrossRef]
79. Coello-Camba, A.; Agustí, S.; Vaqué, D.; Holding, J.; Arrieta, J.M.; Wassmann, P.; Duarte, C.M. Experimental Assessment of Temperature Thresholds for Arctic Phytoplankton Communities. *Estuaries Coasts* **2015**, *38*, 873–885. [CrossRef]
80. Coello-Camba, A.; Agustí, S. Thermal Thresholds of Phytoplankton Growth in Polar Waters and Their Consequences for a Warming Polar Ocean. *Front. Mar. Sci.* **2017**, *4*, 168. [CrossRef]
81. Stawiarski, B.; Buitenhuis, E.T.; Le Quéré, C. The Physiological Response of Picophytoplankton to Temperature and Its Model Representation. *Front. Mar. Sci.* **2016**, *3*, 164. [CrossRef]

82. Bishop, I.W.; Anderson, S.I.; Collins, S.; Rynearson, T.A. Thermal trait variation may buffer Southern Ocean phytoplankton from anthropogenic warming. *Glob. Chang. Biol.* **2022**, *28*, 5755–5767. [CrossRef] [PubMed]
83. Schiffrine, N.; Tremblay, J.É.; Babin, M. Interactive effects of temperature and nitrogen source on the elemental stoichiometry of a polar diatom. *Limnol. Oceanogr.* **2022**, *67*, 2750–2762. [CrossRef]
84. Godhe, A.; Rynearson, T. The role of intraspecific variation in the ecological and evolutionary success of diatoms in changing environments. *Philos. Trans. R. Soc. B Biol. Sci.* **2017**, *372*, 20160399. [CrossRef]
85. Nöthig, E.-M.; Bracher, A.; Engel, A.; Metfies, K.; Niehoff, B.; Peeken, I.; Bauerfeind, E.; Cherkasheva, A.; Gäbler-Schwarz, S.; Hardge, K.; et al. Summertime plankton ecology in Fram Strait—A compilation of long- and short-term observations. *Polar Res.* **2015**, *34*, 23349. [CrossRef]
86. Szeligowska, M.; Trudnowska, E.; Boehnke, R.; Dąbrowska, A.M.; Wiktor, J.M.; Sagan, S.; Błachowiak-Samołyk, K. Spatial Patterns of Particles and Plankton in the Warming Arctic Fjord (Isfjorden, West Spitsbergen) in Seven Consecutive Mid-Summers (2013–2019). *Front. Mar. Sci.* **2020**, *7*, 584. [CrossRef]
87. Metfies, K.; von Appen, W.-J.; Kilias, E.; Nicolaus, A.; Nöthig, E.-M. Biogeography and Photosynthetic Biomass of Arctic Marine Pico-Eukaroytes during Summer of the Record Sea Ice Minimum 2012. *PLoS ONE* **2016**, *11*, e0148512. [CrossRef]
88. Lampe, V.; Nöthig, E.-M.; Schartau, M. Spatio-Temporal Variations in Community Size Structure of Arctic Protist Plankton in the Fram Strait. *Front. Mar. Sci.* **2021**, *7*, 579880. [CrossRef]
89. Worden, A.Z.; Lee, J.H.; Mock, T.; Rouzé, P.; Simmons, M.P.; Aerts, A.L.; Allen, A.E.; Cuvelier, M.L.; Derelle, E.; Everett, M.V.; et al. Green evolution and dynamic adaptations revealed by genomes of the marine picoeukaryotes Micromonas. *Science* **2009**, *324*, 268–272. [CrossRef] [PubMed]
90. Balzano, S.; Marie, D.; Gourvil, P.; Vaulot, D. Composition of the summer photosynthetic pico and nanoplankton communities in the Beaufort Sea assessed by T-RFLP and sequences of the 18S rRNA gene from flow cytometry sorted samples. *ISME J.* **2012**, *6*, 1480–1498. [CrossRef] [PubMed]
91. Hoppe, C.J.M.; Flintrop, C.M.; Rost, B. The Arctic picoeukaryote Micromonas pusilla benefits synergistically from warming and ocean acidification. *Biogeosciences* **2018**, *15*, 4353–4365. [CrossRef]
92. Benner, I.; Irwin, A.J.; Finkel, Z.V. Capacity of the common Arctic picoeukaryote Micromonas to adapt to a warming ocean. *Limnol. Oceanogr. Lett.* **2020**, *5*, 221–227. [CrossRef]
93. Lovejoy, C.; Vincent, W.F.; Bonilla, S.; Roy, S.; Martineau, M.-J.; Terrado, R.; Potvin, M.; Massana, R.; Pedrós-Alió, C. Distribution, phylogeny, and growth of cold-adapted picoprasinophytes in Arctic Seas. *J. Phycol.* **2007**, *43*, 78–89. [CrossRef]
94. Berge, J.; Renaud, P.E.; Darnis, G.; Cottier, F.; Last, K.; Gabrielsen, T.M.; Johnsen, G.; Seuthe, L.; Weslawski, J.M.; Leu, E.; et al. In the dark: A review of ecosystem processes during the Arctic polar night. *Prog. Oceanogr.* **2015**, *139*, 258–271. [CrossRef]
95. Bachy, C.; Sudek, L.; Choi, C.J.; Eckmann, C.A.; Nöthig, E.-M.; Metfies, K.; Worden, A.Z. Phytoplankton Surveys in the Arctic Fram Strait Demonstrate the Tiny Eukaryotic Alga Micromonas and Other Picoprasinophytes Contribute to Deep Sea Export. *Microorganisms* **2022**, *10*, 961. [CrossRef]
96. Aalto, N.J.; Schweitzer, H.D.; Krsmanovic, S.; Campbell, K.; Bernstein, H.C. Diversity and Selection of Surface Marine Microbiomes in the Atlantic-Influenced Arctic. *Front. Microbiol.* **2022**, *13*, 892634. [CrossRef]
97. Piontek, J.; Sperling, M.; Nöthig, E.; Engel, A. Regulation of bacterioplankton activity in Fram Strait (Arctic Ocean) during early summer: The role of organic matter supply and temperature. *J. Mar. Syst.* **2014**, *132*, 83–94. [CrossRef]
98. Kirchman, D.L. The ecology of Cytophaga–Flavobacteria in aquatic environments. *FEMS Microbioliol. Ecol.* **2002**, *39*, 91–100. [CrossRef]
99. Salazar, G.; Cornejo-Castillo, F.; Benítez-Barrios, V.; Fraile-Nuez, E.; Álvarez-Salgado, X.A.; Duarte, C.M.; Gasol, J.M.; Acinas, S.G. Global diversity and biogeography of deep-sea pelagic prokaryotes. *ISME J.* **2016**, *10*, 596–608. [CrossRef]
100. Buchan, A.; LeCleir, G.R.; Gulvik, C.A.; González, J.M. Master recyclers: Features and functions of bacteria associated with phytoplankton blooms. *Nat. Rev. Microbiol.* **2014**, *12*, 686–698. [CrossRef]
101. Sipler, R.E.; Kellogg, C.T.E.; Connelly, T.L.; Roberts, Q.N.; Yager, P.L.; Bronk, D.A. Microbial Community Response to Terrestrially Derived Dissolved Organic Matter in the Coastal Arctic. *Front. Microbiol.* **2022**, *13*, 1018. [CrossRef] [PubMed]
102. Underwood, G.J.C.; Michel, C.; Meisterhans, G.; Niemi, A.; Belzile, C.; Witt, M.; Dumbrell, A.J.; Koch, B.P. Organic matter from Arctic sea-ice loss alters bacterial community structure and function. *Nat. Clim. Chang.* **2019**, *9*, 170–176. [CrossRef]
103. von Jackowski, A.; Becker, K.W.; Wietz, M.; Bienhold, C.; Zancker, B.; Nothig, E.M.; Engel, A. Variations of microbial communities and substrate regimes in the eastern Fram Strait between summer and fall. *Environ. Microbioly* **2022**, *24*, 4124–4136. [CrossRef] [PubMed]
104. Liu, Y.; Blain, S.; Crispi, O.; Rembauville, M.; Obernosterer, I. Seasonal dynamics of prokaryotes and their associations with diatoms in the Southern Ocean as revealed by an autonomous sampler. *Environ. Microbiol.* **2020**, *22*, 3968–3984. [CrossRef] [PubMed]
105. Gärdes, A.; Kaeppel, E.; Shehzad, A.; Seebah, S.; Teeling, H.; Yarza, P.; Glöckner, F.O.; Grossart, H.P.; Ullrich, M.S. Complete genome sequence of Marinobacter adhaerens type strain (HP15), a diatom-interacting marine microorganism. *Stand. Genom. Sci.* **2010**, *3*, 97–107. [CrossRef]
106. Fontanez, K.M.; Eppley, J.M.; Samo, T.J.; Karl, D.M.; DeLong, E.F. Microbial community structure and function on sinking particles in the North Pacific Subtropical Gyre. *Front. Microbiol.* **2015**, *6*, 469. [CrossRef]

107. Pérez-Hernández, M.D.; Pickart, R.S.; Pavlov, V.; Våge, K.; Ingvaldsen, R.; Sundfjord, A.; Renner, A.H.H.; Torres, D.J.; and Erofeeva, S.Y. The Atlantic Water boundary current north of Svalbard in late summer. *J. Geophys. Res. Oceans* **2017**, *122*, 2269–2290. [CrossRef]
108. Hoppmann, M.M. CTD Raw Data Files from POLARSTERN Cruise PS126. 2022. Available online: https://adc.met.no/metsis/metadata/PANGAEA_940754 (accessed on 19 January 2023).

Supplementary material of publication I

Table S 1: Sequencing statistics from the DADA2 pipeline for all samples after each filtering step and the ratio of final reads to raw reads. The reads containing meaningful taxa were used for downstream analyses.

Sample	Library	Raw	Pre-filtered	Primer-filtered	Quality-filtered	Denoised	Merged	Chimera-filtered	Dominant ASVs	Meaningful taxa	Ratio final/raw
t-0 A	18S rRNA	105972	102129	101733	77265	77221	74725	74167	73760	73732	0.70
t-0 B	18S rRNA	106366	102537	102119	76975	76908	74855	74373	74088	53001	0.50
t-0 C	18S rRNA	115389	111281	110791	84438	84389	81729	80820	80435	80412	0.70
t-fin 2°C A	18S rRNA	161197	155275	154551	113570	113431	109554	107588	107250	92958	0.58
t-fin 2°C B	18S rRNA	150115	144823	144142	112997	112890	109254	107668	107277	107219	0.71
t-fin 2°C C	18S rRNA	149148	143591	142947	109079	108969	105497	103219	102934	92662	0.62
t-fin 6°C A	18S rRNA	139390	134525	133877	100796	100694	97286	95952	95522	95221	0.68
t-fin 6°C B	18S rRNA	146305	141118	140473	107167	107060	103657	102096	101633	99875	0.68
t-fin 6°C C	18S rRNA	134361	129531	128985	95384	95294	92221	91059	90784	76884	0.57
t-fin 9°C A	18S rRNA	70900	68296	68027	51772	51726	49549	49095	48993	48731	0.69
t-fin 9°C B	18S rRNA	101178	97537	97104	74486	74421	71485	70477	70228	69701	0.69
t-fin 9°C C	18S rRNA	55008	52572	52330	31691	31668	30279	29721	29671	29553	0.54
t-0 A	16S rRNA	75650	74686	70544	65573	65500	65048	64791	64497	NA	0.85
t-0 B	16S rRNA	60056	58741	55408	50859	50768	50376	50213	49899	NA	0.83
t-0 C	16S rRNA	85422	82774	76936	71775	71691	71260	70942	70533	NA	0.83
t-fin 2°C A	16S rRNA	71273	68709	64370	59131	59072	58664	58536	58321	NA	0.82
t-fin 2°C B	16S rRNA	53276	50612	47129	44205	44162	43952	43865	43652	NA	0.82
t-fin 2°C C	16S rRNA	49802	48099	45192	42014	41968	41707	41616	41474	NA	0.83
t-fin 6°C A	16S rRNA	60990	60498	55802	52285	52219	51878	51352	51074	NA	0.84
t-fin 6°C B	16S rRNA	62537	62039	57526	54097	54014	53611	53157	52897	NA	0.85
t-fin 6°C C	16S rRNA	64960	64300	59607	55494	55399	54954	54311	53994	NA	0.83
t-fin 9°C A	16S rRNA	60505	57263	53879	50355	50260	49954	49926	49440	NA	0.82
t-fin 9°C B	16S rRNA	72004	68234	63706	60142	60057	59595	59518	59126	NA	0.82
t-fin 9°C C	16S rRNA	33611	33486	30968	28255	28187	27956	27948	27767	NA	0.83

Table S 2: Classification of the ASV-based taxonomic groups into three different size classes, four different trophic modes and three different thermal niches. Groups which could not clearly be classified are noted as "uncategorized".

Taxonomic identity	Size	Trophy	Thermal niche	Taxonomic identity	Size	Trophy	Thermal niche
Acanthoecida	uncategorized	heterotroph	Arctic-temperate	MAST-1B	uncategorized	heterotroph	Arctic-temperate
Attheya longicornis	nano	phototroph	Arctic-temperate	MAST-1C	uncategorized	heterotroph	Arctic
Attheya septentrionalis	nano	phototroph	Arctic	MAST-2D	uncategorized	heterotroph	Arctic
Balechina pachydermata	nano	phototroph	Arctic-temperate	MAST-3D	uncategorized	heterotroph	Arctic-temperate
Biecheleria	nano	phototroph	Arctic-temperate	MAST-3I	uncategorized	heterotroph	Arctic-temperate
CCW10-lineage	uncategorized	heterotroph	Arctic	MAST-3L	uncategorized	heterotroph	Arctic-temperate
Chaetoceros cinctus	nano	phototroph	Arctic	MAST-7A	uncategorized	heterotroph	Arctic
Chaetoceros contortus	nano	phototroph	Arctic-temperate	MAST-8A	uncategorized	heterotroph	Arctic-temperate
Chaetoceros danicus	nano	phototroph	Arctic-temperate	MAST-8D	uncategorized	heterotroph	Arctic-temperate

Taxonomic identity	Size	Trophy	Thermal niche	Taxonomic identity	Size	Trophy	Thermal niche
Chaetoceros debilis	micro	phototroph	Arctic-temperate	Mataza-lineage	uncategorized	heterotroph	Arctic
Chaetoceros diadema	micro	phototroph	Arctic-temperate	Micromonas commoda	pico	phototroph	Arctic-temperate
Chaetoceros gelidus	nano	phototroph	Arctic-temperate	Micromonas polaris	pico	phototroph	Arctic
Chaetoceros neogracilis	nano	phototroph	Arctic	MOCH-2	uncategorized	uncategorized	cosmopolitan
Chaetoceros peruvianus	micro	phototroph	Arctic-temperate	Navicula	uncategorized	phototroph	Arctic-temperate
Chrysochromulina	nano	phototroph	Arctic	Naviculaceae	uncategorized	phototroph	Arctic
Chrysophyceae Clade-C	uncategorized	phototroph	Arctic-temperate	Nitzschia	uncategorized	phototroph	Arctic
Chrysophyceae Clade-H	uncategorized	phototroph	Arctic-temperate	Novel-clade-2	uncategorized	uncategorized	Arctic
Chrysophyceae Clade-I	uncategorized	phototroph	Arctic-temperate	Parmales env 3B	uncategorized	mixotroph	Arctic-temperate
Chrysophyceae	uncategorized	phototroph	Arctic	Pedinellales	uncategorized	mixotroph	Arctic-temperate
Chytriodinium roseum	nano	parasitic	cosmopolitan	Pentapharsodinium	uncategorized	uncategorized	Arctic
Cryothecomonas aestivalis	nano	mixotroph	Arctic	Pentapharsodinium tyrrhenicum	micro	parasitic	Arctic
Cryothecomonas-lineage	uncategorized	mixotroph	Arctic	Phaeocystis pouchetii	nano	phototroph	Arctic
Delomila confervacea	nano	phototroph	Arctic	Phaeocystis	nano	phototroph	cosmopolitan
Dictyocha speculum	micro	phototroph	Arctic-temperate	Picozoa	Pico	heterotroph	Arctic-temperate
Dino-Group-I-Clade-1	nano	parasitic	Arctic-temperate	Plagioselmis prolonga	nano	phototroph	cosmopolitan
Dino-Group-I-Clade-4	nano	parasitic	Arctic-temperate	Pleurostomatida	uncategorized	heterotroph	Arctic
Dino-Group-I-Clade-5	nano	parasitic	cosmopolitan	Polarella glacialis	nano	phototroph	Arctic
Dino-Group-II-Clade-1	nano	parasitic	cosmopolitan	Porosira glacialis	micro	phototroph	Arctic
Dino-Group-II-Clade-14	nano	parasitic	cosmopolitan	Prorocentrum cordatum	nano	mixotroph	Arctic-temperate
Dino-Group-II-Clade-23	nano	parasitic	Arctic-temperate	Prorocentrum	uncategorized	uncategorized	cosmopolitan
Dino-Group-II-Clade-47	nano	parasitic	Arctic-temperate	Protaspa-lineage	uncategorized	heterotroph	Arctic
Dino-Group-II-Clade-52	nano	parasitic	cosmopolitan	Prymnesiophyceae Clade E	uncategorized	uncategorized	Arctic
Dino-Group-III	nano	parasitic	cosmopolitan	Pseudo-nitzschia delicatissima	micro	phototroph	Arctic-temperate
Dinophyceae	uncategorized	uncategorized	cosmopolitan	Pseudo-nitzschia multiseries	micro	phototroph	cosmopolitan
Dolichomastigaceae-B	uncategorized	phototroph	Arctic-temperate	Pseudo-nitzschia seriata	micro	phototroph	Arctic-temperate
Ebria tripartita	micro	heterotroph	Arctic-temperate	Pseudo-nitzschia	micro	phototroph	cosmopolitan
Eucampia	micro	phototroph	Arctic	Pterosperma cristatum	nano	phototroph	uncategorized
Filosa-Thecofilosea	micro	heterotroph	Arctic	Pyramimonadales	uncategorized	phototroph	Arctic-temperate
Florenciellales	uncategorized	phototroph	Arctic	Rhizosolenia imbricata shrubsolei	micro	phototroph	Arctic-temperate
Fragilariopsis cylindrus	micro	phototroph	Arctic	Scrippsiella acuminata	micro	mixotroph	Arctic-temperate
Fragilariopsis sublineata	micro	phototroph	Arctic	Skeletonema marinoi	nano	phototroph	Arctic-temperate
Gymnodinium dorsalisulcum	micro	uncategorized	cosmopolitan	Stephanoecidae Group D	uncategorized	heterotroph	Arctic-temperate
Gymnodinium	micro	uncategorized	cosmopolitan	Stephanoecidae Group H	uncategorized	heterotroph	Arctic-temperate
Gyrodinium dominans	micro	heterotroph	Arctic-temperate	Stramenopiles	uncategorized	heterotroph	Arctic-temperate
Gyrodinium fusiforme	nano	heterotroph	cosmopolitan	Strobilidiidae A	uncategorized	heterotroph	Arctic
Gyrodinium helveticum	micro	heterotroph	Arctic-temperate	Strombidiida B	uncategorized	heterotroph	Arctic-temperate
Gyrodinium	uncategorized	heterotroph	Arctic-temperate	Strombidiida F	uncategorized	heterotroph	Arctic-temperate
Gyrodinium spirale	micro	heterotroph	Arctic-temperate	Strombidiidae H	uncategorized	heterotroph	Arctic

Taxonomic identity	Size	Trophy	Thermal niche	Taxonomic identity	Size	Trophy	Thermal niche
Haptophyta Clade HAP5	uncategorized	phototroph	cosmopolitan	Strombidiidae M	uncategorized	heterotroph	Arctic-temperate
heterotrophcapsa nei/rotundata	nano	mixotroph	cosmopolitan	Strombidinopsis	uncategorized	heterotroph	Arctic-temperate
heterotrophcapsa pygmaea	nano	uncategorized	Arctic-temperate	Strombidium capitatum	micro	heterotroph	Arctic
Hypotrichia	uncategorized	heterotroph	Arctic	TAGIRI1-lineage	uncategorized	heterotroph	Arctic
Islandinium minutum	micro	heterotroph	Arctic	Teleaula gracilis	nano	mixotroph	cosmopolitan
Islandinium tricingulatum	micro	heterotroph	Arctic	Telonemia-Group-1	uncategorized	uncategorized	Arctic-temperate
Karlodinium veneficum	nano	mixotroph	Arctic-temperate	Thalassiosira antarctica	nano	phototroph	Arctic
Laboea strobila	micro	mixotroph	Arctic-temperate	Thalassiosira concaviuscula	uncategorized	phototroph	Arctic-temperate
Labyrinthulomycetes LAB14	uncategorized	uncategorized	Arctic	Thalassiosira hispida	uncategorized	phototroph	Arctic
Leegaardiella	micro	heterotroph	Arctic-temperate	Thalassiosira nordenskioeldii	nano	phototroph	Arctic-temperate
Mantoniella squamata	nano	mixotroph	Arctic-temperate	Thalassiosira rotula	micro	phototroph	Arctic-temperate
MAST-12A	uncategorized	heterotroph	Arctic-temperate	Ventricleftida	uncategorized	heterotroph	Arctic
MAST-1A	uncategorized	heterotroph	Arctic-temperate	Not identified	uncategorized	uncategorized	uncategorized

Table S 3: Details of biomass and diversity parameters at tfin for each temperature.

Temp. [°C]	Chl*a* [µg/L]	POC [µg/L]	PON [µg/L]	Chl*a*:POC [g:g]	C:N [mol:mol]	D2	RichnessE	EvennessE	RichnessP	EvennessP
2	1.9 ± 0.6	74 ± 7.8	15.3 ± 2	0.03 ± 0.01	5.6 ± 0.7	8815 ± 222	211 ± 3	0.59 ± 0.01	180 ± 8	0.45 ± 0.04
6	2.5 ± 0.6	153.6 ± 84	30.8 ± 15.4	0.02 ± 0.01	5.8 ± 0.5	6641 ± 788	218 ± 2	0.63 ± 0.02	201 ± 2	0.55 ± 0.01
9	24.4 ± 24.7	312.4 ± 274.8	56.9 ± 52.5	0.09 ± 0.05	6.4 ± 0.2	6018 ± 529	124 ± 21	0.61 ± 0.04	179 ± 19	0.54 ± 0.04

Table S 4: P-values of the pairwise t-tests after bonferroni correction for each temperature pair and biomass or diversity parameter

Pairs	Chl*a*:POC	C:N	D2	RichnessE	EvennessE	RichnessP	EvennessP
2°C-6°C	0.751	1	0.072	1	0.218	0.253	0.032 *
2°C-9°C	0.048 *	0.433	0.008 **	0.001 ***	0.88	1	0.065
6°C-9°C	0.011 *	0.749	0.437	0.001 ***	1	0.213	1

Table S 5: Carbonate chemistry and dissolved nutrients of all three treatments at the end of experimental incubation (n=3).

Temp. [°C]	pH	TA [µmol/kg]	TA drift to t-0 [%]	pCO2 [µatm]	HCO3 [µmol/kgSW]	CO3 [µmol/kgSW]	CO2 [µmol/kgSW]	DIC [µmol/kgSW]	PO4 [µmol/L)	SiO4 [µmol/L]	NO2 [µmol/L]	NO3 [µmol/L]	NH4 [µmol/L]
2	8.09 ± 0.0	2260 ± 6	0.7	373 ± 1	2004 ± 6	98.1 ± 0.3	21.8 ± 21.8	2124 ± 6	4.9 ± 0.1	32.1 ± 0.6	0.1 ± 0	62.8 ± 2.2	0.7 ± 0.1
6	8.04 ± 0.0	2260 ± 3	0.1	512 ± 55	2028 ± 17	89.3 ± 7.7	23.7 ± 25.8	2144 ± 12	4.9 ± 0.1	32.1 ± 0.3	0.1 ± 0	63.0 ± 1.7	0.7 ± 0.3
9	8.15 ± 0.1	2260 ± 10	-0.1	440 ± 107	1966 ± 53	113.0 ± 24.1	24.4 ± 20.0	2101 ± 34	4.7 ± 0.1	25.8 ± 4.1	0.1 ± 0	57.5 ± 5.4	0.2 ± 0

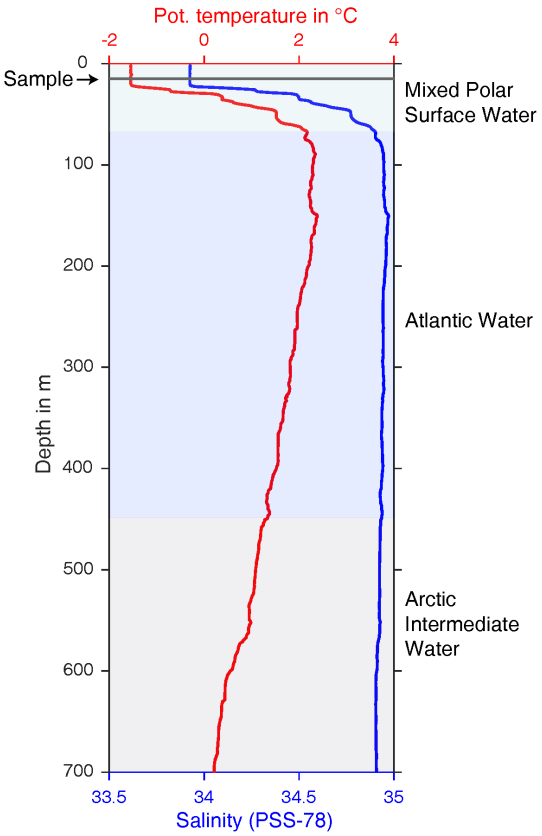


Figure S 1: The temperature and salinity profile at the sampling site HG-IV. The 15 m sampling depth is marked by the horizontal line. The three dominant water masses in the region (modified after [107] are indicated by the shaded areas.

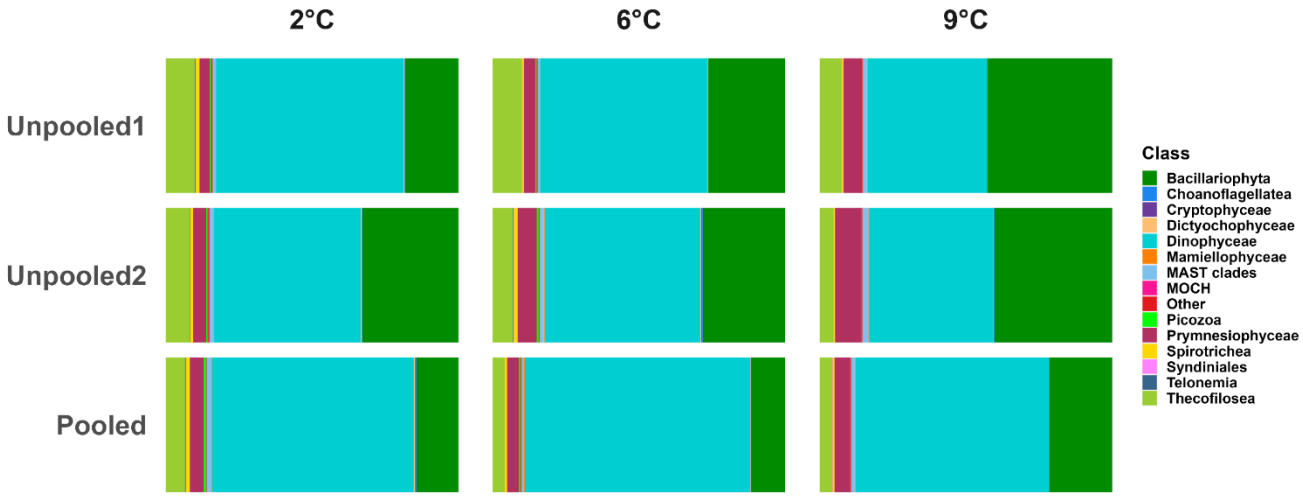


Figure S 2: Replicate-merged bar graphs of the ASV-based class composition after three days for the two unpooled treatments (Unpooled1 = seawater, Unpooled2: 1:5 diluted seawater) and the resulting pools of each temperature that continued the experimental incubation.

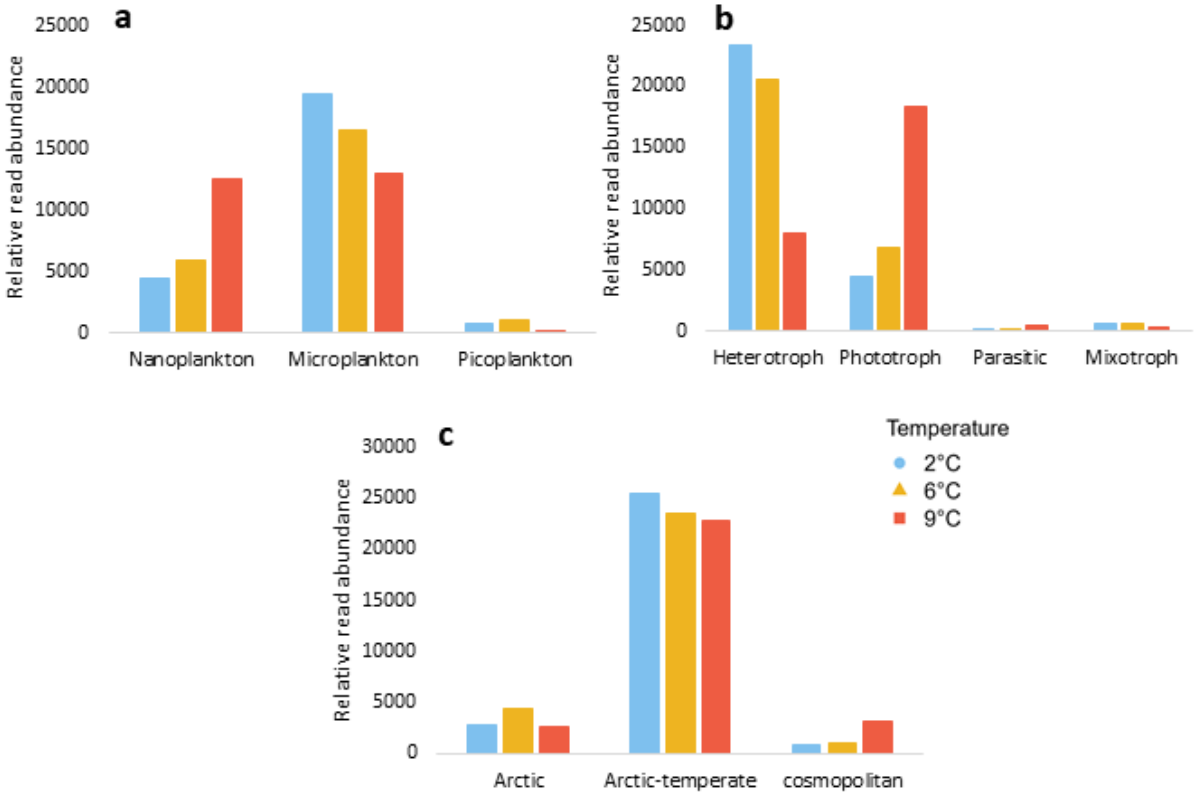


Figure S 3: Bar plot of the mean relative read abundances for each temperature and a) size groups, b) trophic modes and c) thermal niches.

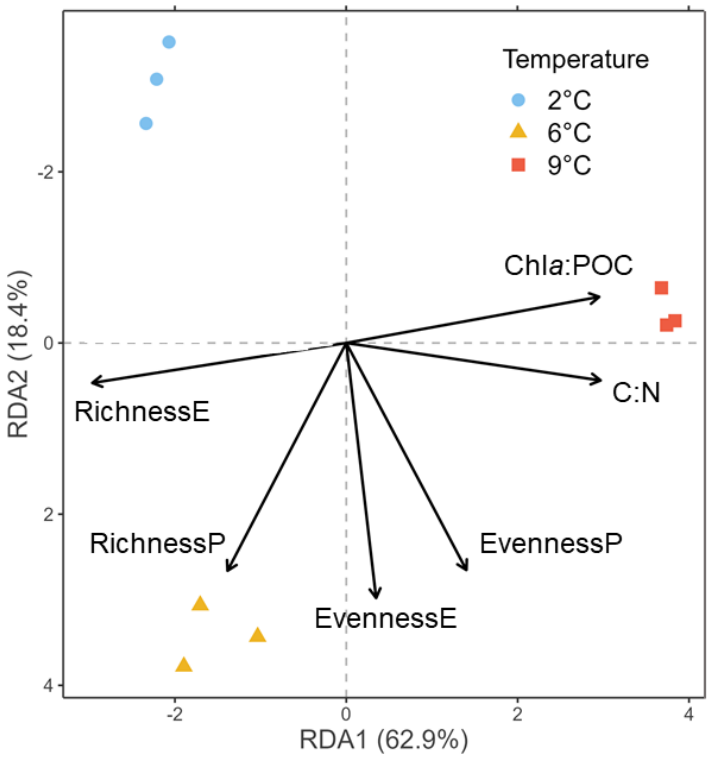


Figure S 4: RDA plot of the ASVs of each temperature constrained by the biomass and diversity parameters without D2.

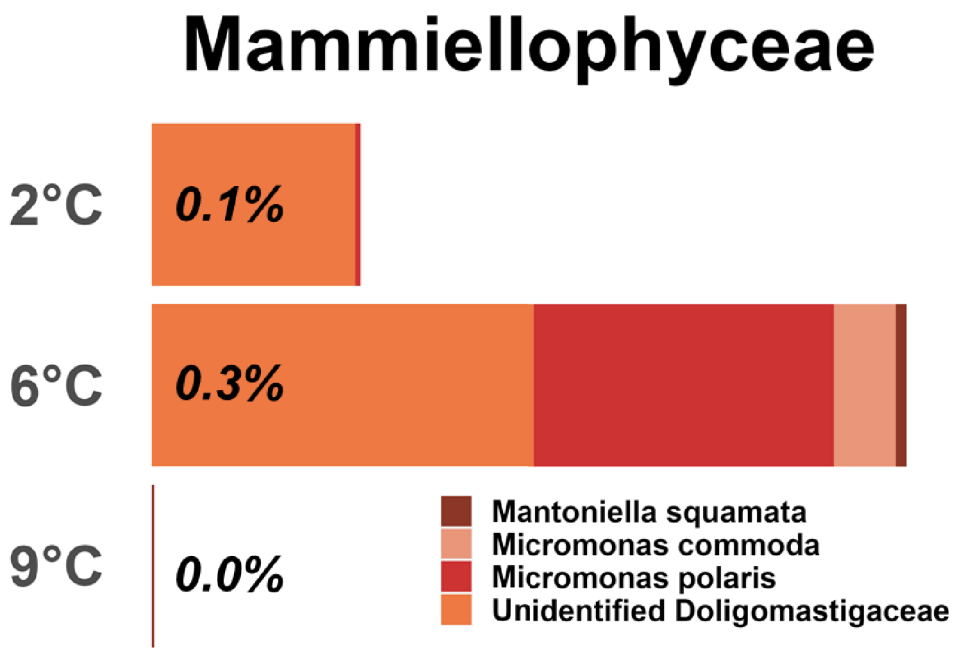


Figure S 5: Relative contribution and species composition of the class of Mammiellophyceae at all treatment temperatures after ten days.

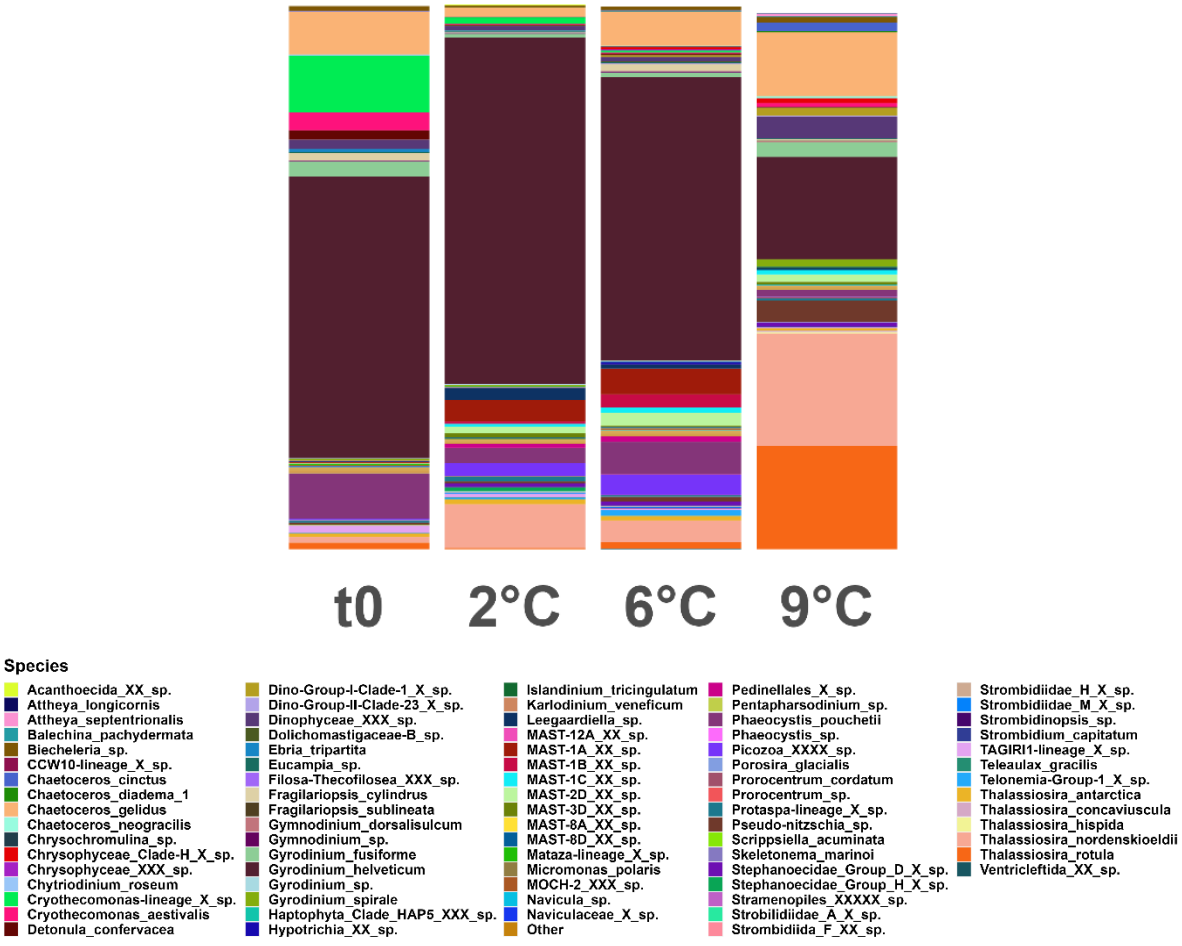
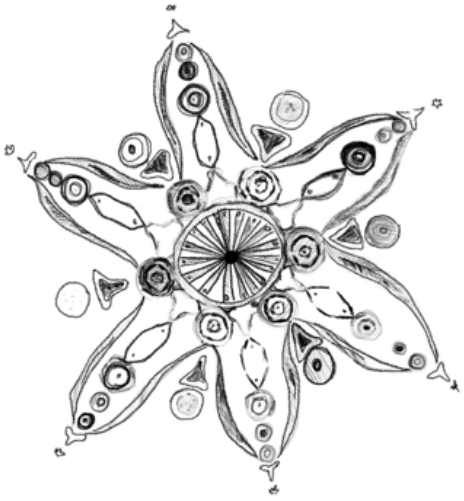


Figure S 6: ASV-based eukaryotic community composition on species level at the start (t0) and at all treatment temperatures after ten days (tfin). ASVs with an abundance of less than 50 reads among all temperatures were categorized as "other".

3

Temperate spring community



3.1: PUBLICATION II

Warming increases the compositional and functional variability of a temperate protist community

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Warming increases the compositional and functional variability of a temperate protist community

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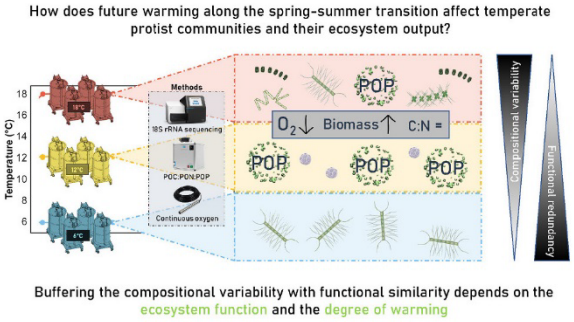
HIGHLIGHTS

- The species' thermal traits primarily drive community reorganisation under warming
- Many North Sea protists tolerate and coexist at temperatures of + 12 °C
- Biomass accumulation in- and oxygen production decreases with warming
- Temperature increases the compositional and functional variability
- *Phaeocystis globosa* drives functional dissimilarity regarding the C:P but not the C:N ratio

GRAPHICAL ABSTRACT



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ABSTRACT

Phototrophic protists are a fundamental component of the world's oceans by serving as the primary source of energy, oxygen, and organic nutrients for the entire ecosystem. Due to the high thermal seasonality of their habitat, temperate protists could harbour many well-adapted species that tolerate ocean warming. However, these species may not sustain ecosystem functions equally well. To address these uncertainties, we conducted a 30-day mesocosm experiment to investigate how moderate (12 °C) and substantial (18 °C) warming compared to ambient conditions (6 °C) affect the composition (18S rRNA metabarcoding) and ecosystem functions (biomass, gross oxygen productivity, nutritional quality – C:N and C:P ratio) of a North Sea spring bloom community. Our results revealed warming-driven shifts in dominant protist groups, with haptophytes thriving at 12 °C and diatoms at 18 °C. Species responses primarily depended on the species' thermal traits, with indirect temperature effects on grazing being less relevant and phosphorus acting as a critical modulator. The species *Phaeocystis globosa* showed highest biomass on low phosphate concentrations and relatively increased in some replicates of

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both warming treatments. In line with this, the C:P ratio varied more with the presence of *P. globosa* than with temperature. Examining further ecosystem responses under warming, our study revealed lowered gross oxygen productivity but increased biomass accumulation whereas the C:N ratio remained unaltered. Although North Sea species exhibited resilience to elevated temperatures, a diminished functional similarity and heightened compositional variability indicate potential ecosystem repercussions for higher trophic levels. In conclusion, our research stresses the multifaceted nature of temperature effects on protist communities, emphasising the need for a holistic understanding that encompasses trait-based responses, indirect effects, and functional dynamics in the face of exacerbating temperature changes.

1. Introduction

Phototrophic protists play a central role in the ecosystem by providing energy, oxygen and organic nutrients for organisms higher up the food chain (Naselli-Flores and Padisák, 2023). Especially in coastal temperate areas, their habitat is characterised by a high seasonality in temperature. For instance, a typical North Sea spring bloom spans from early March to late April, during which the temperature gradually increases from around 6 °C to approximately 12 °C (Käse et al., 2020). A few months later in August, temperature peaks near 18 °C (Wiltshire and Manly, 2004). Many species are adapted to this wide temperature range and therefore often reside below their optimum temperature for growth (Boyd et al., 2013; Giesler et al., 2023). Despite this variability, most experiments investigating warming effects on temperate plankton employ treatments of +3 °C to +6 °C (Happe et al., 2024), which fall well within the range of what they naturally experience (Wiltshire and Manly, 2004). These temperatures may represent the average increases projected for the North Sea (IPCC, 2021); however, they do not encompass heatwaves, which are anticipated to become more frequent, intense, and long-lasting in the upcoming decades (Oliver et al., 2019; Sánchez-Benítez et al., 2022). Many studies also tend to overlook the seasonal dynamics in the field (Gerhard et al., 2023) and, instead, capture a snapshot of a specific point in the year (but see Staehr and Sand-Jensen, 2006). Consequently, the outcomes of these experiments heavily depend on the selection of the starting point, possibly failing to fully represent the actual temperature variations that temperate protist communities will encounter in the future.

Temperature can affect protist community structure via different ways. One of them is through its direct effect on the physiology of single organisms by increasing all metabolic rates until they reach an optimum temperature and then drop (Raven and Geider, 1988). This relationship is described by thermal performance curves (TPCs) and mostly expressed in terms of growth rate (Thomas et al., 2012). Although it can be expected that many temperate phototrophic species tolerate a wide range of temperatures in single strain incubations (Boyd et al., 2013), interspecific competition may alter the response on the community level (Huertas et al., 2011; Denny, 2017). The reason for this lies in species-specific TPC characteristics such as the temperature optimum, the thermal breadth and the maximum growth rate at a given temperature, leading to different growth increments under warming and ultimately entailing species sorting (Bestion et al., 2018; Anderson et al., 2021; Wieczynski et al., 2021). Furthermore, warming can have indirect effects such as stronger grazing (Gibert, 2019) or the acceleration of nutrient incorporation causing earlier limitation (Berges et al., 2002), which in turn affects the TPC of organisms by lowering their thermal optimum or limits (Thomas et al., 2017). Variations in all relevant traits can translate into temperature-induced community shifts, reflected in the compositional transition from spring to summer and between years with different mean temperatures (Alvarez-Fernandez et al., 2012; Bruhn et al., 2021).

Shifts in the community composition of phototrophic protists may mediate a changed output for the ecosystem (Di Pane et al., 2022). But even when the composition remains the same, temperature can alter attributes relevant for higher trophic levels such as the provision of oxygen, energy and organic nutrients (Naselli-Flores and Padisák, 2023). These ecosystem functions stem from a variety of cellular processes and could therefore respond differently to changes in temperature. Functions that are mainly driven by biophysical mechanisms, such as oxygen production, may be affected less strongly compared to functions that rather depend on biochemical reactions, like biomass accumulation (Falkowski and Raven, 2007; Rehder et al., 2023). Another trait tightly linked to temperature is the stoichiometry, specifically the cellular carbon to nutrient ratios (i.e. C:N and C:P), which are used as proxies for the nutritional quality for heterotrophic organisms (van de Waal et al., 2010). While warming favours the investment in nitrogen-rich proteins over phosphorus-rich ribosomes and thus may raise the cellular C:P ratio more than the C:N ratio (Woods et al., 2003; Armin and Inomura, 2021), both ratios could generally increase due to indirect temperature effects such as faster nutrient drawdown (De Senerpont Domis et al., 2014; Matsumoto et al., 2020). Ultimately, all of these processes depend on the environmental context, leading to great variations between the warming responses observed for ecosystem functions (Lewandowska et al., 2014; Striebel et al., 2016).

Differences in functional traits in combination with warming can increase the variability of a system. For example, small variations in the abundance of a species with a high nutrient uptake affinity can quickly build up to large differences when temperatures rise and then affect the surrounding conditions, e.g. induce earlier nutrient limitation (Serra-Pompei et al., 2019). In a setting where this species was slightly less abundant, another species with different traits could outcompete it and nutrient limitation might be induced later. The lag between these scenarios creates a phase with varying competition conditions and could shift the system to fundamentally different compositional states. Consequently, the existing stochasticity within planktonic systems can create several different trajectories and thereby lead to a higher unpredictability (Huisman and Weissing, 2001; Pálffy et al., 2021; Rogers et al., 2022). However, an increased compositional variability does not necessarily result in a higher variability of the respective ecosystem functions as it might be buffered by functional similarity between different species (Eisenhauer et al., 2023). While this has been shown to increase ecosystem stability to abiotic stressors (Biggs et al., 2020), temperature may context-dependently compromise this capacity (Garcia et al., 2018; Zhong et al., 2020).

Despite the urgent need to understand warming responses for projecting future ecosystem properties, current studies on temperate communities do not cover the full potential natural temperature range (Gerhard et al., 2023). To fill this gap, we mechanistically investigated the effect of warming on temperate protist communities in an indoor-mesocosm setting, covering three temperatures from the start of the spring bloom up to the summer peak. The aim was to experimentally determine the compositional output including its variability and to assess potential consequences for ecosystem functions provided to the North Sea by the spring bloom community. We hypothesized that warming leads to a relative increase in species with the respective thermal niche; a higher oxygen production and biomass accumulation but lower nutritional quality; and finally, an increasing compositional and functional variability that depends on the temperature increment, resulting in functional similarity of the communities under moderate but not under substantial warming.

2. Material & methods

2.1. Seawater collection and experimental set-up

The experiment was carried out in the mesocosm facility of the Institute for Chemistry and Biology of the Marine Environment (ICBM) in Wilhelmshaven in March/April 2022. Experimental units were the Planktotrons – twelve stainless steel indoor-mesocosms (Gall et al., 2017). A total of 8000 L of surface seawater was collected from the open North Sea, 60 km off the German coast at the long-term ecological research station Helgoland Roads (DEIMS ID: https://deims.org/1e96ef9b-0915-4661-849f-b3a72f5aa9b1) during a cruise with the German *RV Heincke* on March 6th, 2022. The water was pumped with a diaphragm pump through a 200 μm mesh (reducing the abundance of larger grazers) into eight acid-cleaned 1000 L polyethylene Intermediate Bulk Containers (IBC, AUER Packaging GmbH, Amerang, Germany). On March 7th, we filled the mesocosms by evenly spreading 75 L from each IBC tank into each mesocosm via gravity, resulting in a total of 600 L per mesocosm.

The experiment was conducted using three different temperature treatments, with a replication of four. Upon incubation, the water temperature of all twelve tanks was set to 6 °C, which is representative of the water temperature for the North Sea in March over the last 12 years (based on Helgoland Roads LTER time series) and close to the field temperature during water collection (5.4 °C). As we wanted to cover a wide temperature range, we aimed our highest temperature treatment to be around the optimum temperature for growth (e.g. the highest possible temperature before community growth deteriorates). Therefore, we determined the temperature reaction norm of the phototrophic community at the start of the incubation with a thermal performance curve (TPC) assay. This was started one day after filling the mesocosms (March 8th) by taking a pooled water sample from all mesocosms that was filled into 40 mL cell culture flasks (SARSTEDT, Nümbrecht, Germany) and randomly spread across ten temperatures (3 °C to 30 °C in 3 °C steps) in triplicates. The incubation of the units was achieved by placing them on heating/cooling mats (Inkbird, Shenzhen, China) in different temperature rooms under the same light conditions as the mesocosms. Fluorescence (395/680 nm excitation/emission) was measured daily for eight days to determine growth using a SYNERGY H1 microplate reader (BioTek, Winooski, Vermont, USA) and the thermal performance curve was fitted according to the model by Thomas et al. (2017) using the "rTPC" package (Padfield and O'Sullivan, 2022). We chose the treatment temperatures to span the minimum (6 °C) and optimum temperature (18 °C) for growth, as well as an intermediate temperature (12 °C; Fig. S1). We performed a gradual temperature increase by 1 °C per day starting 24 h after the mesocosms have been filled (incubation days 0–12; Fig. S2). During this phase, temperatures and replicates behaved similarly in terms of community composition as well as ecosystem functions (Figs. S10–14). Therefore, and for comparability, we focus on the period from day 13 onwards.

All parameters that required water to be taken out (DNA, particulate nutrients, dissolved nutrients) were sampled every 3rd day using a self-built tube of the same height as the mesocosms to equally obtain water from all depths. This water was poured into a sample-rinsed bucket, from which subsamples were taken for each parameter after gentle hand-stirring. The starting conditions were assessed on the 8th of March by combining ~4 L of water from each mesocosm and taking technical triplicates from this pool for each parameter. From then onwards, each mesocosm was sampled individually.

2.2. Abiotic conditions

Non-invasive parameters (temperature, pH, salinity) were sampled daily at 10 am. To promote water convection, an integrated, mechanically driven mixing paddle with silicone lips homogenised the water column every two hours at a slow speed to prevent disturbing fragile organisms. Water temperatures were measured constantly with built-in PT100 sensors (Temperature Control, Donaueschingen, Germany) to confirm and adjust the target temperatures in each tank (Fig. S2). Two LED units (IT2040, Evergrow, Shenzhen, China) above each mesocosm were set to a 12:12 h day-night cycle (07:00–19:00 UTC light) with an intensity of 180 μmol photons $m^{-2} s^{-1}$ (spherical PAR-sensor (US-SQS/L, Walz, Effeltrich, Germany) connected to a LI-250 A (Li-Cor, Lincoln, NE, USA)). These settings were selected based on the natural conditions at Helgoland Roads during this time of the year (Wiltshire, 2008, 2010). Although the light intensity changed in all mesocosms over time (potentially due to biomass acuumulation), we observed no differences between the treatments, excluding light as a confounding factor (Fig. S3). Translucent float glass plates (Pilkington Optiwhite, Tokyo, Japan) were placed on top of the mesocosms to prevent evaporation, outgassing, and contamination. Daily salinity measurements (WTW IDS TetraCon, Xylem Analytics, Rye Brook, NY, USA) indicated no differences between temperatures (Fig. S4).

The experiment was conducted under ambient nutrient conditions. To monitor the concentrations of dissolved nitrate and phosphate, a subsample was filtered through a 0.2 μm polyethersulfon syringe filter (Sartorius, Göttingen, Germany) every third day and stored at −20 °C until the colorimetric measurement on a continuous flow analyser (Euro EA 3000, HEKAtech GmbH, Wegberg, Germany). Samples for dissolved silicate were taken every other day and quantified by molybdate reaction according to Wetzel and Likens (2003).

The pH was measured daily (WTW Multi 3630 IDS, Xylem Analytics, Rye Brook, NY, USA). Samples for total alkalinity were taken at the beginning, middle (day 15), and end (day 27) of the incubation by filtering a 100 mL subsample through a 0.2 μm polyethersulfon syringe filter (Sartorius, Göttingen, Germany) into a borosilicate bottle. The sample was kept at 4 °C until it was measured by duplicate potentiometric titration using a TitroLine alphaplus autosampler (Schott Instruments, Mainz, Germany) and subsequent correction with certified reference materials from A. Dickson (Scripps Institution of Oceanography, San Diego, CA, USA). The concentration of dissolved inorganic carbon (DIC) was calculated for incubation day 0, 15, and 27 using the software CO_2sys (Pierrot et al., 2011) with dissociation constants of carbonic acid by Mehrbach et al. (1973), refitted by Dickson and Millero (1987).

2.3. Grazing assessment

Despite the initial filtering procedure, mesozooplankton appeared in all mesocosms, possibly due to early developmental stages (<200 μm) passing the mesh during water collection. Therefore, ~ 8 L water from each mesocosm were filtered through a 200 μm mesh and any mesozooplankton were transferred into 250 mL brown glass bottles before being fixed with Lugol's iodine solution at the end of the experiment (incubation day 27). Mesozooplankton was enumerated and identified to the lowest possible taxonomic level based on scientific literature (Conway, 2012) using a stereo-microscope (S8 AP0O, Leica, Wetzlar, Germany).

Additionally, protist herbivorous-induced mortality (m) rates were measured with the dilution method (Landry and Hassett, 1982) in a two-point modification (Chen, 2015a; Landry et al., 2022) using undiluted (100 %) and diluted (30 %) seawater. Samples from each mesocosm were pooled together for each temperature treatment. From the original water, we prepared 500 mL undiluted (100 %) and diluted (30 %) samples (two technical replicates per temperature treatment at 0 h (t0) and one for 24 h (tf)). Following Landry and Hassett (1982) and Chen (2015a) net phototrophic growth rate (k) was calculated as:

$$k = ln\left(\mathrm{Chla_{tf}}/\mathrm{Chla_{t0}}\right)/\mathrm{t}$$

where $\mathrm{Chla_{tf}}$ and $\mathrm{Chla_{t0}}$ are the Chl*a* concentration measured at the end (tf) and at the beginning (t0) of the incubation period, respectively, and t

represents the duration of the incubation period (24 h). From both rates (that is, k_{30} and k_{100}), we calculated grazing of protist herbivores as:

$$m = (k_{30} - k_{100})/(1 - \times)$$

with $\times$ being the dilution factor used.

2.4. Community composition and diversity

Protist community composition was assessed via 18S rRNA gene amplicon sequencing of the V4 region as described by Ahme et al. (2023) and the results were validated qualitatively using light microscopy (fixed with 1 % Lugol's solution). Briefly, a subsample of 500 mL was gently vacuum-filtered (< − 200 mbar) onto 0.8 μm polycarbonate filters (Nucleopore, Whatman, Maidstone, UK), which were stored in extraction buffer at −80 °C. DNA extraction was performed according to the manufacturer's protocol (NucleoSpin Soil extraction kit, Macherey-Nagel GmbH, Düren, Germany). All samples were normalised to 5 ng μL^{-1} before generating amplicons of the variable region 4 (V4) of the 18S rRNA gene following the standard protocol (16S Metagenomic Sequencing Library Preparation, Part #15044223 Rev. B. Illumina, San Diego, CA, USA). To best target the phototrophic community, we chose the forward and reverese primers of Bradley et al. (2016). Using the Nextera XT Index Kit v2 Set A primers (Illumina, San Diego, CA, USA), single samples were indexed and the barcoded amplicons were pooled equimolarly into one library. The library was sequenced with a 2 × 300 bp paired-end setup on a MiSeq sequencer (Illumina, San Diego, CA, USA) and generated amplicon reads which were demultiplexed by the Generate FASTQ workflow of the MiSeq software.

Primers were removed with v2.8 cutadapt (Martin, 2011) and the data was further processed with v1.18 DADA2 (Callahan et al., 2016). Forward reads were quality-trimmed after 240 and reverse reads after 210 base pairs. Sequences were denoised and paired-end reads merged with a minimum overlap of 20 base pairs. Subsequently, chimeras were predicted and removed (Table S1). The resulting amplicon sequence variants (ASVs) were taxonomically annotated using the protist reference databases v4.12.0 PR2 (Guillou et al., 2013). Samples with a sequencing depth outside of the 90 % quantile range were removed, sufficient depth was confirmed using rarefaction curves (Fig. S5) and all samples were scaled to the lowest depth (Beule and Karlovsky, 2020). ASVs with a count of fewer than ten reads in replicate sample means were excluded, as well as metazoans, fungi, plastids and nuclei. All larger hetero- and mixotrophic taxa were separately analysed and grouped based on their primary feeding strategy (de Vargas et al., 2015; Ramond et al., 2018; Adl et al., 2019). For an assessment of diversity of each sample, we calculated species richness as number of species in each sample, species evenness (Pielou, 1966) and the Shannon index (Ortiz-Burgos, 2016). Processing of the data was performed using R v4.21 (RCoreTeam, 2022) with RStudio v2022.07.2 (RStudioTeam, 2022) and the packages v0.2.3 SRS (Heidrich et al., 2021), v1.40.0 phyloseq (McMurdie and Holmes, 2013), v0.0.22 microbial (Guo and Gao, 2022), and v4.2.6 propr (Quinn et al., 2017).

The replicates diverged in terms of their community composition and therefore no analyses were performed to compare mean compositions between temperature treatments. To get a measure for compositional dissimilarity between replicates, the pairwise Aitchinson distances were calculated between all replicates for each temperature and day. Then, the distances of the replicates to their centroids in multivariate space of a principal coordinate ordination were calculated with the betadisper function (vegan v2.6–2) as described by Anderson et al. (2006) and Pálffy et al. (2021). The compositional variability at a constant temperature of 6 °C represents the "baseline"-variability within our experimental incubation. An increase in variability under warming (to 12 °C or 18 °C) compared to 6 °C indicates a temperature-driven effect rather than experimental duration/bottle effects. Therefore, we consider compositional variability to be driven by temperature if the beta-dispersion at a given warming treatment is significantly higher than at 6 °C.

2.5. Ecosystem functions

To assess the effect of temperature on different ecosystem functions, we chose several proxies: the concentration of particulate organic carbon (POC) for community biomass (Andersson and Rudehäll, 1993), the molar ratios between POC and particulate organic nitrogen (PON)/phosphorus (POP) for nutritional quality (Thomas et al., 2022), as well as the rate of change of dissolved oxygen per POC for gross oxygen productivity (GOP; Sanz-Martín et al., 2019). Furthermore, samples for chlorophyll *a* (Chl*a*) were taken as a proxy to track the development of the phototrophic biomass throughout the experiment.

For Chl*a*, POC/PON and POP, subsamples were filtered onto pre-combusted glass-fibre filters (GF/F Whatman, Maidstone, UK), and kept frozen until processing. Filters for Chl*a* were extracted according to the method of Thrane et al. (2015) and measured using a microplate reader (614 nm/680 nm; SYNERGY H1, BioTek, Winooski, Vermont, USA). For POC/PON, filters were dried and measured with an elemental analyser (Flash EA 1112, Thermo Scientific, Waltham, MA, USA). The POP filters were quantified by molybdate reaction after digestion with a potassium peroxydisulfate solution (Wetzel and Likens, 2003). Particulate nutrient ratios were calculated by dividing the molar masses of the respective nutrients.

Dissolved oxygen concentration in mmol m^{-3} was measured continuously by the built-in OXYBase WR-RS485-L5 sensors (PreSens, Regensburg, Germany), calibrated by PreSens. Daily community production and respiration were calculated via the slope of the linear regression of oxygen concentration over the light period (09:00–19:00) and dark period (19:00–09:00), respectively. Daily GOP was then obtained by summing community production and respiration for each day, as described by Sanz-Martín et al. (2019). To account for differences in biomass, we normalised the daily GOP to POC.

2.6. Statistics

A two-way repeated measures ANOVA (rmANOVA) was conducted to assess the effect of temperature, time and their interaction on the Shannon diversity, Chl*a*, ecosystem functions and the compositional variability. Normality was confirmed visually using quantile-quantile-plots. Sphericity was tested using Mauchly's test, and whenever it was violated, a Greenhouse-Geisser correction was applied. In analysing the beta-dispersion and Shannon diversity, day 24 had to be excluded because of too few data points. If a main effect of either temperature or time and no interaction was observed, pairwise *t*-tests were performed and the *p*-values were adjusted using the Bonferroni correction. For both, a main effect and a significant interaction, a one-way ANOVA of the main effect variable was performed before pairwise t-testing. All data are shown as arithmetic mean with one standard deviation in parentheses and analyses were conducted with a significance level of 0.05, using the v0.7.0 R package rstatix (Kassambara, 2021).

3. Results

3.1. Bloom development and abiotic conditions

Chl*a* was significantly higher at 18 °C than both at 6 °C ($p < .001$) and 12 °C ($p < .001$) without any effect of time (Table 1, Fig. 1). Both phosphate and nitrate concentrations showed no temperature effect but decreased over time, while silicate concentrations showed both main effects of temperature and time and an interaction (Table 1, Fig. 1). Silicate concentrations showed significant effects of temperature and time as well as an interaction (Table 1, Fig. 1). They decreased slightly at 6 °C and strongest at 18 °C (all $p < .001$), whereas the concentrations at 12 °C remained constant over time. Between replicates, phosphate and

Table 1
Results of the two-way rmANOVA for temperature, time, and their interactive effects on chlorophyll *a*, dissolved nutrients, diversity parameter and ecosystem functions. Dfn is the degree of freedom for the numerator of the F ratio, and DFd is for the denominator. Significant effects are highlighted in bold.

Parameter	Effect	DFn	DFd	*F*	*p*
	Temperature	2.00	9.00	30.5	**<0.001**
Chlorophyll *a*	Time	1.99	17.89	2.3	0.134
	Temperature:Time	3.98	17.89	1.3	0.293
	Temperature	2.00	9.00	1.5	0.275
Nitrate	Time	1.88	16.91	8.3	**0.003**
	Temperature:Time	3.76	16.91	1.9	0.158
	Temperature	2.00	9.00	2.0	0.197
Phosphate	Time	4.00	36.00	6.0	**<0.001**
	Temperature:Time	8.00	36.00	0.2	0.995
	Temperature	2.00	9.00	15.8	**0.001**
Silicate	Time	2.07	18.61	55.4	**<0.001**
	Temperature:Time	4.13	18.61	10.2	**<0.001**
	Temperature	2.00	3.00	2.1	0.268
Micrograzing rate	Time	1.00	3.00	1.3	0.343
	Temperature:Time	2.00	3.00	384.7	**<0.001**
	Temperature	2.00	8.00	0.097	0.909
Shannon index	Time	3.00	24.00	9.497	**<0.001**
	Temperature:Time	6.00	24.00	7.572	**<0.001**
	Temperature	2.00	8.00	1.118	0.373
Richness	Time	3.00	24.00	7.576	**<0.001**
	Temperature:Time	6.00	24.00	0.616	0.715
	Temperature	2.00	8.00	0.056	0.946
Evenness	Time	3.00	24.00	5.635	**0.005**
	Temperature:Time	6.00	24.00	9.818	**<0.001**
	Temperature	2.00	9.00	4.9	**0.037**
Biomass	Time	4.00	36.00	14	**<0.001**
	Temperature:Time	8.00	36.00	2.1	0.059
	Temperature	2.00	8.00	7.3	**0.015**
GOP	Time	4.00	32.00	6.2	**0.005**
	Temperature:Time	8.00	32.00	1.4	0.220
	Temperature	2.00	9.00	3.6	0.070
C:N	Time	4.00	36.00	11.8	**<0.001**
	Temperature:Time	8.00	36.00	3.4	**0.006**
	Temperature	2.00	9.00	0.2	0.791
C:P	Time	4.00	36.00	33.5	**<0.001**
	Temperature:Time	8.00	36.00	3.9	**0.002**

silicate concentrations remained similar but they diverged in terms of nitrate, with replicates C and D at 12 °C and C at 18 °C decreasing more (Fig. S6). The pH increased from 8.09 (sd 0.01) to 8.36 (sd 0.05) in all mesocosms during the whole incubation period, but again replicates C and D at 12 °C and replicate C at 18 °C stood out by increasing the pH to 8.55–8.63 (Fig. S7). The same replicates additionally had the strongest decrease of DIC down to a minimum of 1920.17–1823.79 mmol kg SW ^{1} (Fig. S7). The DIC concentration in all other mesocosms decreased to a lesser extent, i.e. 2032.87 (sd 31.75) mmol kg SW ^{1}.

3.2. Grazing impact

In terms of mesozooplankton, we found no significant differences between the temperature treatments regarding their abundances (Kruskal-Wallis test, $\chi 2\ (2) = 1.08$, $p = .5836$) or composition (Fig. S8). The micro-grazing rates showed a significant interaction between temperature and time (Table 1, Fig. S9). Pairwise t-tests revealed a significant decrease over time at 6 °C ($p = .008$) but an increase at 12 °C ($p = .053$) and 18 °C ($p = .061$). Thus, the micro-grazing rates at 6 °C were initially higher compared to 12 and 18 °C ($p_{6-12} = 0.021$, $p_{6-18} = 0.012$) but lower at the end ($p_{6-12} = 0.039$, $p_{6-18} = 0.012$). Between the two warming treatments, we found no significant differences at any time ($p_{15} = 0.669$, $p_{27} = 0.198$). The community composition was consistent between replicates at 6 °C, but became more variable under warming (Fig. S10).

3.3. Community composition and diversity

On phylum level, the main response pattern showed diatoms to be dominant at 6 °C and 18 °C, whereas they relatively decreased at 12 °C and instead haptophytes and dictyochophytes increased their relative abundance (Fig. S11). At 18 °C, either green algae or haptophytes comprised the rest of the community. Dinoflagellates showed similarly low relative abundances between all temperatures except for larger shares in the warming treatments, especially replicate C and D at 18 °C (*Prorocentrum* sp.; Fig. S12).

On lower taxonomic levels, the responses were more complex (Fig. 2). At 6 °C, the species *Chaetoceros debilis* relatively increased during the incubation while *Minidiscus variabilis*, *Skeletonema marinoi*, and *Thalassiosira punctigera* relatively decreased (Fig. S12). Additionally, the haptophyte *Phaeocystis globosa* had a low but stable relative contribution (Fig. S12). At 12 °C, *Dictyocha speculum* relatively increased in replicates A and B while the haptophytes were either dominated by *Gephyrocapsa oceanica* (replicate B), by *P. globosa* (replicates C & D) or switched from *P. globosa* to *G. oceanica* over time (replicate A; Fig. 2 & S12). At 18 °C, diatoms comprised more different species compared to 6 °C (several *Chaetoceros* species, *Dytilum brightwellii*, *T. punctigera* etc.; Fig. S12). In replicate A, *G. oceanica* was the main residual species whereas in replicate C it was *P. globosa*. In replicates B and D, *Pyramimonas* sp. made up a major part (Fig. 2, S12).

The Shannon diversity showed a significant effect of time and an interaction between time and temperature (Table 1, Fig. 3a). It stayed stable under warming and decreased over time at 6 °C ($p_{15-27} = 0.002$, $p_{18-27} = 0.003$), leading to 6 °C having an initially higher but at the end lower Shannon diversity compared to the warming treatments. This was mainly driven by differences in species evenness (Fig. 3c) as opposed to species richness (Fig. 3b), which only showed significant decreases over time at all temperatures (Table 1). The mean beta-dispersion was significantly higher at 18 °C but not at 12 °C compared to 6 °C (Table S2). Ellipsoids in multivariate space were more circular at 6 °C compared to 12, indicating that the differences between replicates at 6 °C were equal while at 12 °C two replicates each were more similar to each other but more dissimilar to the other pair (Fig. S13).

3.4. Ecosystem functions

Biomass (as POC) and gross oxygen productivity (GOP) were the only two ecosystem functions that significantly differed between temperatures (Table 1, Fig. 4a & b). Pairwise comparisons between the temperatures revealed biomass to be significantly higher at 18 °C than both at 6 °C ($p < .001$) and 12 °C ($p = .018$), but similar between 6 and 12 °C ($p = .340$). GOP was significantly higher at 6 °C compared to 12 °C ($p < .001$) and 18 °C ($p = .002$).

All four ecosystem functions showed a significant effect of time (Table 1). Pairwise *t*-tests for biomass across the timepoints showed an increase at all temperatures from the first two sampling days (day 15 and 18) towards the last two sampling days (day 24 and 27; $p_{15-24} = 0.032$, $p_{15-27} = 0.013$, $p_{18-24} = 0.024$, $p_{18-27} = 0.005$). GOP decreased over time but only from sampling day 21 to sampling day 27 ($p = .035$). Both particulate nutrient ratios additionally exhibited a significant interaction between time and temperature (Table 1). The C:N ratio only significantly decreased over time at 12 °C (Fig. 4c; $p = .003$), while the C:P significantly increased over time at all temperatures (Fig. 4d; 6 °C: $p_{C:P} = .003$, all other $p < .001$).

Inspecting differences within the temperatures, the mesocosms in which *P. globosa* made up a large proportion (12 °C C & D, 18 °C C) had higher values of biomass, the C:P ratio and pH and lower concentrations of nitrate compared to the other replicates at the same temperature (Fig. S14). Towards the end, replicate A at 18 °C also increased its biomass more compared to other replicates of the same temperature.

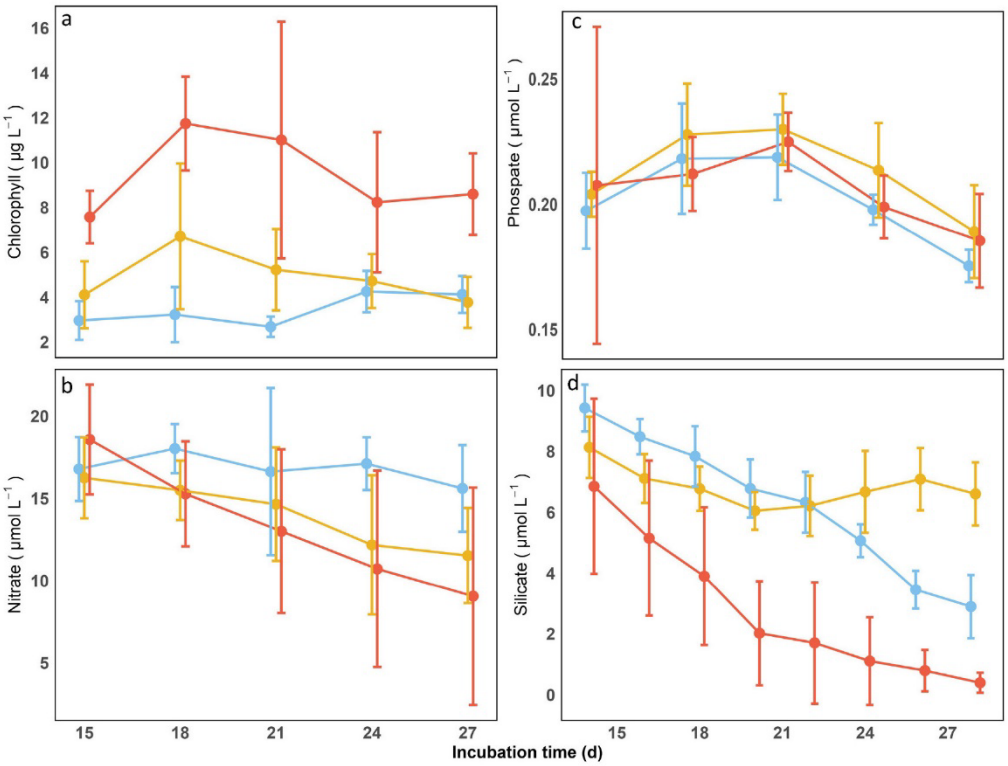

Fig. 1. Chlorophyll a (a), nitrate (b), phosphate (c), and silicate (d) of each temperature over time. Dots represent the arithmetic mean of the temperatures (6 °C: blue, 12 °C: yellow, 18 °C: red) and error bars the standard deviation.

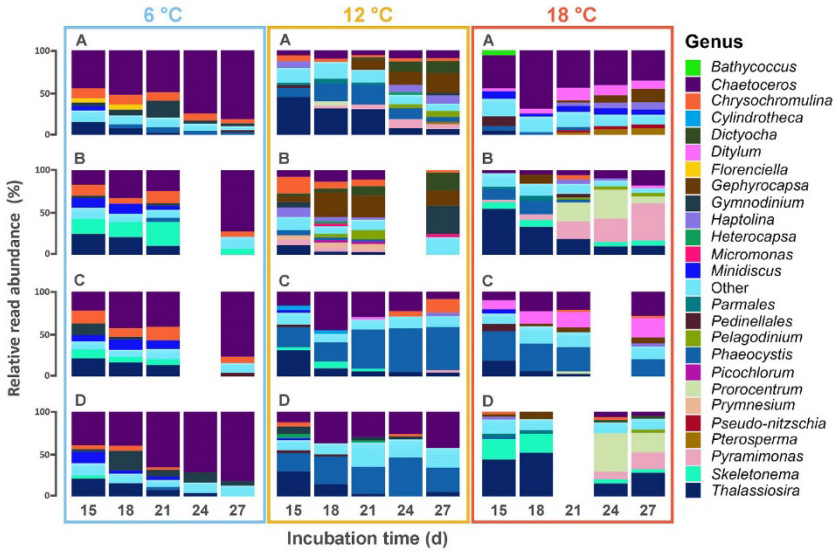

Fig. 2. Metabarcoding-based phototrophic community composition on genus level over time for all replicates (A-D; vertical alignment) and temperatures (6–18 °C; horizontal alignment). For readability, ASVs with an abundance of fewer than 150 reads among temperatures were categorized as "other".

4. Discussion

The aim of this study was to mechanistically investigate warming effects on the composition and resulting ecosystem functions of a temperate protist community. Our results indicate that thermal traits are the most important factor for community reorganisation but can be amended by nutrients (here phosphorus) as modulators. Overall, we observed a high capacity of many North Sea species to tolerate and coexist at increased temperatures. Resulting ecosystem functions showed both warming-driven as well as species-specific responses and, due to reduced functional similarity, consequences for the ecosystem may be severe.

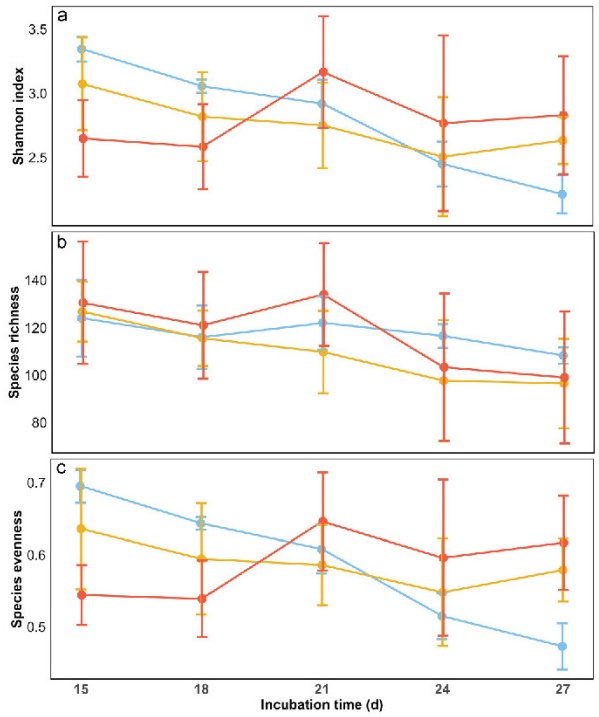

Fig. 3. Shannon index (a), species richness (b), and species evenness (c) of each temperature over time. Dots represent the arithmetic mean of the temperatures (6 °C: blue, 12 °C: yellow, 18 °C: red) and error bars the standard deviation.

Thermal traits are the main driver of community reorganisation under warming

At 6 °C, the species evenness was lower than under warming towards the end of the incubation. This indicates that 6 °C low temperatures to pose a higher selective pressure for species-sorting and dominance. A study by Anderson and Rynearson (2020) on temperate diatoms supports this argument by showing that community dynamics were driven more by thermal limits than thermal optima. Furthermore, we could not confirm previous projections on declining diversity with warming (Benedetti et al., 2021; Henson et al., 2021; Ahme et al., 2023). A potential explanation is that most of these studies depict Arctic communities with narrower thermal breadths, while temperate species usually reside far below their optimum temperature (Thomas et al., 2012). This likely enables many species to prevail under warming, as long as their (comparably high) thermal limits are not reached.

We observed a clear separation of the community composition at the phylum level. Diatoms dominated the communities at 6 °C and 18 °C while at 12 °C, it was largely haptophytes (either *P. globosa* or *Gephyrocapsa oceanica*). *Phaeocystis* spp. are known to decrease grazing pressure via large mucus-embedded colonies or potential toxicants (Stelfox-Widdicombe et al., 2004; Nejstgaard et al., 2007; Liang et al., 2020) and some studies pose that warming disproportionally favours heterotrophs, increasing top-down control (Chen et al., 2012; Boscolo-Galazzo et al., 2018). However, there were no significant differences in micro-grazing or mesozooplankton abundances and neither a clear pattern of certain grazer groups between 12 and 18 °C. In our experiment, grazing pressure can thus be excluded to drive the community composition under warming, consistent with the idea that the temperature-grazing relationship depends on other factors like nutrient levels (Chen et al., 2012).

The ability of diatoms to dominate communities both at low and high temperatures might be indicative of the high thermal niche diversity and a wider thermal breadth compared to haptophytes (Chen, 2015b;

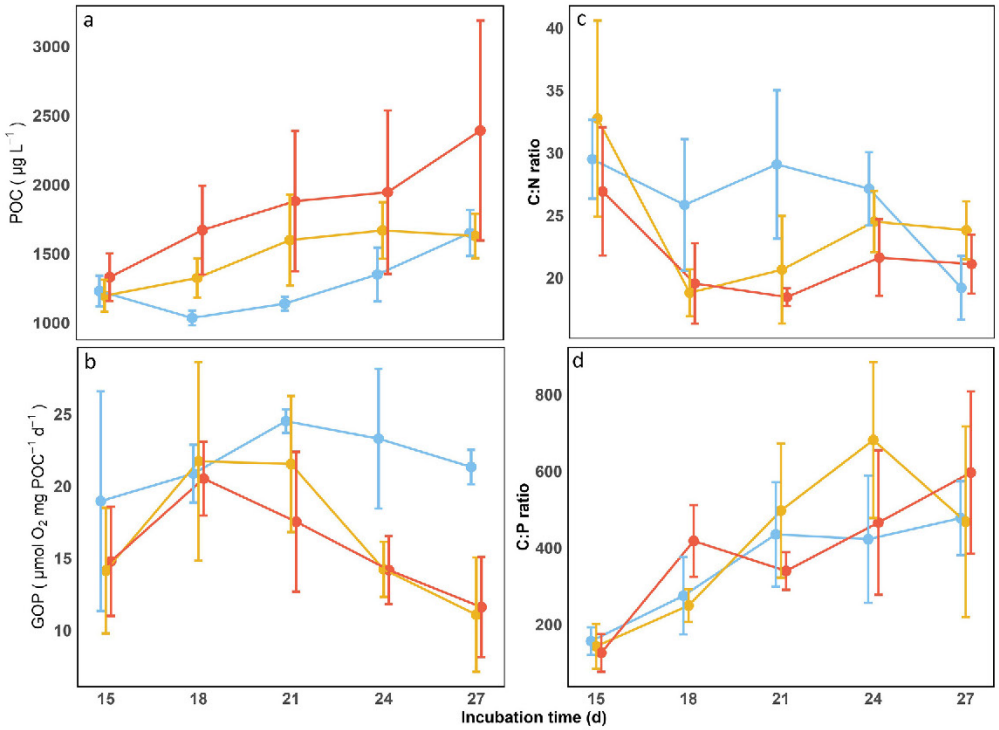

Fig. 4. Ecosystem function values of each temperature over time for (**a**) biomass, (**b**) GOP, (**c**) the C:N ratio, and (**d**) the C:P ratio. Dots represent the arithmetic mean of the temperatures (6 °C: blue, 12 °C: yellow, 18 °C: red) and error bars the standard deviation.

Anderson et al., 2021). For haptophytes, warming may have alleviated potential temperature limitation at 6 °C explaining their dominance at 12 °C (Wang et al., 2010; Wang et al., 2024). However, they could not outcompete diatoms anymore at 18 °C, despite having their optima at temperatures >15 °C (Wang et al., 2010; Müller et al., 2021). Indeed, diatoms are known to deal better with temperatures that exceed the multiyear upper temperature limit of the community at a given point of year (Kling et al., 2020), which could be reflected in the high number of different diatom species at 18 °C. Furthermore, the highest competitive abilities for nutrients are shown to be at colder temperatures compared to growth rate optima (Sunday et al., 2023). Considering the low phosphate concentrations in our experiment, this could have contributed to the dominance of haptophytes at 12 °C. Coccolithophores and *Phaeocystis* are known for thriving under low inorganic phosphorus concentrations (McKew et al., 2015; Moreno et al., 2022) and several studies indicate *Phaeocystis* to outcompete diatoms under inorganic phosphorus depletion under intermediate temperatures (Mori et al., 2021; Breton et al., 2022; Chai et al., 2023). At 18 °C, the competitive ability of haptophytes for low nutrient concentrations may have diminished and the higher growth rates of diatoms became more prevalent (Kremer et al., 2017; Sunday et al., 2023). Therefore, we found the main compositional patterns to arise from thermal traits, while nutrients acted as modulators.

Further support for the importance of thermal traits arises from the species level composition. At 6 °C, the diatom *C. debilis* increasingly dominated over time, reflecting its thermal niche as it is mainly found in colder waters (Ahyong et al., 2022). On the other hand, the decreasing *T. punctigera* is rather common in warmer waters (Ahyong et al., 2022). This is in line with *T. punctigera* being the dominant species in one replicate at 18 °C (C) while in two other replicates (B & D) *Pyramimonas* sp. and *Prorocentrum* sp. made up a large share, consistent with their thermal optima near 18 °C (Thomas et al., 2012; Edullantes et al., 2023). Furthermore, *D. speculum* is known to grow between 11 °C and 15 °C (Henriksen et al., 1993) and had a large contribution in two replicates (A & B) at 12 °C. Overall, our results show that literature-derived thermal traits can be considered a good predictor for community reorganisation under warming.

Temperature-dependence of ecosystem functions is mediated by the presence of Phaeocystis

The higher biomass accumulation under warming is consistent with other studies (De Senerpont Domis et al., 2014; Lewandowska et al., 2014) and reflects the seasonal dynamics in the field (González-Gil et al., 2022). Taken together with the higher species evenness, it may indicate a higher niche complementarity (Zhang et al., 2012). Another explanation could be temperature-stimulated higher carbon fixation rates of all community members taken together as shown by De Senerpont Domis et al. (2014) who also observed increased biomass despite changes in community composition.

The response of gross oxygen productivity (GOP) deviated from expectations. Instead of increasing with temperature, it was significantly lower at 12 °C and 18 °C compared to 6 °C towards the end of the incubation. One potential reason is an enhanced respiration rate of heterotrophs (Yvon-Durocher et al., 2012), exceeding the oxygen production by phototrophs. Indeed, the higher grazing rates observed at 12 °C and 18 °C can be a proxy for higher heterotrophic biomass (Freibott et al., 2016; Cabrerizo and al., in prep.) and therefore might underpin this theory. But even in phototrophs, the ratio of respiration to photosynthesis can increase with temperature (Bozzato et al., 2019; Bestion et al., 2020). Interestingly, there was no further decrease in GOP with temperature. As there were no differences in grazing rates or heterotrophic community composition between 12 °C and 18 °C, this can only be explained by differences in phototrophic community composition based on species- and size-specific variations in metabolic rates (López-Sandoval et al., 2014; Chen and Laws, 2017).

The C:N ratio was observed to be unresponsive to warming, confirming other studies (Yvon-Durocher et al., 2017; Verbeek et al., 2018). This may be based on the tight coupling between nitrate uptake and carbon assimilation as well as a limited ability to store surplus nitrogen in many species (Frost et al., 2023). On the other hand, the C:P ratio showed no mean differences among temperatures but consistently increased over time, implying an enhanced resource use efficiency over the incubation period (Breton et al., 2022) that could be based on species sorting (Verbeek et al., 2018). However, considering the higher C:P ratio of *Phaeocystis*-containing mesocosms which increased under warming, consequences regarding the nutritional quality for higher trophic levels cannot be ruled out (Bukovinszky et al., 2012).

Generally, the mesocosms with major proportions of *P. globosa* behaved differently compared to the other replicates. With higher relative amounts of *P. globosa*, more biomass was built up via more DIC uptake from the water, which resulted in a higher pH. The C:N ratio remained similar to the other replicates via a higher nitrate uptake. However, the C:P ratio was much higher, indicating that *P. globoa* could build up more organic carbon and nitrogen on the same amount of phosphorus compared to other species. This is consistent with other studies (Smith and Trimborn, 2024) and stresses *P. globosas* high capability of growing on low inorganic phosphorus levels, potentially because it better exploits the advantages of alkaline phosphatases (van Boekel and Veldhuis, 1990; Veldhuis et al., 1991). While the biomass of the *Phaeocystis*-dominated replicate at 18 °C was much higher than at 12 °C, the C:P ratio was similar between them, indicating a potential upper threshold of phosphorus resource use efficiency.

Temperature increases compositional variability and decreases functional similarity

The compositional variability increased with warming, which confirms the expectation that the enhanced growth rates until the community T_{opt} increase the potential for small differences in abundances to amplify. This is supported by the results of Pálffy et al. (2021), as they observed higher compositional variability of pioneer communities with warming. We also found an increased functional variability, which was mainly induced by *P. globosa*. But also the non-*Phaeocystis* containing replicate A at 18 °C experienced a stronger biomass increase than the other replicates towards the end (Fig. S14). This indicates that substantial warming (i.e. +12 °C) lowered the functional similarity even more than a moderate temperature increase (i.e. +6 °C). Consistently, modelling studies have observed increased functional uncertainty with warming (Laufkötter et al., 2015; Dutkiewicz et al., 2013; Sarker et al., 2020). Considering the high patchiness of plankton communities in the ocean (Robinson et al., 2021), this stresses the importance of experimentally covering a broad range of potential starting communities that may yield different functional outputs.

Overall, we observed that some functional responses appear universal between different organisms, while others exhibit species-dependence. Especially the C:N ratio can be considered robust across different community compositions. The communities additionally appeared to be functionally similar in terms of GOP under warming, which is in line with the findings of López-Sandoval et al. (2014). However, biomass and the C:P ratio depended on the exact community composition, consistent with the idea that functional and taxonomic diversity can covary (Ramond et al., 2019). A balanced nutrient supply to higher trophic levels under warming can therefore only be assumed for nitrogen, but not for phosphorus. Accordingly, functional similarity can buffer compositional differences only for specific functions (Biggs et al., 2020). However, it has to be kept in mind that the nutrient regime may modulate responses (Fetzer et al., 2015), yielding different results when nutrients are replete (Hoppe et al., 2018). We thus support the notion that functional similarity depends on the ecological context and can differ between ecosystem functions (Meyer et al., 2018; Eisenhauer et al., 2023).

5. Ecological implications and conclusion

From our study, we can derive several implications for resulting ecosystem processes. Firstly, higher temperatures may induce nutrient limitation of either nitrogen or phosphorus, depending on the community composition and the nutrient availability. Considering that phosphorus limitation is increasingly common for the North Sea (Grizzetti et al., 2012; Breton et al., 2022; Rönn et al., 2023), we expect the C:P ratio to increase, potentially limiting the growth of organisms higher up the food chain. Secondly, gross oxygen production could decrease, although this is likely no major problem in most areas of the shallow and well-mixed North Sea. Lastly, we discovered that the presence of *P. globosa* has the potential to shift the ecosystem to an alternative state with implications for the entire food web and biogeochemical cycles. It has to be noted that our incubation only lasted for a month so that we could not capture the aspect of evolution. As this can change the outcome of warming responses (Barton et al., 2020), longer-term incubations and field monitoring are needed to complement our results and infer consequences for the future North Sea more realistically.

In conclusion, our study demonstrates that thermal traits well explain community restructuring, modulated by nutrient-related traits. Considering the strong selective pressure posed by the lowest temperature, the temperature drop at the end of potential heatwaves requires more scientific attention. Furthermore, the degree of warming also determined the development of haptophyte vs. diatom-dominated communities and thereby may affect higher trophic levels and biogeochemical cycles, but the mechanisms for this are still poorly understood and need further investigation. While warming partly affected the mean differences between temperatures, the most striking result of this study was the increased compositional and partly functional variability at higher temperatures. Overall, we can conclude that stronger warming likely results in a less predictable ecosystem and an increased probability of fundamental shifts.

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CRediT authorship contribution statement

Antonia Ahme: Writing – review & editing, Writing – original draft, Visualization, Software, Resources, Project administration, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Anika Happe:** Writing – review & editing, Validation, Methodology, Investigation, Data curation, Conceptualization. **Maren Striebel:** Writing – review & editing, Validation, Supervision, Resources, Project administration, Funding acquisition, Conceptualization. **Marco J. Cabrerizo:** Writing – review & editing, Visualization, Methodology, Investigation, Data curation. **Markus Olsson:** Writing – review & editing, Validation, Methodology, Investigation, Formal analysis, Data curation. **Jakob Giesler:** Writing – review & editing, Validation, Formal analysis, Data curation. **Ruben Schulte-Hillen:** Writing – review & editing, Methodology, Investigation. **Alexander Sentimenti:** Writing – review & editing, Methodology, Investigation. **Nancy Kühne:** Writing – review & editing, Methodology, Investigation. **Uwe John:** Writing – review & editing, Validation, Supervision, Resources, Funding acquisition, Conceptualization.

