# Impacts of warming and increasing $pCO_2$ on natural phytoplankton communities

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# Zusammenfassung

Nach Vorhersagen der IPCC (2014), wird die Oberflächentemperatur der Ozeane voraussichtlich um 3-5°C ansteigen, während gleichzeitig damit gerechnet wird, dass sich die $CO_2$ Konzentration im Wasser bis zum Jahre 2100 verdoppelt haben wird. Zahlreiche Experimente stellten den Einfluss von Erwärmung bzw. vom Anstieg des $CO_2$ Gehalts im Wassers auf die Biomasse, das Wachstum und die chemische Zusammensetzung des Phytoplanktons fest. Die Auswirkungen beider Klimafaktoren zusammen sind jedoch größtenteils noch unbekannt, besonders hinsichtlich natürlicher Phytoplanktongemeinschaften. Da das Phytoplankton die Basis des pelagischen Nahrungsnetzes bildet, wird vermutet, dass sich Veränderungen in deren Artengemeinschaft und in deren Biomasse aufgrund des Klimawandels auf die Futterverfügbarkeit und die trophischen Beziehungen im Ökosystem auswirken werden.

Um die gemeinsamen Einflüsse von Erwärmung und Anstieg des $pCO_2$ Gehalts auf natürliche Phytoplanktongemeinschaften zu untersuchen, führte ich drei Mesokosmenexperimente durch. Es war des Weiteren mein Ziel herauszufinden, ob saisonale Blütenereignisse, mit ihren charakteristischen Unterschieden in der Artenzusammensetzung und in den Wachstumsbedingungen des Phytoplanktons, in ihren Antworten auf die Klimaveränderung variieren.

Im ersten Kapitel (Kapitel I) untersuchte ich die Einflüsse des Klimawandels auf eine typische Diatomeen-dominierte Herbstblüte der Ostsee. Meine Ergebnisse zeigten eine temperatur-bedingte frühere Blüte und eine Abnahme der Phytoplankton-biomasse. Der Phytoplanktonkohlenstoffgehalt, zum Beispiel, sank mit Anstieg der Temperatur um mehr als die Hälfte ab. Keine Hinweise dagegen konnten für einen direkten Einfluss des steigenden $pCO_2$ Gehalts oder dessen Interaktion mit Erwärmung gefunden werden.

Im zweiten Kapitel (Kapitel II) konnte ich nachweisen, wie bereits in Kapitel I vermutet, dass Erwärmung zu einer Verstärkung des Fraßdruckes seitens der Copepoden (Mesozooplankton) führt. Des Weiteren zeigten meine Ergebnisse, dass Auswirkungen von Erwärmung auf die nächst höhere trophische Ebene übertragen werden können. Auf diese Weise veränderte sich die Sommer-Phytoplanktongemeinschaft von einem “bottom-up” zu einem vornehmlichen “top-down” kontrollierten System. Dies zeigte sich in einer signifikanten Abnahme der

Phytoplanktonbiomasse und in einem Anstieg der Zooplanktonabundanz bei steigender Temperatur. Hohe $pCO_2$ Werte beeinflussten hingegen Copepoden Nauplien negativ. Deren Abnahme der Abundanz und eine Verringerung ihres Fraßes spiegelte sich in einer signifikanten Zunahme der Phytoplanktonbiomasse in den kalten $CO_2$-erhöhten Mesokosmen wieder.
Im dritten Kapitel (Kapitel III) untersuchte ich experimentell die Auswirkungen der Klimaveränderung auf die Fettsäurezusammensetzung und den Fettsäuregehalt zweier natürlicher Sommer-Phytoplanktongemeinschaften. Meine Ergebnisse zeigten, dass Erwärmung das Potenzial aufweist, den Gehalt an essenziellen mehrfach ungesättigten Fettsäuren (PUFAs) im Phytoplankton zu verändern. Dies lässt eine möglichen Beeinflussung der Futterqualität für höhere trophische Ebenen vermuten, wenngleich sich das Ausmaß der Effekte zwischen den beiden Sommerstudien unterschied. Entgegen der allgemeinen Theorie korrelierten die Veränderungen in den Fettsäuren als Reaktion auf die Klimaveränderung nicht mit entsprechenden Veränderungen in der taxonomischen Zusammensetzung des Phytoplanktons.

Insgesamt zeigen meine Ergebnisse, dass die ansteigende Wassertemperatur potenziell die chemische Zusammensetzung des Phytoplanktons verändern kann, was sich möglicherweise auf höhere trophische Ebenen auswirken wird. Des Weiteren belegen meine Studien, dass Erwärmung zu einem erhöhten Fraßdruck führt und die trophischen Beziehungen zwischen dem Phytoplankton und deren Fraßfeinden verschieben kann. Steigende $pCO_2$ Werte, indessen, scheinen die Biomasse und die chemischen Zusammensetzung natürlicher Phytoplanktongemeinschaften nur in geringerem Maße zu beeinflussen.

# Summary

Following the predictions of the IPCC (2014), the water surface temperature in the oceans is proposed to increase by 3-5°C, while at the same time $CO_2$ concentrations in the water are expected to double until the year 2100. A large number of experiments observed effects of warming or rising $CO_2$ concentrations in the water on phytoplankton's biomass, growth and chemical composition. The combined effects of both climate change factors are to a large extent still unclear, especially for natural phytoplankton communities. As the phytoplankton represents the base of the pelagic food web, changes in their community composition and biomass due to climate change are supposed to affect food availability and trophic relations in the ecosystem.

To address the combined effects of warming and rising $pCO_2$ on natural plankton communities, I conducted three mesocosm experiments. Furthermore, I wanted to find out, if seasonal bloom events with their characteristic differences in phytoplankton species composition and growth conditions vary in their responses to climate change.

In the first chapter, I investigated the effects of climate change on a typical diatom-dominated autumn bloom of the Baltic Sea. My results showed a temperature-induced earlier bloom-time and a time-dependent decrease in all phytoplankton biomass parameters. Phytoplankton carbon, for instance, declined by more than half with increasing temperature. No evidences, instead, were found for a direct effect of rising $pCO_2$ or an interaction with warming.

In chapter II, I provided evidence for my suggestion of chapter I that warming enhances the grazing pressure of copepods (meso-zooplankton). Furthermore, my results showed that warming effects can be translated to the next higher trophic level by switching a summer plankton community from a bottom-up to a mainly top-down controlled system. This was reflected by a significant decrease in phytoplankton carbon and an increase in zooplankton abundance under higher temperature. High $pCO_2$ levels, instead, indicated a negative impact on copepod nauplii. Their decrease in abundance and their release from grazing was mirrored by a significant increase in phytoplankton carbon in the cold high $pCO_2$ mesocosms.

In the third chapter (chapter III), I intended to experimentally explore the effects of climate change on the fatty acid composition and contents of two natural

phytoplankton summer communities. My results showed that warming has the potential to change the content of essential polyunsaturated fatty acids (PUFAs). This suggest a possible impact on the food quality for higher trophic levels, however, the magnitude of the effects differed between the two studies. Contrasting to a general assumption, the observed changes in fatty acids due to climate change could not be related to changes in taxonomic composition.

Overall my results point out that an increase of the water temperature has the potential to influence phytoplankton's chemical composition, possibly affecting higher trophic levels. Moreover, my studies evidence that warming enhances grazing pressure and can alter trophic relations between phytoplankton and their grazers in the pelagic food web. Increasing $pCO_2$ concentrations, instead, seem to affect biomass and chemical composition of natural phytoplankton communities only to a lesser extent.

# Introduction

## The role of the phytoplankton

Phytoplankton are the dominant phytosynthetic producers in the ocean and represent an extreme phylogenetic diversity including pigmented protists (algae) and cyanobacteria. They form the primary producer level in the elemental and nutrient cycles and represent the foundation of the energy transfer in the pelagic system (Sommer et al. 2012b). Their net primary production (NPP) in the euphotic pelagic zone of the oceans contributes nearly half of the production of organic matter on Earth, although they represent only 0.2 % of global primary producer biomass (Field et al. 1998). Especially phytoplankton communities in the cold and higher latitude regions are highly productive. Between 50 and 85 % of the total atmospheric oxygen are approximately produced by these organisms in marine and aquatic environments (Field et al. 1998). Phytoplankton can be classified taxonomically in the five main groups: diatoms (Bacillariophyceae), cyanobacteria (Cyanophyceae), chlorophytes (Chlorophyceae), Prymnesiophyta and dinoflagellates. Their ecological role differs due to their function and their biogeochemical signature.

## Seasonal succession and trophic link

At high latitudes and in seasonally stratified waters with nutrient impoverishment during summer the phytoplankton spring bloom is usually the seasonal maximum of primary production (Sommer et al. 2012b), which provides most of the energy and organic matter for higher trophic levels like zooplankton and fish. The spring bloom is mainly dominated by diatoms, the preferred food source for zooplankton copepods. In the Baltic Sea further important seasonal peak events go along with changes in nutrient availability and species composition (Wasmund et al. 2008). In summer, when nitrogen is limited, picoplankton (<2 µm) but also flagellates and large nitrogen fixing filamentous cyanobacteria dominate the phytoplankton. Especially in the Central Baltic Sea filamentous cyanobacteria can occur in large, often toxic, blooms. The autumn biomass peak is again dominated by diatoms, providing energy resources for the overwintering zooplankton (Wasmund et al. 2008). The annual cycle of phytoplankton blooms and their species composition can be attributed to temperature, the availability of light and nutrient supply (bottom-up control) but also to the strength of top-down control by grazing pressure of the micro- and

mesozooplankton. Food availability is assumed to control the development of zooplankton grazers. Thus, their peaks follow with some delay the biomass of phytoplankton (Sommer et al. 2012b). Phytoplankton species composition and species sizes play a major role in the phytoplankton – zooplankton interactions in terms of food availability. Further, food quality governed by the chemical composition of the phytoplankton, e.g. fatty acid composition and C:N:P ratio, might affect grazer's growth, survival and reproduction success.

The increase of greenhouse gases in the atmosphere leads to ocean surface warming and rising $pCO_2$ and subsequent changes in stratification, nutrient supply and light availability. Together, these changes are predicted to alter phytoplankton physiology, species abundances and, thus, community composition and global biogeochemical cycling (Litchman et al. 2015). This might lead to changes in the food chain length and the trophic interactions in the marine plankton food web.

## Phytoplankton communities under climate change

Human industrial activities and the emission of fossil fuels have increased the atmospheric $CO_2$ partial pressure since the beginning of the industrial period, which caused an increased uptake of $CO_2$ by the world-wide ocean surface water. The increase of $H^+$ ions results in an incremental acidification of the surface water with an already today observed overall decline of 0.1 pH units since the pre-industrial period (Caldeira & Wicket 2005). Ocean surface $pCO_2$ is prospected to double from current values of approximately 390 µatm to 700 µatm while the pH is expecetd to decrease to 0.5 by the year 2100 (IPCC 2014). The estimated average global ocean surface temperature has already increased by 0.6°C (Hoegh-Guldberg & Bruno 2010) in the last 100 years and is predicted to further increase by even 3-5°C by the year 2100 (IPPC 2014).

Across marine species, rising $pCO_2$ has been observed to act as a stressor, in particular for calcifiers, but it can also have a fertilizing effect in case $CO_2$ is a limiting resource (Kroeker et al. 2012, 2013). The increased inorganic carbon concentrations in the water are supposed to reduce the energetic costs for the phytoplankton's effective carbon concentrating mechanisms (CCM) due to a lower electrochemical gradient through the cell. This might be one of the underlying mechanisms for the benefits of phytoplankton from rising $pCO_2$. However, the efficiency of the CCMs seems to differ strongly among species (Burkhardt et al. 2001; Rost et al. 2008), size

classes and phytoplankton groups (Reinfelder 2011; Raven & Beardall 2014), which might affect species composition and abundance in phytoplankton communities under increasing $pCO_2$.

The effects of rising seawater temperature vary strongly among phytoplankton taxonomic groups and even species therein (Litchman et al. 2015). Warming-induced changes in species distribution, taxonomic community composition as well as phenology in phytoplankton communities are proposed to affect the food quantity and availability for higher trophic levels (e.g. Garzke 2014; Lewandowska et al. 2014; Paul et al. 2015). Further, warming effects were observed to vary regionally and / or with seasonal phytoplankton bloom events, depending on the prevailing nutrient conditions (Lewandowska et al. 2014, Paul et al. accepted). Under nutrient deplete conditions, e.g. oligotrophic open oceans or seasonally stratified seas such as the Baltic Sea in summer, systems are mainly bottom-up controlled via nutrient supply. In these systems the phytoplankton was observed to increase in response to higher seawater temperatures (Taucher et al. 2012; Suikkanen et al. 2013; Lewandowska et al. 2014). Smaller species and nitrogen fixing cyanobacteria are supposed to be favored under such conditions. Therefor they can increase in abundances at the expense of other groups like diatoms (Litchman et al. 2015). Instead, under nutrient replete conditions, found in most coastal regions in spring and autumn, phytoplankton blooms with high diatom abundances are mainly top-down controlled via zooplankton grazing. Such regions are supposed to react to warming with earlier onsets of phytoplankton blooms and decreased phytoplankton biomass due to intensified grazing pressure (O'Connor et al. 2009; Sommer & Lewandowska 2011).

## Climate change and the chemical composition of the phytoplankton

Climate change is expected to directly affect phytoplankton's chemical composition in terms of stoichiometry and fatty acid composition, which likely has significant impacts on herbivorous consumers. Further, the chemical composition of phytoplankton communities can be indirectly affected by changes in the physiological state and the taxonomic composition due to changes in the environmental conditions (Leu et al. 2012). Rising $pCO_2$, for instance, was shown to rise elemental carbon to nitrogen (C:N) ratios (Tortell et al. 2000; Riebesell et al. 2007; Eggers et al. 2014) and elemental carbon to phosphorus (C:P) ratios (Schulz et al. 2013) in experimental studies using phytoplankton communities. However, other ones found no effects on

C:N:P ratios. Warming also seems to affect stoichiometry in natural phytoplankton communities, but the magnitude varies strongly between studies (Wohlers-Zöllner et al. 2012, Paul et al. 2015) and within temporal successions from pre-bloom to bloom and senescence conditions. Overall, the direction of changes in food quality is still unclear.

Food quality as determined by the fatty acid composition is expected to downgrade under rising $pCO_2$ and ocean surface warming. Phytoplankton organisms are supposed to accumulate saturated fatty acids by simultaneously decreasing the amount of essential polyunsaturated fatty acids (PUFAs) under rising $pCO_2$ to regulate the internal cell homeostasis (Rossoll et al. 2012). Warming is also hypothesized to alter the fatty acid chain lengths and the degree of saturations (Dalsgaard et al. 2003), reducing the content of PUFAs. This might lead to a mismatch in PUFA supply as metazoans (here: zooplankton, heterotrophic nanoflagellates) have to take up PUFAs with the food. In contrast to phytoplankton, metazoa cannot synthesize PUFAs *de novo* at rates sufficient to meet their metabolic demands (Brett & Müller-Navarra 1997).

Interaction effects among the major stressors such as rising $pCO_2$, warming and changes in light availability and nutrient supply on the marine plankton system are to a large extend still unexplored. Especially the effects of simultaneous warming and rising $pCO_2$ on the phytoplankton community composition and biomass development largely remain to be experimentally tested. Single effects observed for warming and rising $pCO_2$ might be strengthened, reduced or even cancel each other out under combined climate change conditions. Further it is still unknown if and in which way possible effects on phytoplankton biomass, taxonomic and chemical composition are transmitted to the higher trophic levels like the zooplankton.

# Thesis outline

This thesis is divided into three chapters. Each chapter represents the results of independent studies, addressing the combined effects of warming and rising $pCO_2$ on natural plankton communities of Kiel Fjord (western Baltic Sea) from different seasonal bloom events. This outline gives a short overview of the motivation for the single experimental studies. All experiments were conducted using a large scale mesocosm facility to control the manipulated environmental factors. My aim was to find out in which way both factors of climate change simultaneously affect natural phytoplankton communities regarding biomass, species- and chemical composition. Further, I investigated the possibility of a transmission of climate change effects on the phytoplankton to higher trophic levels in the food chain.

## Chapter 1

This first chapter investigates the effects of climate change on a typical diatom-dominated autumn bloom. Since the effects of warming and rising $pCO_2$ have usually been studied independently, I also was interested in the interaction effects of both climate change factors. Therefore I used a mesocosm experiment with a full factorial design, crossing two temperatures (9°C and 15°C) with two $pCO_2$ levels (target values: 560 and 1400 µatm). Temperature is known to strongly affect species metabolism, leading to increasing growth rates, faster development and higher metabolic demand with an overall increased grazing pressure by top-predators. In that way I hypothesized that warming leads to a decreased phytoplankton biomass and earlier bloom time. Rising $pCO_2$ was hypothesized to have positive effects on diatom dominated communities, which should be reflected in a higher phytoplankton biomass. Due to that I also hypothesized that warming and rising $pCO_2$ have interactive effects on phytoplankton biomass**.** The chemical composition of the phytoplankton in terms of their cellular stoichiometry was additionally proposed to be affected by climate change.

## Chapter 2

Chapter two investigates the effects of future climate change on a coastal summer plankton community under natural nutrient limited conditions. In order to test the effects of warming and rising $pCO_2$ simultaneously, I conducted a mesocosm

experiment using a full factorial design by crossing two temperatures (16.5°C and 22.5°C) with six $pCO_2$ target levels, ranging from 500 to 3000 µatm. Compared to bloom events in spring and autumn, phytoplankton summer communities are generally known to be stronger bottom up regulated via nutrient supply than top-down controlled via grazing. I hypothesized that higher surface temperatures and increasing $CO_2$ concentrations in the water both lead to increased phytoplankton biomass and carbon to nutrient ratios. Further it was hypothesized that changes in phytoplankton biomass and stoichiometry translate to the next trophic level, visible in the zooplankton's abundance and resource use efficiency (RUE).

## Chapter 3

In the third chapter the focus was related to the impact of climate change on the fatty acid composition of two natural Baltic Sea summer plankton communities under nutrient limited conditions. The studies were conducted in the years 2013 and 2014. Both communities were treated by the same multi-factorial design, crossing two temperatures with six target $pCO_2$ levels, ranging from 500 to 3000 µatm. My aim was to identify, in which way warming and rising $pCO_2$ affect phytoplankton's fatty acid composition and total fatty acid content. A focus was put on the effects of climate change on the polyunsaturated fatty acids (PUFAs). They are essential for all species and have to be taken up through the food chain by higher trophic levels. Further, changes in the fatty acids due to warming and / or rising $pCO_2$ were expected to reflect changes in the taxonomic composition of the phytoplankton community, as especially PUFAs can be used as taxonomic markers.

BIOACID Indoor-mesocosms

# Chapter I

## Warming but not enhanced $CO_2$ concentration quantitatively and qualitatively affects phytoplankton biomass


### Abstract

We investigated the impacts of predicted ocean acidification and future warming on the quantity and nutritional quality of a natural phytoplankton autumn bloom in a mesocosm experiment. Since the effects of $CO_2$-enrichment and temperature have usually been studied independently, we were also interested in the interactive effects of both aspects of climate change. Therefore, we used a factorial design with two temperature and two acidification levels in a mesocosm experiment with a Baltic Sea phytoplankton community. Our results show a significant time-dependent influence of warming on phytoplankton carbon, chlorophyll *a* as well as POC. Phytoplankton carbon for instance decreased by more than a half with increasing temperature at bloom time. Additionally, elemental carbon to phosphorus ratios (C:P) increased significantly by approximately 5-8 % under warming. Impacts of $CO_2$ or synergetic effects of warming and acidification could not be detected. We suggest that temperature-induced stronger grazing pressure was responsible for the significant decline in phytoplankton biomass. Our results suggest that biological effects of warming on Baltic Sea phytoplankton are considerable and will likely have fundamental consequences for the trophic transfer in the pelagic food-web.

## Introduction

Ocean acidification, also known as “the other $CO_2$ problem” is caused by increasing uptake of $CO_2$ by the surface water due to the rising atmospheric $CO_2$ partial pressure. The uptake of $CO_2$ leads to increased aqueous $CO_2$, bicarbonate ($HCO_3^-$), and hydrogen ion ($H^+$) concentrations, while the concentration of carbonate ions ($CO_3^{2-}$) declines. The increase of $H^+$ ions causes the acidification of the surface water with an overall decline of 0.1 pH units since the pre-industrial period (Caldeira & Wicket 2005) associated with a substantial decrease in carbonate ion concentration by 30 % (Hoegh-Guldberg & Bruno 2010). As atmospheric $CO_2$ is predicted to rise from current values of approximately 390 µatm to values of 700 µatm at the end of 21$^{st}$ Century (IS92a scenario; Meehl et al. 2007), pH will decrease further by 0.3-0.4 units (Hama et al. 2012). Parallel to ocean acidification, sea surface temperature has already increased by 0.6°C in the last 100 years (Hoegh-Guldberg & Bruno 2010). A doubling of atmospheric $CO_2$ in the 21$^{st}$ century is predicted to go along with a rise of an estimated average global ocean surface temperature of even 2-4.5°C (IPPC 2014).

So far, only a few studies have analyzed the combined effects of both factors on marine primary producers (Hare et al. 2007; Feng et al. 2008, 2009; Torstensson et al. 2012), although sea surface pH and temperature will change in parallel in a future ‘greenhouse’ world. Instead many studies have addressed the biological effects of either ocean acidification or warming in particular on phytoplankton species composition and biomass. These studies indicate that $CO_2$ can act as a stressor, in particular for calcifiers, but it can also have a fertilizing effect in case $CO_2$ is a limiting resource. Across marine species, altered survival, calcification, growth, development and abundance in response to acidification could be observed (Kroeker et al. 2012, 2013). The magnitude of responses, however, significantly varied among species. Whereas growth of calcifying taxa was on average negatively affected by rising $CO_2$, growth of fleshy algae and diatoms increased (Kroeker et al. 2013). In natural Antarctic phytoplankton communities, Tortell et al. (2008) found an increase in growth of larger chain-forming diatoms, resulting in a species compositional shift from prior dominating small pennate diatoms (*Pseudo-nitzschia subcurvata*) to large centric species (*Chaetoceros* spp.). Concordantly, in natural oceanic phytoplankton assemblages Eggers et al. (2014) found a $CO_2$ induced increase of total phytoplankton biomass that was driven by a shift towards large sized diatoms, esp.