Declaration of competing interest

Anika Happe, Marco J. Cabrerizo, Markus Olsson reports financial support was provided by European Commission. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

The DNA data are available from the European Nucleotide Archive at EMBL-EBI under the accession ID PRJEB72441 (https://www.ebi.ac.uk/ena/browser/view/PRJEB72441) and was submitted via GFBio (Diepenbroek et al., 2014). The TPC data can be obtained from Ahme et al. (2023a) and all other data from Ahme et al. (2023b). Abiotic conditions at Helgoland Roads are available in the Data Publisher for Earth & Environmental Science PANGAEA, at https://doi.pangaea.de/10.1594/PANGAEA.960375, or will be shared on reasonable request to LTER.HRSR@awi.de. Code to produce the graphs and results of the manuscript can be found online on GitHub: https://github.com/AntoniaAhme/TopTronsMesocosmIncubation (accessed on 06.11.2023).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171971.

References

Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A., et al., 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. The Journal of Eukaryotic Microbiology 66 (1), 4–119. https://doi.org/10.1111/jeu.12691.

Ahme, A., Von Jackowski, A., McPherson, R.A., Wolf, K.K.E., Hoppmann, M., Neuhaus, S., et al., 2023. Winners and losers of atlantification: the degree of ocean warming affects the structure of arctic microbial communities. Genes [Online] 14 (3).

Ahyong, S., Boyko, C.B., Bailly, N., Bernot, J., Bieler, R., Brandão, S.N., et al., 2022. World Register of Marine Species (WoRMS). WoRMS Editorial Board.

Alvarez-Fernandez, S., Lindeboom, H., Meesters, E., 2012. Temporal changes in plankton of the North Sea: community shifts and environmental drivers. Marine Ecology Progress Series 462, 21–38. https://doi.org/10.3354/meps09817.

Anderson, M.J., Ellingsen, K.E., McArdle, B.H., 2006. Multivariate dispersion as a measure of beta diversity. Ecology Letters 9 (6), 683–693. https://doi.org/10.1111/j.1461-0248.2006.00926.x.

Anderson, S.I., Rynearson, T.A., 2020. Variability approaching the thermal limits can drive diatom community dynamics. Limnology and Oceanography 65 (9), 1961–1973. https://doi.org/10.1002/lno.11430.

Anderson, S.I., Barton, A.D., Clayton, S., Dutkiewicz, S., Rynearson, T.A., 2021. Marine phytoplankton functional types exhibit diverse responses to thermal change. Nature Communications 12 (1), 6413. https://doi.org/10.1038/s41467-021-26651-8.

Andersson, A., Rudehäll, Å., 1993. Proportion of plankton biomass in particulate organic carbon in the northern Baltic Sea. Marine Ecology Progress Series 95, 133–139. https://doi.org/10.3354/meps0 95133.

Armin, G., Inomura, K., 2021. Modelled temperature dependencies of macromolecular allocation and elemental stoichiometry in phytoplankton. Computational and

Structural Biotechnology Journal 19, 5421–5427. https://doi.org/10.1016/j.csbj.2021.09.028.
Barton, S., Jenkins, J., Buckling, A., Schaum, C.E., Smirnoff, N., Raven, J.A., Yvon-Durocher, G., 2020. Evolutionary temperature compensation of carbon fixation in marine phytoplankton. Ecology Letters 23, 722–733.
Benedetti, F., Vogt, M., Elizondo, U.H., Righetti, D., Zimmermann, N.E., Gruber, N., 2021. Major restructuring of marine plankton assemblages under global warming. Nature Communications 12 (1), 5226. https://doi.org/10.1038/s41467-021-25385-x.
Berges, J., Varela, D., Harrison, P., 2002. Effects of temperature on growth rate, cell composition and nitrogen metabolism in the marine diatom Thalassiosira pseudonana (Bacillariophyceae). Marine Ecology Progress Series 225, 139–146. https://doi.org/10.3354/meps225139.
Bestion, E., García-Carreras, B., Schaum, C.E., Pawar, S., Yvon-Durocher, G., 2018. Metabolic traits predict the effects of warming on phytoplankton competition. Ecology Letters 21 (5), 655–664. https://doi.org/10.1111/ele.12932.
Bestion, E., Barton, S., Garcia, F.C., Warfield, R., Yvon-Durocher, G., 2020. Abrupt declines in marine phytoplankton production driven by warming and biodiversity loss in a microcosm experiment. Ecology Letters 23 (3), 457–466. https://doi.org/10.1111/ele.13444.
Beule, L., Karlovsky, P., 2020. Improved normalization of species count data in ecology by scaling with ranked subsampling (SRS): application to microbial communities. PeerJ 8, e9593. https://doi.org/10.7717/peerj.9593.
Biggs, C.R., Yeager, L.A., Bolser, D.G., Bonsell, C., Dichiera, A.M., Hou, Z., et al., 2020. Does functional redundancy affect ecological stability and resilience? A review and meta-analysis. Ecosphere 11 (7), e03184. https://doi.org/10.1002/ecs2.3184.
van Boekel, W.H.M., Veldhuis, M.J.W., 1990. Regulation of alkaline phosphatase synthesis in Phaeocystis sp. Marine Ecology Progress Series 61 (3), 281–289.
Boscolo-Galazzo, F., Crichton, K.A., Barker, S., Pearson, P.N., 2018. Temperature dependency of metabolic rates in the upper ocean: a positive feedback to global climate change? Global and Planetary Change 170, 201–212. https://doi.org/10.1016/j.gloplacha.2018.08.017.
Boyd, P.W., Rynearson, T.A., Armstrong, E.A., Fu, F., Hayashi, K., Hu, Z., et al., 2013. Marine phytoplankton temperature versus growth responses from polar to tropical waters–outcome of a scientific community-wide study. PLoS One 8 (5), e63091. https://doi.org/10.1371/journal.pone.006 3091.
Bozzato, D., Jakob, T., Wilhelm, C., 2019. Effects of temperature and salinity on respiratory losses and the ratio of photosynthesis to respiration in representative Antarctic phytoplankton species. PLoS One 14 (10), e0224101. https://doi.org/10.1371/journal.pone.0224101.
Bradley, I.M., Pinto, A.J., Guest, J.S., 2016. Design and evaluation of illumina MiSeq-compatible, 18S rRNA gene-specific primers for improved characterization of mixed phototrophic communities. Applied and Environmental Microbiology 82 (19), 5878–5891. https://doi.org/10.1128/AEM.01630-16.
Breton, E., Goberville, E., Sautour, B., Ouadi, A., Skouroliakou, D.-I., Seuront, L., et al., 2022. Multiple phytoplankton community responses to environmental change in a temperate coastal system: a trait-based approach. Frontiers in Marine Science 9. https://doi.org/10.3389/fmars.2022.9144 75.
Bruhn, C.S., Wohlrab, S., Krock, B., Lundholm, N., John, U., 2021. Seasonal plankton succession is in accordance with phycotoxin occurrence in Disko Bay, West Greenland. Harmful Algae 103. https://doi.org/10.1016/j.hal .2021.101978.
Bukovinszky, T., Verschoor, A.M., Helmsing, N.R., Bezemer, T.M., Bakker, E.S., Vos, M., et al., 2012. The good, the bad and the plenty: interactive effects of food quality and quantity on the growth of different daphnia species. PLoS One 7 (9), e42966. https://doi.org/10.1371/journal.pone. 0042966.
Callahan, B.J., McMurdie, P.J., Rosen, M.J., Han, A.W., Johnson, A.J., Holmes, S.P., 2016. DADA2: high-resolution sample inference from Illumina amplicon data. Nature Methods 13 (7), 581–583. https://doi.org/10.1038/nmeth.3869.
Chai, X., Zheng, L., Liu, J., Zhan, J., Song, L., 2023. Comparison of photosynthetic responses between haptophyte Phaeocystis globosa and diatom Skeletonema costatum under phosphorus limitation. Frontiers in Microbiology 14. https://doi.org/10.3389/fmicb.2023.1085176.
Chen, B., 2015a. Assessing the accuracy of the "two-point" dilution technique. Limnology and Oceanography: Methods 13 (10), 521–526. https://doi.org/10.1002/lom3.10044.
Chen, B., 2015b. Patterns of thermal limits of phytoplankton. Journal of Plankton Research 37 (2), 285–292. https://doi.org/10.1093/plankt/fbv009.
Chen, B., Laws, E.A., 2017. Is there a difference of temperature sensitivity between marine phytoplankton and heterotrophs? Limnology and Oceanography 62 (2), 806–817. https://doi.org/10.1002/lno.10462.
Chen, B., Landry, M.R., Huang, B., Liu, H., 2012. Does warming enhance the effect of microzooplankton grazing on marine phytoplankton in the ocean? Limnology and Oceanography 57 (2), 519–526. https://doi.org/10.4319/lo.2012.57.2.0519.
Conway, D.V.P., 2012. Identification of the copepodite developmental stages of twenty-six North Atlantic copepods. In: Occasional Publication of the Marine Biological Association.
De Senerpont Domis, L.N., Van de Waal, D.B., Helmsing, N.R., Van Donk, E., Mooij, W. M., 2014. Community stoichiometry in a changing world: combined effects of warming and eutrophication on phytoplankton dynamics. Ecology 95 (6), 1485–1495. https://doi.org/10.1890/13-1251.1.
Denny, M., 2017. The fallacy of the average: on the ubiquity, utility and continuing novelty of Jensen's inequality. The Journal of Experimental Biology 220 (Pt 2), 139–146. https://doi.org/10.1242/jeb.140368.
Di Pane, J., Wiltshire, K.H., McLean, M., Boersma, M., Meunier, C.L., 2022. Environmentally induced functional shifts in phytoplankton and their potential consequences for ecosystem functioning. Global Change Biology 28 (8), 2804–2819. https://doi.org/10.1111/gcb.16098.
Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. Deep Sea Research Part A. Oceanographic Research Papers 34 (10), 1733–1743. https://doi.org/10.1016/0198-0149(87)90021-5.
Dutkiewicz, S., Scott, J.R., Follows, M.J., 2013. Winners and losers: ecological and biogeo- chemical changes in a warming ocean. Global Biogeochemical Cycles 27 (2), 463–477. https://doi.org/10.1002/gbc.20042.
Edullantes, B., Low-Decarie, E., Steinke, M., Cameron, T., 2023. Comparison of thermal traits between non-toxic and potentially toxic marine phytoplankton: implications to their responses to ocean warming. Journal of Experimental Marine Biology and Ecology 562, 151883. https://doi.org/10.1016/j.jembe.2023.151883.
Eisenhauer, N., Hines, J., Maestre, F.T., Rillig, M.C., 2023. Reconsidering functional redundancy in biodiversity research. npj Biodiversity 2 (1), 9. https://doi.org/10.1038/s44185-023-00015-5.
Falkowski, P.G., Raven, J.A., 2007. Aquatic Photosynthesis, Second edition. Princeton University Press.
Fetzer, I., Johst, K., Schäwe, R., Banitz, T., Harms, H., Chatzinotas, A., 2015. The extent of functional redundancy changes as species' roles shift in different environments. Proceedings of the National Academy of Sciences 112 (48), 14888–14893. https://doi.org/10.1073/pnas.1505587112.
Freibott, A., Taylor, A.G., Selph, K.E., Liu, H., Zhang, W., Landry, M.R., 2016. Biomass and composition of protistan grazers and heterotrophic bacteria in the Costa Rica dome during summer 2010. Journal of Plankton Research 38 (2), 230–243. https://doi.org/10.1093/plankt/fbv107.
Frost, P.C., Pearce, N.J.T., Berger, S.A., Gessner, M.O., Makower, A.K., Marzetz, V., et al., 2023. Interactive effects of nitrogen and phosphorus on growth and stoichiometry of lake phytoplankton. Limnology and Oceanography 68 (5), 1172–1184. https://doi.org/10.1002/lno.12337.
Gall, A., Uebel, U., Ebensen, U., Hillebrand, H., Meier, S., Singer, G., et al., 2017. Planktotrons: a novel indoor mesocosm facility for aquatic biodiversity and food web research. Limnology and Oceanography: Methods 15 (7), 663–677. https://doi.org/10.1002/lom3.10196.
Garcia, F.C., Bestion, E., Warfield, R., Yvon-Durocher, G., 2018. Changes in temperature alter the relationship between biodiversity and ecosystem functioning. Proceedings of the National Academy of Sciences of the United States of America 115 (43), 10989–10999. https://doi.org/10.1073/pnas.1805518115.
Gerhard, M., Koussoroplis, A.-M., Raatz, M., Pansch, C., Fey, S.B., Vajedsamiei, J., et al., 2023. Environmental variability in aquatic ecosystems: avenues for future multifactorial experiments. Limnology and Oceanography Letters 8 (2), 247–266. https://doi.org/10.1002/lol2.10286.
Gibert, J.P., 2019. Temperature directly and indirectly influences food web structure. Scientific Reports 9 (1), 5312. https://doi.org/10.1038/s41598-019-41783-0.
Giesler, J.K., Harder, T., Wohlrab, S., 2023. Microbiome and photoperiod interactively determine thermal sensitivity of polar and temperate diatoms. Biology Letters 19 (11), 20230151. https://doi.org/10.1098/rsbl.2023.0151.
González-Gil, R., Banas, N.S., Bresnan, E., Heath, M.R., 2022. The onset of the spring phyto- plankton bloom in the coastal North Sea supports the disturbance recovery hypothesis. Biogeosciences 19 (9), 2417–2426. https://doi.org/10.5194/bg-19-2417-2022.
Grizzetti, B., Bouraoui, F., Aloe, A., 2012. Changes of nitrogen and phosphorus loads to European seas. Global Change Biology 18 (2), 769–782. https://doi.org/10.1111/j.1365-2486.2011. 02576.x.
Guillou, L., Bachar, D., Audic, S., Bass, D., Berney, C., Bittner, L., et al., 2013. The Protist ribosomal reference database (PR2): a catalog of unicellular eukaryote small sub-unit rRNA sequences with curated taxonomy. Nucleic Acids Research 41 (Database issue), D597–D604. https://doi.org/10.1093/nar/gks1160.
Guo, K., Gao, P., 2022. microbial: Do 16s Data Analysis and Generate Figures.
Happe, A., Ahme, A., Cabrerizo, M.J., Gerhard, M., John, U., Striebel, M., 2024. Do the Rate of Temperature Change and Timing of Nutrient Availability Impact the Growth and Stoichio- Metry of a Natural Marine Phytoplankton Community? (under review).
Heidrich, V., Karlovsky, P., Beule, L., 2021. 'SRS' R package and 'q2-SRS' QIIME 2 plugin: normalization of microbiome data using scaling with ranked subsampling (SRS). Applied Sciences 11 (23), 11473. https://doi.org/10.3390/app112311473.
Henriksen, P., Knipschildt, F., Moestrup, Ø., Thomsen, H.A., 1993. Autecology, life history and toxicology of the silicoflagellate Dictyocha speculum (Silicoflagellata, Dictyochophyceae). Phycologia 32 (1), 29–39. https://doi.org/10.2216/i0031-8884-32-1-29.1.
Henson, S.A., Cael, B.B., Allen, S.R., Dutkiewicz, S., 2021. Future phytoplankton diversity in a changing climate. Nature Communications 12 (1), 5372. https://doi.org/10.1038/s41467-021-25699-w.
Hoppe, C.J.M., Wolf, K.K.E., Schuback, N., Tortell, P.D., Rost, B., 2018. Compensation of ocean acidification effects in Arctic phytoplankton assemblages. Nature Climate Change 8 (6), 529–533. https://doi.org/10.1038/s41558-018-0142-9.
Huertas, I.E., Rouco, M., López-Rodas, V., Costas, E., 2011. Warming will affect phytoplankton differently: evidence through a mechanistic approach. Proceedings of the Royal Society B: Biological Sciences 278 (1724), 3534–3543. https://doi.org/10.1098/rspb.2011.0160.
Huisman, J., Weissing, F.J., 2001. Biological conditions for oscillations and chaos generated by multispecies competition. Ecology 82 (10), 2682–2695. https://doi.org/10.1890/0012-9658(2001)082[2682:BCFOAC]2.0.CO;2.
IPCC, 2021. In: Masson-Delmotte, V., Zhai, P., Pirani, A., Connors, S.L., Péan, C., Berger, S., Caud, N., Chen, Y., Goldfarb, L., Gomis, M.I., Huang, M., Leitzell, K., Lonnoy, E., Matthews, J.B.R., Maycock, T.K., Waterfield, T., Yelekçi, O., Yu, R., Zhou, B. (Eds.), Climate Change 2021: The Physical Science Basis. Contribution of

Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change.
Käse, L., Kraberg, A.C., Metfies, K., Neuhaus, S., Sprong, P.A.A., Fuchs, B.M., et al., 2020. Rapid succession drives spring community dynamics of small protists at Helgoland roads, North Sea. Journal of Plankton Research 42 (3), 305–319. https://doi.org/10.1093/plankt/fbaa017.
Kassambara, A., 2021. rstatix: pipe-friendly framework for basic statistical tests.
Kling, J.D., Lee, M.D., Fu, F., Phan, M.D., Wang, X., Qu, P., et al., 2020. Transient exposure to novel high temperatures reshapes coastal phytoplankton communities. ISME 14 (2), 413–424. https://doi.org/10.1038/s41396-019-0525-6.
Kremer, C.T., Thomas, M.K., Litchman, E., 2017. Temperature- and size-scaling of phytoplankton population growth rates: reconciling the Eppley curve and the metabolic theory of ecology. Limnology and Oceanography 62 (4), 1658–1670. https://doi.org/10.1002/lno.10523.
Landry, M.R., Hassett, R.P., 1982. Estimating the grazing impact of marine micro-zooplankton. Marine Biology 67, 283–288. https://doi.org/10.1007/BF00397668.
Landry, M.R., Selph, K.E., Hood, R.R., Davies, C.H., Beckley, L.E., 2022. Low temperature sensitivity of picophytoplankton P: B ratios and growth rates across a natural 10°C tempera- ture gradient in the oligotrophic Indian Ocean. Limnology and Oceanography Letters 7, 112–121. https://doi.org/10.1002/lol2.10224.
Laufkötter, C., Vogt, M., Gruber, N., Aita-Noguchi, M., Aumont, O., Bopp, L., et al., 2015. Drivers and uncertainties of future global marine primary production in marine ecosystem models. Biogeosciences 12 (23), 6955–6984. https://doi.org/10.5194/bg-12-6955-2015.
Lewandowska, A.M., Boyce, D.G., Hofmann, M., Matthiessen, B., Sommer, U., Worm, B., 2014. Effects of sea surface warming on marine plankton. Ecology Letters 17 (5), 614–623. https://doi.org/10.1111/ele.12265.
Liang, Y., Guo, H., Liao, Q., Zhang, X., Huang, K., 2020. Growth performance, phenotypic traits, and antioxidant responses of the rotifer Brachionus plicatilis under different proportions of Phaeocystis globosa. Ecotoxicology and Environmental Safety 202, 110963. https://doi.org/10.1016/j.ecoenv.2020.110963.
López-Sandoval, D.C., Rodríguez-Ramos, T., Cermeño, P., Sobrino, C., Marañón, E., 2014. Photosynthesis and respiration in marine phytoplankton: relationship with cell size, taxonomic affiliation, and growth phase. Journal of Experimental Marine Biology and Ecology 457, 151–159. https://doi.org/10.1016/j.jembe.2014.04.013.
Martin, M., 2011. Cutadapt removes adapter sequences from high-throughput sequencing reads. Next Generation Sequencing Data Analysis 17 (1). https://doi.org/10.14806/ej.17.1.200.
Matsumoto, K., Tanioka, T., Rickaby, R., 2020. Linkages between dynamic phytoplankton c:N:P and the ocean carbon cycle under climate change. Oceanography 33 (2), 44–52. https://doi.org/10.5670/oceanog.2020.203.
McKew, B.A., Metodieva, G., Raines, C.A., Metodiev, M.V., Geider, R.J., 2015. Acclimation of Emiliania huxleyi (1516) to nutrient limitation involves precise modification of the proteome to scavenge alternative sources of N and P. Environmental Microbiology 17 (10), 4050–4062. https://doi.org/10.1111/1462-2920.12957.
McMurdie, P.J., Holmes, S., 2013. Phyloseq: an R package for reproducible interactive analysis and graphics of microbiome census data. PLoS One 8 (4), e61217.
Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicx, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. Limnology and Oceanography 18 (6), 897–907. https://doi.org/10.4319/lo.1973.18.6.0897.
Meyer, S.T., Ptacnik, R., Hillebrand, H., Bessler, H., Buchmann, N., Ebeling, A., Eisenhauer, N., Engels, C., Fischer, M., Halle, S., Klein, A.-M., Oelmann, Y., Roscher, C., Rottstock, T., Scherber, C., Scheu, S., Schmid, B., Schulze, E.-D., Temperton, V.M., Tscharntke, T., Voigt, W., Weigelt, A., Wilcke, W., Weisser, W.W., 2018. Biodiversity–multifunctionality relationships depend on identity and number of measured functions. Nature Ecology & Evolution 2, 44–49.
Moreno, H.D., Köring, M., Di Pane, J., Tremblay, N., Wiltshire, K.H., Boersma, M., et al., 2022. An integrated multiple driver mesocosm experiment reveals the effect of global change on planktonic food web structure. Communications Biology 5 (1), 179. https://doi.org/10.1038/s42003-022-03105-5.
Mori, C., Beck, M., Striebel, M., Merder, J., Schnetger, B., Dittmar, T., et al., 2021. Biogeochemical cycling of molybdenum and thallium during a phytoplankton summer bloom: a mesocosm study. Marine Chemistry 229, 103910. https://doi.org/10.1016/j.marchem.2020.103910.
Müller, M.N., Blanco-Ameijeiras, S., Stoll, H.M., Mendez-Vicente, A., Lebrato, M., 2021. Temperature induced physiological reaction norms of the coccolithophore Gephyrocapsa oceanica and resulting coccolith Sr/ca and mg/ca ratios. Frontiers in Earth Science 9. https://doi.org/10.3389/feart.2021.582521.
Naselli-Flores, L., Padisák, J., 2023. Ecosystem services provided by marine and freshwater phytoplankton. Hydrobiologia 850 (12), 2691–2706. https://doi.org/10.1007/s10750-022-04795-y.
Nejstgaard, J.C., Tang, K.W., Steinke, M., Dutz, J., Koski, M., Antajan, E., , et al.van Leeuwe, M.A., Stefels, J., Belviso, S., Lancelot, C., Verity, P.G., Gieskes, W.W.C., 2007. Zooplankton grazing on Phaeocystis: a quantitative review and future challenges. In: Phaeocystis, Major Link in the Biogeochemical Cycling of Climate-Relevant Elements. Springer Netherlands, Dordrecht, pp. 147–172.
Oliver, E.C.J., Burrows, M.T., Donat, M.G., Sen Gupta, A., Alexander, L.V., Perkins-Kirkpatrick, S.E., et al., 2019. Projected marine heatwaves in the 21st century and the potential for ecological impact. Frontiers in Marine Science 6. https://doi.org/10.3389/fmars.2019.00734.
Ortiz-Burgos, S., 2016. Shannon-weaver diversity index. In: Kennish, M.J. (Ed.), Encyclopedia of Estuaries. Springer Netherlands, Dordrecht, pp. 572–573.
Padfield, D., O'Sullivan, H., 2022. rTPC: functions for fitting thermal performance curves. R package version 1.0.2. https://github.com/padpadpadpad/rTPC.
Pálffy, K., Kovács, A.W., Kardos, V., Hausz, I., Boros, G., 2021. Elevated temperature results in higher compositional variability of pioneer phytoplankton communities in a mesocosm system. Journal of Plankton Research 43 (2), 142–155. https://doi.org/10.1093/plankt/fbab013.
Pielou, E.C., 1966. The measurement of diversity in different types of biological collections. Journal of Theoretical Biology 13, 131–144. https://doi.org/10.1016/0022-5193(66)90013-0.
Pierrot, D., Wallace, D.W.R., Lewis, E.R., Pierrot, D., Wallace, R., Wallace, D.W.R., et al., 2011. MS excel program developed for CO2 system calculations.
Quinn, T., Richardson, M.F., Lovell, D., Crowley, T., 2017. Propr: an R-package for identifying proportionally abundant features using compositional data analysis. Scientific Reports 7, 16252. https://doi.org/10.1038/s41598-017-16520-0.
Ramond, P., Siano, R., Sourisseau, M., 2018. Functional Traits of Marine Protists. SEANOE. https://doi.org/10.17882/51662.
Ramond, P., Sourisseau, M., Simon, N., Romac, S., Schmitt, S., Rigaut-Jalabert, F., et al., 2019. Coupling between taxonomic and functional diversity in protistan coastal communities. Environmental Microbiology 21 (2), 730–749. https://doi.org/10.1111/1462-2920.14537.
Raven, J.A., Geider, R.J., 1988. Temperature and algal growth. The New Phytologist 110 (4), 441–461. https://doi.org/10.1111/j.1469-8137.1988.tb00282.x.
RCoreTeam, 2022. R: A Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/.
Rehder, L., Rost, B., Rokitta, S.D., 2023. Abrupt and acclimation responses to changing temperature elicit divergent physiological effects in the diatom Phaeodactylum tricornutum. The New Phytologist 239 (3), 1005–1013. https://doi.org/10.1111/nph.18982.
Robinson, K.L., Sponaugle, S., Luo, J.Y., Gleiber, M.R., Cowen, R.K., 2021. Big or small, patchy all: resolution of marine plankton patch structure at micro- to submesoscales for 36 taxa. Science Advances 7 (47), eabk2904. https://doi.org/10.1126/sciadv.abk2904.
Rogers, T.L., Johnson, B.J., Munch, S.B., 2022. Chaos is not rare in natural ecosystems. Nature Ecology & Evolution 6 (8), 1105–1111. https://doi.org/10.1038/s41559-022-01787-y.
Rönn, L., Antonucci di Carvalho, J., Blauw, A., Hillebrand, H., Kerimoglu, O., Lenhart, H., et al., 2023. Harmonisation of the phytoplanktonassessment in the German and Dutch Wadden Sea. Interreg V A project "Wasserqualität-Waterkwaliteit" - Synthesis Report.
RStudioTeam, 2022. RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA. http://www.rstudio.com/.
Sánchez-Benítez, A., Goessling, H., Pithan, F., Semmler, T., Jung, T., 2022. The July 2019 european heat wave in a warmer climate: storyline scenarios with a coupled model using spectral nudging. Journal of Climate 35 (8), 2373–2390. https://doi.org/10.1175/JCLI-D-21-0573.1.
Sanz-Martín, M., Vernet, M., Cape, M.R., Mesa, E., Delgado-Huertas, A., Reigstad, M., et al., 2019. Relationship between carbon- and oxygen-based primary productivity in the Arctic Ocean, Svalbard archipelago. Frontiers in Marine Science 6. https://doi.org/10.3389/fmars.2019.00468.
Sarker, S., Yadav, A.K., Akter, M., Shahadat Hossain, M., Chowdhury, S.R., Kabir, M.A., et al., 2020. Rising temperature and marine plankton community dynamics: is warming bad? Ecological Complexity 43, 100857. https://doi.org/10.1016/j.ecocom.2020.100857.
Serra-Pompei, C., Hagstrom, G.I., Visser, A.W., Andersen, K.H., 2019. Resource limitation determines temperature response of unicellular plankton communities. Limnology and Oceanography 64 (4), 1627–1640. https://doi.org/10.1002/lno.11140.
Smith, W.O., Trimborn, S., 2024. Phaeocystis: a global enigma. Annual Review of Marine Science 16 (1), 417–441. https://doi.org/10.1146/annurev-marine-022223-025031.
Staehr, P.A., Sand-Jensen, K., 2006. Seasonal changes in temperature and nutrient control of photosynthesis, respiration and growth of natural phytoplankton communities. Freshwater Biology 51 (2), 249–262. https://doi.org/10.1111/j.1365-2427.2005.01490.x.
Stelfox-Widdicombe, C.E., Archer, S.D., Burkill, P.H., Stefels, J., 2004. Microzooplankton grazing in Phaeocystis and diatom-dominated waters in the southern North Sea in spring. Journal of Sea Research 51 (1), 37–51. https://doi.org/10.1016/j.seares.2003.04.004.
Striebel, M., Schabhüttl, S., Hodapp, D., Hingsamer, P., Hillebrand, H., 2016. Phytoplankton responses to temperature increases are constrained by abiotic conditions and community composition. Oecologia 182 (3), 815–827. https://doi.org/10.1007/s00442-016-3693-3.
Sunday, J.M., Bernhardt, J.R., Harley, C.D.G., O'Connor, M.I., 2023. Temperature dependence of competitive ability is cold-shifted compared to that of growth rate in marine phytoplankton. Ecology Letters n/a (n/a). https://doi.org/10.1111/ele.14337.
Thomas, M.K., Kremer, C.T., Klausmeier, C.A., Litchman, E., 2012. A global pattern of thermal adaptation in marine phytoplankton. Science 338 (6110), 1085–1088. https://doi.org/10.1126/science.1224836.
Thomas, M.K., Aranguren-Gassis, M., Kremer, C.T., Gould, M.R., Anderson, K., Klausmeier, C.A., et al., 2017. Temperature–nutrient interactions exacerbate sensitivity to warming in phytoplank- ton. Global Change Biology 23 (8), 3269–3280. https://doi.org/10.1111/gcb.13641.
Thomas, P.K., Kunze, C., Van de Waal, D.B., Hillebrand, H., Striebel, M., 2022. Elemental and biochemical nutrient limitation of zooplankton: a meta-analysis. Ecology Letters 0 (0), 1–17. https://doi.org/10.1111/ele.14125.
Thrane, J.-E., Kyle, M., Striebel, M., Haande, S., Grung, M., Rohrlack, T., et al., 2015. Spectro- photometric analysis of pigments: a critical assessment of a high-throughput

method for analysis of algal pigment mixtures by spectral deconvolution. PLoS One 10 (9), e0137645. https://doi.org/10.1371/journal.pone.0137645.
de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., et al., 2015. Eukaryotic plankton diversity in the sunlit ocean. Science 348 (6237), 1261605. https://doi.org/10.1126/science.1261605.
Veldhuis, M.J.W., Colijn, F., Admiraal, W., 1991. Phosphate utilization in Phaeocystis pouchetii (haptophyceae). Marine Ecology 12 (1), 53–62. https://doi.org/10.1111/j.1439-0485.1991.tb00083.x.
Verbeek, L., Gall, A., Hillebrand, H., Striebel, M., 2018. Warming and oligotrophication cause shifts in freshwater phytoplankton communities. Global Change Biology 24 (10), 4532–4543. https://doi.org/10.1111/gcb.14337.
van de Waal, D.B., Verschoor, A.M., Verspagen, J.M., van Donk, E., Huisman, J., 2010. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Frontiers in Ecology and the Environment 8 (3), 145–152. https://doi.org/10.1890/080178.
Wang, J., Zeng, C., Feng, Y., 2024. Meta-analysis reveals responses of coccolithophores and diatoms to warming. Marine Environmental Research 193, 106275. https://doi.org/10.1016/j.marenvres.2023.106275.
Wang, X., Tang, K.W., Wang, Y., Smith, W.O., 2010. Temperature effects on growth, colony development and carbon partitioning in three Phaeocystis species. Aquatic Biology 9 (3), 239–249. https://doi.org/10.3354/ab00256.
Wetzel, R.G., Likens, G.E., 2003. Limnological Analyses. Springer-Verlag, New York.
Wieczynski, D.J., Singla, P., Doan, A., Singleton, A., Han, Z.-Y., Votzke, S., et al., 2021. Linking species traits and demography to explain complex temperature responses across levels of organization. Proceedings of the National Academy of Sciences 118 (42), e2104863118. https://doi.org/10.1073/pnas.2104863118.
Wiltshire, K.H., 2008. Hydrochemistry at time series station Helgoland roads, North Sea, in 2007. PANGAEA. https://doi.org/10.1594/PANGAEA.683880.
Wiltshire, K.H., 2010. Hydrochemistry at time series station Helgoland roads, North Sea, in 2009. PANGAEA. https://doi.org/10.1594/PANGAEA.737388.
Wiltshire, K.H., Manly, B.F.J., 2004. The warming trend at Helgoland roads, North Sea: phytoplankton response. Helgoland Marine Research 58 (4), 269–273. https://doi.org/10.1007/s10152-004-0196-0.
Woods, H.A., Makino, W., Cotner, J.B., Hobbie, S.E., Harrison, J.F., Acharya, K., et al., 2003. Temperature and the chemical composition of poikilothermic organisms. Functional Ecology 17 (2), 237–245.
Yvon-Durocher, G., Caffrey, J.M., Cescatti, A., Dossena, M., Giorgio, P.D., Gasol, J.M., et al., 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. Nature 487 (7408), 472–476. https://doi.org/10.1038/nature11205.
Yvon-Durocher, G., Schaum, C.E., Trimmer, M., 2017. The temperature dependence of phytoplankton stoichiometry: investigating the roles of species sorting and local adaptation. Frontiers in Microbiology 8 (OCT). https://doi.org/10.3389/fmicb.2017.02003.
Zhang, Y., Chen, H.Y.H., Reich, P.B., 2012. Forest productivity increases with evenness, species richness and trait variation: a global meta-analysis. Journal of Ecology 100 (3), 742–749. https://doi.org/10.1111/j.1365-2745.2011.01944.x.
Zhong, D., Listmann, L., Santelia, M.E., Schaum, C.E., 2020. Functional redundancy in natural pico-phytoplankton communities depends on temperature and biogeography. Biology Letters 16 (8), 20200330. https://doi.org/10.1098/rsbl.2020.0330.

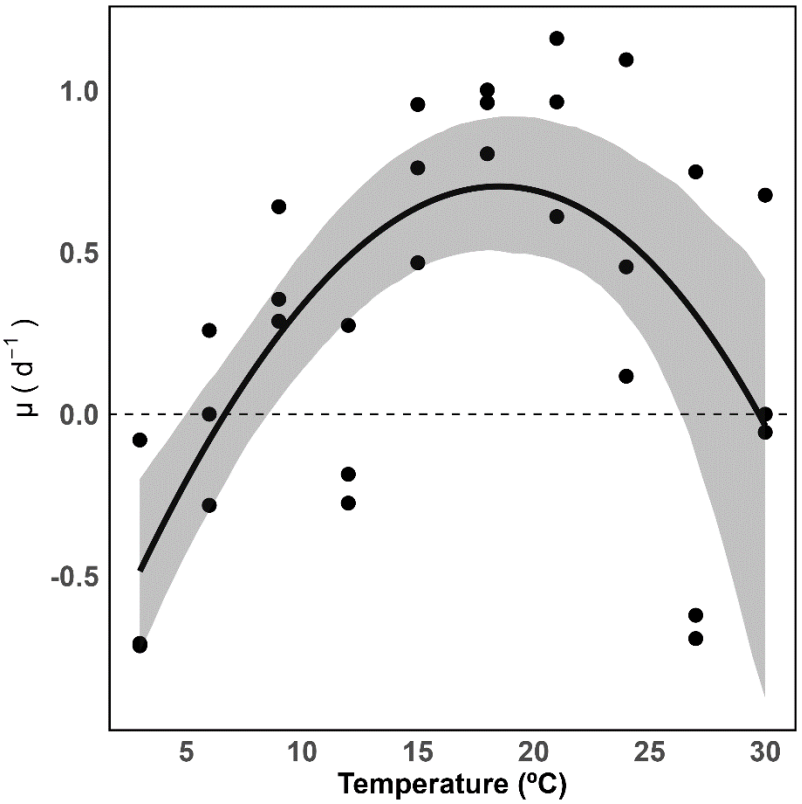

Figure S1: Thermal performance curve of the starting community with the growth rate (μ) across experimental temperatures. The line represents the fit by Thomas et al. (2017) and the grey shaded areas the 95% confidence interval predicted through bootstrapping.

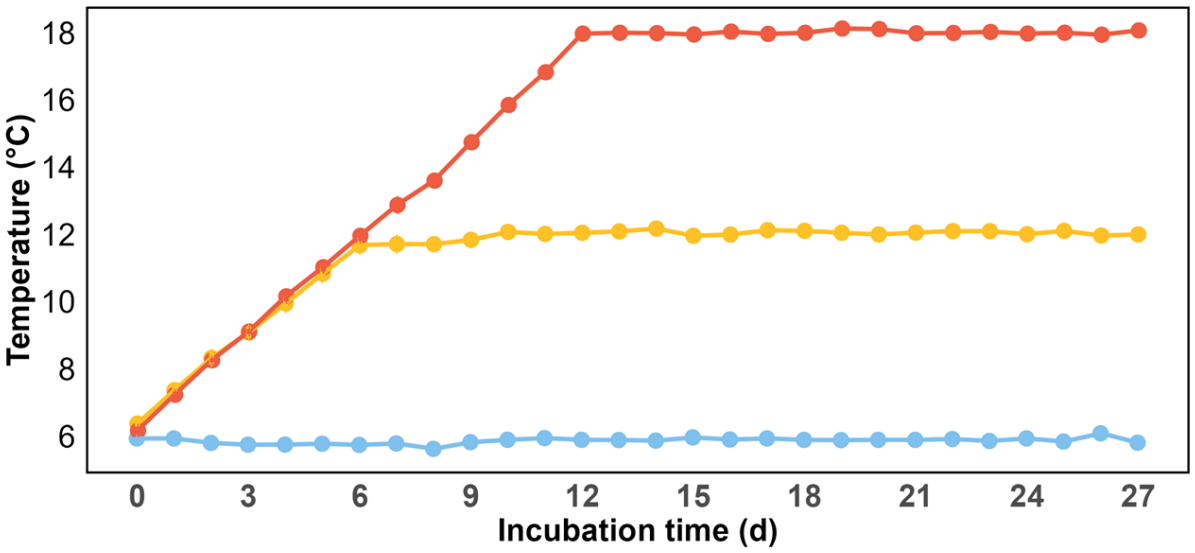

Figure S2: Daily measured temperature over time. Dots represent the arithmetic mean of the temperatures (6 °C: blue, 12 °C: yellow, 18 °C: red) and error bars the standard deviation.

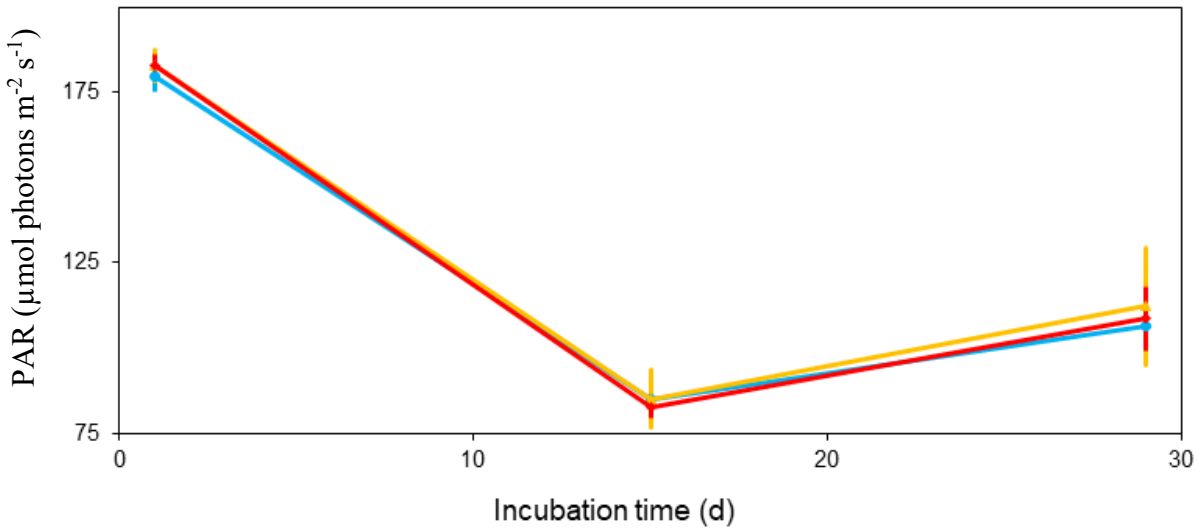

Figure S3: Photosynthetic active radiation (PAR) at 10 cm below the surface over time. Dots represent the arithmetic mean of the temperatures (6 °C: blue, 12 °C: yellow, 18 °C: red) and error bars the standard deviation.

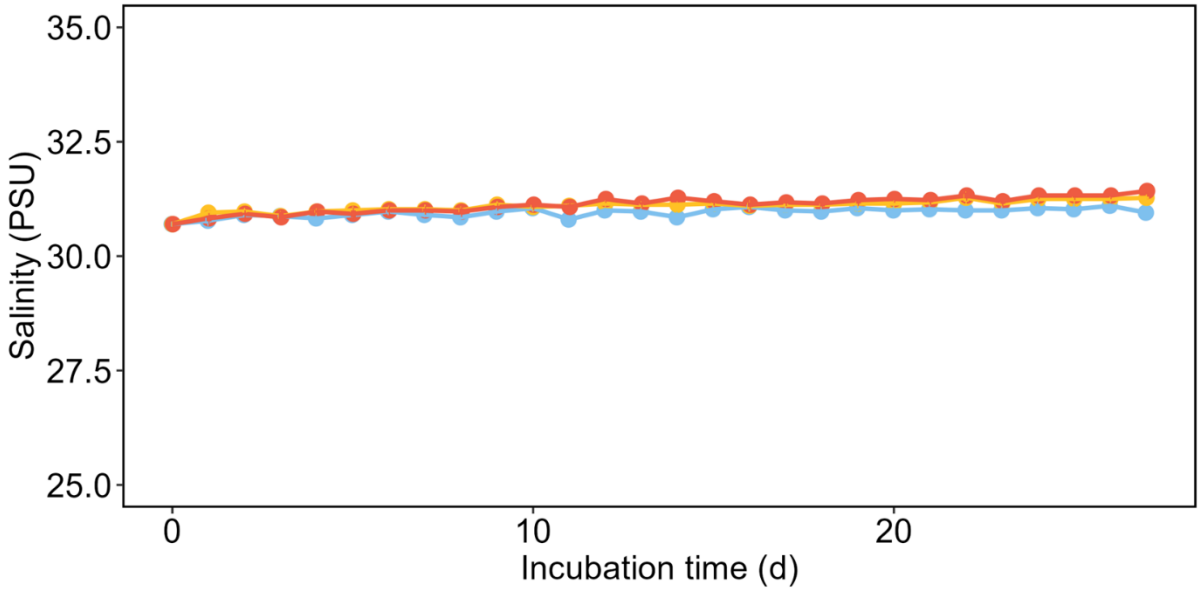

Figure S4: Daily measured salinity over time. Dots represent the arithmetic mean of the temperatures (6 °C: blue, 12 °C: yellow, 18 °C: red) and error bars the standard deviation.

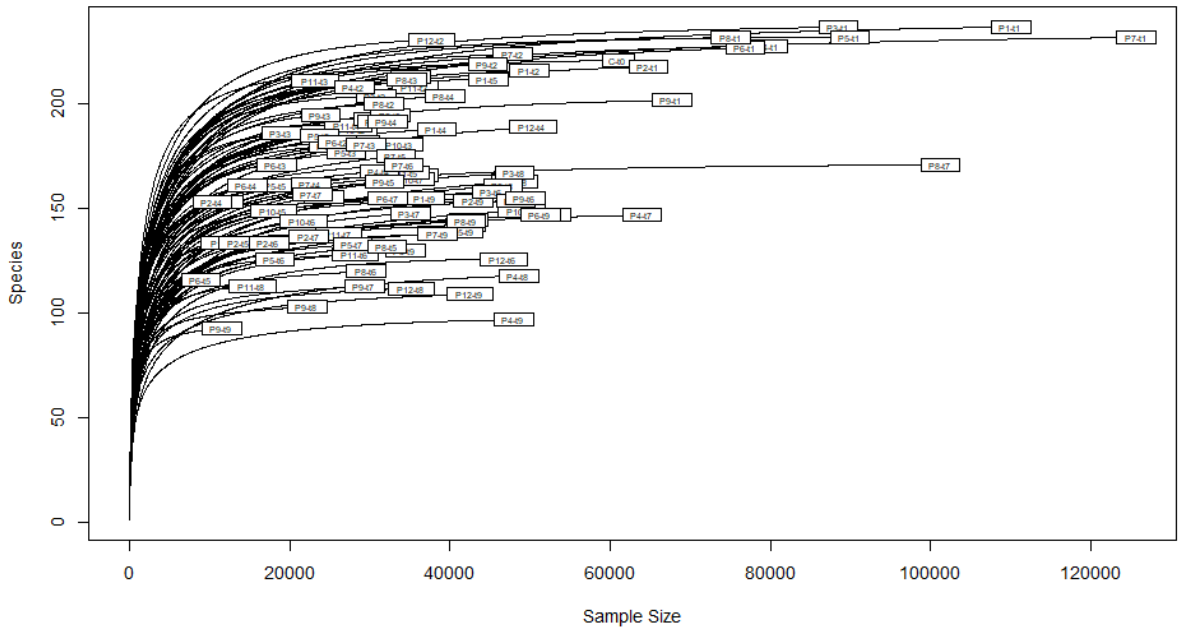

Figure S5: Rarefaction curves of the raw read counts for all samples.

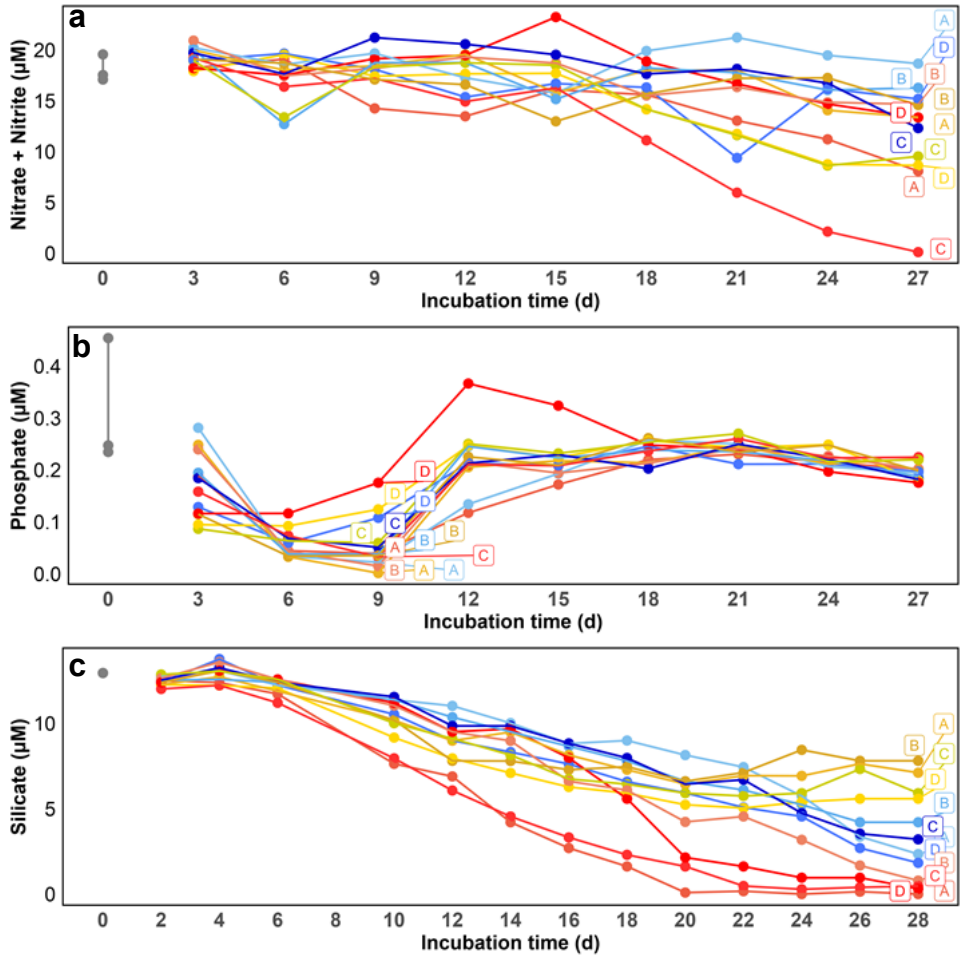

*Figure S6: Development of each replicate mesocosm (A-D) over time for (**a**) nitrate + nitrite, (**b**) phosphate, (**c**) silicate. Colours denote the temperature treatments (6 °C: blue, 12 °C: yellow, 18 °C: red).*

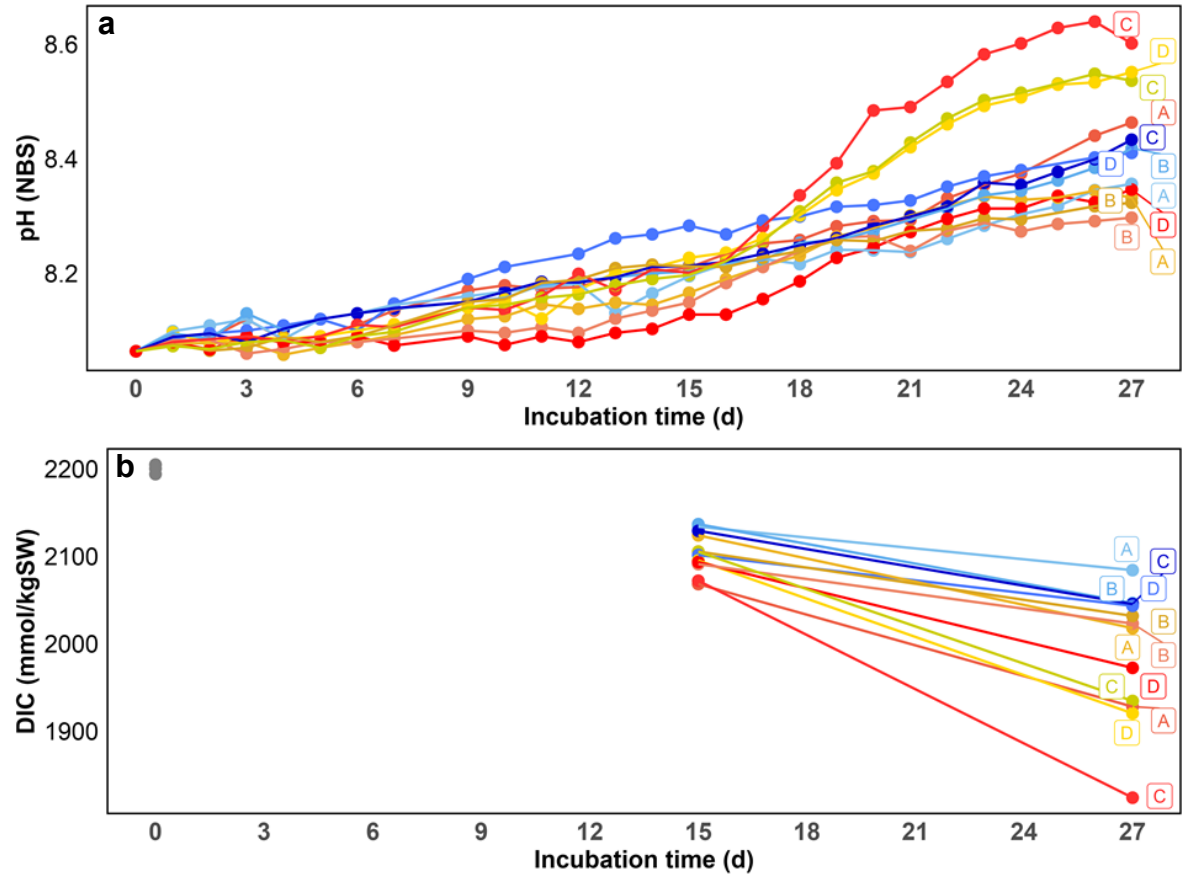

*Figure S7: Development of each replicate mesocosm (A-D) over time for (**a**) the pH and (**b**) dissolved inorganic carbon (DIC). Colours denote the temperature treatments (6 °C: blue, 12 °C: yellow, 18 °C: red, t0: grey).*

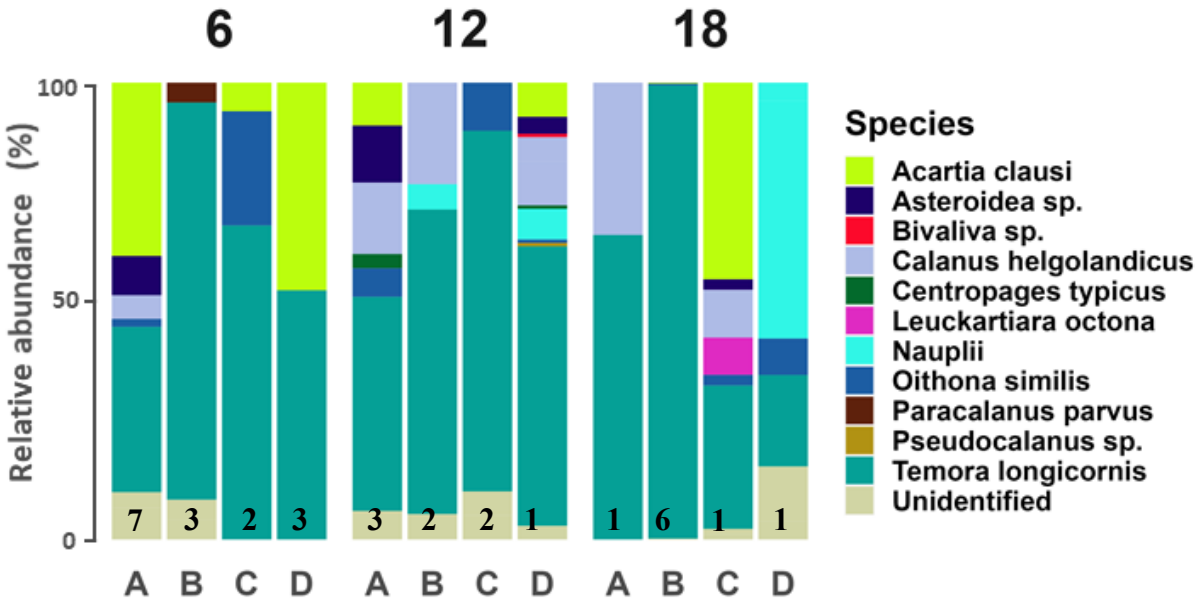

Figure S8: Microscopy-based mesozooplankton community composition and total mesozooplankton abundance L^{-1} (numbers on bar graphs) on species level at day 27 for all replicate mesocosms (A-D) of the temperature treatments (6, 12 and 18 °C).

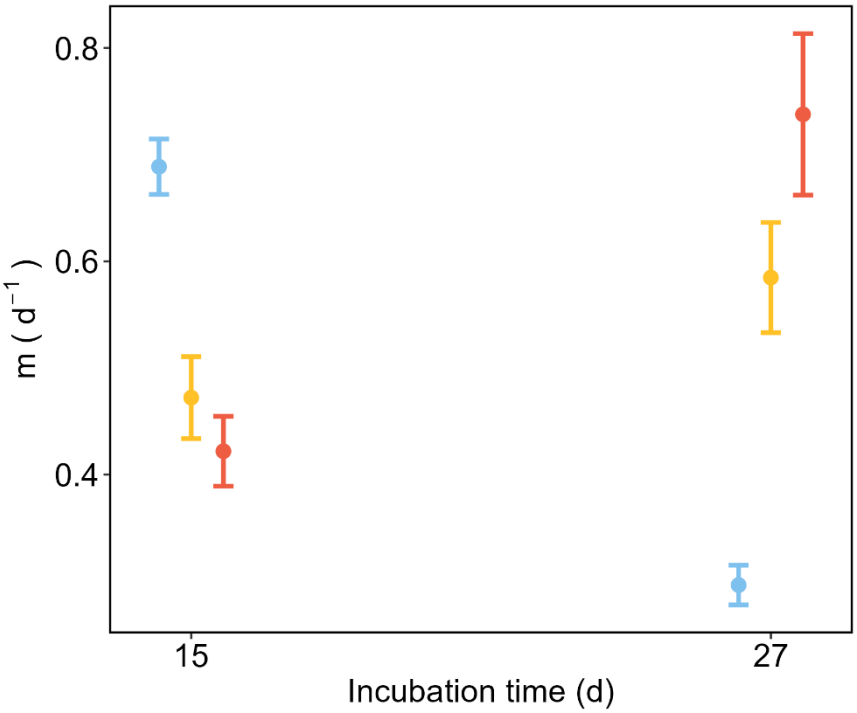

Figure S9: Pooled micro-grazing rates (m) per day at the start (incubation day 15) and the end (incubation day 27) of the experiment for the three treatment temperatures (blue = 6 °C, yellow = 12 °C, red = 18 °C). Dots represent the mean and error bars the standard deviation of the replicates.

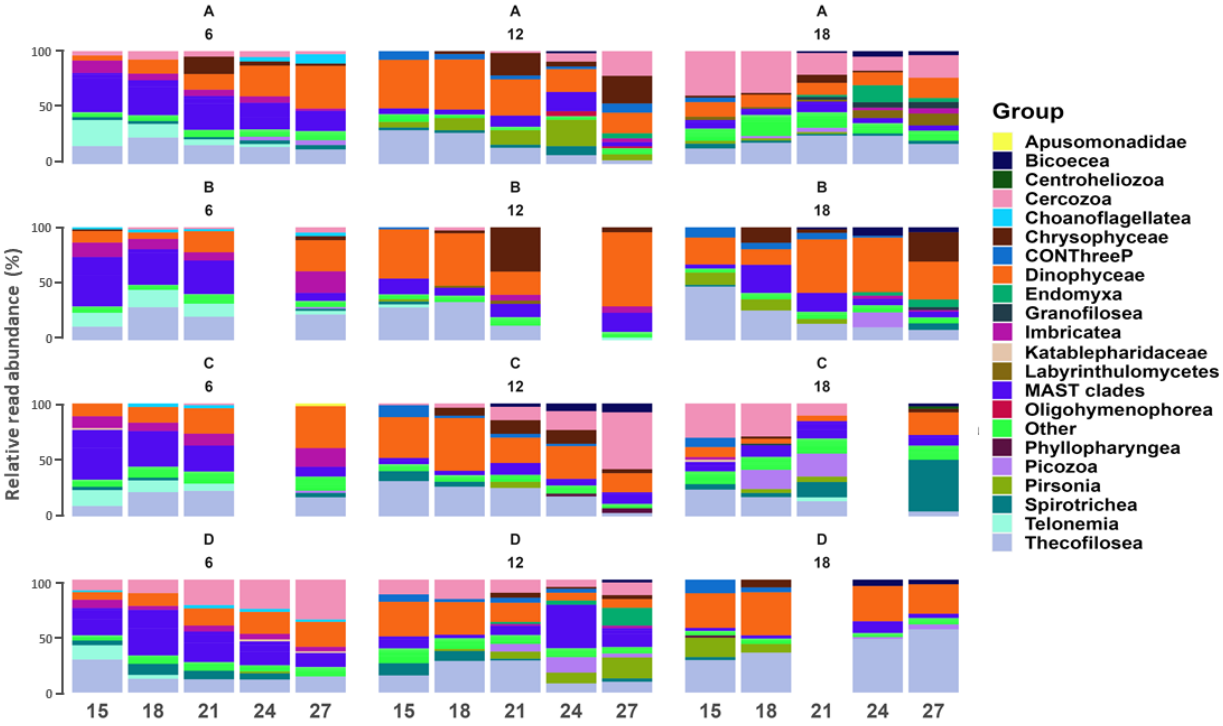

Figure S10: Metabarcoding-based heterotrophic protist community composition on phylum level over time for all replicates (horizontal alignment) and temperatures (vertical alignment). ASVs which could not be annotated were categorized as "other".

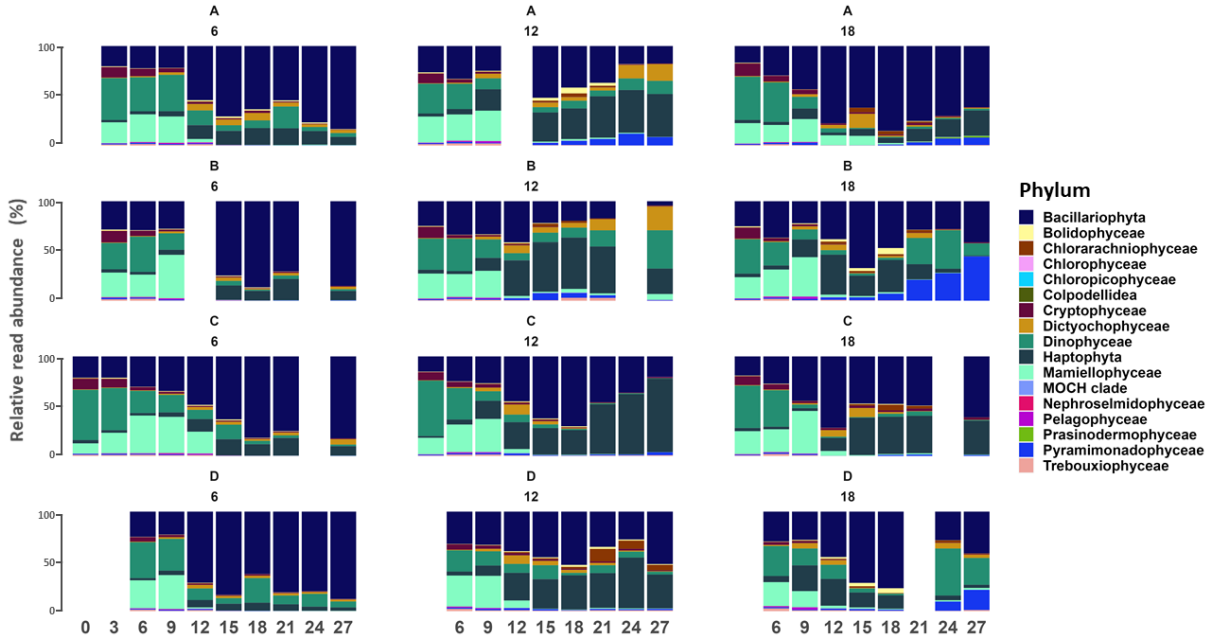

Figure S11: Metabarcoding-based phytoplankton community composition on phylum level over time for all replicates (horizontal alignment) and temperatures (vertical alignment). ASVs which could not be annotated were categorized as "other".

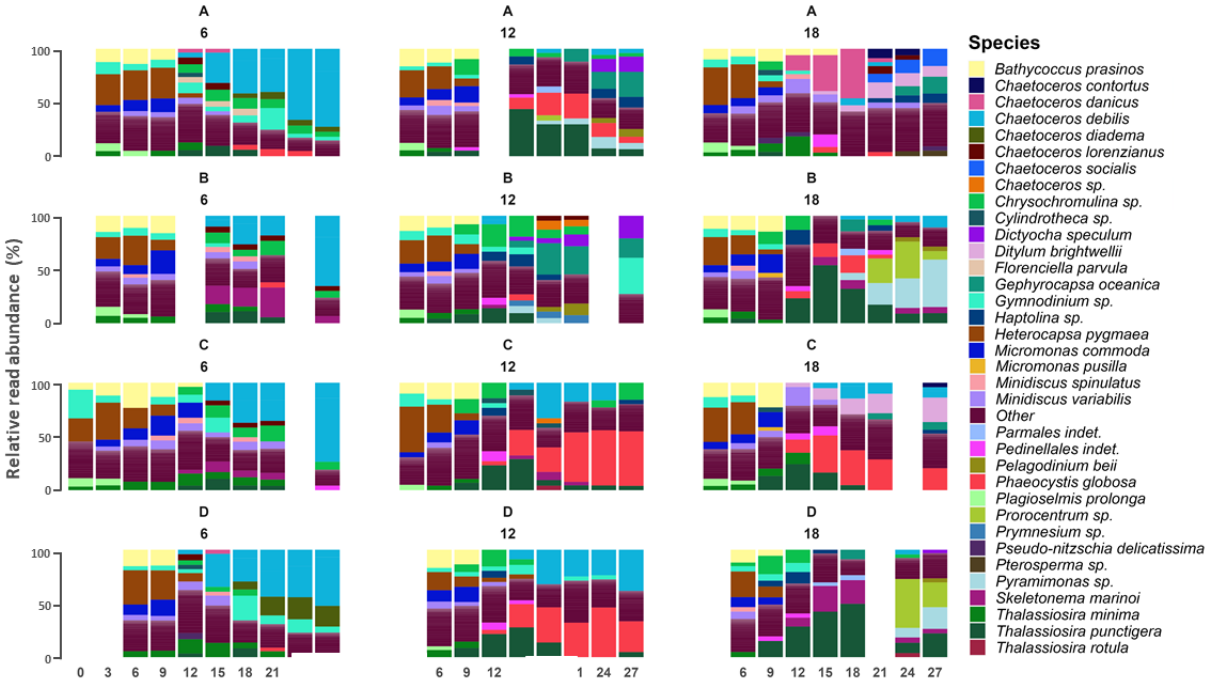

Figure S12: Metabarcoding-based phytoplankton community composition on species level over time for all replicates (horizontal alignment) and temperatures (vertical alignment). ASVs with an abundance of fewer than 200 reads among temperatures were categorized as "other".

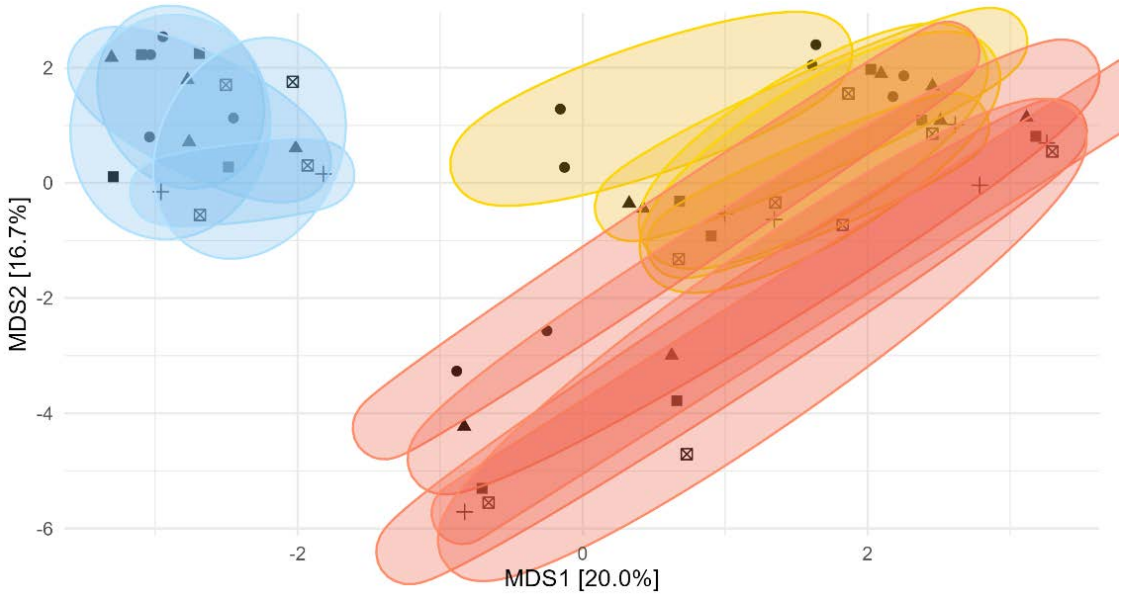

Figure S13: Principal component analysis (PCoA) using euclidean distances of the CLR-transformed ASV-based species composition at the different temperatures (blue = 6 °C; yellow = 12 °C; red = 18 °C) on all sampling days (circle = day 15; triangle = day 18; square = day 21; cross = day 24; cross in square = day 27) of the experiment, including 55 samples and 379 taxa. Ellipsoids are grouped per temperature and day.

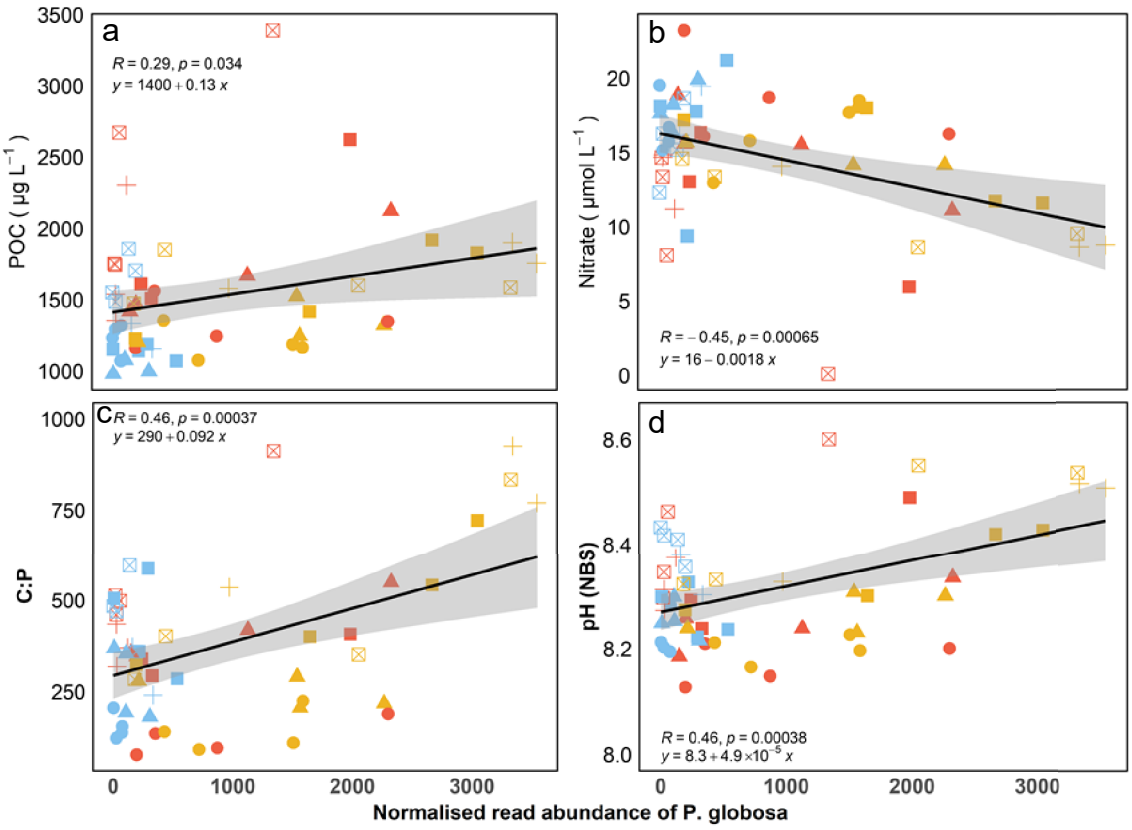

Figure S14: POC (a), nitrate (b), the C:P ratio (c) and pH (d) per normalised read abundance of Phaeocystis globosa of the different temperatures (blue = 6 °C; yellow = 12 °C; red = 18 °C) on all sampling days (circle = day 15; triangle = day 18; square = day 21; cross = day 24; cross in square = day 27) of the experiment. Fitted linear regressions with approximate 95% point-wise confidence intervals (grey-shaded areas).

Table S 1: Sequencing statistics from the DADA2 pipeline for all samples after each filtering step and the ratio of final reads to raw reads.

Sample	Raw	Primer/Quality-filtered	Denoised	Merged	Chimera-filtered
6°C C Day 0	425746	265061	264737	262032	259640
18°C A Day 3	405838	247022	246447	243937	239755
18°C A Day 6	160562	95963	95600	93431	90872
18°C A Day 9	102683	61644	61380	59906	58866
18°C A Day 12	142835	88403	87965	86194	84454
18°C A Day 15	134250	80175	79835	77258	75657
18°C A Day 18	86662	52945	52687	50639	49745
18°C A Day 21	93995	57148	56883	54475	54031
18°C A Day 24	139649	77712	77384	73608	72909
18°C A Day 27	115802	67453	67170	65011	64191
6°C D Day 6	101620	60386	60120	59052	57085
6°C D Day 9	131791	76415	76166	74695	72979
6°C D Day 12	99537	58427	58246	57235	56491
6°C D Day 15	169255	86546	86244	84321	82773
6°C D Day 18	117443	69041	68767	67349	66007
6°C D Day 21	155635	88882	88597	86654	84566
6°C D Day 24	147561	89048	88665	86671	84107
6°C D Day 27	145536	83179	82951	81274	78878
12°C D Day 6	138838	86583	86261	84553	82168
12°C D Day 9	122961	75312	74992	73469	71506
12°C D Day 12	133665	78459	78153	76698	75177
12°C D Day 15	118082	69209	68992	67710	66242
12°C D Day 18	116100	68284	68096	66863	64969
12°C D Day 21	102708	59095	58966	58024	57635
12°C D Day 24	120073	70615	70520	70004	68694
12°C D Day 27	111097	63771	63557	62116	60521
18°C D Day 6	135631	82357	82013	80353	78369
18°C D Day 9	135503	81081	80822	79305	77787
18°C D Day 12	157516	98183	97866	96133	93736
18°C D Day 15	125748	75330	75072	73541	71081
18°C D Day 18	106690	64888	64757	63904	62449
18°C D Day 24	115471	66265	65982	63528	62098
18°C D Day 27	148832	85134	84803	75721	73806
6°C A Day 3	307239	187409	187098	185232	183159
6°C A Day 6	119999	69097	68830	67706	66348
6°C A Day 9	141878	80111	79864	78415	76727
6°C A Day 12	106713	63144	62784	61476	60243
6°C A Day 15	186801	107411	106967	104628	100223
6°C A Day 18	111252	65288	65054	63802	62354
6°C A Day 21	98008	54593	54445	53537	52516
6°C A Day 24	152000	81374	81188	79932	77887
6°C A Day 27	116779	66817	66579	65365	63090
12°C A Day 3	456422	259825	259475	257458	253694
12°C A Day 6	100454	62083	61836	60783	59778

12°C A Day 9	110346	64363	64158	63359	62536
12°C A Day 15	126387	70925	70710	69692	67818
12°C A Day 18	115119	69438	69174	67733	65884
12°C A Day 21	103373	60236	60090	59018	57568
12°C A Day 24	156179	92955	92613	90401	88178
12°C A Day 27	104101	59451	59320	58509	57845
18°C B Day 3	376605	225059	224694	222779	219061
18°C B Day 6	122624	71025	70789	69781	68614
18°C B Day 9	159773	94340	93957	91774	89681
18°C B Day 12	110663	66770	66562	65450	64396
18°C B Day 15	127156	69998	69789	67099	65132
18°C B Day 18	121547	69327	69106	67571	65841
18°C B Day 21	175716	100953	100453	97120	91833
18°C B Day 24	135735	75061	74814	70642	69142
18°C B Day 27	125271	70848	70573	62528	60871
6°C B Day 3	414007	247889	247522	245381	242480
6°C B Day 6	114226	63945	63703	62569	61082
6°C B Day 9	96637	59888	59766	59115	58696
6°C B Day 15	136918	82285	82071	80276	78129
6°C B Day 18	105543	61796	61611	60337	59372
6°C B Day 21	125533	71498	71173	69487	67191
6°C B Day 27	142827	88894	88515	86203	82380
6°C C Day 3	317774	188965	188607	186618	182933
6°C C Day 6	108781	52841	52655	51625	50526
6°C C Day 9	90464	55488	55272	54388	53490
6°C C Day 12	99031	55578	55403	54537	53826
6°C C Day 15	62634	35897	35751	35010	34240
6°C C Day 18	133589	72373	72089	70545	68635
6°C C Day 21	149600	87522	87202	85243	82898
6°C C Day 27	146385	89992	89741	87675	84056
18°C C Day 3	368426	207634	207140	204327	201073
18°C C Day 6	180547	106326	105862	103219	98931
18°C C Day 9	103966	64664	64453	63314	62486
18°C C Day 12	95492	56775	56560	55639	54900
18°C C Day 15	115196	69015	68777	65616	64504
18°C C Day 18	150658	87754	87431	84225	82856
18°C C Day 21	133553	81400	81119	79673	78389
18°C C Day 27	110909	67327	67175	66370	65749
12°C B Day 3	366019	217754	217210	214702	209318
12°C B Day 6	129842	77721	77469	76263	74475
12°C B Day 9	129893	79099	78821	77230	75547
12°C B Day 12	194761	113334	112911	110306	106205
12°C B Day 15	126304	74598	74408	73478	71909
12°C B Day 18	99809	56551	56407	55862	55280
12°C B Day 21	394299	225346	224989	222553	218581
12°C B Day 27	141807	82718	82459	80410	78029
12°C C Day 3	434730	257556	257130	244469	236088
12°C C Day 6	144605	88861	88487	86557	84177

12°C C Day 9	108637	66463	66159	64564	63030
12°C C Day 12	126494	78525	78203	76641	74575
12°C C Day 15	100736	59221	59027	58098	56771
12°C C Day 18	115039	66469	66276	64998	63174
12°C C Day 21	79631	45682	45605	43922	43655
12°C C Day 24	93860	52975	52868	48776	48304
12°C C Day 27	86529	47376	47280	38509	38064

Table S 2: Results of the two-way rmANOVA regarding the effect of temperature, time, and their interactive effects on the mean beta-dispersions of the Aitchinson distances during the experiment phase. Dfn is the degree of freedom for the numerator of the F ratio, and DFd is for the denominator. Significant effects are highlighted in bold.

Parameter	Effect	DFn	DFd	*F*	*p*
6 °C – 12 °C	Temperature	1	6	3.466	.112
	Time	3	18	1.752	.192
	Temperature:Time	3	18	0.432	.733
6 °C – 18 °C	Temperature	1	5	12.241	**0.017**
	Time	1.21	6.04	0.004	0.969
	Temperature:Time	1.21	6.04	0.150	0.754

3.2: PUBLICATION III

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

Under review in Limnology & Oceanography

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, 12, and 18ºC), 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 set-ups lead to contrasting directions of outcome.

Introduction

Increasing temperature and changes in nutrient regimes are among the most prevalent abiotic pressures of the last decades (IPCC 2023; Malone and Newton 2020). Both drivers exert a strong impact on phytoplankton growth (Anderson et al. 2022; Thomas et al. 2017) 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 (Hessen et al. 2013; Sterner and Elser 2002) and the carbon export out of the pelagic zone (Kwiatkowski et al. 2018). Future scenarios predict various possible combinations of temperature and nutrient availability e.g., 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., Bestion et al. 2018; Boyd et al. 2015). 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) (Marañón et al. 2018; Thrane et al. 2017), 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×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 and supporting information S1, Fig. 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 (Paul et al. 2021) and 2.5 °C day^{-1} (Soulié et al. 2023), but more often as an abrupt temperature exposure (72/86 studies) i.e., directly placing the community on the experimental temperature below or above ambient conditions (e.g., Menden-Deuer et al. 2018; Moreau et al. 2014; Sommer and Lewandowska 2011). 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, +4 or +6 °C, but also up to a temperature of +11.8 °C compared to ambient conditions (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 (Fey et al. 2021; Kremer et al. 2018). 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 (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 e.g., 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 (Brooks and Crowe 2019; Gunderson et al. 2016). 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 nutria

ent 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 versus 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.

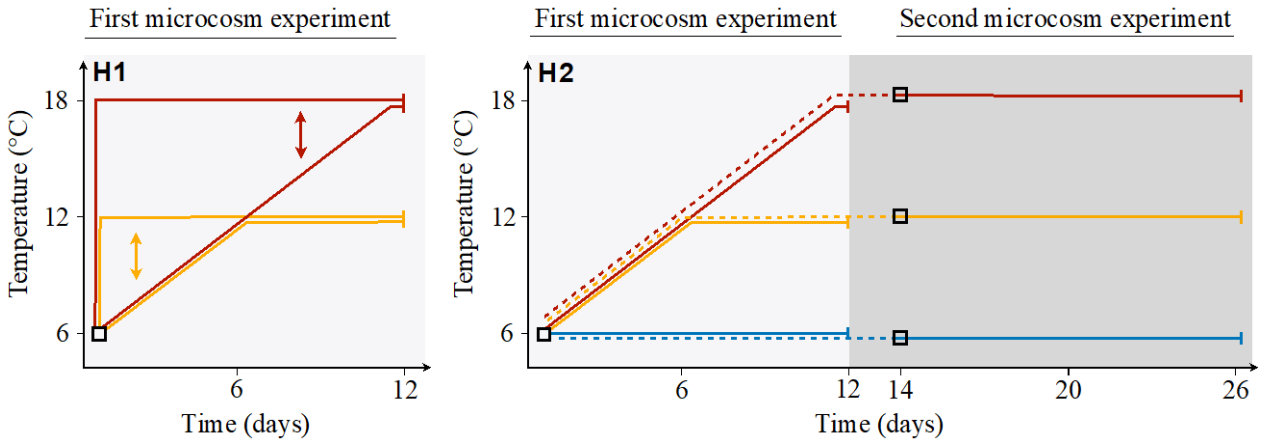

Figure 1: Conceptual overview of experimental temperature treatments applied for testing the hypotheses (H1, H2). The line colors represent the final temperatures at 6 (blue), 12 (orange), and 18 °C (red). For H1, an abrupt temperature exposure and a gradual temperature increase were applied. The black 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.

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.

Experimental Design

The initial plankton community originated from surface seawater 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 6th March 2022 at 05:00h (UTC) using a diaphragm pump and filtered through a 200-µm mesh to reduce mesozooplankton. The water was transported using eight 1000 L polyethylene Intermediate Bulk Containers (IBC, AUER Packaging GmbH, Amerang, Germany) 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 ± 0.13 µg chlorophyll-*a* L^{-1}.

The collected seawater was used to set up a mesocosm experiment (analysed in Ahme et al. 2024) and simultaneously run bottle incubations (microcosms) on the 8th of March 2022. The effect of two gradual temperature increase scenarios (12 and 18 °C in steps of 1 °C day^{-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 s^{-1} m^{-2} from LED units (IT2040, Evergrow, Shenzhen, China) and a day-night cycle of 12h:12h 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 and 18 °C) either as an abrupt exposure or as a gradual increase (1°C day^{-1}) and supplied with a wide gradient of N:P supply ratios (Table 1) as a unique pulse at the start of the incubation.

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.

	P (µmol L^{-1})				
N (µmol 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 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, 12 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 × 5 N levels × 5 P levels × 2 replicates). Both microcosm experiments were terminated after 12 days.

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 µmol N L^{-1} and 0.31 to 3.64 µmol 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, Wegberg, Germany). The ambient nutrient conditions were 0.31 µmol P L^{-1} and 18.07 µmol N L^{-1} for the first microcosm experiment (Table 1), but differed between the temperature levels at 6 °C (0.21 µmol P L^{-1}, 16.78 µmol N L^{-1}), 12 °C (0.20 µmol P L^{-1}, 11.48 µmol N L^{-1}) and 18 °C (0.20 µmol P L^{-1}, 18.58 µmol 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 ~ 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 days, the experiments were terminated and one replicate was filtered onto pre-combusted acid-washed glass microfiber filters (Whatman® GF/C, USA) 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, Walthman, MA, USA). The filters for particulate organic phosphorus (POP) were pre-combusted 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 of the respective starting communities was analyzed via V4 region of the 18S rRNA gene metabarcoding. Samples for DNA were taken and processed as described in (Ahme et al. 2023b, Fig. S2.3). The validity of the 18S rRNA metabarcoding was qualitatively post-evaluated via light microscopy screening using the method by Utermöhl (1958). The initial starting community was dominated by Dinophyceae with further abundant groups being Bacillariophyceae, Syndiniales, Cryptophyceae, Spirotrichea and Thecofilosea (Fig. S2.3). The thermal performance curve (TPC) of the start community (Ahme et al. 2023a) showed a thermal optimum at 18 °C (corresponding to the highest experimental temperature) and a temperature range between 7 and 29 °C (Fig. S2.4).

A temperature-dependent community shift has been observed after the community has experienced the gradual temperature increase in the mesocosms (thermal acclimation for the bottle incubations) under ambient nutrients. With increasing temperature level, the abundance of Bacillariophyceae increased and even dominated the highest temperature level. Instead, Dinophyceae made up a large fraction in the 12°C temperature level while they were also abundant in the other treatments in intermediate relative abundances. Furthermore, the 12 and 18°C treatments also showed an increased proportion of Haptophyceae (mostly *Phaeocystis globosa*) (Fig. S2.3).

Statistical Analyses

Linear growth rates μ (day^{-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 fluorescence at the chosen start (t0) and endpoint (t1) of the first experiment. The two points have been chosen as the exponential growth phase i.e., 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, 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 (Fig. SS.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 to 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 $\log10(\mu_1/\mu_2)$, 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 $\log10(\mu_1/\mu_2)$, 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 warming.

For all following analyses, the applied nutrient ratios were categorized into nitrogen-limited (final N:P ratio $\leq$ 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 and 18 °C) of the first experiment have been performed (μ, particulate N:P ratio ~ temperature level * N:P supply ratio * rate of temperature change; and LRRt ~ N:P supply ratio * temperature level). For the statistical analyses of H2, generalized linear models (μ, particulate N:P ratio ~ temperature level * N:P supply ratio * nutrient availability during temperature change; and LRRn ~ 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 (Tables S2.1-2.5). 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, S2.11). Additionally, phosphorus-limited growth conditions significantly decreased community μ compared to both other nutrient conditions (Fig. 3, 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 breath) (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 (Fig. S2.12, Tables 2, 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 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 LRRt.

		μ			LRRt		N:P ratio		
Effect	Df	*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	

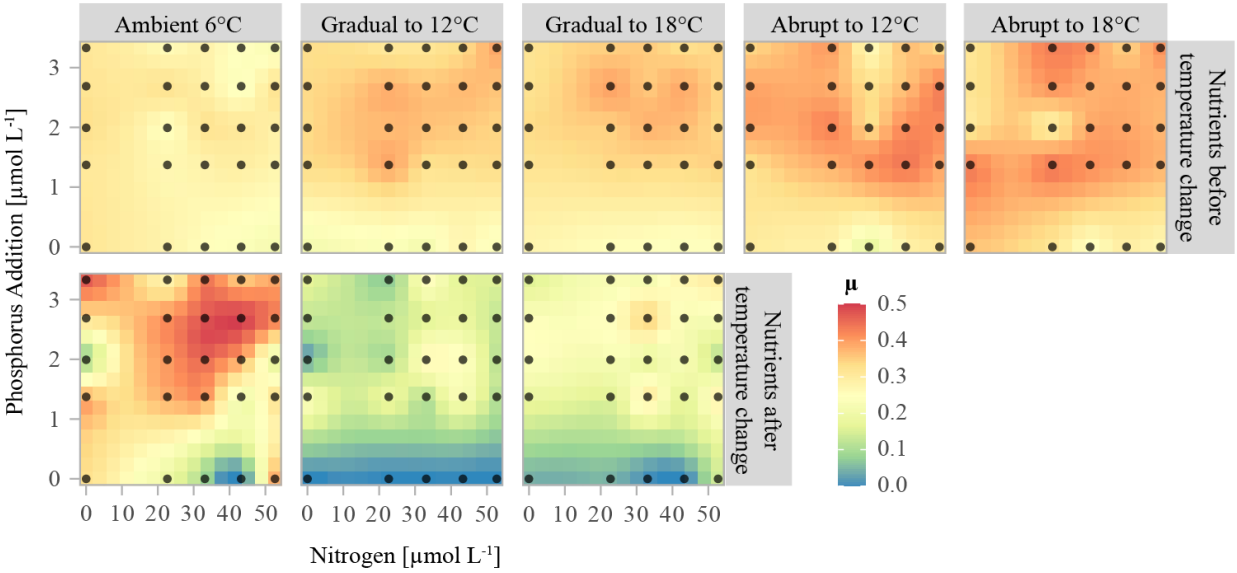

Figure 2: Interpolated response surfaces of the growth rate (μ) over nitrogen and phosphorus supply (μmol L^{-1}). All values below 0 have been set equal to 0. The points mark the tested experimental conditions. The rows represent the first experiment with nutrients added during the temperature change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

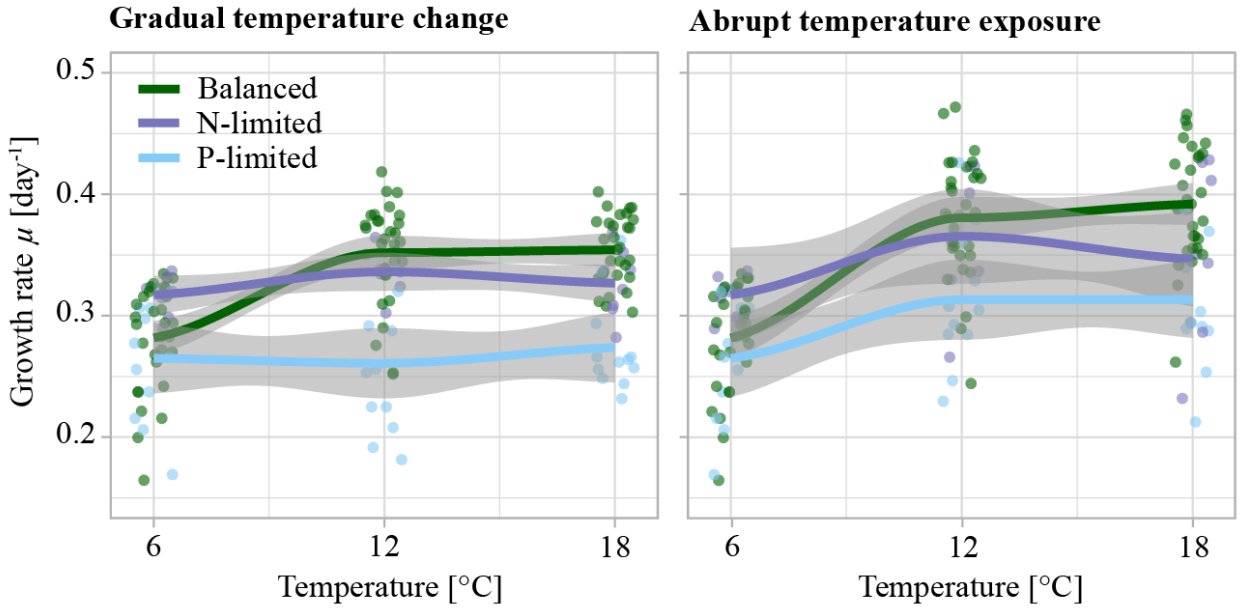

Figure 3: Growth rate (day^{-1}) of the phytoplankton community across experimental temperatures. Colors indicate a balanced (green), N-limited (purple), or P-limited (blue) nutrient supply. Each point represents an individual observation. The grey areas show smoothed conditional means with a sensitivity of 0.8 and a GAM fit.

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 and 18 °C compared to 6 °C, while adding nutrients after the temperature change reversed this effect (Figs. 2, 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. Further, it is evident from the measured background concentrations of dissolved phosphorus at the respective start conditions (0.31 µmol L^{-1} in the first experiment and 0.21 µmol L^{-1} in the second experiment) and the growth response of the treatments without nutrient 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 ºC, 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).

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 LRRn.

		μ			LRRn			N:P ratio		
Effect	Df	*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	*

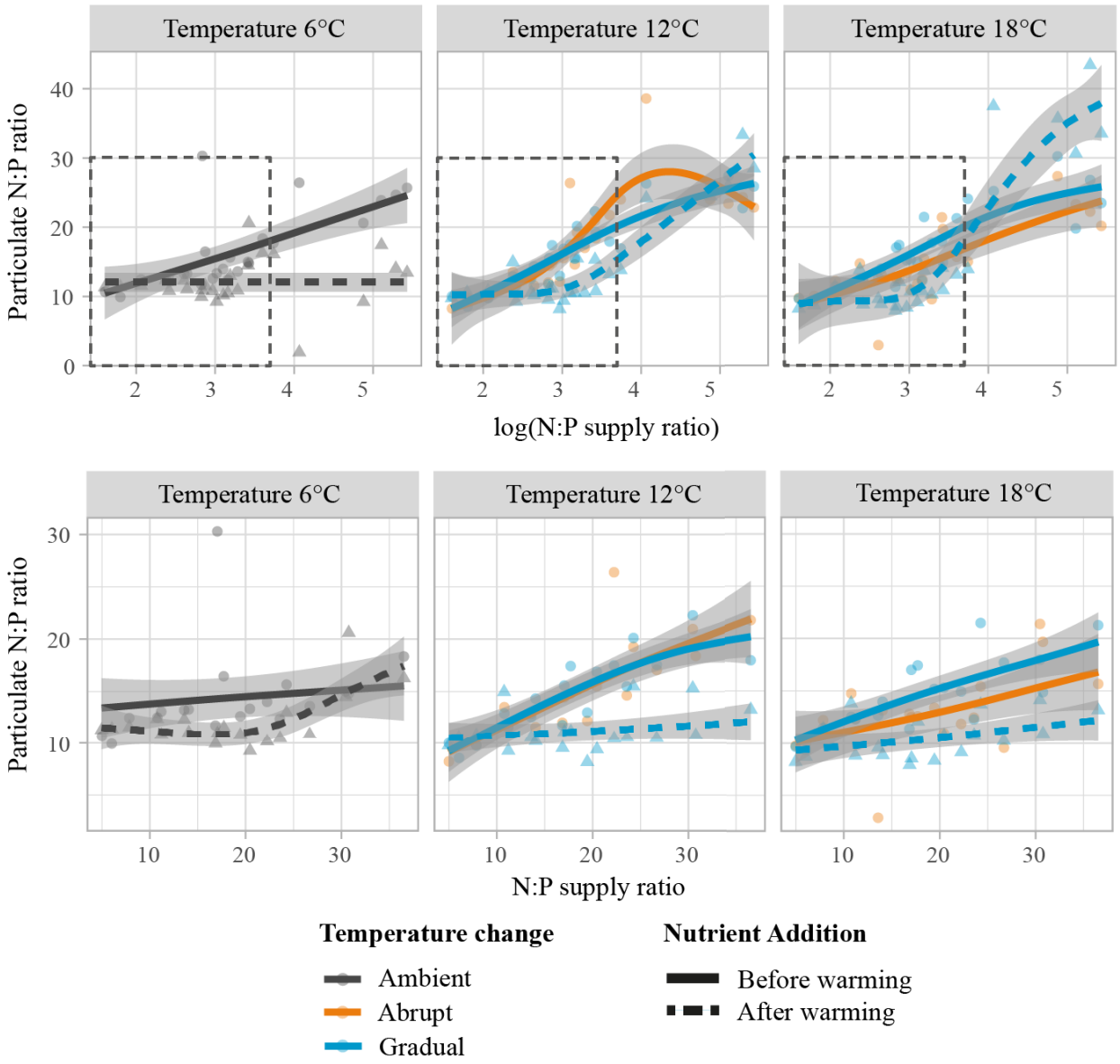

Figure 4: Phytoplankton final particulate N:P ratios across N:P supply ratios (including background concentration) and experimental temperatures. The upper panels include all treatments on a logarithmic scale (to visualize the effects of very high N:P supply ratios), the lower panels focus on the low 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).

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. Firstly, the rate of temperature change influences how phytoplankton respond to warming i.e., abrupt temperature exposure

overestimates the phytoplankton growth rates when compared with those obtained under a gradual temperature increase. Secondly, the timing of nutrient availability (under a balanced N:P 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., Fey et al. 2021; Kremer et al. 2018). 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 towards 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 decrease community growth equally among the temperature treatments (which partly rejects H1b). In line, Anderson et al. (2022) also found (gradual) warming to be beneficial for community growth only under replete nutrient conditions but to be reduced by > 220 % under nutrient depletion. 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. Further, 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 and functional composition during the acclimation period (i.e., period of gradual increase). In this study, the 12 °C treatment showed a relatively high proportion of mixotrophic dinoflagellates, while 18 °C showed an autotrophic diatom-dominated community after acclimation i.e., at the start of the second experiment. 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 (Aranguren-Gassis et al. 2019; Bestion et al. 2018; Fernandez-Gonzalez 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 (Aranguren-Gassis et al. 2019;

Fernandez-Gonzalez et al. 2020; Thomas et al. 2017), 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 positive effect of balanced N:P supply ratios was only found in the ambient temperature treatment in which autotrophs dominated the community ready to thrive when nutrient limitation was alleviated. Although metabolic rates increase with warming, it is well-stated that heterotrophic processes (e.g., grazing) are more temperature-sensitive than autotrophic ones (e.g., phytoplankton growth) (Brown et al. 2004). This pattern can also be supported by the fact that growth of heterotrophs is more constrained than growth of autotrophs under colder temperatures (Rose and Caron 2007). Linking this to the naturally occurring phytoplankton blooms in the North Sea, a relatable pattern with a diatom-dominated spring bloom in colder, nutrient-richer waters and a dinoflagellate-dominated summer bloom in warmer, stratified waters with low nutrient levels can be observed (reviewed by Lin et al. 2016). However, experiments have shown that dinoflagellates can outcompete diatoms under high nitrate and high temperature conditions (Bi et al. 2021) which has already been observed in the Baltic Sea with an increase in relative proportions of dinoflagellates during spring blooms (Spilling et al. 2018). Overall, our findings reveal that the timing of nutrient addition is important and can even lead to a completely reversed outcome when disregarded.

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, 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 over-representation 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 (versus 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 versus 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.

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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. Available as a pre-print at SSRN: http://dx.doi.org/10.2139/ssrn.4717787.

Ahme, A. and others 2023a. Thermal performance assay of a plankton community from Helgoland Roads for an indoor mesocosm experiment in March 2022 In PANGAEA [ed.].

Ahme, A. and others 2023b. Winners and losers of atlantification: The degree of ocean warming affects the structure of Arctic microbial communities. Genes 14: 623.

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.

Bi, R. and others 2021. Responses of Marine Diatom-Dinoflagellate Competition to Multiple Environmental Drivers: Abundance, Elemental, and Biochemical Aspects. Front Microbiol 12: 731786.

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.

Boyd, P. W. and others 2015. Physiological responses of a Southern Ocean diatom to complex future ocean conditions. Nat. Clim. Change 6: 207-213.

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.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology 85: 1771-1789.

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(6): 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.

Fernandez-Gonzalez, C., M. Perez-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(4): 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. 9999: 1-13.

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(11): 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. Ann. 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. PNAS 101: 8998-9002.

IPCC. 2023. Climate change 2023. Synthesis report. Summary for policymakers.

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 reinhartdii. Proc. Royal Soc. B. 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 Biogeochemical Cycles 32: 516-528.

Lin, S., R. W. Litaker, and W. G. Sunda. 2016. Phosphorus physiological ecology and molecular mechanisms in marine phytoplankton. J. Phycol. 52: 10-36.

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. P. Lorenzo, P. Cermeno, and B. Mourino-Carballido. 2018. Nutrient limitation suppresses the temperature dependence of phytoplankton metabolic rates. ISME Journal 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 Biology 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 CO(2). 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, Vienna, Austria. URL https://www.R-project.org/.

Rabalais, N. N., R. E. Turner, R. J. Díaz, and R. D. Justić. 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 tricornutum. 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.

Rose, J. M., and D. A. Caron. 2007. Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. Limnol. Oceanogr. 52: 886-895.

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.

Spilling, K. and others 2018. Shifting diatom—dinoflagellate dominance during spring bloom in the Baltic Sea and its potential effects on biogeochemical cycling. Front Mar Sci 5.

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 University Press.

Striebel, M., S. Schabhuttl, 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. and others 2017. Temperature-nutrient interactions exacerbate sensitivity to warming in phytoplankton. Glob. Change Biol. 23: 3269-3280.

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.

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. Annual Review of Ecology and Systematics 13: 349-372.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytopankton-Methodik. Mitt Int Ver Limnol 9: 1-38.

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, New York, New York, USA.

Wickham, H. 2016. ggplot2: elegant graphics for data analysis. Springer-Verlag New York.

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., 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.

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.

Supplementary material of publication III

Search terms for the systematic literature map
Here, we report on the search terms and used database as required by item 5 of the PRISMA-EcoEvo statement (O'Dea et al. 2021).

The following part is taken from the method section of the paper: The following search string was applied on 28th of June 2023: TS = phytoplankton AND (marine OR coastal) AND (experiment* OR laboratory OR mesocosm* OR microcosm* OR incubation*) AND ("thermal stress" OR "heat stress" OR "temperature stress" OR "increas* temperature" OR "heat wave" OR "heatwave*" OR "extreme temperature*" OR "temperature change" OR "chang* temperature*" OR "temperature increase" OR "thermal* chang*" OR "temperature gradient" OR "different temperature*" OR "heat disturbance" OR "temperature disturbance" OR "warming"). The search aimed to cover all published work for all years available. The resulting records were downloaded as an Excel-file and manually analyzed. The full list of all included studies can be found at the end of this document.

Flow chart of study selection process
This paragraph reports the study selection process in concert with item 19 of the PRISMA-EcoEvo statement (O'Dea et al. 2021). The PRISMA-style flowchart follows the suggested format (Fig. 3 in O'Dea et al. 2021). The exclusion reasons in the abstract screening phase were if a study was not conducted on eukaryotic, autotrophic phytoplankton in a marine environment, was not experimental or clearly did not have a temperature change treatment included. Further exclusion criteria on the full-text level were if a study was only conducted on monocultures or artificial phytoplankton communities and if it was designed to be a temperature shock experiment. With this, a database for monoculture studies was also created, which is available on request, but was not used for the analysis. Temperature shock experiments were excluded since the experimental designs are intentionally conducted as abrupt temperature exposures. This resulted in 81 studies with 83 different experimental designs considered for the analysis. If several temperature levels were applied in a study, only information on the highest temperature treatment was extracted. All exclusion and data extraction was done manually by one person. Eight studies (10%) were checked for accuracy by a co-author.

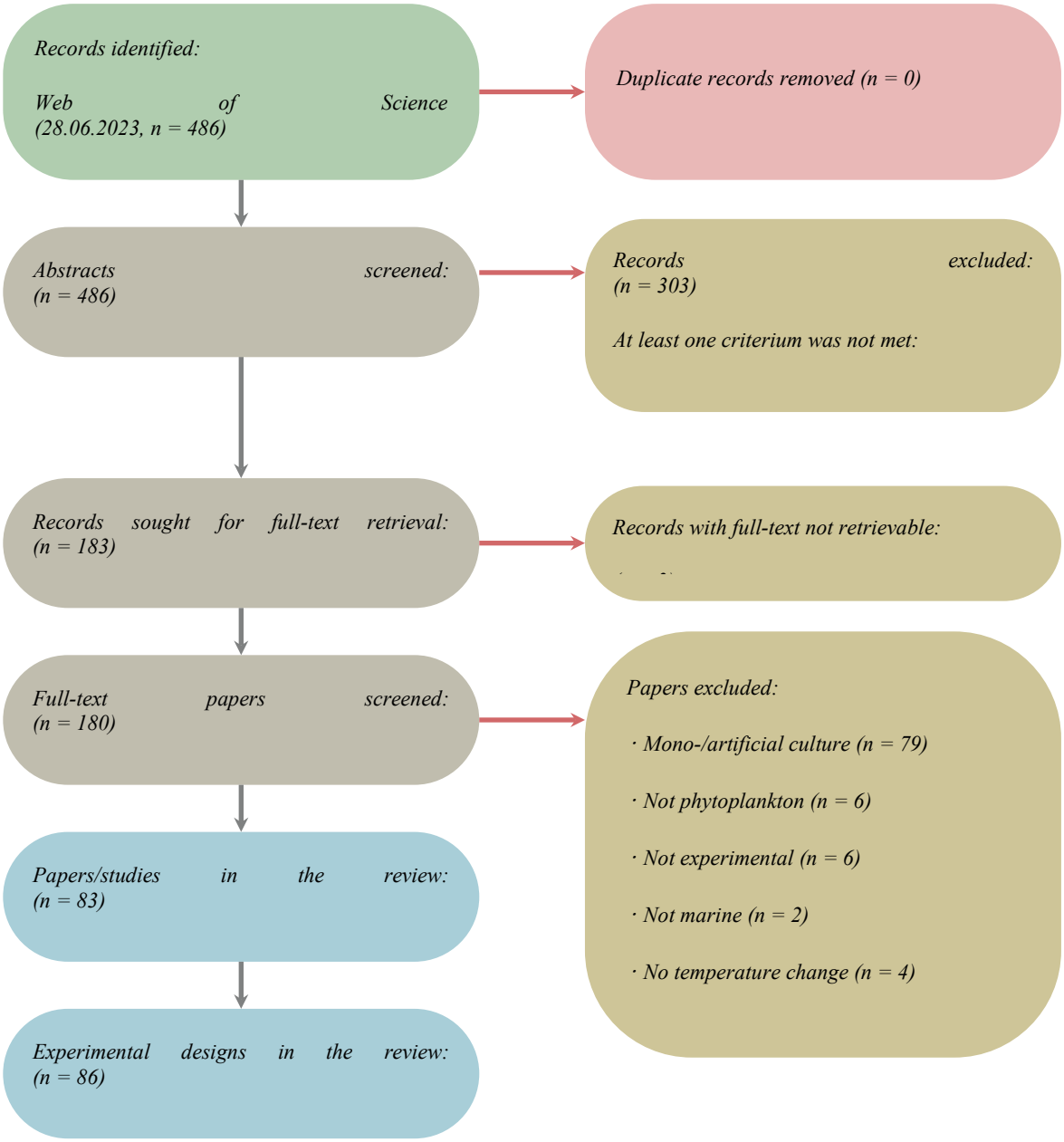

Figure S1: PRISMA-style flowchart of the study selection process conducted for the systematic literature review. The colors of the boxes indicate the step within the selection process i.e., green shows the step of study identification, red indicates the step of removing duplicates, grey is the screening step, yellow marks the excluded papers and blue the included papers. n stands for the number of studies or papers. This flowchart is adapted from Fig. 3 in O'Dea et al. *(2021).*

Data Extraction

The following information was extracted from the included papers:

Aspect	Categories
Type of temperature change	• *Abrupt*: Experiments that were either (i) programmed to be as fast as possible and happened within one day, (ii) conducted as bottle incubations in climate chambers or a water bath and the type of temperature change was not stated otherwise. If the study mentioned a gradual temperature increase, but it happened within 24 hours, it is still considered abrupt, but it is mentioned in the comments. • *Unclear (Abrupt)*: It was only mentioned that temperature was changed and potentially which heating element has been used, but not how it was programmed. • *Gradual*: The temperature increase was programmed and conducted over more than one day, even though it was a technical increase (mesocosms were programmed to increase as fast as possible but it took more than one day). • *Acclimation*: The temperature was applied and the experiment started after the temperature was reached and it was explicitly mentioned that this was used for acclimation of the community. • *Mixed*: Different types of temperature change have been used together (e.g., simultaneously or sequentially). • *Unclear*: It was mentioned that temperature change was conducted but it was not clearly stated how and the experimental design did not hint which type of temperature change has been used.
Rate of temperature change	This is the rate of temperature change per day. For the abrupt temperature exposure designs, this refers to the final experimental temperature. For gradually increasing temperature designs, this is the increase in temperature per day.
Nutrient condition	• *Ambient*: No nutrients have been added to the incubation. They were grown under ambient nutrient conditions in the sea water. • *Unclear (Ambient)*: It was not clearly mentioned, but assumed from the experimental design that the study was performed under ambient nutrient conditions. • *Ambient-adapted*: Nutrients (nitrogen and/or phosphorus) have been added to compensate for unusually low ambient concentrations or to achieve better comparability to a reference year or experiment that used ambient conditions. • *Enriched*: This applied if nitrogen and phosphorus have been added to the incubations in concentrations above the (usual) ambient conditions. • *Manipulated*: The concentration or ratio of nutrients (nitrogen and/or phosphorus) was manipulated as an experimental treatment. This also applied if the incubations were nutrient-enriched and only had a non-enriched control. If e.g., only the source of nitrogen was manipulated, this was not counted as it does not meet the scope of the study. • *Unclear*: The nutrient conditions were not described and they were not to be clearly assumed from the experimental design.
N:P supply ratio manipulation	This is identified as TRUE if the experimental design included a manipulation of the N:P supply ratio.

List of studies included in the systematic map

Authors	Article Title	Source Title	Year	DOI
Aberle, N; Bauer, B; Lewandowska, A; Gaedke, U; Sommer, U	Warming induces shifts in microzooplankton phenology and reduces time-lags between phytoplankton and protozoan production	MARINE BIOLOGY	2012	10.1007/s00227-012-1947-0
Ahme, A; Von Jackowski, A; McPherson, RA; Wolf, KKE; Hoppmann, M; Neuhaus, S; John, U	Winners and losers of atlantification: the degree of ocean warming affects the structure of Arctic microbial communities	GENES	2023	10.3390/genes14030623
Antacli, JC; Hernando, MP; De Troch, M; Malanga, G; Mendiolar, M; Hernandez, DR; Varela, DE; Antoni, J; Sahade, RJ; Schloss, IR	Ocean warming and freshening effects on lipid metabolism in coastal Antarctic phytoplankton assemblages dominated by sub-Antarctic species	SCIENCE OF THE TOTAL ENVIRONMENT	2021	10.1016/j.scitotenv.2021.147879
Arandia-Gorostidi, N; Weber, PK; Alonso-Saez, L; Moran, XAG; Mayali, X	Elevated temperature increases carbon and nitrogen fluxes between phytoplankton and heterotrophic bacteria through physical attachment	ISME JOURNAL	2017	10.1038/ismej.2016.156
Berner, C; Bertos-Fortis, M; Pinhassi, J; Legrand, C	Response of microbial communities to changing climate conditions during summer cyanobacterial blooms in the Baltic Sea	FRONTIERS IN MICROBIOLOGY	2018	10.3389/fmicb.2018.01562
Biermann, A; Engel, A; Riebesell, U	Changes in organic matter cycling in a plankton community exposed to warming under different light intensities	JOURNAL OF PLANKTON RESEARCH	2014	10.1093/plankt/fbu005
Cabrerizo, MJ; Maranon, E; Fernandez-Gonzalez, C; Alonso-Nunez, A; Larsson, H; Aranguren-Gassis, M	Temperature fluctuation attenuates the effects of warming in estuarine microbial plankton communities	FRONTIERS IN MARINE SCIENCE	2021	10.3389/fmars.2021.656282
Cabrerizo, MJ; Medina-Sanchez, JM; Gonzalez-Olalla, JM; Sanchez-Gomez, D; Carrillo, P	Microbial plankton responses to multiple environmental drivers in marine ecosystems with different phosphorus limitation degrees	SCIENCE OF THE TOTAL ENVIRONMENT	2022	10.1016/j.scitotenv.2021.151491
Calbet, A; Sazhin, AF; Nejstgaard, JC; Berger, SA; Tait, ZS; Olmos, L; Sousoni, D; Isari, S; Martinez, RA; Bouquet, JM; Thompson, EM; Bamstedt, U; Jakobsen, HH	Future climate scenarios for a coastal productive planktonic food web resulting in microplankton phenology changes and decreased trophic transfer efficiency	PLOS ONE	2014	10.1371/journal.pone.0094388
Courboules, J; Mostajir, B; Trombetta, T; Mas, S; Vidussi, F	Warming disadvantages phytoplankton and benefits bacteria during a spring bloom in the Mediterranean Thau Lagoon	FRONTIERS IN MARINE SCIENCE	2022	10.3389/fmars.2022.878938
Courboules, J; Vidussi, F; Soulie, T; Mas, S; Pecqueur, D; Mostajir, B	Effects of experimental warming on small phytoplankton, bacteria and viruses in autumn in the Mediterranean coastal Thau Lagoon	AQUATIC ECOLOGY	2021	10.1007/s10452-021-09852-7
D'Amario, B; Perez, C; Grelaud, M; Pitta, P; Krasakopoulou, E; Ziveri, P	Coccolithophore community response to ocean acidification and warming in the Eastern Mediterranean Sea: results from a mesocosm experiment	SCIENTIFIC REPORTS	2020	10.1038/s41598-020-69519-5
Domaizon, I; Lepere, C; Debroas, D; Bouvy, M; Ghiglione, JF; Jacquet, S; Bettarel, Y; Bouvier, C; Torreton, JP; Vidussi, F; Mostajir, B; Kirkham, A;	Short-term responses of unicellular planktonic eukaryotes to increases in temperature and UVB radiation	BMC MICROBIOLOGY	2012	10.1186/1471-2180-12-202

LeFloc'h, E; Fouilland, E; Montanie, H; Bouvier, T				
Domingues, RB; Barreto, M; Brotas, V; Galvao, HM; Barbosa, AB	Short-term effects of winter warming and acidification on phytoplankton growth and mortality: more losers than winners in a temperate coastal lagoon	HYDROBIOLOGIA	2021	10.1007/s10750-021-04672-0
Engel, A; Handel, N; Wohlers, J; Lunau, M; Grossart, HP; Sommer, U; Riebesell, U	Effects of sea surface warming on the production and composition of dissolved organic matter during phytoplankton blooms: results from a mesocosm study	JOURNAL OF PLANKTON RESEARCH	2011	10.1093/plankt/fbq122
Engel, FG; Lewandowska, AM; Eggers, SL; Matthiessen, B	Manipulation of non-random species loss in natural phytoplankton: qualitative and quantitative evaluation of different approaches	FRONTIERS IN MARINE SCIENCE	2017	10.3389/fmars.2017.00317
Feng, YY; Chai, F; Wells, ML; Liao, Y; Li, PF; Cai, T; Zhao, T; Fu, FX; Hutchins, DA	The combined effects of increased pCO_2 and warming on a coastal phytoplankton assemblage: from species composition to sinking rate	FRONTIERS IN MARINE SCIENCE	2021	10.3389/fmars.2021.622319
Feng, YY; Hare, CE; Leblanc, K; Rose, JM; Zhang, YH; DiTullio, GR; Lee, PA; Wilhelm, SW; Rowe, JM; Sun, J; Nemcek, N; Gueguen, C; Passow, U; Benner, I; Brown, C; Hutchins, DA	Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. I. The phytoplankton community and biogeochemical response	MARINE ECOLOGY PROGRESS SERIES	2009	10.3354/meps08133
Fernandez-Gonzalez, C; Tarran, GA; Schuback, N; Woodward, EMS; Aristegui, J; Maranon, E	Phytoplankton responses to changing temperature and nutrient availability are consistent across the tropical and subtropical Atlantic	COMMUNICATIONS BIOLOGY	2022	10.1038/s42003-022-03971-z
Fouilland, E; Mostajir, B; Torreton, JP; Bouvy, M; Got, P; Le Floc'h, E; Nouguier, J; Charriere, B; Sempere, R; Vidussi, F	Microbial carbon and nitrogen production under experimental conditions combining warming with increased ultraviolet-B radiation in Mediterranean coastal waters	JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY	2013	10.1016/j.jembe.2012.10.014
Franze, G; Anderson, SI; Kling, JD; Wilburn, P; Hutchins, DA; Litchman, E; Rynearson, TA; Menden-Deuer, S	Interactive effects of nutrients and temperature on herbivorous predation in a coastal plankton community	LIMNOLOGY AND OCEANOGRAPHY	2022	10.1002/lno.12289
Gaedke, U; Ruhenstroth-Bauer, M; Wiegand, I; Tirok, K; Aberle, N; Breithaupt, P; Lengfellner, K; Wohlers, J; Sommer, U	Biotic interactions may overrule direct climate effects on spring phytoplankton dynamics	GLOBAL CHANGE BIOLOGY	2010	10.1111/j.1365-2486.2009.02009.x
Garcia-Corral, LS; Martinez-Ayala, J; Duarte, CM; Agusti, S	Experimental assessment of cumulative temperature and UV-B radiation effects on Mediterranean plankton metabolism	FRONTIERS IN MARINE SCIENCE	2015	10.3389/fmars.2015.00048
Gazeau, F; Ridame, C; Van Wambeke, F; Alliouane, S; Stolpe, C; Irisson, JO; Marro, S; Grisoni, JM; De Liege, G; Nunige, S; Djaoudi, K; Pulido-Villena, E; Dinasquet, J; Obernosterer, I; Catala, P; Guieu, C	Impact of dust addition on Mediterranean plankton communities under present and future conditions of pH and temperature: an experimental overview	BIOGEOSCIENCES	2021	10.5194/bg-18-5011-2021
George, JA; Lonsdale, DJ; Merlo, LR; Gobler, CJ	The interactive roles of temperature, nutrients, and zooplankton grazing in controlling the winter-spring phytoplankton	LIMNOLOGY AND OCEANOGRAPHY	2015	10.1002/lno.10020

	bloom in a temperate, coastal ecosystem, Long Island Sound			
Hernando, M; Schloss, IR; Almandoz, GO; Malanga, G; Varela, DE; De Troch, M	Combined effects of temperature and salinity on fatty acid content and lipid damage in Antarctic phytoplankton	JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY	2018	10.1016/j.jembe.2018.03.004
Hernando, M; Varela, DE; Malanga, G; Almandoz, GO; Schloss, IR	Effects of climate-induced changes in temperature and salinity on phytoplankton physiology and stress responses in coastal Antarctica	JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY	2020	10.1016/j.jembe.2020.151400
Hoppe, HG; Breithaupt, P; Walther, K; Koppe, R; Bleck, S; Sommer, U; Jurgens, K	Climate warming in winter affects the coupling between phytoplankton and bacteria during the spring bloom: a mesocosm study	AQUATIC MICROBIAL ECOLOGY	2008	10.3354/ame01198
Huete-Stauffer, TM; Arandia-Gorostidi, N; Gonzalez-Benitez, N; Diaz-Perez, L; Calvo-Diaz, A; Moran, XAG	Large plankton enhance heterotrophy under experimental warming in a temperate coastal ecosystem	ECOSYSTEMS	2018	10.1007/s10021-017-0208-y
Hyun, B; Kim, JM; Jang, PG; Jang, MC; Choi, KH; Lee, K; Yang, EJ; Noh, JH; Shin, K	The effects of ocean acidification and warming on growth of a natural community of coastal phytoplankton	JOURNAL OF MARINE SCIENCE AND ENGINEERING	2020	10.3390/jmse8100821
Jiang, ZB; Liao, YB; Shou, L; Liu, JJ; Chen, QZ; Zeng, JN; Zhu, GH	Dynamic changes in phytoplankton community structure after thermal shock and chlorination in a subtropical bay: an experimental study	JOURNAL OF MARINE SCIENCE AND TECHNOLOGY-TAIWAN	2014	10.6119/JMST-013-1011-2
Kaur-Kahlon, G; Kumar, S; Rehnstam-Holm, AS; Rai, A; Bhavya, PS; Edler, L; Singh, A; Andersson, B; Karunasagar, I; Ramesh, R; Godhe, A	Response of a coastal tropical pelagic microbial community to changing salinity and temperature	AQUATIC MICROBIAL ECOLOGY	2016	10.3354/ame01785
Keller, AA; Oviatt, CA; Walker, HA; Hawk, JD	Predicted impacts of elevated temperature on the magnitude of the winter-spring phytoplankton bloom in temperate coastal waters: A mesocosm study	LIMNOLOGY AND OCEANOGRAPHY	1999	10.4319/lo.1999.44.2.0344
Kim, JH; Kim, KY; Kang, EJ; Lee, K; Kim, JM; Park, KT; Shin, K; Hyun, B; Jeong, HJ	Enhancement of photosynthetic carbon assimilation efficiency by phytoplankton in the future coastal ocean	BIOGEOSCIENCES	2013	10.5194/bg-10-7525-2013
Kim, JM; Lee, K; Yang, EJ; Shin, K; Noh, JH; Park, KT; Hyun, B; Jeong, HJ; Kim, JH; Kim, KY; Kim, M; Kim, HC; Jang, PG; Jang, MC	Enhanced production of oceanic dimethylsulfide resulting from CO_2-induced grazing activity in a high CO_2 world	ENVIRONMENTAL SCIENCE & TECHNOLOGY	2010	10.1021/es102028k
Kudo, I; Noiri, Y; Nishioka, J; Taira, Y; Kiyosawa, H; Tsuda, A	Phytoplankton community response to Fe and temperature gradients in the NE (SERIES) and NW (SEEDS) subarctic Pacific Ocean	DEEP-SEA RESEARCH PART II-TOPICAL STUDIES IN OCEANOGRAPHY	2006	10.1016/j.dsr2.2006.05.033
Kumar, S; Bhavya, PS; Ramesh, R; Gupta, GVM; Chiriboga, F; Singh, A; Karunasagar, I; Rai, A; Rehnstam-Holm, AS; Edler, L; Godhe, A	Nitrogen uptake potential under different temperature-salinity conditions: Implications for nitrogen cycling under climate change scenarios	MARINE ENVIRONMENTAL RESEARCH	2018	10.1016/j.marenvres.2018.09.001

Lee, KH; Jeong, HJ; Lee, K; Franks, PJS; Seong, KA; Lee, SY; Lee, MJ; Jang, SH; Potvin, E; Lim, AS; Yoon, EY; Du Yoo, Y; Kang, NS; Kim, KY	Effects of warming and eutrophication on coastal phytoplankton production	HARMFUL ALGAE	2019	10.1016/j.hal.2018.11.017
Lewandowska, A; Sommer, U	Climate change and the spring bloom: a mesocosm study on the influence of light and temperature on phytoplankton and mesozooplankton	MARINE ECOLOGY PROGRESS SERIES	2010	10.3354/meps08520
Lewandowska, AM; Boyce, DG; Hofmann, M; Matthiessen, B; Sommer, U; Worm, B	Effects of sea surface warming on marine plankton	ECOLOGY LETTERS	2014	10.1111/ele.12265
Lewandowska, AM; Hillebrand, H; Lengfellner, K; Sommer, U	Temperature effects on phytoplankton diversity - The zooplankton link	JOURNAL OF SEA RESEARCH	2014	10.1016/j.seares.2013.07.003
Liu, KL; Chen, BZ; Zhang, SW; Sato, M; Shi, ZY; Liu, HB	Marine phytoplankton in subtropical coastal waters showing lower thermal sensitivity than microzooplankton	LIMNOLOGY AND OCEANOGRAPHY	2019	10.1002/lno.11101
Lomas, MW; Glibert, PM	Temperature regulation of nitrate uptake: A novel hypothesis about nitrate uptake and reduction in cool-water diatoms	LIMNOLOGY AND OCEANOGRAPHY	1999	10.4319/lo.1999.44.3.0556
Martin, A; McMinn, A; Heath, M; Hegseth, EN; Ryan, KG	The physiological response to increased temperature in over-wintering sea ice algae and phytoplankton in McMurdo Sound, Antarctica and Tromso Sound, Norway	JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY	2012	10.1016/j.jembe.2012.06.006
Menden-Deuer, S; Lawrence, C; Franze, G	Herbivorous protist growth and grazing rates at in situ and artificially elevated temperatures during an Arctic phytoplankton spring bloom	PEERJ	2018	10.7717/peerj.5264
Meyers, M; Decima, M; Law, CS; Gall, M; Barr, N; Miller, MR; Safi, K; Robinson, K; Sabadel, A; Wing, S; Hoffmann, L	No evidence of altered relationship between diet and consumer fatty acid composition in a natural plankton community under combined climate drivers	JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY	2022	10.1016/j.jembe.2022.151734
Moreau, S; Mostajir, B; Almandoz, GO; Demers, S; Hernando, M; Lemarchand, K; Lionard, M; Mercier, B; Roy, S; Schloss, IR; Thyssen, M; Ferreyra, GA	Effects of enhanced temperature and ultraviolet B radiation on a natural plankton community of the Beagle Channel (southern Argentina): a mesocosm study	AQUATIC MICROBIAL ECOLOGY	2014	10.3354/ame01694
Moreno, HD; Koring, M; Di Pane, J; Tremblay, N; Wiltshire, KH; Boersma, M; Meunier, CL	An integrated multiple driver mesocosm experiment reveals the effect of global change on planktonic food web structure	COMMUNICATIONS BIOLOGY	2022	10.1038/s42003-022-03105-5
Murphy, GEP; Romanuk, TN; Worm, B	Cascading effects of climate change on plankton community structure	ECOLOGY AND EVOLUTION	2020	10.1002/ece3.6055
Noiri, Y; Kudo, I; Kiyosawa, H; Nishioka, J; Tsuda, A	Influence of iron and temperature on growth, nutrient utilization ratios and phytoplankton species composition in the western subarctic Pacific Ocean during the SEEDS experiment	PROGRESS IN OCEANOGRAPHY	2005	10.1016/j.pocean.2005.02.006
Park, KT; Lee, K; Shin, K; Yang, EJ; Hyun, B; Kim, JM; Noh, JH; Kim, M; Kong, B; Choi, DH; Choi, SJ; Jang, PG; Jeong, HJ	Direct linkage between dimethyl sulfide production and microzooplankton grazing, resulting from prey composition change under high partial pressure of carbon dioxide conditions	ENVIRONMENTAL SCIENCE & TECHNOLOGY	2014	10.1021/es403351h

Paul, AJ; Sommer, U; Paul, C; Riebesell, U	Baltic Sea diazotrophic cyanobacterium is negatively affected by acidification and warming	MARINE ECOLOGY PROGRESS SERIES	2018	10.3354/meps12632
Paul, C; Matthiessen, B; Sommer, U	Warming, but not enhanced CO_2 concentration, quantitatively and qualitatively affects phytoplankton biomass	MARINE ECOLOGY PROGRESS SERIES	2015	10.3354/meps11264
Paul, C; Sommer, U; Garzke, J; Moustaka-Gouni, M; Paul, A; Matthiessen, B	Effects of increased CO_2 concentration on nutrient limited coastal summer plankton depend on temperature	LIMNOLOGY AND OCEANOGRAPHY	2016	10.1002/lno.10256
Paul, C; Sommer, U; Matthiessen, B	Composition and dominance of edible and inedible phytoplankton predict responses of Baltic Sea summer communities to elevated temperature and CO_2	MICROORGANISMS	2021	10.3390/microorganisms9112294
Peter, KH; Sommer, U	Phytoplankton cell size: intra- and interspecific effects of warming and grazing	PLOS ONE	2012	10.1371/journal.pone.0049632
Pulina, S; Suikkanen, S; Padedda, BM; Brutemark, A; Grubisic, LM; Satta, CT; Caddeo, T; Farina, P; Luglie, A	Responses of a Mediterranean coastal lagoon plankton community to experimental warming	MARINE BIOLOGY	2020	10.1007/s00227-019-3640-z
Remy, M; Hillebrand, H; Floder, S	Stability of marine phytoplankton communities facing stress related to global change: Interactive effects of heat waves and turbidity	JOURNAL OF EXPERIMENTAL MARINE BIOLOGY AND ECOLOGY	2017	10.1016/j.jembe.2017.10.002
Rose, JM; Feng, Y; DiTullio, GR; Dunbar, RB; Hare, CE; Lee, PA; Lohan, M; Long, M; Smith, WO; Sohst, B; Tozzi, S; Zhang, Y; Hutchins, DA	Synergistic effects of iron and temperature on Antarctic phytoplankton and microzooplankton assemblages	BIOGEOSCIENCES	2009	10.5194/bg-6-3131-2009
Rose, JM; Feng, YY; Gobler, CJ; Gutierrez, R; Hare, CE; Leblanc, K; Hutchins, DA	Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. II. Microzooplankton abundance and grazing	MARINE ECOLOGY PROGRESS SERIES	2009	10.3354/meps08134
Sabadel, AJM; Decimal, M; McComb, K; Meyers, M; Barr, N; Ga, M; Safi, K; Law, CS	Amino acid nitrogen stable isotopes as biomarkers of coastal phytoplankton assemblages and food web interactions	MARINE ECOLOGY PROGRESS SERIES	2022	10.3354/meps14046
Sommer, U; Aberle, N; Engel, A; Hansen, T; Lengfellner, K; Sandow, M; Wohlers, J; Zollner, E; Riebesell, U	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	2007	10.1007/s00442-006-0539-4
Sommer, U; Lewandowska, A	Climate change and the phytoplankton spring bloom: warming and overwintering zooplankton have similar effects on phytoplankton	GLOBAL CHANGE BIOLOGY	2011	10.1111/j.1365-2486.2010.02182.x
Sommer, U; Paul, C; Moustaka-Gouni, M	Warming and ocean acidification effects on phytoplankton-from species shifts to size shifts within species in a mesocosm experiment	PLOS ONE	2015	10.1371/journal.pone.0125239
Soulie, T; Vidussi, F; Courboules, J; Mas, S; Mostajir, B	Metabolic responses of plankton to warming during different productive seasons in coastal Mediterranean waters revealed by in situ mesocosm experiments	SCIENTIFIC REPORTS	2022	10.1038/s41598-022-12744-x
Soulie, T; Vidussi, F; Mas, S; Mostajir, B	Functional and structural responses of plankton communities toward consecutive experimental heatwaves in Mediterranean coastal waters	SCIENTIFIC REPORTS	2023	10.1038/s41598-023-35311-4

Soulie, T; Vidussi, F; Mas, S; Mostajir, B	Functional stability of a coastal Mediterranean plankton community during an experimental marine heatwave	FRONTIERS IN MARINE SCIENCE	2022	10.3389/fmars.2022.831496
Spackeen, JL; Sipler, RE; Bertrand, EM; Xu, K; McQuaid, JB; Walworth, NG; Hutchins, DA; Allen, AE; Bronk, DA	Impact of temperature, CO_2, and iron on nutrient uptake by a late-season microbial community from the Ross Sea, Antarctica	AQUATIC MICROBIAL ECOLOGY	2019	10.3354/ame01886
Stefanidou, N; Genitsaris, S; Lopez-Bautista, J; Sommer, U; Moustaka-Gouni, M	Effects of heat shock and salinity changes on coastal Mediterranean phytoplankton in a mesocosm experiment	MARINE BIOLOGY	2018	10.1007/s00227-018-3415-y
Stefanidou, N; Genitsaris, S; Lopez-Bautista, J; Sommer, U; Moustaka-Gouni, M	Response of a coastal Baltic Sea diatom-dominated phytoplankton community to experimental heat shock and changing salinity	OECOLOGIA	2019	10.1007/s00442-019-04502-0
Sultana, R; Casareto, BE; Sohrin, R; Suzuki, T; Alam, MS; Fujimura, H; Suzuki, Y	Response of Subtropical Coastal Sediment Systems of Okinawa, Japan, to Experimental Warming and High pCO_2	FRONTIERS IN MARINE SCIENCE	2016	10.3389/fmars.2016.00100
Tatters, AO; Roleda, MY; Schnetzer, A; Fu, FX; Hurd, CL; Boyd, PW; Caron, DA; Lie, AAY; Hoffmann, LJ; Hutchins, DA	Short- and long-term conditioning of a temperate marine diatom community to acidification and warming	PHILOSOPHICAL TRANSACTIONS OF THE ROYAL SOCIETY B-BIOLOGICAL SCIENCES	2013	10.1098/rstb.2012.0437
Tatters, AO; Schnetzer, A; Xu, K; Walworth, NG; Fu, FX; Spackeen, JL; Sipler, RE; Bertrand, EM; Mcquaid, JB; Allen, AE; Bronk, DA; Gao, KS; Sun, J; Caron, DA; Hutchins, DA	Interactive effects of temperature, CO_2 and nitrogen source on a coastal California diatom assemblage	JOURNAL OF PLANKTON RESEARCH	2018	10.1093/plankt/fbx074
Taucher, J; Schulz, KG; Dittmar, T; Sommer, U; Oschlies, A; Riebesell, U	Enhanced carbon overconsumption in response to increasing temperatures during a mesocosm experiment	BIOGEOSCIENCES	2012	10.5194/bg-9-3531-2012
Tuyet, DTA; Tanaka, T; Sohrin, R; Hao, DM; Nagaosa, K; Kato, K	Effects of warming on microbial communities in the coastal waters of temperate and subtropical zones in the northern hemisphere, with a focus on gammaproteobacteria	JOURNAL OF OCEANOGRAPHY	2015	10.1007/s10872-014-0264-2
Vaquer-Sunyer, R; Conley, DJ; Muthusamy, S; Lindh, MV; Pinhassi, J; Kritzberg, ES	Dissolved organic nitrogen inputs from wastewater treatment plant effluents increase responses of planktonic metabolic rates to warming	ENVIRONMENTAL SCIENCE & TECHNOLOGY	2015	10.1021/acs.est.5b00674
Vaquer-Sunyer, R; Duarte, CM	Experimental evaluation of the response of coastal Mediterranean planktonic and benthic metabolism to warming	ESTUARIES AND COASTS	2013	10.1007/s12237-013-9595-2
Vidussi, F; Mostajir, B; Fouilland, E; Le Floc'h, E; Nouguier, J; Roques, C; Got, P; Thibault-Botha, D; Bouvier, T; Troussellier, M	Effects of experimental warming and increased ultraviolet B radiation on the Mediterranean plankton food web	LIMNOLOGY AND OCEANOGRAPHY	2011	10.4319/lo.2011.56.1.0206
von Scheibner, M; Dorge, P; Biermann, A; Sommer, U; Hoppe, HG; Jurgens, K	Impact of warming on phyto-bacterioplankton coupling and bacterial community composition in experimental mesocosms	ENVIRONMENTAL MICROBIOLOGY	2014	10.1111/1462-2920.12195
von Scheibner, M; Herlemann, DPR; Lewandowska, AM; Jurgens, K	Phyto- and bacterioplankton during early spring conditions in the Baltic Sea and response to short-term experimental warming	FRONTIERS IN MARINE SCIENCE	2018	10.3389/fmars.2018.00231
Wiklund, AKE; Dahlgren, K; Sundelin, B; Andersson, A	Effects of warming and shifts of pelagic food web structure on benthic productivity in a coastal marine system	MARINE ECOLOGY PROGRESS SERIES	2009	10.3354/meps08290

Wohlers, J; Engel, A; Zollner, E; Breithaupt, P; Jurgens, K; Hoppe, HG; Sommer, U; Riebesell, U	Changes in biogenic carbon flow in response to sea surface warming	PROCEEDINGS OF THE NATIONAL ACADEMY OF SCIENCES OF THE UNITED STATES OF AMERICA	2009	10.1073/pnas.0812743106
Zhang, Y; Wang, TF; Li, H; Bao, NN; Hall-Spencer, JM; Gao, KS	Rising levels of temperature and CO_2 antagonistically affect phytoplankton primary productivity in the South China Sea	MARINE ENVIRONMENTAL RESEARCH	2018	10.1016/j.marenvres.2018.08.011

PRISMA-EcoEvo Checklist

Checklist item	Sub-item number	Sub-item	Reported by authors?	Notes
Title and abstract	1,1	Identify the review as a systematic review, meta-analysis, or both	Yes	
	1,2	Summarise the aims and scope of the review	Yes	
	1,3	Describe the data set		Literature map is not main topic
	1,4	State the results of the primary outcome	Yes	
	1,5	State conclusions	Yes	
	1,6	State limitations		Literature map is not main topic
Aims and questions	2,1	Provide a rationale for the review	Yes	As literature map is not the main topic, this is limited.
	2,2	Reference any previous reviews or meta-analyses on the topic	Yes	
	2,3	State the aims and scope of the review (including its generality)	Yes	
	2,4	State the primary questions the review addresses (e.g. which moderators were tested)	Yes	
	2,5	Describe whether effect sizes were derived from experimental and/or observational comparisons		
Review registration	3,1	Register review aims, hypotheses (if applicable), and methods in a time-stamped and publicly accessible archive and provide a link to the registration in the methods section of the manuscript. Ideally registration occurs before the search, but it can be done at any stage before data analysis.	No	The literature map was not pre-registered.
	3,2	Describe deviations from the registered aims and methods	No	
	3,3	Justify deviations from the registered aims and methods	No	
Eligibility criteria	4,1	Report the specific criteria used for including or excluding studies when screening titles and/or abstracts, and full texts, according to the aims of the systematic review (e.g. study design, taxa, data availability)	Yes	In Supplement 1
	4,2	Justify criteria, if necessary (i.e. not obvious from aims and scope)	Yes	

Finding studies	5,1	Define the type of search (e.g. comprehensive search, representative sample)	Yes	
	5,2	State what sources of information were sought (e.g. published and unpublished studies, personal communications)	Yes	
	5,3	Include, for each database searched, the exact search strings used, with keyword combinations and Boolean operators	Yes	
	5,4	Provide enough information to repeat the equivalent search (if possible), including the timespan covered (start and end dates)	Yes	In Supplement 1
Study selection	6,1	Describe how studies were selected for inclusion at each stage of the screening process (e.g. use of decision trees, screening software)	Yes	In Supplement 1
	6,2	Report the number of people involved and how they contributed (e.g. independent parallel screening)	Yes	
Data collection process	7,1	Describe where in the reports data were collected from (e.g. text or figures)	Yes	
	7,2	Describe how data were collected (e.g. software used to digitize figures, external data sources)	Yes	
	7,3	Describe moderator variables that were constructed from collected data (e.g. number of generations calculated from years and average generation time)	Yes	
	7,4	Report how missing or ambiguous information was dealt with during data collection (e.g. authors of original studies were contacted for missing descriptive statistics, and/or effect sizes were calculated from test statistics)	Yes	
	7,5	Report who collected data	Yes	
	7,6	State the number of extractions that were checked for accuracy by co-authors	Yes	
Data items	8,1	Describe the key data sought from each study	Yes	
	8,2	Describe items that do not appear in the main results, or which could not be extracted due to insufficient information		Not applicable
	8,3	Describe main assumptions or simplifications that were made (e.g. categorising both 'length' and 'mass' as 'morphology')	Yes	In Supplement 1
	8,4	Describe the type of replication unit (e.g. individuals, broods, study sites)		Not applicable
Assessment of individual study quality	9,1	Describe whether the quality of studies included in the systematic review or meta-analysis was assessed (e.g. blinded data collection, reporting quality, experimental *versus* observational)		Not applicable
	9,2	Describe how information about study quality was incorporated into analyses (e.g. meta-regression and/or sensitivity analysis)		Not applicable

Effect size measures	10,1	Describe effect size(s) used		Not applicable
	10,2	Provide a reference to the equation of each calculated effect size (e.g. standardised mean difference, log response ratio) and (if applicable) its sampling variance		Not applicable
	10,3	If no reference exists, derive the equations for each effect size and state the assumed sampling distribution(s)		Not applicable
Missing data	11,1	Describe any steps taken to deal with missing data during analysis (e.g. imputation, complete case, subset analysis)		Not applicable
	11,2	Justify the decisions made to deal with missing data		Not applicable
Meta-analytic model description	12,1	Describe the models used for synthesis of effect sizes		Not applicable
	12,2	The most common approach in ecology and evolution will be a random-effects model, often with a hierarchical/multilevel structure. If other types of models are chosen (e.g. common/fixed effects model, unweighted model), provide justification for this choice		Not applicable
Software	13,1	Describe the statistical platform used for inference (e.g. *R*)		Not applicable
	13,2	Describe the packages used to run models		Not applicable
	13,3	Describe the functions used to run models		Not applicable
	13,4	Describe any arguments that differed from the default settings		Not applicable
	13,5	Describe the version numbers of all software used		Not applicable
Non-independence	14,1	Describe the types of non-independence encountered (e.g. phylogenetic, spatial, multiple measurements over time)		Not applicable
	14,2	Describe how non-independence has been handled		Not applicable
	14,3	Justify decisions made		Not applicable
Meta-regression and model selection	15,1	Provide a rationale for the inclusion of moderators (covariates) that were evaluated in meta-regression models		Not applicable
	15,2	Justify the number of parameters estimated in models, in relation to the number of effect sizes and studies (e.g. interaction terms were not included due to insufficient sample sizes)		Not applicable
	15,3	Describe any process of model selection		Not applicable
Publication bias and sensitivity analyses	16,1	Describe assessments of the risk of bias due to missing results (e.g. publication, time-lag, and taxonomic biases)	Yes	
	16,2	Describe any steps taken to investigate the effects of such biases (if present)	Yes	

	16,3	Describe any other analyses of robustness of the results, e.g. due to effect size choice, weighting or analytical model assumptions, inclusion or exclusion of subsets of the data, or the inclusion of alternative moderator variables in meta-regressions		Not applicable
Clarification of *post hoc* analyses	17,1	When hypotheses were formulated after data analysis, this should be acknowledged.		No hypotheses were formulated.
Metadata, data, and code	18,1	Share metadata (i.e. data descriptions)	Yes	In Supplement 1
	18,2	Share data required to reproduce the results presented in the manuscript	Yes	On request as it is a too small data set to publish in a repository
	18,3	Share additional data, including information that was not presented in the manuscript (e.g. raw data used to calculate effect sizes, descriptions of where data were located in papers)		Not applicable
	18,4	Share analysis scripts (or, if a software package with graphical user interface (GUI) was used, then describe full model specification and fully specify choices)		Not applicable
Results of study selection process	19,1	Report the number of studies screened	Yes	In Supplement 1
	19,2	Report the number of studies excluded at each stage of screening	Yes	
	19,3	Report brief reasons for exclusion from the full text stage	Yes	
	19,4	Present a Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA)-like flowchart (www.prisma-statement.org).	Yes	
Sample sizes and study characteristics	20,1	Report the number of studies and effect sizes for data included in meta-analyses		Not applicable
	20,2	Report the number of studies and effect sizes for subsets of data included in meta-regressions		Not applicable
	20,3	Provide a summary of key characteristics for reported outcomes (either in text or figures; e.g. one quarter of effect sizes reported for vertebrates and the rest invertebrates)	Yes	In Supplement 2
	20,4	Provide a summary of limitations of included moderators (e.g. collinearity and overlap between moderators)		Not applicable
	20,5	Provide a summary of characteristics related to individual study quality (risk of bias)		Not applicable
Meta-analysis	21,1	Provide a quantitative synthesis of results across studies, including estimates for the mean effect size, with confidence/credible intervals		Not applicable
Heterogeneity	22,1	Report indicators of heterogeneity in the estimated effect (e.g. I^2, tau^2 and other variance components)		Not applicable

Meta-regression	23,1	Provide estimates of meta-regression slopes (i.e. regression coefficients) and confidence/credible intervals		Not applicable
	23,2	Include estimates and confidence/credible intervals for all moderator variables that were assessed (i.e. complete reporting)		Not applicable
	23,3	Report interactions, if they were included		Not applicable
	23,4	Describe outcomes from model selection, if done (e.g. R2 and AIC)		Not applicable
Outcomes of publication bias and sensitivity analyses	24,1	Provide results for the assessments of the risks of bias (e.g. Egger's regression, funnel plots)		Not applicable
	24,2	Provide results for the robustness of the review's results (e.g. subgroup analyses, meta-regression of study quality, results from alternative methods of analysis, and temporal trends)		Not applicable
Discussion	25,1	Summarise the main findings in terms of the magnitude of effect		Not applicable
	25,2	Summarise the main findings in terms of the precision of effects (e.g. size of confidence intervals, statistical significance)		Not applicable
	25,3	Summarise the main findings in terms of their heterogeneity	Yes	
	25,4	Summarise the main findings in terms of their biological/practical relevance	Yes	
	25,5	Compare results with previous reviews on the topic, if available	Yes	
	25,6	Consider limitations and their influence on the generality of conclusions, such as gaps in the available evidence (e.g. taxonomic and geographical research biases)		Not applicable
Contributions and funding	26,1	Provide names, affiliations, and funding sources of all co-authors	Yes	
	26,2	List the contributions of each co-author	Yes	
	26,3	Provide contact details for the corresponding author	Yes	
	26,4	Disclose any conflicts of interest	Yes	
References	27,1	Provide a reference list of all studies included in the systematic review or meta-analysis	Yes	In Supplement 1
	27,2	List included studies as referenced sources (e.g. rather than listing them in a table or supplement)	No	

References

O'Dea, R. E., M. Lagisz, M. D. Jennions, J. Koricheva, D. W. A. Noble, T. H. Parker, J. Gurevitch, M. J. Page, G. Stewart, D. Moher, and S. Nakagawa. 2021. Preferred reporting items for systematic reviews and meta-analyses in ecology and evolutionary biology: a PRISMA extension. Biol Rev Camb Philos Soc.

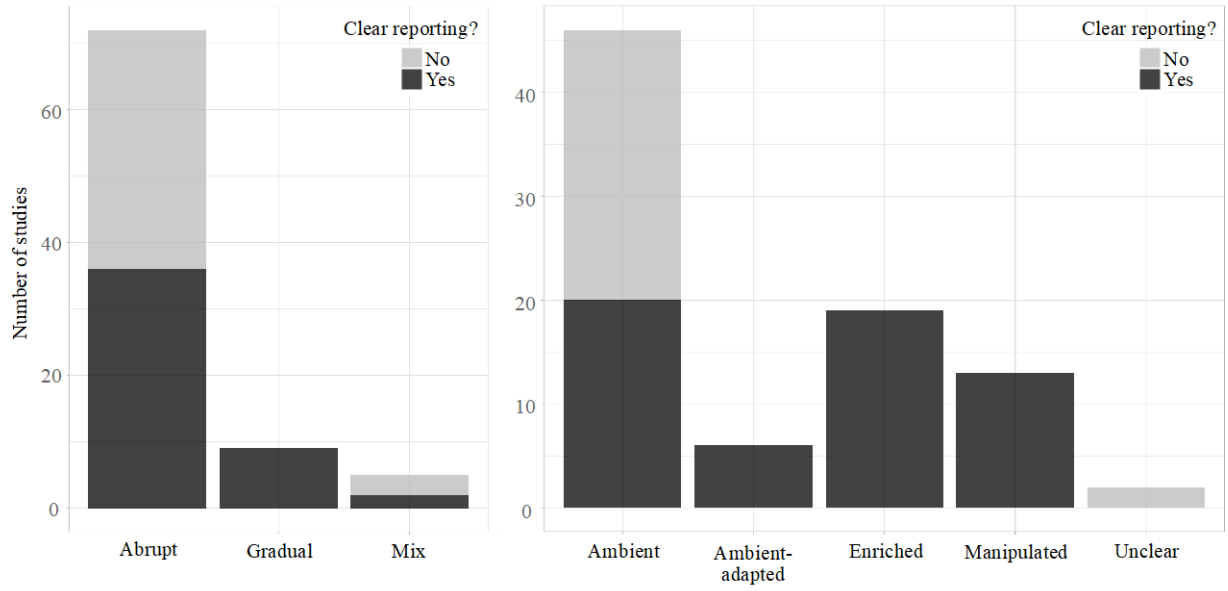

Figure S2.1: Overview of the systematic literature map. The left side shows the type of temperature change (abrupt, gradual, mix) that experimental studies applied. The right side indicates the type of nutrient conditions that were applied. More details about the criteria for the categories can be found in Supplement 1. The colors indicate whether the respective conditions were clearly reported (dark grey) or only to be assumed from the experimental design (light grey).

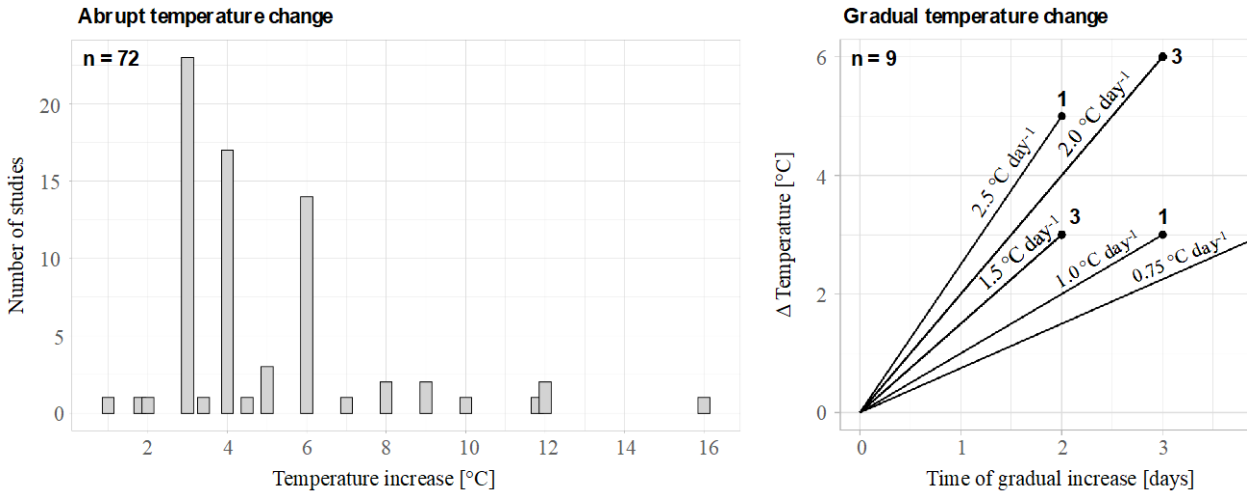

Figure S2.2: Types of temperature increase in experimental studies separated into abrupt (left side) and gradual temperature increases (right side). The temperature increase in the left figure and Δ Temperature in the right figure refer to the highest applied increase (compared to ambient conditions) of the respective study. For gradual temperature increase, the bold values indicate the number of studies that applied the respective gradual increase.

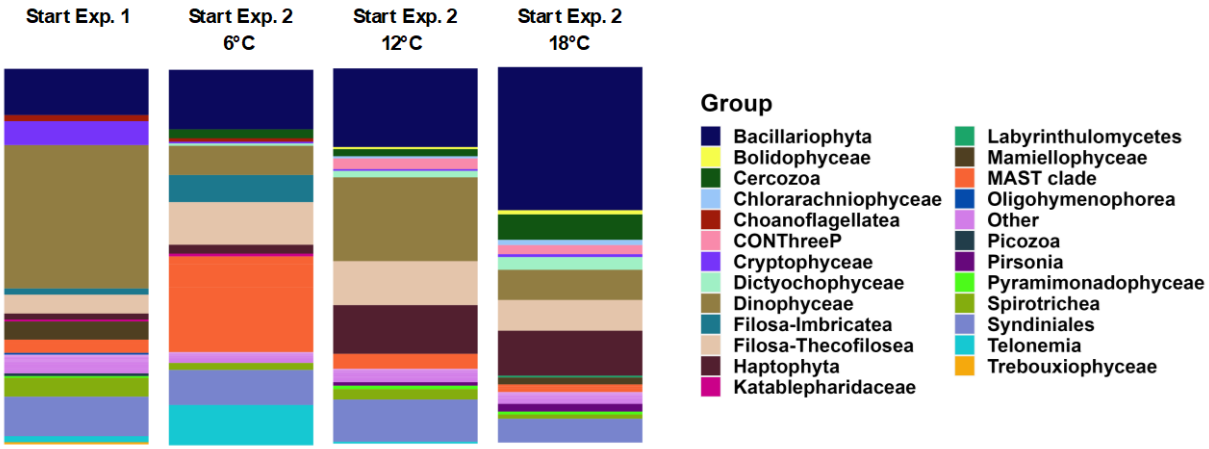

Figure S2.3: Community composition of the start community of both microcosm experiments based on DNA data (18S rRNA sequencing). Experiment 2 is separated into three different temperature treatments: ambient 6 °C, an increase by 1 °C day^{-1} to 12 °C and 18 °C. For readability, ASVs with an abundance of fewer than 100 reads among replicates were categorized as "other". For detailed figures on species level and more information about changes in community composition in the accompanying mesocosm experiment see Ahme et al. *(2024). The sample water was filtered onto 0.8 µm polycarbonate filters (Nucleopore, Whatman, Maidstone, UK) and DNA was extracted using the NucleoSpin Soil extraction Kit (Macherey-Nagel GmbH, Düren, Germany). Using primers targeting the variable region 4 of the 18S rRNA gene (Bradley* et al. *2016), amplicons were generated and sequenced on a MiSeq sequencer following the standard protocol for library preparation and sequencing (Illumina, San Diego, CA, USA). After primer removal, quality-trimming, denoising, and chimera removal, amplicon sequence variants (ASVs) were taxonomically annotated using the protist reference database v4.12.0 PR2 (Guillou* et al. *2013).*

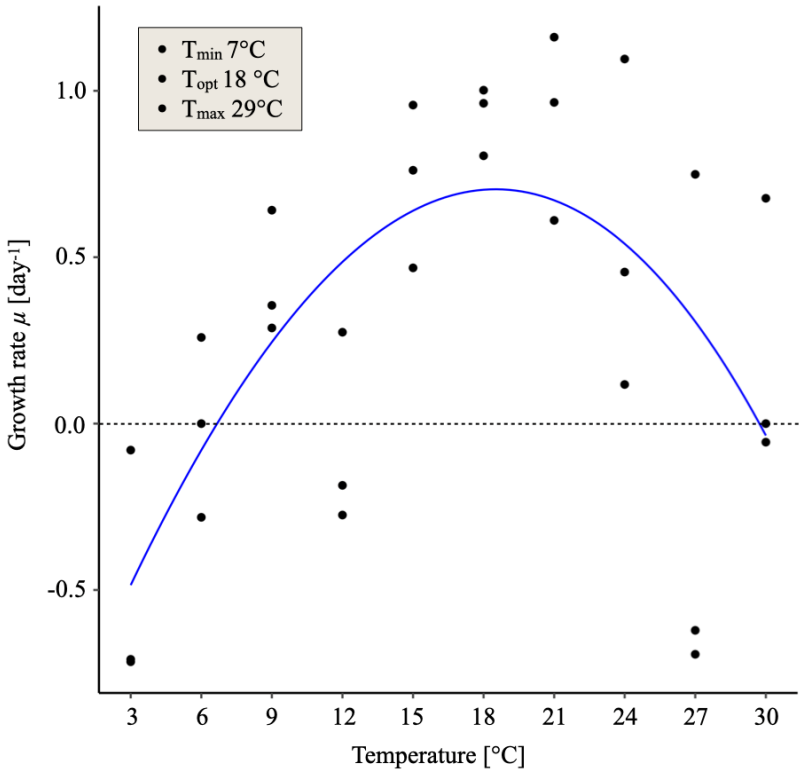

Figure S2.4: Thermal performance curve (TPC) of the phytoplankton community directly after sampling it from the North Sea. The nutrient conditions were kept at ambient. The temperature exposure was done abruptly to temperatures between 3 and 30 °C in steps of 3 °C. The methods for the TPC are described in detail in Ahme et al. *(2023a).*

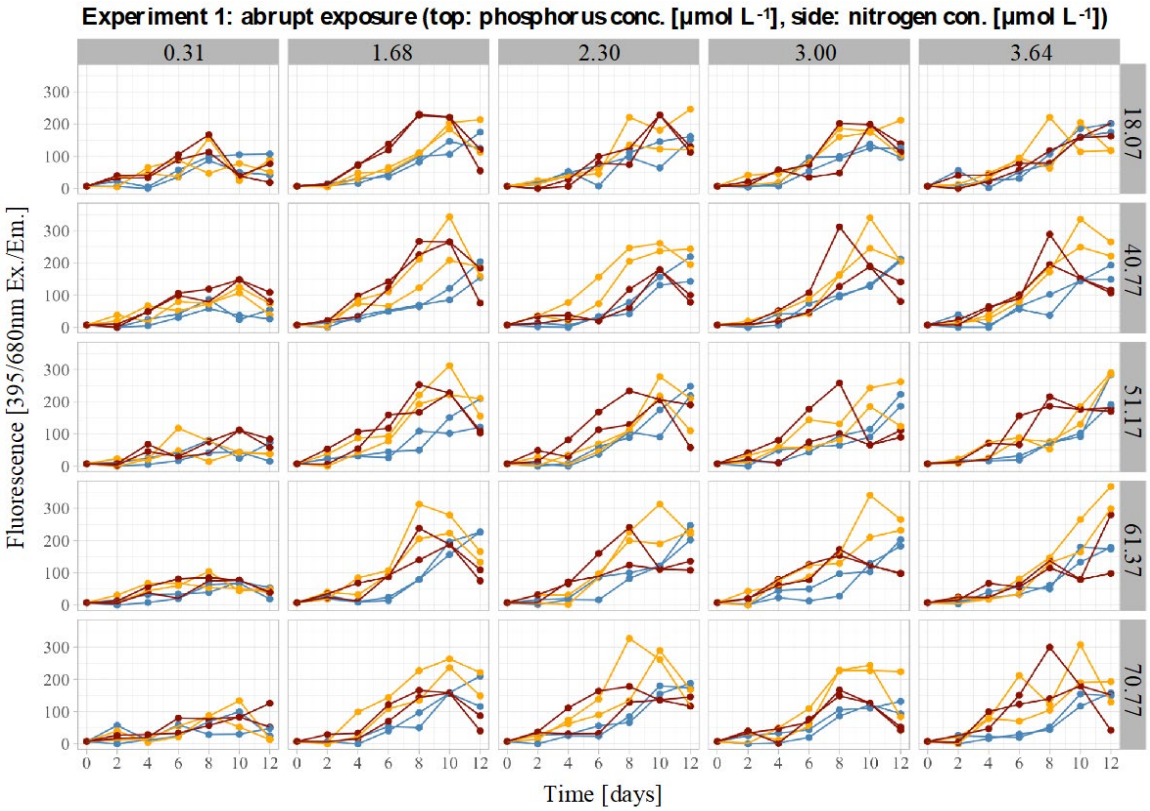

Figure S2.5: Time series of phytoplankton growth as fluorescence at 395/680nm Ex./Em. in the ambient temperature treatment (6°C, blue) and the abruptly exposed temperature treatments (12 °C in orange and 18 °C in red) over a nitrogen (18.07-70.77 µmol L^{-1}) and phosphorus (0.31-3.64 µmol L^{-1}) gradient in the first run of the experiment. The stated values on the facet axes correspond to the final concentrations including background concentration.

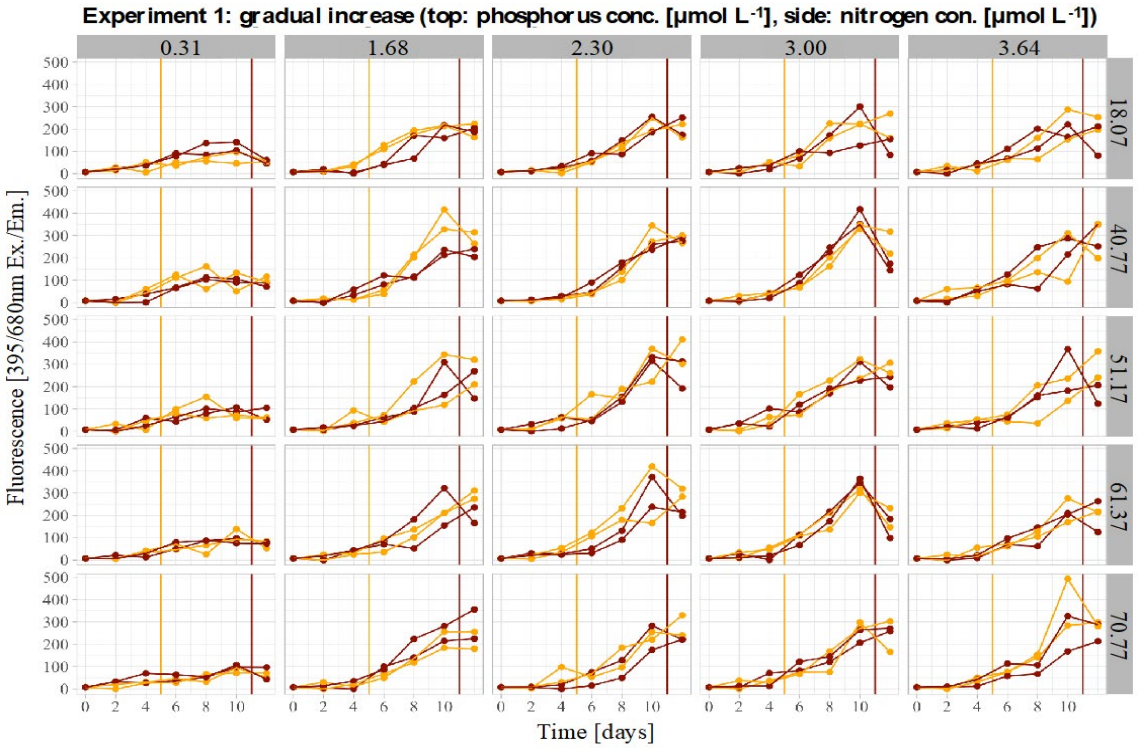

Figure S2.6: Time series of phytoplankton growth as fluorescence at 395/680nm Ex./Em. in the gradually increased temperature treatments of 12 °C (orange) and 18 °C (red) over a nitrogen (18.07-70.77 µmol L^{-1}) and phosphorus (0.31-3.64 µmol L^{-1}) gradient in the first microcosm experiment. The vertical lines indicate the time point at which the respective final experimental has been reached. The stated values on the facet axes correspond to the final concentrations including background concentration.

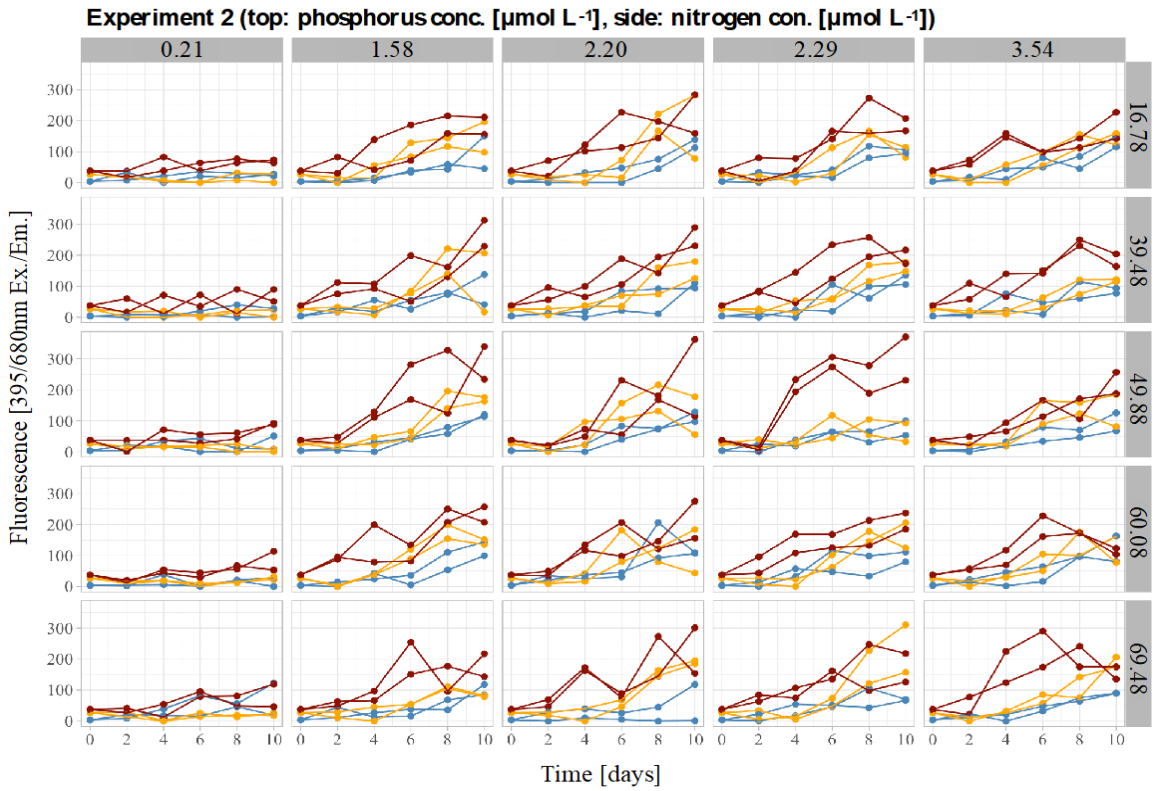

Figure S2.7: Time series of phytoplankton growth as fluorescence at 395/680nm Ex./Em. At the three temperatures 6 °C (blue), 12 °C (orange) and 18 °C (red) in the second experiment over a nitrogen (µmol L^{-1}) and phosphorus (µmol L^{-1}) gradient. This run received the addition of nutrients after thermal acclimation. The stated values on the facet axes correspond to the final concentrations including background concentration for 6 °C (for 12 °C add -5.3 µmol L^{-1} N and 0.0075 µmol L^{-1} P to the shown values, and for 18 °C add 1.80 µmol L^{-1} N and - 0.01 µmol L^{-1} P to the values).

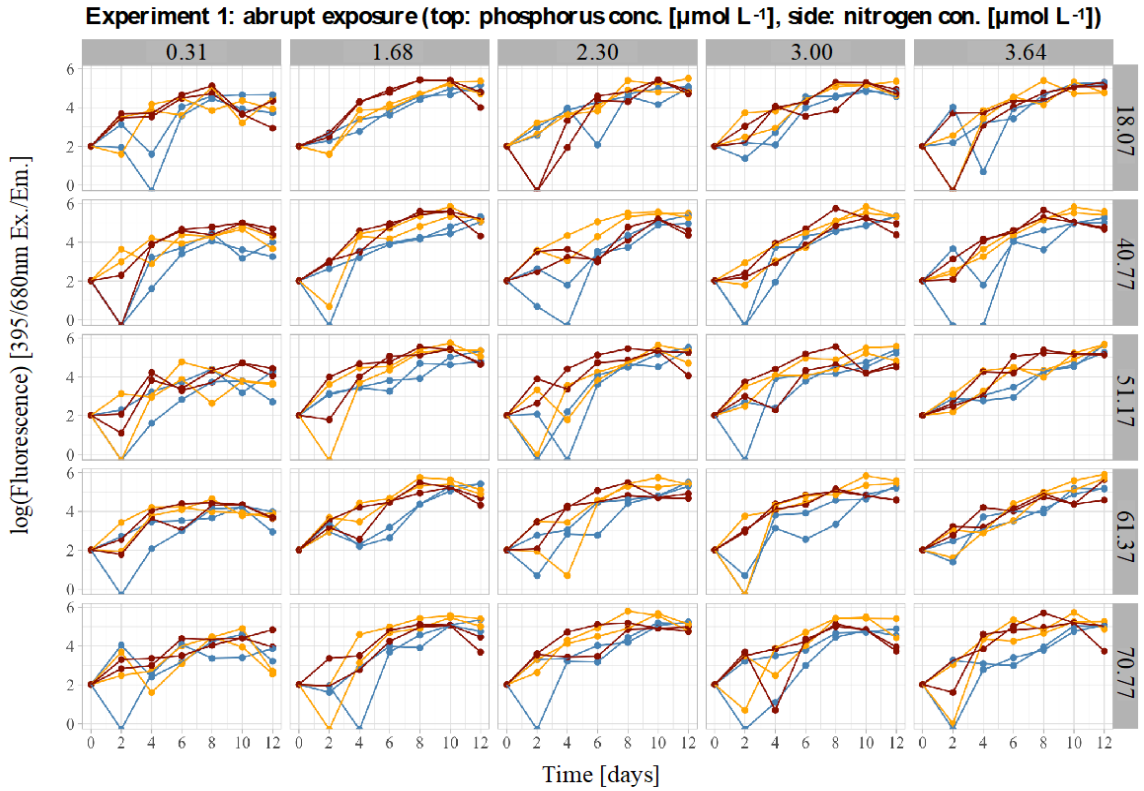

Figure S2.8: Time series of phytoplankton growth as the logarithm of fluorescence at 395/680nm Ex./Em. in the ambient temperature treatment (6°C, blue) and the abruptly exposed temperature treatments (12 °C in orange and 18 °C in red) over a nitrogen (18.07-70.77 µmol L^{-1}) and phosphorus (0.31-3.64 µmol L^{-1}) gradient in the first run of the experiment. The stated values on the facet axes correspond to the final concentrations including background concentration.

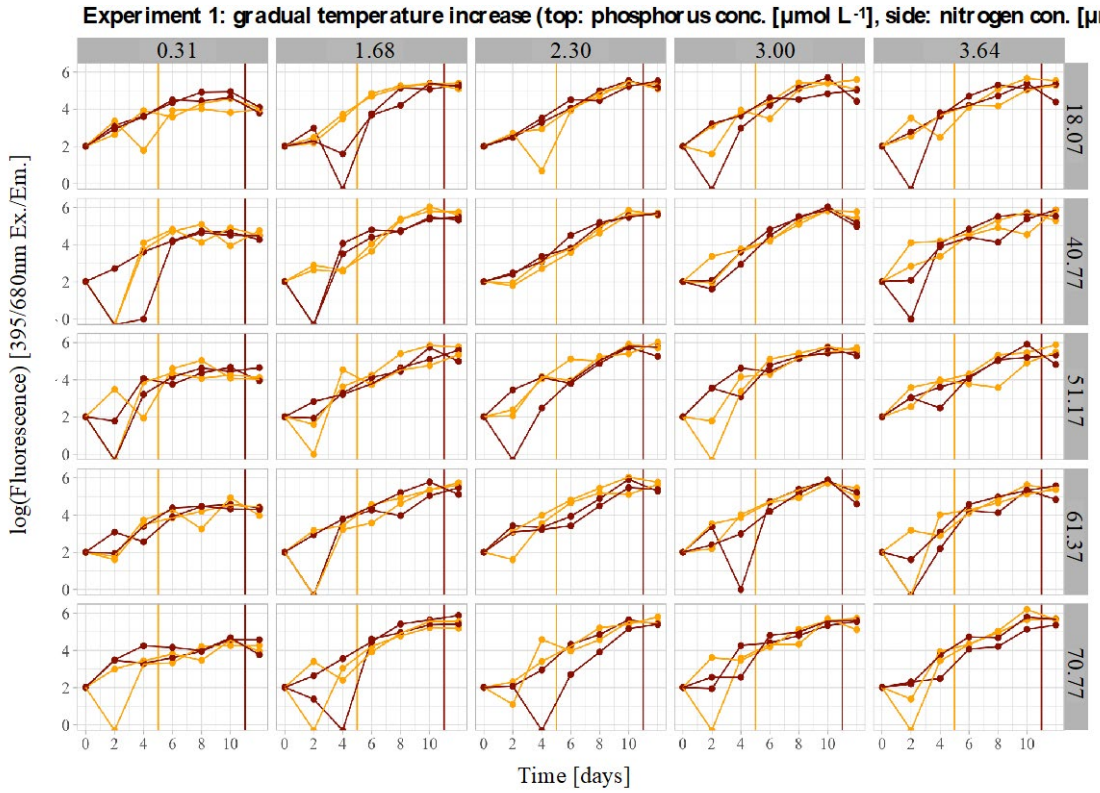

Figure S2.9: Time series of phytoplankton growth as the logarithm of fluorescence at 395/680nm Ex./Em. in the gradually increased temperature treatments of 12 °C (orange) and 18 °C (red) over a nitrogen (18.07-70.77 µmol L^{-1}) and phosphorus (0.31-3.64 µmol L^{-1}) gradient in the first microcosm experiment. The vertical lines indicate the time point at which the respective final experimental has been reached. The stated values on the facet axes correspond to the final concentrations including background concentration.

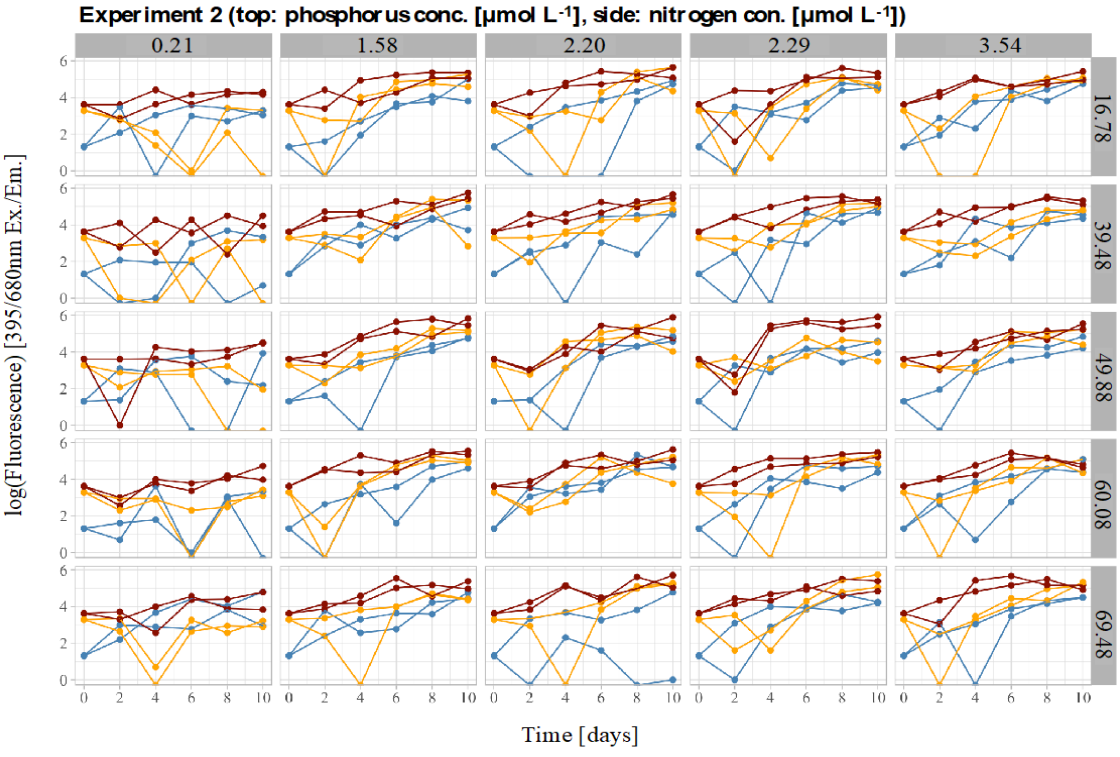

Figure S2.10: Time series of phytoplankton growth as the logarithm of fluorescence at 395/680nm Ex./Em. At the three temperatures 6 °C (blue), 12 °C (orange) and 18 °C (red) in the second experiment over a nitrogen (µmol L^{-1}) and phosphorus (µmol L^{-1}) gradient. This run received the addition of nutrients after thermal acclimation. The stated values on the facet axes correspond to the final concentrations including background concentration for 6 °C (for 12 °C add -5.3 µmol L^{-1} N and 0.0075 µmol L^{-1} P to the shown values, and for 18 °C add 1.80 µmol L^{-1} N and - 0.01 µmol L^{-1} P to the values).

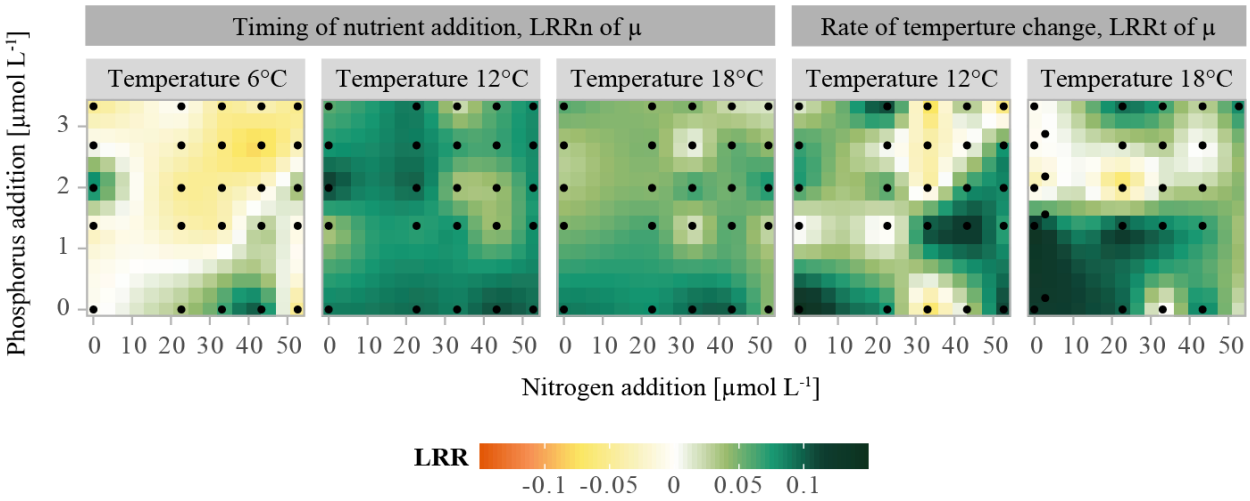

Figure 2.11: Interpolated response surfaces of the LRR for nutrient addition (LRRn: community μ for nutrients added before temperature change divided by community μ for nutrients added after the temperature change) and rate of temperature change (LRRt: community μ for abrupt temperature increase divided by community μ in a gradual temperature change) across nitrogen and phosphorus supply (in μmol L^{-1}). Positive values in the left panels indicate that nutrient availability during temperature change led to higher growth rates. Positive values in the right panels mean that an abrupt temperature exposure led to higher growth rates compared to a gradual temperature increase.

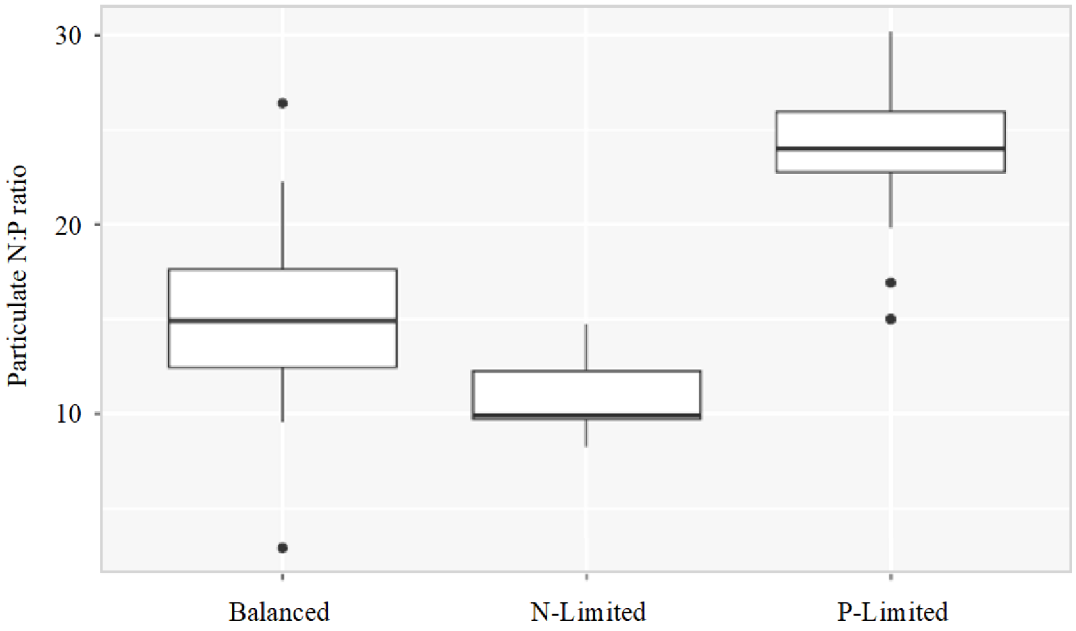

Figure S2.12: The effect of nitrogen:phosphorus (N:P) supply conditions (balanced, nitrogen-limited, phosphorus-limited) on particulate N:P ratios for all abrupt and gradual temperature increase treatments together (since no effect of the rate of temperature increase on particulate N:P ratios has been found). See statistical results in Table S2.2.