*Chaetoceros* spp. and *Thalassiosira constricta*. Contrasting to these observations Schulz et al. (2013) observed no positive $CO_2$ effect on diatom biomass in a natural arctic plankton community. Instead the pico-eukaryote biomass increased under enhanced $CO_2$. However, it was a post-bloom situation that was tested in which the initial diatom abundance was naturally very low (<0.5 µmol C $L^{-1}$).
Increasing $CO_2$ can also increase the efficiency for the phytoplankton to use limiting nutrients to fix carbon. This consequently can result in higher elemental carbon to nitrogen (C:N) ratios (Tortell et al. 2000) and higher elemental nitrogen to phosphorus (N:P) ratios (Tortell et al. 2002). Increased C:N ratios with elevated $CO_2$ were also found by Eggers et al. (2014) in their experiment with natural oceanic phytoplankton communities. In natural phytoplankton assemblages from the arctic, however, a significantly lower C:N ratio could be shown (Schulz et al. 2013). Similarly to the study above (Eggers et al. 2014) a higher elemental ratio of nitrogen to phosphorus (N:P) as well as carbon to phosphorus (C:P) were observed under high $CO_2$ for the arctic assemblage.

Increased water temperature is expected to change the distribution and abundance of phytoplankton communities as well as their phenology and productivity (Hoegh-Guldberg & Bruno 2010). Until now most studies that tested warming on plankton communities have focused on the development of the spring bloom (see Lewandowska & Sommer 2010 and references therein) as it is one of the most important seasonal patterns in pelagic food webs. Mesocosm experiments with Baltic Sea spring phytoplankton showed a significant decrease in the total phytoplankton biomass, as well as a shift towards picophytoplankton and small nanophytoplankton (<5µm) (Sommer & Lengfellner 2008; Lewandoska & Sommer 2010; Sommer & Lewandowska 2010). Both, the decrease in biomass as well as the species shift, were interpreted as footprints of more intensive grazing by copepods and ciliates under warming (Keller et al. 1999; Lewandoska & Sommer 2010). It was also observed that warming accelerated the occurrence of the phytoplankton bloom peak by approximately one day $°C^{-1}$ (Sommer & Lengfellner 2008; Sommer & Lewandowska 2011). Following Eppley (1972) and Torstensson et al. (2012), their observed warming-related altered growth rates may additionally shift the competitive advantage between different algae species.

Although summer experiments with Baltic Sea communities are scarce, results published so far did not confirm the negative relationship between biomass and temperature as reported for the spring bloom (Taucher et al. 2012). Predictions for the influence of rising temperature on autumn phytoplankton communities are completely lacking. Community studies which include changes in the cellular stoichiometry under warming are also rare, even for spring blooms. Wohlers-Zöllner et al. (2012) found a lower mean of particulate C:P ratio with warming in the mesocosm studies with phytoplankton spring communities.

Addressing both factors, $CO_2$ and temperature, simultaneously, the meta-analysis by Kroeker et al. (2013) showed a strong trend towards lower growth rates and development at elevated temperature and $CO_2$, when all marine taxa are pooled together. Additionally the results highlight a trend towards enhanced sensitivity to acidification with warming.

For the phytoplankton in particular, contrasting and species-specific results have been found. The growth rate of the calcifier *Emiliania huxleyi* increased in response to higher temperature as well as to elevated $CO_2$, but an interaction effect among the parameters has not been found (Feng et al. 2008). In contrast, growth rates of the diatom *Navicula directa* increased by 43 % under warming but decreased by 5 % under acidification (Torstensson et al. 2012). In bottle experiments with a natural North Atlantic spring bloom community, a trend of increased total Chl *a* was recognized under greenhouse conditions, i.e. increased temperature and $CO_2$, but no change was observed when just one of the parameters was manipulated (Feng et al. 2009). According to a bottle experiment by Hare et al. (2007) warming alone and in combination with acidification led to substantial increases in carbon fixation rates in a natural Bering Sea summer phytoplankton experiment. Further, a shift from diatom to nanophytoplankton dominance could be detected. However, mesocosm experiment studies based on natural plankton communities combining $CO_2$ and temperature are generally still scarce.

Our study site, Kiel Fjord, Western Baltic Sea, is known as a naturally $CO_2$-enriched area. Here, $CO_2$ concentrations strongly fluctuate and are elevated during large parts of the year (Thomsen et al. 2010, 2013). Due to high primary productivity caused by eutrophication in this area, amplified community respiration leads to a significant consumption of $pO_2$ and at the same time to a strong increase of $CO_2$ below the

thermocline (Helcom 2009; Thomsen et al. 2013). Typical winds from southwest lead to upwelling events of water bodies with high $CO_2$ concentration to the surface of the otherwise seasonally stratified coastal waters (strong temperature and salinity gradients). During summer and autumn temporal $CO_2$ maxima exceed >2300 µatm, leading to a pH <7.5. Average $CO_2$ in summer and autumn is 700 µatm (Thomsen et al. 2010). In this way Kiel Fjord may be seen as an analogue for future more acidic ecosystems (Thomsen et al. 2010).

We set out to test the combination effects of warming and acidification on autumn phytoplankton biomass by crossing the factors temperature (9°C and 15°C) and $CO_2$ (560 µatm and 1400 µatm) to test the following hypotheses: 1. Warming leads to decreasing biomass and earlier bloom time; 2. Rising $CO_2$ will increase phytoplankton biomass; 3. There is a synergetic effect of future warming and acidification on biomass; 4. The quality of phytoplankton biomass, in terms of cellular stoichiometry, is influenced by rising temperature and $CO_2$.

## Material and methods

*Experimental design*

In order to address our hypotheses two different temperature regimes (i.e. 9°C and 15°C) and two $CO_2$ levels (i.e. target values 560 µatm and 1400 µatm $CO_2$) were full-factorially manipulated using natural Baltic Sea phytoplankton assemblages in indoor mesocosms, each with a volume of 1400 L and a surface area of approximately 1.54 m². Each treatment combination was replicated threefold (n=3). The resulting setup of twelve mesocosms was installed in four temperature-controlled culture rooms.

Prior to experimental treatments mesocosms were filled with unfiltered natural seawater (salinity: 19.7) from Kiel Bight, Western Baltic Sea. The water contained the natural autumn plankton community including phytoplankton (photosynthetic bacteria and algae), bacteria and protozoa. To minimize differences among the starting community compositions and densities between the mesocosms, prior to the actual filling water from approximately 2 m depth was gently pumped into a mixing chamber by a rotary pump. From this it was simultaneously filled in each of the mesocosms. Mesozooplankton from net catches (Kiel Bight) was added, mimicking natural densities, i.e. 20 individuals $L^{-1}$. Each mesocosm was covered by a PVC cover (polyvinylchloride, light permeable) containing a sampling port which remained closed between sampling events. After filling (19 October 2012; hereafter called day -3), all

mesocosms had similar temperature and $CO_2$ content. The following three days were used for applying the temperature and $CO_2$ manipulations and reaching divergence between the treatments levels.

The temperature regimes were 9°C and 15°C, representing 3°C above and below the actual water temperature of Kiel Bight on the filling day (day -3). Temperature deviation in a mesocosm between day 0 and day 21 (last experimental day) was maximal ±0.3°C. Maximal temperature deviation between mesocosms of the same temperature treatment was 0.3°C (warm) and 0.4°C (cold). In order to obtain targeted $CO_2$ levels the headspace between cover and water surface received a flow of 30-60 L $h^{-1}$ of two different mixtures of air and $CO_2$ (560 µatm and 1400 µatm $CO_2$). Due to incomplete $CO_2$ equilibration with the headspace, mean values between experimental days 0-21 in the water were for low $CO_2$ 439 µatm (sd=187) and for high $CO_2$ 1040 µatm (sd=210) with maxima of 686 µatm and 1400 µatm during experimental runtime. The average low $CO_2$ value was slightly higher than the mean present day atmospheric level. However, as mentioned before, surface water in Kiel Bight on average exceeds 700 µatm during summer and autumn (2008/2009) (Thomsen et al. 2010). The high $CO_2$ level was conformed to the IPCC prediction (Scenario IS92a, atmospheric $CO_2$: 788 µatm, 2013) for the year 2100, when surface seawater $CO_2$ in the Baltic Sea is suggested to reach 1400 µatm and higher (Thomsen et al. 2010; Melzner et al. 2012). To balance the natural draw down of $CO_2$ by phytoplankton production, over the course of the experiment $CO_2$-enriched water was added to the high $CO_2$ mesocoms at three times (day 7, 11 and 18). For this purpose the same amount of water was taken out of each mesocosm and consecutively filtered (0.2 µm pore size). Afterwards the water was $CO_2$-saturated by bubbling, and retransferred (with a measuring cylinder, beneath the water surface) into the mesocosms. The required volumes were calculated on the basis of DIC (dissolved inorganic carbon) and alkalinity (Table S1, S2).

Over the course of the experiment, light was supplied by computer-controlled light units (GHL Groß Hard- und Softwarelösungen, Kaiserslautern/Germany; Lampunit HL3700 and ProfiluxII). Each light unit consisted of 5 HIBay-LED spotlights (purpose build item of Econlux, 100 W each). Above each of the mesocosms one light unit was installed. Daily irradiance patterns were computer controlled (GHL, Prometheus) and stayed constant over the course of the experiment. The light-dark cycle was 11h 50 min : 12h 10 min. Light supply and day length were aligned to the seasonal light

patterns calculated in the astronomic model of Brock (1981). It conformed to 50 % of solar irradiance of an approximated cloudless 21st September. Daily maximum light intensity was 252 µmol $m^{-2}$ $s^{-1}$, measured in the middle of the water column (0.7 m below PVC cover).

Stirring by an automatic gently moving propeller reduced phytoplankton sedimentation, assured its homogeneous distribution and simulated natural water movement. The experiment was finished after 24 days, when the phytoplankton bloom was terminated. Additionally, wall growth of periphytic microalgae (patches of a thin biofilm in all mesocosms) and sedimentation (mainly rest material of the bloom, appearing long after bloom peak in the last experimental days) became visible and in case of longer runtime this would potentially have influenced the carbon balance and nutrient availability for phytoplankton.

*Sampling and measurements*

Water temperature, salinity and pH were measured daily. For pH measurements the electrode was daily calibrated using standard pH buffers (pH 3; 7; 9, WTW). At least one hour prior to measurements the electrode was placed in the climate room to adapt to the given temperature. Samples for phytoplankton biomass variables i.e. relative fluorescence (as a control, data not shown here), particulate organic carbon (POC), chlorophyll *a* (Chl *a*) and phytoplankton carbon (microscopy and flow cytometer) were taken three times per week (Monday, Wednesday, Friday), which in total resulted in 10 samplings over the course of the experiment. Similarly, samples for inorganic dissolved nutrients, particulate organic phosphorus (POP), particulate organic nitrogen (PON), and as such for building ratios among the particulate elements (i.e. C:N, C:P, N:P) were also taken three times a week.

Carbonate system - For measurements of total dissolved inorganic carbon (DIC) 10 mL samples were filled up into a glass vial (Resteck, Germany) using a peristaltic pump with a flow rate of 6 mL $min^{-1}$. The intake tube of the pump contained a single used syringe filter (0.2 µm, Sartorius). Filtered samples were poisoned with saturated $HgCl_2$ solution (20 µL), crimped with a headspace below one percent and stored dark at 4°C. DIC was measured following Hansen et al. (2013) using a SRI-8610C (Torrence, USA) gas chromatograph. For total alkalinity (TA) 25 mL samples were filtered (Whatman GF/F filter 0.2 µm) and titrated at 20°C with 0.05M HCl-solution (Dickson 1981, Dickson et al. 2003) in an automated titration device (Metrohm Swiss

mode). The remaining carbonate parameter $pCO_2$ was calculated using CO2SYS (Pierrot et al. 2006) and the constants supplied by Hansson (1973) and Mehrbach et al. (1973), that were refitted by Dickson & Millero (1987) and the $KSO_4$ dissociation constant from Dickson (1990).

Measures of phytoplankton biomass - Relative fluorescence was measured immediately after sampling using a fluorometer 10-AU (Turner Design). For Chl *a* measurements, 250 mL water was filtered (Whatmann GF/F filters) and stored at -20°C until analyses took place. Prior to the photometrical measurements (HITACHI, U2900) filters were put into 8 mL acetone (90 %) for 24 h in the dark at 6°C. Chl *a* content was calculated following Jeffrey & Humphrey (1975).

Abundance of small phytoplankton (< 5 µm) was assessed by a flow cytometer (FACScalibur, Becton Dickinson) immediately after sampling, distinguished according to cell size (spherical diameter, FSC) and pigment fluorescence (Chl *a* and phycoerythrine). Larger phytoplankton (>5 µm) were counted microscopically (>100 individuals for common taxa) from Lugol-fixed samples in Utermöhl chambers using an inverted microscope (Utermöhl 1958). Phytoplankton carbon was calculated by first converting cell abundances obtained from flow cytometry and microscopy to biovolume by multiplying cell numbers with linear measurements taking the nearest geometric standard (Hillebrand et al. 1999). Afterwards biovolume was converted into carbon content according to Menden-Deuer & Lessard (2000), i.e. $C=0{,}288V^{0{,}811}$ for diatoms and $C=0.216V^{0.939}$ for other phytoplankton (C=carbon content in pg, V=cell volume in $\mu m^3$). As 180 µm³ is the smallest cell size included in the analysis of Menden-Deuer & Lessard (2000), their non-linear models predict unrealistically high C content for smaller algae. Therefore, conversion factors 0.108 pg C $\mu m^{-3}$ for diatoms and 0.157 pg C $\mu m^{-3}$ for all other organisms were used for phytoplankton cells below 180 $\mu m^3$ (Sommer et al. 2012b).

Particulate organic matter - For POC, PON, and POP 100-250 mL water (volume depending on plankton density) were filtered onto pre-washed (in 5-10 % HCl) and pre-combusted (6h, 550°C) Whatman GF/F filters and. POC and PON were simultaneously determined by an element analyzer (Thermo Scientific Flash 2000). POP was measured colorimetrically at 882 nm, following Hansen & Koroleff (1999).

Dissolved inorganic nutrients – For nitrate/nitrite ($NO_3^-/NO_2^-$), ammonium ($NH_4^+$), silicate ($SiO_4^-$) and phosphate ($PO_4^{3-}$) 20 mL water was filtered through cellulose

acetate filters (Sartorius) and immediately frozen at -20°C. Samples were measured following the protocols of Hansen & Koroleff (1999).

Growth rates were determined for all measures of biomass (i.e. Chl *a*, phytoplankton carbon, POC) by fitting the ascent part of the bloom to the sigmoidal growth model:

$$V = a / (1+((a-b)/b) * 2.71^{(-c*t)}),$$

where V indicates the measure of biomass, t=time; a=maximum biomass (i.e. carrying capacity), b=start biomass, c=growth rate. For this purpose data from the first sampling day until the day after maximum biomass was used. Bloom time was defined as the time, i.e. the day, of highest biomass of each single mesocosm. Out of that mean values of bloom time have been calculated for the four treatments. For maximum biomass the highest measured value of each mesocosm was taken during bloom peak, independent of the experimental day.

*Data analysis*

In order to test for treatment effects and to account for possible time dependence of the measured response variables (phytoplankton carbon, Chl *a*, POC, C:N, C:P, N:P) a generalized least squares (gls) model (nlme package, R) with the factors time (continuous), temperature and $CO_2$ (both categorical), and the interactions $CO_2$ x temperature, time x temperature, and time x $CO_2$ was applied. Prior to analyses the optimal variance-covariate structure was determined by using maximum-likelihood (ML) estimation. All model residuals were checked for normality and transformed if required. Potential heterogeneity of variances was tested using Fligner-test. Prior to conducting the gls we have also tested the above mentioned factors including their interactions using a linear mixed effect model which particularly allows to additionally test the effect “mesocosm.ID” on all response variables using the nlme-package in R (Pinheiro et al. 2013). As no random mesocosm ID effect could be detected (standard deviation <0.5), we decided to apply the gls for the consecutive analyses.

To account for resulting significant interactions among the manipulated factors and time a two way ANOVA with the factors temperature and $CO_2$ and their interaction was calculated on growth rate, maximum biomass as well as on bloom peak time for all measures of biomass. All statistical analysis were conducted using R version

Ri386 3.1.0 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria).

## Results

The measured starting pH (day -3) in all treatments was 7.8 (with exception of M11: 8.0; M1: 7.63). Over the course of the experiment pH increased under low $CO_2$ conditions (grand mean over time course and replicate mesocosm: 7.97, sd=0.16) and decreased under high $CO_2$ condition (grand mean over time course and replicate mesocosm: 7.61, sd=0.12) (Fig. 1 a). The calculated $pCO_2$ increased in all acidified mesocosms up to sampling day 7 (Fig. 1 b). The decrease of $pCO_2$ from day 9 on motivated us to add the $CO_2$ enriched water, which is reflected by the subsequent fluctuations of $pCO_2$ and pH (Fig. 1 a, b). Mean $pCO_2$ values (grand means over time course and replicate mesocosm) were 439 µatm (sd=187) for low $CO_2$ and 1040 µatm (sd=210) for high $CO_2$, respectively (Fig. 1 b).

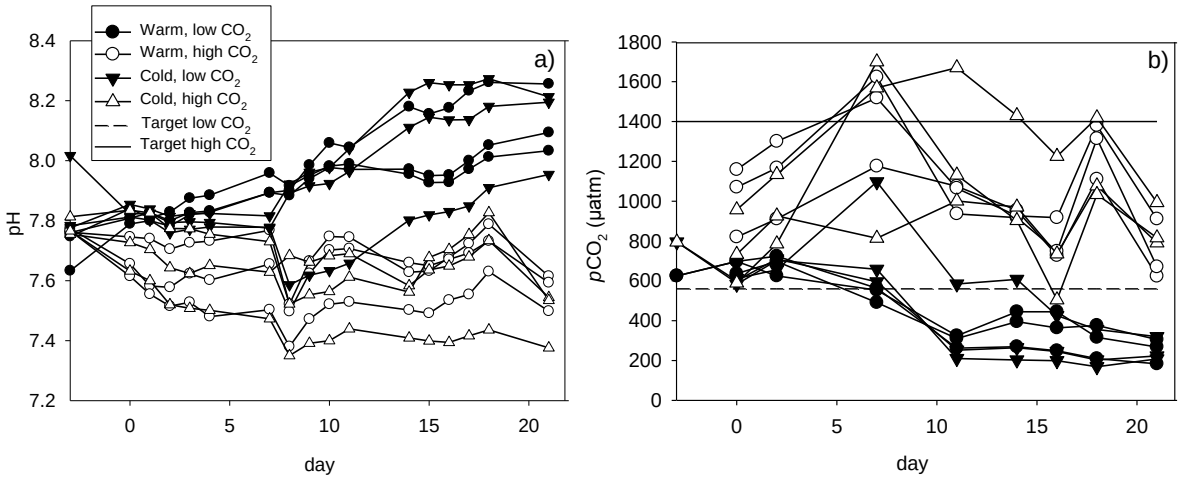


**Fig. 1.** Time course of a) pH and b) $pCO_2$ in µatm in each of the replicated mesoscosms. For symbol attribution to treatment combination see legend.

*Phytoplankton growth and biomass*

Time course - All measures of biomass (i.e. phytoplankton carbon, Chl *a*, POC) naturally were affected by the experimental time due to the build-up of the blooms and significantly decreased in response to the warming treatment. The latter effect, however, depended on the time of the experiment (significant interaction term time x temperature Fig 2 a-c; Table 1). In other words warming negatively affected phytoplankton biomass during the bloom, but not at times of low biomass in the post-

bloom situation. Maximum values of phytoplankton carbon (but not Chl *a* and POC) were marginal significantly lower in the warm treatments compared to the cold ones (Fig. 3 a-c; Table 2). $CO_2$ did not affect phytoplankton biomass as a main or interaction effect with temperature or time (Fig. 2 a-c; Fig. 3 a-c; Table 1, 2).

Bloom time of phytoplankton carbon met our expectation that warming led to a significantly earlier biomass peak by two to three days (Fig. 2 a; Table 2). Phytoplankton carbon started below 10 µg C $L^{-1}$ (Fig. 2 a). Highest values were reached under cold condition at day 16-18, in the warm mesocosms between day 11 and 14. The other measures of phytoplankton biomass (i.e. Chl *a*, POC) did not show altered timing of bloom in response to the manipulated factors (Fig. 2 b, c; Table 2). Chl *a* concentration started below 1 mg $m^{-3}$ and reached the peaks between the days 11-18 (Fig. 2 b). POC tended to increase earlier under warm condition (Fig. 2 c). The highest values, however, were reached between the days 14-18 for all treatments.

Growth rate - In most of the cases the fit of the S-curve was sufficient to calculate growth rates from the start of the experiment to the peaks. There was no significant temperature or $CO_2$ effect on growth rates of all biomass measures (Table 2). In general phytoplankton showed two to three doublings per day during growth phase (Fig. 4 a-c). Phytoplankton carbon and POC, however, showed a slight trend towards faster growth under warming conditions (Fig. 4 a, c), but standard deviations were high. An interaction effect among temperature and $CO_2$ was not found (Table 1).

*Phytoplankton chemical composition*

The C:N ratios significantly increased over the course of the experiment independently of the manipulated factors (Fig. 2 d; Table 1). The C:P ratios were significantly higher at higher temperature, but again the effect depended on experimental time (significant interaction time x temperature, Fig. 2 e; Table 1). N:P ratios were not affected by the manipulated factors or time (Fig. 2 f; Table 1).