*Table S2.1: Post-hoc test for Generalized Linear Model (μ ~ temperature level * final N:P supply ratio * rate of temperature change). Asterisks indicate significance (p > 0.05).*

Term	Group 1	Group 2	Estimate	Conf. Low	Conf. High	P (adjusted)	Significance
Temp	12	18	3.08e-3	-0.00981	0.0160	6.38e-1	
Ratio	Balanced	N-Limited	-2.60e-2	-0.0476	-0.00431	1.41e-2	*
Ratio	Balanced	P-Limited	-7.99e-2	-0.0986	-0.0612	0	****
Ratio	N-Limited	P-Limited	-5.39e-2	-0.0789	-0.0290	2.40e-6	****
Temp. Rate	Abrupt	Gradual	-3.49e-2	-0.0478	-0.0220	2.71e-7	****

*Table S2.2: Post-hoc test for Generalized Linear Model (particulate N:P ratio ~ temperature level * final N:P supply ratio * rate of temperature change). Asterisks indicate significance (p > 0.05).*

Term	Group 1	Group 2	Estimate	Conf. Low	Conf. High	P (adjusted)	Significance
Temp	12	18	-0.938	-2.34	0.459	1.86e-1	
Ratio	Balanced	N-Limited	-4.55	-6.90	-2.20	3.88e-5	****
Ratio	Balanced	P-Limited	8.41	6.36	10.5	3.09e-10	****
Ratio	N-Limited	P-Limited	13.0	10.2	15.7	3.09e-10	****
Temp. Rate	Abrupt	Gradual	1.12	-0.279	2.52	1.15e-1	

*Table S2.3: Post-hoc test for Generalized Linear Model (μ ~ temperature level * final N:P supply ratio * nutrient availability during temperature change). Asterisks indicate significance (p > 0.05).*

Term	Group 1	Group 2	Estimate	Conf. Low	Conf. High	P (adjusted)	Significance
Temp	6	12	-0.450	-0.575	-0.325	4.50e-13	****
Temp	6	18	-0.264	-0.389	-0.139	3.22e-6	****
Temp	12	18	0.186	0.0620	0.310	1.39e-3	**
Ratio	Balanced	N-Limited	-0.0686	-0.212	0.0749	4.99e-1	
Ratio	Balanced	P-Limited	-0.592	-0.715	-0.469	4.25e-13	****
Ratio	N-Limited	P-Limited	-0.523	-0.688	-0.359	3.09e-12	****
Nut. Av.	Before	After	-0.417	-0.502	-0.332	4.35e-13	****

*Table S2.4: Post-hoc test for Generalized Linear Model (LRRn ~ final N:P supply ratio * nutrient availability during temperature change). Asterisks indicate significance (p > 0.05).*

Term	Group 1	Group 2	Estimate	Conf. Low	Conf. High	P (adjusted)	Significance
Temp	6	12	0.0890	0.0709	0.107	1.89e-11	****
Temp	6	18	0.0664	0.0481	0.0847	2.55e-11	****
Temp	12	18	-0.0226	-0.0403	-0.00488	8.94e-3	**
Ratio	Balanced	N-Limited	0.00106	-0.0215	0.0236	9.93e-1	
Ratio	Balanced	P-Limited	0.0404	0.0224	0.0583	3.37e-6	****
Ratio	N-Limited	P-Limited	0.0393	0.0135	0.0651	1.5e-3	**

*Table S2.5: Post-hoc test for Generalized Linear Model (particulate N:P ratio ~ temperature level * final N:P supply ratio * nutrient availability during temperature change). Asterisks indicate significance (p > 0.05).*

Term	Group 1	Group 2	Estimate	Conf. Low	Conf. High	P (adjusted)	Significance
Temp	6	12	0.0747	-0.0207	0.170	1.56e-1	
Temp	6	18	0.0764	-0.0190	0.172	1.43e-1	
Temp	12	18	0.00172	-0.0927	0.0961	9.99e-1	
Ratio	Balanced	N-Limited	-0.188	-0.296	-0.0790	2.15e-4	***
Ratio	Balanced	P-Limited	0.578	0.484	0.672	3.55e-15	****
Ratio	N-Limited	P-Limited	0.766	0.641	0.891	3.55e-15	****
Nut. Av.	Before	After	-0.202	-0.267	-0.138	7.47e-9	****

3.3: PUBLICATION IV

Moderate and extreme warming under a varied resource supply alter the microzooplankton-phytoplankton coupling in marine communities

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Moderate and extreme warming under a varied resource supply alter the microzooplankton-phytoplankton coupling in marine communities

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Abstract

Marine heatwaves are among the most common extreme events on Earth; however, it is barely known how a moderate or extreme warming impacts the trophic interactions, and thus, the trophic transfer efficiency 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 *vs.* 12 ºC above ambient temperature), covering a nitrogen-to-phosphorus gradient from nutrient saturated to limited conditions. We addressed how both drivers, temperature and nutrients, altered the community structure and how these changes mediated the phytoplankton growth (μ) and microzooplankton grazing (m) rates, thus the biomass available for higher trophic levels. Moderate and extreme warming effects on the microzooplankton-phytoplankton relationship differed and were mediated by time. The trophic interaction was weakened due to an uncoupling (μ outpaced m) between phytoplankton growth and microzooplankton grazing, regardless of the warming treatment first (mid-term of the experiment) but a strengthening of the trophic interaction, by increased grazing under extreme warming, after an acclimation period. A strengthened trophic interaction entails a shift towards a higher trophic transfer efficiency but potentially lower carbon export, as warming intensifies, over time. 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 towards mixotrophy or that the temperatures experienced by grazers were suboptimal compared to its prey. Finally, our findings showed that temperature was the main driver whereas the resources availability exerted a minor role on this trophic interaction. By considering that climate change will intensify in the future, food webs could be less productive but more efficient, and thus, potentially support a higher secondary production in a warmer world.

Introduction

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 the metabolic theory of ecology (Brown et al. 2004), and evidence in terrestrial (Allen et al. 2005; Allen & Gillooly 2009), freshwater (O'Connor et al. 2009; Schaum et al. 2018), and marine (López-Urrutia et al. 2006; Liu et al. 2019) ecosystems, the thermal dependence of heterotrophic processes [e.g. grazing rates (m)] is higher than that of autotrophic ones [e.g. phytoplankton growth rates (μ)]. However, findings by Chen & Laws (2017) and Wang et al. (2019) showed that autotrophic processes can be as sensitive to temperature as heterotrophic ones, an even such sensitivity can be larger in autotrophic processes over seasonal scales (Liu et al. 2019). Additionally, the thermal dependence of metabolism is dependent on the resource availability, being stimulated as resources availability increases (Hayashida et al. 2020; Cabrerizo & Marañón 2021a), and weaker (Liu et al. 2021), and even suppressed when they are limiting (Marañón et al. 2018). Therefore, the temperature-resources interplay and the potential differential thermal sensitivity of auto- and heterotrophic processes may determine the fate of primary production in ecosystems, with most of it likely exported to deep waters in high-temperature and resource conditions (i.e. a weakened food web coupling), but the opposite occurring i.e. enhanced trophic transfer (i.e. a strengthened grazer-prey coupling) under high-temperature and low-nutrient availability (Franzè et al. 2023).

The ecological relevance of microzooplankton grazing pressure on the carbon cycling is well-stated, representing the consumption of the ~60% of the global primary production (Schmoker et al. 2013). Despite the pivotal role of this trophic interaction, most studies evaluating its sensitivity to temperature (and resource availability) have considered the spatial variability (Calbet & Landry 2004; Landry et al. 2022; Liu et al. 2023) and m and μ separately (Liu et al. 2019), whereas changes over time and in the trophic coupling, in particular under global change scenarios and extreme weather events, have received scarce attention. The importance of temporal dynamics has been stressed by Anderson & Harvey (2019) to provide more accurate predictions of the primary production fate and related food web processes. Also, Li et al. (2013) have evidenced that the space-for-time substitution allows to infer the structural and compositional state of the plankton communities from observations across geographical gradients. More recently, Cabrerizo & Marañón (2021b) have emphasized that addressing the grazing pressure (i.e. m:μ ratio), rather than both processes in isolation, allows to obtain a more integrative view on how thermal dependence affects trophic interactions in food webs.

Plankton are sentinel organisms to track the impacts of global change on aquatic ecosystems. Phytoplankton play a key role in regulating organic matter and nutrient cycling, trophic interactions and atmosphere-water gas exchange (Iversen 2023), hence any alteration on this trophic compartment may be propagated (and amplified) at higher trophic levels. For instance, recent results have evidenced that heatwave events prompt shifts in the metabolic balance towards heterotrophy, and favored pico- and nanoplanktonic species (Soulié et al. 2022; Soulié et al. 2023). Zhao et al. (2023) showed that warming erodes the ecosystems stability, whereas Courboulès et al. (2022) found that warming reduces phytoplankton biomass and increases the heterotroph to autotroph ratios, triggering a strengthening of the microbial loop compared to the grazing chain. More recently, Vad et al. (2023) found that herbivorous ciliates collapsed and bacterivorous taxa dominated, resulting in a weakened top-down control mediated by a negative impact of a short experimental heatwave. By contrast, other studies have shown that warming can also increase C:nutrient ratios (Biermann et al. 2015; De Senerpont Domis et al. 2014), primary productivity and biodiversity (Yvon-Durocher et al. 2015). Observational and mesocosms studies have reported that warming increases the proportion of smaller phytoplankton cells in communities (Mousing et al. 2014; Yvon-Durocher et al. 2011), and reduces the contribution of mixotrophs although it does not affect the relative biomass of heterotrophs (Jassey et al. 2015). Finally, warming strengthens the producer-grazer (or predator) interaction (Garzke et al. 2019; Schaum et al. 2018), but reduces the energy transfer efficiency to higher trophic levels (Ullah et al. 2018).

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?; and (c) are such effects on microzooplankton grazing consistent once the communities reached the final temperature level and after an acclimation period?. We predict an increased energy transfer efficiency by an accentuated grazing pressure under extreme compared to moderate and control temperature due to microzooplankton grazing will increase more than phytoplankton growth due to its higher (hetero- vs. autotrophic processes) thermal dependence. To address these research gaps, we performed a mesocosms experiment in which plankton communities were exposed to moderate (+6 ºC) and extreme (+12 ºC) warming in respect to *in situ* conditions (6 °C) in two consecutive phases: a ramping phase in which temperature increased by 1 ºC per day until it reached the target treatment temperature (6, and

12 days, respectively), and a constant temperature phase (from day 12 to day 27) in which communities were exposed to warming treatments at different levels. We used a natural marine plankton community from the North Sea and examined these warming effects on different biological organization levels: total biomass, stoichiometry [particulate organic carbon (POC), nitrogen (PON) and phosphorus (POP) ratios], community size- (micro-, nano-, and picoplankton) and trophic- (auto-, mixo-, 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.

Material and methods

Experimental setup of the mesocosm experiment

On 6th March 2022, an 8000 L surface water sample was taken at Helgoland Roads station (54º 11' 17.88" N, 7º 54' E), filtered through a 200 μm mesh to exclude mesozooplankton, and placed the following day into 12 indoor mesocosms (Planktotrons, 600-L stainless steel indoor-mesocosms). On 8th March, the plankton communities were exposed (in quadruplicate) to three temperature treatments: 6 (control temperature), 12 (moderate), and 18 ºC (extreme) over 27 days. We chose 6 ºC as the ambient temperature as it resembles the temperature registered during the sampling day (5.4 ºC) 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 ºC 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 ºC 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 ºC per day until target temperatures were reached for the two warming treatments, and then maintained constant over the experimental period (Fig. S1A). Built-in rotors with silicon lips at the side, top, and bottom, gently rotate 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 LEDs units (IT2040, Evergrow, Shenzhen, China) above each Planktotron, with mean surface irradiances of 181.80 ± 1.76 μmol photons m^{-2} s^{-1} over a 12:12 h light-dark cycle. Translucent float glass panels (Pilkington Optiwhite, Tokyo, Japan) 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, Rye Brook, NY, USA) 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 meso-zooplankton 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 meso-zooplankton 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, Donaueschingen, Germany) whereas light intensity was monitored manually each other day using a Photosynthetically Active Radiation light meter (LI-COR LI-250A, LI-COR Biosciences, USA). All other response variables, except the micro-grazing incubations (see below) were measured every three days (10 times in total) over the experimental period. Early in the morning (9:00 am), integrated water column samples were taken from each Planktotron once the mixing process was completed using a customized Polyvinyl Chloride (PVC) 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, Wegberg, Germany), whereas silicate was quantified by the molybdate reaction following standard protocols (Wetzel 2001). Samples for POC, PON, and POP as well as chlorophyll a (Chl *a*) concentrations were filtered on pre-combusted and acid-washed glass-fiber GF/C filters (Whatman Inc. UK) and stored at -80 ºC until analyzed. POC and PON filters were measured with a CHN elemental analyzer (ThermoFisher Scientific Inc., Flash EA 1112, USA), and POP 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 ºC for 24 h in darkness. Samples were measured in a multi-plate reader (SYNERGY H1, BioTek, Winooski, Vermont, USA) 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 pico- (0.2-2 µm), nano (2-20 µm), and microplankton (20-200 µm), and the trophic mode between hetero-, mixo-, and autotrophs. 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 (WoRMS, http://marinespecies.ogr), Nordic Microalgae (http://nordicmicroalgae.org), and PlanktonNet (htt://planktonnet.awi.net) databases, and Olenina et al. (2006). For species where the required information was not available, we did specific literature searchers (until 12 September 2023) using SCOPUS as search engine (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 incubations

Phytoplankton growth and protist herbivorous-induced mortality rates were measured with the dilution method (Landry & Hassett 1982) in a two-point modification (Menden-Deuer et al. 2018; Anderson & Harvey 2019; Landry et al. 2022) using undiluted (100%) and diluted (30%) seawater. The validity of this approach, compared with the traditional multi-point dilution approach, has been demonstrated in several studies which have evidenced indistinguishable growth and grazing rates with both methodologies (Worden & Binder 2003; Chen 2015; Morison & Menden-Deuer 2017). The dilution factor used was based on previous results by Chen (2015) which showed that setting up a highly diluted bottle (≤ 30%) and treating the net μ of this bottle as the instantaneous μ yields the more accurate estimates.

To do that, samples were taken from each mesocosm, and pooled together for each temperature treatment. From this water, we prepared 500-mL undiluted (100%) and diluted (30%) samples (2 technical replicates per temperature and dilution treatment at the start (t0) without nutrients enrichment and only one (with nutrients enrichment) after 24 hs of incubations (tf)). tf 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. 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. N concentrations added, as $NaNO_3$, ranged between 0-52.70 µM, and those of P, added as hydrated NaH_2PO_4, between 0-3.3 µM. The generated N:P ratios were categorized into N-

limited (N:P < 11), balanced (N:P = 12-39), and P-limited (N:P > 40). Ultimately, the final nutrient supply consisted of the nutrients added plus the background concentrations existing in the seawater at the start of each incubation. To generate the necessary seawater to be mixed with the samples for the diluted treatment (150 mL per bottle), additional 75 L of seawater from a nearby coastal site at the ICBM were filtered through 0.2-µm polycarbonate filters (Millipore, USA), sterilized for 15 min at 121 ºC, and stored in darkness at 4 ºC. 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, Austria), 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 & Hasset (1982) and Chen (2015), the net phytoplankton growth rate (k) was calculated as:

$$k = \ln (\text{Chl}a_{tf} / \text{Chl}a_{t0}) / t$$

where $\text{Chl}a_{tf}$ and $\text{Chl}a_{t0}$ are the Chl *a* concentrations measured at the end (t_f) and at the beginning (t_0) of the incubation period, respectively, and t 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:

$$m = (k_{30} - k_{100}) / (1 - \times)$$

with × 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). RUE 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 function of N (than P) availability (see Fig. S1B-D; Palomares-García et al. 2006). The relative contribution (%) of each size fraction (micro-, nano- and picoplankton) and trophic mode (auto-, hetero-, 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 ASVs (i.e. micro- + nano- + picoplankton for size structure, and auto- + hetero- + mixotrophs for trophic mode). The predator:prey availability ratio over the experimental period was calculated as the quotient between the ASVs of autotroph *versus* those of hetero- plus mixotrophs (see Ahme et al. 2023b and supplementary material and methods for more details). We grouped hetero- and mixotrophs as several experimental findings indicate that mixotrophs behave as heterotrophs, and heterotrophy increases with warming (Wilken et al. 2013; Cabrerizo et al. 2019; Lepori-Bui et al. 2022).

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 & Landry 2004; Anderson & Harvey 2019; Cabrerizo & Marañón 2021b).

A repeated measures (RM) one-way analysis of the variance 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 RM 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 analysis were used to assess the relationship between μ and m over the experimental N:P ratio gradient considered and for each temperature treatment, and Chl *a versus* 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 before ANOVA and regression analysis. 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 Cran Team 2022) with RStudio v. 2023.09.0.

Results

Plankton community size-structure and trophic modes in the mesocosms

The community size-structure was dominated by nanoplankton species regardless of the temperature treatment and over the experimental period (Fig. 1A; Table S2). This group contributes between ~70 (6 ºC) and 61 and 82% (12 and 18 vs. 6ºC ºC) to the total community at days 0 and 27, respectively, whereas microplankton account for ~36% (day 0) and 17-30% (day 27, 6-12 and 18 ºC, respectively). The contribution of picoplankton to the community was minor over time and regardless of the temperature treatment (< 3% of the total; Fig. 1A). When we grouped the species into trophic modes, our results showed that autotrophs had the maximum contribution to the community at the beginning (> 50%; days 3, 6 and 9) and at the end of the experimental period (up to 60%; days 24 and 27) under control temperature (Fig. 1B). 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 in the middle of the experiment, in particular under control, and the lowest contribution to the total community 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 (Fig. S2A) and the total species richness (Fig. S2B), although without significant differences within temperature treatments (C:Chl*a*: *F*-value = 0.58, *p*-value = 0.59; Richness: *F*-value = 0.18, *p*-value = 0.84).

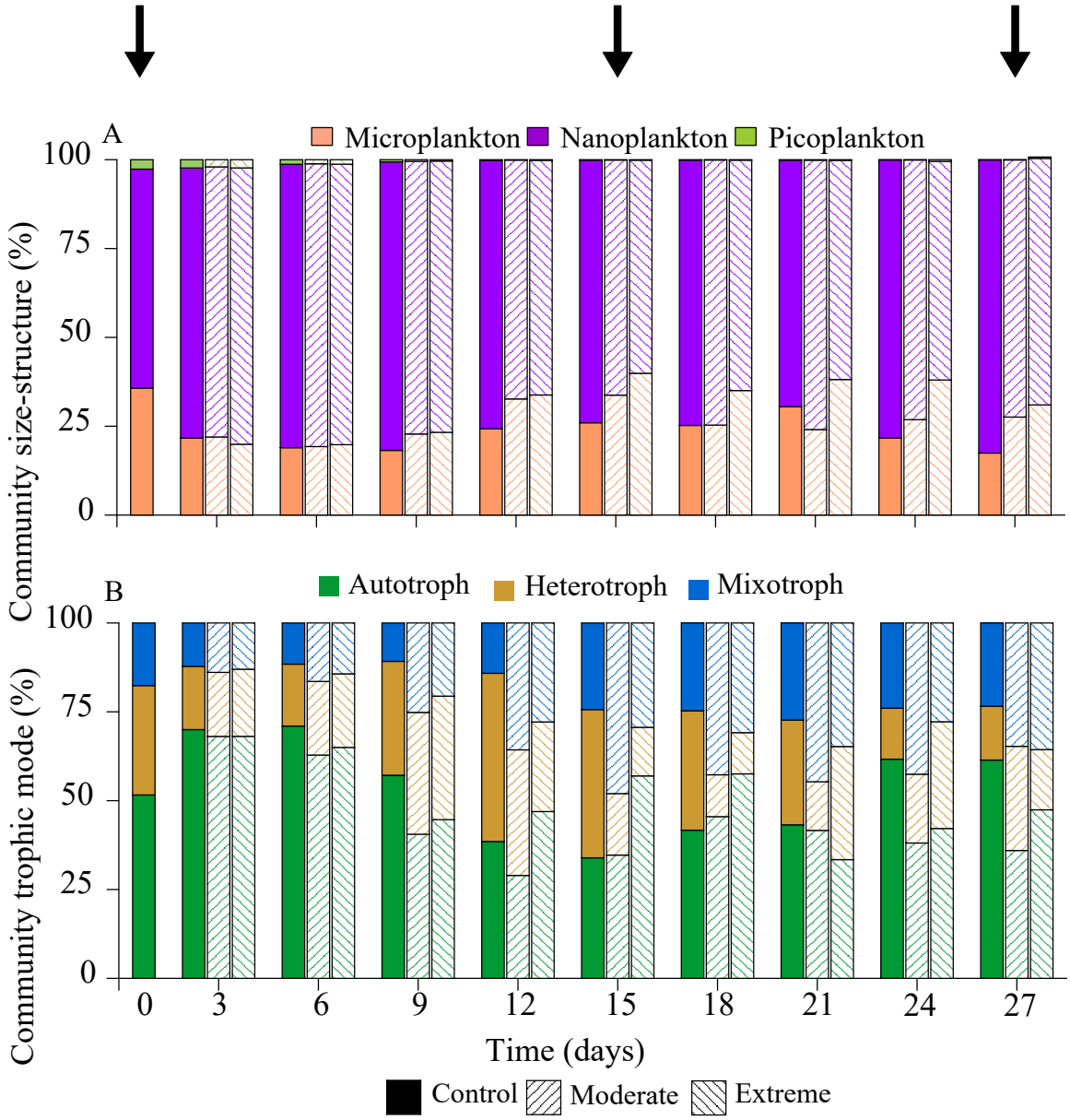

Figure 1: Mean relative contribution (%) of micro-, nano- and picoplankton (A), and auto-, hetero-, and mixotrophs (B) to the total community in plankton communities exposed to three temperature treatments (control, 6; moderate, 12; and extreme, 18 ºC) over the experimental period. Black arrows represent the micro-grazing incubation days.

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 but then, they increased above 1 (i.e. predators > prey availability), in particular in the moderate warming treatment at day 12, where maximum values were measured (ca. 3; Fig. 2A). From mid-experiment, the values decreased in all treatments but highest ratios occurred under extreme temperature, followed by moderate and control temperature treatments. The changing ratios translated into marked variations in the accumulation rates over time (Fig. 2B). These rates significantly increased under moderate and extreme warming treatments at day 15 respect

initial conditions (Table S2), reaching values between 0.5-1 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 respect extreme temperature conditions (Fig. 2B).

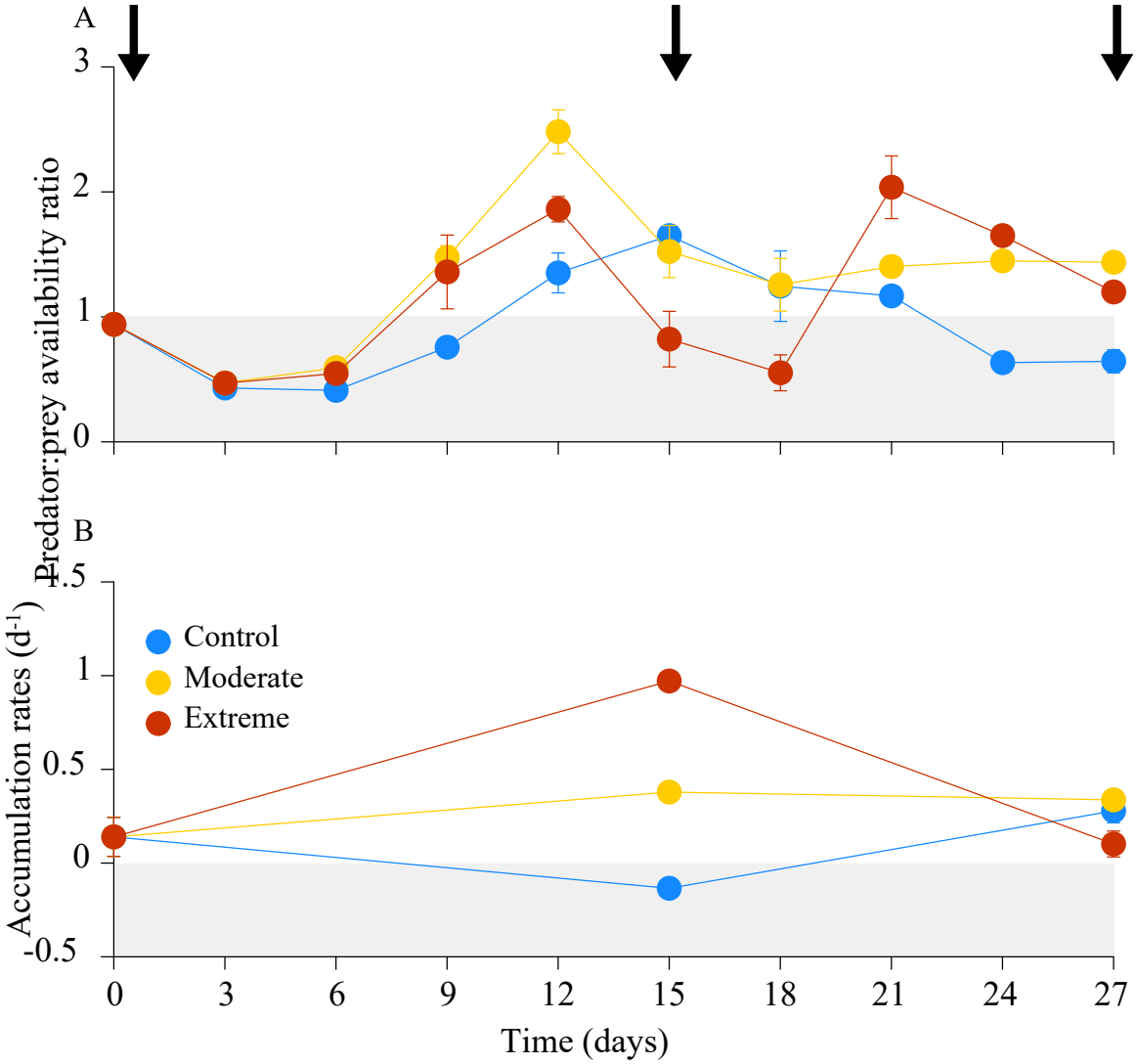

Figure 2: Mean (±SD) predator (hetero- plus mixotrophs) and prey (autotrophs) availability ratios (A), and accumulation rates in plankton communities exposed to three temperature treatments (control, 6; moderate, 12; and extreme, 18 ºC) over the experimental period. Black arrows represent the micro-grazing incubation days. In those symbols where not standard deviation appears is due to bars being smaller than the symbol size.

The relationship between μ and m shows a strong trophic coupling (values around the 1:1 line; R = 0.74, F_{23}-value = 9.08, p-value < 0.01) at day 0 and that it was not influenced by the N:P supply ratio (a similar response pattern was observed at days 15 and 27; Fig. 3A). Based on the slope of the linear regression (and the $m{:}\mu$ ratios; Fig. S3A), microzooplankton consumption accounted, on average, 87% of phytoplankton growth i.e. grazing exerted a strong top-down control on primary production available. At day 15, we observed that such trophic coupling accentuated under ambient conditions (R = 0.94, F_{23}-value = 168.89, p-value <0.0001), reaching values > 120% (Fig. S3B), whereas it weakened under extreme warming conditions (R = 0.91, F_{23}-value = 105.70, p-value <0.0001; Fig. 3B). In the warming conditions, the $m{:}\mu$ ratios ranged, on average, between 24% (extreme warming) and 51% (moderate warming) (Fig. S3B). At day 27, we observed that the weakened trophic coupling only maintained under moderate warming (R = 0.90, F_{23}-value = 88.35, p-value <0.0001; Fig. 3C); however, the mean $m{:}\mu$ ratios were ~50% under control and moderate temperature but increased up to 72% under the extreme one, with independence of the N:P ratio considered (Fig. S3C).

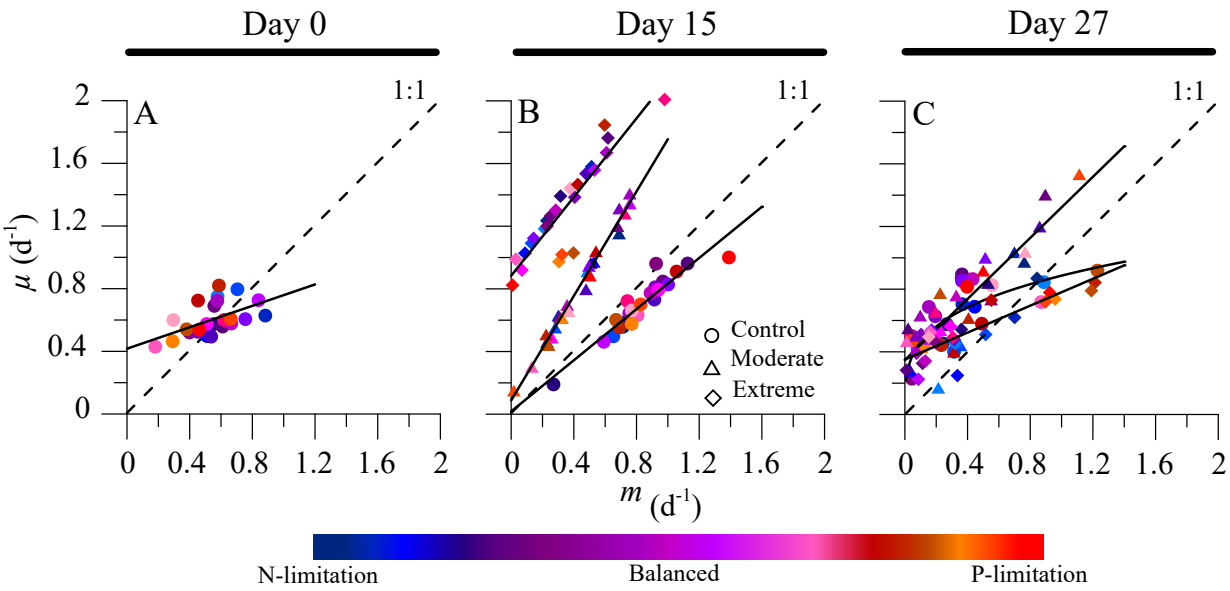

Figure 3: Mean instantaneous phytoplankton growth (μ) versus microzooplankton grazing (m) rates at days 0, 15, and 27 in plankton communities exposed to three temperature treatments (control, 6; moderate, 12; and extreme, 18 ºC) over a dissolved nitrogen:phosphorus (N:P) ratios gradient (see supplementary figure 3 for specific ratios). The dashed line represents the 1:1 relationship and the solid lines are the linear or non-linear fits for each temperature treatment.

Discussion

Our work shows that moderate and extreme warming exert contrasting effects on the herbivorous protist-prey relationship mediated by the temporal response scale, from a weakening of the trophic interaction at the start due to an uncoupling between both trophic levels ($\mu > m$) up to a strengthening of it under extreme warming at the end of the experiment.

Moreover, the effects of temperature on this trophic interaction exceeded those exerted by a N- or P-limiting or balanced resources supply. This dominant effect of temperature, compared to the N:P supply ratios, on microzooplankton-phytoplankton can be due to that the incubation period was too short to observe a detectable change, in terms of biomass, in the phytoplankton community, and subsequently in grazers due to the resources supply (Cáceres et al. 2013; Landry et al. 2022). Thus, and in contrast to previous observational (Marañón et al. 2014), experimental and modeling (O'Connor et al. 2009; O'Connor et al. 2011) results, our findings suggest that the variability in plankton rates is mainly driven by temperature, whereas the resource supply, both limiting and balanced, had a minor role.

Microzooplankton-phytoplankton coupling under warming: Transient responses

A continuous temperature increase (1 ºC d^{-1}) prompted a decoupling in the microzooplankton-phytoplankton relationship (when compared with control conditions, $m{:}\mu$ =120%) due to a weakening in the trophic interaction strength i.e. phytoplankton growth outpaced grazing (Fig. 3B). A weakening in the trophic interaction entails a decrease in the trophic transfer efficiency towards higher trophic levels but an enhanced carbon export out of the euphotic zone due to increased accumulation rates (Fig. 2B; Franzè et al. 2023). Part of the available production could be consumed by mesozooplankton even though global- and regional scale analysis show that its contribution to the total grazing represents only ~22-25% of the total primary production (Calbet 2001; Landry et al. 2020). Our estimates [mean $m{:}\mu$ = 51% (moderate) and 24% (extreme)] are lower compared to prior observational (> 60%; Palomares-García et al. 2006; Schmoker et al. 2013; Steinberg & Landry 2017) and experimental (> 90%; Rose et al. 2009; Menden-Deuer et al. 2018; Horn et al. 2020) works, but higher than recently reported by Franzé *et al*. (2023) in a coastal ecosystem when communities were exposed to interactions between warming and nutrient enrichment. These authors argued that the absence of any detectable grazing pressure was due to two concurrent facts: changes in species composition towards less palatable/edible species (Anderson et al. 2022), and to phytoplankton growth rates. In our case, we find support for the first fact as the proportion of these species (mainly chain-forming diatoms) was higher under extreme compared to control or moderate warming (~26 vs. 18 or 14%, respectively; Ahme et al. 2024). This higher contribution of larger cells to the total community is consistent with the reported results of a five years warming study in experimental ponds (Yvon-Durocher et al. 2015), although its authors attributed these changes to a potential stronger top-down control under warming compared to ambient temperature conditions. Also,

our results are in line with the second proposed argument, as μ_{max} (1.8 d^{-1}) exceeded by 2-fold the m_{max} (< 0.8 d^{-1}) (Fig. 3). A higher μ_{max} is also consistent with the enhanced accumulation rates observed under warming conditions (Fig. 2B) and is in line with previous results by Mojica et al. (2021) who found that increased accumulation denotes faster rates of change in phytoplankton division rates.

Two additional plausible explanations for the reductions found in grazing pressure could be a switch toward mixotrophy and that the temperatures experienced by grazers were more suboptimal than for their prey. Although mixotrophy was not directly quantified in our study, we found that mixotrophs contribute between 30-50% to the total community under warming conditions. The most benefited groups were dinophyta, haptophyta, and unidentified MAST clade species, all of which have well-known phagotrophic species able to grow as photo-heterotrophs (Wilken et al. 2014; Mitra et al. 2016; Labarre et al. 2021). A potentially higher phototrophic activity subsequently would explain why despite the increase in grazers (by 37 and 84% under moderate vs. extreme warming compared to control; Fig. 2A) mediated by increased availability of prey biomass and RUE_N (Fig. S4B), the grazing pressure was lower. In relation to the second explanation, there is evidence in coastal ecosystems showing that microzooplankton has optimal temperatures > 3 ºC higher than that of phytoplankton (Liu et al. 2019). By considering that our phytoplankton community had their growth optimum at 18 ºC (Ahme et al. 2024), which matches with the extreme warming treatment, and the second explanation posed, we speculate that grazers were growing under more suboptimal temperatures than their prey.

Microzooplankton-phytoplankton coupling under warming: Acclimation responses

Once communities were exposed to the two warming regimes for more than two weeks, grazing pressure was maintained under control and moderate but accentuated under extreme [72 (day 27) vs. 25 (day 12) % of the total community production consumed] conditions. Following the reasoning presented above, this differential response pattern between immediate and acclimation timeframes entails a shift towards a strengthening of the trophic coupling, and potentially, a higher energy transfer efficiency to other trophic levels but lower C export, as warming intensifies. This increased grazing pressure under warmer conditions agrees with the idea by Rose and Caron (2007) that acclimated herbivorous protist growth rates increase faster than do those of phototrophs at temperatures above 15 ºC. The discrepancy between grazing pressure observed between immediately heated and acclimated communities under extreme warming (but not under moderate) could be due to a decrease in microzooplankton grazing

pressure with increasing temperature when Chl *a* concentrations are low (i.e. values ~3 μg L^{-1}) but increases when they are high (oligo- vs. eutrophic systems; Chen et al. 2012). The underlying cause is that the lower microzooplankton biomass with increasing temperature (also observed in our work, extreme vs. moderate; Fig. 2A) overweighs the effect of higher activation energy of hetero- compared to autotrophic processes. By contrast, Franzè & Menden-Deuer (2020) have suggested that it can be due to depressed grazing rates because herbivorous protists need a longer time to acclimate to the thermal environment experienced than phytoplankton. In our case, we did not observed depressed grazing rates under extreme warming conditions, as they were in the range measured in the other two temperature treatments, and observed in previous studies in other temperate (Calbet & Landry 2004; Teixeira et al. 2011; Anderson et al. 2018), tropical (Cáceres et al. 2013; Anjusha et al. 2018), and polar ecosystems (Gutiérrez-Rodríguez et al. 2020; Gutiérrez-Rodríguez et al. 2023; Liu et al. 2023). Finally, we can discard that the increased grazing pressure observed at day 27 was due to a higher energetic demand mediated by a lowered prey nutritional quality, as previous studies showed (Mitra & Flynn 2005; De Senerpont Domis et al. 2014; Duarte-Moreno et al. 2022), because we did not find differences in the community food quality i.e. C:N (values ranged 12.09±1.08 and 14.29±1.81), as well as on N:P and C:P (Fig. S4) ratios, among the three temperature treatments.

Conclusions

Concerning the major key question posed about the interacting effects of warming and resource supply on marine plankton and the trophic interactions within food webs: *What are the effects of moderate and extreme warming on the microzooplankton-phytoplankton coupling?*. Our findings suggest, partially in contrast to what we predicted, that temperature changes, that usually occur during extreme warming events (e.g. heatwaves, Smale et al. 2019), or diel thermal fluctuations (Simolo & Corti 2022) can exert a dual role on auto- and heterotrophic protists. From the primary producer perspective, a potential direct beneficial effect on their growth i.e. higher rates, and an indirect one i.e. reduced grazing by herbivorous protists, could result in a win-win effect for them, triggering sudden phytoplankton blooms, and increasing the carbon sequestration and its exportation to deep waters. This argumentation is consistent with the fact that blooms 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 both trophic compartments, phyto- and microzooplankton, are acclimated to the thermal changes experienced, grazing pressure accentuated, that is, there was a strengthening of the trophic interaction, in particular under the extreme warming, and the trophic transfer efficiency boosted. This evidences that no universal assumption can be made about the role (and the relative contribution) that grazers play in marine food webs. Because ocean-scale models and climate change scenarios assume a constant grazing pressure, our findings support the idea that grazing still remains as the largest source of uncertainty for marine carbon cycling (Rohr et al. 2023). Moreover, assuming a constant grazing implies to consider a fixed thermal sensitivity. This argumentation has been recently questioned for primary producers by Anderson et al. (2024), evidencing that the use of a constant thermal sensitivity for different phytoplankton groups leads to unrealistic communities, and significantly alters their competitive ability. Due to this aspect remains unevaluated for grazers, it ultimately also impacts our current estimates of biogeochemical processes (e.g. carbon export).

Therefore, by considering that ecosystems will be exposed to an increasingly occurrence of extreme weather events, and if evolutionary time scales do not compensate for the differential and variable thermal sensitivity on growth and grazing (auto- vs. heterotrophic metabolism) reported here over time, we predict that food webs could be less productive but more efficient, and thus could potentially support a higher secondary production in the future.

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Data availability statement

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

References

Ahme A, Happe A, Striebel M et al. (2024) Warming increases the compositional and functional variability of temperate coastal protist communities. *under review*. http://dx.doi.org/10.2139/ssrn.4717787.

Ahme A, Happe A, Striebel M et al. (2023a) Temperature effects on a plankton community from Helgoland Roads tested in an indoor mesocosm experiment in March 2022. PANGAEA. https://doi.org/10.1594/PANGAEA.961155.

Ahme A, Von Jackowski A, Mcpherson RA, Wolf KKE, Hoppmann M, Neuhaus S, John U (2023b) Winners and lossers of atlantification: The degree of ocean warming affects the structure of Arctic microbial communities. Genes, **14**, 623.

Allen AP, Gillooly JF (2009) Towards an integration of ecological stoichiometry and the metabolic theory of ecology to better understand nutrient cycling. Ecology Letters, **12**, 369-384.

Allen AP, Gillooly JF, Brown JH (2005) Linking the global carbon cycle to individual metabolism. Functional Ecology, **19**, 202-213.

Anderson SI, Franzè G, Kling JD et al. (2022) The interactive effects of temperature and nutrients on a spring phytoplankton community. Limnology and Oceanography, **67**, 634-645.

Anderson SI, Fronda C, Barton AD, Clayton S, Rynearson TA, Dutkiewicz S (2024) Phytoplankton thermal trait parameterization alters community structure and biogeochemical processes in a modeled ocean. Global Change Biology, **30**, e17093.

Anderson SR, Diou-Cass QP, Harvey EL (2018) Short-term estimates of phytoplankton growth and mortality in a tidal estuary. Limnology and Oceanography, **63**, 2411-2422.

Anderson SR, Harvey EL (2019) Seasonal variability and drivers of microzooplankton grazing and phytoplankton growth in a subtropical estuary. Frontiers in Marine Science, **6**, 174.

Anjusha A, Jyothibabu R, Savitha KMM, Albin KJ (2018) Seasonal variation of phytoplankton growth and microzooplankton grazing in a tropical coastal water (off Kochi), Southwest coast of India. Continental Shelf Research, **171**, 12-20.

Biermann A, Lewandowska AM, Engel A, Riebesell U (2015) Organic matter partitioning and stoichiometry in response to rising water temperature and copepod grazing. Marine Ecology Progress Series, **522**, 49-65.

Brown JP, Gillooly F, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. Ecology, **85**, 1771-1789.

Cabrerizo MJ, González-Olalla JM, Hinojosa-López VJ, Peralta-Cornejo FJ, Carrillo P (2019) A shifting balance: responses of mixotrophic marine algae to cooling and warming under UVR. New Phytologist, **221**, 1317-1327.

Cabrerizo MJ, Marañón E (2021a) Geographical and seasonal thermal sensitivity of grazing pressure by microzooplankton in contrasting marine ecosystems. Frontiers in Microbiology, **12**, 679863.

Cabrerizo MJ, Marañón E (2021b) Grazing pressure is independent of prey size in a generalist herbivorous protist: Insights from experimental temperature gradients. Microbial Ecology, **81**, 553-562.

Cáceres C, González-Taboada F, Höfer J, Anadón R (2013) Phytoplankton growth and microzooplankton grazing in the subtropical Northeast Atlantic. PLoS One, **8**, 1-13.

Calbet A (2001) Mesozooplankton grazing effect on primary production: A global comparative analysis in marine ecosystems. Limnology and Oceanography, **46**, 1824-1830.

Calbet A, Landry MR (2004) Phytoplankton growth, microzooplankton grazing, and carbon cycling in marine systems. Limnology and Oceanography, **49**, 51-57.

Courboulès J, Mostajir B, Trombetta T, Mas S, Vidussi F (2022) Warming disadvantages phytoplankton and benefits bacteria during a spring bloom in the Mediterranean Thau lagoon. Frontiers in Marine Science, **9**, 878938.

Chen B (2015) Assessing the accuracy of the "two-point" dilution technique. Limnology and Oceanography: Methods, **13**, 521-526.

Chen B, Landry MR, Huang B, Liu H (2012) Does warming enhance the effect of microzooplankton grazing on marine phytoplankton in the ocean? Limnology and Oceanography, **57**, 519-526.

Chen B, Laws EA (2017) Is there a difference of temperature sensitivity between marine phytoplankton and heterotrophs? Limnology and Oceanography, **62**, 806-817.

Dai Y, Yang S, Zhao D et al. (2023) Coastal phytoplankton blooms expand and intensify in the 21st century. Nature, **615**, 280-284.

De Senerpont Domis L, Van De Waal DB, Helmsing NR, Van Donk E, Mooij WM (2014) Community stoichiometry in a changing world: Combined effects of warming and eutrophication on phytoplankton dynamics. Ecology, **95**, 1485-1495.

Franzè G, Anderson SI, Kling JD et al. (2023) Interactive effects of nutrients and temperature on herbivorous predation in a coastal plankton community. Limnology and Oceanography, **68**, S144-S157.

Franzè G, Menden-Deuer S (2020) Common temperature-growth dependency and acclimation response in three herbivorous protists. Marine Ecology Progress Series, **634**, 1-13.

Garzke J, Connor SJ, Sommer U, O'connor MI (2019) Trophic interactions modify the temperature dependence of community biomass and ecosystem function. PLoS Biology, **17**, e2006806.

Gerhard M, Koussoroplis AM, Hillebrand H, Striebel M (2019) Phytoplankton community responses to temperature fluctuations under different nutrient concentrations and stoichiometry. Ecology, **100**, e02834.

Gillooly F, Brown JH, West GB, Savage VM, Charnov EL (2001) Effect of size and temperature on metabolic rate. Science, **293**, 2248-2251.

Gutiérrez-Rodríguez A, Latasa M, Safi K, Pinkerton MH, Nodder SD (2023) Decoupled growth and grazing rates of diatoms and green algae drive increased phytoplankton productivity on HNLC sub-Antarctic plateaux. Limnology and Oceanography Letters, **8**, 896-905.

Gutiérrez-Rodríguez A, Safi K, Fernández D et al. (2020) Decoupling between phytoplankton growth and microzooplankton grazing enhances productivity in subantarctic waters on Campbell Plateau, southeast of New Zealand. Journal of Geophysical Research: Oceans, **125**, e2019JC015550.

Hayashida H, Matear RJ, Strutton PG (2020) Background nutrient concentration determines phytoplankton bloom response to marine heatwaves. Global Change Biology, **26**, 4800-4811.

Hodapp D, Hillebrand H, Striebel M (2019) Unifying the concept of resource use efficiency in Ecology. Frontiers in Ecology and Evolution, **6**, 00233.

Horn HG, Boersma M, Garzke J, Sommer U, Aberle N (2020) High CO_2 and warming affect microzooplankton food web dynamics in a Baltic Sea summer plankton community. Marine Biology, **167**, 69.

Iversen MH (2023) Carbon export in the ocean: A biologist's perspective. Annual Review of Marine Science, **15**, 357-381.

Jassey VEJ, Signarbieux C, Hättenschwiler S et al. (2015) An unexpected role for mixotrophs in the response of peatland carbon cycling to climate warming. Scientific Reports, **5**, 16931.

Labarre A, López-Escardó D, Latorre F et al. (2021) Comparative genomics reveals new functional insights in uncultured MAST species. The ISME Journal, **15**, 1767-1781.

Landry MR, Hassett RP (1982) Estimating the grazing impact of marine micro-zooplankton. Marine Biology, **67**, 283-288.

Landry MR, Hood, R. R., Davies CH (2020) Mesozooplankton biomass and temperature-enhanced grazing along a 110°E transect in the eastern Indian Ocean. Marine Ecology Progress Series, **649**, 1-19.

Landry MR, Selph KE, Hood RR, Davies CH, Beckley LE (2022) Low temperature sensitivity of picophytoplankton P : B ratios and growth rates across a natural 10°C temperature gradient in the oligotrophic Indian Ocean. Limnology and Oceanography: Letters, **7**, 112-121.

Lepori-Bui M, Paight C, Ebenhard E, Mertz CM, Moeller HV (2022) Evidence for evolutionary adaptation of mixotrophic nanoflagellates to warmer temperatures. Global Change Biology, **28**, 7094-7107.

Li WKW, Carmack EC, Mclaughlin FA, Nelson RJ, Williams WJ (2013) Space-for-time substitution in predicting the state of picoplankton and nanoplankton in a changing Arctic Ocean. Journal of Geophysical Research: Oceans, **118**, 5750-5759.

Liu K, Chen B, Zhang S, Sato M, Shi Z, Liu H (2019) Marine phytoplankton in subtropical coastal waters showing lower thermal sensitivity than microzooplankton. Limnology and Oceanography, **64**, 1103-1119.

Liu K, Nishioka J, Chen B et al. (2023) Role of nutrients and temperature in shaping distinct summer phytoplankton and microzooplankton population dynamics in thewestern North Pacific and Bering Sea. Limnology and Oceanography, **68**, 649-665.

Liu K, Suzuki K, Chen B, Liu H (2021) Are temperature sensitivities of *Prochlorococcus* and *Synechococcus* impacted by nutrient availability in the subtropical northwest Pacific? Limnology and Oceanography, **66**, 639-651.

López-Urrutia A, San Martín E, Harris RP, Irigoien X (2006) Scaling the metabolic balance of the oceans. Proceedings of the National Academy of Sciences, **103**, 8739-8744.

Marañón E, Cermeño P, Huete-Ortega M, López-Sandoval DC, Mouriño-Carballido B, Rodríguez-Ramos T (2014) Resource supply overrides temperature as a controlling factor of marine phytoplankton growth. PLoS One, **9**, e99312.

Marañón E, Lorenzo MP, Cermeño P, Mouriño-Carballido B (2018) Nutrient limitation suppresses the temperature dependence of phytoplankton metabolic rates. The ISME Journal, **12**, 1836-1845.

Menden-Deuer S, Lawrence C, Franzè G (2018) Herbivorous protist growth and grazing rates at in situ and artificially elevated temperatures during an Arctic phytoplankton spring bloom. PeerJ, **6**, e5264.

Mitra A, Flynn KJ (2005) Predator–prey interactions: Is 'ecological stoichiometry' sufficient when good food goes bad? Journal of Plankton Research, **27**, 393-399.

Mitra A, Flynn KJ, Tillmann U et al. (2016) Defining planktonic protist functional groups on mechanisms for energy and nutrient acquisition: Incorporation of diverse mixotrophic strategies. Protist, **167**, 106-120.

Mojica KDA, Behrenfeld MJ, Clay M, Brussaard CPD (2021) Spring accumulation rates in North Atlantic phytoplankton communities linked to alterations in the balance between division and loss. Frontiers in Microbiology, **12**, 706137.

Morison F, Menden-Deuer S (2017) Doing more with less?: Balancing sampling resolution and effort in measurements of protistan growth and grazing-rates. Limnology and Oceanography: Methods, **15**, 794-809.

Mousing EA, Ellegaard M, Richardson K (2014) Global patterns in phytoplankton community size structure — evidence for a direct temperature effect. Marine Ecology Progress Series, **497**, 25-38.

O'connor MI, Gilbert B, Brown CJ (2011) Theoretical predictions for how temperature affects the dynamics of interacting herbivores and plants. The American Naturalist, **178**, 626-638.

O'connor MI, Piehler MF, Leech DM, Anton A, Bruno JF (2009) Warming and resource availability shift food web structure and metabolism. PLoS Biology, **7**, e1000178.

Olenina I, Hajdu S, Edler L et al. (2006) *Biovolumes and size-classes of phytoplankton in the Baltic Sea,* Helsinki, Finland, Baltic Marine Environment Protection Commission.

Palomares-García R, Bustillos-Guzmán JJ, López-Cortés D (2006) Pigment-specific rates of phytoplankton growth and microzooplankton grazing in a subtropical lagoon. Journal of Plankton Research, **28**, 1217-1232.

Rockström J, Gupta J, Qin D et al. (2023) Safe and just Earth system boundaries. Nature, **619**, 102-111.

Rohr T, Richardson AJ, Lenton A, Chamberlain MA, Shadwick EH (2023) Zooplankton grazing is the largest source of uncertainty for marine carbon cycling in CMIP6 models. Communications Earth and Environment, **4**, 212.

Rose JM, Caron DA (2007) Does low temperature constrain the growth rates of heterotrophic protists?. Evidence and implications for algal blooms in cold waters. Limnology and Oceanography, **52**, 886-895.

Rose JM, Feng Y, Gobler CJ, Gutiérrez R, Hare CE, Leblanc K, Hutchins DA (2009) Effects of increased pCO_2 and temperature on the North Atlantic spring bloom. II. Microzooplankton abundance and grazing. Marine Ecology Progress Series, **388**, 27-40.

Schaum CE, Team SR, Ffrench-Constant R, Lowe C, Ólafsson JS, Padfield D, Yvon-Durocher G (2018) Temperature-driven selection on metabolic traits increases the strength of an algal–grazer interaction in naturally warmed streams. Global Change Biology, **24**, 1793-1803.

Schmoker C, Hernández-León S, Calbet A (2013) Microzooplankton grazing in the oceans: impacts, data variability, knowledge gaps and future directions. Journal of Plankton Research, **35**, 691-706.

Simolo C, Corti S (2022) Quantifying the role of variability in future intensification of heat extremes. Nature Communications, **13**, 7930.

Smale DA, Wernberg T, Oliver ECJ et al. (2019) Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nature Climate Change, **9**, 306-312.

Soulié T, Vidussi F, Mas S, Mostajir B (2022) Functional stability of a coastal Mediterranean plankton community during an experimental marine heatwave. Frontiers in Marine Science, **9**, 831496.

Soulié T, Vidussi F, Mas S, Mostajir B (2023) Functional and structural responses of plankton communities toward consecutive experimental heatwaves in Mediterranean coastal waters. Scientific Reports, **13**, 8050.

Steinberg DK, Landry MR (2017) Zooplankton and the ocean carbon cycle. Annual Review of Marine Science, **9**, 413-444.

Team Cran R (2022) *A language and environment for statistical computing. R foundation for statistical computing,* Viena (Austria).

Teixeira IG, Figueiras FG, Crespo BG, Piedracoba S (2011) Microzooplankton feeding impact in a coastal upwelling system on the NW Iberian margin: the Ría de Vigo. Estuarine, Coastal and Shelf Science, **91**, 110-120.

Thrane J-E, Kyle M, Striebel M, Haande S, Grung M, Rohrlack T, Andersen T (2015) Spectrophotometric analysis of pigments: A critical assessment of a high-throughput method for analysis of algal pigment mixtures by spectral deconvolution. PLoS One, **10**, e0137645.

Ullah H, Nagelkerken I, Goldenberg SU, Fordham DA (2018) Climate change could drive marine food web collapse through altered trophic flows and cyanobacterial proliferation. PLoS Biology, **16**, e2003446.

Vad CF, Hanny-Endrédi A, Kratina P et al. (2023) Spatial insurance against a heatwave differs between trophic levels in experimental aquatic communities. Global Change Biology, **29**, 3054-3071.

Wang Q, Lyu Z, Omar S, Cornell S, Yang Z, Montagnes DJS (2019) Predicting temperature impacts on aquatic productivity: Questioning the metabolic theory of ecology's "canonical" activation energies. Limnology and Oceanography, **64**, 1172-1185.

Wetzel RG (2001) *Limnology: Lake and River Ecosystems,* London, UK, Academic Press.

Wilken S, Huisman J, Naus-Wiezer S, Van Donk E (2013) Mixotrophic organisms become more heterotrophic with rising temperature. Ecology Letters, **16**, 225-233.

Wilken S, Schuurmans JM, Matthijs HCP (2014) Do mixotrophs grow as photoheterotrophs? Photophysiological acclimation of the chrysophyte *Ochromonas danica* after feeding. New Phytologist, **204**, 882-889.

Wiltshire KH, Ecker U, Kirstein IV (2013) Hydrochemistry at time series station Helgoland Roads, North Sea since 2001. (ed Helgoland AWI-BI) pp Page, PANGAEA.

Worden AZ, Binder BJ (2003) Application of dilution experiments for measuring growth and mortality rates among Prochlorococcus and Synechococcus populations in oligotrophic environments Aquatic Microbial Ecology, **30**, 159-174.

Yvon-Durocher G, Allen AP, Cellamare M et al. (2015) Five years of experimental warming increases the biodiversity and productivity of phytoplankton. PLoS Biology, **13**, e1002324.

Yvon-Durocher G, Montoya JM, Trimmer M, Woodward G (2011) Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems. Global Change Biology, **17**, 1681-1694.

Zhao Q, Van Den Brink PJ, Xu C et al. (2023) Relationships of temperature and biodiversity with stability of natural aquatic food webs. Nature Communications, **14**, 3507.

Supplementary material of publication IV

Supplementary methods: taxonomic composition in the mesocosms

Five hundred mL samples were gently vacuum-filtered (< 200 mbar) onto 0.8-µm polycarbonate filters (Nucleopore, Whatman, Maidstone, UK), which were put into 700 µL of warm extraction buffer, vortexed, and stored at – 80 °C. Extraction of the DNA was performed according to the manufacturer's protocol (NucleoSpin Soil extraction kit, Macherey-Nagel GmbH, Düren, Germany) after disrupting the cells using a MagNa Lyser (Roche Diagnostics, Basel, Switzerland). The DNA concentration was quantified with a NanoDrop 8000 spectrophotometer (ThermoFisher Scientific, Waltham, MA, USA) and all samples were normalised to 5 ng μL^{-1}. Following the standard protocol for amplicon library preparation (16S Metagenomic Sequencing Library Preparation, Part #15044223 Rev. B. Illumina, San Diego, CA, USA), amplicons of the variable region 4 (V4) of the 18S rRNA gene were generated. To best target the autotrophic community, we chose the forward primer CCAGCASCYGCGGTAATTCC and reverse primer ACTTTCGTTCTTGAT of Bradley et al. (2016), both including an Illumina tail. Using the Nextera XT Index Kit v2 Set A primers (Illumina, San Diego, CA, USA), single samples were indexed and subsequently the resulting libraries were pooled. Sequencing was performed on a MiSeq sequencer (Illumina, San Diego, CA, USA), resulting in 300 base pair paired-end gene amplicon reads, which were demultiplexed by the Generate FASTQ workflow of the MiSeq software. Primers were removed with v2.8 cutadapt (Martin 2011) and the data was further processed with v1.18 DADA2 (Callahan et al. 2016) following the protocol described in Ahme et al. (2023; 2024).

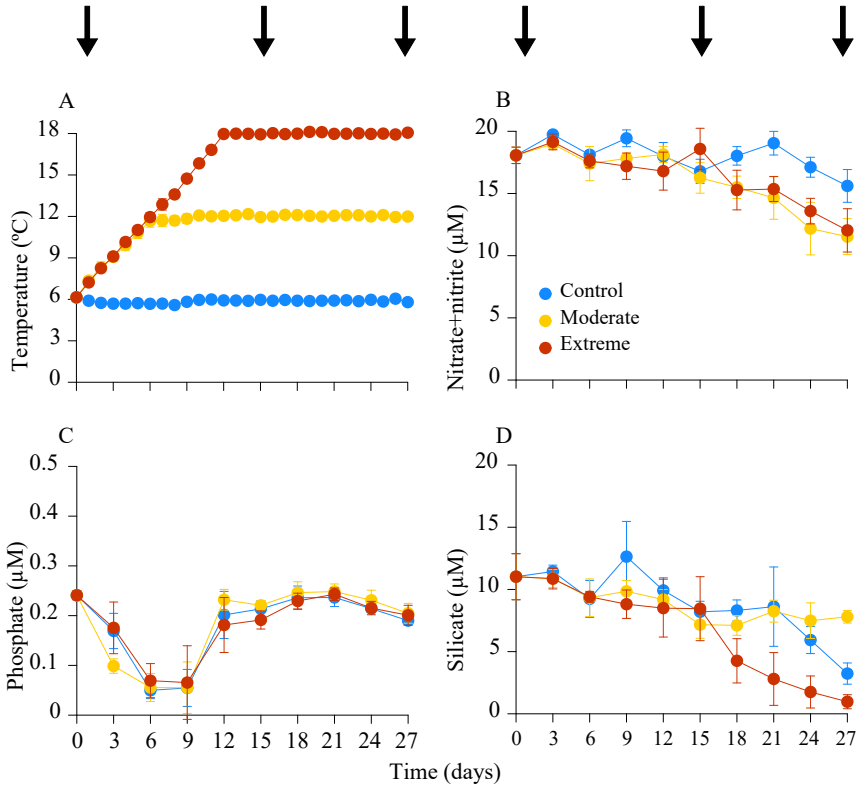


Figure S1: Mean (±SD) temperature (A), nitrate+nitrite (B), phosphate (C), and silicate (D) concentrations measured in the Planktotrons under three temperature treatments (control, 6; moderate, 12; and extreme, 18°C) over the experimental period. Black arrows represent the micro-grazing incubation days.

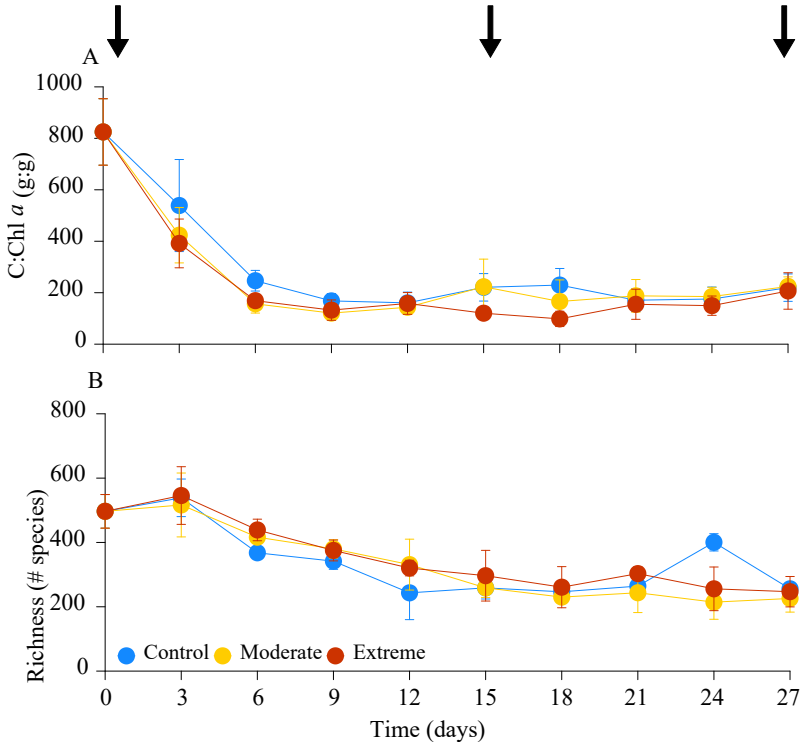


Figure S2: Mean (±SD) carbon:chlorophyll a (C:Chl a) ratios (A) and species richness (B) in plankton communities exposed to three temperature (control, 6; moderate, 12; and extreme, 18ºC) (6, 12, and 18ºC) over the experimental period. Black arrows represent the micrograzing incubation days.

Supplementary results: community biomass, resource use efficiency, and stoichiometry in the mesocosms

Warming had a significant effect on Chl *a*, RUE_N, N:P and C:P ratios (Fig. S4; Table S2). Regardless of the temperature treatment, Chl *a* (and POC, between 400-1600 µmol C L^{-1}; Fig. S5) concentrations increased up to day 12; by contrast, from here to the end of the experiment, their concentrations significantly increased only under extreme warming (Fig. S4A). The RUE_N was almost constant until day 15 with no significant differences among temperature treatments (LSD *post hoc* test, $p > 0.05$; Fig. S4B). However, later during the experiment, it increased reaching values ~12, 6 and 4 under extreme, moderate, and control conditions, respectively, due to a significant temperature × time interaction (Table S2). No significant differences were found for RUE_P among temperature treatments over the experimental period (*F*-test = 0.34, df = 2, *p*-value = 0.73; Fig. S6). N:P and C:P ratios remained relatively stable during the first part of the experiment, whereas during the second half, the 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. S4C). No significant differences between treatments existed for C:P ratio although their values increased from day 18 (Fig. S4D).

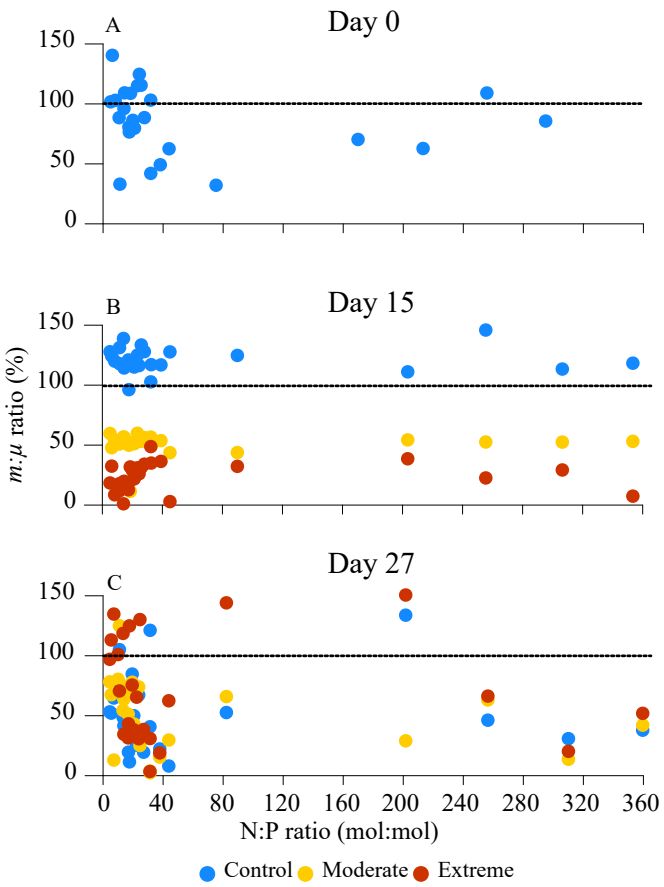


Figure S3: Mean instantaneous phytoplankton growth (μ) and microzooplankton grazing (m) ratios (i.e. grazing pressure, %) at the initial time (day 0), once the communities reached the target temperature treatments (day 15), and after the acclimation period (day 27) in plankton communities exposed to three temperature treatments (control, 6; moderate, 12; and extreme, 18ºC) over a dissolved nitrogen:phosphorus (N:P) ratio gradient between 0 and 360. The dashed line (m:μ = 100) denotes that all primary production generated was consumed by microzooplankton grazing.

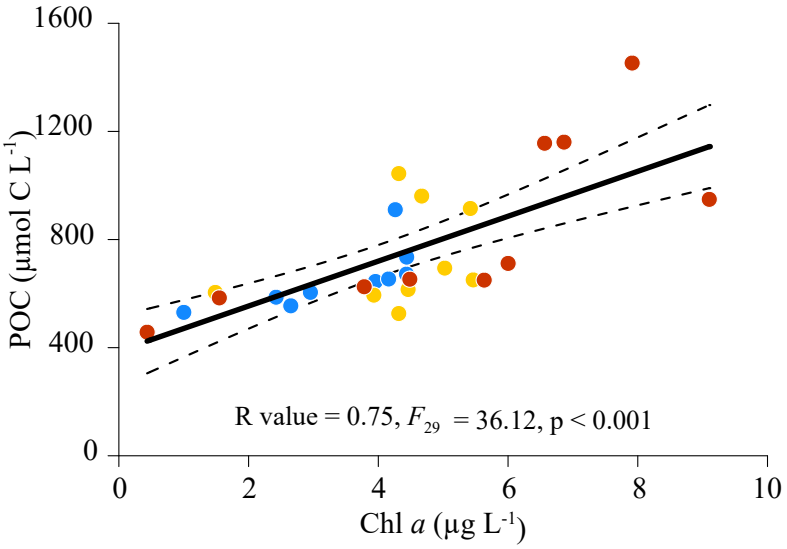


Figure S5: Relationship between mean particulate organic carbon (POC) versus chlorophyll a (Chl a) concentrations under control (6ºC), moderate (12ºC), and extreme (18ºC) temperatures over the experimental period. Solid and dashed lines represent the linear fit and 95% confidence interval, respectively.

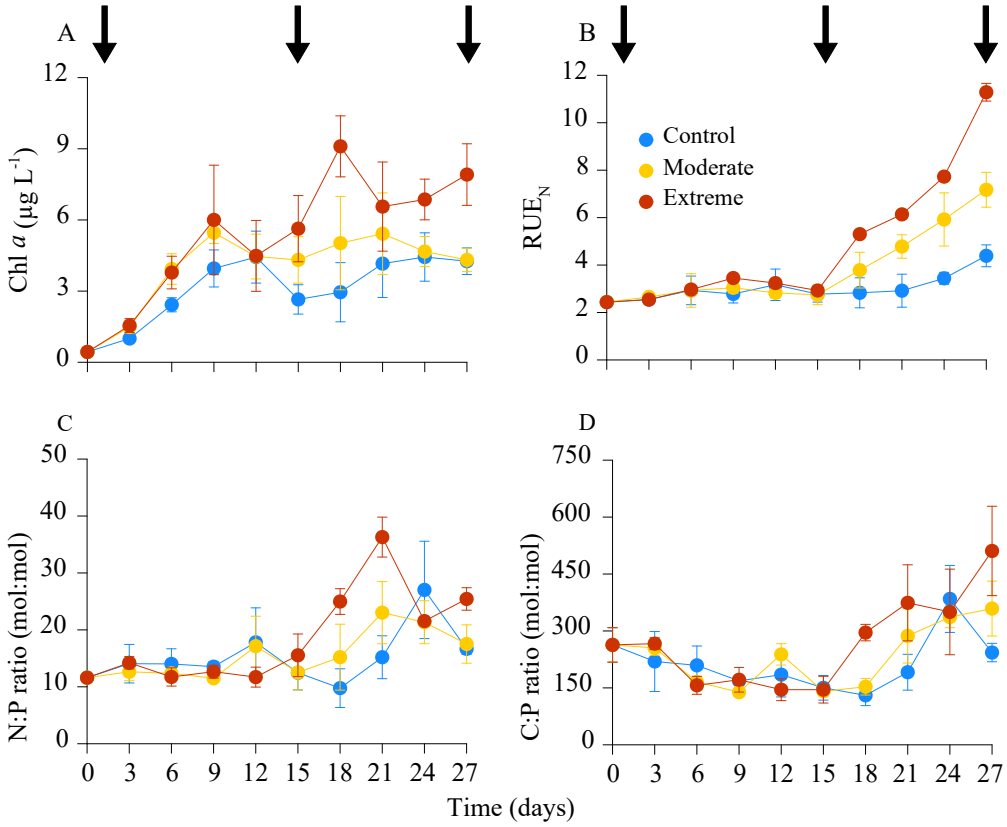


Figure S4: Mean (±SD) chlorophyll a (Chl a) (A), nitrogen-specific resource use efficiency (RUE$_N$) (B), particulate organic nitrogen:phosphorus (N:P) (C) and particulate organic carbon:phosphorus (C:P) (D) ratios in plankton communities exposed to three temperature treatments (control, 6; moderate, 12; and extreme, 18 ºC) over the experimental period. Black arrows represent the micro-grazing incubation days.

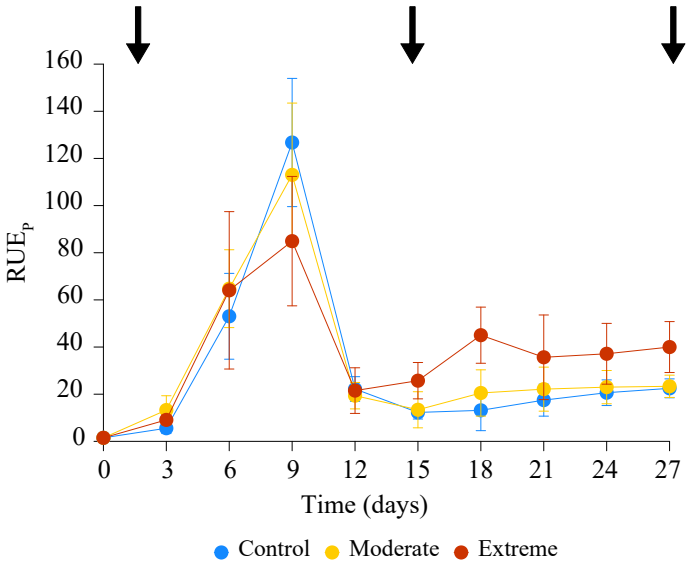


Figure S6: Mean (±SD) phosphorus-specific resource use efficiency (RUE$_P$) in plankton communities exposed to three temperature treatments (control, 6; moderate, 12; and extreme, 18 ºC) over the experimental period. Black arrows represent the micro-grazing incubation days.

Table S1: Mean cell size and trophic mode from published literature for particular species present in our samples and not listed in the public databases consulted (see material and methods section for a detailed description).

Group	Species	Authors	Year	Journal	Vol.	Pag.	Trophic mode	Cell size (µm)
Cryptophyta	*Geminigera cryophila*	Van de Hoff et al.	2020	*J. Phycol.*	56	1028-1038	Mixotroph	9-13
	Goniomonas amphinema	Martín-Cereceda et al.	2009	*J. Euk. Microb.*	57	159-170	Mixotroph	2.50
	Hemiselmis sp.	Shuwang et al.	2021	*Biology*	10	1280.00	Autotroph	0.2-0.6
	Rhodomonas sp.	Lutsos et al.	2022	*J. App. Phycol.*	34	1215-1226	Mixotroph	7-14
	Rhodomonas sp.	Stumpp et al.	2019	*Meth. Cell Biol. (Ch. 16)*	150	391-409	Mixotroph	7-14
Dinophyta	*Abedinium dasypus*	Cooney et al.	2020	*Gen. Biol. Evol.*	12	2417-2428	Autotroph	75-150
	Amphidoma languida	Tilman et al.	2021	*Mar. Biodiv. Rec.*	8	1-11	Autotroph	10.50
	Amylax buxus	Gil Pak et al.	2013	*J. Euk. Microb.*	60	363-376	Mixotroph	0-62
	Ankistrodinium semilunatum	Hoppenrath et al.	2012	*J. Phycol.*	48	1143-1152	Autotroph	29-60
	Ansanella granifera	Lee et al.	2014	*Algae*	29	137-152	Mixotroph	20
	Apocalathium aciculiferum	Annenkova et al.	2015	*The ISME J.*	9	1821-1834	Autotroph	27
	Biecheleria sp.	Raho et al.	2018	*Eur. J. Phycol.*	53	99-113	Mixotroph	9-15
	Biecheleria sp.	Sunesen et al.	2023	*Darwiniana*	11	337-346	Mixotroph	8-15.2
	Biecheleria pachydermata	Gómez et al.	2015	*J. Phycol.*	51	1088-1105	Mixotroph	90-120
	Bastodinium contortum	Alves de Sousa et al.	2011	*Biogeosci.*	8	2125-2136		7-18
	Chytriodinium roseum	Dogiel et al.	1906	*Mitt aus der Zoll*	18	1-45	Mixotroph?	50
	Dactylodinium arachnoides	Lum et al.	2019	*Phycologia*	58	661-674	Mixotroph?	24.70
	Dinophysis acuminata	Mackenzie et al.	2019	*Toxins*	11	74	Mixotroph	41.30
	Dinophysis norwegica	Carvalho et al.	2008	*Harm. Algae*	7	17	Mixotroph	25-70
	Dinophysis caudata	Fabro et al.	2016	*Harm. Algae*	59	31-41	Mixotroph	14
	Dinophysis lenticula	Yurimoto et al.	2015	*Int. J. Aquat. Res.*	7	133-141	Mixotroph	30-40
	Dinophysis caspica	Haifeng et al.	2016	*Phycol. Res.*	64	251-258	Mixotroph	30-38

	Eudubosquella sp.	Coats et al.	2012	*J. Euk. Microb.*	59	1-11	Heterotroph	7-10
	Fragilidium sp.	Skovgaard et al.	1996	*Mar. Ecol. Progr. Ser.*	143	247-253	Autotroph	35-50
	Hematodinium sp.	Hudson & Shields	1994	*Dis. Aquat. Org.*	19	109-119	Heterotroph	6-16
	Islandinium sp.	Kawami et al.	2009	*Phycol. Res.*	57	259-267	Heterotroph	25-35
	Karlodinium sp.	Benico et al.	2020	*J. Phycol.*	56	1264-1282	Heterotroph	25
	Lepidodinium sp.	Roux et al.	2023	*Progr. Oceanog.*	212	102999	Autotroph	> 20
	Levanderina fissa	Gárate-Lizárraga et al.	2014	*Cicimar Oceánidas*	29		Autotroph	30-60
	Nematodinium sp.	García-Portela et al.	2023	*Phycologia*	*in press*		Heterotroph	25.8-38.6
	Nusuttodinium acidotum	Takano et al.	2014	*Protist*	165	759-778	Heterotroph	21-32
	Pelagodinium beii	Siano et al.	2010	*Protist*	161	385-399	Autotroph	8.8-11.4
	Pfiesteria sp.	Jeong et al.	2006	*Aquat. Microb. Ecol.*	44	263-278	Autotroph	9-17
	Polarella glacialis	Montesor et al.	1999	*J. Phycol.*	35	186-197	Autotroph	10-15
	Stoeckeria algicida	Jeong et al.	2005	*J. Euk. Microb.*	52	382-390	Heterotroph	7-20
	Syndinium sp.	Skovgaard et al.	2005	*Protist*	156	413-423	Heterotroph	5
	Takayama sp.	Iwataki et al.	2022	*Harm. Algae*	114	102204	Heterotrophi	35.2-48.8
	Thecadinium inclinatum	René & Hoppenrath	2019	*Harm. Algae*	84	161-171	Heterotroph	43-60
	Warnowia sp.	García-Portela et al.	2023	*Phycologia*	*in press*		Heterotroph	25.8-38.6
	Yihiella yeonensis	Jang et al.	2017	*J. Phycol.*	53	131-155	Heterotroph	7-11
Haptophyta	*Braarudosphaera bigelowii*	Hagino et al.	2013	*PloS One*	8	e81749	Mixotroph	15
	Ancyromonas kenti	Lee	2015	*Acta Protozool.*	54	53-76	Heterotroph	4-6
Ochrophyta	*Clathromonas butcheri*	Hernández-Becerril et al.	2019	*Nova Hed.*	148	1-10	Mixotroph	20
	Florenciella parvula	Li et al.	2021	*Limnol. Oceanogr.*	66	47-60	Heterotroph	3-6

	Minuntocellus polymorphus	Hernández-Márquez et al.	2023	*Botanica Marina*	66	1-10	Autotroph	< 6
	Spumela sp.	Jeong et al.	2021	*Front. Plant Sci.*	12	758067	Heterotroph	< 10
	Pierrecomperia sp.	Sabbe et al.	2010	*Vieu et Milieu*	60	243-256	Autotroph	5.7-15.6
	Pteridomonas danica	Pelegrí et al.	1999	*Microb. Ecol.*	37	276-284	Autotroph	4
	Tenuicylindrus belgicus	Naujappa et al.	2013	*J. Phyco.*	49	917-936	Autotroph	23.6-50
	Triparma sp.	Ichinomiya et al.	2016	*The ISME J.*	10	2419-2434	Autotroph	< 2
Opalozoa	*Mast's clade*	Lin et al.	2012	*App. Environ. Microb.*	78	3387-3399	Hetero/ mixotroph	2.2-4.9
	Adriamonas peritocrescens	Verhagen et al.	1994	*Eur. J. Prot.*	30	295-308	Heterotroph	8.00
	Caecitellum paraparvulum	Haussmann et al.	2006	*Acta Protozool.*	45	415-431	Heterotroph	2.5-4.5
	Cafileria marina	Jirsová et al.	2019	*Microorganisms*	7	240.00	Heterotroph	5-6
	Picomonas judraskeda	Seenivasan et al.	2013	*PloS One*	8	e59565	Heterotroph	2.5-3.8
	Prasinodermacoloniale sp.	Hasegawa et al.	1996	*Phycologia*	35	170-176	Autotroph	< 2
	Rhodelphis marinus	Gawryluk et a.	2019	*Nature*	572	240-243	Heterotroph	10-13
	Aplanochytrium sp.	Bahnweg & Sparrow Jr.	1972	*Arch. Microbiol.*	81	45-49	Heterotroph	2.4-5
	Diplophrys sp.	Anderson & Cavalier-Smith	2012	*Acta Protozool.*	51	291-304	Heterotroph	10-20
Ciliophora	*Mesodinium rubrum*	Labucis et al.	2023	*Oceanologia*	65	398-409	Mixotroph	16-33

Table S2: Results of the repeated measures one-way (or two-way analysis; for community cell size and trophic mode) of the variance for the effects of temperature [and temperature × cell size (i.e. micro-, nano-, and picophytoplankton) or trophic mode (auto-, mixo-, and heterotrophs] over time on chlorophyll a (Chl a), nitrogen-specific resource use efficiency, nitrogen:phosphorus (N:P) and carbon:phosphorus (C:P) ratios, relative contribution (%) of cell size and trophic mode fractions to the total community, predator:prey availability ratios, accumulation rates, inorganic nutrients concentrations, C:Chl a ratios, and total species richness. df, F-value, and p-value mean the degrees of freedom, F-Snedecor test values, and significance values.

Response variable	df	*F*-value	*p*-value
Chl *a*	18	5.89	< 0.0001
RUE_N	18	18.38	< 0.0001
N:P ratio	18	14.78	< 0.0001
C:P ratio	18	11.22	< 0.0001
Community cell size	36	1.77	< 0.01
Community trophic mode	36	11.74	< 0.0001
Predator:prey ratio	18	50.91	< 0.0001
Accumulation rates	4	662.22	< 0.0001
NO_3^-+NO_2^-	18	4.61	< 0.0001
PO_4^{3-}	18	3.61	< 0.001
SiO_2^-	18	16.3	< 0.0001
C:Chl *a* ratio	18	1.99	< 0.05
Total richness	18	8.08	< 0.0001

Supplementary references

Ahme A, Happe A, Striebel M et al. (2024) Warming increases the compositional and functional variability of temperate coastal protist communities. *under review*. http://dx.doi.org/10.2139/ssrn.4717787.

Ahme A, Von Jackowski A, Mcpherson RA, Wolf KKE, Hoppmann M, Neuhaus S, John U (2023) Winners and Lossers of Atlantification: The degree of ocean warming affects the structure of Arctic microbial communities. Genes, **14**, 623.

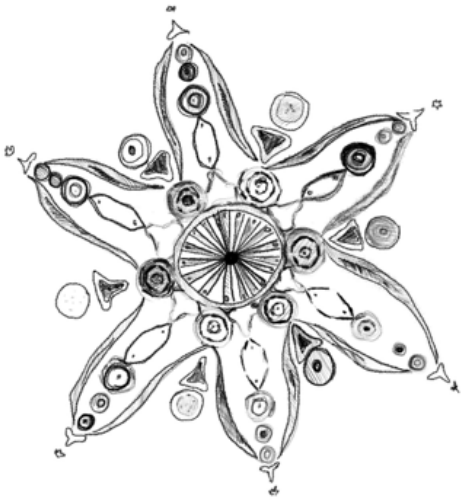
Bradley IM, Pinto AJ, Guest JS (2016) Design and evaluation of Illumina MiSeq-Compatible, 18S rRNA gene-specific primers for improved characterization of mixed phototrophic communities. Applied and Environmental Microbiology, **82**, 5878 - 5891.

Callahan BJ, Mcmurdie PJ, Rosen MJ, Han AW, Johnson AJ, Holmes SP (2016) DADA2: High-resolution sample inference from Illumina amplicon data. Nature Methods, **13**, 581-583.

Martin M (2011) Cutadapt removes adapter sequences from high-throughput sequencing reads. EMBnet Journal, **17**, 10-12.

4

Temperate summer community



4.1: PUBLICATION V

Microbial meltdown: Concurrent global change and heatwaves disturb phototrophic more than heterotrophic protist diversity

To be submitted

Microbial meltdown: Concurrent global change and heatwaves disturb phototrophic more than heterotrophic protist diversity

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Abstract

Anthropogenic pressures are affecting eukaryotic plankton communities in multiple ways, for example via ocean warming, acidification and rising N:P ratios. Global change is also characterized by an increase in the probability of marine heatwaves, but the impacts of these concurrent changes on protist diversity have rarely been assessed. To tackle this gap, we conducted a mesocosm experiment during which we tested the influence of a marine heatwave under ambient and future environmental conditions on a North Sea summer plankton community. We analysed the influence of these environmental conditions on protist diversity via 18S rRNA amplicon sequencing. Our results indicate that global change compromises the diversity of both heterotrophic and phototrophic protists, with particularly pronounced impacts on phototrophic organisms. While heterotrophs remained largely unaffected by heatwaves, phototrophic diversity declined, especially during cooling, and only recovered under ambient but not under global change conditions. Furthermore, the global change scenario induced a shift from nano- to pico-sized phototrophs and increased the abundance of harmful algae bloom species and parasites, whereas heatwaves only affected phototrophs by increasing the number of marine ochrophytes. The coccolithophore *Gephyrocapsa oceanica* developed a bloom in all mesocosms and seemed to profit from global change conditions even under heatwaves. We show that both changing baseline conditions as well as extreme events can interact in shaping the diversity of eukaryotic plankton communities.

Introduction

Coastal marine ecosystems are facing escalating anthropogenic pressures, particularly due to climate change which encompasses a variety of consequences. These include rising temperatures (IPCC, 2023), a lowered pH due to a higher CO_2 partial pressure (Raupach et al., 2007) as well as an increasing duration, frequency and intensity of marine heatwaves (Frölicher et al., 2018; Laufkötter et al., 2020). Simultaneously, intensified agriculture, management efforts and stronger terrestrial runoff may aggravate relative phosphorus limitation by increasing the dissolved N:P ratio in coastal systems like the North Sea (Grizzetti et al., 2012). The impacts of these pressures on ecological processes are projected to be persistent through the following centuries (Oliver et al., 2019), thereby threatening organisms pivotal to marine ecosystems, including planktonic protists (Raven and Beardall, 2021; Salmaso and Tolotti, 2021). North Sea protists usually reside below their optimum temperature for growth (Thomas et al., 2012) and, therefore, warming may decrease their diversity via increasing growth rates and thus a stronger competition for resources. Moreover, marine heatwaves in addition to mean warming can create periods with temperature conditions that exceed a species' thermal limit (Smale et al., 2019) which can further alter the community composition towards a reduced richness and evenness. Therefore, although protists are known to adapt to environmental change quickly (Padfield et al., 2016; O'Donnell et al., 2019), extreme events and the interactive effects of multiple drivers can push this capacity beyond its limits (Boyd et al., 2018; Hayashida et al., 2020; Vinton et al., 2022; Sauterey et al., 2023).

Protists use different feeding strategies, ranging from photoautotrophy and heterotrophy to a combination of the two, i.e. mixotrophy (Chakraborty et al., 2017). The diversity of these groups may be affected differently by global change due to variations in their functional traits. Although the thermal reaction norms of photo- and heterotrophs are comparable in shape (Boyd et al., 2013; Calbet and Saiz, 2022; Ferreira et al., 2022), core parameters such as the temperature optimum or activation energy can vary (Regaudie-de-Gioux and Duarte, 2012; Boscolo-Galazzo et al., 2018). Phototrophs may reach thermal limits earlier than heterotrophs (Liu et al., 2018), resulting in a more significant reduction in species richness and a higher turnover rate under warming and concurrent heatwaves (Hillebrand et al., 2012). While changes in dissolved nutrient ratios could foster competitive exclusion in phototrophs (Tilman et al., 1982), heterotrophic responses are more complex. Furthermore, a higher partial pressure of CO_2 may benefit certain phototrophic species with less efficient carbon concentration mechanisms (Rost et al., 2008; Velthuis et al., 2022), likely lowering species evenness. In addition, all these parameters can interact with each other (Thomas et al., 2017; Litchman and Thomas, 2023;

Kilner et al., 2024) and environmentally induced changes in the composition of one group can have significant impacts on the other (Lewandowska et al., 2014). Specific heterotrophic protists can increase phototrophic evenness by grazing or infecting the most abundant groups (Hillebrand et al., 2007) or decrease their richness through selective feeding (Liu et al., 2014; Anderson and Harvey, 2019; Gutiérrez-Rodríguez et al., 2023). Conversely, heterotrophic diversity can be affected by the nutritional quality or the abundance of their phototrophic prey (John and Davidson, 2001; Tillmann, 2004; Deng et al., 2023).

Maintaining a high diversity is crucial for sustaining ecosystem functions (Tilman et al., 2014; Bestion et al., 2021). However, ongoing anthropogenic pressures have the potential to significantly alter the diversity of eukaryotic plankton communities, potentially impairing their capacity to buffer against concurrent perturbations (Bestion et al., 2020). Despite the urgent need to investigate the interactive effects of various environmental changes on protist diversity, to the best of our knowledge, no study to date has investigated how marine heatwaves modulate the effects of global change scenarios. Therefore, we aimed to assess the concurrent and single effects of global change and marine heatwaves on the diversity and composition of a North Sea summer protist community in an experimentally robust multiple-driver approach.

Material & Methods

Experimental set-up

To assess the joint impact of global change and marine heatwaves on a North Sea plankton community, an integrated multiple-driver experiment was conducted at the mesocosm facility of the Alfred Wegener Institute, Wadden Sea Station on the Island of Sylt (Pansch et al., 2016). Detailed methods can be found in (Meunier et al., in prep.). Briefly, the initial Plankton community was sampled from the surface water on the 1st of September 2021 at the long-term ecological research station Sylt Roads (DEIMS ID: https://deims.org/9d5e3aae-d569-4571-8058-96d5bafda2e7) during a cruise with the RV *Mya II*. The seawater was evenly distributed among 16 mesocosms (520 L each), which were incubated for 27 days in quadruplicates at four different scenarios. These included an ambient control (ambient), an integrated future scenario based on projections for the year 2100 (ERCP 8.5; + 3 °C, N:P of 25, pCO2 of 1000 ppm; Grizzetti et al., 2012; IPCC, 2021; Moreno et al., 2022), and another set of each with a marine heatwave (ambient HW, ERCP 8.5 HW). The baseline temperatures of all scenarios were adjusted daily to mirror the development of the field temperatures at the sampling location. Marine heatwave scenarios were chosen based on in-situ data obtained from the Sylt Roads time series (Rick et al., 2023) and calculated according to Hobday et al. (2016), resulting in a

five-day heatwave of + 2 °C that was achieved by gradual in- and decrease of 1 °C d^{-1} and conducted from incubation day 9 to 17.

Community composition and diversity

Protist community composition and diversity were assessed using 18S rRNA metabarcoding. Every Monday, Wednesday and Friday, a 500 mL subsample from each mesocosm was pre-filtered through a 150 µM net, vacuum-filtered (< - 200 mbar) onto a 3 µm polycarbonate filter (Nucleopore, Whatman, Maidstone, UK) and stored at – 20 °C. DNA extraction was conducted according to the manufacturer's protocol (NucleoSpin Soil extraction kit, Macherey-Nagel GmbH, Düren, Germany) and concentrations were normalised to 5 ng μL^{-1}. Amplicons of the variable region 4 of the 18S rRNA gene were generated following the standard protocol for amplicon library preparation (16S Metagenomic Sequencing Library Preparation, Part #15044223 Rev. B. Illumina, San Diego, CA, USA) with the primers CCAGCASCYGCGGTAATTCC and ACTTTCGTTCTTGAT (Bradley et al., 2016) and the primers from the Nextera XT Index Kit v2 Set A (Illumina, San Diego, CA, USA). After producing 300 base pair paired-end gene amplicon reads on a MiSeq sequencer (Illumina, San Diego, CA, USA), bioinformatic processing of the FASTQ files was performed as described in Ahme et al. (in prep.) including a step to confirm sufficient sequencing depth via rarefaction (Figure S1). Differentiation into phototrophic and heterotrophic protists was based on the functional assignment of Adl et al. (2019) and Ramond et al. (2018). Mixotrophic protists were grouped according to their primary feeding strategy, i.e. whether they were obligately or facultatively photo- or heterotroph (de Vargas et al., 2015; Adl et al., 2019). Diversity parameters (species richness, species evenness, Shannon index) were calculated using the *richness* function of the microbial package (v0.0.22; Guo and Gao, 2022).

Analysis and statistics

Data analysis was conducted in R (v4.21; RCoreTeam, 2022) with RStudio (v2022.07.2; RStudioTeam, 2022). To test the effects of global change and marine heatwaves on diversity, we calculated the log response ratio (LRR) of all diversity parameters according to Urrutia-Cordero et al. (2021) for each incubation day and all three treatments ($LRR_{ERCP\ 8.5}$ & $LRR_{ambient\ HW}$ & $LRR_{ERCP\ 8.5\ HW}$). For $LRR_{ERCP\ 8.5}$ and $LRR_{ambient\ HW}$, the ambient replicates and for $LRR_{ERCP\ 8.5\ HW}$ the ERCP 8.5 replicates were used as control. For the HWs, we excluded the first three time points sampled before the temperature was ramped up. We fitted GAMs to all LRRs and identified significant in- or decrease periods. This involved estimating the first

derivatives with the method of finite differences, followed by assessing whether the slope significantly differed from zero, as proposed by Simpson (2018). All code and the citation report of used packages are available via GitHub (https://github.com/AntoniaAhme/HeatwaveNowTomorrowProtists).

Results

The Shannon diversity was generally lower for phototrophic (1.3 to 3.6; Table S1, Figure S2a), compared to heterotrophic protists (1.9 – 4.1; Table S2), for which an initial rise in diversity occurred in all treatments (Figure S2b). For phototrophic protists, the $LRR_{ERCP\ 8.5}$ significantly decreased from showing positive to negative effects between incubation days 6 and 18 and then remained below zero despite a short increase towards the end (Figure 1a). The $LRR_{ERCP\ 8.5}$ of heterotrophic protists showed a sharp in- and decrease between incubation days 0 and 15 to slightly negative values. Then, it increased back to positive values towards the last six days (Figure 1b). This pattern in the heterotrophic $LRR_{ERCP\ 8.5}$ was mainly driven by increasing diversity in the ambient control. In contrast, the diversity in the global change scenario remained similar in the first half of the incubation (Figure S2b). For phototrophic organisms, the first derivative estimations revealed significant decreases of the LRRs for both scenarios during the HW and cooling phase of the HWs, which significantly increased again in the recovery phase only under ambient conditions (Figure 2a). The LRRs of the heterotrophic diversity fluctuated around zero in the HW treatments but showed a slight decrease towards the end of the incubation in the $LRR_{ERCP\ 8.5\ HW}$ (Figure 2b).