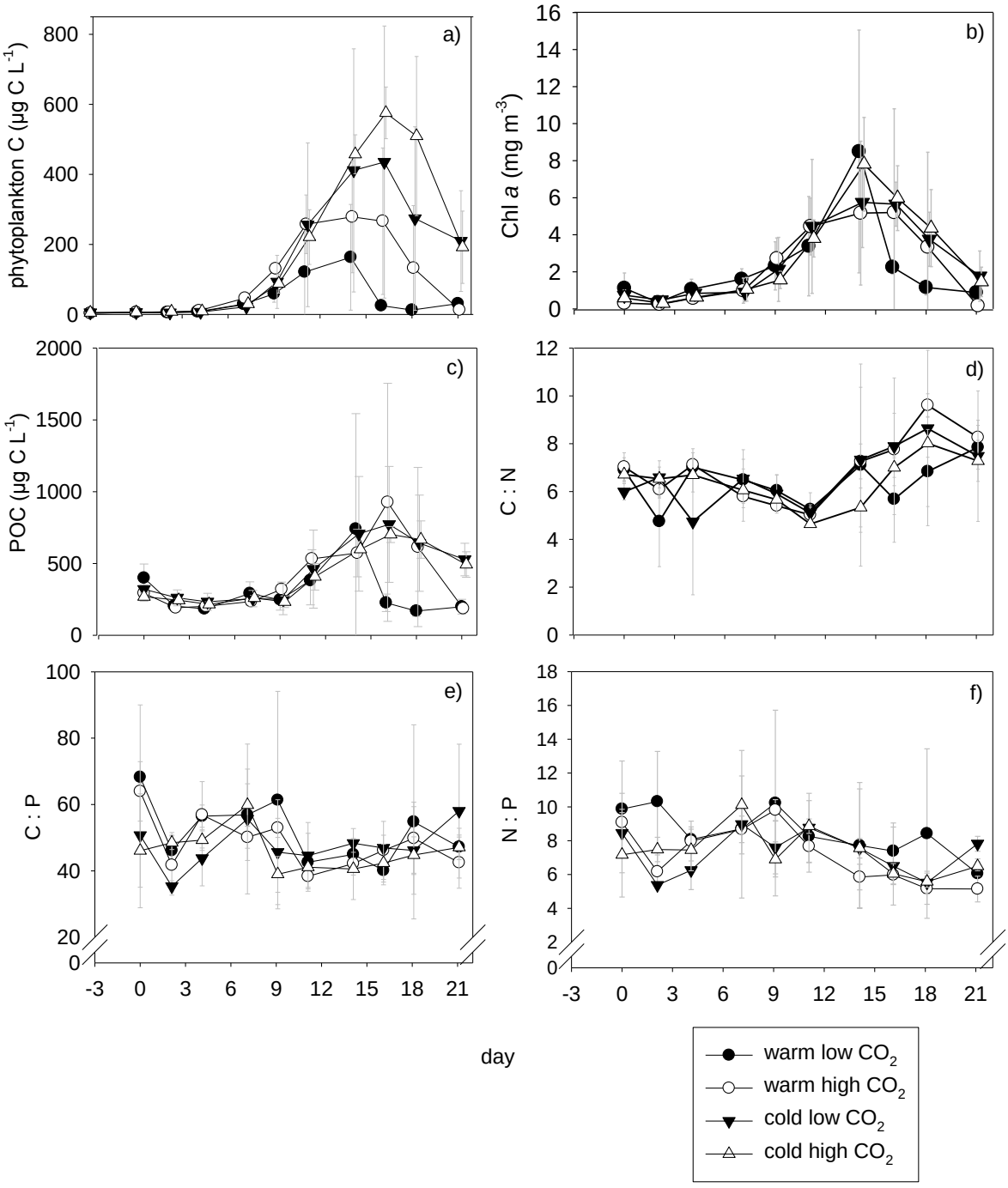


**Fig. 2.** Time course of a) phytoplankton carbon (µg C $L^{-1}$), b) chlorophyll *a* (Chl *a*, mg $m^{-3}$), c) particulate organic carbon (POC, µg C $L^{-1}$), d) C:N, e) C:P, f) N:P. Vertical error bars denote standard error from triplicate samples. For symbol attribution to treatment combination see legend.

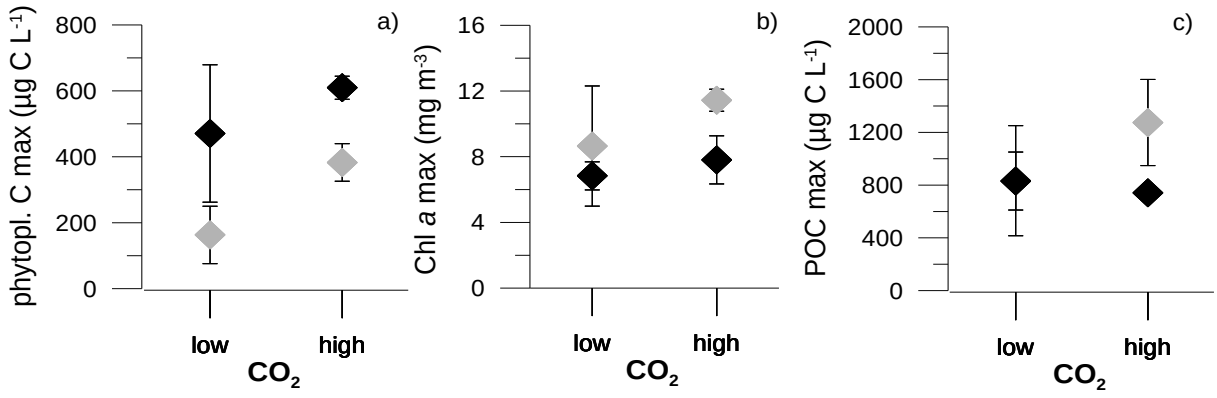


**Fig. 3.** Maximum values of a) phytoplankton carbon (µg C $L^{-1}$), b) chlorophyll *a* (Chl *a,* mg $m^{-3}$), c) particulate organic carbon (POC, µg C $L^{-1}$). Vertical error bars denote standard error from triplicate samples. Warm mesocosms: grey symbols; cold mesocosms: black symbols.

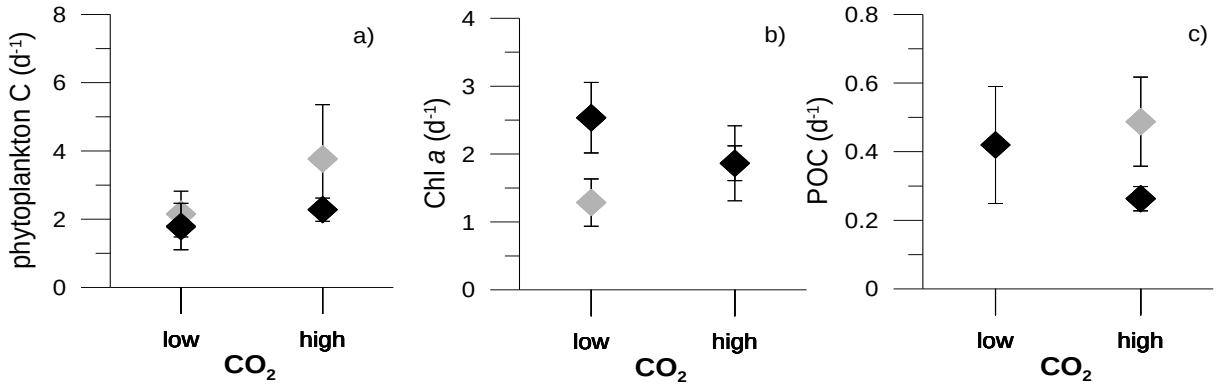


**Fig. 4.** Growth rate calculated from the biomass parameters a) phytoplankton carbon, b) chlorophyll *a* (Chl *a,* mg $m^{-3}$), c) particulate organic carbon (POC, µg C $L^{-1}$). Vertical error bars denote standard error from triplicate samples. Warm mesocosms: grey symbols; cold mesocosms: black symbols.

*Dissolved inorganic nutrients*

The average initial nitrate/nitrite and ammonium concentrations were 3.7 µmol $L^{-1}$ and 4 µmol $L^{-1}$, respectively. The average initial silicate concentration was 19 µmol $L^{-1}$. Phosphate concentration was initially 1.5 µmol $L^{-1}$. Dissolved nutrient concentrations started to decline at the onset of the blooms. Whereas nitrate/nitrite and ammonium were depleted in all treatments by the end of the experiments, phosphate was still available at termination. Silicate was depleted at the end only in the warm and high $CO_2$ treatment. In the other treatment combinations silicate was still available. Temporal developments of all measured dissolved inorganic nutrients are shown in Figure S1 a-d.

**Table 1.** Results of generalized least squares models (gls) testing for the effects of temperature (T), $CO_2$, time as well as the interaction of temperature and $CO_2$ (T x $CO_2$), time and temperature (time x T) and time and $CO_2$ on phytoplankton carbon (phytopl. C), Chl *a*, POC, C:N, C:P, N:P. Significant results are highlighted. *p ≤ 0.05, **p < 0.01, ***p < 0.001

| Response variable | factor | df | t-value | p |
|---|---|---|---|---|
| Log Phytoplankton C | T | 125 | 0.996 | 0.321 |
| | $CO_2$ | 125 | -0.916 | 0.361 |
| | time | 125 | 9.504 | **<0.001*** ** |
| | T x $CO_2$ | 125 | -0.005 | 0.996 |
| | time x T | 125 | -3.694 | **<0.001*** ** |
| | time x $CO_2$ | 125 | -0.899 | 0.371 |
| Log Chl *a* (mg $m^{-3}$) | T | 113 | 1.197 | 0.234 |
| | $CO_2$ | 113 | 0.436 | 0.663 |
| | time | 113 | 4.856 | **<0.001*** ** |
| | T x $CO_2$ | 113 | 0.005 | 0.996 |
| | time x T | 113 | -2.002 | **0.047*** |
| | time x $CO_2$ | 113 | -0.673 | 0.502 |
| Log POC (µg C $L^{-1}$) | T | 112 | 1.843 | 0.068 |
| | $CO_2$ | 112 | 1.453 | 0.149 |
| | time | 112 | 5.699 | **<0.001*** ** |
| | T x $CO_2$ | 112 | -1.428 | 0.156 |
| | time x T | 112 | -3.354 | **0.001*** ** |
| | time x $CO_2$ | 112 | -1.876 | 0.063 |
| C:N | T | 111 | 1.034 | 0.303 |
| | $CO_2$ | 111 | 0.359 | 0.720 |
| | time | 111 | 2.100 | 0.038* |
| | T x $CO_2$ | 111 | -1.316 | 0.191 |
| | time x T | 111 | -0.126 | 0.900 |
| | time x $CO_2$ | 111 | 0.086 | 0.931 |
| C:P | T | 112 | 2.190 | **0.031*** |
| | $CO_2$ | 112 | -0.179 | 0.858 |
| | time | 112 | -0.249 | 0.804 |
| | T x $CO_2$ | 112 | 0.480 | 0.632 |
| | Time x T | 112 | -2.197 | **0.030*** |
| | Time x $CO_2$ | 112 | 0.741 | 0.460 |
| N:P | T | 111 | 0.140 | 0.900 |
| | $CO_2$ | 111 | 0.969 | 0.335 |
| | time | 111 | -1.290 | 0.200 |
| | T x $CO_2$ | 111 | 0.135 | 0.892 |
| | Time x T | 111 | -0.321 | 0.749 |
| | time x $CO_2$ | 111 | -0.283 | 0.778 |

**Table 2.** Results of two-way ANOVA for the effects of temperature (T), $CO_2$ and the interaction of temperature and $CO_2$ (T x $CO_2$) on phytoplankton carbon (phytopl. C), Chl *a,* and POC according to bloom time, maximum values (max) and growth rates per day ($d^{-1}$). Significant results are highlighted. *p ≤ 0.05, **p < 0.01, ***p < 0.001

| Response variable | factor | df | MS | F | p |
|---|---|---|---|---|---|
| bloom time phytopl. C | T | 1.8 | 18.8 | 6.42 | **0.035*** |
| | $CO_2$ | 1.8 | 0.08 | 0.03 | 0.871 |
| | T x $CO_2$ | 1.8 | 0.75 | 0.26 | 0.626 |
| bloom time Chl *a* | T | 1.8 | 4.08 | 0.92 | 0.364 |
| | $CO_2$ | 1.8 | 0.75 | 0.17 | 0.691 |
| | T x $CO_2$ | 1.8 | 2.08 | 0.47 | 0.511 |
| bloom time POC | T | 1.8 | 16.33 | 3.06 | 0.118 |
| | $CO_2$ | 1.8 | 5.33 | 1.00 | 0.347 |
| | T x $CO_2$ | 1.8 | 8.33 | 1.56 | 0.247 |
| phytoplankton C max | T | 1.8 | 21428 | 5.14 | **0.053** |
| (µg C $L^{-1}$) | $CO_2$ | 1.8 | 96380 | 2.31 | 0.167 |
| | T x $CO_2$ | 1.8 | 4916 | 0.12 | 0.740 |
| Chl *a* max | T | 1.8 | 22.3 | 1.78 | 0.219 |
| (mg $m^{-3}$) | $CO_2$ | 1.8 | 10.6 | 0.85 | 0.384 |
| | T x $CO_2$ | 1.8 | 2.49 | 0.20 | 0.667 |
| POC max | T | 1.8 | 215548 | 0.87 | 0.377 |
| (µg C $L^{-1}$) | $CO_2$ | 1.8 | 92681 | 0.37 | 0.557 |
| | T x $CO_2$ | 1.8 | 211421 | 0.86 | 0.382 |
| growth rate phytopl. C | T | 1.8 | 2.06 | 0.65 | 0.451 |
| ($d^{-1}$) | $CO_2$ | 1.8 | 2.67 | 0.84 | 0.395 |
| | T x $CO_2$ | 1.8 | 0.76 | 0.24 | 0.643 |
| growth rate Chl *a* | T | 1.8 | 1.04 | 2.18 | 0.184 |
| ($d^{-1}$) | $CO_2$ | 1.8 | 0.006 | 0.01 | 0.916 |
| | T x $CO_2$ | 1.8 | 1.04 | 2.18 | 0.183 |
| growth rate POC | T | 1.8 | 0.007 | 2.11 | 0.207 |
| ($d^{-1}$) | $CO_2$ | 1.8 | 0.010 | 3.26 | 0.131 |
| | T x $CO_2$ | 1.8 | 0.0005 | 0.16 | 0.711 |

## Discussion

The influence of multiple stressors related to global change such as increasing temperatures and $CO_2$ concentration was hypothesized to synergistically affect phytoplankton biomass. In our study, testing both factors on a Baltic Sea autumn bloom, however, we could show that only warming but not $CO_2$ significantly affected

phytoplankton biomass, bloom time and biochemical composition. In particular warming led to an earlier bloom and overall decline of phytoplankton biomass but to higher C:P ratios.

*Phytoplankton growth and biomass*

One reason for the absence of the $CO_2$ effect might be pre-adaptation to increased $CO_2$ levels of Baltic Sea phytoplankton communities because of pronounced natural short-term and seasonal fluctuations of $CO_2$ concentrations. Natural conditions in Kiel Fjord vary by ca. 0.7 pH units and $pCO_2$ can reach short term peak values of 4000 µam in summer (Thomsen et al. 2010), the latter being the consequence of upwelling of respiration dominated deep water. Adaptation to a wide $pCO_2$ range for coastal phytoplankton in natural acidified waters was already suggested to explain weak responsiveness of phytoplankton to $CO_2$-enrichment (Feely et al. (2008) for the Pacific coast, Rossoll et al. (2013) for a summer Baltic Sea bloom). In fact, evolutionary adaptation via genotypic selection could be shown for the calcifier *Emiliania huxleyi* after 500 generations (Lohbeck et al. 2012). The coastal Baltic Sea short term variability by far exceeds the atmospheric signal of 700 µatm $pCO_2$ (IPCC) for the end of the 21$^{st}$ century. Therefore, future mean conditions may not have dramatic influence on diatom-dominated autumn blooms. However, impacts of future maximal values which will probably exceed present day ones cannot be excluded. Another possible reason for the absence of a main and interaction effect of $CO_2$ on phytoplankton growth and biomass might be that a potential positive fertilizing effect of $CO_2$, in particular on diatom biomass, remained below the level of detection because biomass was generally kept low by the presence of grazers. Zooplankton grazing in our system might potentially have masked an enhanced phytoplankton growth due to increased $CO_2$ concentration. This suggestion could be supported by the tendency of a $CO_2$-fertilizing effect in the phytoplankton carbon data (time course, maximum phytoplankton carbon, growth rate; see Fig. 2 a, 3 a, 4 a). Here, under both temperature treatments biomass and growth rate trended to be on average higher under high $CO_2$ concentration. In fact in studies that found a positive effect of $CO_2$ on phytoplankton community growth or biomass, mesograzers were excluded prior to experimental treatments (Tortell et al. 2008, Eggers et al. 2014, Feng et al. 2009). A thorough test for the suggested grazer effect would be a factorial experimental design manipulating $CO_2$ in the absence and presence of grazers.

The earlier onset of the phytoplankton blooms with higher temperature can be explained by the fact that temperature is a major environmental factor controlling organisms' metabolic rates and thus the start of biological processes in nearly all living species (Brown et al. 2004). Although sampling at only every other day potentially decreases the strength of our result, it is consistent with previous studies finding earlier bloom times due to temperature increase (Sommer & Lengfellner 2008; Lewandowska & Sommer 2010).

The time-dependent biomass decrease in phytoplankton due to increased temperature, i.e. a temperature effect during bloom, largely matches the assumption that temperature has the potential to strengthen grazing because it is known to more strongly enhance heterotrophic than autotrophic processes (O'Connor et al. 2009). Our results also match the majority of the studies published so far from the same geographical region that have investigated temperature effects on the spring bloom (Lewandowska & Sommer 2010; Sommer & Lewandowska 2011; Sommer et al. 2012a). Under spring conditions, likewise to our experimental autumn conditions, nutrients were replete to assume favorable growth conditions. At the same time the elevated temperature increased copepod grazing rates and changed the bottom-up to top-down control of the phytoplankton biomass (Lewandoska & Sommer 2010; Sommer et al. 2012a; Keller et al. 1999). Indeed, in our experiment the development of the copepods (Garzke 2014) was faster at warmer temperatures. The metabolic demands of heterotrophs and hence feeding rates are known to rise with temperature (Brown et al. 2004, O`Connor et al. 2009), which underpins the suggested increased grazing pressure, and in that way the observed lower phytoplankton biomass. However, as mentioned before, unfortunately it was not possible to verify this effect with the present experimental design.

Changes in species composition during bloom time in response to temperature or $CO_2$ as a reason for changes in the biomass can be excluded in our study. The most dominant taxa (diatoms and cryptophytes with on average 83 % and 10.5 % of total biovolume, respectively) and species with the highest cell abundance (*Skeletonema marinoi*, *Teleaulax acuta*) showed no significant response to temperature or $CO_2$ (Sommer et al. 2015). Only pico-plankton like pico-chlorophytes and pico-cyanobacteria showed a significantly higher abundance with warming, but their contribution to total biomass was very low (<1 %).

Nutrient limitation as a reason for lower phytoplankton biomass in response to warming can also be excluded. Average phosphate concentration (1.5 µmol $L^{-1}$) matched the Kiel Fjord annual mean of 1.12 µmol $L^{-1}$ (Nausch et al. 2011). Concentrations of ammonium and nitrite/nitrate were also high enough to preclude nutrient limitation until biomass peak was reached.

The opposite, i.e. increasing biomass with warming, was found for two experimental early summer blooms (Taucher et al. 2012; Lewandowska et al. 2014) as well as for a Baltic Sea long-term field study (1979-2011) by Suikkanen et al. (2013). In summer conditions nutrient concentrations are naturally low. Lewandowska et al. (2014) suggest that under such conditions phytoplankton are mainly controlled by the rate of nutrient delivery via reduced mixing and not by grazing. Warming under such conditions has positive effects on phytoplankton biomass. Suikkanen et al. (2013) also suggested that warming was the key environmental factor explaining the general increase in total phytoplankton biomass in northern summer Baltic Sea communities during the last decades.

To the best of our knowledge as one of the first our study has compared three different biomass parameters in response to manipulated climate change. Here we could prove a time-dependent temperature effect for all three proxies (Chl *a*, phytoplankton carbon, POC), but with unequal effect strength. For these differences it should be kept in mind that no biomass parameter is perfect. Actual phytoplankton might have different cell volume to carbon relationships as shown in the data base of Menden-Deuer & Lessard (2000) which might have affected our measure of microscopically derived phytoplankton carbon. POC contains a lot of non-phytoplankton carbon (detritus, bacteria, heterotrophic protists), and the Chl *a* content of biomass is subject to taxonomic and physiological variability (Moline & Prezelin 2000), i.e. underestimating diatom biomass.

*Phytoplankton chemical composition*

We did not find any significant $CO_2$ effect or an interaction effect of warming and acidification on the C:N:P ratios. As suggested before, the reason might be the pre-adaption of phytoplankton on high $CO_2$ levels in the Baltic Sea. This might explain the difference to studies on oceanic phytoplankton communities (Tortell et al. 2000;, Eggers et al. 2014) which found significantly increased C:N ratios in response to increased $CO_2$.

Instead, warming led to a higher amount of carbon accumulation per unit phosphorus (increased C:P ratio), i.e. higher temperature seems to allow the phytoplankton to yield a higher C-based biomass per unit P. Reasons can be physiological, as C accumulation might have been faster than P accumulation under warming due to a metabolic stimulation of carbon uptake processes. An explanation would be a temperature-induced higher POC content as shown in De Senerpont Domis et al. (2014). This, however, was not found in this experiment. An increase in POC was potentially masked by high grazing pressure in warm treatments. The POP content instead did not differ between the treatments (gls; $t \geq -1.3$; $p \leq 0.16$), pointing to the fact that P uptake processes were not stimulated by temperature. De Senerpont Domis et al. (2014) additionally explained the observed higher C:P ratios with a higher nutrient use efficiency to fix carbon under higher temperature through phenotypic adaptation of the entire community. They also suggested that colder temperature contributed to lower C:P ratios by reallocation of nutrients to cellular compounds such as RNA and proteins. However, as we did not measure such values we cannot prove this suggestion for the experiment.

In contrast to the results we observed, the particulate matter C:P decreased in three of four experiments with Baltic Sea phytoplankton spring communities (Wohlers-Zöllner et al. 2012). There it was suggested that the turnover dynamics of organic phosphorus compounds shifted with warming (Wohlers-Zöllner et al. 2012). The faster replenishment of the POP pool was explained by temperature stimulation of the phosphorus cycling. In our study the particulate C:P ratios were generally low and clearly below Redfield ratio. However, dissolved inorganic phosphorus (DIP) concentrations were high throughout the experiment (0.4 - 1.68 µmol $L^{-1}$), hence phosphorus was not limiting. Therefore a potential stimulation of the phosphorus cycling with warming could not have had consequences for our autumn bloom.

*Potential consequences of our results for the planktonic food web*

Based on our results we suggest that the food web in terms of food quality will be less affected by warming and / or acidification. The increasing C:P ratios in response to warming are most probably also not deleterious for zooplankton feeding, because even the highest ratios were clearly lower than usual C:P ratios in copepods (Sommer & Stibor 2002), the dominant group of marine mesozooplankton. Thus, potential mineral nutrient limitation for higher trophic levels due to stoichiometrically

imbalanced food (Sterner & Hessen 1994; Elser et al. 2001) can be excluded for any treatment combination in our study.

## Conclusion

Our results show that ongoing ocean acidification seems to be less important for phytoplankton than ocean warming. We agree with Havenhand (2012) that most ecologically important groups in the Baltic Sea food web seem to be more or less robust to future acidification. The concurrent effects of warming in the present study suggest a stimulation of phytoplankton blooms which at the same time is subject to strong top-down control by the zooplankton. However, even mesocosm experiments with natural phytoplankton assemblages and their grazers as presented in this study represent snapshots of rapidly manipulated climate change effects. In that way the simulated temperature changes reflect climate processes that in natural systems develop over decades and hence ignore longer acclimation potential of biological communities. Nevertheless our results contribute to a deeper understanding of the relative importance of different aspects of climate change on phytoplankton blooms, which will be essential for predicting the effects of climate change in more detail. To further refine the understanding of multiple climate change factors effects on phytoplankton, future research should more thoroughly investigate the effects on different seasonal bloom events and the role of consumers.

# Chapter II

## Effects of increased $CO_2$ concentration on nutrient limited coastal summer plankton depend on temperature


### Abstract

Increasing seawater temperature and $CO_2$ concentrations both are expected to increase coastal phytoplankton biomass and carbon to nutrient ratios in nutrient limited seasonally stratified summer conditions. This is because temperature enhances phytoplankton growth while grazing is suggested to be reduced during such bottom-up controlled situations. In addition, enhanced $CO_2$ concentrations potentially favor phytoplankton species, that otherwise depend on costly carbon concentrating mechanisms (CCM). The trophic consequences for consumers under such conditions, however, remain little understood. We set out to experimentally explore the combined effects of increasing temperature and $CO_2$ concentration for phytoplankton biomass and stoichiometry and the consequences for trophic transfer (here for copepods) on a natural nutrient limited Baltic Sea summer plankton community. The results show, that warming effects were translated to the next trophic level by switching the system from a bottom-up controlled to a mainly top-down controlled one. This was reflected in significantly down-grazed phytoplankton and increased zooplankton abundance in the warm temperature treatment (22.5°C). Additionally, at low temperature (16.5°C) rising $CO_2$ concentrations significantly increased phytoplankton biomass. The latter effect however, was due to direct negative impact of $CO_2$ on copepod nauplii which released phytoplankton from grazing in the cold but not in the warm treatments. Our results suggest that future seawater warming has the potential to switch trophic relations between phytoplankton and their grazers under nutrient limited conditions with the consequence of potentially disguising $CO_2$ effects on coastal phytoplankton biomass.

## Introduction

Increasing atmospheric $CO_2$ is predicted to rise from current values of approximately 390 µatm to values of 700 µatm by the end of the 21$^{st}$ century (IPCC 2013). As a consequence, surface seawater pH will decrease by 0.3-0.4 units. Simultaneously with rising $pCO_2$, ocean sea surface temperature is predicted to increase up to 3-5 °C by the year 2100 (IPCC 2014). Consequences for the planktonic system remain unclear as only few studies have analyzed the combined effect of warming and acidification on natural plankton communities (Hare et al. 2007; Feng et al. 2009; Paul et al. 2015). In particular the question if there is a trophic transfer of climate change effects, i.e. warming and / or rising $pCO_2$, from phytoplankton to zooplankton due to possible changes in the food quantity and quality remains unanswered.

A meta-analysis revealed that phytoplankton groups like diatoms seem to overall profit in terms of growth rates and photosynthetic rates from higher $pCO_2$ (Kroeker et al. 2013). $CO_2$, in particular for larger cells, can be limiting at ambient concentrations (Murata et al. 2002). A rise in diatom biomass in response to high $CO_2$ concentrations has been shown to result in an increase in total phytoplankton biomass of natural diatom dominated phytoplankton communities (Tortell et al. 2008; Eggers et al. 2014). As diatoms are a preferred food source for zooplankton, in particular for copepods, higher food availability is a potential consequence of increasing $pCO_2$. External inorganic carbon concentrations are predicted to increase with rising $pCO_2$, which is proposed to reduce the metabolic costs for the phytoplankton's effective carbon concentrating mechanisms (CCM) due to a lower electrochemical potential gradient between the medium and the Rubisco active site. This was suggested as one possible underlying mechanism why phytoplankton profit from high $pCO_2$ (Raven 1991). However, as CCM efficiency seems to be different among species (Burkhardt et al. 2001; Rost et al. 2008), size classes and phytoplankton groups (Reinfelder 2011; Raven & Beardall 2014), unequal benefits from increased $CO_2$ concentration might affect phytoplankton species composition and consequently the food availability for the zooplankton in an additional way. Filamentous nitrogen fixing cyanobacteria, a typical group in summer phytoplankton blooms in the Baltic Sea, also contain CCMs. However, there have been mixed responses in physiological studies on *Nodularia spumigena* to increased $pCO_2$ (Czerny et al. 2009; Wannicke et al. 2012, Eichner et al. 2014). As they are a less preferred food source for copepods, a potential change in biomass would play a minor part in the food web interactions.

Studies using natural plankton communities have shown that elevated $pCO_2$ can result in significantly higher elemental carbon to nitrogen (C:N) (Tortell et al. 2000; Riebesell et al. 2007; Eggers et al. 2014) and higher elemental carbon to phosphorus (C:P) ratios (Schulz et al. 2013). This potentially leads to altered food quality for herbivorous consumers (Malzahn & Boersma 2012). Other studies, however, did not find any significant (Paul et al. 2015) or even negative (Schulz et al. 2013) responses to $pCO_2$ in phytoplankton C:N ratios.

Warming seawater is known to affect species distribution, community composition as well as phenology in the phytoplankton, potentially leading to changes in the food quantity and quality for copepods (e.g. Garzke 2014; Lewandowska et al. 2014; Paul et al. 2015). It has been shown that temperature effects on phytoplankton differ among regions / seasons with nutrient deplete and nutrient replete conditions (Lewandowska et al. 2014). Nutrient deplete conditions refer to the oligotrophic open ocean or seasonally stratified shelf seas such as the Baltic Sea in summer. It has been suggested that such systems are mainly bottom-up controlled and as such phytoplankton is expected to increase in response to higher seawater temperatures (Taucher et al. 2012; Suikkanen et al. 2013; Lewandowska et al. 2014). For the phytoplankton's consumers this potentially means higher food availability. Nutrient replete conditions are found in most coastal regions or seasonally mixed shelf seas. Prior to grazing such conditions are characterized by phytoplankton blooms that are often dominated by larger diatoms. A number of experiments showed that such systems are mainly top-down controlled (e.g. O'Connor et al. 2009; Sommer & Lewandowska 2011). Phytoplankton blooms showed earlier onsets and decreased biomass in response to increasing temperature. The latter was explained by temperature induced intensified grazing (O' Connor et al. 2009; Gaedke et al. 2010; Sommer & Lewandowska 2011). The picture regarding temperature effects on phytoplankton stoichiometry remains incomplete, in particular for bottom-up regulated phytoplankton in nutrient deplete areas / seasons. For nutrient replete conditions, however, C:P ratios have been shown to either decrease (Wohlers-Zöllner et al. 2012) or increase (Paul et al. 2015) with increasing seawater temperature, whereas C:N ratios did not change with warming. In the first case, i.e. decreasing C:P ratios, the underlying mechanism was an overall stimulation of the phosphorus turnover due to enhanced activity of the bacterial enzyme APA (alkaline phosphatase) with

warming, facilitating a faster replenishment of the inorganic phosphorus pool. This increased phosphorus availability may have stimulated phosphate assimilation by phytoplankton (Wohlers-Zöllner et al. 2011, 2012). In the second case, i.e. increasing C:P ratios, it was suggested that warming led to greater carbon accumulation per unit phosphorus (Paul et al. 2015). These hitherto ambiguous results on phytoplankton stoichiometry can mean both an increase and decrease of food quality in response to warming. Clarifying this response of food quality is of particular importance as warming is expected to raise the copepod's P-demand due to higher growth rates (Elser et al. 2000).

We set out to test if there is a trophic transfer of the combined effects of seawater warming and increased $pCO_2$ from phytoplankton to zooplankton in a natural coastal summer plankton community. We hypothesize that warming and raising $pCO_2$ lead to (1) increased phytoplankton biomass as well as to a (2) higher elemental carbon to nutrient stoichiometry, and (3) that the changes in phytoplankton biomass and stoichiometry translate to the next trophic level, named the zooplankton's abundance and resource use efficiency (RUE).

## Material and Methods

*Experimental set-up*

In order to address our hypotheses we manipulated a natural summer Baltic Sea plankton assemblage by crossing two different temperature regimes (with a difference of 6°C) with six $CO_2$ levels, target levels ranging from 500 to 3000 µatm. The resulting multi-factorial set-up of twelve mesocosms, each containing a volume of 1400 L with a surface area of approximately 1.54 m², was installed in four temperature-controlled culture rooms. The mesocosms were filled on 13 August 2013 with natural summer plankton including phytoplankton (cyanobacteria and algae), bacteria and protozoa from approximately 2 m depth in Kiel Fjord (western Baltic Sea). In order to ensure homogeneous distribution of the plankton among the twelve mesososms, the water was simultaneously pumped into all mesocosms by using a rotary pump spreading the water over a distributor. In order to mimic the typical composition of a Baltic Sea summer bloom, the filamentous cyanobacterium *Nodularia spumigena* was added to each mesocosm prior to the first sampling on 14 August 2013 (hereafter referred to as day -2). *Nodularia* was cultured at 18°C in a

temperature-controlled room with 150 µmol Phot $m^{-2}$ $s^{-1}$. 1.75 L *Nodularia* culture was added to each mesocosm which resulted in a concentration of approximately 5160 cells $L^{-1}$ at the onset of the experiment. Mesozooplankton from vertical net catches (Kiel Bight, 10 m depth) were added to each of the mesocosms on 15 August 2013 (hereafter referred to as day -1). Prior to the addition mesozooplankton organisms (i.e. male and female individuals of all stages from nauplii to adults) were kept in 10 L buckets for 24 hours to acclimate and to separate living from dead animals. Dead animals were removed from the buckets and the final density was estimated (Garzke et al. 2015). To mimic natural mesozooplankton densities for this region and season, 20 individuals per liter (ind. $L^{-1}$) were introduced to each mesocosm (Behrends 1996). After filling, all mesocosms still had the same temperature and $pCO_2$ level (540 µatm; 19.5°C). These values were consistent with the ones measured for Kiel Fjord at filling day. Over the following two days (day -2, day -1) temperature and $CO_2$ were manipulated gradually until target values were reached. Experimental onset with fully manipulated treatments (16 August 2013) is hereafter referred to as day 0.

The temperature treatments included two levels with 16.5°C and 22.5°C (hereafter referred to as cold and warm treatments, respectively) and represented 3°C above and below the actual water temperature of Kiel Fjord on the filling day (19.5°C). The temperatures were manipulated by adjusting room temperature to the respective target levels with a maximum standard deviation of 0.4°C between mesocosms of the same temperature treatment. The temperature treatments lie within the range of natural average summer sea surface temperatures of the coastal western Baltic Sea in August, measured from 1957 to 2013 (mean temperature at 1m depth, Boknis Eck: 17.75°C, sd = 2.39°C, variance = 5.75°C; Lennartz et al. 2014).

The $pCO_2$ treatments included six levels with the target values of 500, 1000, 1500, 2000, 2500 and 3000 µatm. The lowest $pCO_2$ target value (500 µatm, Fig. S5) represented the mean present $pCO_2$ concentration in Kiel Fjord during summer (Thomsen et al. 2010). The highest target value (3000 µatm, Fig. S5) represented actual Kiel Fjord maximum values (>2300 µatm), which can be temporarily observed in summer for several days (Thomsen et al. 2010). In Kiel Fjord, these upwelling events of water masses enriched with high concentrations of dissolved inorganic carbon (relative to concentrations in the surface water), are caused by strong south-westerly winds which push the otherwise seasonally stratified coastal water with strong temperature and salinity gradients out of the Fjord (Thomsen et al. 2010).

Intermediate target $pCO_2$ values (i.e. 1000, 1500, 2000, 2500 µatm) conformed to predictions for coastal upwelling areas with highly temporal variable $pCO_2$ values, however, strongly exceeding the worst case scenario forecast for surface open oceans (IPCC 2014).

For manipulating the target $pCO_2$ values and for subsequent balancing of the natural $CO_2$ drawdown due to phytoplankton primary production, 0.2 µm filtered seawater from Kiel Fjord (taken at filling day, stored under cold and dark conditions) was enriched with $CO_2$ and the required volume was added to the mesocosms' centre (with a flexible tube) three times per week after sampling (see below) was completed. Seawater $CO_2$ enrichment was prepared by bubbling the water with $CO_2$ gas (99.9 % $CO_2$) for at least 6 hours until saturation. The required volume of enriched water for each mesocosm (< 2 L) was calculated using CO2SYS (Lewis and Wallace 1998) on the basis of the measured concentrations of dissolved inorganic carbon (DIC) and total alkalinity (TA).

Each mesocosm was covered by a PVC cover (polyvinylchloride, light permeable), containing a sampling port which remained closed between sampling events. This maintained a small headspace above each mesocosm's water surface to reduce outgassing of $CO_2$. Above each of the mesocosms a computer controlled light unit (GHL Groß Hard- und Softwarelösungen, Kaiserslautern/Germany) consisting of 5 HIBay-LED spotlights (100 W each, Lampunit HL3700 and ProfiluxII) was installed. Light intensity and day length were calculated with the astronomic model of Brock (1981) and aligned to the natural seasonal light patterns. Light intensity conformed to 40 % of solar irradiance of an approximated cloudless day to account for the shallow water depth. The light:dark cycle was 14h:3min : 9h:57min with a simulated sundown and sunrise of approximately 2 hours. Maximum light intensity was on average 382.7 µmol µmol Phot $m^{-2}$ $s^{-1}$ (LICOR Li-250A light meter) at the water surface.

In order to reduce phytoplankton sedimentation and to assure its homogeneous distribution and simulate natural water movement, the water was gently stirred by an electrical propeller. The experiment was finished after 28 days, when the phytoplankton bloom was terminated.

*Sampling and measurements*

Sampling for DIC took place three times a week (Monday, Wednesday, Friday) directly from the mesocosms, always prior to all other samplings in order to minimize loss of DIC through outgazing. Samples for total alkalinity (TA) were also taken three times a week directly out of the mesocosms. Salinity and water temperature were measured daily directly in the mesocosms. Phytoplankton, particulate organic carbon (POC), particulate organic nitrogen (PON), particulate organic phosphorus (POP), dissolved inorganic nutrients (i.e. nitrate/nitrite ($NO_3^-$/$NO_2^-$), ammonium ($NH_4^+$), silicate ($SiO_4^-$), phosphate ($PO_4^{3-}$) were sampled three times per week (Monday, Wednesday, Friday). For this purpose 20L were taken out of each mesocosm (from around 0.50 m below water surface, using a flexible tube) and filled into a plastic container. Shortly after, the water sample was separated for subsequent analyses of each parameter. Mesozooplankton was sampled weekly (Friday) directly out of the mesocosms by taking three vertical net hauls with a hand-held plankton net (64 µm mesh size, 12 cm diameter, net hauls from mesocosm bottom to surface), respectively. Each net haul sampled a volume of 5.1 L. Sampling for salinity, temperature, phytoplankton and DIC started at day -2. Onset for sampling of all other parameters was day 0.

Carbonate system – DIC water samples were gently pressure-filtered (0.2 µm, Sarstedt Filtropur) and collected into 50 mL gas tight vessels with at least 100 mL of overflow directly out of the mesocosms. DIC was analysed by infrared detection of $CO_2$ by a LICOR LI-7000 on an AIRICA system (MARIANDA, Kiel). Samples for total alkalinity (TA) analyses were sterile filtered as for DIC but were collected in polyethylene containers (200 mL). TA samples were analysed by open-cell potentiometric titration on an auto-sampler (Metrohm 869 Sample Changer and 907 Titrando Dosing unit) according to Dickson et al. (2007). Certified reference material provided by Andrew Dickson (Scripps Institute for Oceanography of the University of California, San Diego) was used to correct for any drift during analyses within a run. The remaining carbonate parameter $pCO_2$ was calculated from DIC and TA using CO2SYS (Lewis & Wallace 1998; Pierrot et al. 2006) and the carbonic acid dissociation constants of Millero et al. (2006). For calculated $pCO_2$ data in details please see Fig. S5.

Contrary to temperature, the factor $CO_2$ underlies strong natural biological feedback. The fluctuations and thus deviations from the target $pCO_2$ levels (Fig. S5) are mainly due to rapid $CO_2$ draw-down through phytoplankton growth and photosynthesis, which naturally changes the concentration of the inorganic carbon species over the course of time (Rost et al. 2008).

Measurement of phytoplankton abundance and biomass – For the abundance of small phytoplankton (< 5 µm), 3 mL of pre-filtered water (64 µm mesh) were fixed with formalin in a cryovial, flash frozen in liquid nitrogen and kept frozen in at -20°C until measurement on a flow cytometer (FASCalibur, Becton Dickinson). The small phytoplankton (< 5µm) was distinguished according to size and pigment fluorescence (chlorophyll *a* and phycoerythrin). Additionally, flow-cytometric categories were matched to taxa identified by fluorescence microscopy under blue and green excitation at 1000 fold magnification on the basis of size and correlations between abundances. For abundance of larger phytoplankton species (>5 µm), 100 mL of sample was Lugol-fixed and stored in the dark. With an inverted light microscope species were determined to the species level and counted using the Utermöhl technique (Utermöhl 1958).

Total phytoplankton carbon (total phytoplankton C) was calculated as a measure of phytoplankton biomass. For this purpose the biovolume of each species (identified by flow cytometry and microscopy) was assessed by taking the respective nearest geometric standard (Hillebrand et al. 1999). Afterwards, the species' biovolumes were converted into carbon content according to Menden-Deuer & Lessard (2000), i.e. $C=0.288V^{0,811}$ for diatoms and $C=0.216V^{0.939}$ for other phytoplankton (C=carbon content in pg, V=cell volume in µm³). As 180 µm³ is the smallest cell size included in the analysis of Menden-Deuer & Lessard (2000), their non-linear models predict unrealistically high C content for smaller algae. Therefore, conversion factors 0.108 pg C $\mu m^{-3}$ for diatoms and 0.157 pg C $\mu m^{-3}$ for all other organisms were used for phytoplankton cells below 180 µm³ (Sommer et al. 2012a). In a final step the calculated carbon content for each species was multiplied with its respective cell abundance.

Phytoplankton species size is a critical factor for feeding relationships and trophic connections (Boyce et al. 2015). To account for such differences in the feeding relationship due to phytoplankton size in the mesocosms, total phytoplankton C was

separated into edible phytoplankton carbon (edible phytoplankton C) and inedible phytoplankton carbon (inedible phytoplankton C) for zooplankton, here copepods. Accordingly, very small (< 5 µm, pico-plankton) and very large phytoplankton (cells > 70 µm length, here mainly *Rhizosolenia* sp., large filamentous cyanobacteria, *Ceratium* sp.), which are known to be less preferred by copepods (Sommer et al. 2001; Sommer et al. 2005; Sommer & Sommer 2006), were summarized as inedible phytoplankton C. All phytoplankton species with cell sizes between 5 µm and 70 µm were hereafter classified as edible and hence contributed to edible phytoplankton C.

Measures of zooplankton abundance and resource use efficiency (RUE) – The sampled meso-zooplankton was immediately Lugol fixed and stored in the dark. Copepods were counted and identified to the genus level and developmental stage. Out of that, total and stage-specific zooplankton (copepod) abundances were calculated. The latter were separated into nauplii (including nauplii stages 1-6), copepodite (including copepodid stage 1-5), and adult (including copepodid stage 6) abundances. Samples with high copepod abundances were, prior to analyses, divided with a sample splitter (Hydro-Bios, Kiel, Germany), such that a quarter of the total sample volume was used for counting.

Zooplankton RUE was calculated from total zooplankton abundance (ind. $L^{-1}$) per unit edible phytoplankton C (µg $L^{-1}$) for each available sampling day (partly following Filstrup et al. 2014). We used zooplankton abundance instead of biomass (sensu Filstrup et al. 2014) because the mesozooplankton community was largely dominated by *Acartia* sp. The very few individuals of medium-sized but very carbon-rich *Centropages* sp., present only at the beginning of the experiment, would have led to an overestimation of zooplankton biomass during the bloom (see data analysis below).

POC, PON, and POP – 100-250 mL water sample (volume depending on plankton density) were filtered onto pre-washed (in 5-10 % HCl) and pre-combusted (6h, 550°C) Whatman GF/F filters and immediately frozen at -20°C. POC and PON were simultaneously determined by an element analyzer (Thermo Scientific Flash 2000). POP was measured colorimetrically at 882 nm, following Hansen & Koroleff (1999).

Molar ratios (mol:mol) among particulate C:N, C:P and N:P were build out of these measurements.