Regarding species richness, the heterotrophic LRRs first decreased but then increased again in the ERCP 8.5 scenario, and showed no response under HWs (Figure S3b, S4b). Richness LRRs for phototrophs decreased both in the ERCP 8.5 scenario and in the recovery period of ERCP 8.5 HW scenario (Figure S3a, S4a). Generally, the pattern of species evenness LRRs was more similar to the one of the Shannon index LRRs (Figure 1, 2, S5, S6). This indicates the evenness to be a stronger driver of diversity differences over time for both the ERCP 8.5 scenario (Figure S3, S5) and the HWs (Figure S4, S6) than the species richness.

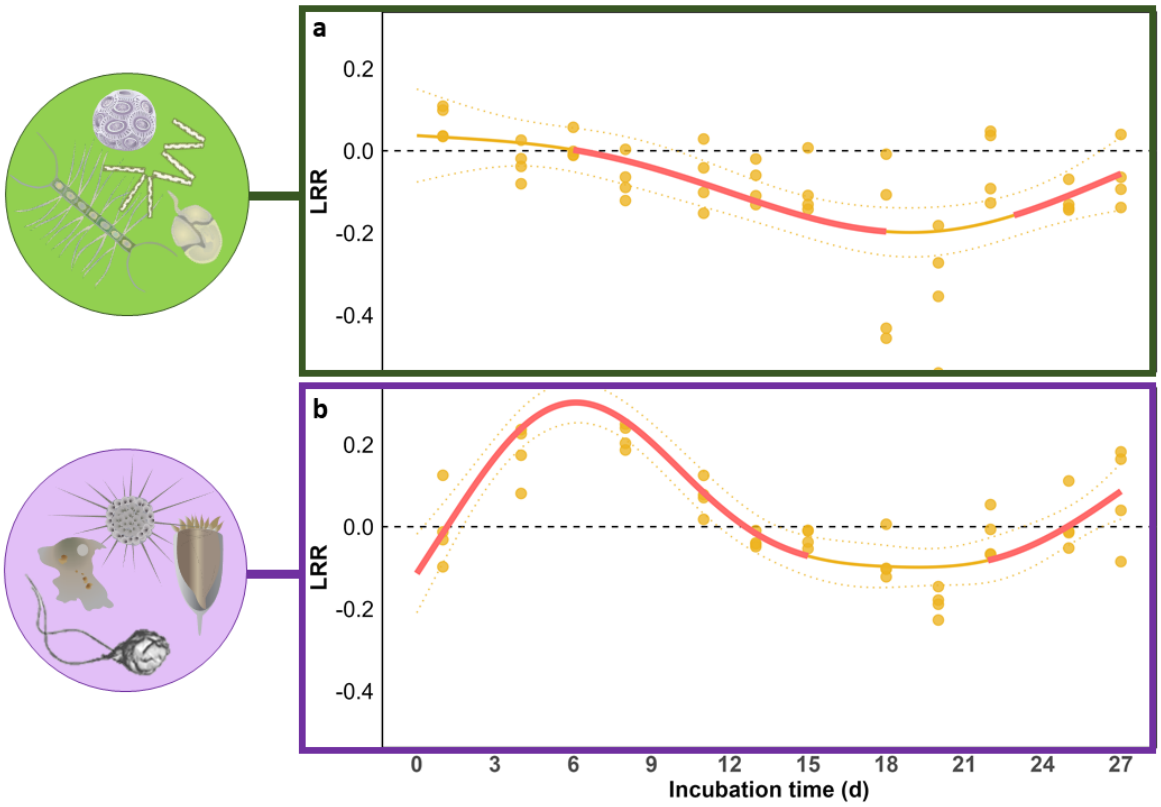

*Figure 1: Log response ratios (LRR) of the Shannon index for (**a**) phototrophic and (**b**) heterotrophic protists for the ERCP 8.5 scenario over time. Each point represents an individual observation. Fitted thin-plate spline with approximate 95% point-wise confidence interval (dotted lines). The thick orange line represents phases of significant change.*

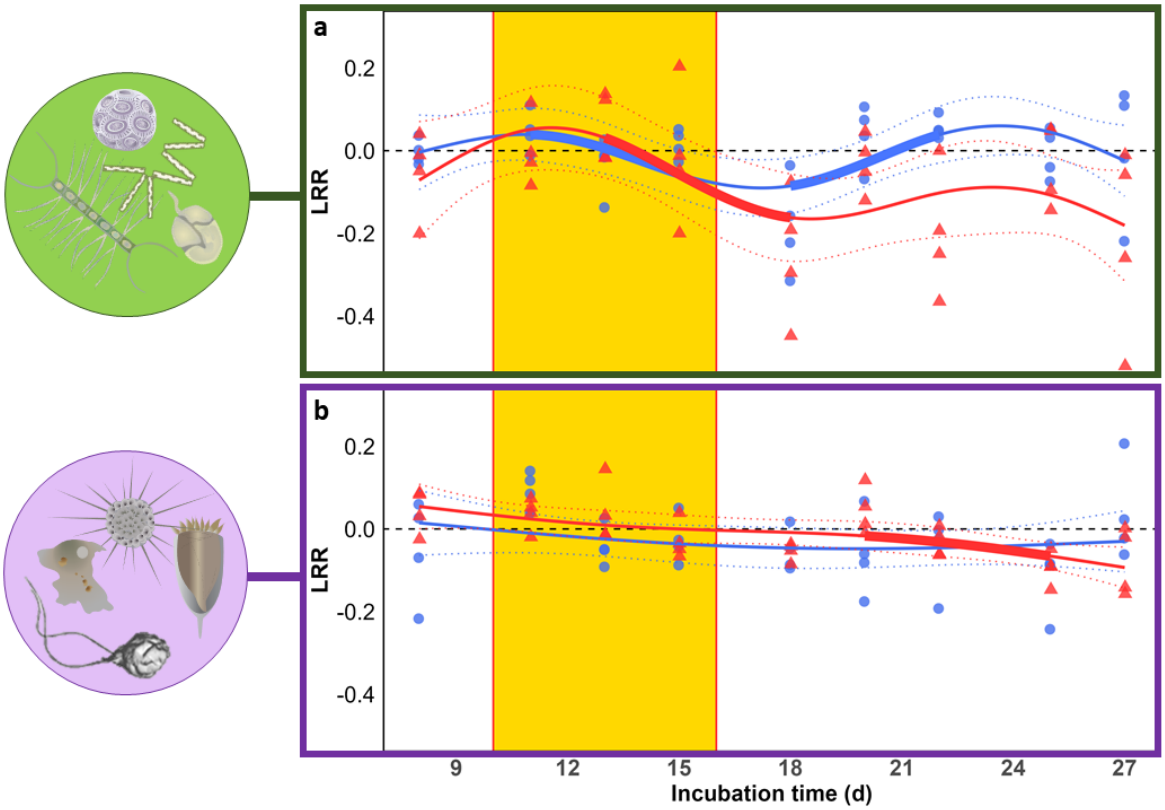

*Figure 2: Log response ratios (LRR) of the Shannon index for (**a**) phototrophic and (**b**) heterotrophic protists for the HWs over time. Each point represents an individual observation (blue = ambient HW, red = ERCP 8.5 HW). Fitted thin-plate spline with approximate 95% point-wise confidence interval (dotted lines). The yellow rectangle indicates the HW period. Thick parts of the line represent phases of significant change*

There were no overall dominance shifts in composition between the scenarios. However, we observed notable differences in the residual communities. All treatments and replicates were increasingly dominated by the calcifying haptophyte *Gephyrocapsa oceanica* over time, but more so in the ERCP 8.5 scenarios (Figure 3). The major fraction of heterotrophic protists was composed of members of the protaspa lineage (Figure 4). Residual differences towards the end of the incubation were more pronounced between the ambient and ERCP 8.5 treatments than between these and the HWs. Among the phototrophic protists, we observed relatively more *Aureococcus anophagepherens* and *Bathycoccus prasinos* but relatively fewer *Picochlorum sp.*, *Minidiscus variabilis*, *Pelagodinium beii* in the ERCP 8.5 scenarios, while the HW treatments differed in terms of more undetermined marine ochrophytes (MOCH; Figure 3). In the ERCP 8.5 scenarios, heterotrophic communities showed relatively more *Eurychasma dicksonii* and *Selenidium* but fewer members of the marine stramenopiles (MAST) clade and the tagiri lineage compared to ambient conditions. In contrast, there were no striking differences observed in the HW scenarios (Figure 4).

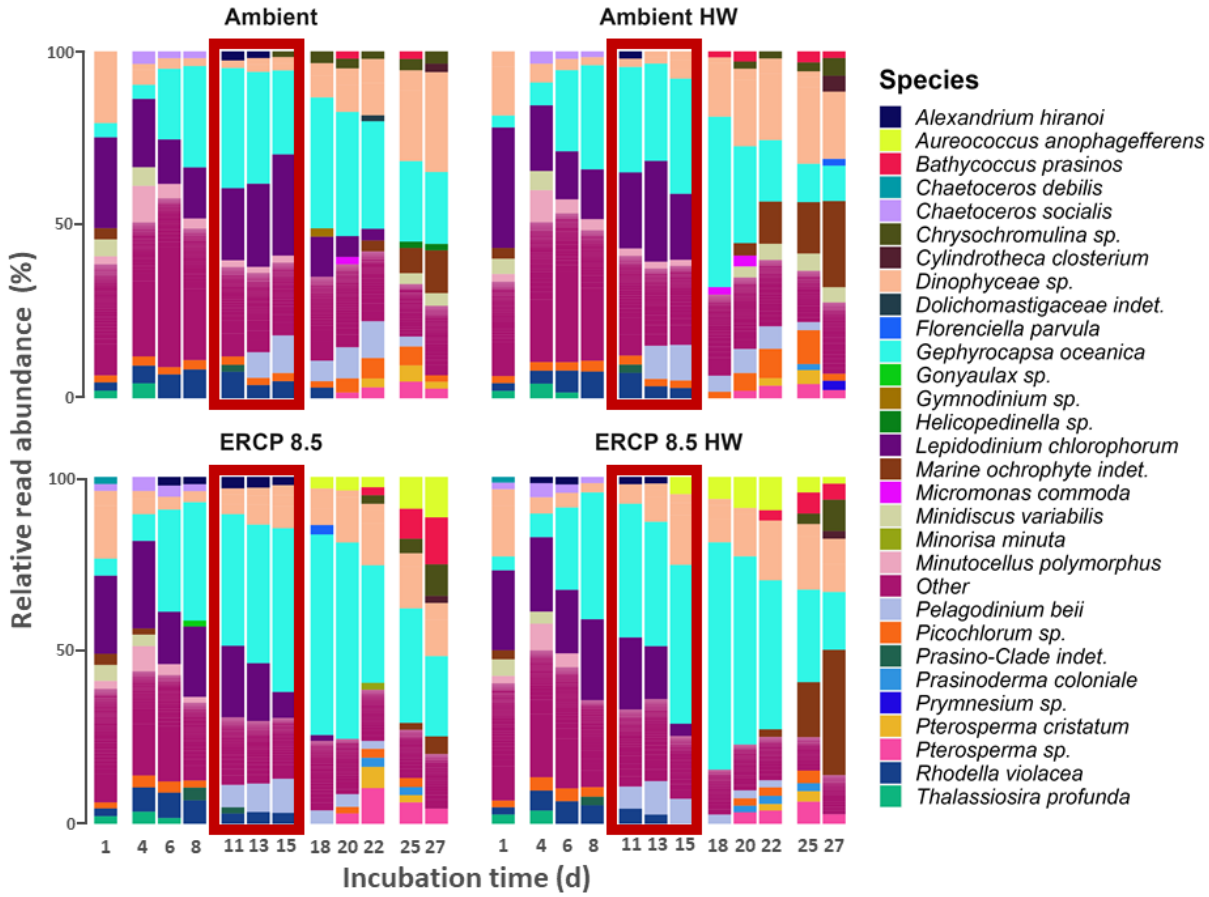

Figure 3: Mean metabarcoding-based phototrophic community composition on species level over time for all scenarios. The red square indicates the time of the HW for the HW scenarios. For readability, ASVs with an abundance of fewer than 100 reads among temperatures were categorized as "other".

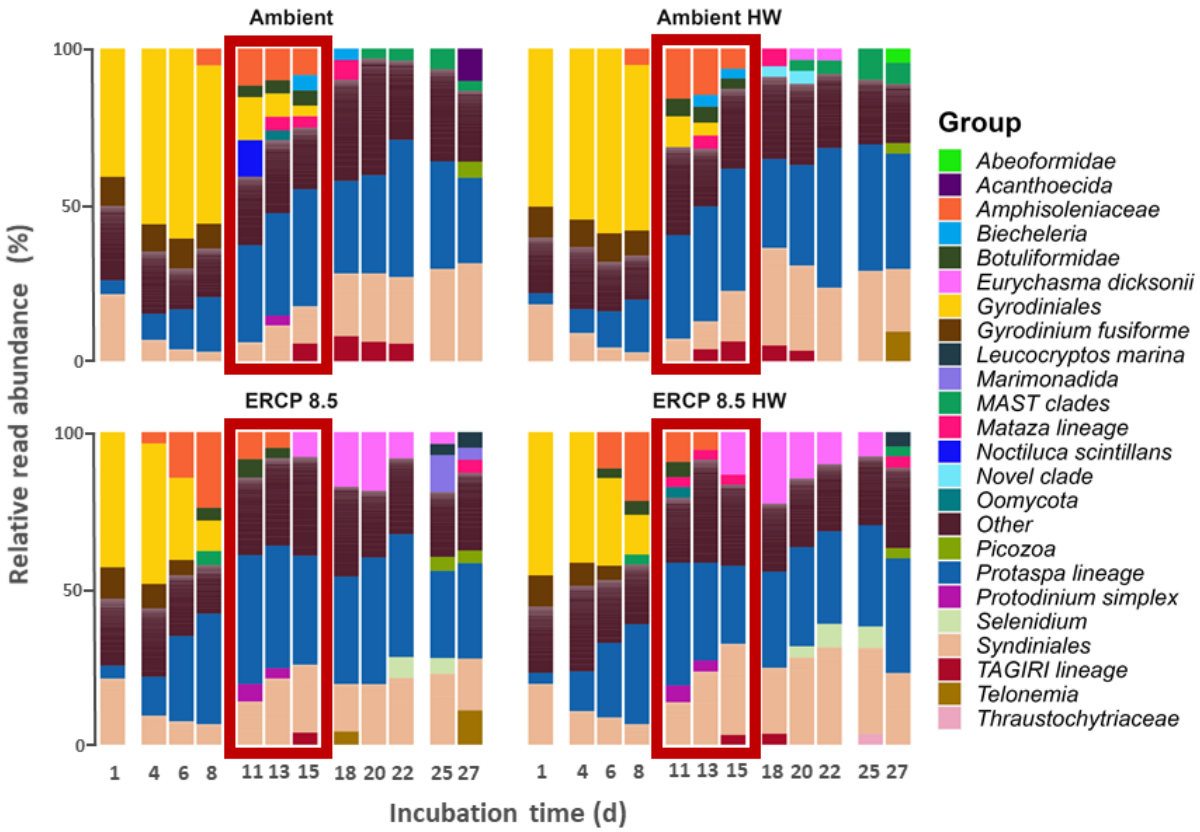

Figure 4: Mean metabarcoding-based heterotrophic community composition on species level over time for all scenarios. The red square indicates the timing of the marine HW for the HW scenarios. For readability, ASVs with an abundance of fewer than 100 reads among temperatures were categorized as "other".

Discussion

Our study revealed negative effects of the ERCP 8.5 and HW scenarios on the diversity of phototrophic protists, whereas heterotrophic diversity remained largely uncompromised. Shifts in community composition were subtler compared to the overall diversity, but especially in the ERCP 8.5 scenario, both trophic groups showed compositional responses with potential ecological implications. Furthermore, we identified critical heatwave phases in which the phototrophic diversity appeared to be more susceptible and these varied between ambient conditions and the ERCP 8.5 scenario. Differential effects on species richness and evenness as well as potential reasons for the phototrophic-heterotrophic divide will be discussed below.

Global change impairs phototrophic more than heterotrophic diversity

The decline in phototrophic diversity in the ERCP 8.5 scenario compared to ambient conditions was driven by decreases in both, species evenness and richness. The reduced evenness of the community was mainly due to the dominance of the coccolithophore *Gephyrocapsa oceanica*, which is consistent with previous research (Feng et al., 2009; Rivero-Calle et al., 2015; Moreno

et al., 2022). They are known for their high competitive ability under low phosphorus concentrations (McKew et al., 2015), high CO_2 partial pressures (Langer et al., 2009; Reinfelder, 2010) and increasing temperatures (Wang et al., 2024). Furthermore, warming in combination with nutrient limitation can decrease species richness by increasing the growth rates, leading to faster competitive exclusion and higher extinction rates (Gerhard et al., 2019; Gerhard et al., 2022). Small species thrive under these conditions due to their higher surface-to-volume ratios, faster division rates and lower nutrient requirements (Marañón, 2015; Hillebrand et al., 2022; Moreno et al., 2022; Kilner et al., 2024). Indeed, we observed a shift towards fewer and smaller species, with the nanoplanktonic species *Picochlorum sp.*, *Minidiscus variabilis*, and *Pelagodinium beii* (Kaczmarska et al., 2009; Potvin et al., 2015; Markina, 2020) being replaced by the pico-sized harmful algae bloom species *Aureococcus anophagepherens* and by *Bathycoccus prasinos* (Gobler and Sunda, 2012; Grimsley et al., 2015). Overall, it seems that many phototrophic organisms encountered environmental conditions that approached or exceeded their physiological limits, resulting in reduced species richness and evenness.

Although the ERCP 8.5 scenario initially had a negative effect on species richness within heterotrophic protists, it later became positive, and their evenness fluctuated from positive to negative back to neutral. This heterotrophic resilience can be explained by the fact that they are more constrained by cold than warm temperatures (Rose and Caron, 2007) and have higher thermal growth optima than phototrophic protists (Liu et al., 2018). The higher evenness at the beginning may be attributed to the faster decline of *Gyrodiniales* in the ERCP 8.5 scenarios, which is consistent with their thermal sensitivity (Calbet et al., 2022; Calbet and Saiz, 2022). Subsequently, the evenness decreased with the decline of MAST species and the tagiri lineage, while it fostered the relative abundance of *Eurychasma dicksonii* and *Selenidium*. This indicates a shift from microzooplankton species (Massana et al., 2009; Lin et al., 2012) to parasites that can infect brown algae (*E. dicksonii*; Tsirigoti et al., 2015) and marine invertebrates (*Selenidium*; Wakeman and Leander, 2013). Indeed, studies show that the response of certain MAST species varies with temperature (Lin et al., 2022; Kang and Kang, 2023), and that marine parasites are expected to increase with global change (Byers, 2021). Despite the potentially greater food availability provided by smaller phototrophic species, there was a decrease in heterotrophic species richness. One possible explanation is that certain specialised predators may have lost their specific prey species, despite not being limited by general food availability (Grinienė et al., 2016; Shemi et al., 2021). This is consistent with the delayed richness response of heterotrophs compared to phototrophs. Overall, while the ERCP 8.5 scenario led to the

extinction of some heterotrophic protists, it also benefited other species, resulting in no net effect on diversity at the end of the incubation period.

Marine heatwaves disturb phototrophic diversity especially under concurrent global change

Considering the HW scenarios, heterotrophic diversity was even less responsive both under ambient and ERCP 8.5 conditions. This resistance indicates that marine heatwaves did not pose a strong selective pressure on heterotrophs. It has to be noted that we observed a slight decrease of heterotrophic diversity towards the end of the incubation that was based on a slightly lowered species evenness. However, from the compositional data, we can derive that no major dominances occurred. To our knowledge, our study was the first to investigate the influence of heatwaves on heterotrophic protist diversity. Although some studies in lakes indicate that mesozooplankton dynamics can be affected by heatwaves (Roth et al., 2022; Hermann et al., 2023), others also only found minor impacts (Işkın et al., 2020) which is in line with the assumption that temperature was not a negative driver of heterotrophic diversity. An explanation could be their high thermal limits (Martinez, 1980; Caron and Hutchins, 2012; Ferreira et al., 2022) which are consistent with the natural succession pattern throughout the year, in which heterotrophic protists dominate summer communities (Wiltshire et al., 2015; Scharfe and Wiltshire, 2019) and might therefore be better adapted to periods of elevated temperatures.

In contrast, phototrophs showed strong diversity responses in the HW scenarios, which differed between ambient and the ERCP 8.5 conditions. Diversity changes under ambient conditions were driven by variances in both, species evenness, which decreased under warming and then increased again after the heatwave, and richness, which only decreased during the heatwave. Although no major groups were found to have increased during the ambient HW scenario, the decrease in species evenness and richness may indicate a high number of rarer species that are acclimated to ambient temperature conditions without the possibility of heat-tolerant species invading and compensating the loss (Burgmer and Hillebrand, 2011). Previous studies have also noted a decrease in phototrophic species richness under fluctuating temperatures and aquatic heatwaves (Rasconi et al., 2017; Stefanidou et al., 2018; Stefanidou et al., 2019). Additionally, the Shannon index decreased during cooling in ambient conditions but directly increased, suggesting that some time is required to adjust any cooling-induced physiological imbalances (Rehder et al., 2023). Following the heatwave, comparable groups to those in the ambient control exhibited a relative increase, reducing the dominance of *Gephyrocapsa oceanica* and restoring species evenness. This suggests a high potential for

recovery under ambient temperature conditions, similar to the recovery of functional parameters such as gross oxygen production observed by Soulié et al. (2022) and to the high resistance of phototrophs to moderate heatwaves found by Remy et al. (2017).

However, in the ERCP 8.5 HW scenario, phototrophic diversity did not recover after the heatwave. Evenness decreased strongest during the cooling phase of the heatwave and remained low thereafter, while species richness drastically declined towards the end of the experiment. The higher temperature of the ERCP 8.5 scenario likely prompted a pre-selection for warm-tolerant species, preventing the diversity from decreasing during the heatwave but rather entailing the reduced evenness when temperatures cooled down again. This is supported by Samuels et al. (2021), who found that growing above the thermal optimum before a marine heatwave can intensify negative effects of subsequent marine heatwaves and thereby induce increased mortality rates. Although the stable species richness during the heatwave indicates that no thermal limits were reached, it is not unlikely that the temperature of ~ 22 °C was still above the optimum for many temperate phototrophs (Thomas et al., 2012). But contrary to the response under ambient conditions, the effect on evenness remained negative, mainly due to the strong relative increase in uncultured MOCH species. While their occurrence and ecology are not well described yet, they seem to cope well with the challenges imposed by marine heatwaves in both scenarios. Other studies also noted negative interactions between temperature changes and nutrient shifts on community diversity (Thomas et al., 2017; Hayashida et al., 2020), consistent with the decreased species richness towards the end of the incubation observed in our study. A possible explanation is that in many species, the unbalanced nutrient supply can impair physiological re-adjustments like the degradation of reactive oxygen species or the downregulation of the metabolism after the temperature drop (Gerhard et al., 2019; Rehder et al., 2023; Happe et al., in prep.). Finally, our results align well with the body of literature that proposes diversity to act as a buffer against further environmental changes (Tilman et al., 2014; Bestion et al., 2020) since the decreased species richness in the ERCP 8.5 scenario likely reduced the number of species that could endure the physiological challenges imposed by marine heatwaves. This is also in line with the observation that concurrent stressors can have exacerbating effects on community diversity (Boyd et al., 2018; Seifert et al., 2020) and has been observed by other aquatic heatwave studies that investigated the additional effects of changes in salinity (Stefanidou et al., 2018), turbidity (Remy et al., 2017), nutrients (Filiz et al., 2020) and by applying several consecutive heatwaves (Soulié et al., 2023).

Conclusion and ecological implications

Our study represents a systematic and statistically robust analysis of the impact of global change and marine heatwaves on different trophic groups and therefore enhances our knowledge on future protist diversity. First and foremost, our results indicate that protist diversity is generally affected by global change, but phototrophs appear to be more susceptible to concurrent shifts in temperature, nutrients and CO_2 than heterotrophs. Due to the decreased diversity, the capacity of phototrophs to recover from marine heatwaves was impaired by the applied ERCP 8.5 scenario, whereas heterotrophs remained largely uncompromised. The respective compositional shifts could alter biogeochemical cycles, increase harmful algae blooms and raise pathogenic activity. Although the effects on heterotrophic diversity were minor, changes in the phototrophic community may cascade up to heterotrophic protists and even higher trophic levels in the long term. Therefore, we expect consequences for the whole ecosystem, if anthropogenic pressures continue to increase. Future studies could try to disentangle the relative effects of each global change driver, to gain a mechanistic understanding of future changes in protist diversity.

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Data availability statement

The DNA data are available from the European Nucleotide Archive at EMBL-EBI under the accession ID PRJEB66280 (https://www.ebi.ac.uk/ena/browser/view/PRJEB66280) and were submitted via GFBio (Diepenbroek et al., 2014). Code to produce the graphs and results of the manuscript can be found online on GitHub: https://github.com/AntoniaAhme/HeatwaveNowTomorrowProtists (last accessed on 02.01.2024).

References

Adl, S.M., Bass, D., Lane, C.E., Lukeš, J., Schoch, C.L., Smirnov, A., et al. (2019). Revisions to the classification, nomenclature, and diversity of eukaryotes. *Journal of Eukaryotic Microbiology* 66(1)**,** 4-119. doi: 10.1111/jeu.12691.

Anderson, S.R., and Harvey, E.L. (2019). Seasonal Variability and Drivers of Microzooplankton Grazing and Phytoplankton Growth in a Subtropical Estuary. *Frontiers in Marine Science* 6. doi: 10.3389/fmars.2019.00174.

Bestion, E., Barton, S., Garcia, F.C., Warfield, R., and Yvon-Durocher, G. (2020). Abrupt declines in marine phytoplankton production driven by warming and biodiversity loss in a microcosm experiment. *Ecology Letters* 23(3)**,** 457-466. doi: 10.1111/ele.13444.

Bestion, E., Haegeman, B., Alvarez Codesal, S., Garreau, A., Huet, M., Barton, S., et al. (2021). Phytoplankton biodiversity is more important for ecosystem functioning in highly variable thermal environments. *Proceedings of the National Academy of Sciences* 118(35)**,** e2019591118. doi: doi:10.1073/pnas.2019591118.

Boscolo-Galazzo, F., Crichton, K.A., Barker, S., and Pearson, P.N. (2018). Temperature dependency of metabolic rates in the upper ocean: A positive feedback to global climate change? *Global and Planetary Change* 170**,** 201-212. doi: 10.1016/j.gloplacha.2018.08.017.

Boyd, P.W., Collins, S., Dupont, S., Fabricius, K., Gattuso, J.-P., Havenhand, J., et al. (2018). Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change—A review. *Global Change Biology* 24(6)**,** 2239-2261. doi: 10.1111/gcb.14102.

Boyd, P.W., Rynearson, T.A., Armstrong, E.A., Fu, F., Hayashi, K., Hu, Z., et al. (2013). Marine phytoplankton temperature versus growth responses from polar to tropical waters--outcome of a scientific community-wide study. *PLoS One* 8(5)**,** e63091. doi: 10.1371/journal.pone.0063091.

Bradley, I.M., Pinto, A.J., and Guest, J.S. (2016). Design and evaluation of illumina MiSeq-compatible, 18S rRNA gene-specific primers for improved characterization of mixed phototrophic communities. *Applied and Environmental Microbiology* 82(19)**,** 5878-5891. doi: 10.1128/AEM.01630-16.

Burgmer, T., and Hillebrand, H. (2011). Temperature mean and variance alter phytoplankton biomass and biodiversity in a long-term microcosm experiment. *Oikos* 120(6)**,** 922-933. doi: https://doi.org/10.1111/j.1600-0706.2010.19301.x.

Byers, J.E. (2021). Marine Parasites and Disease in the Era of Global Climate Change. *Annual Review of Marine Science* 13(1)**,** 397-420. doi: 10.1146/annurev-marine-031920-100429.

Calbet, A., Martínez, R.A., Saiz, E., and Alcaraz, M. (2022). Effects of Temperature on the Bioenergetics of the Marine Protozoans Gyrodinium dominans and Oxyrrhis marina. *Frontiers in Marine Science* 9. doi: 10.3389/fmars.2022.901096.

Calbet, A., and Saiz, E. (2022). Thermal Acclimation and Adaptation in Marine Protozooplankton and Mixoplankton. *Frontiers in Microbiology* 13. doi: 10.3389/fmicb.2022.832810.

Caron, D.A., and Hutchins, D.A. (2012). The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps. *Journal of Plankton Research* 35(2)**,** 235-252. doi: 10.1093/plankt/fbs091.

Chakraborty, S., Nielsen, L.T., and Andersen, K.H. (2017). Trophic Strategies of Unicellular Plankton. *The American Naturalist* 189(4)**,** E77-E90. doi: 10.1086/690764.

de Vargas, C., Audic, S., Henry, N., Decelle, J., Mahé, F., Logares, R., et al. (2015). Eukaryotic plankton diversity in the sunlit ocean. *Science* 348(6237)**,** 1261605. doi: doi:10.1126/science.1261605.

Deng, L., Cheung, S., Xu, Z., Liu, K., and Liu, H. (2023). Microzooplankton Grazing Exerts a Strong Top-Down Control on Unicellular Cyanobacterial Diazotrophs. *Journal of Geophysical Research: Biogeosciences* 128(12), e2023JG007824. doi: https://doi.org/10.1029/2023JG007824.

Diepenbroek, M., Glöckner, F., Grobe, P., Güntsch, A., Huber, R., König-Ries, B., et al. (2014). "Towards an Integrated Biodiversity and Ecological Research Data Management and Archiving Platform: The German Federation for the Curation of Biological Data (GFBio)," in *Informatik 2014 – Big Data Komplexität meistern,* ed. G.L. Plödereder E., Schneider E., Ull D. GI-Edition: Lecture Notes in Informatics (LNI) – Proceedings. ed (Bonn: Köllen Verlag), 1711–1724.

Feng, Y., Hare, C.E., Leblanc, K., Rose, J.M., Zhang, Y., DiTullio, G.R., et al. (2009). Effects of increased pCO2 and temperature on the North Atlantic spring bloom. I. The phytoplankton community and biogeochemical response. *Marine Ecology Progress Series* 388, 13-25.

Ferreira, G.D., Grigoropoulou, A., Saiz, E., and Calbet, A. (2022). The effect of short-term temperature exposure on vital physiological processes of mixoplankton and protozooplankton. *Marine Environmental Research* 179, 105693. doi: https://doi.org/10.1016/j.marenvres.2022.105693.

Filiz, N., Işkın, U., Beklioğlu, M., Öğlü, B., Cao, Y., Davidson, T.A., et al. (2020). Phytoplankton community response to nutrients, temperatures, and a heat wave in shallow lakes: An experimental approach. *Water (Switzerland)* 12(12). doi: 10.3390/w12123394.

Frölicher, T.L., Fischer, E.M., and Gruber, N. (2018). Marine heatwaves under global warming. *Nature* 560(7718), 360-364. doi: 10.1038/s41586-018-0383-9.

Gerhard, M., Koussoroplis, A.M., Hillebrand, H., and Striebel, M. (2019). Phytoplankton community responses to temperature fluctuations under different nutrient concentrations and stoichiometry. *Ecology* 100(11), e02834. doi: 10.1002/ecy.2834.

Gerhard, M., Schlenker, A., Hillebrand, H., and Striebel, M. (2022). Environmental stoichiometry mediates phytoplankton diversity effects on communities' resource use efficiency and biomass. *Journal of Ecology* 110(2), 430-442. doi: https://doi.org/10.1111/1365-2745.13811.

Gobler, C.J., and Sunda, W.G. (2012). Ecosystem disruptive algal blooms of the brown tide species, Aureococcus anophagefferens and Aureoumbra lagunensis. *Harmful Algae* 14, 36-45. doi: https://doi.org/10.1016/j.hal.2011.10.013.

Grimsley, N., Yau, S., Piganeau, G., and Moreau, H. (2015). "Typical Features of Genomes in the Mamiellophyceae," in *Marine Protists: Diversity and Dynamics,* eds. S. Ohtsuka, T. Suzaki, T. Horiguchi, N. Suzuki & F. Not. (Tokyo: Springer Japan), 107-127.

Grinienė, E., Šulčius, S., and Kuosa, H. (2016). Size-selective microzooplankton grazing on the phytoplankton in the Curonian Lagoon (SE Baltic Sea). *Oceanologia* 58(4), 292-301. doi: https://doi.org/10.1016/j.oceano.2016.05.002.

Grizzetti, B., Bouraoui, F., and Aloe, A. (2012). Changes of nitrogen and phosphorus loads to European seas. *Global Change Biology* 18(2), 769-782. doi: 10.1111/j.1365-2486.2011.02576.x.

Guo, K., and Gao, P. (2022). "microbial: Do 16s Data Analysis and Generate Figures".).

Gutiérrez-Rodríguez, A., Latasa, M., Safi, K., Pinkerton, M.H., and Nodder, S.D. (2023). Decoupled growth and grazing rates of diatoms and green algae drive increased phytoplankton productivity on HNLC sub-Antarctic plateaux. *Limnology and Oceanography Letters* 8(6), 896-905. doi: https://doi.org/10.1002/lol2.10355.

Happe, A., Ahme, A., Cabrerizo, M.J., Gerhard, M., John, U., and Striebel, M. (in prep.). Do the rate of temperature change and timing of nutrient availability impact the growth and stoichiometry of a natural marine phytoplankton community? .

Hayashida, H., Matear, R.J., and Strutton, P.G. (2020). Background nutrient concentration determines phytoplankton bloom response to marine heatwaves. *Global Change Biology* 26(9), 4800-4811. doi: https://doi.org/10.1111/gcb.15255.

Hermann, M., Peeters, E.T.H.M., and Van den Brink, P.J. (2023). Heatwaves, elevated temperatures, and a pesticide cause interactive effects on multi-trophic levels of a freshwater ecosystem. *Environmental Pollution* 327, 121498. doi: https://doi.org/10.1016/j.envpol.2023.121498.

Hillebrand, H., Antonucci Di Carvalho, J., Dajka, J.-C., Dürselen, C.-D., Kerimoglu, O., Kuczynski, L., et al. (2022). Temporal declines in Wadden Sea phytoplankton cell volumes observed within and across species. *Limnology and Oceanography* 67(2), 468-481. doi: https://doi.org/10.1002/lno.12005.

Hillebrand, H., Burgmer, T., and Biermann, E. (2012). Running to stand still: temperature effects on species richness, species turnover, and functional community dynamics. *Marine Biology* 159(11), 2415-2422. doi: 10.1007/s00227-011-1827-z.

Hillebrand, H., Gruner, D.S., Borer, E.T., Bracken, M.E.S., Cleland, E.E., Elser, J.J., et al. (2007). Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. *Proceedings of the National Academy of Sciences* 104(26), 10904-10909. doi: doi:10.1073/pnas.0701918104.

Hobday, A.J., Alexander, L.V., Perkins, S.E., Smale, D.A., Straub, S.C., Oliver, E.C.J., et al. (2016). A hierarchical approach to defining marine heatwaves. *Progress in Oceanography* 141, 227-238. doi: https://doi.org/10.1016/j.pocean.2015.12.014.

IPCC (2021). "Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)]".).

IPCC (2023). "Summary for Policymakers," in *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,* ed. H.L.a.J. Romero. (Geneva, Switzerland: IPCC), 1-34.

Işkın, U., Filiz, N., Cao, Y., Neif, É.M., Öğlü, B., Lauridsen, T.L., et al. (2020). Impact of Nutrients, Temperatures, and a Heat Wave on Zooplankton Community Structure: An Experimental Approach. *Water* 12(12), 3416.

John, E.H., and Davidson, K. (2001). Prey selectivity and the influence of prey carbon:nitrogen ratio on microflagellate grazing. *Journal of Experimental Marine Biology and Ecology* 260(1), 93-111. doi: https://doi.org/10.1016/S0022-0981(01)00244-1.

Kaczmarska, I., Lovejoy, C., Potvin, M., and Macgillivary, M. (2009). Morphological and molecular characteristics of selected species of Minidiscus (Bacillariophyta, Thalassiosiraceae). *European Journal of Phycology* 44(4), 461-475. doi: 10.1080/09670260902855873.

Kang, Y., and Kang, C.-K. (2023). Coupling and decoupling of marine stramenopiles and cyanobacteria in eutrophic coastal waters of Korea. *Science of The Total Environment* 893, 164927. doi: https://doi.org/10.1016/j.scitotenv.2023.164927.

Kilner, C.L., Carrell, A.A., Wieczynski, D.J., Votzke, S., DeWitt, K., Yammine, A., et al. (2024). Temperature and CO2 interactively drive shifts in the compositional and functional structure of peatland protist communities. *Global Change Biology* 30(3), e17203. doi: https://doi.org/10.1111/gcb.17203.

Langer, G., Nehrke, G., Probert, I., Ly, J., and Ziveri, P. (2009). Strain-specific responses of Emiliania huxleyi to changing seawater carbonate chemistry. *Biogeosciences* 6(11), 2637-2646. doi: 10.5194/bg-6-2637-2009.

Laufkötter, C., Zscheischler, J., and Frölicher, T.L. (2020). High-impact marine heatwaves attributable to human-induced global warming. *Science* 369(6511). doi: 10.1126/science.aba0690.

Lewandowska, A.M., Hillebrand, H., Lengfellner, K., and Sommer, U. (2014). Temperature effects on phytoplankton diversity — The zooplankton link. *Journal of Sea Research* 85**,** 359-364. doi: https://doi.org/10.1016/j.seares.2013.07.003.

Lin, Y.-C., Campbell, T., Chung, C.-C., Gong, G.-C., Chiang, K.-P., and Worden, A.Z. (2012). Distribution Patterns and Phylogeny of Marine Stramenopiles in the North Pacific Ocean. *Applied and Environmental Microbiology* 78(9)**,** 3387-3399. doi: doi:10.1128/AEM.06952-11.

Lin, Y.-C., Chin, C.-P., Yang, J.W., Chiang, K.-P., Hsieh, C.-h., Gong, G.-C., et al. (2022). How Communities of Marine Stramenopiles Varied with Environmental and Biological Variables in the Subtropical Northwestern Pacific Ocean. *Microbial Ecology* 83(4)**,** 916-928. doi: 10.1007/s00248-021-01788-7.

Litchman, E., and Thomas, M.K. (2023). Are we underestimating the ecological and evolutionary effects of warming? Interactions with other environmental drivers may increase species vulnerability to high temperatures. *Oikos* 2023(2)**,** e09155. doi: https://doi.org/10.1111/oik.09155.

Liu, K., Chen, B., Zhang, S., Sato, M., Shi, Z., and Liu, H. (2018). Marine phytoplankton in subtropical coastal waters showing lower thermal sensitivity than microzooplankton. *Limnology and Oceanography* 64(3)**,** 1103-1119. doi: 10.1002/lno.11101.

Liu, X., Tang, C.H., and Wong, C.K. (2014). Microzooplankton grazing and selective feeding during bloom periods in the Tolo Harbour area as revealed by HPLC pigment analysis. *Journal of Sea Research* 90**,** 83-94. doi: https://doi.org/10.1016/j.seares.2014.02.017.

Marañón, E. (2015). Cell size as a key determinant of phytoplankton metabolism and community structure. *Annual Review of Marine Science* 7(1)**,** 241-264. doi: 10.1146/annurev-marine-010814-015955.

Markina, Z.V. (2020). The Growth, Population Size Structure, Physiological Changes, and Ultrastructure of the Microalga Picochlorum maculatum (Chlorophyta) under Sublethal Copper Concentrations. *Russian Journal of Marine Biology* 46(5)**,** 346-350. doi: 10.1134/S1063074020050077.

Martinez, E.A. (1980). Sensitivity of marine ciliates (Protozoa, ciliophora) to high thermal stress. *Estuarine and Coastal Marine Science* 10(4)**,** 369-IN361. doi: https://doi.org/10.1016/S0302-3524(80)80117-4.

Massana, R., Unrein, F., Rodríguez-Martínez, R., Forn, I., Lefort, T., Pinhassi, J., et al. (2009). Grazing rates and functional diversity of uncultured heterotrophic flagellates. *The ISME Journal* 3(5)**,** 588-596. doi: 10.1038/ismej.2008.130.

McKew, B.A., Metodieva, G., Raines, C.A., Metodiev, M.V., and Geider, R.J. (2015). Acclimation of Emiliania huxleyi (1516) to nutrient limitation involves precise modification of the proteome to scavenge alternative sources of N and P. *Environmental Microbiology* 17(10)**,** 4050-4062. doi: 10.1111/1462-2920.12957.

Meunier, C.L., Schmidt, J., Ahme, A., and Kirstein, I. (in prep.). Plankton communities today and tomorrow – impacts of global change and marine heatwaves in a multiple-driver mesocosm experiment.

Moreno, H.D., Köring, M., Di Pane, J., Tremblay, N., Wiltshire, K.H., Boersma, M., et al. (2022). An integrated multiple driver mesocosm experiment reveals the effect of global change on planktonic food web structure. *Communications Biology* 5(1)**,** 179. doi: 10.1038/s42003-022-03105-5.

O'Donnell, D.R., Du, Z.-y., and Litchman, E. (2019). Experimental evolution of phytoplankton fatty acid thermal reaction norms. *Evolutionary Applications* 12(6)**,** 1201-1211. doi: https://doi.org/10.1111/eva.12798.

Oliver, E.C.J., Burrows, M.T., Donat, M.G., Sen Gupta, A., Alexander, L.V., Perkins-Kirkpatrick, S.E., et al. (2019). Projected marine heatwaves in the 21st century and the potential for ecological impact. *Frontiers in Marine Science* 6. doi: 10.3389/fmars.2019.00734.

Padfield, D., Yvon-Durocher, G., Buckling, A., Jennings, S., and Yvon-Durocher, G. (2016). Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton. *Ecology Letters* 19(2)**,** 133-142. doi: https://doi.org/10.1111/ele.12545.

Pansch, A., Winde, V., Asmus, R., and Asmus, H. (2016). Tidal benthic mesocosms simulating future climate change scenarios in the field of marine ecology. *Limnology and Oceanography: Methods* 14(4)**,** 257-267. doi: https://doi.org/10.1002/lom3.10086.

Potvin, E., Jeong, H.J., Kang, N.S., Noh, J.H., and Yang, E.J. (2015). Morphology, molecular phylogeny, and pigment characterization of an isolate of the dinoflagellate Pelagodinium bei from Korean waters. *Algae* 30(3)**,** 183-195. doi: 10.4490/algae.2015.30.3.183.

[Dataset] Ramond, P., Siano, R., and Sourisseau, M. (2018). *Functional traits of marine protists.* doi: 10.17882/51662.

Rasconi, S., Winter, K., and Kainz, M.J. (2017). Temperature increase and fluctuation induce phytoplankton biodiversity loss - Evidence from a multi-seasonal mesocosm experiment. *Ecol Evol* 7(9)**,** 2936-2946. doi: 10.1002/ece3.2889.

Raupach, M.R., Marland, G., Ciais, P., Le Quéré, C., Canadell, J.G., Klepper, G., et al. (2007). Global and regional drivers of accelerating CO2 emissions. *Proceedings of the National Academy of Sciences* 104(24)**,** 10288-10293. doi: doi:10.1073/pnas.0700609104.

Raven, J.A., and Beardall, J. (2021). Influence of global environmental Change on plankton. *Journal of Plankton Research* 43(6)**,** 779-800. doi: 10.1093/plankt/fbab075.

RCoreTeam (2022). "R: A language and environment for statistical computing. URL: https://www.R-project.org/". (Vienna, Austria: R Foundation for Statistical Computing).

Regaudie-de-Gioux, A., and Duarte, C.M. (2012). Temperature dependence of planktonic metabolism in the ocean. *Global Biogeochemical Cycles* 26(1). doi: 10.1029/2010gb003907.

Rehder, L., Rost, B., and Rokitta, S.D. (2023). Abrupt and acclimation responses to changing temperature elicit divergent physiological effects in the diatom Phaeodactylum tricornutum. *New Phytologist* 239(3)**,** 1005-1013. doi: 10.1111/nph.18982.

Reinfelder, J.R. (2010). Carbon Concentrating Mechanisms in Eukaryotic Marine Phytoplankton. *Annual Review of Marine Science* 3(1)**,** 291-315. doi: 10.1146/annurev-marine-120709-142720.

Remy, M., Hillebrand, H., and Flöder, S. (2017). Stability of marine phytoplankton communities facing stress related to global change: Interactive effects of heat waves and turbidity. *Journal of Experimental Marine Biology and Ecology* 497**,** 219-229. doi: 10.1016/j.jembe.2017.10.002.

Rick, J.J., Scharfe, M., Romanova, T., van Beusekom, J.E.E., Asmus, R., Asmus, H., et al. (2023). An evaluation of long-term physical and hydrochemical measurements at the Sylt Roads Marine Observatory (1973–2019), Wadden Sea, North Sea. *Earth Syst. Sci. Data* 15(3)**,** 1037-1057. doi: 10.5194/essd-15-1037-2023.

Rivero-Calle, S., Gnanadesikan, A., Del Castillo, C.E., Balch, W.M., and Guikema, S.D. (2015). Multidecadal increase in North Atlantic coccolithophores and the potential role of rising CO2. *Science* 350(6267)**,** 1533-1537. doi: doi:10.1126/science.aaa8026.

Rose, J.M., and Caron, D.A. (2007). Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. *Limnology and Oceanography* 52(2)**,** 886-895. doi: 10.4319/lo.2007.52.2.0886.

Rost, B., Zondervan, I., and Wolf-Gladrow, D. (2008). Sensitivity of phytoplankton to future changes in ocean carbonate chemistry: Current knowledge, contradictions and research directions. *Marine Ecology Progress Series* 373**,** 227-237. doi: 10.3354/meps07776.

Roth, S.K., Polazzo, F., García-Astillero, A., Cherta, L., Sobek, A., and Rico, A. (2022). Multiple stressor effects of a heatwave and a herbicide on zooplankton communities: Implications of global climate change. *Frontiers in Environmental Science* 10. doi: 10.3389/fenvs.2022.920010.

RStudioTeam (2022). "RStudio: Integrated development environment for R. URL: http://www.rstudio.com/". (Boston, MA: RStudio, PBC).

Salmaso, N., and Tolotti, M. (2021). Phytoplankton and anthropogenic changes in pelagic environments. *Hydrobiologia* 848(1)**,** 251-284. doi: 10.1007/s10750-020-04323-w.

Samuels, T., Rynearson, T.A., and Collins, S. (2021). Surviving Heatwaves: Thermal Experience Predicts Life and Death in a Southern Ocean Diatom. *Frontiers in Marine Science* 8(9). doi: 10.3389/fmars.2021.600343.

Sauterey, B., Gland, G.L., Cermeño, P., Aumont, O., Lévy, M., and Vallina, S.M. (2023). Phytoplankton adaptive resilience to climate change collapses in case of extreme events – A modeling study. *Ecological Modelling* 483**,** 110437. doi: https://doi.org/10.1016/j.ecolmodel.2023.110437.

Scharfe, M., and Wiltshire, K.H. (2019). Modeling of intra-annual abundance distributions: Constancy and variation in the phenology of marine phytoplankton species over five decades at Helgoland Roads (North Sea). *Ecological Modelling* 404**,** 46-60. doi: https://doi.org/10.1016/j.ecolmodel.2019.01.001.

Seifert, M., Rost, B., Trimborn, S., and Hauck, J. (2020). Meta-analysis of multiple driver effects on marine phytoplankton highlights modulating role of pCO2. *Global Change Biology* 26(12)**,** 6787-6804. doi: 10.1111/gcb.15341.

Shemi, A., Alcolombri, U., Schatz, D., Farstey, V., Vincent, F., Rotkopf, R., et al. (2021). Dimethyl sulfide mediates microbial predator–prey interactions between zooplankton and algae in the ocean. *Nature Microbiology* 6(11)**,** 1357-1366. doi: 10.1038/s41564-021-00971-3.

Simpson, G.L. (2018). Modelling Palaeoecological Time Series Using Generalised Additive Models. *Frontiers in Ecology and Evolution* 6. doi: 10.3389/fevo.2018.00149.

Smale, D.A., Wernberg, T., Oliver, E.C.J., Thomsen, M., Harvey, B.P., Straub, S.C., et al. (2019). Marine heatwaves threaten global biodiversity and the provision of ecosystem services. *Nature Climate Change* 9(4)**,** 306-312. doi: 10.1038/s41558-019-0412-1.

Soulié, T., Vidussi, F., Mas, S., and Mostajir, B. (2022). Functional Stability of a Coastal Mediterranean Plankton Community During an Experimental Marine Heatwave. *Frontiers in Marine Science* 9. doi: 10.3389/fmars.2022.831496.

Soulié, T., Vidussi, F., Mas, S., and Mostajir, B. (2023). Functional and structural responses of plankton communities toward consecutive experimental heatwaves in Mediterranean coastal waters. *Scientific Reports* 13(1)**,** 8050. doi: 10.1038/s41598-023-35311-4.

Stefanidou, N., Genitsaris, S., Lopez-Bautista, J., Sommer, U., and Moustaka-Gouni, M. (2018). Unicellular Eukaryotic Community Response to Temperature and Salinity Variation in Mesocosm Experiments. *Frontiers in Microbiology* 9. doi: 10.3389/fmicb.2018.02444.

Stefanidou, N., Genitsaris, S., Lopez-Bautista, J., Sommer, U., and Moustaka-Gouni, M. (2019). Response of a coastal Baltic Sea diatom-dominated phytoplankton community to experimental heat shock and changing salinity. *Oecologia* 191(2)**,** 461-474. doi: 10.1007/s00442-019-04502-0.

Thomas, M.K., Aranguren-Gassis, M., Kremer, C.T., Gould, M.R., Anderson, K., Klausmeier, C.A., et al. (2017). Temperature–nutrient interactions exacerbate sensitivity to warming in phytoplankton. *Global Change Biology* 23(8)**,** 3269-3280. doi: 10.1111/gcb.13641.

Thomas, M.K., Kremer, C.T., Klausmeier, C.A., and Litchman, E. (2012). A global pattern of thermal adaptation in marine phytoplankton. *Science* 338(6110), 1085-1088. doi: 10.1126/science.1224836.

Tillmann, U. (2004). Interactions between Planktonic Microalgae and Protozoan Grazers. *Journal of Eukaryotic Microbiology* 51(2), 156-168. doi: https://doi.org/10.1111/j.1550-7408.2004.tb00540.x.

Tilman, D., Isbell, F., and Cowles, J.M. (2014). Biodiversity and Ecosystem Functioning. *Annual Review of Ecology, Evolution, and Systematics* 45(1), 471-493. doi: 10.1146/annurev-ecolsys-120213-091917.

Tilman, D., Kilham, S.S., and Kilham, P. (1982). Phytoplankton community ecology: the role of limiting nutrients. *Annual review of ecology and systematics. Volume 13*, 349-372. doi: 10.1146/annurev.es.13.110182.002025.

Tsirigoti, A., Beakes, G.W., Hervé, C., Gachon, C.M.M., and Katsaros, C. (2015). Attachment, penetration and early host defense mechanisms during the infection of filamentous brown algae by Eurychasma dicksonii. *Protoplasma* 252(3), 845-856. doi: 10.1007/s00709-014-0721-1.

Urrutia-Cordero, P., Langenheder, S., Striebel, M., Angeler, David G., Bertilsson, S., Eklöv, P., et al. (2021). Integrating multiple dimensions of ecological stability into a vulnerability framework. *Journal of Ecology* 110(2), 374-386. doi: 10.1111/1365-2745.13804.

Velthuis, M., Keuskamp, J.A., Bakker, E.S., Boersma, M., Sommer, U., van Donk, E., et al. (2022). Differential effects of elevated pCO2 and warming on marine phytoplankton stoichiometry. *Limnology and Oceanography* 67(3), 598-607. doi: https://doi.org/10.1002/lno.12020.

Vinton, A.C., Gascoigne, S.J.L., Sepil, I., and Salguero-Gómez, R. (2022). Plasticity's role in adaptive evolution depends on environmental change components. *Trends in Ecology & Evolution*. doi: https://doi.org/10.1016/j.tree.2022.08.008.

Wakeman, K.C., and Leander, B.S. (2013). Molecular phylogeny of marine gregarine parasites (Apicomplexa) from tube-forming polychaetes (Sabellariidae, Cirratulidae and Serpulidae), including descriptions of two new species of Selenidium. *J Eukaryot Microbiol* 60(5), 514-525. doi: 10.1111/jeu.12059.

Wang, J., Zeng, C., and Feng, Y. (2024). Meta-analysis reveals responses of coccolithophores and diatoms to warming. *Marine Environmental Research* 193, 106275. doi: 10.1016/j.marenvres.2023.106275.

Wiltshire, K.H., Boersma, M., Carstens, K., Kraberg, A.C., Peters, S., and Scharfe, M. (2015). Control of phytoplankton in a shelf sea: Determination of the main drivers based on the Helgoland Roads Time Series. *Journal of Sea Research* 105, 42-52. doi: 10.1016/j.seares.2015.06.022.

Supplementary material of publication V

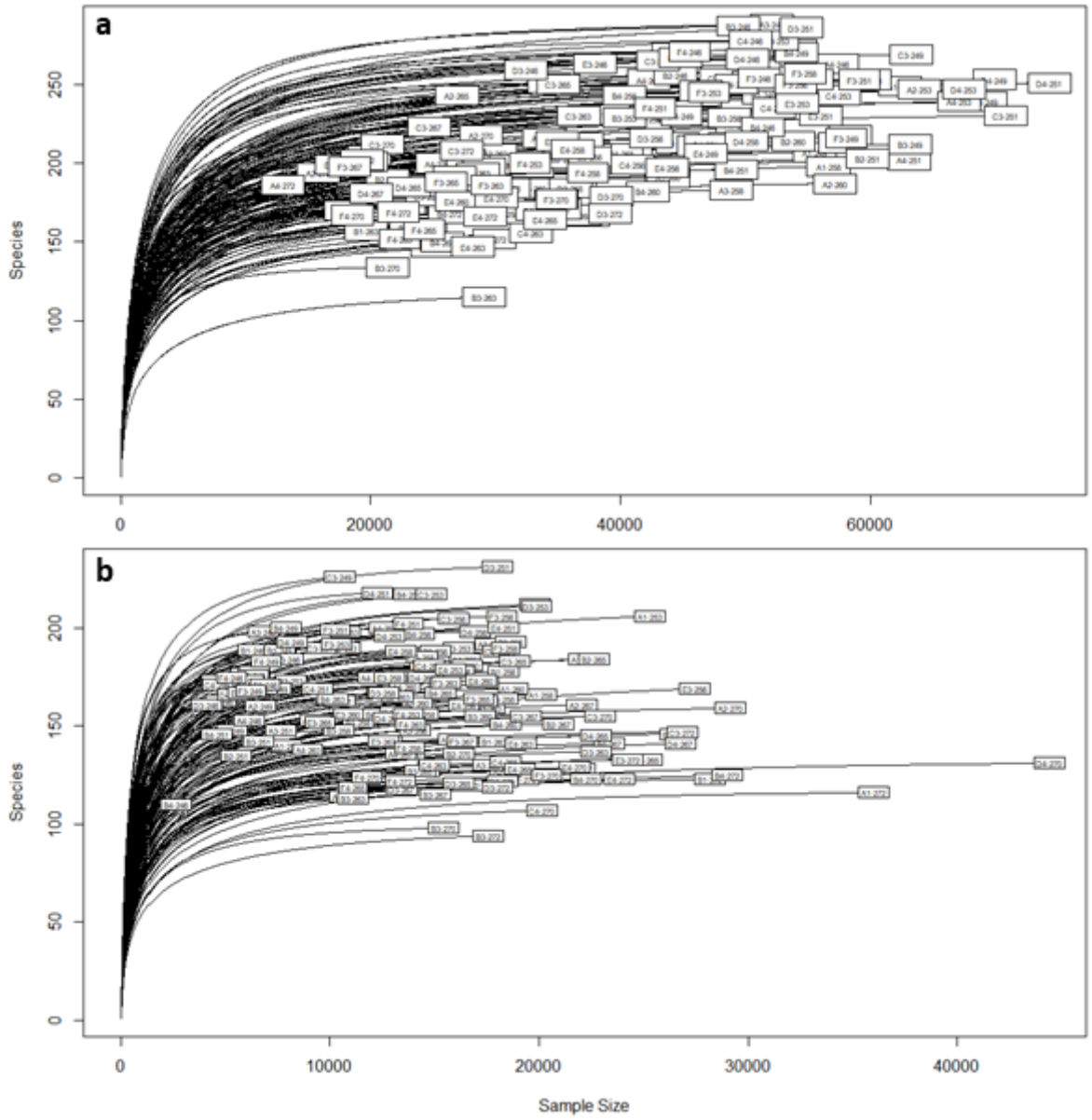

*Figure S1: Rarefaction curves of the raw read counts for (**a**) phototrophic and (**b**) heterotrophic protists.*

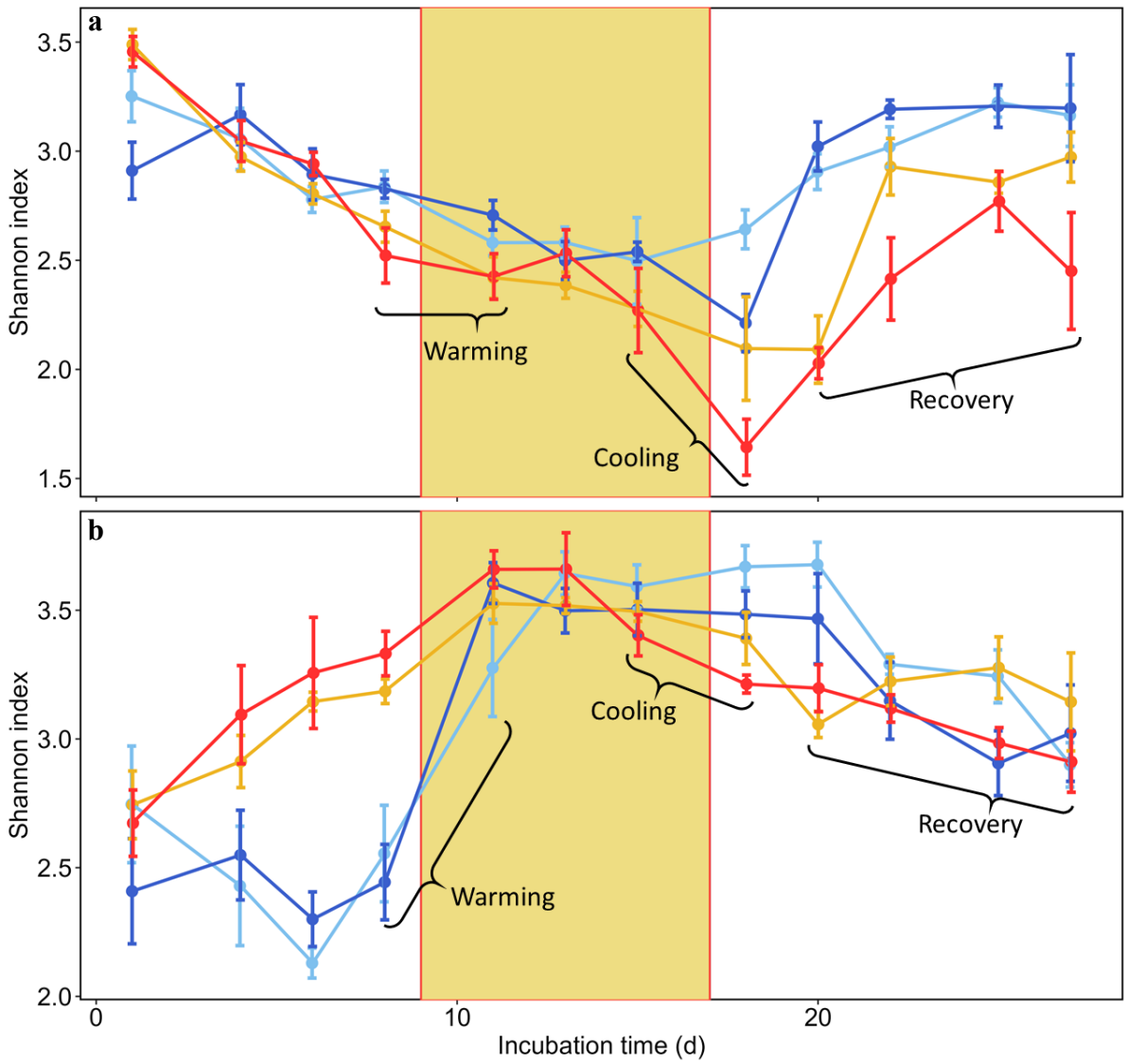

*Figure S2: Shannon diversity index of (**a**) phototrophic and (**b**) heterotrophic protists per treatment (light blue = ambientient, dark blue = ambientient HW, yellow = ERCP 8.5, red = ERCP 8.5 HW) over time for all relevant phases. Dots represent the mean and error bars the standard error of the replicates. The yellow rectangle indicates the heatwave period.*

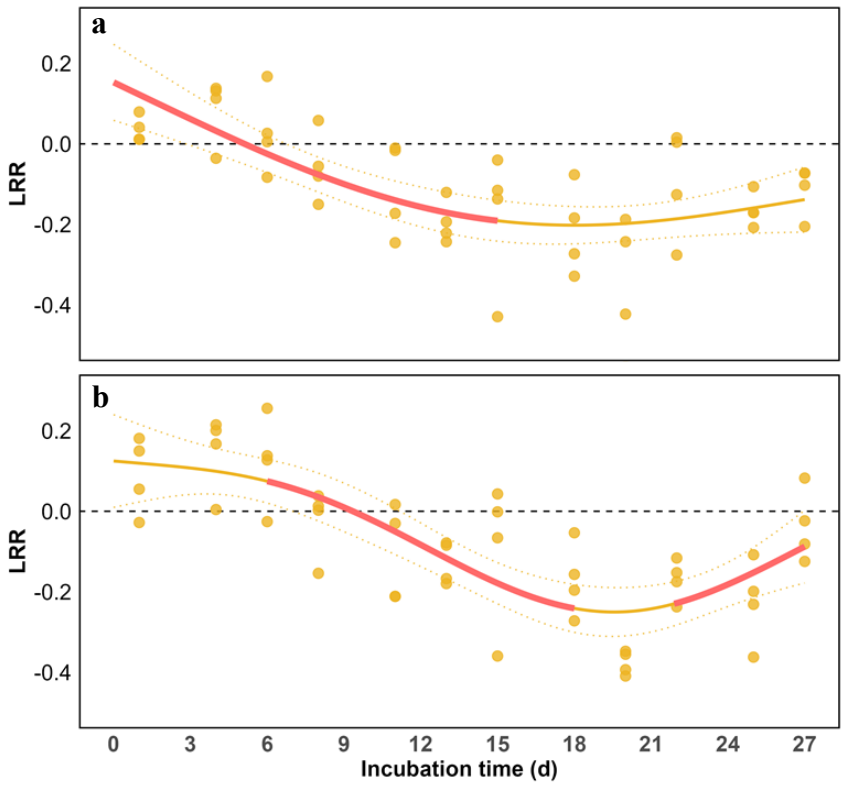

*Figure S3: Log response ratios (LRR) of the species richness for (**a**) phototrophic and (**b**) heterotrophic protists for the global change scenario over time. Each point represents an individual observation. Fitted thin-plate spline with approximate 95% point-wise confidence interval (dotted lines). The thick orange line represents phases of significant change.*

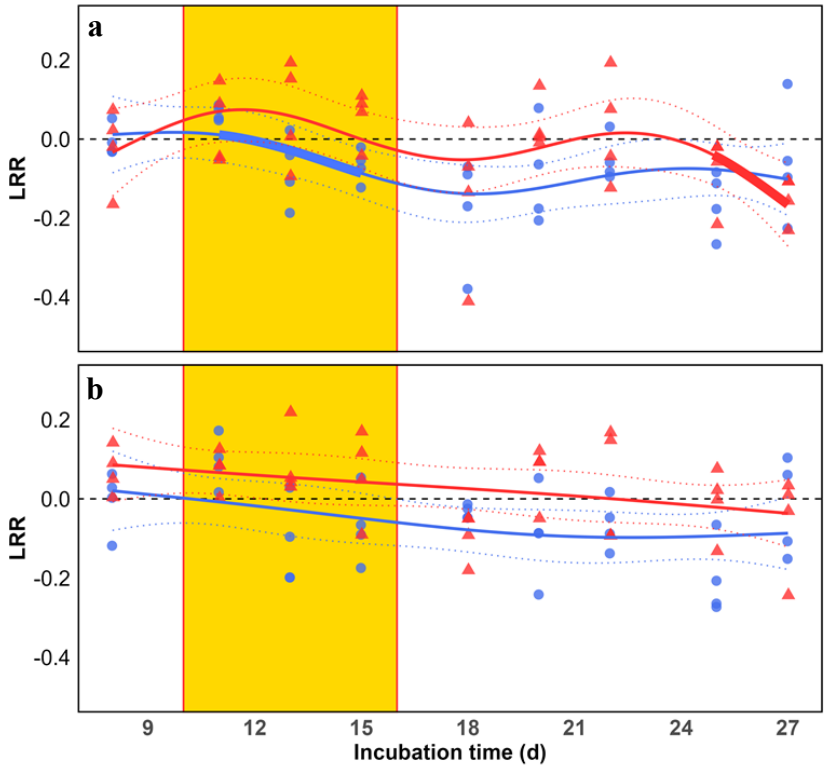

*Figure S4: Log response ratios (LRR) of the species richness for (**a**) phototrophic and (**b**) heterotrophic protists for the heatwaves over time. Each point represents an individual observation (blue = ambientient HW, red = ERCP 8.5 HW). Fitted thin-plate spline with approximate 95% point-wise confidence interval (dotted lines). The yellow rectangle indicates the heatwave period. Thick parts of the line represent phases of significant change.*

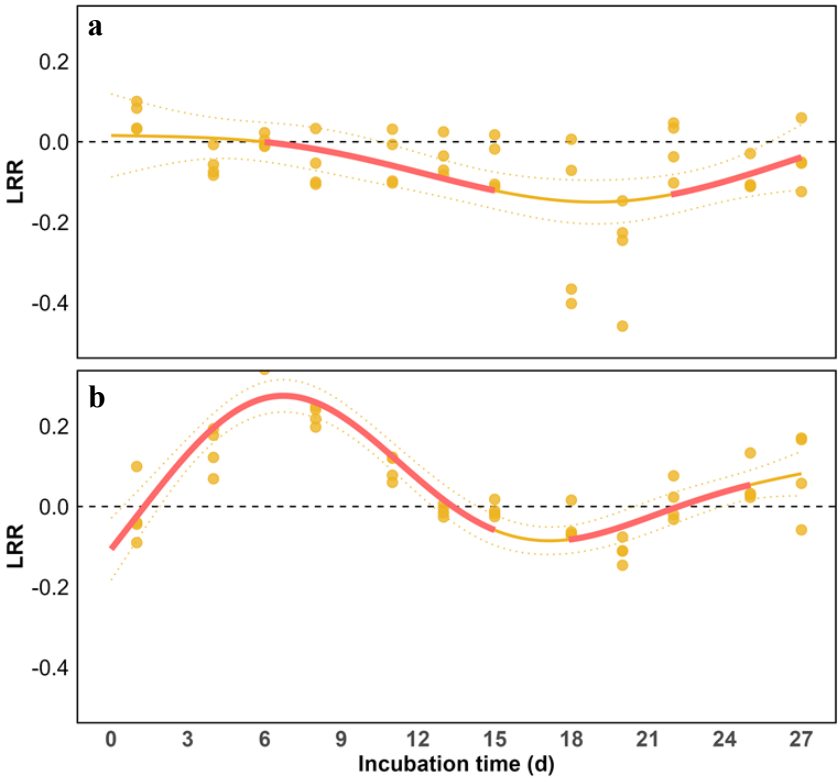

*Figure S5: Log response ratios (LRR) of the species evenness for (**a**) phototrophic and (**b**) heterotrophic protists for the global change scenario over time. Each point represents an individual observation. Fitted thin-plate spline with approximate 95% point-wise confidence interval (dotted lines). The thick orange line represents phases of significant change.*

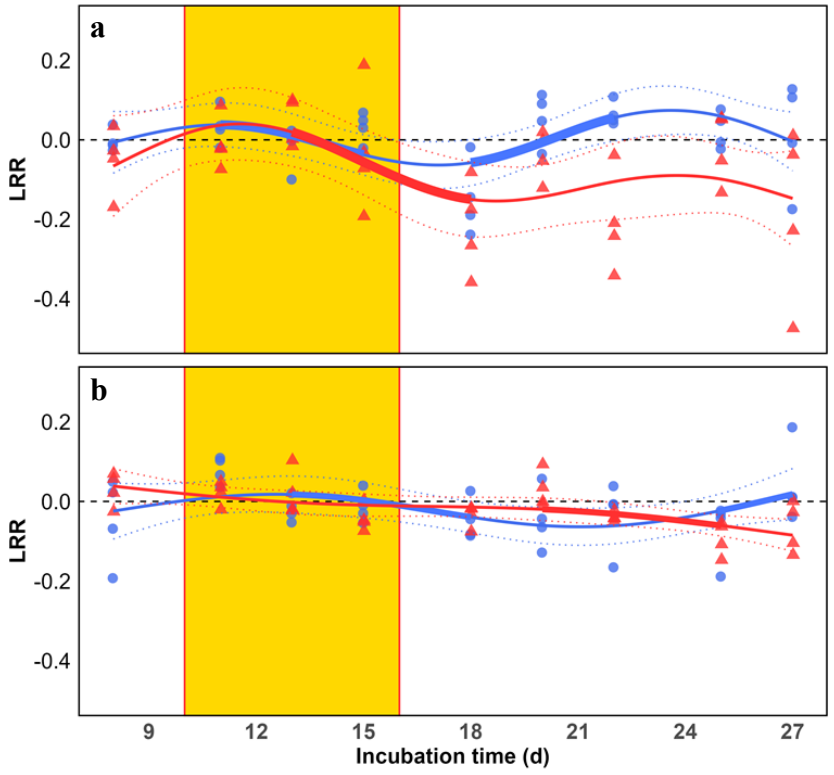

*Figure S6: Log response ratios (LRR) of the species evenness for (**a**) phototrophic and (**b**) heterotrophic protists for the heatwaves over time. Each point represents an individual observation (blue = ambientient HW, red = ERCP 8.5 HW). Fitted thin-plate spline with approximate 95% point-wise confidence interval (dotted lines). The yellow rectangle indicates the heatwave period. Thick parts of the line represent phases of significant change.*

Table S1: Diversity metrics of all treatments, replicates and sampling days for the phototrophic protists.

Treatment	Day	Replicate	Richness	Evenness	Shannon Index
AMBIENT	1	A	199	0.678109042	3.589437864
AMBIENT	1	B	192	0.591063692	3.107514627
AMBIENT	1	C	195	0.584360862	3.081334565
AMBIENT	1	D	201	0.608838398	3.228855666
AMBIENT	4	A	140	0.5671652	2.802727612
AMBIENT	4	B	162	0.627827954	3.1941352
AMBIENT	4	C	186	0.647832916	3.385410706
AMBIENT	6	B	80	0.648678716	2.842527409
AMBIENT	6	C	123	0.55077727	2.650441761
AMBIENT	6	D	138	0.55178946	2.71880665
AMBIENT	8	A	155	0.560119638	2.82492145
AMBIENT	8	B	149	0.583775056	2.921179033
AMBIENT	8	C	163	0.522634313	2.662168635
AMBIENT	8	D	171	0.540029378	2.776649373
AMBIENT	11	A	198	0.55495083	2.934728177
AMBIENT	11	C	170	0.579232619	2.974821978
AMBIENT	11	D	158	0.48773077	2.469183372
AMBIENT	13	A	180	0.499428394	2.593510098
AMBIENT	13	B	184	0.526171719	2.743951715
AMBIENT	13	C	153	0.500226077	2.516356227
AMBIENT	13	D	170	0.477190582	2.450754647
AMBIENT	15	A	180	0.500366864	2.598383537
AMBIENT	15	B	167	0.489431741	2.504908623
AMBIENT	15	C	187	0.529998129	2.77247778
AMBIENT	15	D	132	0.390241951	1.905474147
AMBIENT	18	A	164	0.521379917	2.658967935
AMBIENT	18	B	166	0.54103272	2.765752656
AMBIENT	18	C	171	0.516955122	2.658009313
AMBIENT	18	D	172	0.510504507	2.627819132
AMBIENT	20	A	136	0.49031759	2.408761104
AMBIENT	20	B	187	0.514952026	2.693769983
AMBIENT	20	C	183	0.544556419	2.836859124
AMBIENT	20	D	208	0.550623284	2.938972744
AMBIENT	22	A	190	0.513034547	2.691904618
AMBIENT	22	B	222	0.571670055	3.088548875
AMBIENT	22	C	186	0.555811226	2.904528668
AMBIENT	22	D	184	0.578717659	3.017975413
AMBIENT	25	A	156	0.552260704	2.788837032
AMBIENT	25	B	196	0.613960528	3.240554062
AMBIENT	25	C	181	0.583062645	3.031049431
AMBIENT	27	A	196	0.647318977	3.416623781
AMBIENT	27	B	173	0.602489377	3.104803441
AMBIENT	27	C	201	0.603152538	3.198701814
AMBIENT	27	D	213	0.59207678	3.174296601
AMBIENT+HW	1	A	181	0.639047823	3.322088209
AMBIENT+HW	1	B	172	0.599754904	3.087235056

Treatment	Day	Replicate	Richness	Evenness	Shannon Index
AMBIENT+HW	1	C	188	0.657335365	3.442098489
AMBIENT+HW	1	D	186	0.536168211	2.801879245
AMBIENT+HW	4	A	182	0.61455206	3.198133028
AMBIENT+HW	4	B	150	0.515118669	2.581071783
AMBIENT+HW	4	C	193	0.542523503	2.855133118
AMBIENT+HW	4	D	183	0.577672385	3.009376291
AMBIENT+HW	6	A	150	0.615879845	3.08594929
AMBIENT+HW	6	B	190	0.674047483	3.536743371
AMBIENT+HW	6	C	175	0.613825683	3.170278277
AMBIENT+HW	6	D	149	0.574891915	2.876728273
AMBIENT+HW	8	A	137	0.548537942	2.698796213
AMBIENT+HW	8	B	138	0.545039623	2.685548492
AMBIENT+HW	8	C	174	0.594170436	3.065358138
AMBIENT+HW	8	D	179	0.602602109	3.125929625
AMBIENT+HW	11	A	170	0.534649853	2.745853879
AMBIENT+HW	11	B	185	0.544083194	2.840307872
AMBIENT+HW	11	C	170	0.541676096	2.781939247
AMBIENT+HW	11	D	174	0.57054736	2.943485383
AMBIENT+HW	13	A	177	0.493090727	2.552311434
AMBIENT+HW	13	B	182	0.553762516	2.881783835
AMBIENT+HW	13	C	178	0.516334743	2.67553488
AMBIENT+HW	13	D	184	0.520940122	2.716669269
AMBIENT+HW	15	A	146	0.451581274	2.250503428
AMBIENT+HW	15	B	169	0.497721077	2.553258713
AMBIENT+HW	15	C	158	0.502046378	2.541657498
AMBIENT+HW	18	A	180	0.510580157	2.651420724
AMBIENT+HW	18	B	155	0.481800348	2.429923978
AMBIENT+HW	18	C	147	0.526903158	2.62947469
AMBIENT+HW	18	D	150	0.516687098	2.588930609
AMBIENT+HW	20	A	140	0.507211378	2.506457263
AMBIENT+HW	20	B	158	0.445708014	2.256439177
AMBIENT+HW	20	C	116	0.405593256	1.928024121
AMBIENT+HW	20	D	143	0.426168811	2.115009596
AMBIENT+HW	22	A	155	0.505554334	2.549725426
AMBIENT+HW	22	B	218	0.600114422	3.231313143
AMBIENT+HW	22	C	189	0.574623375	3.012030359
AMBIENT+HW	22	D	164	0.613744188	3.130013379
AMBIENT+HW	25	A	169	0.52893976	2.713407393
AMBIENT+HW	25	B	165	0.648819648	3.312837745
AMBIENT+HW	25	C	163	0.611612277	3.11540016
AMBIENT+HW	25	D	169	0.618780042	3.174278943
AMBIENT+HW	27	A	185	0.606548423	3.166398594
AMBIENT+HW	27	B	180	0.640947548	3.328412958
ERCP 8.5	1	A	150	0.597252787	2.992615893
ERCP 8.5	1	B	175	0.65973394	3.4073846
ERCP 8.5	1	C	164	0.607352272	3.097415462
ERCP 8.5	1	D	172	0.603380581	3.105898208
ERCP 8.5	4	A	145	0.510550225	2.540872532

Treatment	Day	Replicate	Richness	Evenness	Shannon Index
ERCP 8.5	4	B	165	0.690896422	3.527679459
ERCP 8.5	4	C	209	0.676646107	3.614869673
ERCP 8.5	4	D	205	0.681125468	3.625637664
ERCP 8.5	6	A	213	0.669665046	3.590269962
ERCP 8.5	6	B	199	0.637111658	3.372426212
ERCP 8.5	6	C	199	0.635974834	3.366408658
ERCP 8.5	6	D	137	0.573239972	2.820329727
ERCP 8.5	8	A	159	0.618681361	3.13603655
ERCP 8.5	8	B	162	0.578250551	2.941905384
ERCP 8.5	8	C	163	0.588539783	2.997874637
ERCP 8.5	8	D	130	0.564484689	2.747648669
ERCP 8.5	11	A	145	0.557558795	2.774821669
ERCP 8.5	11	B	142	0.555555693	2.753237933
ERCP 8.5	11	C	167	0.574941262	2.942545823
ERCP 8.5	13	A	186	0.496502941	2.594598592
ERCP 8.5	13	B	166	0.520558791	2.661090185
ERCP 8.5	13	C	162	0.493963115	2.513084936
ERCP 8.5	13	D	151	0.567363465	2.846621272
ERCP 8.5	15	A	166	0.456192479	2.332050383
ERCP 8.5	15	B	132	0.454036805	2.216971786
ERCP 8.5	15	C	167	0.519002465	2.656251405
ERCP 8.5	18	A	142	0.499626472	2.476062388
ERCP 8.5	18	B	145	0.465003786	2.314200033
ERCP 8.5	18	C	138	0.45969172	2.265017721
ERCP 8.5	18	D	141	0.511488485	2.531233699
ERCP 8.5	20	A	156	0.481680475	2.432417039
ERCP 8.5	20	B	138	0.439475834	2.165408922
ERCP 8.5	20	D	141	0.442659739	2.190616762
ERCP 8.5	22	A	152	0.500593253	2.514920693
ERCP 8.5	22	C	103	0.483199101	2.239496882
ERCP 8.5	25	A	157	0.518209394	2.620194074
ERCP 8.5	25	D	122	0.356964993	1.714867341
ERCP 8.5	27	A	141	0.479632926	2.373588185
ERCP 8.5	27	B	129	0.344372156	1.673584074
ERCP 8.5	27	C	167	0.473137363	2.421514096
ERCP 8.5	27	D	158	0.436955814	2.212130333
ERCP 8.5+HW	1	A	116	0.428818033	2.038425194
ERCP 8.5+HW	1	B	132	0.346429326	1.691545777
ERCP 8.5+HW	1	C	158	0.52536018	2.659685837
ERCP 8.5+HW	1	D	182	0.602313852	3.134445316
ERCP 8.5+HW	4	A	180	0.609824787	3.166793807
ERCP 8.5+HW	4	D	136	0.560683137	2.754442752
ERCP 8.5+HW	6	A	176	0.547089075	2.828715308
ERCP 8.5+HW	6	B	165	0.546636001	2.791093614
ERCP 8.5+HW	6	C	165	0.54915593	2.803960237
ERCP 8.5+HW	6	D	159	0.593415835	3.007968018
ERCP 8.5+HW	8	A	169	0.5780807	2.96549544
ERCP 8.5+HW	8	B	164	0.645320584	3.291048784

Treatment	Day	Replicate	Richness	Evenness	Shannon Index
ERCP 8.5+HW	8	C	169	0.537036588	2.754943305
ERCP 8.5+HW	8	D	148	0.576355935	2.880172954
ERCP 8.5+HW	11	A	212	0.663242567	3.552716032
ERCP 8.5+HW	11	B	207	0.654435339	3.48991963
ERCP 8.5+HW	11	C	183	0.677699729	3.530467355
ERCP 8.5+HW	11	D	192	0.618173112	3.250042275
ERCP 8.5+HW	13	A	137	0.586353328	2.88484719
ERCP 8.5+HW	13	B	175	0.622348589	3.214297263
ERCP 8.5+HW	13	C	164	0.628076312	3.2031053
ERCP 8.5+HW	13	D	148	0.577090168	2.883842069
ERCP 8.5+HW	15	A	143	0.571124613	2.834402717
ERCP 8.5+HW	15	C	150	0.585140187	2.931924075
ERCP 8.5+HW	15	D	195	0.585976943	3.089856161
ERCP 8.5+HW	18	A	165	0.569880552	2.909779024
ERCP 8.5+HW	18	B	141	0.438985932	2.172435972
ERCP 8.5+HW	18	C	163	0.495950226	2.526246562
ERCP 8.5+HW	18	D	179	0.506114923	2.625413366
ERCP 8.5+HW	20	A	170	0.538188126	2.764025734
ERCP 8.5+HW	20	B	166	0.471040609	2.407953843
ERCP 8.5+HW	20	C	145	0.472630464	2.35215598
ERCP 8.5+HW	20	D	176	0.525651508	2.717872707
ERCP 8.5+HW	22	A	144	0.447704805	2.225009295
ERCP 8.5+HW	22	C	132	0.480102844	2.344247089
ERCP 8.5+HW	25	A	176	0.529400632	2.737257494
ERCP 8.5+HW	25	B	146	0.471325592	2.348901341
ERCP 8.5+HW	25	C	169	0.526194123	2.699322553
ERCP 8.5+HW	25	D	149	0.433811179	2.170767848
ERCP 8.5+HW	27	A	128	0.38440738	1.865156239
ERCP 8.5+HW	27	C	143	0.562808289	2.793130093
ERCP 8.5+HW	27	D	146	0.451581709	2.250505595

Table S2: Diversity metrics of all treatments, replicates and sampling days for the heterotrophic protists.

Treatment	Day	Replicate	Richness	Evenness	Shannon Index
AMBIENT	1	A	1	0.454634137	2.340226707
AMBIENT	1	B	169	0.464464953	2.382658165
AMBIENT	1	C	206	0.566997	3.020889802
AMBIENT	1	D	214	0.603778284	3.23985979
AMBIENT	4	A	175	0.467965185	2.416940021
AMBIENT	4	B	191	0.431521561	2.266469228
AMBIENT	4	C	250	0.555214432	3.065594789
AMBIENT	6	B	81	0.447709857	1.967438204
AMBIENT	6	C	139	0.414546214	2.045567485
AMBIENT	6	D	160	0.40660114	2.06357146
AMBIENT	8	A	194	0.436593829	2.299914364
AMBIENT	8	B	176	0.407898727	2.109033841

Treatment	Day	Replicate	Richness	Evenness	Shannon Index
AMBIENT	8	C	180	0.444964953	2.310683803
AMBIENT	8	D	176	0.439098793	2.27035328
AMBIENT	11	A	223	0.570596433	3.085312924
AMBIENT	11	C	191	0.48605312	2.552883887
AMBIENT	11	D	197	0.622816784	3.290467955
AMBIENT	13	A	198	0.634462415	3.355206671
AMBIENT	13	B	224	0.680805143	3.684276463
AMBIENT	13	C	152	0.551860779	2.772482616
AMBIENT	13	D	173	0.677042875	3.488999359
AMBIENT	15	A	193	0.6672184	3.511363729
AMBIENT	15	B	207	0.716819742	3.822598107
AMBIENT	15	C	193	0.712399094	3.749135723
AMBIENT	15	D	156	0.661907747	3.342538812
AMBIENT	18	A	185	0.696757731	3.637323278
AMBIENT	18	B	198	0.700528317	3.704580803
AMBIENT	18	C	166	0.720637682	3.683891028
AMBIENT	18	D	191	0.732420586	3.846873182
AMBIENT	20	A	170	0.680118611	3.4929521
AMBIENT	20	B	192	0.715826474	3.763454375
AMBIENT	20	C	177	0.690185712	3.572504588
AMBIENT	20	D	208	0.729578442	3.894152718
AMBIENT	22	A	195	0.6920612	3.649238403
AMBIENT	22	B	194	0.701276055	3.694222789
AMBIENT	22	C	162	0.682242123	3.470972524
AMBIENT	22	D	166	0.636041907	3.25143846
AMBIENT	25	A	150	0.64080886	3.210859492
AMBIENT	25	B	159	0.66871038	3.389628855
AMBIENT	25	C	154	0.656860309	3.308574243
AMBIENT	27	A	156	0.657282759	3.319183288
AMBIENT	27	B	146	0.664245171	3.310336632
AMBIENT	27	C	142	0.593408196	2.940828394
AMBIENT	27	D	171	0.661746198	3.402476311
AMBIENT+HW	1	A	121	0.557700611	2.674615318
AMBIENT+HW	1	B	124	0.617124386	2.974713303
AMBIENT+HW	1	C	132	0.587485189	2.868573812
AMBIENT+HW	1	D	135	0.627672628	3.078906713
AMBIENT+HW	4	A	181	0.457453315	2.378069701
AMBIENT+HW	4	B	134	0.382283841	1.87236501
AMBIENT+HW	4	C	188	0.483176672	2.530126599
AMBIENT+HW	4	D	196	0.540706929	2.853913169
AMBIENT+HW	6	A	201	0.480502606	2.548251826
AMBIENT+HW	6	B	236	0.556129358	3.038597272
AMBIENT+HW	6	C	196	0.4271647	2.254624266
AMBIENT+HW	6	D	177	0.454841553	2.354327985
AMBIENT+HW	8	A	145	0.426598305	2.12306618
AMBIENT+HW	8	B	165	0.416220702	2.125200211
AMBIENT+HW	8	C	203	0.451535201	2.399099528
AMBIENT+HW	8	D	216	0.4747248	2.551777969

Treatment	Day	Replicate	Richness	Evenness	Shannon Index
AMBIENT+HW	11	A	171	0.399946396	2.05638981
AMBIENT+HW	11	B	205	0.509497299	2.712059205
AMBIENT+HW	11	C	193	0.452869254	2.383310578
AMBIENT+HW	11	D	198	0.496133996	2.623689052
AMBIENT+HW	13	A	196	0.644720937	3.40291103
AMBIENT+HW	13	B	209	0.6893791	3.682893578
AMBIENT+HW	13	C	214	0.664764748	3.567111693
AMBIENT+HW	13	D	229	0.693786637	3.769843713
AMBIENT+HW	15	A	157	0.684983209	3.463443479
AMBIENT+HW	15	B	174	0.672179318	3.467810274
AMBIENT+HW	15	C	157	0.657342465	3.32368508
AMBIENT+HW	18	A	197	0.707460935	3.737660251
AMBIENT+HW	18	B	186	0.723140752	3.778950377
AMBIENT+HW	18	C	148	0.658535319	3.290840779
AMBIENT+HW	18	D	165	0.674549018	3.444210508
AMBIENT+HW	20	A	161	0.688250435	3.497278765
AMBIENT+HW	20	B	174	0.723765542	3.733946455
AMBIENT+HW	20	C	180	0.674315987	3.501693825
AMBIENT+HW	20	D	178	0.649277099	3.36441339
AMBIENT+HW	22	A	174	0.646835919	3.337062276
AMBIENT+HW	22	B	200	0.742098407	3.931872876
AMBIENT+HW	22	C	174	0.67105686	3.462019448
AMBIENT+HW	22	D	149	0.616468767	3.084776608
AMBIENT+HW	25	A	174	0.656964252	3.389314907
AMBIENT+HW	25	B	160	0.646415779	3.280672435
AMBIENT+HW	25	C	144	0.645731601	3.2091655
AMBIENT+HW	25	D	137	0.551616357	2.713941955
AMBIENT+HW	27	A	150	0.676485133	3.389620286
AMBIENT+HW	27	B	144	0.629145384	3.126735095
ERCP 8.5	1	A	117	0.623678901	2.970067404
ERCP 8.5	1	B	118	0.533254821	2.543990576
ERCP 8.5	1	C	125	0.617615649	2.982042124
ERCP 8.5	1	D	136	0.603754118	2.966035618
ERCP 8.5	4	A	110	0.603102046	2.834869325
ERCP 8.5	4	B	115	0.574245634	2.724756556
ERCP 8.5	4	C	142	0.719262541	3.564540762
ERCP 8.5	4	D	185	0.477116283	2.490716768
ERCP 8.5	6	A	228	0.499416584	2.711505249
ERCP 8.5	6	B	221	0.576582906	3.112488336
ERCP 8.5	6	C	201	0.501716564	2.660755916
ERCP 8.5	6	D	175	0.509992942	2.634004395
ERCP 8.5	8	A	216	0.537844498	2.891063916
ERCP 8.5	8	B	213	0.568313797	3.046896306
ERCP 8.5	8	C	206	0.577652982	3.077663556
ERCP 8.5	8	D	163	0.606096208	3.08730268
ERCP 8.5	11	A	190	0.611687769	3.209540446
ERCP 8.5	11	B	192	0.585145891	3.076401813
ERCP 8.5	11	C	216	0.596621095	3.207004489

Treatment	Day	Replicate	Richness	Evenness	Shannon Index
ERCP 8.5	13	A	200	0.591003093	3.131321953
ERCP 8.5	13	B	195	0.622064713	3.280146955
ERCP 8.5	13	C	193	0.617426481	3.249324286
ERCP 8.5	13	D	165	0.603156765	3.079685553
ERCP 8.5	15	A	187	0.672250899	3.516617472
ERCP 8.5	15	B	156	0.660479202	3.335324868
ERCP 8.5	15	C	196	0.703336039	3.71228826
ERCP 8.5	18	A	156	0.701356964	3.541751677
ERCP 8.5	18	B	176	0.675092135	3.49055308
ERCP 8.5	18	C	177	0.697219822	3.608914197
ERCP 8.5	18	D	162	0.682169441	3.47060275
ERCP 8.5	20	A	160	0.689979222	3.501764482
ERCP 8.5	20	B	184	0.682829542	3.560912194
ERCP 8.5	20	D	165	0.67762566	3.459919673
ERCP 8.5	22	A	176	0.687752153	3.5560115
ERCP 8.5	22	C	123	0.707561173	3.404914808
ERCP 8.5	25	A	173	0.716295757	3.691280904
ERCP 8.5	25	D	139	0.658155072	3.247649047
ERCP 8.5	27	A	150	0.66169173	3.315495935
ERCP 8.5	27	B	156	0.655092922	3.308124925
ERCP 8.5	27	C	128	0.62739174	3.044123709
ERCP 8.5	27	D	134	0.627905154	3.075378856
ERCP 8.5+HW	1	A	126	0.605701709	2.929344216
ERCP 8.5+HW	1	B	133	0.649862162	3.178052857
ERCP 8.5+HW	1	C	135	0.666373872	3.268746947
ERCP 8.5+HW	1	D	132	0.630554118	3.078870859
ERCP 8.5+HW	4	A	140	0.702631004	3.472151178
ERCP 8.5+HW	4	D	124	0.637895712	3.074836943
ERCP 8.5+HW	6	A	126	0.663247292	3.207650876
ERCP 8.5+HW	6	B	122	0.665229032	3.195774269
ERCP 8.5+HW	6	C	107	0.658881421	3.078840102
ERCP 8.5+HW	6	D	138	0.735809337	3.625519268
ERCP 8.5+HW	8	A	113	0.563276943	2.662828559
ERCP 8.5+HW	8	B	125	0.707802402	3.417492061
ERCP 8.5+HW	8	C	118	0.632362255	3.016800885
ERCP 8.5+HW	8	D	139	0.705032074	3.478962391
ERCP 8.5+HW	11	A	218	0.483812156	2.605084164
ERCP 8.5+HW	11	B	193	0.445770587	2.345952497
ERCP 8.5+HW	11	C	204	0.552943681	2.940620846
ERCP 8.5+HW	11	D	216	0.520885392	2.799903999
ERCP 8.5+HW	13	A	201	0.558862369	2.963817543
ERCP 8.5+HW	13	B	248	0.596789458	3.290356156
ERCP 8.5+HW	13	C	234	0.641020751	3.496974041
ERCP 8.5+HW	13	D	181	0.505179618	2.626174746
ERCP 8.5+HW	15	A	177	0.593820791	3.073705329
ERCP 8.5+HW	15	C	199	0.574242591	3.039641079
ERCP 8.5+HW	15	D	254	0.704870426	3.903103166
ERCP 8.5+HW	18	A	211	0.562409808	3.009937507

Treatment	Day	Replicate	Richness	Evenness	Shannon Index
ERCP 8.5+HW	18	B	189	0.59264919	3.106517125
ERCP 8.5+HW	18	C	217	0.643389161	3.461367645
ERCP 8.5+HW	18	D	206	0.651933429	3.473420579
ERCP 8.5+HW	20	A	198	0.621294493	3.285571186
ERCP 8.5+HW	20	B	189	0.699497358	3.666588188
ERCP 8.5+HW	20	C	197	0.718522748	3.796102059
ERCP 8.5+HW	20	D	189	0.708692523	3.714786919
ERCP 8.5+HW	22	A	174	0.670170284	3.457445555
ERCP 8.5+HW	22	C	174	0.672932414	3.471695536
ERCP 8.5+HW	25	A	178	0.70109226	3.63290834
ERCP 8.5+HW	25	B	176	0.670602444	3.467339204
ERCP 8.5+HW	25	C	210	0.760665683	4.0673612
ERCP 8.5+HW	25	D	170	0.657410754	3.376329123
ERCP 8.5+HW	27	A	148	0.65458848	3.271117588
ERCP 8.5+HW	27	C	182	0.639770403	3.329369454
ERCP 8.5+HW	27	D	192	0.691159018	3.633765341

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Plankton communities today and tomorrow – impacts of global change and marine heatwaves in a multiple-driver mesocosm experiment

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Plankton communities today and tomorrow – impacts of global change and marine heatwaves in a multiple-driver mesocosm experiment

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Abstract

Human activities cause simultaneous changes in marine environmental conditions. Future scenarios indicate that temperatures will continue to increase and pH to decrease in the oceans' surface. Moreover, the nitrogen-to-phosphorus ratio has steadily increased in European coastal waters over the past decades, and coastal systems are still receiving N in excess and are becoming increasingly P-limited. Hence, marine biota is exposed to the concurrent effects of multiple anthropogenic drivers, which put marine systems under pressure, potentially affecting community structure and functioning. In the context of global change, marine organisms are subjected not only to gradual average changes in abiotic parameters, but also to an increasing number of heatwaves. However, we still know little about the influence of heatwaves on the structure of marine communities, and experimental studies are needed to test the effect of heatwaves alone and in combination with other environmental drivers. Here, we conducted a mesocosm experiment to assess the potential impact of heatwaves under ambient and future environmental conditions on natural coastal plankton communities. Throughout the experiment, we assessed the abundance and taxonomic composition of multiple trophic levels, including bacterioplankton, phytoplankton, microzooplankton, and mesozooplankton. While we did not observe any effect on phytoplankton total biomass, we identified that future environmental conditions may favour smaller phytoplankton species, and that additional heatwaves may favour small phytoflagellates and coccolithophores. We also observed that future environmental conditions may reduce the abundances and modify the species composition of bacterioplankton, microzooplankton, and mesozooplankton, and that heatwaves exacerbate these effects. To the best of our knowledge, our study is the first to examine the impacts of heatwaves under current and future environmental conditions on a natural multi-trophic marine plankton community. Using this unique approach, we show that the combination of multiple global change drivers have the potential to perturb the entire basis of marine food-webs.