Dissolved inorganic nutrients – For $NO_3^-/NO_2^-$, $NH_4^+$, $SiO_4^-$ and $PO_4^{3-}$ 20 mL water was filtered through cellulose acetate filters (Sartorius, 0.2 µm pore size) and immediately frozen at -20°C. Samples were measured following the protocols of Hansen & Koroleff (1999) with an auto-analyzer (Skalar, $SAN^{PLUS}$; Breda/Netherlands). The detection limit of the auto-analyzer was at a concentration of 0.1 µmol $L^{-1}$.

*Data analysis*

In order to test for treatment effects and to account for possible time dependence of the measured response variables (time-course of: total phytoplankton C, edible phytoplankton C, inedible phytoplankton C, total zooplankton abundance, nauplii abundance, copepodite abundance, adult abundance, zooplankton RUE, C:N, C:P, N:P) a generalized least squares (gls) model (nlme package, R) with the factors time, target $pCO_2$ (both continuous), temperature (categorical), and the interactions $pCO_2$ x temperature, time x temperature, time x $pCO_2$ and time x temperature x $pCO_2$ was applied. Also the time point of the bloom, i.e. the day of highest total phytoplankton C of each mesocosm, was tested by using the gls model. As the time-point of the bloom did not significantly differ between treatments (Table S2), phytoplankton bloom was defined as the period from experimental day 0 to 12 for all mesocosms. Phytoplankton post-bloom was, in that way, defined as the period from experimental day 14 to 28 (Fig. 1). Regarding the zooplankton, the first three samplings (day 0, 7, 14) were related to phytoplankton bloom period, the last two samplings (day 21, 28) to post-bloom. In order to test temperature and $pCO_2$ effects separately during bloom and post-bloom, average values of all response variables (total phytoplankton C, edible phytoplankton C, inedible phytoplankton C, total zooplankton abundance, nauplii abundance, copepodite abundance, adult abundance, zooplankton RUE, C:N, C:P, N:P) have been calculated over bloom and post-bloom period, respectively. Their responses to treatments were tested also using a gls model with the factors temperature, $pCO_2$ and the interaction between temperature and $pCO_2$ (temperature x $pCO_2$). In case a significant interaction effect was detected, separate regression analyses with $pCO_2$ as continuous factor were conducted for the warm and cold

treatments respectively. Prior to all statistical analyses the optimal variance-covariate structure was determined by using Restricted Maximum-Likelihood (REML) estimation. All model residuals were checked for normality using the Shapiro-Wilk test and transformed (log or sqrt) if required. Potential heterogeneity of variances was tested using Fligner-test. Auto-correlation was checked using the Durbin Watson Test. All statistical analysis were conducted using R version Ri386 3.1.0 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria).
To detect and determine the type of trophic control, correlations (correlation factor (r), Frank et al. 2006; Boyce et al. 2015) between various phytoplankton groups (edible phytoplankton C, *Chaetoceros curvisetus*, edible phytoplankton C excluding *Ch. curviset*us) and zooplankton (total zooplankton abundance, nauplii abundance) were calculated using the program STATISTICA (version 8.0).

## Results

*Phytoplankton C and composition*

Over the course of time total phytoplankton C significantly decreased with warming and increased with $pCO_2$ (Fig. 1 a; Fig. S1 a; Table 1). These main effects were reflected in a time dependent and temperature driven interaction with $pCO_2$, with highest phytoplankton C at low temperature (16.5°C) and high $pCO_2$ during the bloom (day 0-12; Fig. 1 b; Table 1) but not during the post-bloom period (day 14-28; Fig. 1 c; Table S2). Overall the edible fraction of total phytoplankton C contributed on average 25 % to total phytoplankton C during bloom. Its contribution differed among temperatures with on average 16 % in the warm and 34 % in the cold treatments (Fig. S2). Despite its relatively low contribution to total phytoplankton C, the edible fraction was identified as responsible for the observed interaction effect among temperature and $pCO_2$. Likewise to total phytoplankton C, edible phytoplankton C was significantly higher at low temperature and high $pCO_2$ over the entire course of time (Fig. 1 d; Fig. S1 c, d; Table 1) and during the bloom and post-bloom period (Fig. 1 e, f; Table 1; Table S3). The inedible fraction of total phytoplankton C showed the same, however non-significant trend over the entire experimental time (Fig. 1 g; Fig. S1 e, f; Table 1) but not during bloom or post bloom (Fig. 1 h, i; Table S2).
Edible phytoplankton composition was identical in all treatments at the start of the experiment, and mainly consisted of *Teleaulax* sp.*, Prorocentrum micans, Heterocapsa triquetra and Ditylum brightwellii,* (Fig 2 a-m). During bloom, species

composition differed considerably between temperature treatments. While *Ch. curvisetus* dominated phytoplankton C during bloom in all cold treatments by 70-80 % (mean: 15-36 µg C $L^{-1}$; Fig. 2 b, d, f, h, k, m), species composition in the warm treatments was more evenly distributed (Fig. 2 a, c, e, g, i, l).The exception was the lowest $pCO_2$ level (target value 500 µatm; Fig. 2 a) at which *Ch. curvisetus* was more abundant (mean = 13 µg C $L^{-1}$ compared to on average 2 µg C $L^{-1}$ in all other $pCO_2$ levels, Fig. 2 c, e, g, i, l).

Over the entire course of the experiment the phytoplankton that was inedible for copepods mainly consisted of pico-cyanobacteria (*Synechocystis* and the pico-colonial *Cyanodictyon*), pico-eukaryotes (*Bathycoccus* sp.) and small haptophytes (*Chrysochromulina* sp.). The small cryptophyte *Plagioselmis* sp. was only abundant during the start. Biomass of the inedible larger-sized filamentous cyanobacteria (*Nodularia spumigena* and *Anabaena* sp.) was generally low and contributed less than 1 % to total phytoplankton C on most of the sample days in all treatments.

*Zooplankton abundance*

During bloom total zooplankton abundance was significantly higher in the warm temperature treatments, and decreased with rising $pCO_2$ levels across both temperature levels (Fig. 1 l; Table 1). However, total zooplankton abundance did neither differ between temperature nor $CO_2$ treatments over the time course and in the post-bloom period (Fig. 1 k, m; Fig. S1 g, h; Table S1, Table S2). Within the zooplankton, the nauplii were most abundant in all treatments (Fig. S3). The abundance of zooplankton nauplii was identified as the driver for the warming and $pCO_2$ effect on total zooplankton abundance, i.e. nauplii abundances were significantly higher in the warm temperature treatments and overall declined with increasing $pCO_2$ during bloom (Fig. 1 o; Table 1). This effect, however, was not observed over the entire course of time and during the post-bloom period (Fig. 1 n, p; Fig. S1 i, l; Table S1, Table S2). The abundance of zooplankton adults and copepodites, were not affected by temperature or $pCO_2$ over the course of time and during bloom (Table S1, Table S2). At post-bloom, however, copepodite abundance was marginally significantly higher at low temperature and overall decreased with rising $pCO_2$ (Table 1).

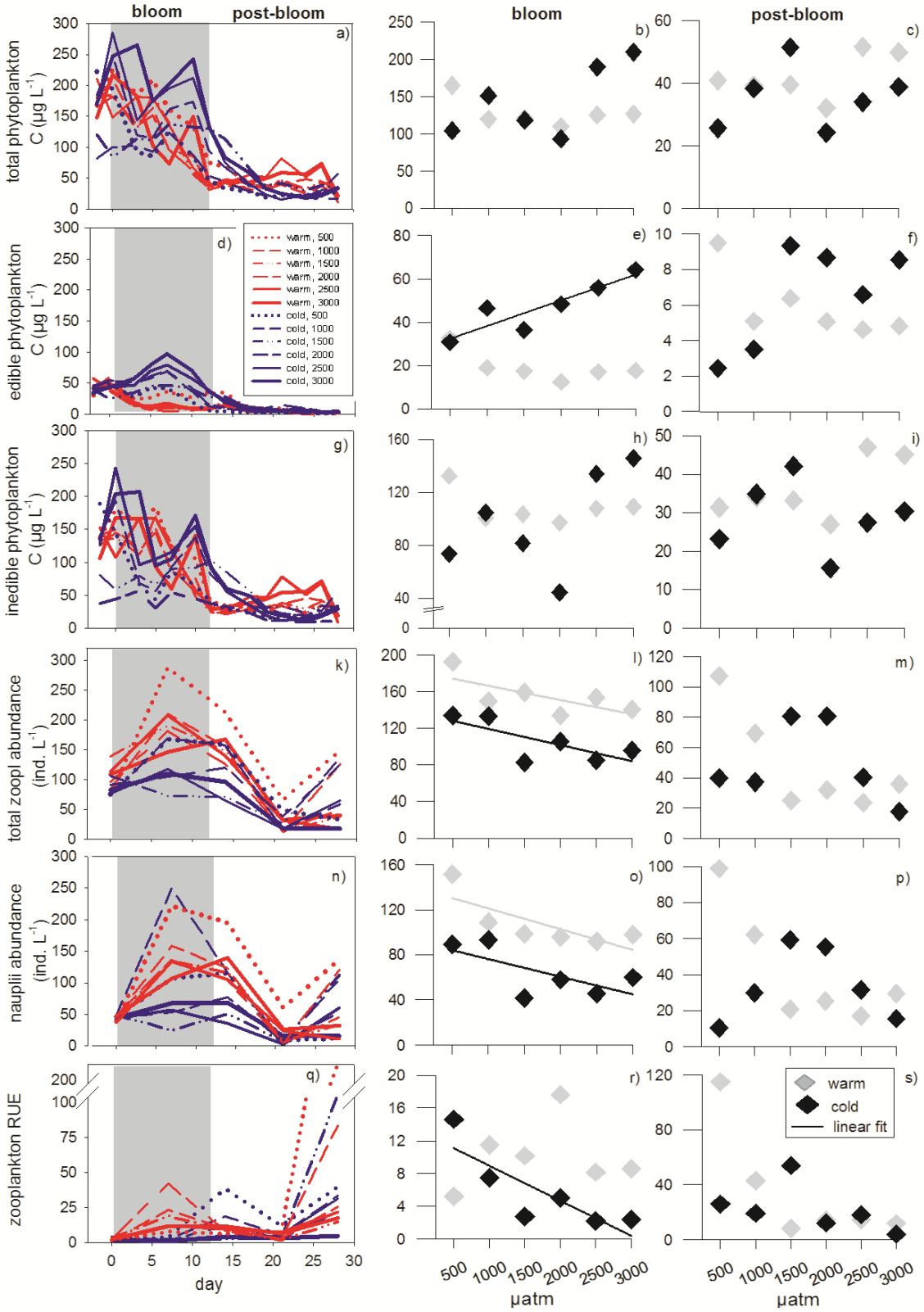


**Fig. 1.** Time-course, bloom and post-bloom period of: a-c) total phytoplankton C, d-f) edible phytoplankton C, g-i) inedible phytoplankton C, k-m) total zooplankton abundance, n-p) nauplii abundance, q-s) zooplankton resource use efficiency (zooplankton RUE). For symbol attribution to treatment combination (temperature treatment, $pCO_2$ target value in µatm) see legends.

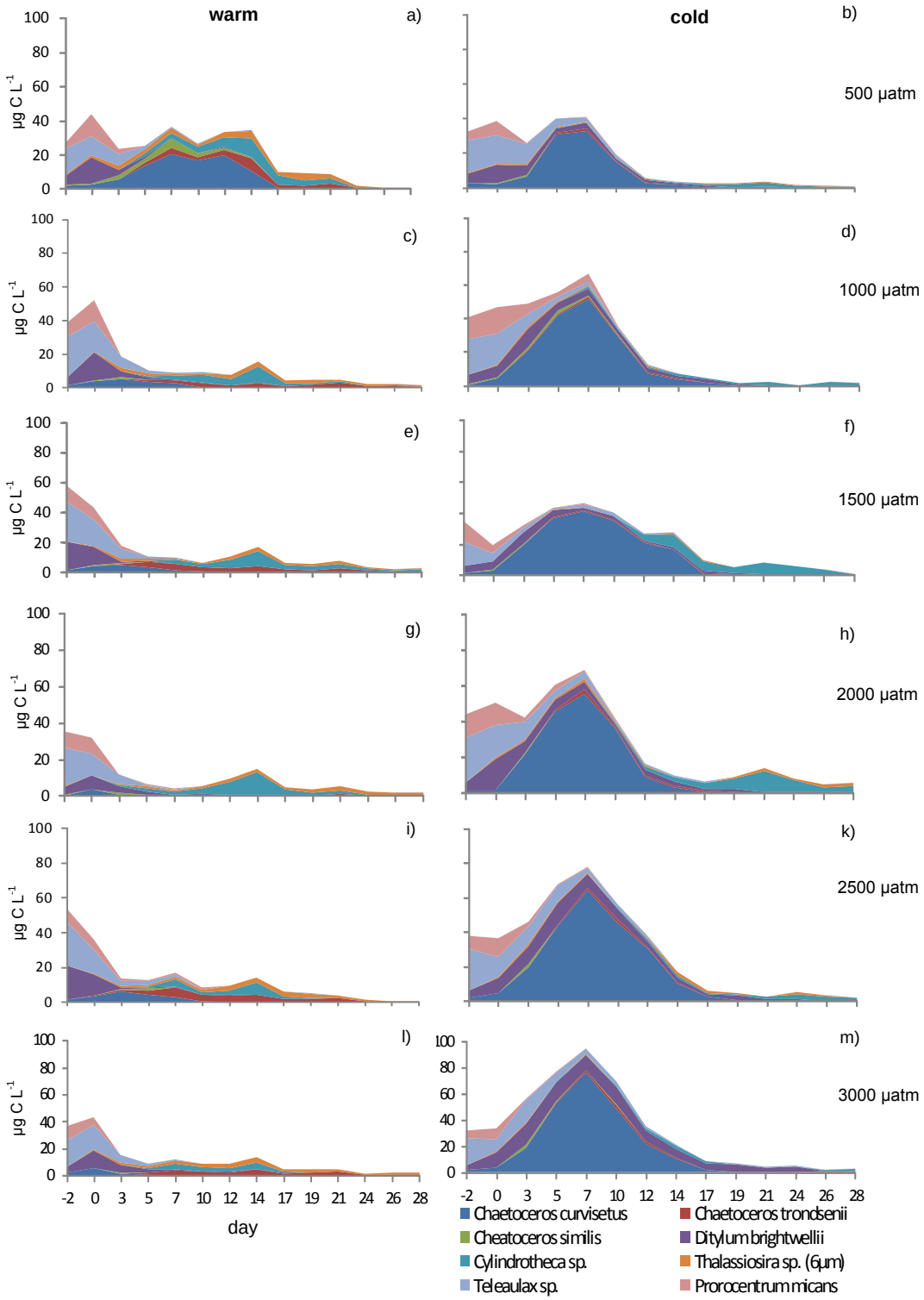


**Fig. 2.** Edible phytoplankton species composition over the course of time for the treatment combinations (temperature treatment, $pCO_2$ target value): a) warm, 500 µatm; b) cold, 500 µatm; c) warm, 1000 µatm; d) cold, 1000µatm; e) warm, 1500 µatm; f) cold, 1500 µatm; g) warm, 2000 µatm; h) cold, 2000 µatm; i) warm, 2500 µatm; k) cold, 2500 µatm; l) warm, 3000 µatm; m) cold, 3000 µatm. Represented here are the 9 most dominant species. For symbol attribution to species see legend.

*Zooplankton RUE*

During phytoplankton bloom there was a significant interaction effect between temperature and $pCO_2$, with lowest zooplankton RUE under low temperature and high $pCO_2$ (Fig. 1 r; Table 1; Table S3). Zooplankton RUE showed no significant differences between treatments over the course of time (Fig. 1 q; Fig. S1 m, n; Table S1) and during the post-bloom period (Fig. 1 s; Table S2).

*Trophic relationships*

In the low temperature treatments, edible phytoplankton was uncorrelated with (log) total zooplankton ($r$ = -0.09; $n$ = 18; $p$ = 0.716) and (log) nauplii abundance ($r$ = -0.20; $n$ = 18; $p$ = 0.422), respectively. Additionally it was tested, if *Ch. curvisetus*, due to its dominance, might have masked a potential trophic relationship at low temperature. Edible phytoplankton C excluding *Ch. curvisetus*, however, remained uncorrelated with total zooplankton abundance ($r$ = -0.37; $n$ = 18; $p$ = 0.133), but showed a negative trend with nauplii abundance ($r$ = -0.435, $n$ = 18, $p$ = 0.07). At high temperature edible phytoplankton C correlated negatively with (log) total zooplankton ($r$ = -0.52; $n$ = 18; $p$ = 0.025) and nauplii abundance ($r$ = -0.53; $n$ = 18; $p$ = 0.023), respectively, suggesting a top-down control of phytoplankton under warming.

*Dissolved inorganic nutrients*

From the beginning of the experiment, the system was nitrogen limited with the average initial total dissolved inorganic nitrogen concentration (including $NO_3^-/NO_2^-$, $NH_4^+$) of 1 µmol $L^{-1}$ (Fig. S4 a, b). Initial average $PO_4^{3-}$ concentration was 0.6 µmol $L^{-1}$ (Fig. S4 c) and $SiO_4^-$ concentration was 11 µmol $L^{-1}$ (Fig. S4 d). Nutrient concentrations declined with the onset of the bloom (day 0), but with no significant differences between treatments during bloom (Fig. S4; Table S2). The $NO_3^-/NO_2^-$ concentration declined below detection limit and ammonium was also depleted by the end of the bloom (Fig. S4 a, b). $PO_4^{3-}$ was still available at the end of the bloom on day 12 (on average 0.4 µmol $L^{-1}$; Fig. S4 c). However, concentrations declined considerably during post-bloom in the cold and high $pCO_2$ mesocosms. Except for one mesocosm (warm, 500 µatm) $SiO_{4-}$ was still available in all treatments at bloom termination (Fig. S4 d). The strong decrease in $SiO_4^-$ under warm 500 µatm might be due to a strong increase in edible phytoplankton C with highest diatom diversity of all treatments.

**Table 1.** Overview of the significant results of generalized least squares models (gls) testing for the effects of temperature (T), $pCO_2$, time, the interaction of temperature and $pCO_2$ (T+$CO_2$), time and temperature (time x T) and time and $pCO_2$ (time x $CO_2$) over the course of time, during bloom and post-bloom on: total phytoplankton C, edible phytoplankton C, inedible phytoplankton C, total zooplankton abundance, nauplii abundance, zooplankton resource use efficiency (zooplankton RUE), N:P. Significant results are in **bold**. *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$.

| Response variable | factor | df residual | t-value | *p* |
|---|---|---|---|---|
| **Time-course** | | | | |
| Total phytoplankton C | T | 160 | 2.629 | **<0.01**** |
| (µg $L^{-1}$) | $CO_2$ | 160 | 3.102 | **<0.01**** |
| | time | 160 | -3.970 | **<0.001***** |
| | T x $CO_2$ | 160 | -3.084 | **<0.01**** |
| | time x T | 160 | -1.739 | **0.072** |
| | time x $CO_2$ | 160 | -1.840 | **0.056*** |
| | time x T x $CO_2$ | 160 | 2.145 | **0.027*** |
| (Log) edible phytoplankton | T | 160 | -0.142 | 0.886 |
| C (µg $L^{-1}$) | $CO_2$ | 160 | 1.396 | 0.164 |
| | time | 160 | -9.618 | **<0.001***** |
| | T x $CO_2$ | 160 | -2.099 | **0.037*** |
| | time x T | 160 | 1.466 | 0.144 |
| | time x $CO_2$ | 160 | 1.861 | 0.064 |
| | time x T x $CO_2$ | 160 | -1.034 | 0.302 |
| (Log) inedible phytoplankton | T | 160 | 1.921 | **0.056*** |
| C (µg $L^{-1}$) | $CO_2$ | 160 | 1.853 | 0.065 |
| | time | 160 | -3.536 | **<0.001***** |
| | T x $CO_2$ | 160 | -1.702 | 0.090 |
| | time x T | 160 | -1.344 | 0.180 |
| | time x $CO_2$ | 160 | -1.696 | 0.091 |
| | time x T x $CO_2$ | 160 | 1.854 | 0.065 |
| **Bloom** | | | | |
| Total phytoplankton C | T | 8 | 1.617 | 0.144 |
| (µg C $L^{-1}$) | $CO_2$ | 8 | 2.463 | **0.039*** |
| | T x $CO_2$ | 8 | -2.267 | **0.053*** |
| Edible phytoplankton C | T | 8 | 0.209 | 0.839 |
| (µg $L^{-1}$) | $CO_2$ | 8 | 4.287 | **<0.01**** |
| | T x $CO_2$ | 8 | -4.282 | **<0.01**** |
| (Log) total zooplankton | T | 8 | 1.553 | **0.003**** |
| abundance (ind. $L^{-1}$) | $CO_2$ | 8 | -2.385 | **0.044*** |
| | T x $CO_2$ | 8 | 0.666 | 0.523 |
| Nauplii abundance (ind. $L^{-1}$) | T | 8 | 4.591 | **0.001**** |
| | $CO_2$ | 8 | -3.118 | **0.012*** |
| | T x $CO_2$ | 8 | -0.242 | 0.698 |
| (Log) RUE | T | 8 | -1.159 | 0.279 |
| | $CO_2$ | 8 | -3.358 | **0.010**** |
| | T x $CO_2$ | 8 | 2.774 | **0.024*** |
| **Post-bloom** | | | | |
| (Log) edible phytoplankton C | T | 8 | 2.876 | **0.020*** |
| (µg $L^{-1}$) | $CO_2$ | 8 | 3.096 | **0.014*** |
| | T x $CO_2$ | 8 | -3.250 | **0.011*** |
| (Log) copepodite abundance | T | 8 | -2.22 | **0.057*** |
| (ind. $L^{-1}$) | $CO_2$ | 8 | -2.591 | **0.032*** |
| | T x $CO_2$ | 8 | 1.644 | 0.138 |
| (Log) N:P | T | 8 | -2.229 | **0.056*** |
| | $CO_2$ | 8 | -0.755 | 0.471 |
| | T x $CO_2$ | 8 | 1.558 | 0.157 |

*Particulate organic matter stoichiometry*

Carbon to nitrogen ratios (C:N) and carbon to phosphorus ratios (C:P) of particulate organic matter did not differ between treatments, neither over the course of time nor during bloom and post-bloom (Fig. 3 a-f; Fig. S6; Table S1; Table S2). Nitrogen to phosphorus ratios (N:P) of particulate organic matter were marginal significantly higher under low temperature during post-bloom (Fig. 3 i; Table S2) but did not differ between treatments over the course of time and during bloom (Fig. 3 g, h; Table S1; Table S2).