Introduction

Human activities and associated increasing greenhouse gas emissions are causing simultaneous changes in a range of marine abiotic parameters. The Intergovernmental Panel on Climate Change (IPCC) developed a suite of different scenarios projecting that, by 2100, temperatures will increase by 0.6–4.0 °C and pH will decrease by 0.1–0.4 units in the oceans' upper hundred meters (Pörtner et al., 2022), depending on humanity's ability to curb greenhouse gas emissions. However, global warming is not uniform, and long-term data series analyses have shown that marine coastal areas are warming at a faster rate than the global average (de Amorim et al., 2023). This observation is particularly important as coastal systems are among the most productive areas in the world, but also among the most sensitive ones to environmental change (Halpern et al., 2008). Numerous studies have shown that warming and acidification may have profound implications for coastal marine ecosystems (Duarte, 2014, Harley et al., 2006, Wernberg et al., 2012), but changes in dissolved nutrient concentrations also may alter the performance and survival of many organisms (Alvarez-Fernandez et al., 2018, Breitburg et al., 2015, Doney, 2010, Meunier et al., 2018). The alteration of coastal biogeochemical cycles is largely driven by nutrient runoffs originating from urban, agricultural, and industrial activities. For instance, the nitrogen-to-phosphorus (N:P) ratio has steadily increased in European coastal waters over the past decades, and coastal systems are becoming increasingly P-limited while receiving N in excess (Balkoni et al., 2023, Peñuelas et al., 2013, Peñuelas et al., 2012, Van Beusekom et al., 2019). Marine organisms are consequently exposed to the concurrent effects of multiple anthropogenic drivers, which put marine systems under pressure, potentially affecting community structure and functioning, and altering the associated ecosystem services (Horn et al., 2021).

In the context of global change, marine organisms are subjected not only to gradual average changes in abiotic parameters, but also to an increasing number of extreme weather events (Pörtner et al., 2022). The most recent IPCC report outlines a rise in the number and intensity of marine heatwaves across the global ocean (Lee et al., 2023). Heatwaves have led to mass mortalities of marine organisms, to reductions in biodiversity in several coastal systems around the world, and have been suggested to increase infections by pathogens such as *Vibrio sp.* (Arias-Ortiz et al., 2018, Brehm et al., 2021, Le Nohaïc et al., 2017, Sanford et al., 2019). Nonetheless, most evidence of marine heatwave impacts concentrates on few long and severe events (McKinstry et al., 2022, Shanks et al., 2020, Ziegler et al., 2023), and most studies focus on the coast of Australia and North America (Joyce et al., 2024). Heatwaves are not uniform on a regional to local scale, and it is important to consider that the seasonality of heatwaves is

an essential aspect determining their impact. This may be especially true in temperate systems which have a high variability in weather conditions, and for short-lived organisms with a limited seasonal window of occurrence, such as plankton. However, we still know little on the influence of heatwaves on the structure of marine communities, and experimental studies are needed to test the effect of abrupt temperature increases alone and in combination with other environmental drivers. Altogether, the combination of short- and long-term changes in physico-chemical conditions exert pressure on coastal marine organisms such as plankton.

Studies have shown that the timing of phytoplankton blooms and of zooplankton development shift in response to temperature changes in temperate regions, which can create a mismatch between food availability and nutritional demands of higher trophic levels (Boersma et al., 2015, Edwards & Richardson, 2004, Hjerne et al., 2019, Sommer & Lewandowska, 2011). Further, altered environmental conditions may increase dissolved organic carbon (DOC) exudation by phytoplankton, which may benefit bacterioplankton and channel more carbon into the microbial loop (Engel et al., 2011, Guo et al., 2022, Moreno et al., 2023). Not only bacterioplankton overall, but also specific groups like the pathogenic genus *Vibrio sp.* may benefit from altered environmental conditions (Brehm et al., 2021, Diner et al., 2021). In addition, elevated seawater temperatures may lead to harmful algal blooms (Coyne et al., 2021, Gu et al., 2022) that also impact the marine food web. Changes in interactions between trophic levels are not constrained to the basis of the food web, and warming, for instance, is known to increase zooplankton nutritional demands and, consequently, grazing pressure on prey communities (Caron & Hutchins, 2013, Castellani et al., 2005, Garrido et al., 2013). While studies testing the influence of single drivers are undeniably important for our understanding of mechanisms driving plankton dynamics, they offer limited realism and large-scale ecological relevance. Indeed, global change is characterized by simultaneous alterations in multiple environmental drivers which interact and affect the physiology and ecology of organisms with potential consequences for entire food webs (Giménez et al., 2021, Todgham & Stillman, 2013). The very few studies addressing the combined effects of different global change drivers on community scales observed high synergy between drivers with, for example, adverse effects on copepod abundance, or shifts in phytoplankton organismal size (Garzke et al., 2015, Moreno et al., 2022, Rose et al., 2009, Sommer et al., 2015). Furthermore, thus far, only a handful of studies assessed the impacts of heatwaves in the context of global change by considering the combined effects of both, long-term average environmental change and of extreme events. The impact of heatwaves on planktonic organisms may be exacerbated under future environmental conditions, if warming, increasing pCO_2, or changes in nutrient availability push planktonic

organisms towards the edge of their tolerance windows. The few studies testing the combined effects of heatwaves and other global change drivers found high synergy between those, leading to changes in community structure and biodiversity loss (Filiz et al., 2020, Remy et al., 2017). Given that global change impacts on plankton biodiversity (Bellard et al., 2012) and community composition and biomass (Greve et al., 2004, Telesh et al., 1999) may alter energy transfer to higher trophic levels and nutrient recycling (Duarte et al., 2009, Elser et al., 2000), there is an urgent need for studies addressing the combined effects of short- and long-term environmental changes on planktonic food webs.

Here, we conducted a mesocosm experiment and applied an integrated multiple driver design to assess the potential impact of heatwaves under ambient and future environmental conditions on natural coastal plankton communities. To represent future environmental conditions, temperature and pH were manipulated based on the Representative Concentration Pathway 8.5 proposed by the IPCC for 2100, and dissolved N:P ratios were increased to simulate the conditions expected in European coastal zones (Grizzetti et al., 2012). Throughout the experiment, we assessed the influence of the different scenarios on the abundance and taxonomic composition of multiple trophic levels, including bacterioplankton, phytoplankton, microzooplankton, and mesozooplankton. While various approaches can be employed to investigate community responses to multiple global change drivers, mesocosm experiments provide the highest level of ecological relevance while still being conducive to experimental manipulations and rigorous replication (Boyd et al., 2018, Stewart et al., 2013). Hence, by incorporating natural assemblages and by manipulating environmental conditions according to realistic scenarios, our mesocosm experiment goes beyond tightly controlled microcosm experiments which suffer from limited realism, and provides unique insights on the influence of marine heatwaves today and tomorrow on the structure of coastal planktonic food webs.

Material and methods

Experimental design

To assess the potential impact of heatwaves under ambient and future environmental conditions on natural coastal plankton communities, we carried out an integrated multiple-driver experiment. Therein, we investigated the response of planktonic communities to four scenarios: An "Ambient" scenario displaying the climatic conditions of today (ambient temperature, pH, pCO_2) and a scenario based on the RCP 8.5 scenario developed by the IPCC for the year 2100 (+3.0 °C, -0.3 pH, pCO_2 = 1000 ppm; IPCC, 2021). As dissolved nutrient concentrations are expected to change towards considerably higher nitrogen to phosphorus ratios (N:P) in coastal

seas (Grizzetti et al., 2012), we extended the RCP scenario (ERCP) to simulate changing nutrient concentrations, with an N:P ratio (molar) of 25, whereas the N:P ratio was adjusted to 16 (Redfield ratio) for the "Ambient" scenario. Each of these two scenarios was either subjected to a heatwave ("Ambient HW", "ERCP 8.5 HW") or not ("Ambient", "ERCP 8.5"). These four scenarios were carried out in four replicates each.

Mesocosm system

The experiment was conducted on the island of Sylt, Germany, at the mesocosm facility of the Wadden Sea Station, Alfred-Wegener Institute for Polar and Marine Research. The general design of the experiment followed that described by Moreno et al. (2022). We used 16 mesocosms which are black double-hulled, insulated, cylindrical tanks, made of UV stabilised high-density polyethylene (HDPE; Spranger Kunststoffe, Plauen, Germany). These tanks are 85 cm high, 170 cm wide and comprise a net volume of 1800 L (Pansch et al., 2016). The tanks were closed with a translucent lid made of HDPE, allowing 90 % of photosynthetically active radiation to pass through. Inside each tank, we placed a transparent bag, made of low-density polyethylene (LDPE) used in the food industry for packaging (POLY-VERPACKUNGEN GmbH, Trappenkamp, Germany), comprising a net volume of 520 L. The tanks were filled with water which surrounded the LDPE bags and served as water bath. To prevent sedimentation of planktonic organisms, the water was gently homogenised with a custom-made propeller controlled by a mortar mixer engine (TC-MX 1400-2 E, Einhell Germany AG, Landau/Isar, Germany) placed on top of each mesocosm tank. This system gently stirred the water column inside the LDPE bags at 50 rpm in a 1-minute-mixing/30-minutes-pause interval, simulating the well-mixed conditions of the sampling location.

Seawater collection

Seawater containing a natural plankton community was collected from the coastal North Sea on the 1st of September 2021 at Sylt Roads Station 1 (55°1'48"N, 8°27'36"E) on board the research vessel Mya II (AWI Sylt, Germany). A 500 L tub hooked to a crane was submerged to collect seawater from the upper three meters. The water was then transferred into 1000 L Intermediate Bulk Containers (IBC, AUER Packaging GmbH, Amerang, Germany) via gravity through a hose to which a 1000 µm mesh was attached to exclude larger organisms. This procedure prevented any disproportionally large impact which larger consumers can have on the rest of the plankton community in a 520 L enclosed water volume. At the mesocosm facility,

we gently homogenized the seawater inside the IBC-tanks with a paddle before transferring it into the LDPE bags. For the filling process, the IBC tanks were lifted by a wheeled loader to allow the water to flow into the mesocosm bags via gravity. Hence, no pumps were used at any moment, which prevented damage to fragile organisms within the planktonic community. A four-way-distributor with four hoses of 13 mm diameter (Gardena Deutschland GmbH, Ulm Germany) was attached to the IBC tank, and a flowmeter was connected to the end of each hose to measure the exact water volume transferred, thereby ensuring an equal distribution of the seawater from each IBC tank. We filled 60 L of seawater simultaneously to four bags, and then filled the next mesocosm quadruplet. This enabled an equal distribution of the water contained in each IBC tank among the sixteen mesocosms. This procedure was repeated until all mesocosm bags were filled with 520 L of seawater.

Setup

Once the filling procedure was completed, we directly measured dissolved inorganic phosphorus (DIP) and dissolved inorganic nitrogen (DIN) concentrations according to the method described in Grasshoff et al. (1999), and we subsequently adjusted the dissolved N:P ratios to 16 (Ambient and Ambient HW) and 25 (ERCP 8.5 and ERCP 8.5 HW). To achieve these ratios while adding the least amount of nutrients possible, we did not add DIP in the ERCP scenarios as the start concentration was 0.33 µmol L^{-1}, and we adjusted the DIP concentration to reach 0.50 µmol L^{-1} in the Ambient scenario. DIN was added to reach 8.24 µmol L^{-1} in all scenarios (Supplementary Tab. 2). Furthermore, we added 1.8 L of CO_2 saturated seawater to the ERCP mesocosm bags to reduce the initial pH values by 0.3 units. During the rest of the experiment, the pH was influenced by the planktonic communities through photosynthesis and respiration, and by the atmospheric pCO_2 above the bags which was adjusted by bubbling the water surrounding the LDPE bags. The Ambient and Ambient HW scenarios were bubbled with pressured air, and the ERCP 8.5 and ERCP 8.5 HW scenarios with 1000 µatm pCO_2 adjusted by a CO_2-mixing facility (GMZ 750, HTK, Hamburg, Germany).

Temperature was regulated by a computerised system (4H-Jena engineering, Jena, Germany), controlling cooling units (Titan 2000 or Titan 4000 Aqua Medic, Bissendorf, Germany) and heating units (Titanium heater 500 W, Aqua Medic, Bissendorf, Germany) placed in the water surrounding the LDPE bags. Thus, temperature inside the bags was regulated indirectly through the surrounding water. On the first day of the experiment, seawater temperature of the Ambient and Ambient HW scenarios was set to the temperature measured

at Sylt Roads station when the seawater was collected, and was progressively increased by 3.0 °C for the ERCP scenarios. The temperature of each experimental day was calculated based on data provided by the ecological long-time series of Sylt Roads (Rick et al., 2023). The average daily temperature during the years of 1986 – 2016 was calculated at the exact same time span as that of the experiment (September 3rd to 30th), and we adjusted the temperature daily during the experiment accordingly (Fig. 1).

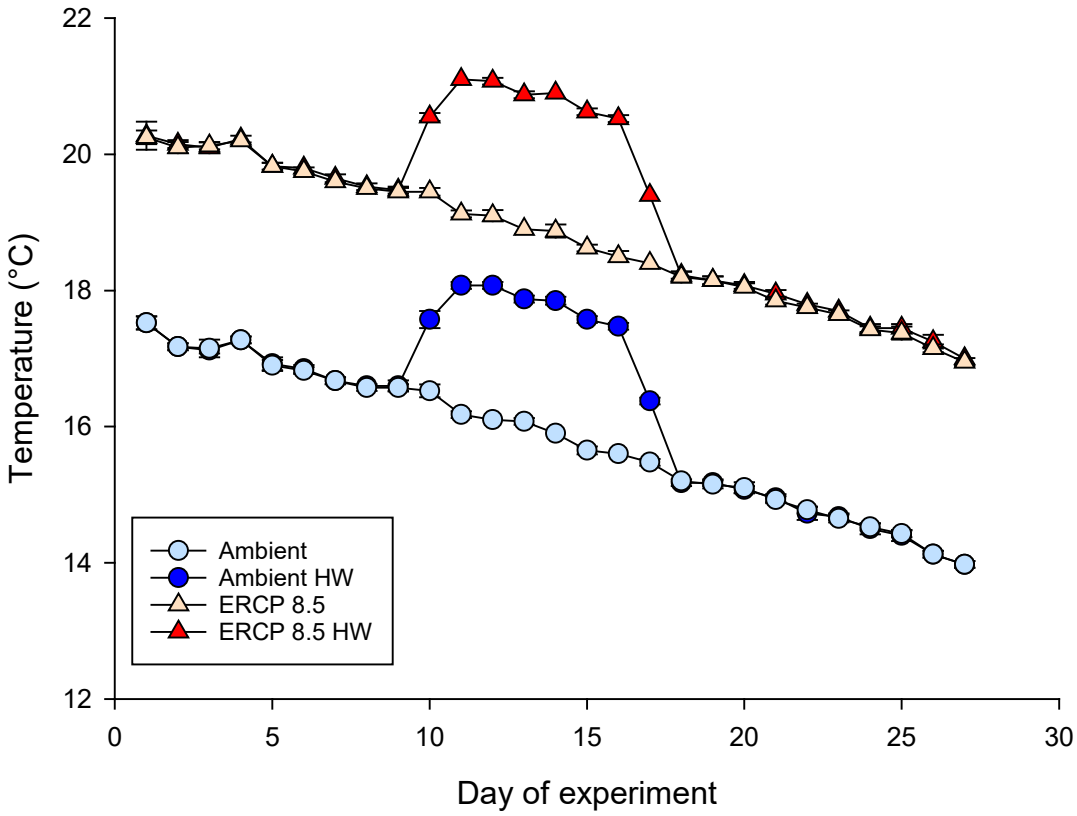

Figure 1: Temperature in the mesocosms throughout the experiment. Different colours and symbols represent the Ambient scenario (circle) and Extended Representative Concentration Pathway (ERCP) scenario (triangle) with and without heatwave (light blue = Ambient, dark blue = Ambient HW, orange = ERCP 8.5, red = ERCP 8.5 HW). Symbols represent means and standard errors of four replicates per scenario.

The intensity and duration of the heatwave were determined based on the definition of marine heatwaves by Hobday et al. (2016), and the calculation of the average marine heatwave intensity in the North Sea. A marine heatwave is defined as at least five consecutive days exceeding the 90th percentile of the climatological average daily temperature (Hobday et al., 2016). Because we did not have sufficient in-situ data to compute heatwave statistics, we retrieved additional data from the online marine heatwave tracker tool (Schlegel, 2020) for the waters surrounding Sylt. This online tool uses NOAA satellite data (Banzon et al., 2016, Banzon et al., 2020, Reynolds et al., 2007) to compute heatwave statistics based on Hobday et al. (2016, 2018)

applying algorithms as described in Schlegel and Smit (2018). We retrieved data on heatwave duration and intensity and identified that the mean intensity of a 5 days heatwave is 2 °C above the climatological mean. This value is similar to the mean intensity for a similar heatwave calculated for the open North Sea at Helgoland Roads (= 1.7 °C), where we have *in situ* data (Giménez et al., 2024), and a 2°C heatwave over a period of 5 days corresponds to a realistic heatwave for the study area. Hence, on day 10 of the experiment, four tanks of each the Ambient and the ERCP 8.5 scenario were subjected to a five-day heatwave in which the water temperature was increased by 2 °C. To minimize the mortality risk from heat-shock and to adjust the temperature realistically, the water temperature was increased gradually by 1 °C on day 9, and by another 1 °C on day 10 of the experiment. Similarly, temperature was decreased gradually by 1 °C on day 16, and by another 1 °C on day 17 to end the heatwave (Fig. 1).

Physical-chemical conditions

Abiotic parameters, namely temperature and pH were measured daily at 9:00 o'clock directly inside the mesocosm bags, with a thermometer Testo-110 (Lenzkirch, Germany) and a WTW pH 340i equipped with a SenTix 81 pH electrode (Letchworth, England), respectively. For total alkalinity (TA), 100 mL of mesocosm water were filtered over 0.2 µm nylon syringe filters (Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany) and filled into 100 mL Schott bottles (Schott AG, Mainz, Germany) with a gastight lid and stored at 4 °C until they were analysed within 36 hours through Gran-titration (Dickson, 1981) using a TitroLine alpha plus (Schott Instruments GmbH, Mainz, Germany). For dissolved nutrients (DIN, DIP, dissolved silica DSi), 10 mL of mesocosm water were filtered over 0.45 µM PTFE syringe filters (Sartorius Lab Instruments GmbH & Co. KG, Göttingen, Germany) into polystyrol tubes (neolab Migge GmbH, Heidelberg, Germany), using the first 2 mL to rinse the filter and the syringe before being directly discarded. The dissolved nutrient samples were stored at 4°C (DSi) and at -20°C (DIN, DIP), until they were analysed spectrophotometrically. Samples for particulate carbon, nitrogen, and phosphorus (C, N, P) were obtained by filtering 200 mL of mesocosm water through acid-washed, precombusted glass microfiber filters (Whatman plc, Kent, United Kingdom). For particulate carbon and nitrogen analysis, the filters were placed into a 6-well plate and stored in a drying cabinet at 60 °C. For particulate phosphate analysis, the filters were placed into 2 mL Eppendorf tubes, and stored at -20 °C. Particulate CN content was later on analysed with an Elementar CN-Analyser (Elementar GmbH, Langenselbold, Germany), while particulate P content was determined according to the method described in Grasshoff et al. (1999).

Planktonic community

To quantify the bacterioplankton abundance, 10 mL of sampled mesocosm water were fixed with 0.2-µm-filtered formaldehyde (1% final concentration, 1 hour at room temperature). Using a standard bottle top set-up (polysulfon), fixed cells were subsequently filtered (≤ 200 mbar) onto 0.2-µm polycarbonate filters (47 mm diameter; Sigma Aldrich, Taufkirchen, Germany), which were placed on 0.45-µm cellulose nitrate support filters (Sigma Aldrich). Total cell counts (TCC) were determined from a cut and 4′,6-diamidino-2-phenylindole (DAPI)-stained filter section and analyzed automatically as described previously (Brüwer et al., 2023). Images were automatically recorded using a Zeiss AxioImager.Z2m microscope with a cooled charged-coupled device (CCD) camera (Zeiss AxioCam MRm, Zeiss Oberkochen, Germany) and the Zeiss AxioVision software with a custom-built macro. Recorded images were analysed in the ACME tool (Bennke et al., 2016). Since this group may benefit from environmental change and severely impact other organisms as well as human health, we quantified the bacterial abundance of the genus *Vibrio* via qPCR. Samples were extracted using DNeasy®PowerWater®Kit. For DNA quantification prior to qPCR, a fluorometric quantification method was applied using the Quant-iTTMPicoGreen®dsDNAassayKit in black bottom 96-well plates with TECAN® infinit200 microplate reader. In order to quantify the amount of DNA in a single *V. alginolyticus* cell, the isolate DSM2171 was utilized as a control value. For the qPCR approach, the LightCycler® 480 SYBR Green I Master kit (Roche) was applied using the oligonucleotide primers Vib-567F and Vib2-r (Supplementary Tab. 1) targeting the 16S rRNA gene covering the whole *Vibrio* genus (Thompson et al., 2004b). The PCR conditions were chosen according to LightCycler® 480 SYBR Green I Master and the specific primer conditions (Thompson et al., 2004b). The concentration, i.e. the number of template molecules in the original sample, was calculated using a standard curve (Bustin et al., 2009, Fraga et al., 2014). *V. alginolyticus* (DSM 2171) was used as an external standard to perform an absolute quantification. Seven standard concentrations in a 10-fold serial dilution were analyzed in duplicate to calculate a standard curve (Supplementary Tab. 2). Further taxonomic analyses of the bacterioplankton community were also conducted but go beyond the scope of the current paper, and will be published at a later stage.

To determine phytoplankton and microzooplankton abundance and species composition, 100 mL of mesocosm water were poured into brown-glass bottles and fixed with 2 mL of Lugol's acid iodine solution. These samples were stored cool and dark and were analysed following the method described in Utermöhl (1958) using an inverted microscope Olympus

CKX41 (Olympus Scientific Solutions, Tokyo, Japan). Planktonic organisms were identified to species level when possible, or pooled into size-shape dependent groups. Biovolume of each phytoplankton and microzooplankton species was calculated from the measurement of cell dimensions using geometric formulae according to (Hillebrand et al., 1999). Cell volume was converted into carbon following the equations of Menden-Deuer and Lessard (2000) for diatoms (pg C $cell^{-1}$ = 0.288 x $V^{0.811}$), dinoflagellates (pg C $cell^{-1}$ = 0.760 x $V^{0.819}$) and other protist plankton with the exception of ciliates (pg C $cell^{-1}$ = 0.216 x $V^{0.939}$), where V is the cell volume in μm^3. Ciliate carbon content was calculated as 0.19 pg C μm^{-3} according to Putt and Stoecker (1989). Since toxin-producing planktonic species which may cause harmful algal blooms may benefit from environmental changes (Coyne et al., 2021, Gu et al., 2022), we also collected samples at the beginning and at the end of the experiment to conduct quantitative and qualitative toxin analyses (see supplementary material).

Samples for mesozooplankton were obtained by sieving 4 L of mesocosm water over a 150 µM mesh. The material captured by the mesh was flushed back into a 200 mL Kautex container (Kautex Textron GmbH & Co. KG, Bonn, Germany) with sterile filtered seawater (0.2 µM) and fixed with 20 mL 37% borax-buffered formol. The mesozooplankton community was determined by counting the whole sample or splitting it up into sub-samples with a Folsom-Splitter, from which at least three were counted (McEwen et al., 1954, Sell & Evans, 1982). The counting took place in a Bogorov chamber under a stereomicroscope (Leica M205; Leica Microsystems GmbH, Wetzlar, Germany) and identification was conducted up to the highest taxonomic level possible, as in Boersma et al. (2015).

Functional groups of the plankton were determined as bacterioplankton, phytoplankton, microzooplankton, and mesozooplankton. The phytoplankton group included diatoms, phytoflagellates, and autotrophic dinoflagellates, according to the descriptions of trophic mode for each species (Kraberg et al., 2010), which were grouped by size in nanophytoplankton (<20µm) and microphytoplankton (>20µm). The microzooplankton group comprised heterotrophic and mixotrophic dinoflagellates and ciliates, including nanociliates (< 20 µm). Mesozooplankton species were all the heterotrophic organisms larger than 200 µm.

Statistical analyses

For all statistical analyses, we used R 4.1.2 with the interface RStudio and the packages "vegan", "dplyr" and "pairwise.adonis2" (Martinez Arbizu, 2020, Oksanen et al., 2007, R Core Team, 2021, Wickham et al., 2018). All statistical tests were conducted at a significance threshold of $\alpha = 0.05$. Until the heatwaves were initiated on day 9, the eight Ambient

mesocosms and the eight ERCP 8.5 mesocosms were replicates. For a clearer depiction of our results, we averaged the data of the eight Ambient and the eight ERCP 8.5 mesocosms for the first 9 days in figures 2-8. For all statistical analyses, we considered four individual replicates per scenario during the entire experiment, each representing one tank of the mesocosm system. To assess the impacts of the different scenarios on planktonic abundances (bacterioplankton, phytoplankton, microzooplankton, mesozooplankton), we fitted general linear models (GLMs). Therefore, a first model of total cumulative abundances depending on scenarios was fitted, allowing us to check for a general scenario effect on planktonic abundances, followed by a second model including scenario and time. By comparing these two models with a likelihood ratio test (LRT), we tested if abundances changed differently in the four scenarios over time. Effects of the ERCP scenarios on the phyto- and microzooplankton species composition and affinity of species to the scenarios were analysed through the principal response curve (PRC) using the 'vegan' R package. This test shows the degree of difference of the community composition over time in the ERCP scenarios in comparison to the Ambient condition, which is set as a control (effect '0'). Species weights are analysed as means of their regression coefficient against the control. When the curve of difference of the ERCP scenario has a positive slope, positive values for species weights represent the affinity of this species to the scenario, whereas negative values would represent the negative effect of the scenario on such species, and vice versa. Differences in mesozooplankton abundance were analysed through Analysis of Variance (ANOVA) followed by a post hoc test (Tukey test). If data was not normally distributed, it was either log-, square-root, or exponentially transformed, depending on skewness.

Results

Physical-chemical conditions

At the onset of the experiment, the seawater had a pH of 8.00. The initial addition of CO_2-saturated water to the ERCP 8.5 and ERCP 8.5 HW mesocosms, and the subsequent control of atmospheric pCO_2 in these scenarios, lowered the pH by 0.10 to 0.15 compared to the Ambient scenarios (Supplementary Tab. 3). While this difference was maintained throughout the entire experiment, the absolute pH values increased from day 1 to day 6, and subsequently decreased until reaching initial values on day 20, where they remained stable until the end of the experiment. Following their initial adjustment, concentrations of dissolved N, P, and Si rapidly decreased until being depleted on day 4-6, and these concentrations remained close to zero until

the end of the experiment (Supplementary Tab. 4). Seston stoichiometry was not significantly different between scenarios (Supplementary Fig. 1). Seston C:N ratios fluctuated between ca. 7 and 11 during the experiment, albeit without any clear temporal trend. Seston C:P and N:P ratios had relatively low initial values, around 70 and 8, respectively, those increased over the first few days of the expeirment, and varied around 100 and 11 throughout the rest of experiment.

Bacterioplankton

We observed a statistically significant effect of the scenarios on bacterioplankton (TCC, Fig. 2) abundances (GLM, Ambient HW $p<0.05$, ERCP 8.5 $p<0.05$, ERCP 8.5 HW $p<0.05$), which fluctuated over time (GLM, $p<0.05$), with an interactive effect of scenario and time (GLM and Likelihood Ratio Test, $p<0.05$). Bacterioplankton abundances rapidly declined at the onset of the experiment, and started increasing again from day 3 to 6 (Fig. 2), reaching substantially higher levels in the Ambient (ca. 5.2 10^6 cells mL^{-1}) than in the ERCP 8.5 scenario (ca. 4 10^6 cells mL^{-1}). From day 6 to 14, bacterioplankton abundances declined in the Ambient scenario before increasing again to form a second bloom, which peaked at 5.5 10^6 cells mL^{-1} on day 18. This second bloom occurred faster in the Ambient HW scenario with a peak on day 16, and, while it collapsed to reach initial abundances of about 2 10^6 cells mL^{-1} in the Ambient scenario, bacterioplankton abundances stabilised around 4 10^6 cells mL^{-1} on day 22 in the Ambient HW scenario. During the heatwave, bacterial abundances increased faster in the ERCP 8.5 HW than in the ERCP 8.5 scenario, but also decreased faster after the heatwave. This bloom was weaker in the ERCP 8.5 and ERCP 8.5 HW scenarios than in the Ambient and Ambient HW scenarios. Bacterial abundances in the ERCP 8.5 and ERCP 8.5 HW stabilized at the same level as in the Ambient HW scenario from day 22. We also observed that the abundances of *Vibrio sp.* fluctuated over time, and that cell concentrations were significantly higher in the ERCP 8.5 and ERCP 8.5 HW scenarios with a bloom between days 8 and 15, than in the other two scenarios (Fig. 2B).

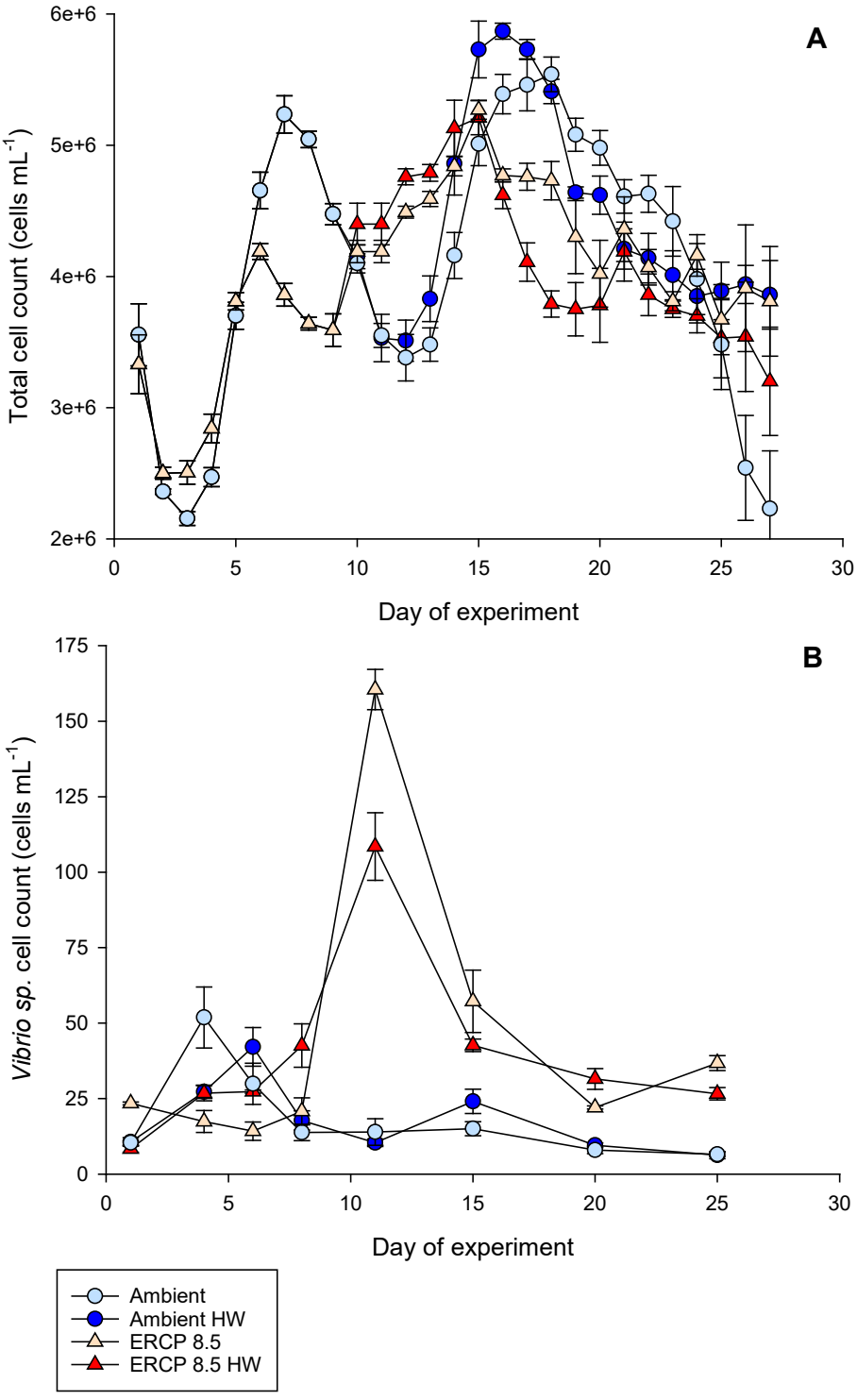

Figure 2: Bacterioplankton abundances in the mesocosms throughout the experiment. Total Cell Counts (TCC) based on DAPI counts (A), and Vibrio sp. cell counts based on qPCR analysis (B). Different colours and symbols represent the Ambient scenario (circle) and Extended Representative Concentration Pathway (ERCP) scenario (triangle) with and without heatwave (light blue = Ambient, dark blue = Ambient HW, orange = ERCP 8.5, red = ERCP 8.5 HW). Symbols represent means and standard errors of four replicates per scenario.

Phytoplankton

In all scenarios, total phytoplankton carbon biomass rapidly increased from 100 µg C L^{-1} at the beginning of the experiment, to reach a maximum of about 350 µg C L^{-1} on day 6, followed by an overall decrease to almost initial concentrations on day 15 where it stayed relatively stable until the end of the experiment (Fig. 3A). Nanophytoplankton and microphytoplankton carbon biomasses followed the same pattern as total phytoplankton carbon biomass throughout the experiment, but from day 10 on, nanophytoplankton carbon biomass remained relatively stable at concentrations twice as high as the initial concentrations, around 100 µg C L^{-1} (Fig. 3B), whereas microphytoplankton carbon biomass continued decreasing until being negligible from day 15 on (Fig. 3C). Overall, we did not observe any statistically significant effect of the scenarios on the total phytoplankton carbon biomass (GLM, Ambient HW $p=0.40$, ERCP 8.5 $p=0.16$, ERCP 8.5 HW $p=0.47$), nanophytoplankton carbon biomass (GLM, Ambient HW $p=0.52$, ERCP 8.5 $p=0.24$, ERCP 8.5 HW $p=0.35$), and microphytoplankton carbon biomass (GLM, Ambient HW $p=0.62$, ERCP 8.5 $p=0.57$, ERCP 8.5 HW $p=0.48$). While total phytoplankton, nanophytoplankton, and microphytoplankton biomasses were affected by time (GLM, $p < 0.001$), there was no interactive effect of scenario over time (GLM and Likelihood Ratio Test, $p=0.553$).

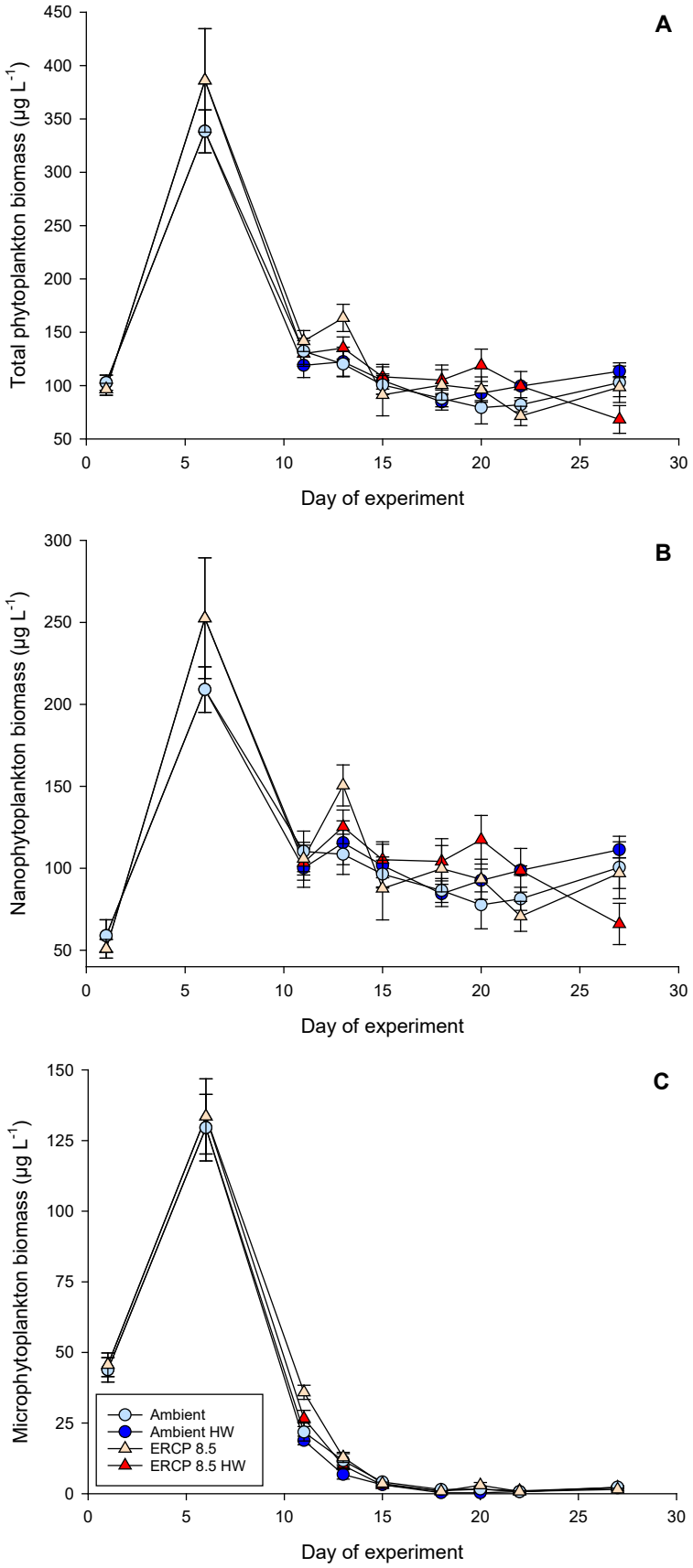

Figure 3: Phytoplankton carbon biomass in the mesocosms throughout the experiment of total phytoplankton (A), and different size classes: nanophytoplankton (B) and microphytoplankton (C). Different colours and symbols represent the Ambient scenario (circle) and Extended Representative Concentration Pathway (ERCP) scenario (triangle) with and without heatwave (light blue = Ambient, dark blue = Ambient HW, orange = ERCP 8.5, red = ERCP 8.5 HW). Symbols represent means and standard errors of four replicates per scenario.

The phytoplankton community was marginally dominated by nanophytoplankton at the onset of the experiment, with the 3 and 5 µm phytoflagellates being the most abundant taxa (Fig. 4). Species of the order *Rhizosoleniales* and the diatom *Lauderia annulata* dominated the microphytoplankton assemblage. In all scenarios, the phytoplankton bloom was characterized by an increase in dominance of *Phaeocystis globosa* and *Chaetoceros protuberans* in the nanophytoplankton assemblage at the expense of phytoflagellates, and by a decrease of *Rhizosoleniales* and an increase of *Leptocylindricus danicus* in the microphytoplankton assemblage (PRC test). Further, nanophytoplankton built up 70% of the total phytoplankton carbon biomass at the peak of the bloom in the ERCP 8.5 and ERCP 8.5 HW scenarios, compared to only 60% in the Ambient and Ambient HW scenarios (Fig. 4). The second half of the experiment was characterized by a dominance of phytoflagellates in all scenarios, whereby the smaller ones (3 µm) were particularly abundant in the Ambient HW and ERCP 8.5 scenarios. We observed a markable increase of the coccolithophore *Gephyrocapsa oceanica* after the phytoplankton bloom, particularly in the ERCP 8.5 HW scenario in which this species made 50% of the total phytoplankton carbon biomass on day 20, compared to only ca. 25% in the other scenarios (Fig. 4, PRC test).

In the net tow sample taken on the initial day for qualitative analysis of phycotoxins, a total of 3.2 ng of domoic acid were detected. While no domoic acid was found in the fraction >200 µm, 55 and 45 % were detected in the 50-200 µm and in the 20-50 µm size fractions, respectively. No domoic acid or other phycotoxins were found (see supplementary material), neither in the 800 L of filtered seawater taken on the initial day, nor in the mesocosm water at the end of the experiment.

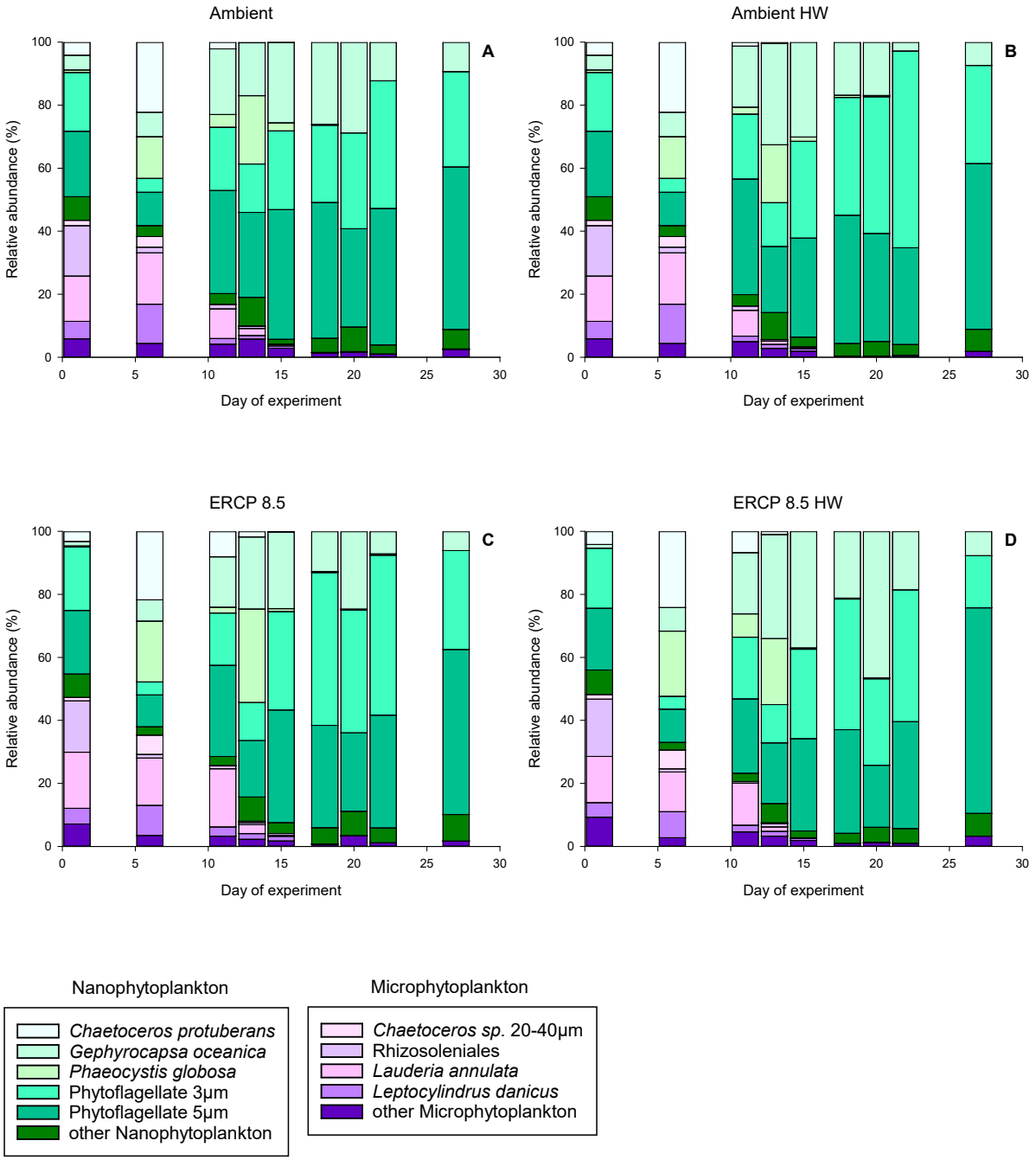

Figure 4: Phytoplankton community composition in the mesocosms throughout the experiment. Different colours represent different phytoplankton size classes (green shades = nanophytoplankton, purple shades = microphytoplankton). The figures represent the four scenarios, Ambient (A), Ambient HW (B), ERCP 8.5 (C), ERCP 8.5 HW (D).

Microzooplankton

In all scenarios, total microzooplankton carbon biomass rapidly increased at the onset of the experiment and reached higher levels in the Ambient and Ambient HW scenarios than in the ERCP 8.5 and ERCP 8.5 HW scenarios (Fig. 5A, GLM, $p<0.05$). In the Ambient scenario, total microzooplankton carbon biomass remained relatively constant around 55 µg C L^{-1} until day 15, after which it quickly declined. This decline occurred 4 days earlier in the other scenarios, and was particularly pronounced in the ERCP 8.5 HW scenario (GLM, $p<0.05$). Hence, total microzooplankton carbon biomass fluctuated over time, it was affected by the scenarios, and an interactive effect of scenario over time was observed (GLM and Likelihood Ratio Test, $p<0.05$). Heterotrophic dinoflagellates largely dominated the microzooplankton community (Fig. 5B), and their carbon biomass was significantly influenced by the scenarios, time, and their interaction (GLM, Ambient HW $p<0.05$, ERCP 8.5 $p<0.05$, ERCP 8.5 HW $p<0.05$; GLM and Likelihood-Ratio Test, $p<0.05$). The carbon biomass of ciliates increased concomitantly to that of dinoflagellates until day 5, after which it declined and remained relatively low from day 15 on (Fig. 5C, GLM $p<0.05$). Ciliate carbon biomass was higher in the Ambient and Ambient HW scenarios than in the ERCP 8.5 and ERCP 8.5 HW scenarios from day 5 to 15 (GLM, Ambient HW $p=0.23$, ERCP 8.5 $p<0.05$, ERCP 8.5 HW $p<0.05$).

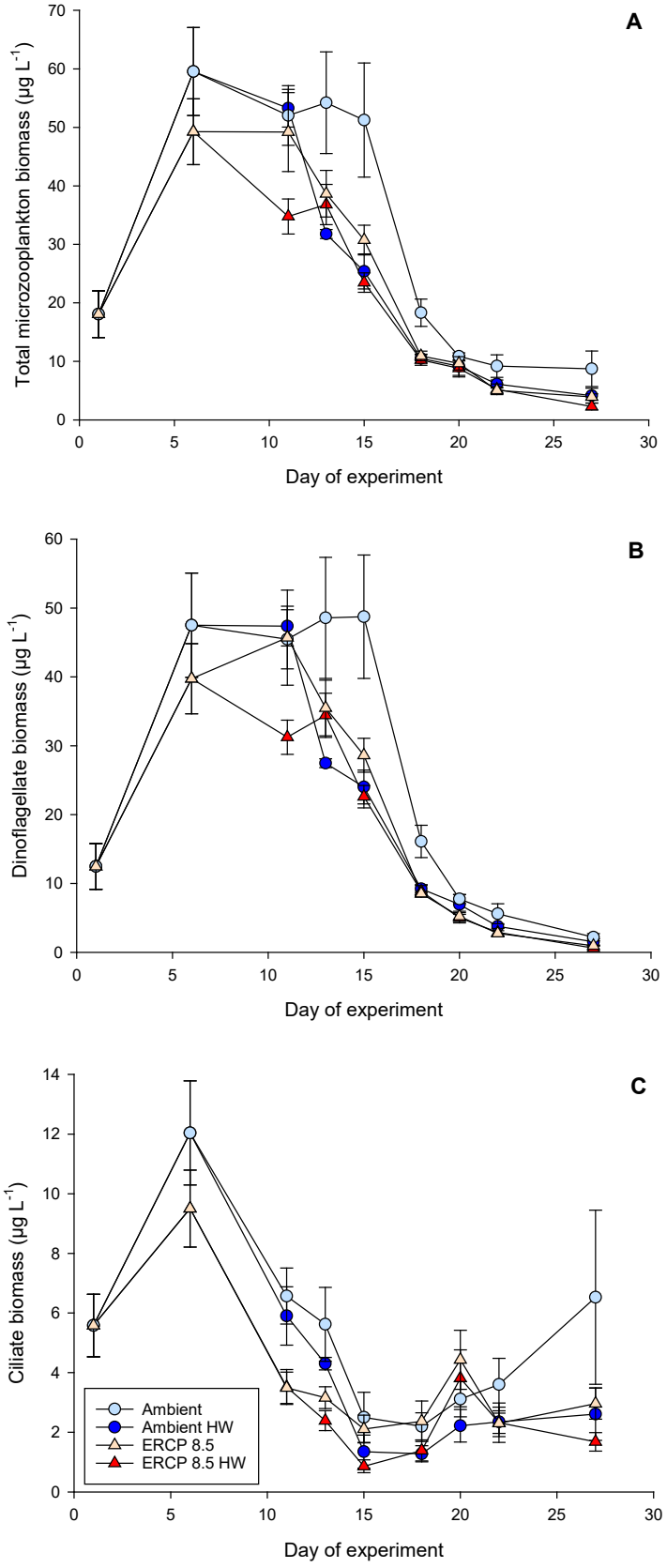

Figure 5: Microzooplankton carbon biomass in the mesocosms throughout the experiment of total microzooplankton (A), and different groups: dinoflagellates (B) and ciliates (C). Different colours and symbols represent the Ambient scenario (circle) and Extended Representative Concentration Pathway (ERCP) scenario (triangle) with and without heatwave (light blue = Ambient, dark blue = Ambient HW, orange = ERCP 8.5, red = ERCP 8.5 HW). Symbols represent means and standard errors of four replicates per scenario.

The microzooplankton community was largely dominated by dinoflagellates, which represented 80-90% of the total microzooplankton carbon biomass in all scenarios until day 20 (Fig. 6). During this period, species of the order Gymnodiniales between 15 and 30 µm dominated the microzooplankton community in all scenarios. Further, *Prorocentrum micans* was more abundant in the Ambient HW and ERCP 8.5 HW scenarios than in the other two scenarios, especially on days 11 to 17, and *Gyrodinium sp.* was more abundant in the Ambient and Ambient HW scenarios than in the ERCP 8.5 and ERCP 8.5 HW scenarios on day 6 (PRC test). From day 20 on, the proportion of ciliates increased, in particular due to an increase of *Strombidium sp.* which was particularly pronounced in the ERCP 8.5 and ERCP 8.5 HW scenarios (Fig. 6, PRC test).

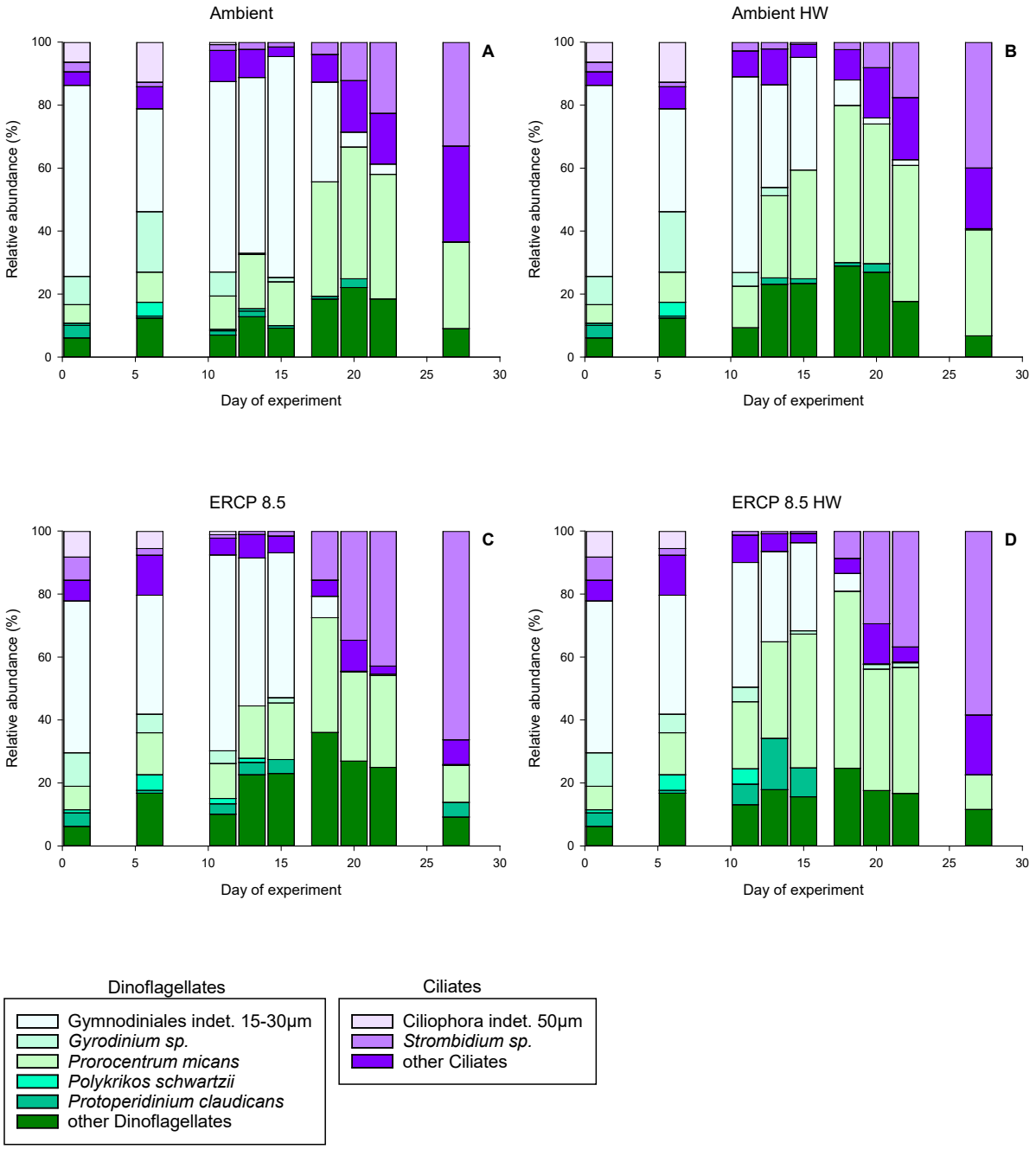

Figure 6: Microzooplankton community composition in the mesocosms throughout the experiment*. Different colours represent different groups (green shades = dinoflagellates, purple shades = ciliates). The figures represent the four scenarios, Ambient (A), Ambient HW (B), ERCP 8.5 (C), ERCP 8.5 HW (D).*

Mesozooplankton

Mesozooplankton abundances significantly varied over time (GLM, $p<0.05$), and increased from 20 to 32 individuals L^{-1} in the ERCP 8.5 HW and from 20 to 41 individuals L^{-1} in the ERCP 8.5 scenarios throughout the experiment (Fig. 7A). Mesozooplankton reached significantly higher abundances in the Ambient and Ambient HW scenario, with maxima of 47 and 62 individuals L^{-1} on day 20, respectively (Fig. 7A; GLM, Ambient HW $p<0.05$, ERCP 8.5 $p<0.05$, ERCP 8.5 HW $p<0.05$). The mesozooplankton community was dominated by *Acartia sp.* copepods in all scenarios (Fig. 8). In terms of taxonomic composition, the mesozooplankton community only differed between scenarios on day 13, on which *Acartia sp.* and *Oithona sp.* were equiproportional in the Ambient scenario, whereas *Acartia sp.* was more abundant in the other three scenarios.

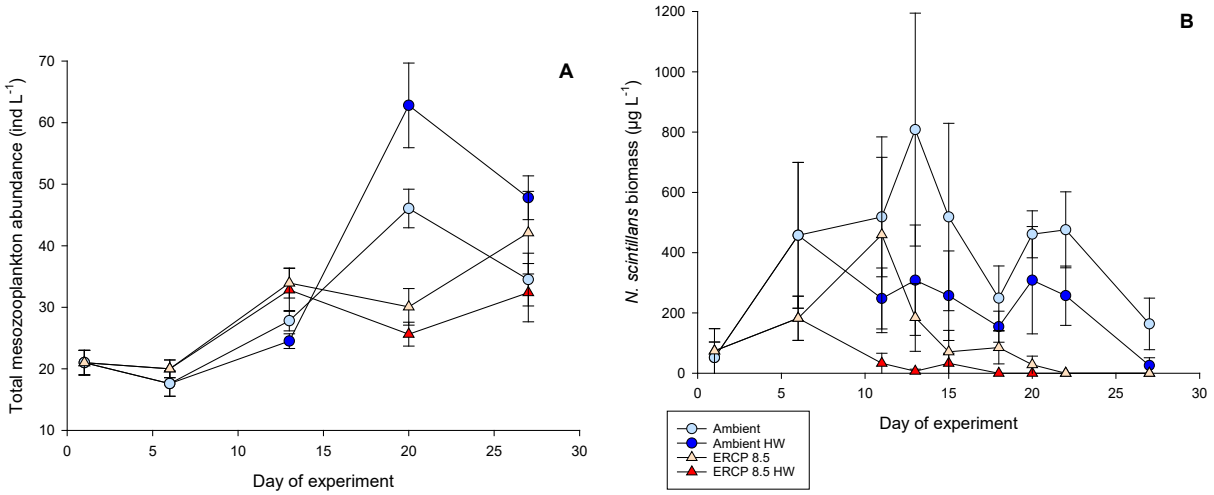

Figure 7: Mesozooplankton abundances in the mesocosms throughout the experiment: total mesozooplankton excluding Noctiluca scintillans (A), carbon biomass of Noctiluca scintillans (B). Different colours and symbols represent the Ambient scenario (circle) and Extended Representative Concentration Pathway (ERCP) scenario (triangle) with and without heatwave (light blue = Ambient, dark blue = Ambient HW, orange = ERCP 8.5, red = ERCP 8.5 HW). Symbols represent means and standard errors of four replicates per scenario.

The carbon biomass of *Noctiluca scintillans* was low at the onset of the experiment, it increased to reach ca. 800 µg L^{-1} on day 13 in the Ambient scenario, and subsequently decreased until the end of the experiment (Fig. 7B). This bloom was much weaker in the Ambient HW scenario, in which the carbon biomass of *Noctiluca scintillans* never exceeded 400 µg L^{-1} (Fig. 7B; GLM, $p<0.05$). In the ERCP 8.5 scenario, the carbon biomass of *Noctiluca scintillans* increased to reach about 400 µg L^{-1} on day 11, but rapidly collapsed afterward and this species was not found anymore after day 22 (Fig.7B; GLM, $p < 0.05$). The ERCP 8.5 HW was the least suitable for *Noctiluca scintillans*, which did not bloom in this scenario, and was not found anymore after the end of the heatwave.

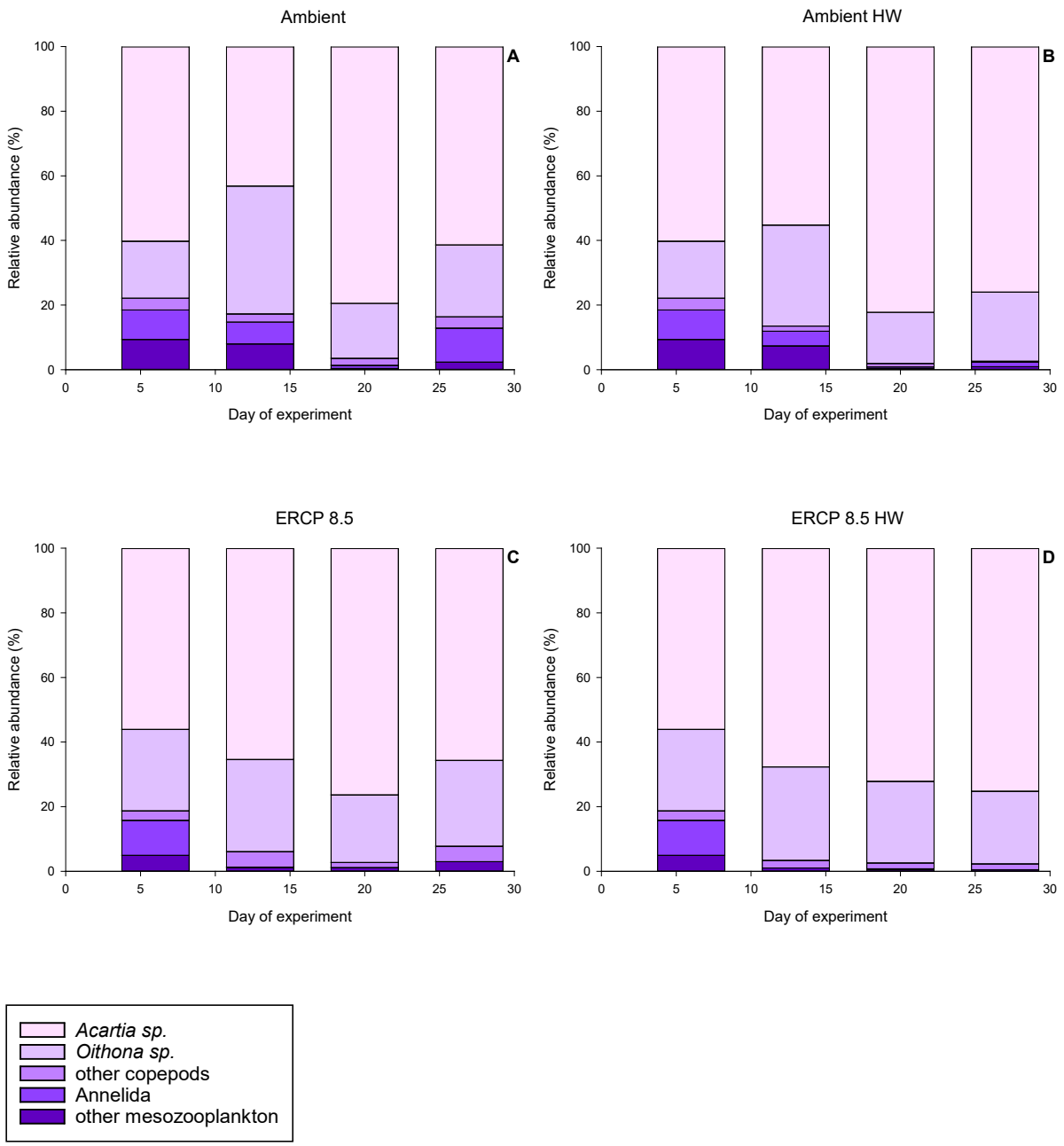

Figure 8: Mesozooplankton community composition in the mesocosms throughout the experiment, excluding Noctiluca scintillans. Different shades of purple represent different mesozooplankton groups. The figures represent the four scenarios, Ambient (A), Ambient HW (B), ERCP 8.5 (C), ERCP 8.5 HW (D).

Discussion

Here, we conducted a mesocosm experiment to study the influence of heatwaves under ambient and future conditions on a coastal plankton community. Our results indicate that these environmental alterations influence the abundance and species composition at several trophic levels, and alter the overall planktonic food web structure. As described by Gimenez et al. (2024), the seasonality of heatwaves is an important aspect which may influence their

ecological impact. Organisms typically occurring during the winter to spring and during the spring to summer seasonal changes are likely adapted to rapidly increasing environmental temperatures. As such, they are less likely to be affected by marine heatwaves than organisms occurring during seasonal changes with cooling trends, namely summer to autumn and autumn to winter (Giménez et al., 2024). Thus, heatwaves occurring in late summer and autumn may have the highest impact on marine organisms, which may be especially true in temperate systems which have a high variability in weather conditions, and for short-lived organisms with a limited seasonal window of occurrence, such as plankton. While our study indicates that the combination of multiple global change drivers has the potential to perturb the entire basis of marine food webs, these results may be context-dependent and should be put in perspective, which we aim to do in the following paragraphs.

Marine heatwaves affect bacterioplankton dynamics and phytoplankton species composition

We observed altered dynamics and reduced abundance of bacterioplankton in the ERCP 8.5 scenario compared to the Ambient scenario. Since bacterial growth is largely influenced by temperature, global change may have a major direct effect on the population dynamics of marine bacteria (Apple et al., 2006, Nedwell, 1999, Pomeroy & Wiebe, 2001). Moreover, lower pH has been suggested to have a secondary effect on bacterial dynamics by affecting bacterivores (Allgaier et al., 2008, Joint et al., 2011). However, in our experiment, microzooplankton was negatively affected by the environmental conditions in the ERCP 8.5 scenario, hence a top-down control is unlikely to explain the lower bacterioplankton abundances we observed. Global change effects on bacterioplankton have been studied primarily in experiments where, for instance, temperature or acidification were manipulated (Allgaier et al., 2008, Grossart et al., 2006, Hoppe et al., 2008, Piontek et al., 2009, Rochelle-Newall et al., 2004). These studies are, unfortunately, rather equivocal, with reports of increasing, decreasing or constant bacterial activities in response to warming and acidification, which led Lindh et al. (2013) to conclude that these global change drivers may only have limited influence on bacterioplankton. Our results contradict this conclusion as we show that the combination of warming, acidification, and increased N:P ratios may substantially alter bacterioplankton dynamics. Phytoplankton blooms release large amounts of carbon-rich substances like carbohydrates, which are used as resources by bacterioplankton (e.g., Teeling et al., 2012). Further, the type of polysaccharides released vary over time depending on the phytoplankton assemblage and nutritional status, which promotes the growth of different bacterial clades (Giljan et al., 2022). Although phytoplankton carbon biomass was not affected

by the different scenarios, we cannot exclude that the quantity and quality of exudates varied under the influence of global change drivers, as was shown by other studies (for review see Thornton, 2014). Certain bacterioplankton populations may be more sensitive to environmental changes than others, altering the overal dynamics and successions within the bacterioplankton community, which may explain the different dynamics we observed between scenarios. For instance, we observed higher abundances of the potentially harmful genus *Vibrio* in the ERCP 8.5 and ERCP 8.5 HW scenarios than in the other two scenarios. These results are significant since some *Vibrio* species are animal pathogens, but also human pathogens which cause wound infections associated with recreational bathing, septicemia, or diarrhea after ingestion of contaminated foods (Thompson et al., 2004a). Moreover, we observed that marine heatwaves exacerbated the effects observed on bacterioplankton, with faster dynamics in the Ambient HW and ERCP 8.5 HW scenarios than in the respective scenarios without heatwave, and we observed lower biomass in the ERCP 8.5 HW than in the ERCP 8.5 scenario. These results are supported by the study of Joint and Smale (2017) in which heterotrophic productivity was quantified across temperature gradients in the western English Channel. This work showed that episodically high temperatures can change nutrient and energy flow patterns through the microbial loop. Altogether, the influence of long-term environmental change and short-term temperature variability on bacterioplankton dynamics and assemblage structure may have important implications for ecosystem functions (Bell et al., 2005, Worm et al., 2006), including alterations of biogeochemical processes (Traving et al., 2021).

We did not observe any effect of the scenarios on phytoplankton biomass, which stands in contrast to many studies that have shown an influence of heatwaves (Arteaga & Rousseaux, 2023, Soulié et al., 2022, Zhan et al., 2023), warming (Behrenfeld et al., 2016, Lewandowska et al., 2014), higher pCO_2 (Bach et al., 2017, Kroeker et al., 2013, Sommer et al., 2015), or dissolved nutrient ratios (Burson et al., 2016, Klausmeier et al., 2004) on phytoplankton biomass. For instance, heatwaves have been shown to increase primary production and growth rates (Arteaga & Rousseaux, 2023, Soulié et al., 2022). Warming is also known to influence phytoplankton physiology directly and to accelerate metabolic processes (Lewandowska et al., 2014, Rehder et al., 2023), especially when resources such as light and nutrients are not limiting (Winder & Sommer, 2012). A near-global ocean physical–biogeochemical model simulation identified that the responses of phytoplankton blooms to marine heatwaves are related to the background nutrient conditions, with heatwaves causing weaker blooms in nutrient-poor waters and stronger ones in nutrient-rich waters (Hayashida et al., 2020). In our experiment, the marine heatwave occurred at the end of the bloom, which may explain why it did not influence the

overall phytoplankton biomass. However, the marine heatwave, as well as the potential future environmental conditions, altered the taxonomic composition of the phytoplankton community, which is in line with recent studies (Moreno et al., 2022, Zhan et al., 2023). The blooming community mainly consisted of diatoms (*Chaetoceros protuberans*, Rhizosoleniales, *Lauderia annulata*) and the haptophyte *Phaeocystis globosa*. We observed a higher relative abundance of nanophytoplankton species in the ERCP 8.5 than in the Ambient scenario, which goes hand in hand with observations that average phytoplankton community cell size decreases under future environmental conditions (Moreno et al., 2022, Sommer et al., 2015). We quantified low amounts of domoic acid in the seawater used to fill the mesocosm. This toxin is produced by diatoms of genus *Pseudo-nitzschia*, which were present in low abundances (<10.000 cell L^{-1}, data not shown) at the beginning of the experiment. *Pseudo-nitzschia* abundances remained low during the entire experiment, which explains that we did not find any domoic acid at the end of the experiment, and suggests that a harmful bloom of this genus may not be triggered by the scenarios tested in our experiment. However, we observed that the marine heatwaves in the Ambient HW and ERCP 8.5 HW scenarios favored small phytoflagellates and the coccolithophore *Gephyrocapsa oceanica*. This is supported by the results of a recent mesocosm study which also found that coccolithophores may benefit from higher temperature, pCO_2 levels, and N:P ratios (Moreno et al., 2022). Further, coccolithophore blooms, which are common during summer or early autumn in temperate regions (Hopkins et al., 2015, León et al., 2018), have increased in intensity over the past decades in the North Atlantic (Rivero-Calle et al., 2015). Changes in blooming patterns of coccolithophores could have considerable impacts on the biological carbon pump and biogeochemical processes of coastal zones (Rost & Riebesell, 2004). Furthermore, alterations of phytoplankton community structure, and an overall increase in the abundance of small phytoplankton species may have consequences for primary consumers.

Marine heatwaves exacerbate the negative effect of future environmental conditions on microzooplankton biomass

Microzooplankton carbon biomass significantly differed between scenarios, with lower biomasses in the ERCP 8.5 than in the Ambient scenario. Further, *Gyrodinium sp.*, a taxon which may be more sensitive to temperature changes than other microzooplankton taxa (Calbet et al., 2022, Calbet & Saiz, 2022), was less abundant in the ERCP 8.5 than in the ambient scenario. This is in contrast with the results of Moreno et al. (2022) who also studied a late-summer plankton community, and observed a significant increase in microzooplankton biomass with warming, acidification, and higher N:P ratios. It is important to note that, in their study,

the positive response of microzooplankton was triggered by a higher prey availability, which was not the case in our experiment. This suggests an interaction between food availability, and nutritional requirements under future environmental conditions. Although we observed an increase in the relative proportion of nanophytoplankton, whose size generally better suits the feeding preference of microzooplankton than microphytoplankton does (Calbet, 2008, Naustvoll, 2000), the overall phytoplankton biomass was not affected by the scenarios. Warming, at least to the extent studied here, usually has a positive effect on microzooplankton growth rates, metabolic activities, and turnover rates, so increased grazing on phytoplankton would be expected (Calbet & Saiz, 2022, Di Pane et al., 2024, López-Abbate, 2021, Rose et al., 2009). Further, elevated temperature and pCO_2 may increase energetic demands (Meunier et al., 2017, O'Connor et al., 2009), and thereby intensify the sensitivity of consumers to food availability. Given the correlation between environmental conditions and metabolic rates, altered metabolic demands of consumers, together with resource quality shifts, may create or increase already existing nutritional mismatches (Cross et al., 2015). Recent work shows that the nutritional requirements of zooplankton, and the resource quality which maximises the growth of these ectotherms, is not constant but rather varies with temperature (Laspoumaderes et al., 2022). However, as seston C:N:P stoichiometry did not vary across scenarios (Supplementary Fig. 1), bottom-up effects were likely driven by resource availability rather than by elemental stoichiometric quality. Interestingly, the negative effect of future environmental conditions on microzooplankton biomass was exacerbated by marine heatwaves. Only *Prorocentrum micans*, a species known to cope well with high temperatures (Abd El Fatah et al., 2022, Zhang et al., 2023), increased in relative abundance in response to the heatwave. To our knowledge, the impact of heatwaves on marine microzooplankton has never been studied before. Nevertheless, heatwaves, as other environmental drivers, may increase the metabolic demands of microzooplankton, and may have negative consequences for overall biomass if prey availability is insufficient. Further, due to their abruptness, heatwaves may act synergistically with other environmental drivers to hamper microzooplankton, which we observed. Microzooplankton is one of the major functional groups in planktonic food webs (Landry & Calbet, 2004a, Landry & Calbet, 2004b), as it facilitates the rapid recycling of nutrients back to primary producers (Calbet & Saiz, 2005, Suzuki et al., 1996). Microzooplankton also link the smaller planktonic unicellular organisms with higher metazoan trophic levels (Löder et al., 2011, Sherr & Sherr, 2007), and contribute substantially to mesozooplankton diets (Kleppel, 1993). Hence, decreases in microzooplankton biomass may

upset the functioning of planktonic food webs, and, for instance, negatively influence secondary consumers (López-Abbate, 2021).

Future environmental conditions impair large grazers, marine heatwaves compromise specific species

We observed substantially lower mesozooplankton abundances in the ERCP 8.5 and ERCP 8.5 HW scenarios than in the Ambient and Ambient HW scenarios. As for microzooplankton, the combination of higher metabolic requirements under altered environmental conditions and low prey availability may have reduced mesozooplankton abundances. While *Acartia sp.* and *Oithona sp.*, the dominant mesozooplankton taxa in our experiment, have been shown to have higher fitness when feeding on larger-sized prey items (Berggreen et al., 1988, Castellani et al., 2005, Støttrup & Jensen, 1990), nanophytoplankton dominated the phytoplankton community. Further, microzooplankton, which make up a significant share of copepods' diet (Calbet & Saiz, 2005, Castellani et al., 2005), were more abundant in the Ambient and Ambient HW scenarios than in the other two scenarios. Our results are supported by a mesocosm study in which mesozooplankton from the Baltic Sea were exposed to a temperature gradient (Garzke et al., 2015). The authors observed significant temperature effects on copepod and copepodite abundances, with lower zooplankton peak abundance in the warmer treatments (Garzke et al., 2015). The marine heatwave had a positive influence on mesozooplankton abundances in the Ambient scenario, but a negative on in the ERCP 8.5 scenario. Similar findings were obtained by Siegle et al. (2018), who observed that, in natural environments, copepods suffered from higher mortality after multiple exposures to warm events. Despite a growing body of literature highlighting that mesozooplankton may adapt to warming (Bitter et al., 2021, Dam, 2013), these results indicate that repeated exposure to sublethal temperatures may reduce thermal tolerance. Batten et al. (2022) examined a zooplankton community of the North East Pacific after the long 2014-2016 marine heatwave, and found that 20 % of the species disappeared from the study region after the heatwave. Hence, specific species may not be resistant towards marine heatwaves, and extinction rates may increase, especially under successive or prolonged warming periods (Hillebrand et al., 2012). In our experiment, we observed a negative effect of warming, acidification, and higher N:P ratios on the abundance of *Noctiluca scintillans*, which was substantially exacerbated by a marine heatwave which entirely suppressed the growth of this species in the ERCP 8.5 HW scenario. Since there was no top-down control on mesozooplankton during the experiment, it is important to note that the negative effects seen here could differ, and potentially be enhanced, in communities in which their predators are

present. Conversely, we suggest that reduced mesozooplankton abundances under future environmental conditions may create suboptimal feeding conditions for higher trophic levels.

Conclusion

In this study, we show that heatwaves under current and future environmental conditions influence the biomass and taxonomic composition of multiple trophic levels, and alter the overall structure of planktonic communities. We observed that future environmental conditions alter bacterioplankton dynamics and reduce their abundances, and that these effects are exacerbated further by a heatwave. While we did not observe any effect on phytoplankton carbon biomass, we observed that future environmental conditions reduce microzooplankton carbon biomass, and that this negative effect is exacerbated by a heatwave. Our results indicate that future environmental conditions may favour smaller phytoplankton species, and that additional heatwaves may favour small phytoflagellates and coccolithophores. We also observed alterations in the composition of microzooplankton assemblages with *Gyrodinium sp.* being less abundant under future environmental conditions, and *Prorocentrum micans* being more abundant in the heatwave scenarios. We identified that mesozooplankton abundances were lower under future conditions, and that a heatwave intensified this negative effect on the biomass of *Noctiluca scintillans*. To the best of our knowledge, our study is the first to examine the impacts of heatwaves under current and future environmental conditions on a natural multi-trophic marine plankton community. Using this unique approach, we show that the combination of multiple global change drivers have the potential to perturb the entire basis of marine food-webs. It is important to note that the seasonality of heatwaves may determine their ecological impacts, and future studies should be conducted with planktonic communities sampled at different times of the year to test the generality of our results.

Acknowledgments

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References

Abd El Fatah HM, Ali DM, Ibrahim M (2022) Seasonal dynamics and ecological drivers of Prorocentrum micans Ehrenberg dinoflagellate blooms in Qarun Lake, Egypt. The Egyptian Journal of Aquatic Research, **48**, 375-382.

Allgaier M, Riebesell U, Vogt M, Thyrhaug R, Grossart H-P (2008) Coupling of heterotrophic bacteria to phytoplankton bloom development at different pCO 2 levels: a mesocosm study. Biogeosciences, **5**, 1007-1022.

Alvarez-Fernandez S, Bach LT, Taucher J et al. (2018) Plankton responses to ocean acidification: The role of nutrient limitation. Progress in Oceanography, **165**, 11-18.

Apple JK, Del Giorgio P, Kemp WM (2006) Temperature regulation of bacterial production, respiration, and growth efficiency in a temperate salt-marsh estuary. Aquatic Microbial Ecology, **43**, 243-254.

Arias-Ortiz A, Serrano O, Masqué P et al. (2018) A marine heatwave drives massive losses from the world's largest seagrass carbon stocks. Nature Climate Change, **8**, 338-344.

Arteaga LA, Rousseaux CS (2023) Impact of Pacific Ocean heatwaves on phytoplankton community composition. Communications Biology, **6**, 263.

Bach LT, Alvarez-Fernandez S, Hornick T, Stuhr A, Riebesell U (2017) Simulated ocean acidification reveals winners and losers in coastal phytoplankton. PLoS ONE, **12**, e0188198.

Balkoni A, Guignard MS, Boersma M, Wiltshire KH (2023) Evaluation of different averaging methods for calculation of ratios in nutrient data. Fundamental and Applied Limnology.

Banzon V, Smith TM, Chin TM, Liu C, Hankins W (2016) A long-term record of blended satellite and in situ sea-surface temperature for climate monitoring, modeling and environmental studies. Earth System Science Data, **8**, 165-176.

Banzon V, Smith TM, Steele M, Huang B, Zhang H-M (2020) Improved estimation of proxy sea surface temperature in the Arctic. Journal of Atmospheric and Oceanic Technology, **37**, 341-349.

Batten SD, Ostle C, Hélaouët P, Walne AW (2022) Responses of Gulf of Alaska plankton communities to a marine heat wave. Deep Sea Research Part II: Topical Studies in Oceanography, **195**, 105002.

Behrenfeld MJ, O'malley RT, Boss ES et al. (2016) Revaluating ocean warming impacts on global phytoplankton. Nature Clim. Change, **6**, 323-330.

Bell T, Newman JA, Silverman BW, Turner SL, Lilley AK (2005) The contribution of species richness and composition to bacterial services. Nature, **436**, 1157-1160.

Bellard C, Bertelsmeier C, Leadley P, Thuiller W, Courchamp F (2012) Impacts of climate change on the future of biodiversity. Ecology Letters, **15**, 365-377.

Bennke CM, Reintjes G, Schattenhofer M, Ellrott A, Wulf J, Zeder M, Fuchs BM (2016) Modification of a high-throughput automatic microbial cell enumeration system for shipboard analyses. Applied and Environmental Microbiology, **82**, 3289-3296.

Berggreen U, Hansen B, Kiørboe T (1988) Food size spectra, ingestion and growth of the copepod Acartia tonsa during development: implications for determination of copepod production. Marine Biology, **99**, 341-352.

Bitter M, Wong J, Dam H et al. (2021) Fluctuating selection and global change: a synthesis and review on disentangling the roles of climate amplitude, predictability and novelty. Proceedings of the Royal Society B, **288**, 20210727.

Boersma M, Wiltshire KH, Kong S-M, Greve W, Renz J (2015) Long-term change in the copepod community in the southern German Bight. Journal of Sea Research, **101**, 41-50.

Boyd PW, Collins S, Dupont S et al. (2018) Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change—a review. Global Change Biology, **24**, 2239-2261.

Brehm TT, Berneking L, Martins MS et al. (2021) Heatwave-associated Vibrio infections in Germany, 2018 and 2019. Eurosurveillance, **26**, 2002041.

Breitburg DL, Salisbury J, Bernhard JM et al. (2015) And on top of all that…: Coping with ocean acidification in the midst of many stressors by denise. Oceanography, **28**, 48-61.

Brüwer JD, Orellana LH, Sidhu C et al. (2023) In situ cell division and mortality rates of SAR11, SAR86, Bacteroidetes, and Aurantivirga during phytoplankton blooms reveal differences in population controls. mSystems, e01287-01222.

Burson A, Stomp M, Akil L, Brussaard CP, Huisman J (2016) Unbalanced reduction of nutrient loads has created an offshore gradient from phosphorus to nitrogen limitation in the N orth S ea. Limnology and Oceanography, **61**, 869-888.

Bustin SA, Benes V, Garson JA et al. (2009) The MIQE Guidelines: M inimum I nformation for Publication of Q uantitative Real-Time PCR E xperiments. pp Page, Oxford University Press.

Calbet A (2008) The trophic roles of microzooplankton in marine systems. ICES Journal of Marine Science: Journal du Conseil, **65**, 325-331.

Calbet A, Martínez RA, Saiz E, Alcaraz M (2022) Effects of temperature on the bioenergetics of the marine protozoans Gyrodinium dominans and Oxyrrhis marina. Frontiers in Marine Science, **9**, 901096.

Calbet A, Saiz E (2005) The ciliate-copepod link in marine ecosystems. Aquatic Microbial Ecology, **38**, 157-167.

Calbet A, Saiz E (2022) Thermal acclimation and adaptation in marine Protozooplankton and Mixoplankton. Frontiers in Microbiology, **13**, 832810.

Caron DA, Hutchins DA (2013) The effects of changing climate on microzooplankton grazing and community structure: drivers, predictions and knowledge gaps. Journal of Plankton Research, **35**, 235-252.

Castellani C, Irigoien X, Harris RP, Lampitt RS (2005) Feeding and egg production of Oithona similis in the North Atlantic. Marine Ecology Progress Series, **288**, 173-182.

Coyne KJ, Salvitti LR, Mangum AM, Ozbay G, Main CR, Kouhanestani ZM, Warner ME (2021) Interactive effects of light, CO2 and temperature on growth and resource partitioning by the mixotrophic dinoflagellate, Karlodinium veneficum. PLoS ONE, **16**, e0259161.

Cross WF, Hood JM, Benstead JP, Huryn AD, Nelson D (2015) Interactions between temperature and nutrients across levels of ecological organization. Global Change Biology, **21**, 1025-1040.

Dam HG (2013) Evolutionary Adaptation of Marine Zooplankton to Global Change. Annual Review of Marine Science, **5**, 349-370.

De Amorim FDLL, Wiltshire KH, Lemke P et al. (2023) Investigation of marine temperature changes across temporal and spatial Gradients: Providing a fundament for studies on the effects of warming on marine ecosystem function and biodiversity. Progress in Oceanography, **216**, 103080.

Di Pane J, Bourdaud P, Horn S, Moreno HD, Meunier CL (2024) Global change alters coastal plankton food webs by promoting the microbial loop: An inverse modelling and network analysis approach on a mesocosm experiment. Science of the Total Environment, 171272.

Dickson AG (1981) An exact definition of total alkalinity and a procedure for the estimation of alkalinity and total inorganic carbon from titration data. Deep Sea Research Part A. Oceanographic Research Papers, **28**, 609-623.

Diner RE, Kaul D, Rabines A, Zheng H, Steele JA, Griffith JF, Allen AE (2021) Pathogenic Vibrio species are associated with distinct environmental niches and planktonic taxa in Southern California (USA) aquatic microbiomes. mSystems, **6**, e00571-00521.

Doney SC (2010) The growing human footprint on coastal and open-ocean biogeochemistry. Science, **328**, 1512-1516.

Duarte CM (2014) Global Change and the Future Ocean: A Grand Challenge for Marine Sciences. Frontiers in Marine Science, **1**.

Duarte CM, Holmer M, Olsen Y et al. (2009) Will the Oceans Help Feed Humanity? Bioscience, **59**, 967-976.

Edwards M, Richardson AJ (2004) Impact of climate change on marine pelagic phenology and trophic mismatch. Nature, **430**, 881-884.

Elser JJ, Fagan WF, Denno RF et al. (2000) Nutritional constraints in terrestrial and freshwater foodwebs. Nature, **408**, 578-580.

Engel A, Händel N, Wohlers J, Lunau M, Grossart H-P, Sommer U, Riebesell U (2011) Effects of sea surface warming on the production and composition of dissolved organic matter during phytoplankton blooms: results from a mesocosm study. Journal of Plankton Research, **33**, 357-372.

Filiz N, Işkın U, Beklioğlu M et al. (2020) Phytoplankton community response to nutrients, temperatures, and a heat wave in shallow lakes: An experimental approach. Water, **12**, 3394.

Fraga D, Meulia T, Fenster S (2014) Real-time PCR. Current protocols essential laboratory techniques, **8**, 10.13. 11-10.13. 40.

Garrido S, Cruz J, Santos AMP, Re P, Saiz E (2013) Effects of temperature, food type and food concentration on the grazing of the calanoid copepod Centropages chierchiae. Journal of Plankton Research, **35**, 843-854.

Garzke J, Ismar SM, Sommer U (2015) Climate change affects low trophic level marine consumers: warming decreases copepod size and abundance. Oecologia, **177**, 849-860.

Giljan G, Arnosti C, Kirstein IV, Amann R, Fuchs BM (2022) Strong seasonal differences of bacterial polysaccharide utilization in the North Sea over an annual cycle. Environmental Microbiology, **24**, 2333-2347.

Giménez L, Boersma M, Wiltshire KH (2024) A multiple baseline approach for marine heatwaves. Limnology and Oceanography.

Giménez L, Chatterjee A, Torres G (2021) A state-space approach to understand responses of organisms, populations and communities to multiple environmental drivers. Communications Biology, **4**, 1142.

Grasshoff K, Ehrhardt M, Kremling K (1999) *Methods of Seawater Analysis*, Wileyh-VCH.

Greve W, Reiners F, Nast J, Hoffmann S (2004) Helgoland Roads meso- and macrozooplankton time-series 1974 to 2004: lessons from 30 years of single spot, high frequency sampling at the only off-shore island of the North Sea. Helgoland marine research, **58**, 274-288.

Grizzetti B, Bouraoui F, Aloe A (2012) Changes of nitrogen and phosphorus loads to European seas. Global Change Biology, **18**, 769-782.

Grossart H-P, Allgaier M, Passow U, Riebesell U (2006) Testing the effect of CO2 concentration on the dynamics of marine heterotrophic bacterioplankton. Limnology and Oceanography, **51**, 1-11.

Gu H, Wu Y, Lü S, Lu D, Tang YZ, Qi Y (2022) Emerging harmful algal bloom species over the last four decades in China. Harmful Algae, **111**, 102059.

Guo K, Chen J, Yuan J, Wang X, Xu S, Hou S, Wang Y (2022) Effects of Temperature on Transparent Exopolymer Particle Production and Organic Carbon Allocation of Four Marine Phytoplankton Species. Biology, **11**, 1056.

Halpern BS, Walbridge S, Selkoe KA et al. (2008) A Global Map of Human Impact on Marine Ecosystems. Science, **319**, 948-952.

Harley CD, Randall Hughes A, Hultgren KM et al. (2006) The impacts of climate change in coastal marine systems. Ecology Letters, **9**, 228-241.

Hayashida H, Matear RJ, Strutton PG (2020) Background nutrient concentration determines phytoplankton bloom response to marine heatwaves. Global Change Biology, **26**, 4800-4811.

Hillebrand H, Burgmer T, Biermann E (2012) Running to stand still: temperature effects on species richness, species turnover, and functional community dynamics. Marine Biology, **159**, 2415-2422.

Hillebrand H, Dürselen C-D, Kirschtel D, Pollingher U, Zohary T (1999) Biovolume calculation for pelagic and benthic microalgae. Journal of Phycology, **35**, 403-424.

Hjerne O, Hajdu S, Larsson U, Downing AS, Winder M (2019) Climate driven changes in timing, composition and magnitude of the Baltic Sea phytoplankton spring bloom. Frontiers in Marine Science, **6**, 482.

Hobday AJ, Alexander LV, Perkins SE et al. (2016) A hierarchical approach to defining marine heatwaves. Progress in Oceanography, **141**, 227-238.

Hobday AJ, Oliver EC, Gupta AS et al. (2018) Categorizing and naming marine heatwaves. Oceanography, **31**, 162-173.

Hopkins J, Henson SA, Painter SC, Tyrrell T, Poulton AJ (2015) Phenological characteristics of global coccolithophore blooms. Global Biogeochemical Cycles, **29**, 239-253.

Hoppe H-G, Breithaupt P, Walther K, Koppe R, Bleck S, Sommer U, Jürgens K (2008) Climate warming in winter affects the coupling between phytoplankton and bacteria during the spring bloom: a mesocosm study. Aquatic Microbial Ecology, **51**, 105-115.

Horn S, Meunier CL, Fofonova V, Sarker S, Pogoda B, Asmus H, Wiltshire KH (2021) Improving model capacities for assessment of climate change impacts on coastal bentho-pelagic food webs and their ecosystem services. Frontiers in Marine Science, **8**, 819.

Joint I, Doney SC, Karl DM (2011) Will ocean acidification affect marine microbes? The ISME journal, **5**, 1-7.

Joint I, Smale DA (2017) Marine heatwaves and optimal temperatures for microbial assemblage activity. FEMS Microbiology Ecology, **93**, fiw243.

Joyce PW, Tong CB, Yip YL, Falkenberg LJ (2024) Marine heatwaves as drivers of biological and ecological change: implications of current research patterns and future opportunities. Marine Biology, **171**, 20.

Klausmeier CA, Litchman E, Levin SA (2004) Phytoplankton growth and stoichiometry under multiple nutrient limitation. Limnology and Oceanography, **49**, 1463-1470.

Kleppel GS (1993) On the diets of calanoid copepods. Marine Ecology Progress Series, **99**, 1-2.

Kraberg A, Baumann M, Dürselen C-D (2010) *Coastal phytoplankton: photo guide for Northern European seas*, Univerza v Ljubljani, Biotehniška fakulteta.

Kroeker KJ, Kordas RL, Crim R et al. (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. Global Change Biology, **19**, 1884-1896.

Landry MR, Calbet A (2004a) Microzooplankton production in the oceans. ICES Journal of Marine Science: Journal du Conseil, **61**, 501-507.

Landry MR, Calbet A (2004b) Significance of predation by protists in aquatic microbial food webs. Journal of Marine Science, **61**, 501-507.

Laspoumaderes C, Meunier CL, Magnin A et al. (2022) A common temperature dependence of nutritional demands in ectotherms. Ecology Letters, **25**, 2189-2202.

Le Nohaïc M, Ross CL, Cornwall CE, Comeau S, Lowe R, Mcculloch MT, Schoepf V (2017) Marine heatwave causes unprecedented regional mass bleaching of thermally resistant corals in northwestern Australia. Scientific Reports, **7**, 14999.

Lee H, Calvin K, Dasgupta D et al. (2023) 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.

León P, Walsham P, Bresnan E, Hartman SE, Hughes S, Mackenzie K, Webster L (2018) Seasonal variability of the carbonate system and coccolithophore Emiliania huxleyi at a Scottish Coastal Observatory monitoring site. Estuarine, Coastal and Shelf Science, **202**, 302-314.

Lewandowska AM, Boyce DG, Hofmann M, Matthiessen B, Sommer U, Worm B (2014) Effects of sea surface warming on marine plankton. Ecology Letters, **17**, 614-623.

Lindh MV, Riemann L, Baltar F, Romero-Oliva C, Salomon PS, Granéli E, Pinhassi J (2013) Consequences of increased temperature and acidification on bacterioplankton community composition during a mesocosm spring bloom in the B altic S ea. Environmental Microbiology Reports, **5**, 252-262.

Löder MGJ, Meunier C, Wiltshire KH, Boersma M, Aberle N (2011) The role of ciliates, heterotrophic dinoflagellates and copepods in structuring spring plankton communities at Helgoland Roads, North Sea. Marine Biology, **158**, 1551-1580.

López-Abbate MC (2021) Microzooplankton Communities in a Changing Ocean: A Risk Assessment. Diversity, **13**, 82.

Martinez Arbizu P (2020) pairwiseAdonis: Pairwise multilevel comparison using adonis. R package version 0.4, **1**.

Mcewen G, Johnson M, Folsom TR (1954) A statistical analysis of the performance of the Folsom plankton sample splitter, based upon test observations. Archiv für Meteorologie, Geophysik und Bioklimatologie, Serie A, **7**, 502-527.

Mckinstry CA, Campbell RW, Holderied K (2022) Influence of the 2014–2016 marine heatwave on seasonal zooplankton community structure and abundance in the lower Cook Inlet, Alaska. Deep Sea Research Part II: Topical Studies in Oceanography, **195**, 105012.

Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. Limnology and Oceanography, **45**, 569-579.

Meunier CL, Algueró-Muñiz M, Horn HG, Lange JaF, Boersma M (2017) Direct and indirect effects of near-future pCO2 levels on zooplankton dynamics. Marine & Freshwater Research, 373-380.

Meunier CL, Alvarez-Fernandez S, Cunha-Dupont AÖ, Geisen C, Malzahn AM, Boersma M, Wiltshire KH (2018) The craving for phosphorus in heterotrophic dinoflagellates and its potential implications for biogeochemical cycles. Limnology and Oceanography, **63**, 1774-1784.

Moreno HD, Köring M, Pane JD, Tremblay N, Wiltshire KH, Boersma M, Meunier CL (2022) An integrated multiple driver mesocosm experiment reveals the effect of global change on planktonic food web structure. Communications Biology, **5**, 1-9.

Moreno HD, Rokitta S, Tremblay N et al. (2023) Higher temperature, increased CO2, and changing nutrient ratios alter the carbon metabolism and induce oxidative stress in a cosmopolitan diatom. Limnology and Oceanography.

Naustvoll L-J (2000) Prey size spectra and food preferences in thecate heterotrophic dinoflagellates. Phycologia, **39**, 187-198.

Nedwell DB (1999) Effect of low temperature on microbial growth: lowered affinity for substrates limits growth at low temperature. FEMS Microbiology Ecology, **30**, 101-111.

O'connor MI, Piehler MF, Leech DM, Anton A, Bruno JF (2009) Warming and resource availability shift food web structure and metabolism. PLoS Biology, **7**, e1000178.

Oksanen J, Kindt R, Legendre P, O'hara B, Stevens MHH, Oksanen MJ, Suggests M (2007) The vegan package. Community ecology package, **10**, 719.

Pansch A, Winde V, Asmus R, Asmus H (2016) Tidal benthic mesocosms simulating future climate change scenarios in the field of marine ecology. Limnology and Oceanography: Methods, **14**, 257-267.

Peñuelas J, Poulter B, Sardans J et al. (2013) Human-induced nitrogen–phosphorus imbalances alter natural and managed ecosystems across the globe. Nature Communications, **4**.

Peñuelas J, Sardans J, Rivas-Ubach A, Janssens IA (2012) The human-induced imbalance between C, N and P in Earth's life system. Global Change Biology, **18**, 3-6.

Pierrot D, Lewis E, Wallace D (2006) CO2SYS MS Excel Program developed for CO2 system calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN.

Piontek J, Händel N, Langer G, Wohlers J, Riebesell U, Engel A (2009) Effects of rising temperature on the formation and microbial degradation of marine diatom aggregates. Aquatic Microbial Ecology, **54**, 305-318.

Pomeroy LR, Wiebe WJ (2001) Temperature and substrates as interactive limiting factors for marine heterotrophic bacteria. Aquatic Microbial Ecology, **23**, 187-204.

Pörtner H-O, Roberts DC, Poloczanska E et al. (2022) IPCC, 2022: Summary for policymakers.

Putt M, Stoecker DK (1989) An experimentally determined carbon : volume ratio for marine "oligotrichous" ciliates from estuarine and coastal waters. Limnology and Oceanography, **34**, 1097-1103.

R Core Team R (2021) R: A language and environment for statistical computing.

Rehder L, Rost B, Rokitta SD (2023) Abrupt and acclimation responses to changing temperature elicit divergent physiological effects in the diatom Phaeodactylum tricornutum. New Phytologist.

Remy M, Hillebrand H, Flöder S (2017) Stability of marine phytoplankton communities facing stress related to global change: Interactive effects of heat waves and turbidity. Journal of Experimental Marine Biology and Ecology, **497**, 219-229.

Reynolds RW, Smith TM, Liu C, Chelton DB, Casey KS, Schlax MG (2007) Daily high-resolution-blended analyses for sea surface temperature. Journal of Climate, **20**, 5473-5496.

Rick JJ, Scharfe M, Romanova T et al. (2023) An evaluation of long-term physical and hydrochemical measurements at the Sylt Roads Marine Observatory (1973–2019), Wadden Sea, North Sea. Earth System Science Data, **15**, 1037-1057.

Rivero-Calle S, Gnanadesikan A, Del Castillo CE, Balch WM, Guikema SD (2015) Multidecadal increase in North Atlantic coccolithophores and the potential role of rising CO2. Science, **350**, 1533-1537.

Rochelle-Newall E, Delille B, Frankignoulle M et al. (2004) Chromophoric dissolved organic matter in experimental mesocosms maintained under different pCO2 levels. Marine Ecology Progress Series, **272**, 25-31.

Rose J, Feng Y, Gobler C, Gutierrez R, Hare C, Leblanc K, Hutchins D (2009) Effects of increased pCO2 and temperature on the North Atlantic spring bloom. II. Microzooplankton abundance and grazing. Marine Ecology Progress Series, **388**, 27-40.

Rost B, Riebesell U (2004) Coccolithophores and the biological pump: responses to environmental changes. In: *Coccolithophores: from molecular processes to global impact.* pp Page., Springer.

Sanford E, Sones JL, García-Reyes M, Goddard JH, Largier JL (2019) Widespread shifts in the coastal biota of northern California during the 2014–2016 marine heatwaves. Scientific Reports, **9**, 4216.

Schlegel R (2020) Marine heatwave tracker. See http://www.marineheatwaves.org/tracker.

Schlegel RW, Smit AJ (2018) heatwaveR: A central algorithm for the detection of heatwaves and cold-spells. Journal of Open Source Software, **3**, 821.

Sell DW, Evans MS (1982) A statistical analysis of subsampling and an evaluation of the Folsom plankton splitter. Hydrobiologia, **94**, 223-230.

Shanks AL, Rasmuson LK, Valley JR et al. (2020) Marine heat waves, climate change, and failed spawning by coastal invertebrates. Limnology and Oceanography, **65**, 627-636.

Sherr EB, Sherr BF (2007) Heterotrophic dinoflagellates: a significant component of microzooplankton biomass and major grazers of diatoms in the sea. Marine Ecology Progress Series, **352**, 187-197.

Siegle MR, Taylor EB, O'connor MI (2018) Prior heat accumulation reduces survival during subsequent experimental heat waves. Journal of Experimental Marine Biology and Ecology, **501**, 109-117.

Sommer U, Lewandowska A (2011) Climate change and the phytoplankton spring bloom: warming and overwintering zooplankton have similar effects on phytoplankton. Global Change Biology, **17**, 154-162.

Sommer U, Paul C, Moustaka-Gouni M (2015) Warming and Ocean Acidification Effects on Phytoplankton?From Species Shifts to Size Shifts within Species in a Mesocosm Experiment. PLoS ONE, **10**, e0125239.

Soulié T, Vidussi F, Mas S, Mostajir B (2022) Functional stability of a coastal Mediterranean plankton community during an experimental marine heatwave. Frontiers in Marine Science, **9**.

Stewart RI, Dossena M, Bohan DA et al. (2013) Mesocosm experiments as a tool for ecological climate-change research. Advances in Ecological Research, **48**, 71-181.

Støttrup JG, Jensen J (1990) Influence of algal diet on feeding and egg-production of the calanoid copepod Acartia tonsa Dana. Journal of Experimental Marine Biology and Ecology, **141**, 87-105.

Suzuki MT, Sherr EB, Sherr BF (1996) Estimation of ammonium regeneration efficiencies associated with bacterivory in pelagic food webs via a 15N tracer method. Journal of Plankton Research, **18**, 411-428.

Teeling H, Fuchs BM, Becher D et al. (2012) Substrate-controlled succession of marine bacterioplankton populations induced by a phytoplankton bloom. Science, **336**, 608-611.

Telesh IV, Alimov AF, Golubkov SM, Nikulina VN, Panov VE (1999) Response of aquatic communities to anthropogenic stress: a comparative study of Neva Bay and the eastern Gulf of Finland. Hydrobiologia, **393**, 95-105.

Thompson FL, Iida T, Swings J (2004a) Biodiversity of vibrios. Microbiology and Molecular Biology Reviews, **68**, 403-431.

Thompson JR, Randa MA, Marcelino LA, Tomita-Mitchell A, Lim E, Polz MF (2004b) Diversity and dynamics of a North Atlantic coastal Vibrio community. Applied and Environmental Microbiology, **70**, 4103-4110.

Thornton DC (2014) Dissolved organic matter (DOM) release by phytoplankton in the contemporary and future ocean. European Journal of Phycology, **49**, 20-46.

Todgham AE, Stillman JH (2013) Physiological responses to shifts in multiple environmental stressors: relevance in a changing world. Integrative and Comparative Biology, **53**, 539-544.

Traving SJ, Kellogg CT, Ross T et al. (2021) Prokaryotic responses to a warm temperature anomaly in northeast subarctic Pacific waters. Communications Biology, **4**, 1217.

Utermöhl H (1958) Zur vervollkommnung der quantitativen phytoplankton-methodik. Mitt. int. Ver. theor. angew. Limnol., **9**, 1-38.

Van Beusekom JE, Carstensen J, Dolch T et al. (2019) Wadden Sea Eutrophication: long-term trends and regional differences. Frontiers in Marine Science, **6**, 370.

Wernberg T, Smale DA, Thomsen MS (2012) A decade of climate change experiments on marine organisms: procedures, patterns and problems. Global Change Biology, **18**, 1491-1498.

Wickham H, François R, Henry L, Müller K (2018) dplyr: A Grammar of Data Manipulation. R package version 0.7. 6. Computer software. https://CRAN.R-project.org/package=dplyr.

Winder M, Sommer U (2012) Phytoplankton response to a changing climate. Hydrobiologia, **698**, 5-16.

Worm B, Barbier EB, Beaumont N et al. (2006) Impacts of biodiversity loss on ocean ecosystem services. Science, **314**, 787-790.

Zhan W, Zhang Y, He Q, Zhan H (2023) Shifting responses of phytoplankton to atmospheric and oceanic forcing in a prolonged marine heatwave. Limnology and Oceanography.

Zhang J, Li X, Wang X, Guan W (2023) Transcriptome analysis of two bloom-forming Prorocentrum species reveals physiological changes related to light and temperature. Harmful Algae, **125**, 102421.

Ziegler SL, Johnson JM, Brooks RO et al. (2023) Marine protected areas, marine heatwaves, and the resilience of nearshore fish communities. Scientific Reports, **13**, 1405.

Supplementary material of publication VI

Supplementary Table 1: Primers for the 16S rRNA gene of the genus Vibrio.

Primer	Sequence	Reference
Vib-567F	GGCGTAAAGCGCATGCAGGT	(Thompson et al., 2004b)
Vib2-r	GAAATTCTACCCCCCTCTACAG	(Thompson et al., 2004b)

Supplementary Table 2: Total DNA amount in the different standard approaches.

genome copies	1	10	10^2	10^3	10^4	10^5	10^6	10^7
Total DNA [ng]	$5*10^{-6}$	$5*10^{-5}$	$5*10^{-4}$	$5*10^{-3}$	$5*10^{-2}$	$5*10^{-1}$	5	50

Supplementary Table 3: Carbonate chemistry in the mesocosms throughout the experiment. pH and total alkalinity (TA) were used to calculate partial pressure of CO_2 (pCO_2)using CO2Sys (Pierrot et al.*, 2006).*

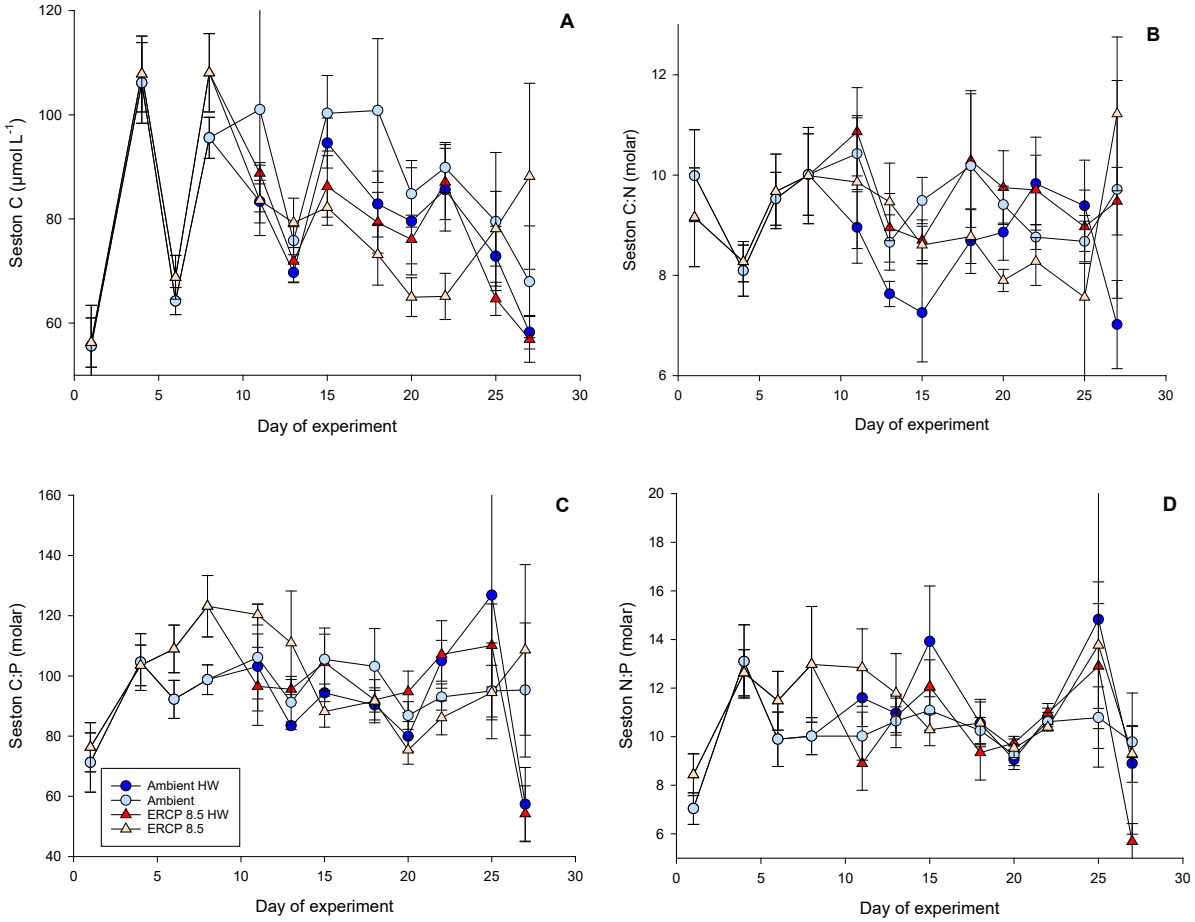
Day of experiment	**Scenario**	**pH**	**TA**	***p*CO₂**
			(µmol $KgSW^{-1}$)	(µatm)
0		8.00±0.00	n.a.	n.a.
1	Ambient	8.05±0.01	2195.2±1.9	433.0±10.7
	Ambient HW	8.04±0.00	2217.6±40.4	447.0±10.4
	ERCP 8.5	7.83±0.02	2206.±6.4	854.2±13.5
	ERCP 8.5 HW	7.83±0.00	2198.1±2.1	851.6±5.6
4	Ambient	8.16±0.02	2189.7±5.3	441.5±15.5
	Ambient HW	8.15±0.02	2215.5±62.6	457.9±29.6
	ERCP 8.5	8.02±0.03	2199.4±7.1	615.6±42.7
	ERCP 8.5 HW	8.01±0.03	2188.0±5.7	634.3±39.1
6	Ambient	8.20±0.02	2232.7±4.8	404.2±15.0
	Ambient HW	8.20±0.02	2226.1±13.8	410.4±15.8
	ERCP 8.5	8.07±0.01	2240.9±3.1	556.2±16.4
	ERCP 8.5 HW	8.07±0.01	2241.4±2.4	556.3±16.2
8	Ambient	8.16±0.01	2220.6±13.4	442.2±10.2
	Ambient HW	8.16±0.02	2214.2±11.1	443.8±16.7
	ERCP 8.5	8.06±0.01	2215.4±15.3	569.5±6.3
	ERCP 8.5 HW	8.05±0.01	2219.3±5.3	584.3±7.2
11	Ambient	8.17±0.03	2208.7±5.9	430.3±27.8
	Ambient HW	8.15±0.05	2258.2±75.7	465.4±46.2
	ERCP 8.5	8.06±0.03	2224.4±6.0	572.6±28.8
	ERCP 8.5 HW	8.05±0.04	2223.7±8.8	587.1±45.8

13	Ambient	8.15±0.01	2199.7±3.1	448.6±8.5
	Ambient HW	8.13±0.01	2205.5±20.8	474.6±11.6
	ERCP 8.5	8.05±0.01	2206.3±6.4	574.2±16.3
	ERCP 8.5 HW	8.01±0.01	2211.9±3.4	636.4±12.1
15	Ambient	8.13±0.01	2211.7±8.1	478.7±3.9
	Ambient HW	8.11±0.02	2233.8±35.1	510.6±25.2
	ERCP 8.5	8.02±0.01	2217.9±2.8	616.1±19.6
	ERCP 8.5 HW	7.98±0.02	2220.3±7.3	685.9±22.2
18	Ambient	8.07±0.01	2200.8±10.3	549.3±9.2
	Ambient HW	8.07±0.02	2229.3±47.2	560.1±23.2
	ERCP 8.5	7.99±0.02	2207.7±8.3	666.6±33.5
	ERCP 8.5 HW	7.96±0.03	2205.5±7.5	706.4±35.7
20	Ambient	8.06±0.02	2196.1±6.7	561.5±18.1
	Ambient HW	8.06±0.02	2265.6±135.8	582.4±29.8
	ERCP 8.5	7.96±0.02	2202.6±3.5	709.4±31.6
	ERCP 8.5 HW	7.96±0.02	2202.1±4.9	713.2±24.7
22	Ambient	8.02±0.02	2195.9±12.5	610.1±22.7
	Ambient HW	8.03±0.03	2195.8±3.9	606.9±32.7
	ERCP 8.5	7.93±0.02	2207.3±6.6	758.6±36.2
	ERCP 8.5 HW	7.92±0.03	2198.2±7.3	773.6±38.7
25	Ambient	8.00±0.01	2197.0±23.2	643.5±19.6
	Ambient HW	8.02±0.03	2203.0±26.3	619.6±31.2
	ERCP 8.5	7.94±0.03	2209.8±3.3	751.3±50.6
	ERCP 8.5 HW	7.91±0.02	2197.8±11.1	801.3±43.3
27	Ambient	8.00±0.02	2201.7±21.8	649.0±28.0
	Ambient HW	8.03±0.01	2253.0±47.7	618.1±9.5
	ERCP 8.5	7.94±0.03	2197.0±33.9	743.3±61.9
	ERCP 8.5 HW	7.91±0.02	2241.5±13.7	816.7±32.9

Supplementary Table 4: Dissolved nutrient concentrations in the mesocosms throughout the experiment. Phosphate (PO_4^{3-}), dissolved inorganic silica (Si), Nitrite (NO_2^-), Nitrate (NO_3^-), Ammonia (NH_4).

Day of experiment	**Scenario**	**PO_4^{3-}** (µmol L^{-1})	**Si** (µmol L^{-1})	**NO_2^-** (µmol L^{-1})	**NO_3^-** (µmol L^{-1})	**NH_4** (µmol L^{-1})
0		0.33±0.01	0.39±0.01	0.00±0.00	0.03±0.00	0.27±0.08
1	Ambient	0.42±0.01	0.39±0.01	0.00±0.01	1.77±0.21	6.10±0.44
	Ambient HW	0.42±0.02	0.40±0.01	0.02±0.03	1.63±0.08	5.91±0.12
	ERCP 8.5	0.25±0.01	0.39±0.01	0.02±0.02	1.71±0.19	6.13±0.28

	ERCP 8.5 HW	0.25±0.00	0.41±0.04	0.01±0.01	1.69±0.08	5.89±0.17
4	Ambient	0.08±0.01	0.00±0.00	0.02±0.02	0.28±0.29	0.59±0.32
	Ambient HW	0.09±0.02	0.00±0.00	0.02±0.01	0.32±0.22	0.51±0.23
	ERCP 8.5	0.04±0.01	0.00±0.00	0.02±0.01	0.08±0.14	0.28±0.17
	ERCP 8.5 HW	0.07±0.04	0.01±0.01	0.02±0.02	0.16±0.21	1.15±1.53
6	Ambient	0.03±0.01	0.00±0.01	0.00±0.00	0.02±0.02	0.36±0.16
	Ambient HW	0.03±0.01	0.00±0.00	0.03±0.02	0.02±0.02	0.34±0.33
	ERCP 8.5	0.04±0.02	0.00±0.00	0.12±0.16	0.06±0.09	0.32±0.30
	ERCP 8.5 HW	0.02±0.01	0.00±0.00	0.01±0.01	0.01±0.01	0.19±0.13
8	Ambient	0.03±0.01	0.01±0.01	0.01±0.01	0.07±0.06	0.33±0.24
	Ambient HW	0.02±0.00	0.01±0.01	0.01±0.02	0.04±0.03	0.19±0.08
	ERCP 8.5	0.03±0.01	0.02±0.02	0.02±0.02	0.07±0.07	0.74±1.16
	ERCP 8.5 HW	0.05±0.04	0.02±0.01	0.01±0.01	0.07±0.07	0.49±0.73
11	Ambient	0.02±0.01	0.05±0.01	0.00±0.00	0.03±0.06	0.23±0.05
	Ambient HW	0.02±0.01	0.07±0.02	0.00±0.00	0.00±0.00	0.26±0.02
	ERCP 8.5	0.03±0.01	0.05±0.01	0.00±0.00	0.00±0.00	0.23±0.03
	ERCP 8.5 HW	0.02±0.01	0.08±0.01	0.00±0.00	0.00±0.00	0.24±0.01
13	Ambient	0.02±0.01	0.09±0.01	0.00±0.00	0.00±0.00	0.34±0.04
	Ambient HW	0.02±0.01	0.12±0.02	0.00±0.00	0.03±0.05	0.41±0.07
	ERCP 8.5	0.01±0.01	0.07±0.02	0.00±0.00	0.00±0.00	0.31±0.04
	ERCP 8.5 HW	0.02±0.00	0.12±0.01	0.00±0.00	0.00±0.00	0.32±0.03
15	Ambient	0.02±0.01	0.13±0.01	0.00±0.00	0.02±0.03	0.32±0.04
	Ambient HW	0.03±0.01	0.19±0.01	0.00±0.00	0.00±0.00	0.50±0.14
	ERCP 8.5	0.04±0.01	0.11±0.01	0.01±0.02	0.12±0.21	0.35±0.06
	ERCP 8.5 HW	0.04±0.00	0.18±0.01	0.00±0.00	0.00±0.00	0.37±0.06
18	Ambient	0.04±0.01	0.17±0.01	0.00±0.00	0.00±0.00	0.35±0.09
	Ambient HW	0.02±0.01	0.24±0.02	0.00±0.00	0.00±0.00	0.38±0.12
	ERCP 8.5	0.01±0.01	0.16±0.03	0.00±0.00	0.02±0.03	0.31±0.06
	ERCP 8.5 HW	0.02±0.02	0.23±0.02	0.00±0.00	0.00±0.00	0.31±0.11
20	Ambient	0.01±0.00	0.20±0.02	0.00±0.00	0.09±0.18	0.51±0.16
	Ambient HW	0.01±0.01	0.25±0.01	0.00±0.00	0.00±0.00	0.65±0.17
	ERCP 8.5	0.02±0.02	0.22±0.05	0.00±0.00	0.00±0.00	0.57±0.12
	ERCP 8.5 HW	0.02±0.01	0.25±0.03	0.00±0.00	0.01±0.02	0.69±0.41
22	Ambient	0.04±0.02	0.20±0.02	0.00±0.00	0.00±0.00	0.40±0.08
	Ambient HW	0.03±0.02	0.25±0.01	0.00±0.00	0.00±0.00	0.35±0.09
	ERCP 8.5	0.04±0.02	0.20±0.02	0.00±0.00	0.00±0.00	0.38±0.14
	ERCP 8.5 HW	0.02±0.01	0.24±0.04	0.00±0.00	0.00±0.00	0.30±0.07
25	Ambient	0.03±0.02	0.17±0.04	0.00±0.00	0.00±0.00	0.56±0.44
	Ambient HW	0.03±0.03	0.22±0.02	0.06±0.11	0.11±0.14	0.49±0.16
	ERCP 8.5	0.01±0.00	0.17±0.03	0.00±0.00	0.00±0.00	0.29±0.05
	ERCP 8.5 HW	0.02±0.02	0.18±0.06	0.09±0.1	0.06±0.06	0.42±0.14
27	Ambient	0.02±0.01	0.08±0.06	0.01±0.02	0.01±0.02	0.44±0.21
	Ambient HW	0.01±0.01	0.17±0.03	0.00±0.01	0.00±0.00	0.28±0.10
	ERCP 8.5	0.01±0.00	0.13±0.02	0.00±0.00	0.00±0.00	0.26±0.05
	ERCP 8.5 HW	0.01±0.00	0.11±0.04	0.00±0.00	0.00±0.00	0.30±0.04



Supplementary Figure 1: Seston carbon concentrations (A) and seston C:N (B), C:P (C), N:P (D) stoichiometry in the mesocosms throughout the experiment. Different colours and symbols represent the Ambient scenario (circle) and Extended Representative Concentration Pathway (ERCP) scenario (triangle) with and without heatwave (light blue = Ambient, dark blue = Ambient HW, orange = ERCP 8.5, red = ERCP 8.5 HW). Symbols represent means and standard errors of four replicates per scenario.

Determination of phycotoxins

During the water collection for the mesocosm experiment, samples were taken for the qualitative detection of phycotoxins by vertical phytoplankton net tows (20 µm mesh size) performed over a depth of 17 m, and 800 L seawater were collected for quantitative phycotoxin analysis. The combined net-tow concentrates and the 800 L seawater were fractionized separately, using a filter tower including 200, 50, and 20 µm mesh sizes. Each size fraction was rinsed into a 45 mL centrifugation tube with 5 µm filtered seawater and centrifuged at 3220 x g and 10 °C for 10 minutes (model 5810R, Eppendorf, Hamburg, Germany), and the supernatants were discarded. The pellets were resuspended in 1-2 mL of filtered seawater, transferred to 2 mL cryovials (Sarstedt micro tube, Nümbrecht, Germany) and centrifuged again at 16,000 x g for 5 minutes (Eppendorf 5415). The remaining fluid was carefully removed with a pipette and the cell pellets were stored at -20 °C until toxin extraction. On the final day of the experiment, 237.5 L of seawater from each mesocosm were size fractionized over a filter tower (200, 50 and 20 µm). Different fractions were transferred to 45 mL centrifugation tubes and dried by centrifugation as mentioned above.

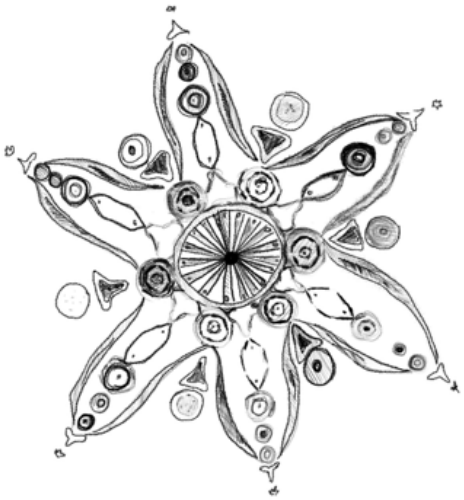
For the extraction of toxins, 500 µL of UHPLC grade methanol and 0.9 g lysing matrix D (Thermo-Savant, Illkich, France) were added to each sample. Cells were lysed by reciprocal shaking in a FastPrep instrument for 45 s at 6.5 m s^{-1} (FastPrep-24, MP Biomedicals, Eschwege, Germany). Subsequently, the samples were centrifuged at 16,000 x g for 15 minutes at 10 °C (Eppendorf 5415) and the supernatants were spin filtered at a cut-off of 0.45 µm (Millipore, Eschborn, Germany) for 30 seconds at 845 x g. The filtrates were transferred to glass vials and stored at -20 °C until analysis by LC-MS/MS. Measurements were performed on a model 1100 LC liquid chromatograph (Agilent, Waldbronn, Germany), coupled to an ABI-Sciex 4000 Q Trap triple-quadrupole mass spectrometer (Applied Biosystems, Darmstadt, Germany). Reversed-phase chromatography was performed on a C_8 column (50 x 2 mm), packed with 3 µm Hypersil beads (Phenomenex, Aschaffenburg, Germany) at 20 °C. Elution was performed with eluent A (water) and eluent B (acetonitrile), both enriched with 50 mM formic acid and 2 mM ammonium formate. Chromatography consisted of 12 minutes column equilibration with 5 % B, a linear gradient to 100 % B within 10 minutes, 6 minutes of isocratic elution with 100 % B and return to initial conditions within 3 minutes, resulting in a total run time of 31 minutes at a constant flow rate of 0.2 mL min^{-1}. For each sample and the standards, 5 µL were injected. Chromatography and mass spectrometry were divided into three periods for different toxins. The initial 8 minutes were for the detection of domoic acid, followed by a 2.5-minute-long period for the measurement of gymnodimines and spirolides and finally a 5.5-minute-long period for goniodomin A, okadaic acid, dinophysistoxins, azaspiracids, pectenotoxins and yessotoxin (Table X). Parameters of the MS/MS were as follows: Ion-Spray-Voltage: 5500 V, temperature: 275 °C, nebulizer gas: 50 psi, auxiliary gas: 50 psi, declustering potential: 50; entrance potential:10 V, exit potential:15 V, curtain gas: 20 psi during the first period, 10 psi during the second and third period.

Detected toxins were quantified against external standards using the software Analyst (version 1.5, Applied Biosystems).

*Table 1: Lipophilic toxins including mass transitions, retention times, and period the samples were screened for. DA=Domoic acid, GYM= Gymnodimine, SPX= Spirolide, GON= Goniodomin, OA= Okadaic acid, DTX=Dinophysistoxin, AZA= Azaspiracid, PTX= Pectenotoxin, YTX=Yessotoxin. *= Standard available. Retention times are only given for standards.*

Toxin	Mass transition (*m/z*)	Retention time (min)	Period	Collision energy (V)	LoD (pg NT^{-1})	LoD (pg L^{-1})
DA*	312→266	6.7	1	20	348	2.89
GYM A*	508→490	8.98	2	40	1.18	0.01
SPX A	692→150		2	55		
SPX 1*	692→164	9.45	2	55	73.6	0.62
SPX B	694→150		2	55		
SPX C	706→164		2	55		
20Me- SPX G	706→164		2	55		
GON A	786→607		3	55		
OA*	822→223	11.09	3	55	4030	34.0
DTX-2	822→223		3	55		
DTX-1*	836→237	12.07	3	55	5560	46.8
AZA-1*	842→824	12.33	3	55	30.5	0.26
PTX-12	874→213		3	55		
PTX-2*	876→213	11.64	3	55	574	4.84
PTX-2-sa	894→213		3	55		
YTX*	1160→965	12.32	3	55	956	8.05

5
Synthesis



This thesis aimed to enhance our understanding of the compositional and functional temperature responses of marine protist communities. Overall, it highlighted the importance of experimentally assessing temperature responses at the community level. Mimicking a natural environment in which interactions can take place enables an identification of the species that become dominant and thus an inference of community reorganisation principles under abiotic changes. Further, this level of complexity allows us to derive potential ecosystem consequences, as species often interactively determine the functional outcome. The discussion will integrate the results obtained in all chapters with the broader literature from different research fields. Overarching patterns are derived by compiling similarities across chapters, and system-specific attributes are inferred from their differences. Each of the six thesis objectives will be addressed in a separate subchapter.

5.1 Overarching patterns under warming

Across all three experiments, overarching patterns could be identified which will be discussed in the following chapters to answer the first four objectives.

5.1.1 Community reorganisation principles

One major goal of this thesis was to identify principles that underlie the observed patterns of community diversity and compositional responses under temperature changes (objective 1). For warming as a single driver, parameters of the species' thermal performance curves (TPC) are likely the most important determinant for community reorganisation (Anderson et al. 2024). These include for example thermal limits, optimum temperatures, growth increments with warming and the thermal breadth (see chapter 1.1.2). All TPC parameters can vary with other traits, such as the trophic mode, the cell size and the thermal history. Therefore, temperature changes can induce community shifts between organisms with different characteristics.

According to the metabolic theory of ecology (MTE, see chapter 1.3.2), the temperature sensitivity of heterotrophic processes is greater than that of phototrophic ones (e.g. Regaudie-de-Gioux and Duarte 2012, Boscolo-Galazzo et al. 2018). Consistent with this, chapter 3.1 shows that oxygen production rates decrease with warming, possibly due to a stronger increase in community respiration compared to photosynthesis. Furthermore, the upper thermal limits of heterotrophic protists appeared to be higher as their diversity was more resistant to warming (chapter 4.1). However, across all three experiments, this did not translate into a relative increase of heterotrophic protists compared to phototrophs under warming (Figure 9).

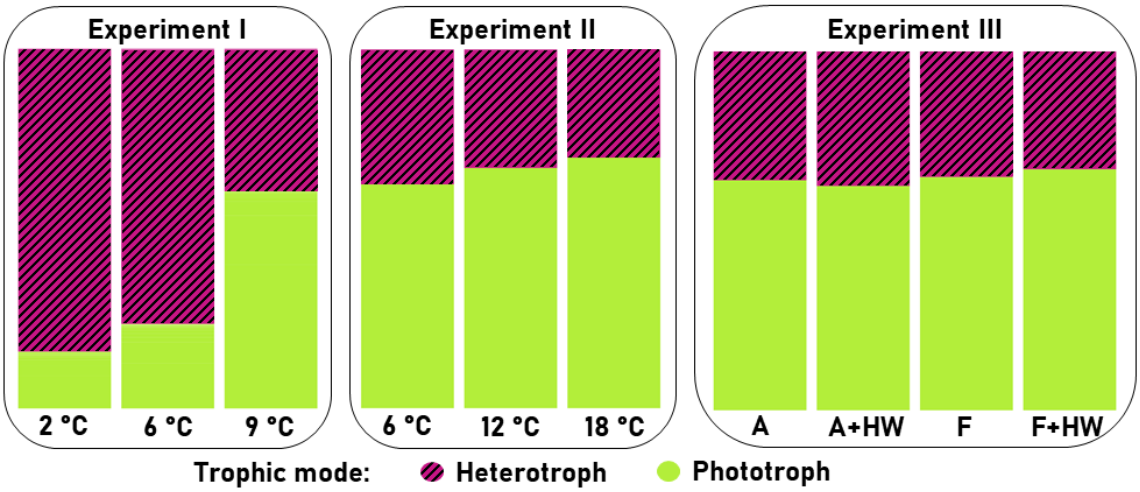


Figure 9: Trophic modes of the protist species assessed via 18S rRNA metabarcoding across all three publications. The data from publication II and III are the mean values per temperature throughout the incubation. A = ambient conditions, A + HW = ambient plus heatwave, F = future scenario (+ 3 °C, pCO2 of 1000 ppm, N:P of 25), F + HW = future scenario plus heatwave.

One reason might be that the differential growth of hetero- vs. phototrophs depends on factors like the season and region (Cabrerizo and Marañón 2021) or the nutrient regime (López-Urrutia 2008, Chen et al. 2012). This thesis found variations among regions, but not among seasons or nutrient regimes. While the proportions among hetero- and phototrophs were similar across different temperatures in both North Sea communities (chapter 3.1 & 4.1), warming induced a decrease of heterotrophs relative to phototrophs in the Arctic community (chapter 2). However, this may also be a confounding result of the different incubation volumes. Furthermore, there was no evidence for differences across different nutrient conditions, i.e. replete (chapter 2), reduced (chapter 3.1 & chapter 4.1 A and A+HW) and limiting (chapter 4.1 F and F+HW). Even under a wide resource supply gradient, there was no effect of different N:P ratios on grazing rates (chapter 3.3). Another reason could be that growth differences between heterotrophic and phototrophic protists only manifest themselves over longer time scales, as their thermal dependencies are potentially closer together than assumed (Wang et al. 2019). However, there is evidence from stream communities that metabolic compensation can constrain differential temperature dependence at the community level in the long term (Padfield et al. 2017). Therefore, it is likely that other covarying traits were simply more important for species sorting processes under warming than the trophic mode.

As there was no clear decrease in cell size with warming in the communities of chapter 2 and 3, this was also not the most important trait, contrasting theoretical considerations. In general, smaller organisms have a higher surface-to-volume ratio, which enables a more efficient uptake of dissolved gases and nutrients per body mass (Lindemann et al. 2016, Deutsch

et al. 2022). Therefore, it is assumed that the mean size within a community decreases with warming as metabolic demands increase (Daufresne et al. 2009, Gardner et al. 2011), exacerbated by the higher growth increments of smaller cells with warming (Kremer et al. 2017). There is a broad body of literature supporting this theory for protist communities based on field observations (Rasconi et al. 2015, Hillebrand et al. 2022), experimental incubations (Atkinson et al. 2003, Lewandowska and Sommer 2010, Yvon-Durocher et al. 2011, Tan et al. 2021) and modelling studies (Vernet et al. 2017, Chen et al. 2020). Still, although some larger species relatively increased at the coldest temperature of the second experiment (chapter 3.1), there was no overall size difference under warming (chapter 4.3). Absent temperature-size relationships have previously been reported for single-species incubations (Fernandez-Gonzalez and Maranon 2021) and have also been found in other organisms such as endo- and ectothermic animals (Riemer et al. 2018, Siepielski et al. 2019). Another theory poses that cells of intermediate size have the highest volume-specific metabolic rates (Marañón et al. 2013, Hillebrand et al. 2021) and that under nutrient-replete conditions these should dominate, which partly aligns with the results from chapter 2. Potentially, the warming-induced size reductions described by the literature are a confounding result of factors that correlate with temperature such as grazing or decreased nutrient concentrations (Peter and Sommer 2012, 2013, Hillebrand et al. 2021). The fact that a clear size pattern could only be observed in chapters 4.1 and 4.2, in which nutrients were manipulated along with temperature, supports this assumption. Thus, like the trophic mode, cell size may not be the most informative trait for community reorganisation when considering temperature as a single driver.

In all three experiments, the species' thermal histories were found to be the best explanation for community shifts under changing temperatures. These histories could be either acute, in terms of thermal acclimation, or longer-term, reflecting the thermal environment to which the species have adapted. In chapter 2, temperate organisms gradually increased under warming regarding their relative abundances and Arctic-adapted species drastically decreased, especially when their thermal limits were reached. This aligns with the biogeographic distribution of diatom genotypes and endemic species in the Svalbard area along a gradient of Atlantic water influence and therefore temperature (Šupraha et al. 2022). Next to an adaptation to higher average temperatures, the invasion potential of temperate species may additionally be based on the thermal fluctuations of their original environment, as has been demonstrated in Baltic Sea communities (Santelia 2022) and vascular plants (Miller et al. 2021). In chapter 3.1, the community TPC indicates that likely no thermal limits were exceeded (Figure S1). Still, the environmental distribution of species well reflected emerging compositional patterns under

warming, with an increase of warm-water and a decrease of cold-water species. Furthermore, chapter 4.1 suggests a pre-selection of species based on their thermal histories and associated acclimation potentials under ambient and warming conditions, since either the warming or cooling of the subsequent heatwave strongly reduced diversity. The relevance of thermal histories is supported by a variety of studies, including compilations of incubation assays (Coello-Camba and Agustí 2017, Bishop et al. 2022, Ye et al. 2023), biogeographic distribution analyses (Thomas et al. 2012, Chen 2015), biodiversity experiments (Bestion et al. 2020, Zhong et al. 2020) and a combination of experimental work and modelling (Bestion et al. 2018, Anderson and Rynearson 2020, Anderson et al. 2024).

However, it should be noted that the species' traits (i.e. trophic mode, cell size, thermal history) are only inferred from the existing literature, as they could not be measured live during the incubations. Since this might distort the actual trait diversity of the communities, I propose new experimental designs to address this limitation in chapter 5.3.

KEY OUTCOME

The thermal histories of the present species primarily drive community reorganisation under warming, with their trophic modes and cell sizes playing minor roles.

5.1.2 Winner species

If we want to gain a better understanding of future ecosystem functioning, we need to identify and characterise those species that prevail under environmental change. Therefore, the second aim of this thesis was to determine potential winners of global warming (objective 2). Regarding the phototrophic community, some overall trends could be derived from the three experiments. Generally, at ambient temperature, diatoms mostly remained dominant (chapter 2 & 3.1) or were able to increase their relative abundance again towards the end of the incubation (chapter 4.1). At temperatures moderately elevated above ambient conditions (experiment I: + 4°C, experiment II: + 6 °C, experiment III: + 3-5 °C), haptophytes increased in their relative abundance. This was either *Phaeocystis pouchetii* (chapter 2), *Phaeocystis globosa* (chapter 3.1), or the coccolithophore *Gephyrocapsa oceanica* (chapters 3.1 & 4.1). Under nutrient-replete conditions (chapter 2), the growth rates of haptophytes were not sufficiently high to outcompete diatoms, but haptophytes were able to dominate in settings in which phosphate was scarce (chapter 3.1) or in which nitrate, silicate and phosphate were depleted (chapter 4.1). At even higher temperatures (experiment I: + 7°C, experiment II: + 12 °C), the relative

contribution of haptophytes diminished and mainly diatoms predominated, regardless of the nutrient conditions. Within the diatoms, a low evenness with one dominating species was observed at ambient incubation temperatures and a higher evenness and species richness under substantial warming for the given habitat (chapters 2 & 3.1).

These findings align with existing literature, illustrating diverse thermal niches of diatoms, with few species thriving at very low temperatures and many that cope with novel high temperatures (Kling et al. 2020, Anderson et al. 2021). However, at certain temperatures, haptophytes outcompete diatoms, especially under low nutrient conditions (Gypens et al. 2007, Nöthig et al. 2015, Mori et al. 2021, Breton et al. 2022). Therefore, in contrast to other studies (Remy et al. 2017, Soulié et al. 2022), haptophytes instead of diatoms dominated under heatwaves in the experiment of this thesis (chapter 4.1), likely due to nutrient depletion (chapter 4.2). This is further evidenced by the higher haptophyte diversity compared to diatoms in oligotrophic oceans such as the Central Pacific (Endo et al. 2018). Indeed, coccolithophores like *Gephyrocapsa oceanica* are known to thrive in environments with high temperatures and low nutrient concentrations (McKew et al. 2015, Moreno et al. 2022, Wang et al. 2024). Additionally, *Phaeocystis globosa* has been shown to endure phosphate limitation (Chai et al. 2023). In comparison to *Gephyrocapsa oceanica*, however, *Phaeocystis spp.* require higher nitrate concentrations and are predominantly observed in spring (Gieskes et al. 2007, Smith and Trimborn 2024). Studies on the colony formation of *Phaeocystis* (Zhang et al. 2020, Cheng et al. 2023) and on its growth under warming (Aflenzer et al. 2023) confirm that *Phaeocystis* only thrives within a narrow temperature range. This is consistent with the species' dominance under intermediate temperature elevations, as observed in this thesis (chapter 2 & 3.1).

In terms of heterotrophic protists, I could not identify any species that profits from warming, although in chapters 3.1 and 4.1 there was a slight increase in potentially parasitic organisms. Still, considering that parasites can change their hosts' thermal performance (Padfield et al. 2020), even small changes in parasite abundances may affect community dynamics in a warmer ocean. Since species belonging to the group of unidentified marine stramenopiles (MAST) consistently diminished with warming (above 6 °C) in all experiments, they are likely to play a minor role in future high-latitude oceans if no warm-water adapted species invade (Lin et al. 2022). Although it has to be noted that micro- or mesocosm incubations can always skew the results by adversely affecting some species more than others (Venrick et al. 1977, Calvo-Díaz et al. 2011), the differences between treatments still allow inferences on potential temperature responses.

KEY OUTCOMES

- Haptophytes like *Phaeocystis spp.* (spring) and *Gephyrocapsa oceanica* (summer) thrive with moderate warming and at low phosphate levels.
- Diatoms dominate under current temperatures and stronger warming, as long as there is sufficient nutrient availability.

5.1.3 Ecosystem consequences

The emerging dominant species often contributes most to the functional output of communities under environmental change (Bestion et al. 2020). Therefore, identifying prevailing species helps to assess the functional consequences of ocean warming for the ecosystem (objective 3). In all experiments, warming induced the dominance of similar protist groups, which makes it logical to assume that there were also similar functional responses. In both spring communities, warming led to a stronger increase in particulate organic carbon, a proxy for overall biomass, and chlorophyll *a*, a proxy for phototrophic biomass (chapters 2 & 3.1). This finding is consistent with observations from high-latitude oceans where protist communities also accumulate biomass from spring to summer due to higher growth rates as temperatures rise (De Senerpont Domis et al. 2014, Lewandowska et al. 2014, González-Gil et al. 2022). However, despite the initial nutrient pulse, there was no increase in particulate organic carbon under warming within the temperate summer community (chapter 4.2). A potential reason is that the temperature optimum of the community may have been surpassed so that warming cannot translate into higher community biomass accumulation.

The communities were able to increase the uptake of nitrogen along with the higher production of particulate organic carbon, resulting in stable C:N ratios across temperatures (chapters 2 & 3.1). The results are consistent with experimental studies in freshwater systems (Yvon-Durocher et al. 2017, Verbeek et al. 2018). Frost et al. (2023) attribute consistent C:N ratios to the close relationship between nitrate uptake and carbon assimilation, as well as the limited capacity of many species to store excess nitrogen. Recent studies also oppose the notion that *Phaeocystis spp.* colonies should have a higher cellular C:N ratio than other species (Smith and Trimborn 2024, and references therein), which is consistent with the findings of chapter 3.1. Temperature did not generally affect C:P ratios either (chapter 3.1 & 4.2) which contrasts previous studies that reported increasing C:P ratios with warming (Toseland et al. 2013, Yvon-Durocher et al. 2015b). However, mesocosms that were warmed and dominated by *Phaeocystis globosa* consistently exhibited higher C:P ratios (chapter 3.1). Studies conducted in the

Southern Ocean have also observed higher C:P and N:P ratios in *Phaeocystis antarctica* compared to diatoms (Arrigo et al. 1999, Arrigo et al. 2002, Zhu et al. 2016) but this has not yet been demonstrated for the North Sea. These higher ratios are potentially based on the carbon- and nitrogen-rich extracellular matrix of *Phaeocystis* colonies (Solomon et al. 2003). Another reason could be that *Phaeocystis spp.* may require less phosphorus per carbon due to lower regulatory costs, resulting in fewer P-rich ribosomes needed for similar metabolic processes compared to other species (McCain et al. 2021).

A warmer future ocean with a higher abundance of *Phaeocystis spp.* could have significant implications for the entire marine food web, biogeochemical cycles and therefore climate change itself (Smith and Trimborn 2024). *Phaeocystis* colonies are generally a poor food source for mesozooplankton (chapter 4.2; Gasparini et al. 2000) which could be exacerbated by the high C:P ratios (Thomas et al. 2022). In addition, *Phaeocystis*-rich aggregates may enhance bacterial degradation and nutrient recycling, which could ultimately dampen carbon export and sequestration (Figure 11; Reigstad and Wassmann 2007, Wolf et al. 2016, Meyer et al. 2022). However, carbon export of *Phaeocystis*-dominated communities can be increased by mixing events and eddies (Lalande et al. 2011, Dall'Olmo et al. 2016, Jones and Smith 2017) or under concurrent high diatom abundances (Le Moigne et al. 2015, Balaguer et al. 2023).

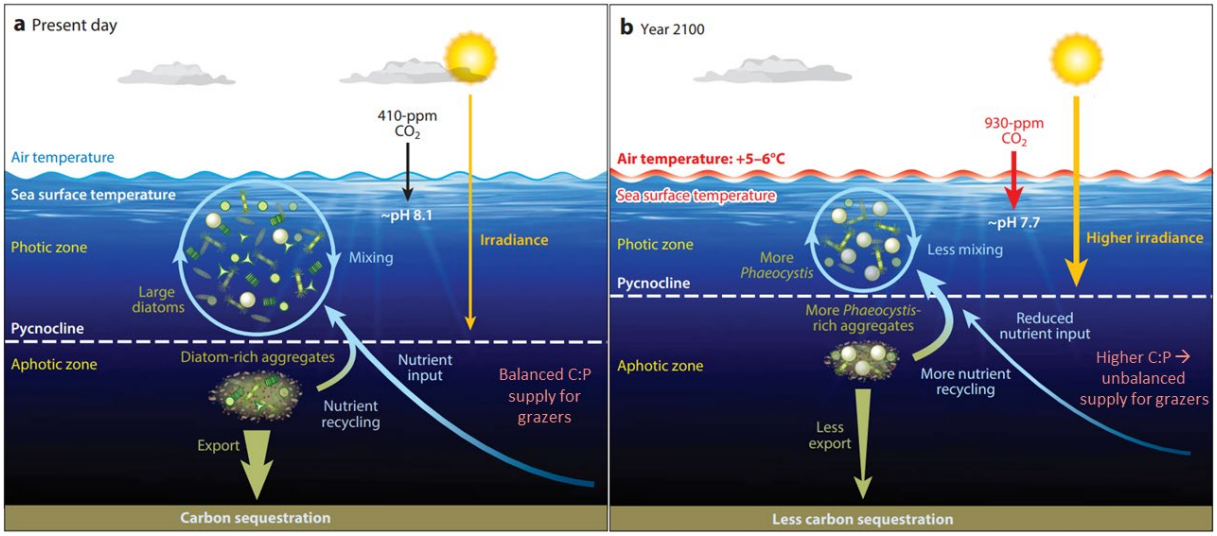


Figure 10: Simplified illustration of the functional impact of (a) diatom-dominated communities (under today's conditions) and (b) Phaeocystis-dominated communities in a potential future scenario. This illustration only displays differences between the two scenarios – factors that remain constant, such as the C:N ratio, are not shown. Modified after Smith and Trimborn (2024).

In the temperate spring community (chapter 3.1), oxygen production was observed to decrease with warming, likely due to respiration increasing faster than photosynthesis (Regaudie-de-

Gioux and Duarte 2012). As there was no relative increase in heterotrophic protists (chapter 5.1.1), either an increase of prokaryotes or the metabolic balance within the phototrophic community must be the basis for this shift. This can occur on a cellular level (Bozzato et al. 2019, Rehder et al. 2023) or due to compositional changes (López-Sandoval et al. 2014, Chen and Laws 2017).

KEY OUTCOMES

- Warming decreases the oxygen production but enhances the biomass accumulation of protist communities in spring.
- The community C:N ratio remains unaffected by warming and the C:P ratio increases with the dominance of *Phaeocystis globosa*.

5.1.4 Modulation by other drivers

While temperature can be the dominating factor for planktonic community composition in the field (Trombetta et al. 2021), other drivers often modulate its responses (Litchman and Thomas 2023). Thus, another objective of this thesis was to evaluate how abiotic factors can modify the community response to temperature (objective 4). This synthesis focuses on the effect of inorganic nutrients, as it was the only driver that played a role across all three experiments and is likely one of the most important modulators for the temperature responses of marine microbial communities (see chapter 1.3.3).

A side experiment of chapter 2 revealed that nitrate concentrations did not significantly alter the compositional response of the phototrophs to temperature in the Arctic community (Appendix 1). This may be because the lowest nitrate concentration was still at 16 µM, and thus nitrogen was not limiting the growth of most species (Eppley et al. 1969, Lewis et al. 2019, Henley et al. 2020). Gerhard et al. (2019) found that the community response to temperature variations can be affected by the absolute concentration of nutrients. However, the responses were measured after the initial supply of nutrients was likely depleted. Therefore, in cases of low or limiting nutrient availability, nutrients seem to influence the community outcome of warming.

In chapter 3.1, very low phosphorus concentrations (< 0.3 µM) appeared to give haptophytes a competitive advantage under intermediate warming. Additionally, although compositional data for chapter 3.2 is unfortunately unavailable, it was shown that the timing of nutrient addition affected the community's growth rate response and their N:P ratio. This may indicate a shift towards different species that are better at exploiting and growing in a specific

nutrient-temperature combination, as observed in other studies (Serra-Pompei et al. 2019, Anderson et al. 2022). One possible explanation for this is that the minimum resource requirement (R*) is at a lower temperature compared to the thermal optimum for growth within many species (Sunday et al. 2023). This means that the competitive outcome changes under warming when nutrients are scarce.

Finally, the compositional changes in the phototrophic community under global change conditions (chapter 4.1) only became apparent after the depletion of dissolved nutrients on incubation day 6 (chapter 4.2). The subsequent heatwave exacerbated the induced dominance of *Gephyrocapsa oceanica*, providing further evidence of the significant impact of nutrient depletion on the temperature response.

KEY OUTCOME

When nutrients become limiting, they change the compositional response of the phototrophic community to temperature.

5.2 System-specific attributes

In addition to overarching patterns, this thesis identified differences between the various investigated scales. The following chapters will compare the community responses from these different settings to answer the last two objectives.

5.2.1 Critical temperatures

The current projections for ocean warming span a wide range of temperatures due to model uncertainties (IPCC 2023). Therefore, to assess future ecosystem states, it is important to identify the temperatures that can cause shifts within a given community setting (objective 5). I found that the upper critical temperatures differ among seasons and habitats, while low temperatures apply a strong selective pressure on all marine microbial communities alike.

In the Arctic community incubation of chapter 2, the Shannon diversity increased at 6 °C (+ 4 °C), but drastically decreased at 9 °C (+ 7 °C). This suggests that many Arctic species have an upper thermal limit between these two temperatures, consistent with findings from other authors (e.g. Coello-Camba 2015, Coello-Camba and Agustí 2017). For Arctic protist diversity, ≥ 9 °C can thus be considered a critical temperature range. However, warming to 6 °C could still induce changes in the ecosystem due to the relatively higher abundance of species such as *Phaeocystis pouchetii* (Appendix 1). In contrast, the diversity of the temperate community in

the spring incubation (chapter 2) did not decrease at 12 °C (+ 6°C) or even at temperatures as high as 18 °C (+ 12 °C). This is not surprising considering the natural development of North Sea water temperatures (Käse et al. 2020). The thermal performance curve of the phototrophic community (chapter 2.1, Figure S1) shows that temperatures only become supra-optimal at 21 °C and lethal at 30 °C, i.e. at temperatures that are not even projected for the North Sea in a worst-case scenario (Schrum et al. 2016). However, the degree of warming determined the relative proportion of *Phaeocystis globosa* to diatoms and the compositional variability, i.e. the relevance of ecological stochasticity, gradually increased (chapter 3.1), congruent with other studies (Henson et al. 2021, Pálffy et al. 2021). Therefore, ecosystem changes may occur at + 6 °C, even though + 12 °C cannot be considered critical for the North Sea protists themselves. In summer, a diversity decline can occur with much smaller temperature increases. Chapter 4.1 demonstrates that the diversity of phototrophic organisms in a North Sea summer community is resilient under a marine heatwave alone (18 °C, + 2 °C), but compromised by global change (19 °C, + 3 °C above ambient), and also by both applied together (21.1 °C, + 5 °C). As all tested scenarios negatively affected other trophic levels (bacterio-, and mesozooplankton; chapter 4.2), even small temperature increases can have critical functional impacts in temperate summer communities. It is important to note that the temperature levels applied in this thesis were not gradual and recent studies have rightly questioned the concept of tipping points in ecological systems (Hillebrand et al. 2020, Hillebrand et al. 2023). While I argue that the thermal limits of specific species do set temperature thresholds to the diversity of protist communities, they may not entail abrupt ecosystem shifts. As evident from all three experiments, gradual functional changes likely already occur at various points below these thresholds, especially under multiple drivers and regarding multiple ecosystem functions.

Low temperatures, whether in the form of the ambient control (Chapter 2 - Appendix 2 & Chapter 3.1) or the temperature drop at the end of the heatwave (Chapter 4.1), resulted in lower species evenness but not lower richness across all three experiments. This suggests that while most species were able to survive the low temperatures, only a few were competitive enough to dominate the communities. Furthermore, many temperate species only started increasing at 9 °C in the mixed community from the Fram Strait (chapter 2). Accordingly, the thermal performance curve of the North Sea community in chapter 3.1 (Figure S1) shows that the lower thermal limit for phototrophic growth is at 6 °C, a temperature often observed at the onset of the bloom in the field (Käse et al. 2020). Therefore, this thesis contradicts the idea that light alleviation mainly triggers the spring bloom. Together with the fact that many species can likely make use of very low light intensities (Raven et al. 2000, Hoppe 2021), I propose

temperature is the primary abiotic driver of bloom initiation in the North Sea. This has significant implications for future bloom phenology and can cause potential trophic mismatches (Edwards and Richardson 2004) if spring temperatures increase earlier in the year (Schrum et al. 2016). However, there are also scenarios in which Northern European temperatures may rapidly decrease due to a slowdown of the Atlantic meridional overturning circulation (Ditlevsen and Ditlevsen 2023). Although decreasing water temperatures are not the most likely scenario, other factors can exacerbate their adverse effects on protist community composition and functions (Hoppe et al. 2018). Therefore, it is necessary to conduct further scientific research on cold temperatures to fully understand their effects. The same accounts for the temperature drop at the end of marine heatwaves, as these will become more frequent and intense by the end of the century (Oliver et al. 2019).

KEY OUTCOMES

- Critical warming for adverse effects on ecosystem functions varies by season and habitat, being consistently lower than the threshold compromising protist diversity.
- Cold temperatures are a fundamental constraint on protist diversity.
- Temperature rather than light drives the initiation of the North Sea spring bloom.

5.2.2 Susceptibility among seasons and habitats

The final goal of this thesis was to identify marine microbial communities that may be particularly vulnerable to environmental change and to determine underlying factors (objective 6). According to the critical temperatures outlined in chapter 5.2.1, marine protist communities from Arctic spring and temperate summer are more susceptible to warming than those from temperate spring. Some studies suggest that a higher diversity can increase the resistance of biological communities to thermal fluctuations (Lehman and Tilman 2000, Bestion et al. 2020, Bestion et al. 2021). However, the lower species richness and Shannon diversity of the starting community in the temperate spring community (richness: 172, Shannon: 3.2) compared to the summer (richness: 217, Shannon: 3.6) and Arctic communities (richness: 218, Shannon: 3.4) indicate that this cannot be considered a significant factor. Other possible explanations are a higher intraspecific diversity (Wolf et al. 2018) or that species in the spring community were already better adapted to the new conditions (Huertas et al. 2011), consistent with the key outcome of chapter 5.1.1. Considering the environmental history of these communities, it is

evident that temperate spring communities face significantly higher temperature variations compared to Arctic or summer communities (Timmermans and Labe 2020, Rick et al. 2023). Therefore, temperate spring organisms may be more plastic and quicker to adjust their physiology to warming than the more stenothermic Arctic and summer species, which have historically experienced and adapted to more stable temperatures (Schaum et al. 2022). Another consequence of these environmental histories are narrower thermal breadths (Li 1985, Stock et al. 2019), which means that Arctic and summer species would reach their upper thermal limits faster (Chen 2015). Therefore, temperatures can quickly become supra-optimal, so that even small degrees of warming can drastically reduce performance (Martin and Huey 2008, Kingsolver 2009). Especially in an invasion context like the Fram Strait, this can foster the communities' vulnerability by making them less competitive against species advected from lower latitudes (chapter 2), which has also been observed for amphipods (Schröter et al. 2019, Murray et al. 2023), other metazoans (Ingvaldsen et al. 2021) and in comparable Antarctic regions (Antoni et al. 2024).

Despite not being compromised in their overall diversity, chapter 3.1 demonstrates that the North Sea spring community exhibited the lowest functional redundancy. Warming resulted in variable community compositions that, depending on the dominant species, entailed changes in certain functional outputs (C:P, biomass accumulation). This supports the notion that functional redundancy can vary between different ecosystem functions (Meyer et al. 2018) but contradicts the findings of Zhong et al. (2020), who observed that a cooler and less variable environmental history leads to lower functional redundancy, particularly under warming. One reason for these deviations could be the difference in species richness among the habitats. In this thesis, the habitat with the most variable thermal history (temperate spring) had the community with the lowest species richness. Contrarily, in the study of Zhong et al. (2020), the species richness was consistently higher in the community from the more variable habitat. Therefore, the initial species richness may be a crucial factor for functional redundancy in protists under perturbations, as demonstrated in prokaryotic communities (Fetzer et al. 2015, Garcia et al. 2018, Sierocinski et al. 2018). However, a comprehensive analysis of ecosystem vulnerability would require the consideration of additional dimensions of ecological stability such as the recovery potential or temporal stability (Hillebrand et al. 2018, Urrutia-Cordero et al. 2021).

KEY OUTCOME

Protist communities from the Arctic Ocean and temperate summer are more vulnerable to ocean warming than those from temperate spring, but these exhibit the highest compositional variability and the lowest functional redundancy.

5.3 Future perspectives

The results of this thesis enhanced our knowledge of temperature responses in natural protist communities by integrating insights from diverse environmental settings in micro- and mesocosm incubations. However, several methodological gaps persist, and new research questions have emerged. This chapter outlines the most promising avenues for future research and presents new conceptual ideas to elucidate the complex nature of marine microbial communities under environmental change.

As with many studies examining microbial community responses, the functional traits of the species in this thesis were inferred from the literature. Although this still provides valuable insights into community dynamics (chapter 5.1.1), functional traits are known to be plastic and can be altered by intra- and interspecific interactions (Wolf et al. 2019, Govaert et al. 2021) as well as the environmental setting (D'Aguillo et al. 2022). Therefore, to obtain a more accurate estimation of the organism's trait space and its dynamics within natural community incubations, new experimental concepts are required. A potential solution to identify the contribution of different trait sets to community reorganisation could be a mechanistic multiple-step approach, including strain assays, modelling and experimental incubations. One implementation involves characterising the trait space of key species through single-strain growth assays under various abiotic drivers. This information can then be used to parameterise competition models that include different driver sets. Finally, these models can be tested in incubation assays of artificial mock communities of these strains. Wieczynski et al. (2021) used a similar approach using cell characteristics like their shape or volume, with promising results. However, even more could be achieved by implementing trait landscapes for each species. Such accurate estimations of species' traits could also be used to better parametrize biogeochemical models and thereby reduce their uncertainty (Sullivan et al. 2024).

Another aspect that can significantly alter an organism's response to environmental changes is evolution (Padfield et al. 2016, Schaum et al. 2022). Certain patterns only emerge when including evolution (Wickman et al. 2024), and thermal responses can even be reversed

in the long term both on the species level (Collins 2016, Barton et al. 2020) or when considering species richness on the ecosystem level (Febvre et al. 2024). Although short-term experiments are important to inform on the species that initially survive new conditions (chapter 1.4.2), they only provide limited insight into long-term realities as species within a community may evolve at different rates (Baltar et al. 2019). Therefore, future studies should aim to include an assessment of varying evolutionary trajectories in natural community experiments. This can be achieved by extending the incubation time in mesocosm experiments, ideally covering an entire season or if financially feasible even years, as demonstrated in the studies by Bach et al. (2016) and Yvon-Durocher et al. (2015a). Differential evolution among various key species can then be tracked via common garden assays, similar to those used in the study of Scheinin et al. (2015). Although tracing evolution may be particularly challenging in complex natural communities compared to artificial communities with only a few species (Faillace and Morin 2020, Lachapelle et al. 2022), it is worth attempting. Gaining insights into real-time evolutionary responses within incubations of natural marine communities can be an important cornerstone for complementing space-for-time (Blois et al. 2013, Lovell et al. 2023) and back-in-time approaches (Franks et al. 2018, Hinners et al. 2019).

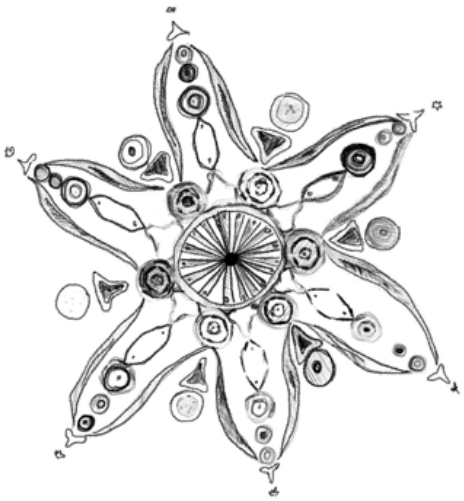
Longer incubation times may also help to distinguish between actual global warming effects and temperature-stimulated accelerated bloom development during the spring-summer transition. To enable a proper cross-comparison of different habitats and seasons, these studies could be conducted community-wide in several mesocosms using the same experimental design and different field communities. This approach is comparable to the work of Boyd et al. (2013), who performed an experiment using the exact same protocol across various labs, and could lead to more generalisable results. The baseline temperature should be adjusted following changes in field temperature (as in chapter 4.2) and sampled communities can be obtained from long-term monitoring stations, such as Helgoland Roads, to provide a 'field control' in addition to the 'ambient mesocosm control'.

The investigation of atlantification in the Arctic Ocean could also benefit from a more realistic approach by strategically combining multiple drivers. Although I have demonstrated that warming alone can provide temperate species with a competitive advantage and increase their invasion potential (chapter 2), this may not hold when other 'fixed' drivers in the Arctic Ocean are considered. It is uncertain whether temperate organisms can cope with the polar environment, which includes lower salinity and prolonged periods of darkness. To address this, the combined impact of temperature and low light/darkness on the competitive abilities of several polar and temperate key species should be investigated in controlled laboratory settings.

Another possible approach is to isolate multiple strains from the same species found in the two different water masses mentioned in chapter 2 (WSC & EGC) and evaluate the impact of their distinct environmental histories on the reaction norms towards light and temperature.

The thesis revealed that low temperatures consistently exert the strongest selective pressure and therefore require further scientific attention. Warming, on the other hand, increases the compositional and functional variability, which can destabilise communities and induce chaotic behaviour (chapter 3.1). A mechanistic assessment of the influence of small abundance differences using artificial communities could provide further insights into the relevance of these stochastic processes. Finally, the critique of the search for tipping points, which may not exist in complex ecological systems (Hillebrand et al. 2020), highlights the need for alternative frameworks to investigate community dynamics in changing environments. In addition to the recommendations proposed by Hillebrand et al. (2023), the scientific focus could be directed towards better characterising compensatory processes on different levels of organisation that accompany gradual ecological changes, such as reductions in antagonistic interactions or increasing evolution (Connell and Ghedini 2015, Hoppe et al. 2018).

6
Conclusion



The diversity of marine protist communities has intrigued and perplexed scientists for many years, especially since the coining of the 'paradox of the plankton' by Hutchinson in 1961. Numerous studies have demonstrated the significance of these communities for the ecosystem, which has stimulated interest in comprehending their complexity (Bachy et al. 2022). Particularly in the context of global change, determining how marine microbes respond to abiotic factors has become a key goal for microbial ecologists (Cavicchioli et al. 2019). This thesis has contributed to our understanding of these communities under global warming on multiple levels (Figure 11). I demonstrated that the thermal histories of community members are more significant for temperature-induced community reorganisation than cell size or trophic mode, across various seasons and habitats. The selection of dominant species is based on the degree of warming, with haptophytes exhibiting a relative increase mainly at temperatures moderately elevated above ambient conditions. Furthermore, this thesis identified common changes in the functional output, a modulating role of nutrient limitation and low temperatures to be a major constraint for marine protist communities. This indicates that temperature is potentially the primary driver for spring bloom initiation. Functional shifts become more prevalent at lower degrees of warming than reductions in biodiversity, which supports the idea that change occurs gradually rather than abruptly in ecological systems. Arctic spring and temperate summer communities were identified to be particularly vulnerable to global warming, likely because they reside near their upper thermal limits. Ultimately, my work highlights the significance of integrating results from various spatial and seasonal scales and thereby emphasises the benefits that could arise from implementing community-wide studies.

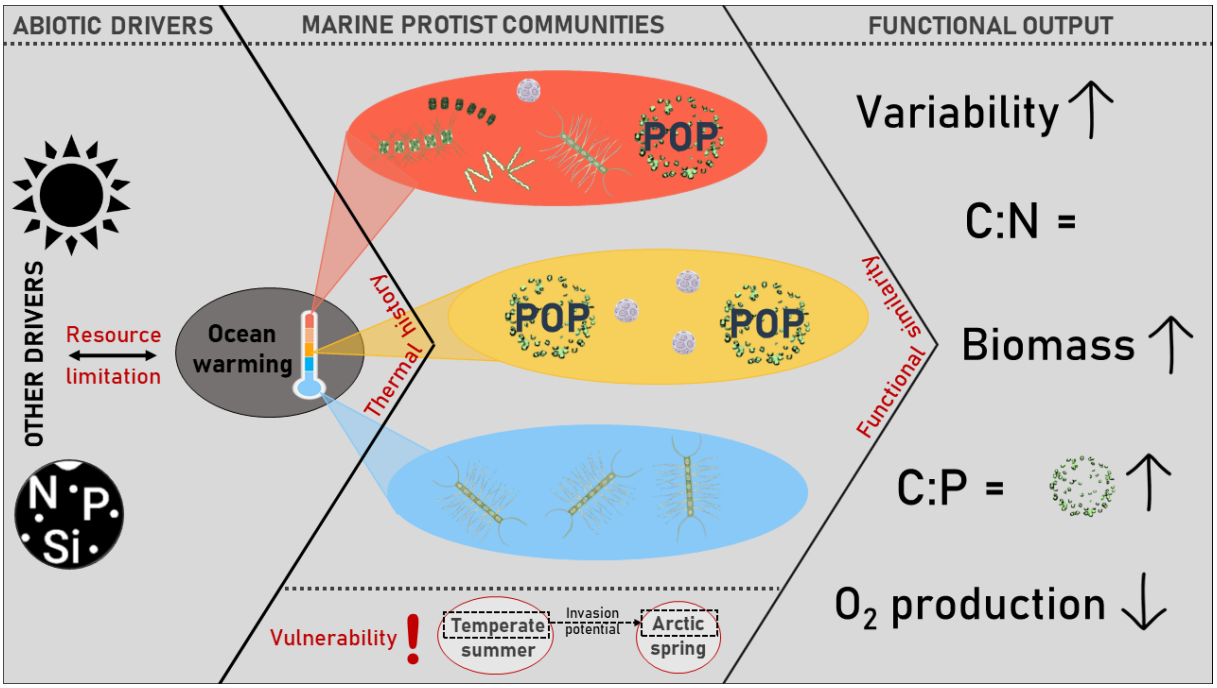
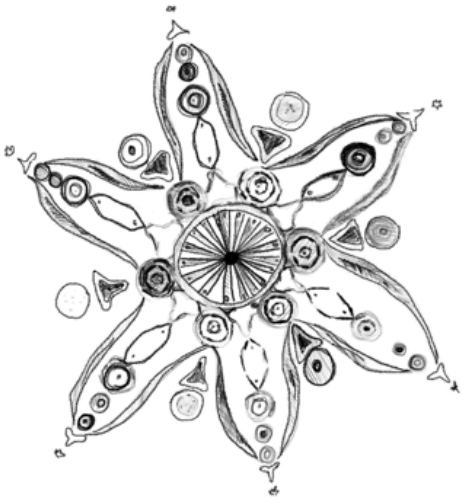


Figure 11: Illustration of summarised key outcomes of this thesis. The response of marine protist communities to ocean warming can be modulated by other drivers, mainly when they become limiting. Temperature-induced compositional reorganisation is primarily based on the thermal history of present species and the strongest selective pressure is posed by low temperatures. Different degrees of warming entail different community compositions, with diverging effects on the resulting functional output, depending on the functional similarity among species. In particular, temperate summer and Arctic spring communities can be considered vulnerable and the degree of warming affects the invasion potential of temperate species for Arctic communities.

7
References



Adl, S. M., D. Bass, C. E. Lane, J. Lukeš, C. L. Schoch, A. Smirnov, S. Agatha, C. Berney, M. W. Brown, F. Burki, P. Cárdenas, I. Čepička, L. Chistyakova, J. del Campo, M. Dunthorn, B. Edvardsen, Y. Eglit, L. Guillou, V. Hampl, A. A. Heiss, M. Hoppenrath, T. Y. James, A. Karnkowska, S. Karpov, E. Kim, M. Kolisko, A. Kudryavtsev, D. J. G. Lahr, E. Lara, L. Le Gall, D. H. Lynn, D. G. Mann, R. Massana, E. A. D. Mitchell, C. Morrow, J. S. Park, J. W. Pawlowski, M. J. Powell, D. J. Richter, S. Rueckert, L. Shadwick, S. Shimano, F. W. Spiegel, G. Torruella, N. Youssef, V. Zlatogursky, and Q. Zhang. 2019. Revisions to the classification, nomenclature, and diversity of eukaryotes. Journal of Eukaryotic Microbiology **66**:4-119.

Aflenzer, H., L. Hoffmann, T. Holmes, K. Wuttig, C. Genovese, and A. R. Bowie. 2023. Effect of dissolved iron (II) and temperature on growth of the Southern Ocean phytoplankton species Fragilariopsis cylindrus and Phaeocystis antarctica. Polar Biology **46**:1163–1173.

Agnihotri, K., and H. Kaur. 2019. The dynamics of viral infection in toxin producing phytoplankton and zooplankton system with time delay. Chaos, Solitons & Fractals **118**:122-133.

Altermatt, F., E. A. Fronhofer, A. Garnier, A. Giometto, F. Hammes, J. Klecka, D. Legrand, E. Mächler, T. M. Massie, F. Pennekamp, M. Plebani, M. Pontarp, N. Schtickzelle, V. Thuillier, and O. L. Petchey. 2015. Big answers from small worlds: a user's guide for protist microcosms as a model system in ecology and evolution. Methods in Ecology and Evolution **6**:218-231.

Alvain, S., C. Moulin, Y. Dandonneau, and H. Loisel. 2008. Seasonal distribution and succession of dominant phytoplankton groups in the global ocean: A satellite view. Global Biogeochemical Cycles **22**:GB3001.

Anderson, S. I., A. D. Barton, S. Clayton, S. Dutkiewicz, and T. A. Rynearson. 2021. Marine phytoplankton functional types exhibit diverse responses to thermal change. Nature Communications **12**:6413.

Anderson, S. I., G. Franzè, J. D. Kling, P. Wilburn, C. T. Kremer, S. Menden-Deuer, E. Litchman, D. A. Hutchins, and T. A. Rynearson. 2022. The interactive effects of temperature and nutrients on a spring phytoplankton community. Limnology and Oceanography **67**:634-645.

Anderson, S. I., C. Fronda, A. D. Barton, S. Clayton, T. A. Rynearson, and S. Dutkiewicz. 2024. Phytoplankton thermal trait parameterization alters community structure and biogeochemical processes in a modeled ocean. Global Change Biology **30**:e17093.

Anderson, S. I., and T. A. Rynearson. 2020. Variability approaching the thermal limits can drive diatom community dynamics. Limnology and Oceanography **65**:1961-1973.

Angilletta, J., and J. Michael. 2009. Thermal Adaptation: A Theoretical and Empirical Synthesis. Oxford University Press.

Antoni, J. S., G. O. Almandoz, J. Goldsmit, M. D. Garcia, X. Flores-Melo, M. P. Hernando, and I. R. Schloss. 2024. Long-term studies on West Antarctic Peninsula phytoplankton blooms suggest range shifts between temperate and polar species. Global Change Biology **30**:e17238.

Aranguren-Gassis, M., C. T. Kremer, C. A. Klausmeier, and E. Litchman. 2019. Nitrogen limitation inhibits marine diatom adaptation to high temperatures. Ecology Letters **22**:1860-1869.

Ardyna, M., and K. R. Arrigo. 2020. Phytoplankton dynamics in a changing Arctic Ocean. Nature Climate Change **10**:892–903.

Ardyna, M., M. Babin, M. Gosselin, E. Devred, L. Rainville, and J.-É. Tremblay. 2014. Recent Arctic Ocean sea ice loss triggers novel fall phytoplankton blooms. Geophysical Research Letters **41**:6207-6212.

Argyle, P. A., J. Hinners, N. G. Walworth, S. Collins, N. M. Levine, and M. A. Doblin. 2021. A High-Throughput Assay for Quantifying Phenotypic Traits of Microalgae. Frontiers in Microbiology **12**:1664-1302X.

Arrigo, K. R., R. B. Dunbar, M. P. Lizotte, and D. H. Robinson. 2002. Taxon-specific differences in C/P and N/P drawdown for phytoplankton in the Ross Sea, Antarctica. Geophysical Research Letters **29**:441-444.

Arrigo, K. R., D. H. Robinson, D. L. Worthen, R. B. Dunbar, G. R. DiTullio, M. VanWoert, and M. P. Lizotte. 1999. Phytoplankton Community Structure and the Drawdown of Nutrients and CO2 in the Southern Ocean. Science **283**:365-367.

Asbjørnsen, H., M. Årthun, Ø. Skagseth, and T. Eldevik. 2020. Mechanisms Underlying Recent Arctic Atlantification. Geophysical Research Letters **47**:e2020GL088036.

Atkinson, D., B. J. Ciotti, and D. J. S. Montagnes. 2003. Protists decrease in size linearly with temperature: ca. 2.5% per °C. Proceedings of the Royal Society of London. Series B: Biological Sciences **270**:2605-2611.

Bach, L. T., P. Stange, J. Taucher, E. P. Achterberg, M. Algueró-Muñiz, H. Horn, M. Esposito, and U. Riebesell. 2019. The Influence of Plankton Community Structure on Sinking Velocity and Remineralization Rate of Marine Aggregates. Global Biogeochemical Cycles **33**:971-994.

Bach, L. T., J. Taucher, T. Boxhammer, A. Ludwig, K. C. The Kristineberg, E. P. Achterberg, M. Algueró-Muñiz, L. G. Anderson, J. Bellworthy, J. Büdenbender, J. Czerny, Y. Ericson, M. Esposito, M. Fischer, M. Haunost, D. Hellemann, H. G. Horn, T. Hornick, J. Meyer, M. Sswat, M. Zark, and U. Riebesell. 2016. Influence of Ocean Acidification on a Natural Winter-to-Summer Plankton Succession: First Insights from a Long-Term Mesocosm Study Draw Attention to Periods of Low Nutrient Concentrations. PLoS ONE **11**:e0159068.

Bachy, C., E. Hehenberger, Y.-C. Ling, D. M. Needham, J. Strauss, S. Wilken, and A. Z. Worden. 2022. Marine Protists: A Hitchhiker's Guide to their Role in the Marine Microbiome. Pages 159-241 *in* L. J. Stal and M. S. Cretoiu, editors. The Marine Microbiome. Springer International Publishing, Cham.

Balaguer, J., F. Koch, C. M. Flintrop, C. Völkner, M. H. Iversen, and S. Trimborn. 2023. Iron and manganese availability drives primary production and carbon export in the Weddell Sea. Current Biology **33**:4405-4414.

Baltar, F., B. Bayer, N. Bednarsek, S. Deppeler, R. Escribano, C. E. Gonzalez, R. L. Hansman, R. K. Mishra, M. A. Moran, D. J. Repeta, C. Robinson, E. Sintes, C. Tamburini, L. E. Valentin, and G. J. Herndl. 2019. Towards Integrating Evolution, Metabolism, and Climate Change Studies of Marine Ecosystems. Trends in Ecology & Evolution **34**:1022-1033.

Bar-On, Y. M., R. Phillips, and R. Milo. 2018. The biomass distribution on Earth. Proceedings of the National Academy of Sciences **115**:6506-6511.

Barkhordarian, A., D. M. Nielsen, D. Olonscheck, and J. Baehr. 2024. Arctic marine heatwaves forced by greenhouse gases and triggered by abrupt sea-ice melt. Communications Earth & Environment **5**:57.

Barton, S., J. Jenkins, A. Buckling, C. E. Schaum, N. Smirnoff, J. A. Raven, and G. Yvon-Durocher. 2020. Evolutionary temperature compensation of carbon fixation in marine phytoplankton. Ecology Letters **23**:722-733.

Becks, L., and H. Arndt. 2008. Transitions from stable equilibria to chaos, and back, in an experimental food web. Ecology **89**:3222-3226.

Becks, L., F. M. Hilker, H. Malchow, K. Jürgens, and H. Arndt. 2005. Experimental demonstration of chaos in a microbial food web. Nature **435**:1226-1229.

Benincà, E., J. Huisman, R. Heerkloss, K. D. Jöhnk, P. Branco, E. H. Van Nes, M. Scheffer, and S. P. Ellner. 2008. Chaos in a long-term experiment with a plankton community. Nature **451**:822-825.

Bestion, E., S. Barton, F. C. Garcia, R. Warfield, and G. Yvon-Durocher. 2020. Abrupt declines in marine phytoplankton production driven by warming and biodiversity loss in a microcosm experiment. Ecology Letters **23**:457-466.

Bestion, E., B. García-Carreras, C. E. Schaum, S. Pawar, and G. Yvon-Durocher. 2018. Metabolic traits predict the effects of warming on phytoplankton competition. Ecology Letters **21**:655-664.

Bestion, E., B. Haegeman, S. Alvarez Codesal, A. Garreau, M. Huet, S. Barton, and J. M. Montoya. 2021. Phytoplankton biodiversity is more important for ecosystem functioning in highly variable thermal environments. Proceedings of the National Academy of Sciences **118**:e2019591118.

Bi, R., Z. Cao, S. M. H. Ismar-Rebitz, U. Sommer, H. Zhang, Y. Ding, and M. Zhao. 2021. Responses of Marine Diatom-Dinoflagellate Competition to Multiple Environmental Drivers: Abundance, Elemental, and Biochemical Aspects. Frontiers in Microbiology **12**:731786.

Bieg, C., and D. Vasseur. 2024. Interactions between temperature and nutrients determine the population dynamics of primary producers. Ecology Letters **27**:e14363.

Biggs, C. R., L. A. Yeager, D. G. Bolser, C. Bonsell, A. M. Dichiera, Z. Hou, S. R. Keyser, A. J. Khursigara, K. Lu, A. F. Muth, B. Negrete Jr., and B. E. Erisman. 2020. Does functional redundancy affect ecological stability and resilience? A review and meta-analysis. Ecosphere **11**:e03184.

Bishop, I. W., S. I. Anderson, S. Collins, and T. A. Rynearson. 2022. Thermal trait variation may buffer Southern Ocean phytoplankton from anthropogenic warming. Global Change Biology **28**:5755-5767.

Blois, J. L., J. W. Williams, M. C. Fitzpatrick, S. T. Jackson, and S. Ferrier. 2013. Space can substitute for time in predicting climate-change effects on biodiversity. Proceedings of the National Academy of Sciences **110**:9374-9379.

Bolnick, D. I., P. Amarasekare, M. S. Araújo, R. Bürger, J. M. Levine, M. Novak, V. H. W. Rudolf, S. J. Schreiber, M. C. Urban, and D. A. Vasseur. 2011. Why intraspecific trait variation matters in community ecology. Trends in Ecology & Evolution **26**:183-192.