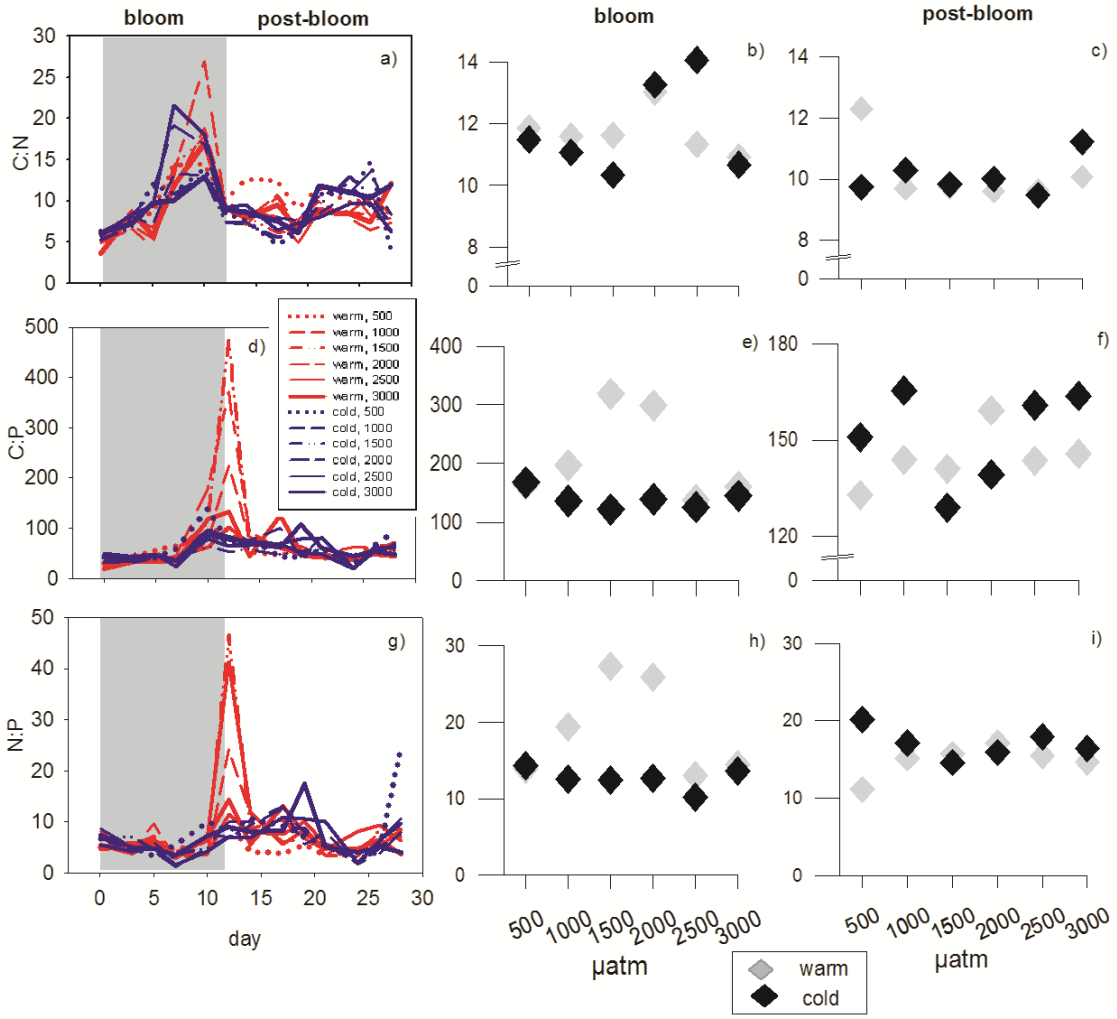


**Fig. 3.** Time-course, bloom and post-bloom of: a-c) C:N (mol:mol), d-f) C:P (mol:mol), g-i) N:P (mol:mol). For symbol attribution to treatment combination (temperature treatment, $pCO_2$ target value in µatm) see legends.

## Discussion

Contrary to predictions for summer plankton communities of coastal and seasonally stratified systems, warming in this study decreased total and the edible fraction of phytoplankton C (partly rejecting hypothesis 1). $PCO_2$ led to an overall increase in total and edible phytoplankton C. This effect, however, was driven by the increase of phytoplankton C with $pCO_2$ in the cold treatments (partly accepting hypothesis 1). Phytoplankton stoichiometry was not affected by the experimental treatments (rejecting hypothesis 2).

The results suggest that warming did not increase phytoplankton C because enhanced phytoplankton growth was masked by intensified grazing. As such the system switched from a bottom up-controlled in the cold treatments to a mainly top-down controlled one in the warm treatments with overall lower (i.e. grazed) phytoplankton C and higher zooplankton (i.e. copepod) abundance (partly accepting hypothesis 3). The positive effect of $CO_2$ on phytoplankton C in the cold treatment, however, did not translate to the next trophic level (partly rejecting hypothesis 3). Instead there seemed to be a direct negative effect of increasing $pCO_2$ on copepods which was reflected in increasing phytoplankton C (i.e less grazed) along with decreasing zooplankton RUE in the cold treatments.

The results of the low temperature treatments in this experimental system are in line with previous observations suggesting that Baltic Sea summer plankton is bottom-up regulated (Taucher et al. 2012; Suikkanen et al. 2013; Lewandowska et al. 2014). This is reflected first, by a limited availability of inorganic nitrogen for the phytoplankton in the whole set-up, and second, by the fact that no trophic relationships between edible phytoplankton and zooplankton could be detected. Declining phytoplankton C in the warm temperature treatments suggests that the bottom-up regulation became overcompensated by temperature intensified grazing (sensu Keller et al. 1999; Brown et al. 2004; O'Connor et al. 2009, Garzke et al. 2015), leading to a mainly top down controlled system with down-grazed phytoplankton and higher zooplankton abundance. The latter are known to compensate temperature induced higher metabolic demands (O'Connor et al. 2007) through increased consumption and feeding rates (Sanford 1999). This is underpinned by significant negative correlations between edible phytoplankton and

zooplankton (sensu Boyce et al. 2015) in the warm treatments. These results are in contrast to the few studies on the effects of warming on Baltic Sea summer plankton that all found increased phytoplankton biomass due to various reasons (Taucher et al. 2012; Suikkanen et al. 2013; Lewandowska et al. 2014)**.** Lewandowska et al. (2014) suggested that the net response of phytoplankton under experimentally nutrient depleted conditions was mainly due to temperature-driven changes in nutrient availability (bottom-up control) instead of direct metabolic effects like a higher metabolic demand by the mesozooplankton, resulting in stronger grazing (top-town control). Moreover, the phytoplankton community in Lewandowska et al. (2014) comprised only very few diatoms, which are the preferred food source for copepods. Instead, it was dominated by small flagellates, which are not consumed by copepods but rather by ciliates. This was proposed to cause a shift in feeding preference of copepods towards ciliates which in turn released phytoplankton from grazing. Likewise, in the present study small, and for copepods inedible, phytoplankton (< 5µm) initially dominated and remained high under both temperatures (Fig. S2). However, the inedible phytoplankton remained unaffected by the manipulated factors during bloom and the overall abundance of microzooplankton was very low (i.e. < 2 ind. $L^{-1}$). Thus, inedible phytoplankton turned out not being responsible for the treatment effects in total phytoplankton C and the potential influence of protist grazing (ciliates, heterotrophic nano-flagellates) can be indirectly neglected. Instead, the edible phytoplankton fraction (mainly consisting of diatoms) was responsible for the responses of total phytoplankton C. In a long-term monitoring study of the northern central Baltic Sea (Suikkanen et al. 2013), seawater warming was identified as the main driver for the observed increase in total phytoplankton biomass mediated by a significant increase in large filamentous cyanobacteria which, however, are less edible for copepods. Although large filamentous cyanobacteria were present in this study, they did not significantly contribute to total phytoplankton C (< 1 %), and thus can be excluded as a reason for the observed increase in total biomass.

The positive effect of $pCO_2$ on total and edible phytoplankton C in the cold treatments is likely indirect due to grazing release caused by a direct negative effect of $pCO_2$ on copepods. Though Acartia sp., the dominant copepod species in this system, was widely considered as not being affected by increasing $pCO_2$ up to levels of 5000 µatm (Kurihara et al. 2004), a recent study showed that $CO_2$ sensitivity of this species occurs and is largely stage-specific. In particular the nauplii showed 100 %

mortality above 2000 µatm (Cripps et al. 2014). Though Cripps et al. (2014) did not give a physiological explanation for their significant findings, they suggest that compensation for $CO_2$ stress consumes additional energy during the critical ontogenetic stage in which the nauplii switch their energy sources from the endogenous yolk to exogenous food (i.e. phytoplankton). In fact the decline of *Acartia* sp. nauplii with increasing $pCO_2$ was responsible for the decrease in total zooplankton abundance in this study. The abundance of nauplii declined by 33 %, suggesting a less significant mortality compared to the results of Cripps et al. (2014). The reason for this might be evolutionary adaptation of *Acartia* sp. due to the naturally high and fluctuating $CO_2$ concentrations in Kiel Fjord. Even today $CO_2$ concentrations in Kiel Fjord temporarily exceed 2300 µatm for several days in summer (Thomsen et al. 2010) which likely selects for more stress tolerant genotypes. However, even in populations with a relatively high proportion of stress tolerant genotypes, juvenile stages might remain the ontogenetic bottleneck in their response to high $pCO_2$ in future (Dupont et al. 2009; Cripps et al. 2014).

Indirect negative impacts on zooplankton by changes in stoichiometrical food quality due to future warming or rising $pCO_2$ can considered as less important based on the results of this study, as neither phytoplankton C:N nor C:P ratios were affected by $CO_2$ or temperature. N:P ratios were marginally lower under warming in the post-bloom period, but still near to the Redfield ratio (16), suggesting no effect on zooplankton nutritional composition. C:N ratios were above Redfield ratio (C:N = 6.6) and above the usual copepods biomass ratio (C:N =4-6, Koski 1999) during bloom and post-bloom in all treatments, but copepods in general and especially *Acartia* sp. belong to high C:N:P species (stoichiometric theory, Andersen & Hessen 1991), which are less likely to be N or P limited.

The decline in zooplankton RUE due to negative $CO_2$ effects in the cold treatments during phytoplankton bloom shows that excess edible phytoplankton was not consumed and thus not transferred to the next trophic level in this bottom-up controlled system (see previous discussion above). In the warm temperature treatments total zooplankton and in particular nauplii abundances also declined with increasing $pCO_2$, however, this was not reflected in reduced grazing (i.e. in increasing phytoplankton C and decreasing zooplankton RUE). The reason might be that, despite of the zooplankton abundance decline with higher $pCO_2$ in the warm treatments, grazing pressure remained sufficient due to generally higher copepod

abundances by on average 49 additional ind. $L^{-1}$ (i.e. 32 % higher abundance; see also Fig. 1 l; Fig. S3). These higher abundances are probably mainly due to warming-related accelerated hatching rates (Holste & Peck 2006), and to a lesser extent to faster transition from stage to stage (Campbell et al. 2001; Hirst & Kiorboe 2002; Leandro et al. 2006), egg production and reproduction (Kordas et al. 2011). On top these higher numbers of individuals likely grazed in faster rates compared to the cold treatments. According to the classic Q10 rule (Prosser 1973) the grazing rates of the copepods should have increased by 1.2 to 1.8 times in response to the experimental temperature manipulation of 6°C. In combination with the increase in abundance this could have resulted in an overall intensified grazing by 2.5 to 3.3 times under warming. Copepod abundances at the highest $pCO_2$ levels (i.e. 2000 to 3000 µatm) were likewise higher by on average 47 ind. $L^{-1}$ resulting in potentially intensified grazing by 2.7 to 3.3 times in the warm compared to the cold treatments. This in total might explain the absence of a (indirect) $CO_2$ effect on phytoplankton C and zooplankton RUE in the warm treatments. Subsequently, the steep copepod decline from sample day 14 to 21 in the warm treatments can be explained by food limitation which is reflected in the down-grazed edible phytoplankton during bloom phase.

While the range of experimental $CO_2$ concentrations exceeded the predictions for the open ocean by the end of this century, it is relevant for the variability in local conditions in Kiel Fjord where this study took place. This is because the surface water $pCO_2$ in coastal upwelling systems (like Kiel Fjord) can be temporarily strongly elevated due to wind-driven upwelling events of $CO_2$ enriched water from deeper layers below the thermocline (e.g. Hansen et al. 1999; Feely et al. 2008). High primary productivity caused by eutrophication leads to enhanced respiration and thus to a large increase of the $CO_2$ concentrations (Helcom 2009; Thomsen et al. 2013) in the deeper layers. Therefore, the results of declining grazing due to high $pCO_2$ cannot be necessarily transferred to other low nutrient region such as the open ocean where ambient and projected $CO_2$ concentrations remain well below most levels used in this study (i.e. 700-1000 µatm, IPCC 2013). These concentrations are not expected to harm zooplankton grazers such as copepods (Cripps et al. 2014).
Increasing seawater $CO_2$ concentration can also act directly on phytoplankton. Firstly, it can stimulate growth, if $CO_2$ is a limiting nutrient. This can occur after intense phytoplankton blooms (Murata et al. 2002). However, such a scenario can be

excluded in this design because the $CO_2$ concentration was regularly adjusted to the experimental target values to counteract uptake by phytoplankton. Secondly, while $CO_2$ can be a limiting factor in terms of its concentration, rising $pCO_2$ can also be profitable for larger phytoplankton cells with active $CO_2$ uptake mechanisms. Most phytoplankton groups and especially diatoms have evolved effective, but energy-demanding carbon concentrating mechanisms (CCM) because passive diffusion of $HCO_3^-$ through membranes is limited by cell volume-surface ratios and the electrochemical potential gradient (negative inside) across cell's plasma membranes (Reinfelder 2011). Increasing $pCO_2$ can potentially mean a reduction of the metabolic costs for the phytoplankton's effective CCM, which was suggested as the underlying mechanism for profiting from high $pCO_2$ by these organisms (Raven 1991). In this study we can indirectly exclude this as a reason for the increased phytoplankton C with increasing $pCO_2$, because neither C:N nor C:P ratios increased during phytoplankton bloom while at the same time dissolved inorganic nitrogen was nearly depleted in all treatments.

## *Conclusion*

Our results point out that the previously suggested discrimination of different responses among nutrient deplete (i.e. mainly bottom-up controlled) and replete (i.e. mainly top-down controlled) conditions in marine plankton to seawater warming is not necessarily clear cut. We showed that warming can switch one condition to the other, i.e. from a bottom up-controlled to a mainly top-down controlled phytoplankton system, with significant implications for their respective responses to the here strongly increased seawater $CO_2$ concentrations. Whereas the described warming effect might be of general importance for future regulation of nutrient-limited plankton systems, the grazing release due to lower zooplankton (copepod) abundance with increasing $pCO_2$ might be more site-specific and as such of higher importance for plankton in seasonally stratified regions with temporary upwelling of $CO_2$ enriched water (Hansen et al. 1999; Feely et al. 2008). In such conditions, warming, however, has the potential to mask $CO_2$ effects (either from bottom-up or top-down) due to generally intensified grazing.

# Chapter III

## Climate change effects on phytoplankton fatty acids


### Abstract

Warming and rising $pCO_2$ can change phytoplankton's fatty acid contents and the taxonomic composition of phytoplankton communities. Until now, studies showed contrasting results regarding the single effects of warming and rising $pCO_2$ on fatty acids. The combined effects of warming and rising $pCO_2$ on fatty acids still remain little understood, although sea surface $pCO_2$ and temperature will change in parallel in a future 'greenhouse' world. We set out to experimentally explore these combined effects on phytoplankton fatty acids and the potential correlated response of taxonomic composition by crossing two temperature regimes with six $pCO_2$ levels using two different natural Baltic Sea summer plankton communities. Our results show that warming changed particular polyunsaturated fatty acids (PUFAs), leading for instance to a decrease in EPA and DHA, but to an increase in ARA and linolenic acid. However, the observed warming-induced changes in fatty acids overall did not correlate with changes in the taxonomic composition of the community. Rising $pCO_2$ affected fatty acids only minor in both of the studies.

We conclude that warming, but not $pCO_2$, can change fatty acid contents of natural phytoplankton communities, and thus potentially affects food quality for higher trophic levels. Nevertheless, temperature effects seem to be complex as they varied strongly between the two experiments.

## Introduction

Fatty acids play a major role in all marine organisms, as they function as energy reserves, membrane components, antioxidants and hormones. In the photosynthetic plankton the fatty acid composition is additionally important for membrane-bound physiological processes and compounds such as the light harvesting complex (LHCS) (Leu et al. 2012; Mironov et al. 2012). The fatty acid composition of the major algae groups vary due to different biosynthetic pathways for fatty acid synthesis. In this way fatty acid composition of phytoplankton communities reflects taxonomic composition (de Carvalho & Caramujo 2014) and can be used as trophic markers (Dalsgaard et al. 2003). Thus, changes in the taxonomic composition of plankton communities due to environmental conditions are proposed to be reflected indirectly by the community's fatty acids. However, fatty acids of a given taxon are also subject to environmental influences (Dalsgaard et al. 2003).

Polyunsaturated fatty acids (PUFAs), i.e. fatty acids containing two or more double bonds, are essential for all animals; however, most of the heterotrophic organisms (here: zooplankton, heterotrophic nanoflagellates) cannot synthesize PUFAs *de novo* at rates sufficient to meet their metabolic demands (Brett & Müller-Navarra 1997). Consequently, the majority of essential PUFAs have to be taken up with the phytoplankton food source. Among major phytoplankton groups, diatoms, a major food source of the mesozooplankton, contain the highest PUFA contents especially eicosapentaenoic acid (EPA, $C_{20:5n3}$) and arachidonic acid (ARA, $C_{20:4n6}$), but low amounts of alpha linolenic acid ($C_{18:3n3}$; Erwin 1973; Dalsgaard et al. 2003). Cyanobacteria and chlorophytes in contrast, both contain relative low amounts of PUFAs, especially of EPA and docosahexaenoic acid (DHA, $C_{22:6n3}$). Chlorophytes contain high proportions of linolenic acid, and linoleic acid ($C_{18:2n6}$), whereas the fatty acid content of cyanobacteria is generally low (Brett & Müller-Navarra 1997; Dalsgaard et al. 2003,). Dinoflagellates are known to be rich in DHA (Ahlgren 1997), whereas EPA accounts for only approximately 10 % of total fatty acids in dinoflagellates and cryptophytes (Ahlgren et al. 1992). In that way fatty acid-associated food quality is an important factor regulating the energy transfer between primary producers and consumers (Müller-Navarra et al. 2004) in the food web. This is especially relevant in coastal and upwelling areas of high and temperate latitudes with seasonal phytoplankton blooms, e.g. in the Baltic Sea, where lipid-rich zooplankton species (mainly copepods) constitute a major vector of energy transfer

to higher trophic levels like fish larvae (Kattner et al. 2007). In particular the PUFAs EPA, DHA and ARA are required for growth and survival of all organisms. Additionally they play a major role for egg production and reproduction success of zooplankton. EPA is even suggested to be one of the key nutritional constituents (Brett & Müller-Navarra 1997). Linolenic acid is a further key nutritional component as it can be converted to EPA and DHA by all omnivore species.

Climate change is assumed to affect the phytoplankton fatty acid composition, leading to a change in the food quality for higher trophic levels (Kattner et al. 2007; Rossoll et al. 2012). Whereas atmospheric $pCO_2$ is prospected to double from current values of approximately 390 µatm to 700 µatm and the pH to decrease by 0.5 until the year 2100 (IPCC 2014, RCP8.5), the estimated average global ocean surface temperature is predicted to increase by even 2-4°C (IPPC 2013). Although sea surface $pCO_2$ and temperature will change in parallel in a future 'greenhouse' world, to the best of our knowledge our study is one of the first analyzing the combined effects of both factors on phytoplankton`s chemical composition.
Enhanced $pCO_2$ is hypothesized to downgrade food quality for higher trophic levels. To regulate the internal cell homeostasis and reduce the fluidity of their membranes, organisms are expected to accumulate saturated fatty acids by simultaneously decreasing PUFAs under elevated $pCO_2$. However, experimental studies so far yielded contrasting results. Whereas an increase in total fatty acids (TFA) but a decrease in PUFA was found in a single species prymnesiophyte culture experiment (Carvalho & Malcata 2005) under high $pCO_2$ levels, an increase in EPA was observed in the PUFA-rich algae *Nannochloropsis* (Hoshida et al. 2005). In laboratory experiments including consumers, significant changes of the concentration and composition of fatty acids in the diatom *Thalassiosira pseudonana* as food algae even translated into limited growth and reproduction of the consumer copepod *Acartia tonsa* at higher $pCO_2$ (Rossoll et al. 2012) More precisely, the food algae cultured under elevated (750 µatm) $pCO_2$ showed a decline in both the total fatty acid content as well as the relative amount of long-chain polyunsaturated fatty acids (PUFAs). In contrast to this simple two-species food chain, no direct effects of rising $pCO_2$ on PUFAs have been found in a mesocosm study containing a natural Arctic plankton community (Leu et al. 2012). Although the content of most PUFAs

correlated with $pCO_2$, this was indirectly caused by changes in the taxonomic community composition (Leu et al. 2012).

Increasing temperature is considered the key factor affecting the fatty acid pattern of phytoplankton by alteration of the fatty acid chain lengths and the degree of saturations (Dalsgaard et al. 2003). Single culture experiments with in total eight marine phytoplankton species revealed significant effects on the fatty acid composition such as a an overall decrease in PUFAs with warming (Thompson et al. 1992). Temperature-dependent modifications such as a decrease in total PUFA and EPA and an increase in total saturated fatty acids (SFAs) have also been reported in a number of other phytoplankton single-species experiments (e.g. Renaud et al. 2002; Hoffmann et al. 2010; Dodson et al. 2014). These results suggest, likewise to the $pCO_2$ effect, a decline in the food quality of phytoplankton due to increasing seawater temperature.

We set out (i) to analyze and compare the fatty acid composition and content of two experimental Baltic Sea phytoplankton summer blooms with natural community composition; and (ii) to investigate if and how manipulated seawater temperature and $pCO_2$ in these two experiments changes the phytoplanktons' fatty acid composition and content. Whereas the first study was conducted in August 2013 and hereafter is referred to as "mid-summer bloom 2013", the second study was conducted at the end of August / beginning of September in 2014 and hereafter is referred to as "late-summer bloom 2014".

Both experimental phytoplankton blooms were nitrogen limited from the beginning on, which is typical for seasonally stratified areas in summer (Sørensen & Sahlsten 1987; Kratzer & Sørensen 2011). While the species pools were also similar in both studies, quantitative species composition differed, i.e. the species' contribution to total phytoplankton carbon (total phytoplankton C). At ambient conditions total phytoplankton C was more than twice as high in late-summer bloom 2014 compared to mid-summer bloom 2013 (Fig. S1 a, b).

## Material and methods

*Experimental design*

Two different temperature regimes were crossed with six $pCO_2$ target levels, ranging from 500 to 3000 µatm. The set-up resulted in twelve mesocosms, installed in four temperature-controlled culture rooms. The mesocosms contained the natural Baltic Sea summer plankton community including phytoplankton (photosynthetic bacteria and algae), bacteria and protozoa. *Nodularia spumigena,* tyical for Baltic summer blooms, was added as a culture to each mesocosm prior the first sampling culture conditions: 18°C, temperature-controlled room, ~150µmol Phot $L^{-1}$). *Nodularia* was added to the mesocosms on 14 August 2013 in mid-summer bloom experiment with a final concentration of approximately 5160 cells $L^{-1}$ per mesocosm. In the late-summer bloom experiment, *Nodularia* was added to the mesocosms with a final concentration of approximately 37450 cells $L^{-1}$ per mesocosm on 1 September 2014.
To minimize differences in the starting community between treatments, the water was pumped by a rotary pump over a distributor in all mesocosms at the same time. After filling, temperature and $CO_2$ was manipulated stepwise. The mesocosms in mid-summer bloom experiment 2013 (August 2013) contained each a volume of 1400 L and had a surface area of approximately 1.54 m². Mesocosms of the late summer bloom experiment 2014 (late August/September 2014) consisted of swimming plastic bags (LDPE, Poly Pack), each with a surface area of approximately 1.3 m² and containing approximately 200 L of natural Baltic Sea water. Each bag was swimming in a 1400 L barrel with a stirrer, containing also the natural Baltic Sea water of the filling day. In both experiments the mesocosms were covered by a PVC cover (polyvinylchloride, light permeable) containing a sampling port which remained closed between sampling events. In order to reduce phytoplankton sedimentation and to assure its homogeneous distribution over the course of experiment, the water was stirred by an automatically gently moving propeller in mid-summer bloom 2013. In the late-summer bloom 2014 the water was mixed once a day before sample taking by moving a Cecchi disk carefully up and down.

The temperature regimes, i.e. 15°C and 21°C (mid-summer bloom 2013) as well as 13°C and 19°C (late-summer bloom 2014), represented 3°C above and below the actual water temperature of Kiel Bight (western Baltic Sea) on the filling day and were hereafter referred to as warm (19°C, 22.5°C) and cold (13°C, 16.5°C) regimes. The

temperature treatments lie within the natural average sea surface temperatures and their fluctuations of the coastal western Baltic Sea in August / September, measured from 1957 to 2013 (mean temperature at 1m depth, Boknis Eck: August: 17.75°C (SD: 2.4); September: 15.55°C (SD: 1.8), Lennartz et al. 2014).

The target $pCO_2$ levels for manipulation were 500, 1000, 1500, 2000, 2500, 3000 µatm in both experiments. The lowest $pCO_2$ regime (Fig. S2 a, b) represented $CO_2$ concentrations close to the minimum of the surface water in Kiel Bight. The highest regimes (Fig. S2 a, b), represented present day maximum values in Kiel Bight (>2300 µatm), which are temporally reached during upwelling events in summer. These upwelling events of water masses in Kiel Bight, enriched with high dissolved inorganic carbon, are caused by strong winds from south-west, whereas otherwise the coastal water is seasonally stratified (strong temperature and salinity gradients; Thomsen et al. 2010). $PCO_2$ values in between (Fig. S2 a, b) conformed to predictions for coastal upwelling areas with highly temporal variable $pCO_2$ values, exceeding strongly even the worst case scenario forecast for open ocean surface waters (IPCC 2014).

For manipulating the target $pCO_2$ values and for subsequent balancing of the natural $CO_2$ drawdown due to phytoplankton primary production, $CO_2$ enriched water (Kiel Bight, 0.2 µm filtered, stored at cool and dark conditions, $CO_2$ saturated by bubbling with $CO_2$ gas) was added to the mesocosms (using a flexible tube), after the sample taking procedure (Monday, Wednesday, Friday). The required volumes were calculated on the basis of dissolved inorganic carbon (DIC) and total alkalinity (TA) using CO2SYS (Lewis & Wallace 1998).

For light supply, above each mesocosm a computer-controlled light unit (GHL Groß Hard- und Softwarelösungen, Kaiserslautern/Germany) was installed, each consisting of 5 HIBay-LED spotlights (purpose build item of Econlux, 100 W each). Day length and light intensity were calculated with the astronomic model of Brock (1981) and aligned to the natural seasonal light patterns. Light conformed to 40 % of solar irradiance of an approximated cloudless day. The light:dark cycle in mid-summer bloom 2013 was 14 h:3 min : 9 h:57 min with a simulated sundown and sunrise of approximately 2 hours. Maximum light intensity was in mean 382.7 µmol photons $m^{-2}$ $s^{-1}$ (LICOR Li-250A light meter) at the water surface. The light:dark cycle in late-summer bloom 2014 was 13 h:40 min : 10 h:20 min with a simulated sundown

and sunrise of approximately 3.5 hours. Maximum light intensity in this experiment was in mean 391,5 µmol photons $m^{-2}$ $s^{-1}$ at the water surface and 275,15 µmol photons $m^{-2}$ $s^{-1}$ in the middle of the water column (0,34 m below surface; LICOR Li-250A light meter; 18.09.2014).

*Sampling and measurements*

Salinity and water temperature were measured daily. Samples for fatty acids were taken once a week (Friday). Samples for total (DIC), phytoplankton species composition and biomass (including flow cytometer and microscope counting), dissolved inorganic nutrients ($NO_3^-$/$NO_2^-$, $NH_4^+$, $PO_4^{3-}$) and particulate organic carbon (POC) have been taken three times a week (Monday, Wednesday, Friday) whereas samples TA were taken once a week (Monday). Experiments were finished after 28 days (mid-summer bloom 2013) and 25 days (late-summer bloom 2014) respectively, when the phytoplankton bloom was terminated.

Carbonate system – In the mid-summer bloom experiment 2013, DIC samples were gently pressure-filtered (0.2 µm, Sarstedt Filtropur) and collected into 50 mL gas tight vessels with at least 100 mL of overflow before sample collection, already described in Paul et al. (accepted). DIC was analysed by infrared detection of $CO_2$ by a LICOR LI-7000 on an AIRICA system (MARIANDA, Kiel). Samples for TA analyses were sterile filtered as for DIC but were collected in polyethylene containers (200 mL). TA samples were analysed by open-cell potentiometric titration on an auto-sampler (Metrohm 869 Sample Changer and 907 Titrando Dosing unit) according to Dickson et al. (2007). Certified reference material provided by Andrew Dickson (Scripps Institute for Oceanography of the University of California, San Diego) was used to correct for any drift during analyses within a run.

In the late-summer bloom experiment 2014, DIC samples were gently pressure-filtered (0.2 µm, Sarstedt Filtropur) and collected into 50 mL gas tight vessels with at least 100 mL of overflow before sample collection. Samples were measured following Hansen et al. (2013) using a SRI-8610C 3 (Torrence, USA) gas chromatograph. For TA 25 mL samples were filtered (Whatman GF/F filter 0.2 µm) and titrated at 20°C with 0.05M HCl-solution 5 (Dickson 1981, Dickson et al. 2003) in an automated titration device (Metrohm Swiss 6 mode). Certified reference material provided by Andrew Dickson (Scripps Institute for Oceanography of the University of California, San Diego) was used to correct for any drift during analyses within a run.

The remaining carbonate parameter $pCO_2$ was calculated under both experiments using CO2SYS (Lewis & Wallace 1998; Pierrot et al. 2006) and the constants supplied by Hansson (1973) and Mehrbach et al. (1973), that were refitted by Dickson & Millero (1987) and the $KSO_4$ dissociation constant from Dickson (1990).

Dissolved inorganic nutrients – For $NO_3^-/NO_2^-$, $NH_4^+$ and $PO_4^{3-}$ 20 mL water was filtered through cellulose acetate filters (Sartorius, 0.2 µm pore size) and immediately frozen at -20°C. Samples were measured following the protocols of Hansen and Koroleff (1999) with an auto-analyzer (Skalar, $SAN^{PLUS}$; Breda/Netherlands). The detection limit of the auto-analyzer was a concentration of 0.1 µmol $L^{-1}$.

Fatty acids – The fatty acids of the plankton (including mainly phytoplankton, but also bacteria, protozoa) were analyzed regarding the fatty acid contents per carbon biomass (ng fatty acid per µg C) and the fatty acid composition (fatty acid content per total fatty acid content (TFA), %). Therefor 100-250 mL (depending on biomass) water was filtered onto pre-washed (in 5-10 % HCl) and pre-combusted (6h, 550°C) Whatman GF/F filters and immediately frozen at -20°C. Filters were extracted in chloroform : dichlormethane : methanol (1:1:1 v/v/v) following Arndt & Sommer (2013). Prior to extraction two internal standards, heneicosanoic acid (C21:0) and FAME - C19:0 were added. Methyl esters were prepared by esterification with toluene and $H_2SO_4$ (1 %) in methanol heated up to 50°C for 12 hours. After extraction with *n*-hexane the fatty acid methyl esters were analyzed with a gas chromatograph (Thermo Scientific Trace GC Ultra with autosampler AS 3000). Peaks were identified by comparison with standard mixtures. For quantifying the fatty acids, each peak area was calculated by fitting to the internal standard C19:0 with a known quantity of 22.26 ng $\mu L^{-1}$. For standardizing them to a biomass, they were related to carbon (POC).

Particulate organic carbon - For POC 100-250 mL water (volume depending on plankton density) were filtered onto pre-washed (in 5-10 % HCl) and pre-combusted (6h, 550°C) Whatman GF/F filters and immediately frozen at -20°C. POC was determined by an element analyzer (Thermo Scientific Flash 2000).

Phytoplankton species composition and biomass – Species composition is here presented as the contribution (%) of species to total phytoplankton carbon (total phytoplankton C) biomass. Species were taxonomically divided into: a) diatoms, b) cyanobacteria (including pico-cyanobacteria (2 µm) and large filamentous ones like *Nodularia spumigena*), c) phototrophic flagellates (including dinoflagellates and cryptophytes) and d) small phytoplankton (<5 µm, containing only chl *a*, included e.g. pico-chlorophytes).

For the abundance of small phytoplankton and pico-cyanobacteria, 3 mL of pre-filtered water (64 µm mesh) were fixed with formalin in a cryovial, flash frozen in liquid nitrogen and kept frozen in at -20°C until measurement on a flow cytometer (FASCalibur, Becton Dickinson). The phytoplankton was distinguished according to size and pigment fluorescence (chlorophyll *a* and phycoerythrine). For abundance of larger phytoplankton species (>5 µm), 100 mL of sample was Lugol-fixed and stored in the dark. With an inverted light microscope species were determined to the species level and counted using the Utermöhl technique (Utermöhl 1958).

For calculating total phytoplankton C the biovolume of each species (identified by flow cytometry and microscopy) was calculated taking the respective nearest geometric standard (Hillebrand et al. 1999). Afterwards, the species' biovolumes were converted into carbon content according to Menden-Deuer & Lessard (2000), i.e. $C=0{,}288V^{0{,}811}$ for diatoms and $C=0.216V^{0.939}$ for other phytoplankton (C=carbon content in pg, V=cell volume in µm³). As 180 µm³ is the smallest cell size included in the analysis of Menden-Deuer and Lessard (2000), their non-linear models predict unrealistically high C content for smaller algae. Therefore, the conversion factors 0.108 pg C $\mu m^{-3}$ for diatoms and 0.157 pg C $\mu m^{-3}$ for all other organisms were used for phytoplankton cells below 180 µm³ (Sommer et al. 2012b). At last, the calculated carbon content for each species was multiplied with its respective cell abundance.

*Data analysis*

In order to test for treatment effects during phytoplankton bloom on the measured and calculated response variables in each experiment itself, a generalized least squares (gls) model (nlme package, R) with the factors target $pCO_2$ (continuous), temperature (categorical), and the interactions $CO_2$ x temperature was applied. As response variables we chose: species composition (% cyanobacteria, diatoms, flagellates and small phytoplankton on total phytoplankton C); TFA, total PUFA,

content of EPA, DHA, ARA, linolenic acid and 18:1n9; percentage of PUFA, MUFA, SFA to TFA, Where a significant interaction effect was detected, separate regression analysis with $pCO_2$ as continuous factor were conducted for warm and cold treatments. Prior to gls models the optimal variance-covariate structure was determined by using Restricted Maximum-likelihood (REML) estimation. All model residuals were checked for normality using Shapiro-Wilk test and transformed (sqrt, log) if required. Potential heterogeneity of variances was tested using Fligner-test. Species' contribution to total phytoplankton C (% cyanobacteria, diatoms, flagellates, small phytoplankton) and the contributions of PUFA, MUFA, SFA to TFA (%) were traditional transformed with arcsine before statistical analyses, to take care of error distributions. All statistical analysis were conducted using R version Ri386 3.1.0 (R Development Core Team, R Foundation for Statistical Computing, Vienna, Austria).
Comparisons between both studies, i.e. mid-summer bloom 2013 and late-summer bloom 2014, were just done qualitatively without using statistics.
Principle component analysis (PCA) was implemented to depict patterns of association between fatty acid composition and species composition. Analyses were done separately for both experiments with the content of EPA, DHA, ARA, linolenic acid and 18:1n9 as independent (active) variables. Species compositions, i.e. the contribution of diatoms, cyanobacteria and flagellates on total phytoplankton C (%), were taken as supplementary variables. The response variables are indicated by arrows, the length of which represent the importance of the variable to explain the variation in the data set (increasing length = increasing importance) (see Fig. 4; Table 1).

Phytoplankton bloom was defined as the period from experimental day 0 to 12 for all mesocosms in the study of 2013, as the time-point of the bloom did not significantly differ between treatments (Fig. S1 a; Table 2). For the same reason**,** phytoplankton bloom in the study of 2014 was defined as the period from experimental day 3 to 13 for all mesocosms (Fig. S1 b; Table 2). Herefore, the time-point of the bloom, i.e. the time-point (day) of highest total phytoplankton C of each mesocosm, was tested for significant differences between treatments by using a gls model.
Ambient conditions were defined as treatments with a combination of cold temperature (15°C mid-summer bloom 2013, 19°C late-summer bloom 2014) and the lowest $pCO_2$ target value, i.e. 500 µatm.

## Results

Both phytoplankton blooms were nitrogen limited from the beginning on (Fig. S3 a, b). At ambient conditions total phytoplankton C was more than twice as high in the late-summer bloom experiment 2014 compared to the mid-summer bloom experiment 2013 (Fig. S1 a, b; Table S1). Under manipulated conditions, highest total phytoplankton C was found under low temperature and high $pCO_2$ in mid-summer bloom 2013 (Fig. S1 a; Table S1). In late-summer bloom 2014 total phytoplankton C was highest under high temperature and high $pCO_2$ (Fig. S1 b; Table S1). Species identities were similar in both studies, whereas species composition, i.e. species' contribution to total phytoplankton C, differed strong between both studies under ambient conditions (Fig 1; 2 a, b). The contributions of diatoms and small phytoplankton to total phytoplankton C were 20 % higher in mid-summer bloom 2013 compared to late-summer bloom 2014 at ambient conditions (Fig. 1 c, d, g, h; 2 a, b), respectively. Flagellates in contrast contributed seven times more in late-summer bloom 2014 compared to mid-summer bloom 2013 (Fig. 1 e, f; 2 a, b).

*Effects of warming and rising $pCO_2$ on species composition*

Mid-summer bloom 2013 - The contributions of cyanobacteria to total phytoplankton C during bloom were significantly higher by on average 10 % in the warm temperature treatments, but did not change with rising $pCO_2$ (Fig. 1 a; 2 a; Table S2). The contributions of diatoms to total phytoplankton C trended to decrease with warming, however, the effect was statistically not significant (Fig. 1 c; 2 a; Table S2). The contributions of flagellates and small phytoplankton to total phytoplankton C were not affected by temperature or $CO_2$ manipulations. (Fig. 1 e, g; 2 a; Table S2).

Late-summer bloom 2014 - The contributions of small phytoplankton to total phytoplankton C were significantly higher by on average 16 % in the warm temperature treatments compared to the cold ones, but were not affected by rising $pCO_2$ during bloom (Fig. 1 h; 2 b; Table S3). The contributions of flagellates to total phytoplankton C were on average 15 % lower in the warm treatments compared to the cold ones (Fig.1 f, 2 b; Table S3). Under both temperature treatments the percentages of flagellates decreased with rising $pCO_2$ (warm: p= 0.021; t= 3.67; df= 6; cold: p= 0.015; t= -4.09; df= 6; Fig. 1 f). The contributions of cyanobacteria to total phytoplankton C significantly increased with rising $pCO_2$ under both temperature treatments (warm: p= 0.001; t= 8.34; df= 6; cold: p= 0.033; t= 3.20; df= 6; Fig. 1 b, 2

b; Table S3). The contributions of diatoms to total phytoplankton C did not differ significantly between treatments (Fig. 1 d; 2 b; Table S3).

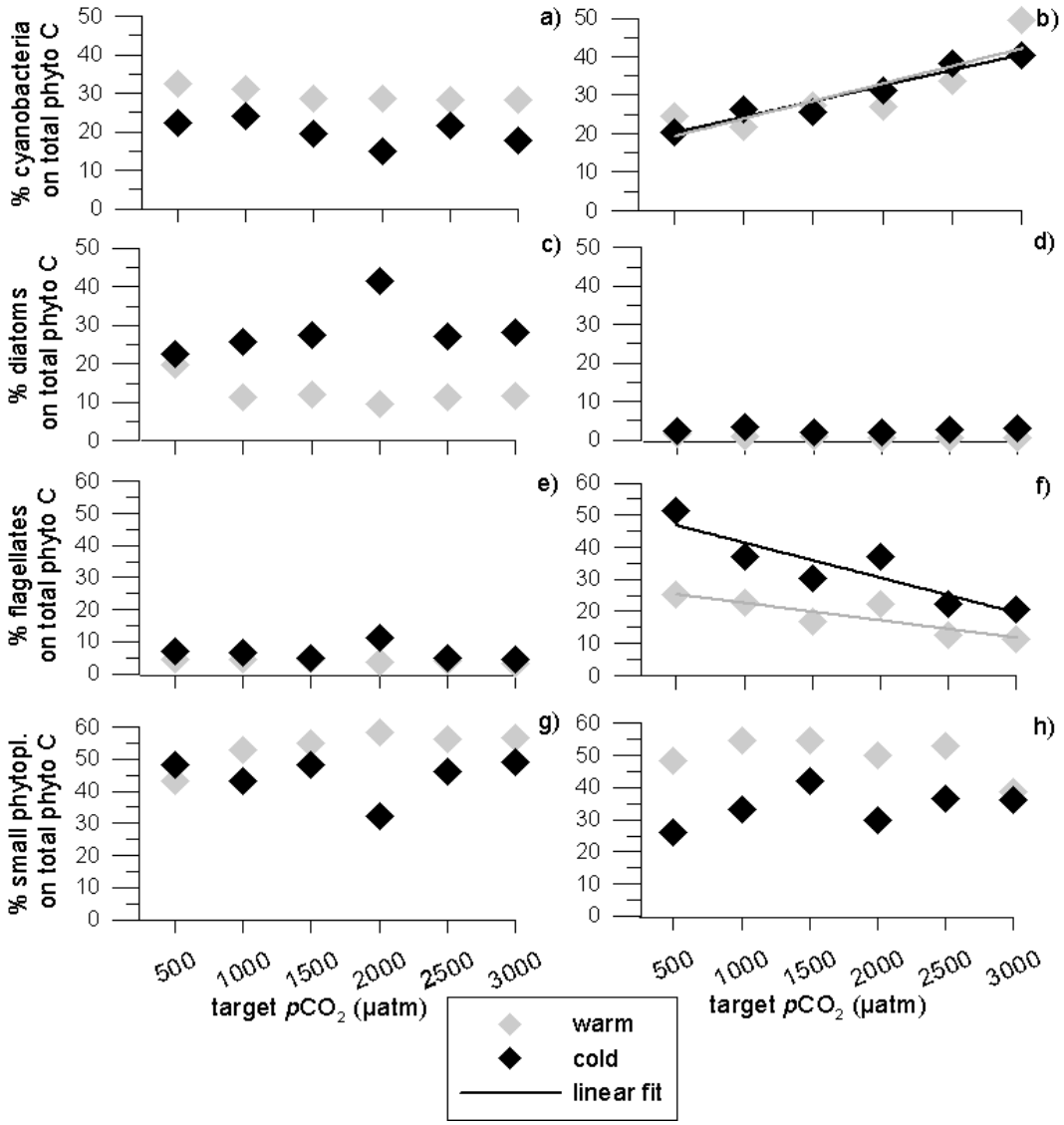


**Fig. 1.** Species composition during phytoplankton bloom: a-b) % cyanobacteria on total phytoplankton C, c-d) % diatoms on total phytoplankton C, e-f) % flagellates on total phytoplankton C, g-h) % small phytoplankton on total phytoplankton C. Diagrams on the left sight represent results of mid-summer bloom 2013, diagrams on the right sight represent results of late-summer bloom 2014. For symbol attribution to treatment combination see legend.