Boscolo-Galazzo, F., K. A. Crichton, S. Barker, and P. N. Pearson. 2018. Temperature dependency of metabolic rates in the upper ocean: A positive feedback to global climate change? Global and Planetary Change **170**:201-212.

Boyd, P. W., S. Collins, S. Dupont, K. Fabricius, J.-P. Gattuso, J. Havenhand, D. A. Hutchins, U. Riebesell, M. S. Rintoul, M. Vichi, H. Biswas, A. Ciotti, K. Gao, M. Gehlen, C. L. Hurd, H. Kurihara, C. M. McGraw, J. M. Navarro, G. E. Nilsson, U. Passow, and H.-O. Pörtner. 2018. Experimental strategies to assess the biological ramifications of multiple drivers of global ocean change - A review. Global Change Biology **24**:2239-2261.

Boyd, P. W., T. A. Rynearson, E. A. Armstrong, F. Fu, K. Hayashi, Z. Hu, D. A. Hutchins, R. M. Kudela, E. Litchman, M. R. Mulholland, U. Passow, R. F. Strzepek, K. A. Whittaker, E. Yu, and M. K. Thomas. 2013. Marine phytoplankton temperature versus growth responses from polar to tropical waters - outcome of a scientific community-wide study. PLoS ONE **8**:e63091.

Bozzato, D., T. Jakob, and C. Wilhelm. 2019. Effects of temperature and salinity on respiratory losses and the ratio of photosynthesis to respiration in representative Antarctic phytoplankton species. PLoS ONE **14**:e0224101.

Breton, E., E. Goberville, B. Sautour, A. Ouadi, D.-I. Skouroliakou, L. Seuront, G. Beaugrand, L. Kléparski, M. Crouvoisier, D. Pecqueur, C. Salmeron, A. Cauvin, A. Poquet, N. Garcia, F. Gohin, and U. Christaki. 2022. Multiple phytoplankton community responses

to environmental change in a temperate coastal system: A trait-based approach. Frontiers in Marine Science **9**:914475.

Briddon, C. L., M. Nicoară, A. Hegedüs, A. Niculea, R. Bellerby, W. Eikrem, B. G. Crespo, S. Dupont, and B. Drugă. 2023. Acclimation to various temperature and pCO2 levels does not impact the competitive ability of two strains of Skeletonema marinoi in natural communities. Frontiers in Marine Science **10**:1197570.

Brockmann, U. H., and G. Kattner. 1997. Winter- to- summer changes of nutrients, dissolved and particulate organic material in the North Sea. Deutsche Hydrografische Zeitschrift **49**:229-242.

Brown, J. H., J. F. Gillooly, A. P. Allen, V. M. Savage, and G. B. West. 2004. Toward a metabolic theory of ecology. Ecology **85**:1771-1789.

Burki, F., A. J. Roger, M. W. Brown, and A. G. B. Simpson. 2020. The New Tree of Eukaryotes. Trends in Ecology & Evolution **35**:43-55.

Cabrerizo, M. J., and E. Marañón. 2021. Geographical and Seasonal Thermal Sensitivity of Grazing Pressure by Microzooplankton in Contrasting Marine Ecosystems. Frontiers in Microbiology **12**:369–384.

Cabrerizo, M. J., J. M. Medina-Sánchez, J. M. González-Olalla, D. Sánchez-Gómez, and P. Carrillo. 2021. Microbial plankton responses to multiple environmental drivers in marine ecosystems with different phosphorus limitation degrees. Science of the Total Environment **816**:151491.

Caldeira, K., and M. E. Wickett. 2003. Anthropogenic carbon and ocean pH. Nature **425**:365-365.

Calvo-Díaz, A., L. Díaz-Pérez, Á. Suárez Luis, G. Morán Xosé Anxelu, E. Teira, and E. Marañón. 2011. Decrease in the Autotrophic-to-Heterotrophic Biomass Ratio of Picoplankton in Oligotrophic Marine Waters Due to Bottle Enclosure. Applied and Environmental Microbiology **77**:5739-5746.

Camarena-Gómez, M. T., T. Lipsewers, J. Piiparinen, E. Eronen-Rasimus, D. Perez-Quemaliños, L. Hoikkala, C. Sobrino, and K. Spilling. 2018. Shifts in phytoplankton community structure modify bacterial production, abundance and community composition. Aquatic Microbial Ecology **81**:149-170.

Carradec, Q., E. Pelletier, C. Da Silva, A. Alberti, Y. Seeleuthner, R. Blanc-Mathieu, G. Lima-Mendez, F. Rocha, L. Tirichine, K. Labadie, A. Kirilovsky, A. Bertrand, S. Engelen, M. A. Madoui, R. Meheust, J. Poulain, S. Romac, D. J. Richter, G. Yoshikawa, C. Dimier, S. Kandels-Lewis, M. Picheral, S. Searson, O. Jaillon, J. M. Aury, E. Karsenti, M. B. Sullivan, S. Sunagawa, P. Bork, F. Not, P. Hingamp, J. Raes, L. Guidi, H. Ogata, C. de Vargas, D. Iudicone, C. Bowler, and P. Wincker. 2018. A global ocean atlas of eukaryotic genes. Nature Communications **9**:373.

Carter-Gates, M., C. Balestreri, S. E. Thorpe, F. Cottier, A. Baylay, T. S. Bibby, C. M. Moore, and D. C. Schroeder. 2020. Implications of increasing Atlantic influence for Arctic microbial community structure. Scientific Reports **10**:19262.

Caruso, T., Y. Chan, D. C. Lacap, M. C. Y. Lau, C. P. McKay, and S. B. Pointing. 2011. Stochastic and deterministic processes interact in the assembly of desert microbial communities on a global scale. The ISME Journal **5**:1406-1413.

Cavicchioli, R., W. J. Ripple, K. N. Timmis, F. Azam, L. R. Bakken, M. Baylis, M. J. Behrenfeld, A. Boetius, P. W. Boyd, A. T. Classen, T. W. Crowther, R. Danovaro, C. M. Foreman, J. Huisman, D. A. Hutchins, J. K. Jansson, D. M. Karl, B. Koskella, D. B. M. Welch, J. B. H. Martiny, M. A. Moran, V. J. Orphan, D. S. Reay, J. V. Remais, V. I. Rich, B. K. Singh, L. Y. Stein, F. J. Stewart, M. B. Sullivan, M. J. H. van Oppen, S. C. Weaver, E. A. Webb, and N. S. Webster. 2019. Scientists' warning to humanity: microorganisms and climate change. Nature Reviews Microbiology **17**:569-586.

Cermeño, P., S. Dutkiewicz, R. P. Harris, M. Follows, O. Schofield, and P. G. Falkowski. 2008. The role of nutricline depth in regulating the ocean carbon cycle. Proceedings of the National Academy of Sciences **105**:20344-20349.

Chai, X., L. Zheng, J. Liu, J. Zhan, and L. Song. 2023. Comparison of photosynthetic responses between haptophyte Phaeocystis globosa and diatom Skeletonema costatum under phosphorus limitation. Frontiers in Microbiology **14**:1085176.

Chakraborty, S., L. T. Nielsen, and K. H. Andersen. 2017. Trophic Strategies of Unicellular Plankton. The American Naturalist **189**:E77-E90.

Chen, B. 2015. Patterns of thermal limits of phytoplankton. Journal of Plankton Research **37**:285-292.

Chen, B., M. R. Landry, B. Huang, and H. Liu. 2012. Does warming enhance the effect of microzooplankton grazing on marine phytoplankton in the ocean? Limnology and Oceanography **57**:519-526.

Chen, B., and E. A. Laws. 2017. Is there a difference of temperature sensitivity between marine phytoplankton and heterotrophs? Limnology and Oceanography **62**:806-817.

Chen, H., K. Ma, Y. Huang, Y. Yang, Z. Ma, and C. Chu. 2021. Salinity Drives Functional and Taxonomic Diversities in Global Water Metagenomes. Frontiers in Microbiology **12**:719725.

Chen, M., M. Fan, and X. Wang. 2020. Effect of temperature on adaptive evolution of phytoplankton cell size. JOURNAL OF APPLIED ANALYSIS AND COMPUTATION **10**:2644-2658.

Cheng, H. M., S. F. Zhang, X. L. Ning, J. X. Peng, D. X. Li, H. Zhang, K. Zhang, L. Lin, S. Q. Liu, W. O. Smith, Jr., and D. Z. Wang. 2023. Elucidating colony bloom formation mechanism of a harmful alga Phaeocystis globosa (Prymnesiophyceae) using metaproteomics. Science of the Total Environment **869**:161846.

Clark, M. S., J. I. Hoffman, L. S. Peck, L. Bargelloni, D. Gande, C. Havermans, B. Meyer, T. Patarnello, T. Phillips, K. R. Stoof-Leichsenring, D. L. J. Vendrami, A. Beck, G. Collins, M. W. Friedrich, K. M. Halanych, J. F. Masello, R. Nagel, K. Norén, C. Printzen, M. B. Ruiz, S. Wohlrab, B. Becker, K. Dumack, F. Ghaderiardakani, K. Glaser, S. Heesch, C. Held, U. John, U. Karsten, S. Kempf, M. Lucassen, A. Paijmans, K. Schimani, A. Wallberg, L. C. Wunder, and T. Mock. 2023. Multi-omics for studying and understanding polar life. Nature Communications **14**:7451.

Coello-Camba, A., and S. Agustí. 2017. Thermal Thresholds of Phytoplankton Growth in Polar Waters and Their Consequences for a Warming Polar Ocean. Frontiers in Marine Science **4**:168.

Coello-Camba, A., Agustí, S., Vaqué, D. et al. 2015. Experimental Assessment of Temperature Thresholds for Arctic Phytoplankton Communities. Estuaries and Coasts **38**:873-885.

Collins, S. 2016. Growth rate evolution in improved environments under Prodigal Son dynamics. Evolutionary Applications **9**:1179-1188.

Collins, S., H. Whittaker, and M. K. Thomas. 2022. The need for unrealistic experiments in global change biology. Current Opinion in Microbiology **68**:102151.

Connell, S. D., and G. Ghedini. 2015. Resisting regime-shifts: the stabilising effect of compensatory processes. Trends in Ecology & Evolution **30**:513-515.

D'Aguillo, M., C. Hazelwood, B. Quarles, and K. Donohue. 2022. Genetic Consequences of Biologically Altered Environments. Journal of Heredity **113**:26-36.

Dall'Olmo, G., J. Dingle, L. Polimene, R. J. Brewin, and H. Claustre. 2016. Substantial energy input to the mesopelagic ecosystem from the seasonal mixed-layer pump. Nature Geosciences **9**:820-823.

Darling, K. F., M. Kucera, and C. M. Wade. 2007. Global molecular phylogeography reveals persistent Arctic circumpolar isolation in a marine planktonic protist. Proceedings of the National Academy of Sciences **104**:5002-5007.

Daufresne, M., K. Lengfellner, and U. Sommer. 2009. Global warming benefits the small in aquatic ecosystems. Proceedings of the National Academy of Sciences **106**:12788-12793.

De Amorim, F. d. L. L., K. H. Wiltshire, P. Lemke, K. Carstens, S. Peters, J. Rick, L. Gimenez, and M. Scharfe. 2023. Investigation of marine temperature changes across temporal and spatial Gradients: Providing a fundament for studies on the effects of warming on marine ecosystem function and biodiversity. Progress in Oceanography **216**:103080.

De Senerpont Domis, L. N., W. M. Mooij, and J. Huisman. 2007. Climate-induced shifts in an experimental phytoplankton community: a mechanistic approach. Hydrobiologia **584**:403-413.

De Senerpont Domis, L. N., D. B. Van de Waal, N. R. Helmsing, E. Van Donk, and W. M. Mooij. 2014. Community stoichiometry in a changing world: combined effects of warming and eutrophication on phytoplankton dynamics. Ecology **95**:1485-1495.

De Vargas, C., S. Audic, N. Henry, J. Decelle, F. Mahé, R. Logares, E. Lara, Ć. Berney, N. Le Bescot, I. Probert, M. Carmichael, J. Poulain, S. Romac, S. Colin, J. M. Aury, L. Bittner, S. Chaffron, M. Dunthorn, S. Engelen, O. Flegontova, L. Guidi, A. Horák, O. Jaillon, G. Lima-Mendez, J. Lukeš, S. Malviya, R. Morard, M. Mulot, E. Scalco, R. Siano, F. Vincent, A. Zingone, C. Dimier, M. Picheral, S. Searson, S. Kandels-Lewis, S. G. Acinas, P. Bork, C. Bowler, G. Gorsky, N. Grimsley, P. Hingamp, D. Iudicone, F. Not, H. Ogata, S. Pesant, J. Raes, M. E. Sieracki, S. Speich, L. Stemmann, S. Sunagawa, J. Weissenbach, P. Wincker, E. Karsenti, E. Boss, M. Follows, L. Karp-Boss, U. Krzic, E. G. Reynaud, C. Sardet, M. B. Sullivan, D. Velayoudon, and C. Tara Oceans. 2015. Eukaryotic plankton diversity in the sunlit ocean. Science **348**:126160.

Deutsch, C., J. L. Penn, W. C. E. P. Verberk, K. Inomura, M.-G. Endress, and J. L. Payne. 2022. Impact of warming on aquatic body sizes explained by metabolic scaling from microbes to macrofauna. Proceedings of the National Academy of Sciences **119**:e2201345119.

Ditlevsen, P., and S. Ditlevsen. 2023. Warning of a forthcoming collapse of the Atlantic meridional overturning circulation. Nature Communications **14**:4254.

Drake, J. A., G. R. Huxel, and C. L. Hewitt. 1996. Microcosms as Models for Generating and Testing Community Theory. Ecology **77**:670-677.

Drew, G. C., E. J. Stevens, and K. C. King. 2021. Microbial evolution and transitions along the parasite-mutualist continuum. Nature Reviews Microbiology **19**:623-638.

Duret, M. T., R. S. Lampitt, and P. Lam. 2020. Eukaryotic influence on the oceanic biological carbon pump in the Scotia Sea as revealed by 18S rRNA gene sequencing of suspended and sinking particles. Limnology and Oceanography **65**:S49-S70.

Dutkiewicz, S., C. L. Follett, M. J. Follows, F. Henderikx-Freitas, F. Ribalet, M. R. Gradoville, S. N. Coesel, H. Farnelid, Z. V. Finkel, A. J. Irwin, O. Jahn, D. M. Karl, J. P. Mattern, A. E. White, J. P. Zehr, and E. V. Armbrust. 2024. Multiple biotic interactions establish phytoplankton community structure across environmental gradients. Limnology and Oceanography **n/a**.

Dutkiewicz, S., J. R. Scott, and M. J. Follows. 2013. Winners and losers: Ecological and biogeochemical changes in a warming ocean. Global Biogeochemical Cycles **27**:463-477.

Edwards, M., and A. J. Richardson. 2004. Impact of climate change on marine pelagic phenology and trophic mismatch. Nature **430**:881-884.

Eisenhauer, N., J. Hines, F. T. Maestre, and M. C. Rillig. 2023. Reconsidering functional redundancy in biodiversity research. npj Biodiversity **2**:9.

Endo, H., H. Ogata, and K. Suzuki. 2018. Contrasting biogeography and diversity patterns between diatoms and haptophytes in the central Pacific Ocean. Scientific Reports **8**:10916.

Eppley, R. W., J. N. Rogers, and J. J. McCarthy. 1969. Half-saturation constants for uptake of nitrate and ammonium by marine phytoplankton. Limnology and Oceanography **14**:912-920.

Evans, S., J. B. H. Martiny, and S. D. Allison. 2017. Effects of dispersal and selection on stochastic assembly in microbial communities. The ISME Journal **11**:176-185.

Faillace, C. A., and P. J. Morin. 2020. Evolution alters post-invasion temporal dynamics in experimental communities. Journal of Animal Ecology **89**:285-298.

Falkowski, P. G., R. T. Barber, and V. Smetacek. 1998. Biogeochemical Controls and Feedbacks on Ocean Primary Production. Science **281**:200-206.

Febvre, C., C. Goldblatt, and R. El-Sabaawi. 2024. Thermal performance of ecosystems: Modelling how physiological responses to temperature scale up in communities. Journal of Theoretical Biology **in press**:111792.

Feng, J., H. Wang, D. Huang, and S. Li. 2006. Alternative attractors in marine ecosystems: A comparative analysis of fishing effects. Ecological Modelling **195**:377-384.

Fernandez-Gonzalez, C., and E. Maranon. 2021. Effect of temperature on the unimodal size scaling of phytoplankton growth. Scientific Reports **11**:953.

Fetzer, I., K. Johst, R. Schäwe, T. Banitz, H. Harms, and A. Chatzinotas. 2015. The extent of functional redundancy changes as species' roles shift in different environments. Proceedings of the National Academy of Sciences **112**:14888-14893.

Field, C. B., M. J. Behrenfeld, J. T. Randerson, and P. Falkowski. 1998. Primary Production of the Biosphere: Integrating Terrestrial and Oceanic Components. Science **281**:237-240.

Filiz, N., U. Işkın, M. Beklioğlu, B. Öğlü, Y. Cao, T. A. Davidson, M. Søndergaard, T. L. Lauridsen, and E. Jeppesen. 2020. Phytoplankton community response to nutrients, temperatures, and a heat wave in shallow lakes: An experimental approach. Water **12**:3394.

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. Journal of Plankton Research **32**:119-137.

Flynn, K. J., A. Mitra, W. H. Wilson, S. A. Kimmance, D. R. Clark, A. Pelusi, and L. Polimene. 2022. 'Boom-and-busted' dynamics of phytoplankton-virus interactions explain the paradox of the plankton. New Phytologist **234**:990-1002.

Fontana, S., O. L. Petchey, and F. Pomati. 2016. Individual-level trait diversity concepts and indices to comprehensively describe community change in multidimensional trait space. Functional Ecology **30**:808-818.

Fontana, S., M. K. Thomas, M. Moldoveanu, P. Spaak, and F. Pomati. 2018. Individual-level trait diversity predicts phytoplankton community properties better than species richness or evenness. The ISME Journal **12**:356-366.

Franks, S. J., E. Hamann, and A. E. Weis. 2018. Using the resurrection approach to understand contemporary evolution in changing environments. Evolutionary Applications **11**:17-28.

Frost, P. C., N. J. T. Pearce, S. A. Berger, M. O. Gessner, A. K. Makower, V. Marzetz, J. C. Nejstgaard, A. Pralle, S. Schälicke, A. Wacker, N. D. Wagner, and M. A. Xenopoulos. 2023. Interactive effects of nitrogen and phosphorus on growth and stoichiometry of lake phytoplankton. Limnology and Oceanography **68**:1172-1184.

Fujiwara, A., S. Nishino, K. Matsuno, J. Onodera, Y. Kawaguchi, T. Hirawake, K. Suzuki, J. Inoue, and T. Kikuchi. 2018. Changes in phytoplankton community structure during wind-induced fall bloom on the central Chukchi shelf. Polar Biology **41**:1279-1295.

Galand, P. E., O. Pereira, C. Hochart, J. C. Auguet, and D. Debroas. 2018. A strong link between marine microbial community composition and function challenges the idea of functional redundancy. ISME Journal **12**:2470-2478.

Gall, A., U. Uebel, U. Ebensen, H. Hillebrand, S. Meier, G. Singer, A. Wacker, and M. Striebel. 2017. Planktotrons: A novel indoor mesocosm facility for aquatic biodiversity and food web research. Limnology and Oceanography: Methods **15**:663-677.

Garcia, F. C., E. Bestion, R. Warfield, and G. Yvon-Durocher. 2018. Changes in temperature alter the relationship between biodiversity and ecosystem functioning. Proceedings of the National Academy of Sciences of the United States of America **115**:10989-10999.

Gardner, J. L., A. Peters, M. R. Kearney, L. Joseph, and R. Heinsohn. 2011. Declining body size: a third universal response to warming? Trends in Ecology & Evolution **26**:285-291.

Gasparini, S., M. H. Daro, E. Antajan, M. Tackx, V. Rousseau, J. Y. Parent, and C. Lancelot. 2000. Mesozooplankton grazing during the Phaeocystis globosa bloom in the southern bight of the North Sea. Journal of Sea Research **43**:345-356.

Gaul, W., and A. N. Antia. 2001. Taxon-specific growth and selective microzooplankton grazing of phytoplankton in the Northeast Atlantic. Journal of Marine Systems **30**:241-261.

Gerhard, M., A.-M. Koussoroplis, M. Raatz, C. Pansch, S. B. Fey, J. Vajedsamiei, M. Calderó-Pascual, D. Cunillera-Montcusí, N. P. D. Juvigny-Khenafou, F. Polazzo, P. K. Thomas, C. C. Symons, M. Beklioğlu, S. A. Berger, R. M. Chefaoui, K. A. Ger, S. Langenheder, J. C. Nejstgaard, R. Ptacnik, and M. Striebel. 2023. Environmental variability in aquatic ecosystems: Avenues for future multifactorial experiments. Limnology and Oceanography Letters **8**:247-266.

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.

Gerhard, M., C. Mori, and M. Striebel. 2021. Nonrandom species loss in phytoplankton communities and its effect on ecosystem functioning. Limnology and Oceanography **66**:779-792.

Gieskes, W. W. C., S. C. Leterme, H. Peletier, M. Edwards, and P. C. Reid. 2007. Phaeocystis Colony Distribution in the North Atlantic Ocean since 1948, and Interpretation of Long-Term Changes in the Phaeocystis Hotspot in the North Sea. Biogeochemistry **83**:49-60.

Giesler, J. K., T. Harder, and S. Wohlrab. 2023. Microbiome and photoperiod interactively determine thermal sensitivity of polar and temperate diatoms. Biology letters **19**:20230151.

Glibert, P. M. 2016. Margalef revisited: A new phytoplankton mandala incorporating twelve dimensions, including nutritional physiology. Harmful Algae **55**:25-30.

Goldford, J. E., N. Lu, D. Bajić, S. Estrela, M. Tikhonov, A. Sanchez-Gorostiaga, D. Segrè, P. Mehta, and A. Sanchez. 2018. Emergent simplicity in microbial community assembly. Science **361**:469-474.

González-Gil, R., N. S. Banas, E. Bresnan, and M. R. Heath. 2022. The onset of the spring phytoplankton bloom in the coastal North Sea supports the Disturbance Recovery Hypothesis. Biogeosciences **19**:2417-2426.

Govaert, L., L. J. Gilarranz, and F. Altermatt. 2021. Competition alters species' plastic and genetic response to environmental change. Scientific Reports **11**:23518.

Graham, D. W., C. W. Knapp, E. S. Van Vleck, K. Bloor, T. B. Lane, and C. E. Graham. 2007. Experimental demonstration of chaotic instability in biological nitrification. The ISME Journal **1**:385-393.

Grattan, L. M., S. Holobaugh, and J. G. Morris, Jr. 2016. Harmful Algal Blooms and Public Health. Harmful Algae **57**:2-8.

Grattepanche, J. D., D. L. Juarez, C. C. Wood, G. B. McManus, and L. A. Katz. 2019. Incubation and grazing effects on spirotrich ciliate diversity inferred from molecular analyses of microcosm experiments. PLoS ONE **14**:e0215874.

Grizzetti, B., F. Bouraoui, and A. Aloe. 2012. Changes of nitrogen and phosphorus loads to European seas. Global Change Biology **18**:769-782.

Guidi, L., L. Stemmann, G. A. Jackson, F. Ibanez, H. Claustre, L. Legendre, M. Picheral, and G. Gorsky. 2009. Effects of phytoplankton community on production, size and export of large aggregates: A world-ocean analysis. Limnology and Oceanography **54**:1951-1963.

Guimarães, P. R. 2020. The Structure of Ecological Networks Across Levels of Organization. Annual Review of Ecology, Evolution, and Systematics **51**:433-460.

Gypens, N., G. Lacroix, and C. Lancelot. 2007. Causes of variability in diatom and Phaeocystis blooms in Belgian coastal waters between 1989 and 2003: A model study. Journal of Sea Research **57**:19-35.

Hall, J. P. J., E. Harrison, and M. A. Brockhurst. 2018. Competitive species interactions constrain abiotic adaptation in a bacterial soil community. Evolution Letters **2**:580-589.

Henley, S. F., M. Porter, L. Hobbs, J. Braun, R. Guillaume-Castel, E. J. Venables, E. Dumont, and F. Cottier. 2020. Nitrate supply and uptake in the Atlantic Arctic sea ice zone: seasonal cycle, mechanisms and drivers. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences **378**:20190361.

Henson, S. A., B. B. Cael, S. R. Allen, and S. Dutkiewicz. 2021. Future phytoplankton diversity in a changing climate. Nature Communications **12**:5372.

Hillebrand, H., E. Acevedo-Trejos, S. D. Moorthi, A. Ryabov, M. Striebel, P. K. Thomas, and M. L. Schneider. 2021. Cell size as driver and sentinel of phytoplankton community structure and functioning. Functional Ecology **36**:276-293.

Hillebrand, H., J. Antonucci Di Carvalho, J.-C. Dajka, C.-D. Dürselen, O. Kerimoglu, L. Kuczynski, L. Rönn, and A. Ryabov. 2022. Temporal declines in Wadden Sea phytoplankton cell volumes observed within and across species. Limnology and Oceanography **67**:468-481.

Hillebrand, H., D. M. Bennett, and M. W. Cadotte. 2008. Consequences of dominance: a review of evenness effects on local and regional ecosystem processes. Ecology **89**:1510-1520.

Hillebrand, H., T. Burgmer, and E. Biermann. 2012. Running to stand still: temperature effects on species richness, species turnover, and functional community dynamics. Marine Biology **159**:2415-2422.

Hillebrand, H., I. Donohue, W. S. Harpole, D. Hodapp, M. Kucera, A. M. Lewandowska, J. Merder, J. M. Montoya, and J. A. Freund. 2020. Thresholds for ecological responses to global change do not emerge from empirical data. Nature Ecology & Evolution **4**:1502-1509.

Hillebrand, H., D. S. Gruner, E. T. Borer, M. E. S. Bracken, E. E. Cleland, J. J. Elser, W. S. Harpole, J. T. Ngai, E. W. Seabloom, J. B. Shurin, and J. E. Smith. 2007. Consumer versus resource control of producer diversity depends on ecosystem type and producer community structure. Proceedings of the National Academy of Sciences **104**:10904-10909.

Hillebrand, H., L. Kuczynski, C. Kunze, M. C. Rillo, and J.-C. Dajka. 2023. Thresholds and tipping points are tempting but not necessarily suitable concepts to address anthropogenic biodiversity change - an intervention. Marine Biodiversity **53**:43.

Hillebrand, H., S. Langenheder, K. Lebret, E. Lindström, Ö. Östman, and M. Striebel. 2018. Decomposing multiple dimensions of stability in global change experiments. Ecology Letters **21**:21-30.

Hinners, J., I. Hense, and A. Kremp. 2019. Modelling phytoplankton adaptation to global warming based on resurrection experiments. Ecological Modelling **400**:27-33.

Hoerstmann, C., S. Wohlrab, and U. John. 2022. Concepts towards Functional Eukaryotic Microbial Biogeography in the Ocean. Journal of Marine Science and Engineering **10**:1730.

Hood, R. R., E. A. Laws, R. A. Armstrong, N. R. Bates, C. W. Brown, C. A. Carlson, F. Chai, S. C. Doney, P. G. Falkowski, R. A. Feely, M. A. M. Friedrichs, M. R. Landry, J. Keith Moore, D. M. Nelson, T. L. Richardson, B. Salihoglu, M. Schartau, D. A. Toole, and J. D. Wiggert. 2006. Pelagic functional group modeling: Progress, challenges and prospects. Deep Sea Research Part II: Topical Studies in Oceanography **53**:459-512.

Hoppe, C. J. M. 2021. Always ready? Primary production of Arctic phytoplankton at the end of the polar night. Limnology and Oceanography Letters **7**:167-174.

Hoppe, C. J. M., K. K. E. Wolf, N. Schuback, P. D. Tortell, and B. Rost. 2018. Compensation of ocean acidification effects in Arctic phytoplankton assemblages. Nature Climate Change **8**:529-533.

Horas, E. L., S. M. Metzger, B. Platzer, J. B. Kelly, and L. Becks. 2022. Context-dependent costs and benefits of endosymbiotic interactions in a ciliate-algae system. Environmental Microbiology **24**:5924-5935.

Huertas, I. E., M. Rouco, V. López-Rodas, and E. Costas. 2011. Warming will affect phytoplankton differently: evidence through a mechanistic approach. Proceedings of the Royal Society B: Biological Sciences **278**:3534-3543.

Huey, R. B., and R. D. Stevenson. 1979. Integrating Thermal Physiology and Ecology of Ectotherms: A Discussion of Approaches. American Zoologist **19**:357-366.

Huisman, J., and F. J. Weissing. 1999. Biodiversity of plankton by species oscillations and chaos. Nature **402**:407-410.

Huisman, J., and F. J. Weissing. 2001. Biological conditions for oscillations and chaos generated by multispecies competition. Ecology **82**:2682-2695.

Hutchinson, G. E. 1961. The Paradox of the Plankton. The American Naturalist **95**:137-145.

Ingvaldsen, R. B., K. M. Assmann, R. Primicerio, M. Fossheim, I. V. Polyakov, and A. V. Dolgov. 2021. Physical manifestations and ecological implications of Arctic Atlantification. Nature Reviews Earth & Environment **2**:874-889.

IPCC. 2014. Climate Change 2014: Synthesis Report. Contribution of Working Groups I, II and III to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change [Core Writing Team, R.K. Pachauri and L.A. Meyer (eds.)]. Geneva, Switzerland.

IPCC. 2021. Climate Change 2021: The Physical Science Basis. Contribution of Working Group I to the Sixth Assessment Report of the Intergovernmental Panel on Climate Change [Masson-Delmotte, V., P. Zhai, A. Pirani, S.L. Connors, C. Péan, S. Berger, N. Caud, Y. Chen, L. Goldfarb, M.I. Gomis, M. Huang, K. Leitzell, E. Lonnoy, J.B.R. Matthews, T.K. Maycock, T. Waterfield, O. Yelekçi, R. Yu, and B. Zhou (eds.)].

IPCC. 2023. Summary for Policymakers. Pages 1-34 *in* H. L. a. J. Romero, editor. 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, Geneva, Switzerland.

Isobe, K., N. J. Bouskill, E. L. Brodie, E. A. Sudderth, and J. B. H. Martiny. 2020. Phylogenetic conservation of soil bacterial responses to simulated global changes. Philosophical Transactions of the Royal Society B: Biological Sciences **375**:20190242.

John, E. H., and K. Davidson. 2001. Prey selectivity and the influence of prey carbon:nitrogen ratio on microflagellate grazing. Journal of Experimental Marine Biology and Ecology **260**:93-111.

Jones, R. M., and W. O. Smith. 2017. The influence of short-term events on the hydrographic and biological structure of the southwestern Ross Sea. Journal of Marine Systems **166**:184-195.

Jurburg, S. D., F. Buscot, A. Chatzinotas, N. M. Chaudhari, A. T. Clark, M. Garbowski, M. Grenié, E. F. Y. Hom, C. Karakoç, S. Marr, S. Neumann, M. Tarkka, N. M. van Dam,

A. Weinhold, and A. Heintz-Buschart. 2022. The community ecology perspective of omics data. Microbiome **10**:225.

Käse, L., A. C. Kraberg, K. Metfies, S. Neuhaus, P. A. A. Sprong, B. M. Fuchs, M. Boersma, and K. H. Wiltshire. 2020. Rapid succession drives spring community dynamics of small protists at Helgoland Roads, North Sea. Journal of Plankton Research **42**:305-319.

Katkov, E., and G. F. Fussmann. 2023. The effect of increasing temperature and pCO2 on experimental pelagic freshwater communities. Limnology and Oceanography **68**:S202-S216.

Katz, M. E., Z. V. Finkel, D. Grzebyk, A. H. Knoll, and P. G. Falkowski. 2004. Evolutionary trajectories and biogeochemical impacts of marine eukaryotic phytoplankton. Annual Review of Ecology, Evolution, and Systematics **35**:523-556.

Keeling, P. J., and F. Burki. 2019. Progress towards the Tree of Eukaryotes. Current Biology **29**:R808-R817.

Kent, M. 2007. Determinism. Oxford University Press.

Kilner, C. L., A. A. Carrell, D. J. Wieczynski, S. Votzke, K. DeWitt, A. Yammine, J. Shaw, D. A. Pelletier, D. J. Weston, and J. P. Gibert. 2024. Temperature and CO2 interactively drive shifts in the compositional and functional structure of peatland protist communities. Global Change Biology **30**:e17203.

Kingsolver, J. G. 2009. The well-temperatured biologist. (American Society of Naturalists Presidential Address). American Naturalist **174**:755-768.

Kling, J. D., M. D. Lee, F. Fu, M. D. Phan, X. Wang, P. Qu, and D. A. Hutchins. 2020. Transient exposure to novel high temperatures reshapes coastal phytoplankton communities. ISME **14**:413-424.

Kling, J. D., M. D. Lee, E. A. Webb, J. T. Coelho, P. Wilburn, S. I. Anderson, Q. Zhou, C. Wang, M. D. Phan, F. Fu, C. T. Kremer, E. Litchman, T. A. Rynearson, and D. A. Hutchins. 2023. Dual thermal ecotypes co-exist within a nearly genetically-identical population of the unicellular marine cyanobacterium Synechococcus. PNAS **120**:e23157011.

Koussoroplis, A.-M., and A. Wacker. 2016. Covariance modulates the effect of joint temperature and food variance on ectotherm life-history traits. Ecology Letters **19**:143-152.

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 reinhartdii. Proceedings of the Royal Society B: Biological Sciences **285**:29321297.

Kremer, C. T., M. K. Thomas, and E. Litchman. 2017. Temperature- and size-scaling of phytoplankton population growth rates: Reconciling the Eppley curve and the metabolic theory of ecology. Limnology and Oceanography **62**:1658-1670.

Lachapelle, J., E. Bestion, E. E. Jackson, and C.-E. Schaum. 2022. Presence of a resident species aids invader evolution. Limnology and Oceanography **67**:2252-2264.

Lalande, C., E. Bauerfeind, and E. M. Nöthig. 2011. Downward particulate organic carbon export at high temporal resolution in the eastern Fram Strait: influence of Atlantic Water on flux composition. Marine Ecology Progress Series **440**:127-136.

Lassen, M. K., K. D. Nielsen, K. Richardson, K. Garde, and L. Schlüter. 2010. The effects of temperature increases on a temperate phytoplankton community - A mesocosm climate change scenario. Journal of Experimental Marine Biology and Ecology **383**:79-88.

Laufkötter, C., M. Vogt, N. Gruber, M. Aita-Noguchi, O. Aumont, L. Bopp, E. Buitenhuis, S. C. Doney, J. Dunne, T. Hashioka, J. Hauck, T. Hirata, J. John, C. Le Quéré, I. D. Lima, H. Nakano, R. Seferian, I. Totterdell, M. Vichi, and C. Völker. 2015. Drivers and uncertainties of future global marine primary production in marine ecosystem models. Biogeosciences **12**:6955-6984.

Laufkötter, C., J. Zscheischler, and T. L. Frölicher. 2020. High-impact marine heatwaves attributable to human-induced global warming. Science **369**:1621-1625.

Laurenceau-Cornec, E. C., T. W. Trull, D. M. Davies, S. G. Bray, J. Doran, F. Planchon, F. Carlotti, M. P. Jouandet, A. J. Cavagna, A. M. Waite, and S. Blain. 2015. The relative importance of phytoplankton aggregates and zooplankton fecal pellets to carbon export: insights from free-drifting sediment trap deployments in naturally iron-fertilised waters near the Kerguelen Plateau. Biogeosciences **12**:1007-1027.

Le Moigne, F. A. C., A. J. Poulton, S. A. Henson, C. J. Daniels, G. M. Fragoso, E. Mitchell, S. Richier, B. C. Russell, H. E. K. Smith, G. A. Tarling, J. R. Young, and M. Zubkov. 2015. Carbon export efficiency and phytoplankton community composition in the Atlantic sector of the Arctic Ocean. Journal of Geophysical Research: Oceans **120**:3896-3912.

Lehman, C. L., and D. Tilman. 2000. Biodiversity, stability, and productivity in competitive communities. American Naturalist **156**:534-552.

Leu, E., C. J. Mundy, P. Assmy, K. Campbell, T. M. Gabrielsen, M. Gosselin, T. Juul-Pedersen, and R. Gradinger. 2015. Arctic spring awakening – Steering principles behind the phenology of vernal ice algal blooms. Progress in Oceanography **139**:151-170.

Lewandowska, A. M., D. G. Boyce, M. Hofmann, B. Matthiessen, U. Sommer, and B. Worm. 2014. Effects of sea surface warming on marine plankton. Ecology Letters **17**:614-623.

Lewandowska, A. M., and U. Sommer. 2010. Climate change and the spring bloom: a mesocosm study on the influence of light and temperature on phytoplankton and mesozooplankton. Marine Ecology Progress Series **405**:101-111.

Lewandowska, A. M., M. Striebel, U. Feudel, H. Hillebrand, and U. Sommer. 2015. The importance of phytoplankton trait variability in spring bloom formation. ICES Journal of Marine Science **72**:1908-1915.

Lewington-Pearce, L., A. Narwani, M. K. Thomas, C. T. Kremer, H. Vogler, and P. Kratina. 2019. Temperature-dependence of minimum resource requirements alters competitive hierarchies in phytoplankton. Oikos **128**:1194-1205.

Lewis, K. M., A. E. Arntsen, P. Coupel, H. Joy-Warren, K. E. Lowry, A. Matsuoka, M. M. Mills, G. L. van Dijken, V. Selz, and K. R. Arrigo. 2019. Photoacclimation of Arctic Ocean phytoplankton to shifting light and nutrient limitation. Limnology and Oceanography **64**:284-301.

Li, F., A. Burger, J. M. Eppley, K. E. Poff, D. M. Karl, and E. F. DeLong. 2023. Planktonic microbial signatures of sinking particle export in the open ocean's interior. Nature Communications **14**:7177.

Li, G., L. Cheng, J. Zhu, K. E. Trenberth, M. E. Mann, and J. P. Abraham. 2020. Increasing ocean stratification over the past half-century. Nature Climate Change **10**:1116-1123.

Li, W. K. W. 1985. Photosynthetic response to temperature of marine phytoplankton along a latitudinal gradient (16°N to 74°N). Deep Sea Research Part A. Oceanographic Research Papers **32**:1381-1391.

Lin, Y.-C., C.-P. Chin, J. W. Yang, K.-P. Chiang, C.-h. Hsieh, G.-C. Gong, C.-Y. Shih, and S.-Y. Chen. 2022. How Communities of Marine Stramenopiles Varied with Environmental and Biological Variables in the Subtropical Northwestern Pacific Ocean. Microbial Ecology **83**:916-928.

Lindemann, C., Ø. Fiksen, K. H. Andersen, and D. L. Aksnes. 2016. Scaling Laws in Phytoplankton Nutrient Uptake Affinity. Frontiers in Marine Science **3**:26.

Listmann, L., G. S. I. Hattich, B. Matthiessen, and T. B. H. Reusch. 2020. Eco-Evolutionary Interaction in Competing Phytoplankton: Nutrient Driven Genotype Sorting Likely Explains Dominance Shift and Species Responses to CO2. Frontiers in Marine Science **7**:634.

Litchman, E., P. de Tezanos Pinto, K. F. Edwards, C. A. Klausmeier, C. T. Kremer, and M. K. Thomas. 2015. Global biogeochemical impacts of phytoplankton: a trait-based perspective. Journal of Ecology **103**:1384-1396.

Litchman, E., P. de Tezanos Pinto, C. A. Klausmeier, M. K. Thomas, and K. Yoshiyama. 2010. Linking traits to species diversity and community structure in phytoplankton. Hydrobiologia **653**:15-28.

Litchman, E., and C. A. Klausmeier. 2008. Trait-Based Community Ecology of Phytoplankton. Annual Review of Ecology, Evolution, and Systematics **39**:615-639.

Litchman, E., C. A. Klausmeier, O. M. Schofield, and P. G. Falkowski. 2007. The role of functional traits and trade-offs in structuring phytoplankton communities: Scaling from cellular to ecosystem level. Ecology Letters **10**:1170-1181.

Litchman, E., M. D. Ohman, and T. Kiørboe. 2013. Trait-based approaches to zooplankton communities. Journal of Plankton Research **35**:473-484.

Litchman, E., and M. K. Thomas. 2023. Are we underestimating the ecological and evolutionary effects of warming? Interactions with other environmental drivers may increase species vulnerability to high temperatures. Oikos **2023**:e09155.

Liu, X., C. H. Tang, and C. K. Wong. 2014. Microzooplankton grazing and selective feeding during bloom periods in the Tolo Harbour area as revealed by HPLC pigment analysis. Journal of Sea Research **90**:83-94.

Ljungström, G., T. J. Langbehn, and C. Jørgensen. 2021. Light and energetics at seasonal extremes limit poleward range shifts. Nature Climate Change **11**:530-536.

Lopes dos Santos, A., C. Gérikas Ribeiro, D. Ong, L. Garczarek, X. L. Shi, S. D. Nodder, D. Vaulot, and A. Gutiérrez-Rodríguez. 2022. Chapter 11 - Phytoplankton diversity and ecology through the lens of high throughput sequencing technologies. Pages 353-413 *in* L. A. Clementson, R. S. Eriksen, and A. Willis, editors. Advances in Phytoplankton Ecology. Elsevier.

López-Sandoval, D. C., T. Rodríguez-Ramos, P. Cermeño, C. Sobrino, and E. Marañón. 2014. Photosynthesis and respiration in marine phytoplankton: Relationship with cell size, taxonomic affiliation, and growth phase. Journal of Experimental Marine Biology and Ecology **457**:151-159.

López-Urrutia, Á. 2008. The metabolic theory of ecology and algal bloom formation. Limnology and Oceanography **53**:2046-2047.

Louca, S., L. W. Parfrey, and M. Doebeli. 2016. Decoupling function and taxonomy in the global ocean microbiome. Science **353**:1272-1277.

Louca, S., M. F. Polz, F. Mazel, M. B. N. Albright, J. A. Huber, M. I. O'Connor, M. Ackermann, A. S. Hahn, D. S. Srivastava, S. A. Crowe, M. Doebeli, and L. W. Parfrey. 2018. Function and functional redundancy in microbial systems. Nature Ecology and Evolution **2**:936-943.

Louthan, A. M., M. L. Peterson, and L. G. Shoemaker. 2021. Climate sensitivity across latitude: scaling physiology to communities. Trends in Ecology & Evolution **36**:931-942.

Lovell, R. S. L., S. Collins, S. H. Martin, A. L. Pigot, and A. B. Phillimore. 2023. Space-for-time substitutions in climate change ecology and evolution. Biological Reviews Cambridge Philosophical Society **98**:2243-2270.

Mäki, A., P. Salmi, A. Mikkonen, A. Kremp, and M. Tiirola. 2017. Sample Preservation, DNA or RNA Extraction and Data Analysis for High-Throughput Phytoplankton Community Sequencing. Frontiers in Microbiology **8**:1848.

Marañón, E. 2015. Cell size as a key determinant of phytoplankton metabolism and community structure. Annual Review of Marine Science **7**:241-264.

Marañón, E., P. Cermeno, D. C. Lopez-Sandoval, T. Rodriguez-Ramos, C. Sobrino, M. Huete-Ortega, J. M. Blanco, and J. Rodriguez. 2013. Unimodal size scaling of phytoplankton growth and the size dependence of nutrient uptake and use. Ecology Letters **16**:371-379.

Marañón, E., M. P. Lorenzo, P. Cermeño, and B. Mouriño-Carballido. 2018. Nutrient limitation suppresses the temperature dependence of phytoplankton metabolic rates. The ISME Journal **12**:1836-1845.

Martin, Tara L., and Raymond B. Huey. 2008. Why "Suboptimal" Is Optimal: Jensen's Inequality and Ectotherm Thermal Preferences. The American Naturalist **171**:E102-E118.

Mayersohn, B., M. Lévy, I. Mangolte, and K. S. Smith. 2022. Emergence of Broadband Variability in a Marine Plankton Model Under External Forcing. Journal of Geophysical Research: Biogeosciences **127**:e2022JG007011.

McCain, J. S. P., A. E. Allen, and E. M. Bertrand. 2021. Proteomic traits vary across taxa in a coastal Antarctic phytoplankton bloom. The ISME Journal **16**:569–579.

McClean, D., V.-P. Friman, A. Finn, L. I. Salzberg, and I. Donohue. 2019. Coping with multiple enemies: pairwise interactions do not predict evolutionary change in complex multitrophic communities. Oikos **128**:1588-1599.

McKew, B. A., G. Metodieva, C. A. Raines, M. V. Metodiev, and R. J. Geider. 2015. Acclimation of Emiliania huxleyi to nutrient limitation involves precise modification of the proteome to scavenge alternative sources of N and P. Environmental Microbiology **17**:4050-4062.

McQuoid, M. R., A. Godhe, and K. Nordberg. 2002. Viability of phytoplankton resting stages in the sediments of a coastal Swedish fjord. European Journal of Phycology **37**:191-201.

Menden-Deuer, S., J. Rowlett, M. Nursultanov, S. Collins, and T. Rynearson. 2021. Biodiversity of marine microbes is safeguarded by phenotypic heterogeneity in ecological traits. PLoS ONE **16**: e0254799.

Meredith, M., M. Sommerkorn, S. Cassotta, C. Derksen, A. Ekaykin, A. Hollowed, G. Kofinas, A. Mackintosh, J. Melbourne-Thomas, M.M.C. Muelbert, G. Ottersen, H. Pritchard, and E.A.G. Schuur. 2019. Polar Regions. Pages 203-320 *in* D. C. R. H.-O. Pörtner, V. MassonDelmotte, P. Zhai, M. Tignor, E. Poloczanska, K. Mintenbeck, A. Alegría, M. Nicolai, A. Okem, J. Petzold, B. Rama, N.M. Weyer, editor. IPCC Special Report on the Ocean and Cryosphere in a Changing Climate. Cambridge University Press, Cambridge, UK and New York, NY, USA.

Meyer, M. G., R. M. Jones, and W. O. Smith Jr. 2022. Quantifying Seasonal Particulate Organic Carbon Concentrations and Export Potential in the Southwestern Ross Sea Using Autonomous Gliders. Journal of Geophysical Research: Oceans **127**:e2022JC018798.

Meyer, S. T., R. Ptacnik, H. Hillebrand, H. Bessler, N. Buchmann, A. Ebeling, N. Eisenhauer, C. Engels, M. Fischer, S. Halle, A.-M. Klein, Y. Oelmann, C. Roscher, T. Rottstock, C. Scherber, S. Scheu, B. Schmid, E.-D. Schulze, V. M. Temperton, T. Tscharntke, W. Voigt, A. Weigelt, W. Wilcke, and W. W. Weisser. 2018. Biodiversity–multifunctionality relationships depend on identity and number of measured functions. Nature Ecology & Evolution **2**:44-49.

Miller, A. D., H. Inamine, A. Buckling, S. H. Roxburgh, and K. Shea. 2021. How disturbance history alters invasion success: biotic legacies and regime change. Ecology Letters **24**:687-697.

Mock, T., W. Boulton, J.-P. Balmonte, K. Barry, S. Bertilsson, J. Bowman, M. Buck, G. Bratbak, E. J. Chamberlain, M. Cunliffe, J. Creamean, O. Ebenhöh, S. L. Eggers, A. A. Fong, J. Gardner, R. Gradinger, M. A. Granskog, C. Havermans, T. Hill, C. J. M. Hoppe, K. Korte, A. Larsen, O. Müller, A. Nicolaus, E. Oldenburg, O. Popa, S. Rogge, H. Schäfer, K. Shoemaker, P. Snoeijs-Leijonmalm, A. Torstensson, K. Valentin, A. Vader, K. Barry, I. M. A. Chen, A. Clum, A. Copeland, C. Daum, E. Eloe-Fadrosh, B. Foster, B. Foster, I. V. Grigoriev, M. Huntemann, N. Ivanova, A. Kuo, N. C. Kyrpides, S. Mukherjee, K. Palaniappan, T. B. K. Reddy, A. Salamov, S. Roux, N. Varghese, T. Woyke, D. Wu, R. M. Leggett, V. Moulton, and K. Metfies. 2022. Multiomics in the

central Arctic Ocean for benchmarking biodiversity change. PLoS Biology **20**:e3001835.

Møller, E. F., A. Christensen, J. Larsen, K. D. Mankoff, M. H. Ribergaard, M. Sejr, P. Wallhead, and M. Maar. 2023. The sensitivity of primary productivity in Disko Bay, a coastal Arctic ecosystem, to changes in freshwater discharge and sea ice cover. Ocean Sciences **19**:403-420.

Monod, J. 1949. The growth of bacterial cultures. Annual Review of Microbiology **3**:371-394.

Moreno, H. D., M. Köring, J. Di Pane, N. Tremblay, K. H. Wiltshire, M. Boersma, and C. L. Meunier. 2022. An integrated multiple driver mesocosm experiment reveals the effect of global change on planktonic food web structure. Communications Biology **5**:179.

Mori, C., M. Beck, M. Striebel, J. Merder, B. Schnetger, T. Dittmar, K. Pahnke, and H.-J. Brumsack. 2021. Biogeochemical cycling of molybdenum and thallium during a phytoplankton summer bloom: A mesocosm study. Marine Chemistry **229**:103910.

Murray, A., K. Præbel, A. Desiderato, H. Auel, and C. Havermans. 2023. Phylogeography and molecular diversity of two highly abundant Themisto amphipod species in a rapidly changing Arctic Ocean. Ecology and Evolution **13**:e10359.

Naselli-Flores, L., and J. Padisák. 2023. Ecosystem services provided by marine and freshwater phytoplankton. Hydrobiologia **850**:2691-2706.

Nazarimehr, F., S. Jafari, S. M. R. Hashemi Golpayegani, and J. C. Sprott. 2017. Can Lyapunov exponent predict critical transitions in biological systems? Nonlinear Dynamics **88**:1493-1500.

Ning, D. L., Y. Deng, J. M. Tiedje, and J. Z. Zhou. 2019. A general framework for quantitatively assessing ecological stochasticity. Proceedings of the National Academy of Sciences of the United States of America **116**:16892-16898.

Noh, K.-M., J.-H. Oh, H.-G. Lim, H. Song, and J.-S. Kug. 2024. Role of Atlantification in Enhanced Primary Productivity in the Barents Sea. Earth's Future **12**:e2023EF003709.

Northfield, T. D., J. Ripa, L. A. Nell, and A. R. Ives. 2021. Coevolution, diversification and alternative states in two-trophic communities. Ecology Letters **24**:269-278.

Nöthig, E. M., A. Bracher, A. Engel, K. Metfies, B. Niehoff, I. Peeken, E. Bauerfeind, A. Cherkasheva, S. Gäbler-Schwarz, K. Hardge, E. Kilias, A. Kraft, Y. M. Kidane, C. Lalande, J. Piontek, K. Thomisch, and M. Wurst. 2015. Summertime plankton ecology in fram strait-a compilation of long-and short-term observations. Polar Research **34**:23349.

O'Malley, M. A., A. G. B. Simpson, and A. J. Roger. 2013. The other eukaryotes in light of evolutionary protistology. Biology & Philosophy **28**:299-330.

Oliver, E. C. J., M. T. Burrows, M. G. Donat, A. Sen Gupta, L. V. Alexander, S. E. Perkins-Kirkpatrick, J. A. Benthuysen, A. J. Hobday, N. J. Holbrook, P. J. Moore, M. S. Thomsen, T. Wernberg, and D. A. Smale. 2019. Projected marine heatwaves in the 21st century and the potential for ecological impact. Frontiers in Marine Science **6**:734.

Ortiz-Burgos, S. 2016. Shannon-Weaver Diversity Index. Pages 572-573 *in* M. J. Kennish, editor. Encyclopedia of Estuaries. Springer Netherlands, Dordrecht.

OSPAR. 2010. Quality Status Report 2010, Chapter 3: Climate Change. London.

Padfield, D., M. Castledine, and A. Buckling. 2020. Temperature-dependent changes to host-parasite interactions alter the thermal performance of a bacterial host. ISME **14**:389-398.

Padfield, D., C. Lowe, A. Buckling, R. Ffrench-Constant, T. Student Research, S. Jennings, F. Shelley, J. S. Ólafsson, and G. Yvon-Durocher. 2017. Metabolic compensation constrains the temperature dependence of gross primary production. Ecology Letters **20**:1250-1260.

Padfield, D., G. Yvon-Durocher, A. Buckling, S. Jennings, and G. Yvon-Durocher. 2016. Rapid evolution of metabolic traits explains thermal adaptation in phytoplankton. Ecology Letters **19**:133-142.

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. Journal of Plankton Research **43**:142-155.

Pan, B. J., M. Vernet, R. A. Reynolds, and B. G. Mitchell. 2019. The optical and biological properties of glacial meltwater in an Antarctic fjord. PLoS ONE **14**:e0211107.

Petchey, O. L., and K. J. Gaston. 2006. Functional diversity: back to basics and looking forward. Ecology Letters **9**:741-758.

Peter, K. H., and U. Sommer. 2012. Phytoplankton Cell Size: Intra- and Interspecific Effects of Warming and Grazing. PLoS ONE **7**:e49632.

Peter, K. H., and U. Sommer. 2013. Phytoplankton Cell Size Reduction in Response to Warming Mediated by Nutrient Limitation. PLoS ONE **8**:e71528.

Pielou, E. C. 1966. The measurement of diversity in different types of biological collections. Journal of Theoretical Biology **13**:131-144.

Polyakov, I. V., A. V. Pnyushkov, M. B. Alkire, I. M. Ashik, T. M. Baumann, E. C. Carmack, I. Goszczko, J. Guthrie, V. V. Ivanov, T. Kanzow, R. Krishfield, R. Kwok, A. Sundfjord, J. Morison, R. Rember, and A. Yulin. 2017. Greater role for Atlantic inflows on sea-ice loss in the Eurasian Basin of the Arctic Ocean. Science **356**:285-291.

Previdi, M., K. L. Smith, and L. M. Polvani. 2021. Arctic amplification of climate change: a review of underlying mechanisms. Environmental Research Letters **16**:093003.

Props, R., P. Monsieurs, M. Mysara, L. Clement, N. Boon, and D. Hodgson. 2016. Measuring the biodiversity of microbial communities by flow cytometry. Methods in Ecology and Evolution **7**:1376-1385.

Quigg, A., Z. V. Finkel, A. J. Irwin, Y. Rosenthal, T.-Y. Ho, J. R. Reinfelder, O. Schofield, F. M. M. Morel, and P. G. Falkowski. 2003. The evolutionary inheritance of elemental stoichiometry in marine phytoplankton. Nature **425**:291-294.

Ramond, P., R. Siano, M. Sourisseau, and R. Logares. 2023. Assembly processes and functional diversity of marine protists and their rare biosphere. Environmental Microbiome **18**:59.

Ramond, P., M. Sourisseau, N. Simon, S. Romac, S. Schmitt, F. Rigaut-Jalabert, N. Henry, C. de Vargas, and R. Siano. 2019. Coupling between taxonomic and functional diversity in protistan coastal communities. Environmental Microbiology **21**:730-749.

Rantanen, M., A. Y. Karpechko, A. Lipponen, K. Nordling, O. Hyvärinen, K. Ruosteenoja, T. Vihma, and A. Laaksonen. 2022. The Arctic has warmed nearly four times faster than the globe since 1979. Communications Earth & Environment **3**:168.

Rasconi, S., A. Gall, K. Winter, and M. J. Kainz. 2015. Increasing Water Temperature Triggers Dominance of Small Freshwater Plankton. PLoS ONE **10**:e0140449.

Rasconi, S., K. Winter, and M. J. Kainz. 2017. Temperature increase and fluctuation induce phytoplankton biodiversity loss - Evidence from a multi-seasonal mesocosm experiment. Ecology & Evolution **7**:2936-2946.

Raven, J. A., J. E. Kübler, and J. Beardall. 2000. Put out the light, and then put out the light. Journal of the Marine Biological Association of the United Kingdom **80**:1-25.

Regaudie-de-Gioux, A., and C. M. Duarte. 2012. Temperature dependence of planktonic metabolism in the ocean. Global Biogeochemical Cycles **26**:GB1015.

Rehder, L., B. Rost, and S. D. Rokitta. 2023. Abrupt and acclimation responses to changing temperature elicit divergent physiological effects in the diatom Phaeodactylum tricornutum. New Phytologist **239**:1005-1013.

Reigstad, M., and P. Wassmann. 2007. Does Phaeocystis spp. contribute significantly to vertical export of organic carbon? Pages 217-234 *in* M. A. van Leeuwe, J. Stefels, S. Belviso,

C. Lancelot, P. G. Verity, and W. W. C. Gieskes, editors. Phaeocystis, major link in the biogeochemical cycling of climate-relevant elements. Springer Netherlands, Dordrecht.

Remy, M., H. Hillebrand, and S. Flöder. 2017. Stability of marine phytoplankton communities facing stress related to global change: Interactive effects of heat waves and turbidity. Journal of Experimental Marine Biology and Ecology **497**:219-229.

Richardson, K., and J. Bendtsen. 2017. Photosynthetic oxygen production in a warmer ocean: the Sargasso Sea as a case study. Philosophical Transactions of the Royal Society A: Mathematical, Physical and Engineering Sciences **375**:20160329.

Rick, J. J., M. Scharfe, T. Romanova, J. E. E. van Beusekom, R. Asmus, H. Asmus, F. Mielck, A. Kamp, R. Sieger, and K. H. Wiltshire. 2023. An evaluation of long-term physical and hydrochemical measurements at the Sylt Roads Marine Observatory (1973–2019), Wadden Sea, North Sea. Earth System Science Data **15**:1037-1057.

Riemer, K., R. P. Guralnick, and E. P. White. 2018. No general relationship between mass and temperature in endothermic species. eLife **7**:e27166.

Rogers, T. L., B. J. Johnson, and S. B. Munch. 2022. Chaos is not rare in natural ecosystems. Nature Ecology & Evolution **6**:1105-1111.

Rose, J. M., and D. A. Caron. 2007. Does low temperature constrain the growth rates of heterotrophic protists? Evidence and implications for algal blooms in cold waters. Limnology and Oceanography **52**:886-895.

Rose, J. M., Y. Feng, G. R. DiTullio, R. B. Dunbar, C. E. Hare, P. A. Lee, M. Lohan, M. Long, J. W. O. Smith, B. Sohst, S. Tozzi, Y. Zhang, and D. A. Hutchins. 2009. Synergistic effects of iron and temperature on Antarctic phytoplankton and microzooplankton assemblages. Biogeosciences **6**:3131-3147.

Röthig, T., S. M. Trevathan-Tackett, C. R. Voolstra, C. Ross, S. Chaffron, P. J. Durack, L. M. Warmuth, and M. Sweet. 2023. Human-induced salinity changes impact marine organisms and ecosystems. Global Change Biology **29**:4731-4749.

Roy, S., and S. Majumdar. 2022. Chaos, Stochasticity and Noise. Pages 145-154 *in* S. Roy and S. Majumdar, editors. Noise and Randomness in Living System. Springer Singapore, Singapore.

Russell, B. D., C. D. G. Harley, T. Wernberg, N. Mieszkowska, S. Widdicombe, J. M. Hall-Spencer, and S. D. Connell. 2011. Predicting ecosystem shifts requires new approaches that integrate the effects of climate change across entire systems. Biology letters **8**:164-166.

Samuels, T., T. A. Rynearson, and S. Collins. 2021. Surviving Heatwaves: Thermal Experience Predicts Life and Death in a Southern Ocean Diatom. Frontiers in Marine Science **8**.

Santelia, M. E. 2022. Stranger things happen at sea: Phytoplankton phenotypic plasticity in response to thermal predictability across different timescales. PhD. University of Hamburg, Hamburg.

Sanz, J. L. 2011. Microorganism. Pages 1061-1061 *in* M. Gargaud, R. Amils, J. C. Quintanilla, H. J. Cleaves, W. M. Irvine, D. L. Pinti, and M. Viso, editors. Encyclopedia of Astrobiology. Springer Berlin Heidelberg, Berlin, Heidelberg.

Säterberg, T., and K. McCann. 2021. Detecting alternative attractors in ecosystem dynamics. Communications Biology **4**:975.

Schaum, C.-E., A. Buckling, N. Smirnoff, and G. Yvon-Durocher. 2022. Evolution of thermal tolerance and phenotypic plasticity under rapid and slow temperature fluctuations. Proceedings of the Royal Society B: Biological Sciences **289**:20220834.

Schaum, C.-E., B. Rost, A. J. Millar, and S. Collins. 2013. Variation in plastic responses of a globally distributed picoplankton species to ocean acidification. Nature Climate Change **3**:298-302.

Scheinin, M., U. Riebesell, T. A. Rynearson, K. T. Lohbeck, and S. Collins. 2015. Experimental evolution gone wild. Journal of the Royal Society Interface **12**:25833241.

Schmoker, C., S. Hernández-León, and A. Calbet. 2013. Microzooplankton grazing in the oceans: Impacts, data variability, knowledge gaps and future directions. Journal of Plankton Research **35**:691-706.

Schröter, F., C. Havermans, A. Kraft, N. Knüppel, A. Beszczynska-Möller, E. Bauerfeind, and E. M. Nöthig. 2019. Pelagic amphipods in the eastern fram strait with continuing presence of Themisto compressa based on sediment trap time series. Frontiers in Marine Science **6**:311.

Schrum, C., J. Lowe, H. E. M. Meier, I. Grabemann, J. Holt, M. Mathis, T. Pohlmann, M. D. Skogen, A. Sterl, and S. Wakelin. 2016. Projected Change—North Sea. Pages 175-217 *in* M. Quante and F. Colijn, editors. North Sea Region Climate Change Assessment. Springer International Publishing, Cham.

Seifert, M., C. Nissen, B. Rost, M. Vogt, C. Völker, and J. Hauck. 2023. Interaction matters: Bottom-up driver interdependencies alter the projected response of phytoplankton communities to climate change. Global Change Biology **29**:4234-4258.

Seifert, M., B. Rost, S. Trimborn, and J. Hauck. 2020. Meta-analysis of multiple driver effects on marine phytoplankton highlights modulating role of pCO2. Global Change Biology **26**:6787-6804.

Serra-Pompei, C., G. I. Hagstrom, A. W. Visser, and K. H. Andersen. 2019. Resource limitation determines temperature response of unicellular plankton communities. Limnology and Oceanography **64**:1627-1640.

Siepielski, A. M., M. B. Morrissey, S. M. Carlson, C. D. Francis, J. G. Kingsolver, K. D. Whitney, and L. E. B. Kruuk. 2019. No evidence that warmer temperatures are associated with selection for smaller body sizes. Proceedings of the Royal Society B: Biological Sciences **286**:20191332.

Sierocinski, P., F. Bayer, G. Yvon-Durocher, M. Burdon, T. Grosskopf, M. Alston, D. Swarbreck, P. J. Hobbs, O. S. Soyer, and A. Buckling. 2018. Biodiversity-function relationships in methanogenic communities. Molecular Ecology **27**:4641-4651.

Smith, L. 2007. Chaos: a very short introduction. OUP Oxford.

Smith, W. O., and S. Trimborn. 2024. Phaeocystis: A Global Enigma. Annual Review of Marine Science **16**:417-441.

Solomon, C. M., E. J. Lessard, R. G. Keil, and F. M. S. 2003. Characterization of extracellular polymers of Phaeocystis globosa and P. antarctica. Marine Ecology Progress Series **250**:81-89.

Sommer, U. 2002. Competition and Coexistence in Plankton Communities. Pages 79-108 *in* U. Sommer and B. Worm, editors. Competition and Coexistence. Springer Berlin Heidelberg, Berlin, Heidelberg.

Sommer, U., C. Paul, and M. Moustaka-Gouni. 2015. Warming and ocean acidification effects on phytoplankton - From species shifts to size shifts within species in a mesocosm experiment. PLoS ONE **10**:e0125239.

Sommeria-Klein, G., R. Watteaux, F. M. Ibarbalz, J. J. P. Karlusich, D. Iudicone, C. Bowler, and H. Morlon. 2021. Global drivers of eukaryotic plankton biogeography in the sunlit ocean. Science **374**:594-599.

Soulié, T., F. Vidussi, S. Mas, and B. Mostajir. 2022. Functional Stability of a Coastal Mediterranean Plankton Community During an Experimental Marine Heatwave. Frontiers in Marine Science **9**:831496.

Staehr, P. A., and K. Sand-Jensen. 2006. Seasonal changes in temperature and nutrient control of photosynthesis, respiration and growth of natural phytoplankton communities. Freshwater Biology **51**:249-262.

Stefanidou, N., S. Genitsaris, J. Lopez-Bautista, U. Sommer, and M. Moustaka-Gouni. 2018. Unicellular Eukaryotic Community Response to Temperature and Salinity Variation in Mesocosm Experiments. Frontiers in Microbiology **9**:2444.

Stefanidou, N., S. Genitsaris, J. Lopez-Bautista, U. Sommer, and M. Moustaka-Gouni. 2019. Response of a coastal Baltic Sea diatom-dominated phytoplankton community to experimental heat shock and changing salinity. Oecologia **191**:461-474.

Stegen, J. C., X. Lin, A. E. Konopka, and J. K. Fredrickson. 2012. Stochastic and deterministic assembly processes in subsurface microbial communities. The ISME Journal **6**:1653-1664.

Stewart, R. I. A., M. Dossena, D. A. Bohan, E. Jeppesen, R. L. Kordas, M. E. Ledger, M. Meerhoff, B. Moss, C. Mulder, J. B. Shurin, B. Suttle, R. Thompson, M. Trimmer, and G. Woodward. 2013. Chapter Two - Mesocosm Experiments as a Tool for Ecological Climate-Change Research. Pages 71-181 *in* G. Woodward and E. J. O'Gorman, editors. Advances in Ecological Research. Academic Press.

Stock, W., B. Vanelslander, F. Rüdiger, K. Sabbe, W. Vyverman, and U. Karsten. 2019. Thermal Niche Differentiation in the Benthic Diatom Cylindrotheca closterium (Bacillariophyceae) Complex. Frontiers in Microbiology **10**:1395.

Striebel, M., S. Schabhüttl, 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.

Strom, S. L., and K. A. Fredrickson. 2008. Intense stratification leads to phytoplankton nutrient limitation and reduced microzooplankton grazing in the southeastern Bering Sea. Deep Sea Research Part II: Topical Studies in Oceanography **55**:1761-1774.

Sullivan, M. R., F. W. Primeau, G. I. Hagstrom, W.-L. Wang, and A. C. Martiny. 2024. Integrating Trait-Based Stoichiometry in a Biogeochemical Inverse Model Reveals Links Between Phytoplankton Physiology and Global Carbon Export. Global Biogeochemical Cycles **38**:e2023GB007986.

Sunagawa, S., L. P. Coelho, S. Chaffron, J. R. Kultima, K. Labadie, G. Salazar, B. Djahanschiri, G. Zeller, D. R. Mende, A. Alberti, F. M. Cornejo-Castillo, P. I. Costea, C. Cruaud, F. d'Ovidio, S. Engelen, I. Ferrera, J. M. Gasol, L. Guidi, F. Hildebrand, F. Kokoszka, C. Lepoivre, G. Lima-Mendez, J. Poulain, B. T. Poulos, M. Royo-Llonch, H. Sarmento, S. Vieira-Silva, C. Dimier, M. Picheral, S. Searson, S. Kandels-Lewis, C. Bowler, C. de Vargas, G. Gorsky, N. Grimsley, P. Hingamp, D. Iudicone, O. Jaillon, F. Not, H. Ogata, S. Pesant, S. Speich, L. Stemmann, M. B. Sullivan, J. Weissenbach, P. Wincker, E. Karsenti, J. Raes, S. G. Acinas, and P. Bork. 2015. Structure and function of the global ocean microbiome. Science **348**:1261359.

Sunday, J. M., J. R. Bernhardt, C. D. G. Harley, and M. I. O'Connor. 2023. Temperature dependence of competitive ability is cold-shifted compared to that of growth rate in marine phytoplankton. Ecology Letters **27**:e14337.

Šupraha, L., K. Klemm, S. Gran-Stadniczeñko, C. Hörstmann, D. Vaulot, B. Edvardsen, and U. John. 2022. Diversity and biogeography of planktonic diatoms in Svalbard fjords: The role of dispersal and Arctic endemism in phytoplankton community structuring. Elementa: Science of the Anthropocene **10**:00117.

Suter, E. A., M. Pachiadaki, G. T. Taylor, and V. P. Edgcomb. 2022. Eukaryotic Parasites Are Integral to a Productive Microbial Food Web in Oxygen-Depleted Waters. Frontiers in Microbiology **12**:764605.

Tan, H., A. G. Hirst, D. Atkinson, and P. Kratina. 2021. Body size and shape responses to warming and resource competition. Functional Ecology **35**:1460-1469.

Tappan, H. 1979. Plankton. Pages 615-622 Paleontology. Springer Berlin Heidelberg, Berlin, Heidelberg.

Teichert, N., M. Lepage, A. Sagouis, A. Borja, G. Chust, M. T. Ferreira, S. Pasquaud, R. Schinegger, P. Segurado, and C. Argillier. 2017. Functional redundancy and sensitivity of fish assemblages in European rivers, lakes and estuarine ecosystems. Scientific Reports **7**:17611.

Telesh, I. V., H. Schubert, K. D. Joehnk, R. Heerkloss, R. Schumann, M. Feike, A. Schoor, and S. O. Skarlato. 2019. Chaos theory discloses triggers and drivers of plankton dynamics in stable environment. Scientific Reports **9**:20351.

Thomas, M. K., M. Aranguren-Gassis, C. T. Kremer, M. R. Gould, K. Anderson, C. A. Klausmeier, and E. Litchman. 2017. Temperature–nutrient interactions exacerbate sensitivity to warming in phytoplankton. Global Change Biology **23**:3269-3280.

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 R. Ranjan. 2024. Designing More Informative Multiple-Driver Experiments. Annual Review of Marine Science **16**:513-536.

Thomas, P. K., C. Kunze, D. B. Van de Waal, H. Hillebrand, and M. Striebel. 2022. Elemental and biochemical nutrient limitation of zooplankton: A meta-analysis. Ecology Letters **0**:1-17.

Tillmann, U. 2004. Interactions between Planktonic Microalgae and Protozoan Grazers. Journal of Eukaryotic Microbiology **51**:156-168.

Tilman, D., F. Isbell, and J. M. Cowles. 2014. Biodiversity and Ecosystem Functioning. Annual Review of Ecology, Evolution, and Systematics **45**:471-493.

Timmermans, M.-L., and Z. Labe. 2020. Arctic Report Card 2020: Sea Surface Temperature.

Toseland, A., S. J. Daines, J. R. Clark, A. Kirkham, J. Strauss, C. Uhlig, T. M. Lenton, K. Valentin, G. A. Pearson, V. Moulton, and T. Mock. 2013. The impact of temperature on marine phytoplankton resource allocation and metabolism. Nature Climate Change **3**:979-984.

Tréguer, P., C. Bowler, B. Moriceau, S. Dutkiewicz, M. Gehlen, O. Aumont, L. Bittner, R. Dugdale, Z. Finkel, D. Iudicone, O. Jahn, L. Guidi, M. Lasbleiz, K. Leblanc, M. Levy, and P. Pondaven. 2018. Influence of diatom diversity on the ocean biological carbon pump. Nature Geoscience **11**:27-37.

Trombetta, T., F. Vidussi, C. Roques, S. Mas, M. Scotti, and B. Mostajir. 2021. Co-occurrence networks reveal the central role of temperature in structuring the plankton community of the Thau Lagoon. Scientific Reports **11**:17675.

Tuerena, R. E., C. Mahaffey, S. F. Henley, C. de la Vega, L. Norman, T. Brand, T. Sanders, M. Debyser, K. Dähnke, J. Braun, and C. März. 2022. Nutrient pathways and their susceptibility to past and future change in the Eurasian Arctic Ocean. Ambio **51**:355-369.

Urrutia-Cordero, P., S. Langenheder, M. Striebel, David G. Angeler, S. Bertilsson, P. Eklöv, L. A. Hansson, E. Kelpsiene, H. Laudon, M. Lundgren, L. Parkefelt, I. Donohue, and H. Hillebrand. 2021. Integrating multiple dimensions of ecological stability into a vulnerability framework. Journal of Ecology **110**:374-386.

Utermöhl, H. 1958. Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. Internationale Vereinigung für Theoretische und Angewandte Limnologie: Mitteilungen **9**:1-38.

Vallina, S. M., P. Cermeno, S. Dutkiewicz, M. Loreau, and J. M. Montoya. 2017. Phytoplankton functional diversity increases ecosystem productivity and stability. Ecological Modelling **361**:184-196.

van de Poll, W. H., G. Kulk, P. D. Rozema, C. P. D. Brussaard, R. J. W. Visser, and A. G. J. Buma. 2018. Contrasting glacial meltwater effects on post-bloom phytoplankton on temporal and spatial scales in Kongsfjorden, Spitsbergen. Elementa: Science of the Anthropocene **6**:50.

van de Waal, D. B., and M. Boersma. 2012. Ecological stoichiometry in aquatic ecosystems.

van de Waal, D. B., A. M. Verschoor, J. M. Verspagen, E. van Donk, and J. Huisman. 2010. Climate-driven changes in the ecological stoichiometry of aquatic ecosystems. Frontiers in Ecology and the Environment **8**:145-152.

van der Loos, L. M., and R. Nijland. 2021. Biases in bulk: DNA metabarcoding of marine communities and the methodology involved. Molecular Ecology **30**:3270-3288.

van Nes, E. H., and M. Scheffer. 2004. Large species shifts triggered by small forces. American Naturalist **164**:255-266.

Vandermeer, J. 1993. Loose Coupling of Predator-Prey Cycles: Entrainment, Chaos, and Intermittency in the Classic Macarthur Consumer-Resource Equations. The American Naturalist **141**:687-716.

Venrick, E. L., J. R. Beers, and J. F. Heinbokel. 1977. Possible consequences of containing microplankton for physiological rate measurements. Journal of Experimental Marine Biology and Ecology **26**:55-76.

Verbeek, L., A. Gall, H. Hillebrand, and M. Striebel. 2018. Warming and oligotrophication cause shifts in freshwater phytoplankton communities. Global Change Biology **24**:4532-4543.

Verghese, J., J. Abrams, Y. Wang, and K. A. Morano. 2012. Biology of the heat shock response and protein chaperones: budding yeast (Saccharomyces cerevisiae) as a model system. Microbioly Molecular Biology Reviews **76**:115-158.

Vernet, M., T. L. Richardson, K. Metfies, N. Eva-Maria, and I. Peeken. 2017. Models of plankton community changes during a warm water anomaly in Arctic waters show altered trophic pathways with minimal changes in carbon export. Frontiers in Marine Science **4**:160.

Volpe, C., O. Vadstein, G. Andersen, and T. Andersen. 2021. Nanocosm: A well plate photobioreactor for environmental and biotechnological studies. Lab on a Chip **21**:2027-2039.

Walther, G.-R. 2010. Community and ecosystem responses to recent climate change. Philosophical Transactions of the Royal Society B: Biological Sciences **365**:2019-2024.

Wang, J., C. Zeng, and Y. Feng. 2024. Meta-analysis reveals responses of coccolithophores and diatoms to warming. Marine Environmental Research **193**:106275.

Wang, Q., Z. Lyu, S. Omar, S. Cornell, Z. Yang, and D. J. S. Montagnes. 2019. Predicting temperature impacts on aquatic productivity: Questioning the metabolic theory of ecology's "canonical" activation energies. Limnology and Oceanography **64**:1172-1185.

Wang, X., K. W. Tang, Y. Wang, and W. O. Smith. 2010. Temperature effects on growth, colony development and carbon partitioning in three Phaeocystis species. Aquatic Biology **9**:239-249.

Ward, B. A., B. B. Cael, S. Collins, and C. R. Young. 2021. Selective constraints on global plankton dispersal. Proceedings of the National Academy of Sciences **118**:e2007388118.

Wells, M. L., B. Karlson, A. Wulff, R. Kudela, C. Trick, V. Asnaghi, E. Berdalet, W. Cochlan, K. Davidson, M. De Rijcke, S. Dutkiewicz, G. Hallegraeff, K. J. Flynn, C. Legrand, H. Paerl, J. Silke, S. Suikkanen, P. Thompson, and V. L. Trainer. 2020. Future HAB science: Directions and challenges in a changing climate. Harmful Algae **91**:101632.

Wetzel, R. G. 2001. Protists: Key Ecosystem Regulators. BioScience **51**:997-997.

Wickman, J., E. Litchman, and C. A. Klausmeier. 2024. Eco-evolutionary emergence of macroecological scaling in plankton communities. Science **383**:777-782.

Wieczynski, D. J., P. Singla, A. Doan, A. Singleton, Z.-Y. Han, S. Votzke, A. Yammine, and J. P. Gibert. 2021. Linking species traits and demography to explain complex temperature responses across levels of organization. Proceedings of the National Academy of Sciences **118**:e2104863118.

Wiltshire, K. H., M. Boersma, K. Carstens, A. C. Kraberg, S. Peters, and M. Scharfe. 2015. Control of phytoplankton in a shelf sea: Determination of the main drivers based on the Helgoland Roads Time Series. Journal of Sea Research **105**:42-52.

Wiltshire, K. H., and B. F. J. Manly. 2004. The warming trend at Helgoland Roads, North Sea: phytoplankton response. Helgoland Marine Research **58**:269-273.

Winter, C., T. Bouvier, G. Weinbauer Markus, and T. F. Thingstad. 2010. Trade-Offs between Competition and Defense Specialists among Unicellular Planktonic Organisms: the "Killing the Winner" Hypothesis Revisited. Microbiology and Molecular Biology Reviews **74**:42-57.

Wolf-Gladrow, D. A., R. E. Zeebe, C. Klaas, A. Körtzinger, and A. G. Dickson. 2007. Total alkalinity: The explicit conservative expression and its application to biogeochemical processes. Marine Chemistry **106**:287-300.

Wolf, C., M. Iversen, C. Klaas, and K. Metfies. 2016. Limited sinking of Phaeocystis during a 12 days sediment trap study. Molecular Ecology **25**:3428-3435.

Wolf, K. K. E., C. J. M. Hoppe, and B. Rost. 2018. Resilience by diversity: Large intraspecific differences in climate change responses of an Arctic diatom. Limnology and Oceanography **63**:397-411.

Wolf, K. K. E., E. Romanelli, B. Rost, U. John, S. Collins, H. Weigand, and C. J. M. Hoppe. 2019. Company matters: The presence of other genotypes alters traits and intraspecific selection in an Arctic diatom under climate change. Global Change Biology **25**:2869-2884.

Worden, A. Z., M. J. Follows, S. J. Giovannoni, S. Wilken, A. E. Zimmerman, and P. J. Keeling. 2015. Rethinking the marine carbon cycle: Factoring in the multifarious lifestyles of microbes. Science **347**:1257594.

Wu, X., H. Liu, Z. Ru, G. Tu, L. Xing, and Y. Ding. 2021. Meta-analysis of the response of marine phytoplankton to nutrient addition and seawater warming. Marine Environmental Research **168**:105294.

Ye, M., M. Xiao, S. Zhang, J. Huang, J. Lin, Y. Lu, S. Liang, J. Zhao, X. Dai, L. Xu, M. Li, Y. Zhou, S. Overmans, J. Xia, and P. Jin. 2023. Multi-trait analysis reveals large interspecific differences for phytoplankton in response to thermal change. Marine Environmental Research **188**:106008.

Yeh, Y. C., D. M. Needham, E. T. Sieradzki, and J. A. Fuhrman. 2018. Taxon Disappearance from Microbiome Analysis Reinforces the Value of Mock Communities as a Standard in Every Sequencing Run. mSystems **3**:101128.

Yvon-Durocher, G., A. P. Allen, M. Cellamare, M. Dossena, K. J. Gaston, M. Leitao, J. M. Montoya, D. C. Reuman, G. Woodward, and M. Trimmer. 2015a. Five Years of Experimental Warming Increases the Biodiversity and Productivity of Phytoplankton. PLoS Biology **13**:e1002324.

Yvon-Durocher, G., J. M. Caffrey, A. Cescatti, M. Dossena, P. d. Giorgio, J. M. Gasol, J. M. Montoya, J. Pumpanen, P. A. Staehr, M. Trimmer, G. Woodward, and A. P. Allen. 2012. Reconciling the temperature dependence of respiration across timescales and ecosystem types. Nature **487**:472-476.

Yvon-Durocher, G., M. Dossena, M. Trimmer, G. Woodward, and A. P. Allen. 2015b. Temperature and the biogeography of algal stoichiometry. Global Ecology and Biogeography **24**:562-570.

Yvon-Durocher, G., J. M. Montoya, M. Trimmer, and G. U. Y. Woodward. 2011. Warming alters the size spectrum and shifts the distribution of biomass in freshwater ecosystems. Global Change Biology **17**:1681-1694.

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. Frontiers in Microbiology **8**:2003.

Zélé, F., S. Magalhães, S. Kéfi, and A. B. Duncan. 2018. Ecology and evolution of facilitation among symbionts. Nature Communications **9**:4869.

Zhang, S. F., K. Zhang, H. M. Cheng, L. Lin, and D. Z. Wang. 2020. Comparative transcriptomics reveals colony formation mechanism of a harmful algal bloom species Phaeocystis globosa. Science of the Total Environment **719**:137454.

Zhang, Y., R. Klapper, K. T. Lohbeck, L. T. Bach, K. G. Schulz, T. B. H. Reusch, and U. Riebesell. 2014. Between- and within-population variations in thermal reaction norms of the coccolithophore Emiliania huxleyi. Limnology and Oceanography **59**:1570-1580.

Zhang, Y., Y. Zhuang, Z. Ji, J. Chen, Y. Bai, B. Wang, and H. Jin. 2023. Impacts of Atlantic water intrusion on interannual variability of the phytoplankton community structure in the summer season of Kongsfjorden, Svalbard under rapid Arctic change. Marine Environmental Research:106195.

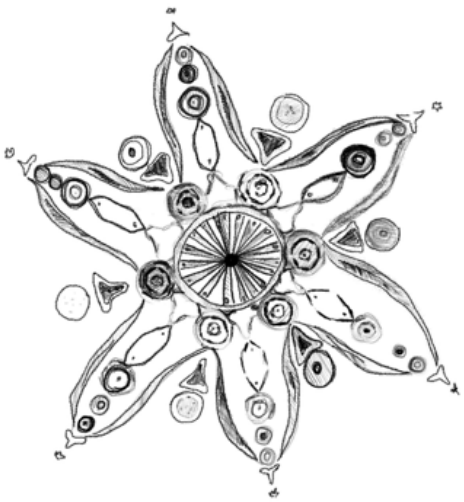
Zhong, D., L. Listmann, M. E. Santelia, and C. E. Schaum. 2020. Functional redundancy in natural pico-phytoplankton communities depends on temperature and biogeography. Biology letters **16**:20200330.

Zhou, J., Y. Deng, P. Zhang, K. Xue, Y. Liang, J. D. Van Nostrand, Y. Yang, Z. He, L. Wu, D. A. Stahl, T. C. Hazen, J. M. Tiedje, and A. P. Arkin. 2014. Stochasticity, succession, and environmental perturbations in a fluidic ecosystem. Proceedings of the National Academy of Sciences **111**:E836-E845.

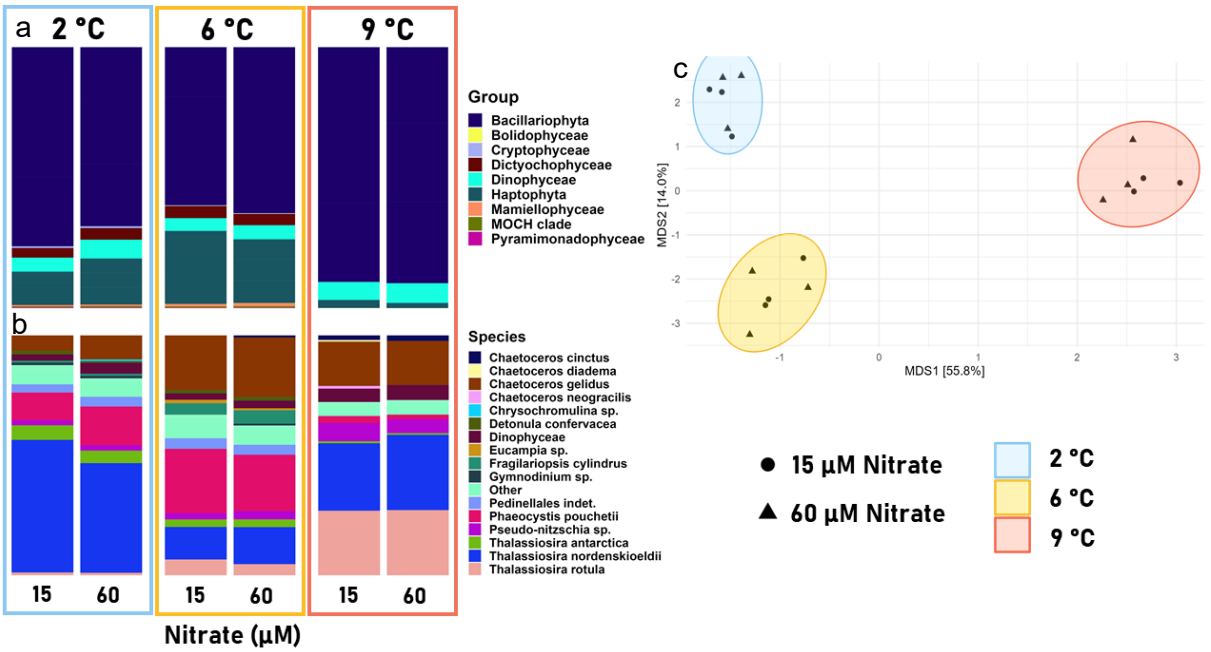
Zhou, J., and D. Ning. 2017. Stochastic community assembly: Does it matter in microbial ecology? Microbiology and Molecular Biology Reviews **81**:101128.

Zhu, Z., K. Xu, F. Fu, J. L. Spackeen, D. A. Bronk, and D. A. Hutchins. 2016. A comparative study of iron and temperature interactive effects on diatoms and Phaeocystis antarctica from the Ross Sea, Antarctica. Marine Ecology Progress Series **550**:39-51.

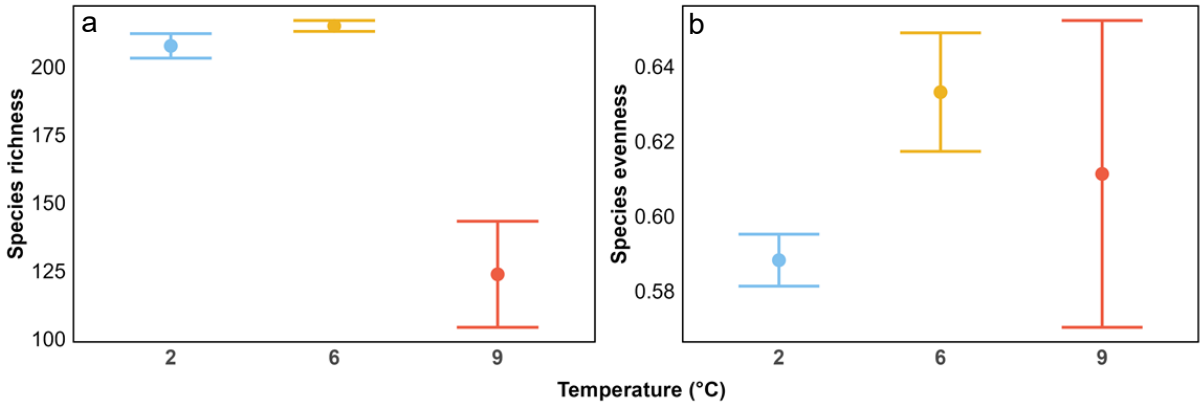
8
Appendix



8.1 Appendix figures



Appendix 1: Results of the 18S rRNA gene sequencing of a side experiment of publication I (chapter 2) which compared low (15 µM) with high (60 µM) nitrate levels. (a) Group-level bar plots of the protist community composition across temperatures and nitrate levels, (b) species-level bar plots of the phototrophy community composition and (c) multidimensional scaling plot of the different communities.



Appendix 2: Diversity metrics ((a) species richness and (b) species evenness) across temperature levels of the community experiment of chapter 2. Dots represent the mean values of the replicates (n = 3) and the error bars represent +/- one standard deviation.

8.2 List of figures

8.3 Declaration

Versicherung an Eides Statt

Ich, Antonia Ahme,

versichere an Eides Statt durch meine Unterschrift, dass ich die vorstehende Arbeit selbständig und ohne fremde Hilfe angefertigt und alle Stellen, die ich wörtlich dem Sinne nach aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe, mich auch keiner anderen als der angegebenen Literatur oder sonstiger Hilfsmittel bedient habe.

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Ort, Datum Unterschrift

8.4 Contribution

Declaration on the contribution of the candidate to a multi-author article/manuscript. Contribution of the candidate in % of the total workload (up to 100% for each of the following categories).

Chapter 2, Publication I

Experimental concept and design: ca. 85%

Experimental work and/or acquisition of (experimental) data: ca. 95%

Data analysis and interpretation: ca. 70%

Preparation of Figures and Tables: ca. 70%

Drafting of the manuscript: ca. 95%

Chapter 3, Publication II

Experimental concept and design: ca. 90%

Experimental work and/or acquisition of (experimental) data: ca. 70%

Data analysis and interpretation: ca. 90%

Preparation of Figures and Tables: ca. 100%

Drafting of the manuscript: ca. 95%

Chapter 3, Publication III

Experimental concept and design: ca. 60%

Experimental work and/or acquisition of (experimental) data: ca. 25%

Data analysis and interpretation: ca. 20%

Preparation of Figures and Tables: ca. 5%

Drafting the manuscript: ca. 5%

Revising the manuscript: ca. 30%

Chapter 3, Publication IV

Experimental concept and design: ca. 50%

Experimental work and/or acquisition of (experimental) data: ca. 25%

Data analysis and interpretation: ca. 20%

Preparation of Figures and Tables: ca. 5%

Drafting the manuscript: ca. 5%

Revising the manuscript: ca. 30%

Chapter 4, Publication V

Experimental concept and design: ca. 10%

Experimental work and/or acquisition of (experimental) data: ca. 30%

Data analysis and interpretation: ca. 90%

Preparation of Figures and Tables: ca. 100%

Drafting of the manuscript: ca. 95%

Chapter 4, Publication VI

Experimental concept and design: ca. 0%

Experimental work and/or acquisition of (experimental) data: ca. 20%

Data analysis and interpretation: ca. 10%

Preparation of Figures and Tables: ca. 5%

Drafting the manuscript: ca. 5%

Revising the manuscript: ca. 20%

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Ort, Datum Unterschrift

2024

Antonia Ahme