*Fatty acid content and composition under ambient conditions*

Under ambient conditions the content of TFA (Fig. 2 c, d; black diamonds at 500 µatm $pCO_2$) was by 11 % lower while the content of total PUFA (Fig. 3 a, b) was by 23 % higher in the mid-summer bloom 2013 compared to late-summer bloom 2014.

The fatty acid composition between the two studies also differed. The relative contribution of MUFA to TFA was by 15 % lower in mid-summer 2013 (Fig. 2 e, f). At the same time the contribution of PUFA to TFA was by 9 % higher (Fig. 2 g, h). The relative contributions of SFA were similar between studies (Fig. 2 i, k). Among the different most important species of PUFA only the contents of linolenic acid and ARA differed strong between the two studies (Fig. 3 c-k). Whereas linolenic acid content was more than twice as high in mid-summer bloom 2013 (Fig. 3 g, h), ARA was generally low in content and nearly disappeared compared to late summer bloom 2014 (Fig. 3 i, k). The contents of 18:1n9 were similar between both studies (Fig. 3 l, m).

*Effects of warming and rising $pCO_2$ on fatty acid content and composition*

Mid-summer bloom 2013 - During the phytoplankton bloom, the TFA content showed a significant interaction effect of temperature and $pCO_2$ (Fig. 2c; Table S2), leading to highest fatty acid contents under high temperature and the highest $pCO_2$ levels (warm x $CO_2$: p= 0.018; t= 3.876; df= 6; cold x $CO_2$: p= 0.947, t= 0.071, df= 6). The fatty acid composition, i.e. the relative contributions of MUFA, PUFA and SFA to TFA (Fig. 2 e, g, i; Table S2) was not affected by the treatments. The contents of total PUFA as well as the contents of the most important PUFAs EPA, DHA and linolenic acid did also not differ between treatments (Fig. 3 a, c, e, g; Table S2). The contents of ARA, instead, showed an interaction effect of warming and rising $pCO_2$ (Fig. 3 i; Table S2), leading to significantly higher ARA with rising $pCO_2$ in the warm temperature treatments (p= 0.02; t= 3.67; df= 6) but not in the cold ones (p= 0.85; t= -0.20; df= 6). The contents of 18:1n9 did not differ between treatments (Fig. 3 l; Table S2).

Late-summer bloom 2014 - Neither the TFA contents (Fig. 2 d; Tab 2), nor the contents of total PUFA (Fig. 3 b, Table S3) or the fatty acid composition (Fig. 2 f, h, k; Table S3) differed between treatments during bloom. However, treatment effects occurred for the contents of the most important essential PUFAs. The contents of EPA (Fig. 2 d; Table S3) and DHA (Fig. 3 f; Table S3) decreased significantly under warming to only half of the content of the cold treatments. Instead, linolenic acid was on average almost twice as high in the warm temperature treatments compared to the cold ones (Fig. 3 h; Table S3) and increased significantly with rising $pCO_2$ under cold temperature (warm x $CO_2$: p= 0.585; t= 0.058; df= 6; cold x $CO_2$: p= 0.018; t=

3.870; df= 6). The contents of ARA were also twice as high in the warm temperature treatments (Fig. 3 k; Table S3) compared to the cold ones. Instead, the contents of 18:1n9 were reduced to less than the half under warming (Fig. 3 m; Table S3).

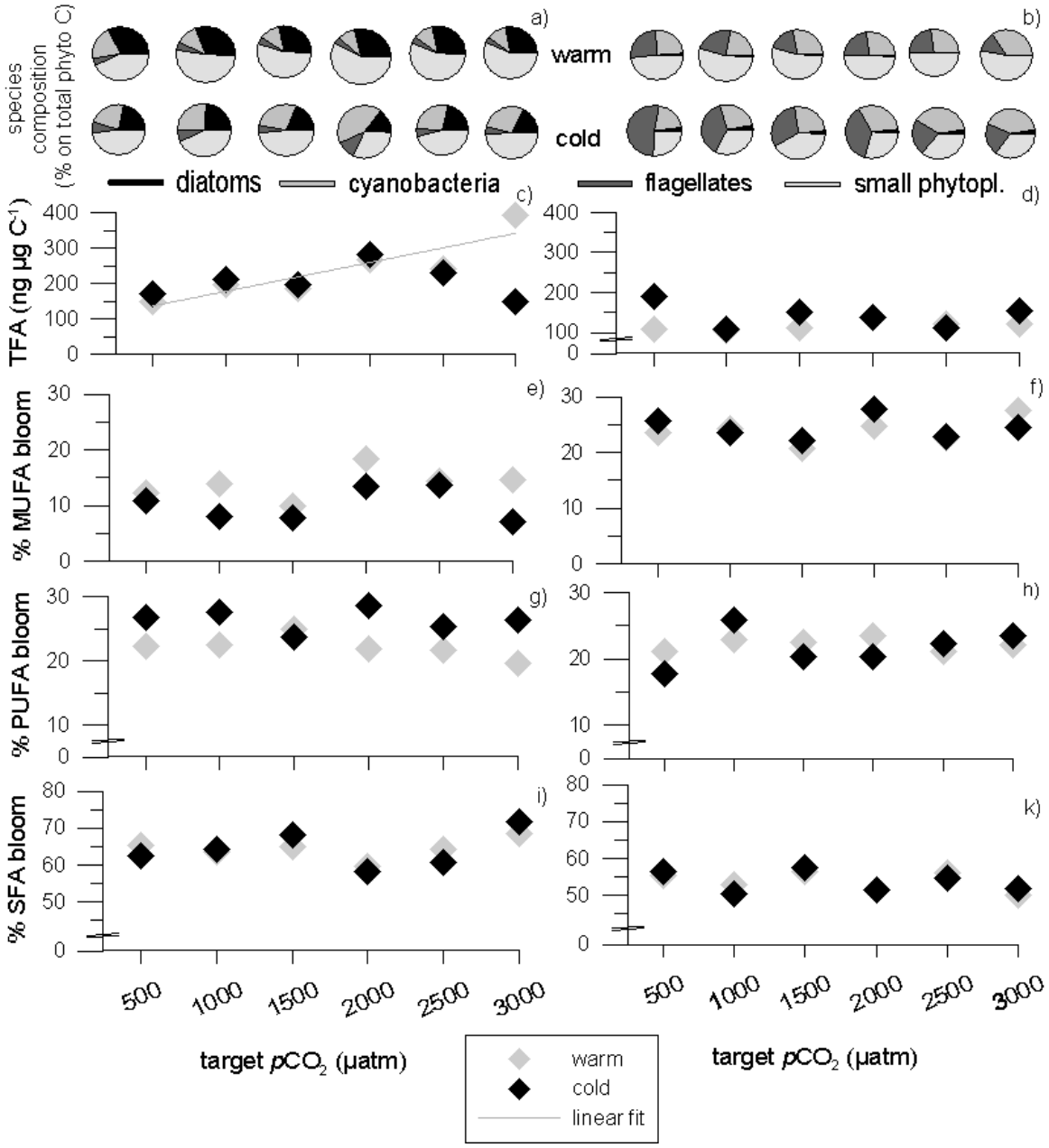


**Fig. 2.** Species composition and fatty acid composition during phytoplankton bloom: a-b) species composition (% on total phytoplankton C), c-d) total fatty acid content (TFA, ng µg $C^{-1}$), e-f) % of monounsatturated fatty acids (MUFA) on TFA, g-h) % polyounsatturated fatty acids (PUFA) on TFA, i-k) % satturated fatty acids (SFA) on TFA. Diagrams on the left side: mid-summer bloom 2013, diagrams on the right side: late-summer bloom 2014. For symbol attribution to treatment combination see legend. Cyanob. = cyanobacteria; small phytopl. = small phytoplankton.

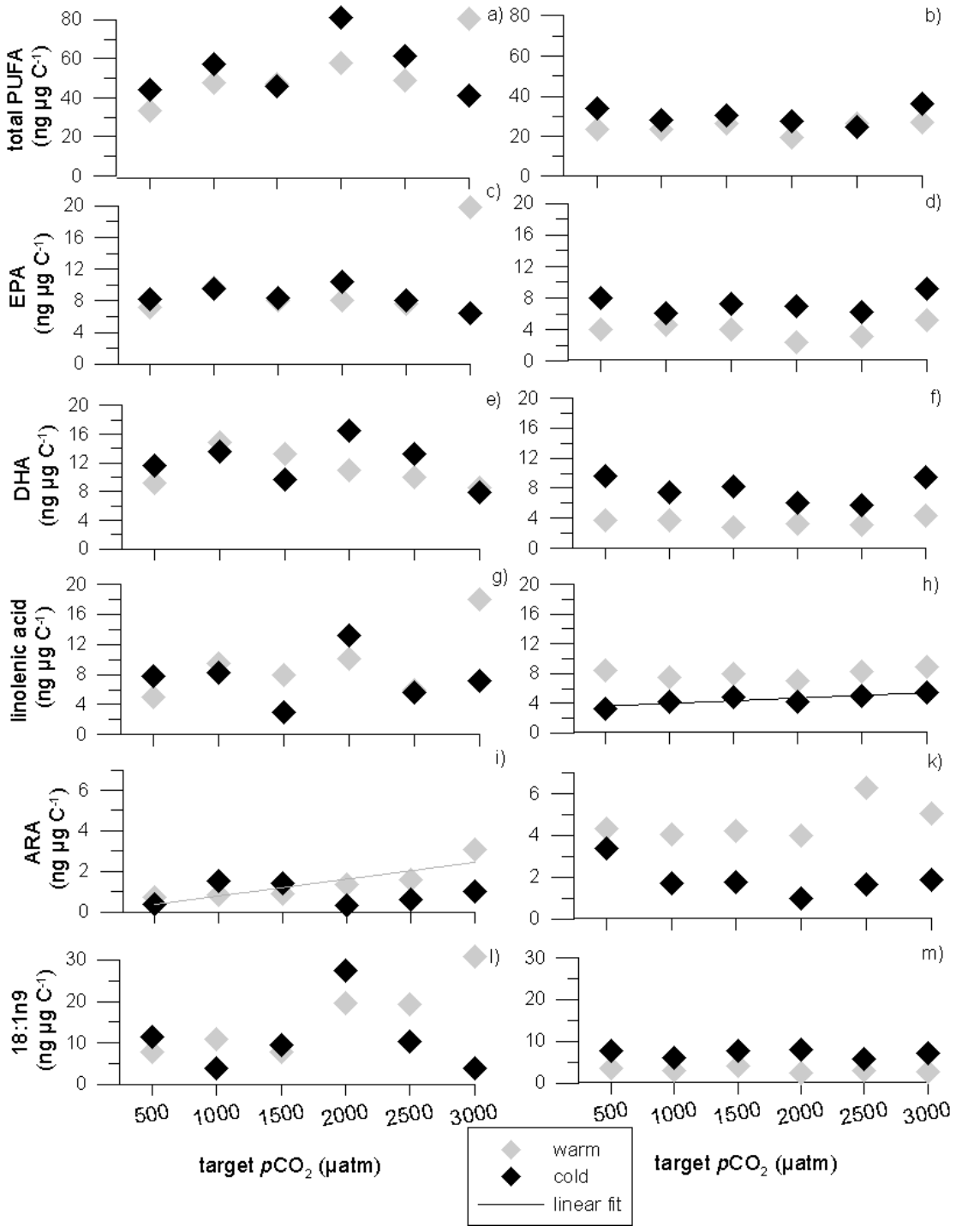


**Fig. 3**. Fatty acid contents during phytoplankton bloom period: a-b) total polyunsatturated fatty acids (PUFA, ng µg $C^{-1}$), c-d) EPA (ng µg $C^{-1}$), e-f) DHA (ng µg $C^{-1}$), g-h) linolenic acid (ng µg $C^{-1}$), i-k) ARA (ng µg $C^{-1}$) and l-m) 18:1n9 (ng µg $C^{-1}$). Diagrams on the left side: mid-summer bloom 2013, diagrams on the right side: late-summer bloom 2014. For symbol attribution to treatment combination see legend.

*Relationship between fatty acids and species composition*

**Table 1**. Eigenvectors of the Principle Component Analyses (PCA) of mid-summer bloom 2013 and late-summer bloom 2014.

| Variable | Factor 1 | Factor 2 |
|---|---|---|
| **Mid-summer bloom 2013** | | |
| EPA | -0.549 | -0.008 |
| DHA | 0.076 | -0.809 |
| Linolenic acid | -0.512 | -0.284 |
| ARA | -0.450 | 0.452 |
| 18:1n9 | -0.476 | -0.243 |
| **Late-summer bloom 2014** | | |
| EPA | 0.446 | 0.489 |
| DHA | 0.452 | 0.461 |
| Linolenic acid | -0.442 | 0.315 |
| ARA | -0.420 | 0.670 |
| 18:1n9 | 0.469 | -0.010 |

Mid-summer bloom 2013 - PCA displayed associations between fatty acids variability and phytoplankton taxonomic composition. The first two principal component factors (PC factor 1 and 2) explained 87.36 % of the total variance (factor 1: 54 %; factor 2: 23 %) (Fig. 4 a). Results showed that EPA was negatively correlated with factor 1, while DHA showed a negative correlation with factor 2 but only a slightly positive one with factor 1. Likewise, a same pattern of variability was also displayed by flagellates (Fig. 4 a; Table 1). The fatty acids EPA, ARA, 18:1n9 and linolenic acid showed strong negative correlations to factor 1, but this did not show a close distribution with cyanobacteria's and diatoms' contribution in the biplot projection (Fig. 4a; Table 1).

Late-summer bloom 2014 - The PCA factors 1 and 2 explained together 93.53 % of the variance in the data set (factor 1: 71 %; factor 2: 17 %; Fig. 4b). The fatty acids EPA, DHA and 18:1n9 displayed a strongly positive correlation with factor 1. Likewise, diatoms and flagellates showed a similar pattern (Fig. 4 b; Table 1). ARA and linolenic acid both were strongly negative correlated to factor 1 (Fig. 4 b; Table 1) displaying a similar pattern as picoplankton.

*Dissolved inorganic nutrients*

During mid-summer bloom 2013 the $NO_3^-/NO_2^-$ concentrations declined below detection limit and ammonium was also depleted by the end of bloom in all treatments (Fig. S3 a, c; Table S2). At late-summer bloom 2014 total dissolved inorganic nitrogen concentrations ($NO_3^-/NO_2^-$, $NH_4^+$) were also similar between treatments (Fig. S3 b, d; Table S3). Average bloom $PO_4^{3-}$ concentrations were similar in all treatments in mid-summer bloom 2013 (Fig S3 e; Table S2), whereas they slightly differ between temperature treatments in late-summer bloom 2014 (Fig. S 3f;

Table S3). However, phosphate was not depleted in any treatment and remained available by the end of bloom (Fig S3 e, f).

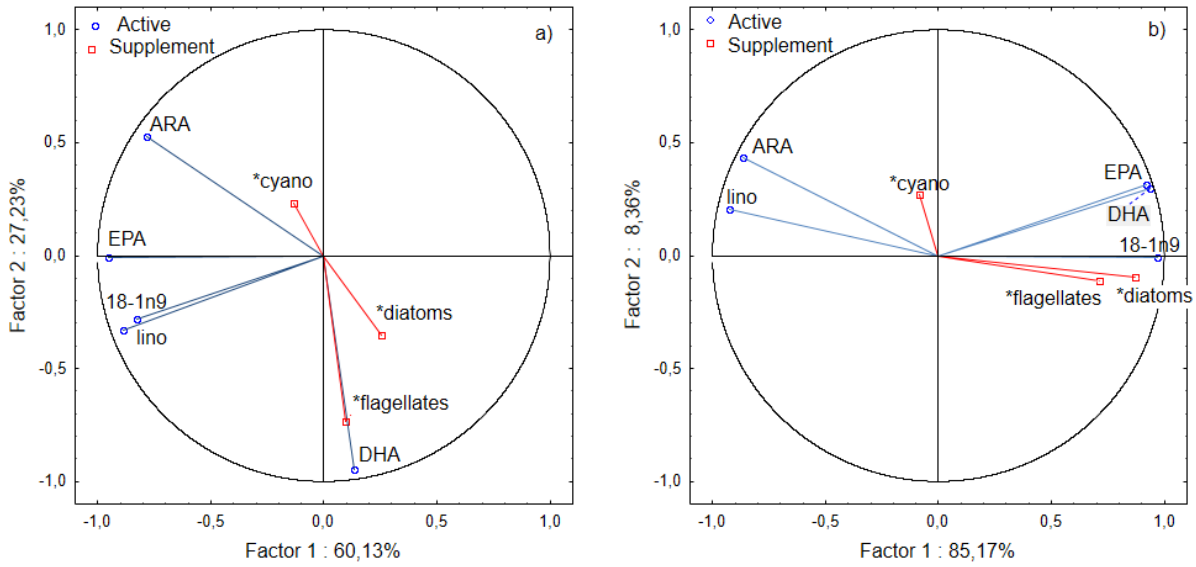


**Fig. 4.** Plots of the Principle Component Analyses (PCA): a) mid-summer bloom 2013, b) late-summer bloom 2014. lino = linolenic acid; cyano = cyanobacteria. For symbol attribution to treatment combination see legend.

## Discussion

*Fatty acids and species composition under ambient conditions*

Overall, the differences in the fatty acid composition and contents among the two studies only partly reflect the phytoplankton taxonomic composition under ambient conditions. The relatively low contribution of PUFAs under ambient conditions (~31 %, SD=18.5) in both of the studies might be due to the dominance of cyanobacteria, small phytoplankton < 5µm and flagellates, typical for Baltic Sea nutrient limited summer conditions. The higher total and relative content of PUFAs in mid-summer bloom 2013 under ambient conditions might be explained by the higher relative abundance of diatoms by 20 % compared to late-summer bloom 2014. The higher content (17 %) of flagellate marker fatty acid DHA in mid-summer bloom 2013, instead, did not match the considerably lower contribution of flagellates. The equal contents of the cyanobacteria marker 18:1n9 in both studies seem to coincide with the equal relative abundance of cyanobacteria in both studies.

*Fatty acids and species composition in response to warming and rising $pCO_2$*

The response of fatty acids to warming and $pCO_2$ could even less be explained by shifts in the taxonomic composition of the phytoplankton due to treatments in both of the studies. This contradicts a comprehensive meta-study, which identified phytoplankton taxonomic group even as three to four times stronger than different environmental factors and growth conditions to explain variation in the fatty acids (Galloway & Winder 2015). In the studies here, the observed effects in the fatty acids might be more explainable by direct physiological responses of the phytoplankton to climate change. Warming, for instance, was found to lead to a reduction in the number of the longest and the most unsaturated fatty acids when tested on single species, i.e. to a decline in PUFAs (Dodson et al. 2014). Increased $pCO_2$ has the potential to raise the carbon fixation in cells, which might lead to more carbon that can be allocated for fatty acid synthesis (Carvalho & Malcata 2005).

While particular essential were mostly affected by temperature, i.e. decreases in EPA and DHA and increases in ARA and linolenic acid, the contribution of PUFA to TFA, but also total PUFA content, remained unaffected by treatments. However, total PUFA contains other PUFA species, which were not analysed in detail but might have affected the overall response to treatments. The contribution of MUFA and SFA to TFA did also not differ between treatments in both studies. These observations contradict to the generally accepted theory that SFA increases with warming to raise the membrane melting temperature (e.g. Fuschino et al. 2011; Dodson et al. 2014), and to maintain average membrane lipid order (fluidity) by simultaneous reduction in PUFAs (Lynch & Thompson 1982; Mortensen et al. 1988).

The observed declines in EPA and DHA with warming in mid-summer bloom 2014 are in line with several studies using single species (Renaud et al. 2002; Dodson et al. 2014,), possibly overall negatively affecting food quality for higher trophic levels. In contrast, other studies using single-species observed no effects or increased contents of EPA and / or DHA with warming (e.g. Thompson et al. 1992). As already mentioned, such a decrease with warming was probably a physiological response, which led to a reduction of, at least some, of the longest and the most unsaturated fatty acids (Dodson et al. 2014). Effects of changes in taxonomic composition can be excluded for EPA, as its content never corresponded to changing diatom abundance due to temperature and vice versa. For instance, the tendency for a lower

contribution of diatoms to total phytoplankton C with warming was not at all reflected by EPA content in mid-summer bloom 2013. The decline in DHA with warming in late-summer bloom 2014 might have been a combination of physically responses of the community in the fatty acids and taxonomic composition. The DHA-rich flagellates' relative contribution decreased by only 15 % with warming, which likely not completely explains the 50 % decline of DHA.

The contents of ARA and linolenic acid doubled with warming in late-summer bloom 2014, showing a contrasting physiological response compared to the other PUFA species. A similar contrast in the response of single PUFA species was found in a meta-study by Arts et al. (2015). Here, diatoms and chlorophytes showed also only an overall increase in ARA with warming, whereas all the others tended to decrease. However, species composition cannot be used to account for increases in our study as the small phytoplankton included also other species than linolenic acid-rich pico-chlorophytes. Nevertheless, an influence cannot be totally excluded.

The strong decrease in the cyanobacteria marker fatty acid 18:1n9 seems to be also a physiological response to warming. Unfortunately our data lacks further explanations and similar studies are, to the best of our knowledge, missing. 18:1n9 was not at all correlated with the warming induced change of cyanobacteria contribution to total phytoplankton C in both studies. However, effects for the food chain seems to be minor, as cyanobacteria are not a preferred food source for higher trophic levels like copepods and overall represent low-quality food due to their minor amount of PUFAs (Brett & Müller-Navarra 1997 and references therein).

Generally our results suggest that rising $pCO_2$ has only minor effects on fatty acids in natural phytoplankton communities. Rising $pCO_2$ only significantly increased linolenic acid in the cold temperature treatments in late-summer bloom 2014, which was possibly directly physiologically induced by a raise in carbon fixation, leading to more carbon, allocated for fatty acid synthesis (Carvalho & Malcata 2005). However, this was not observed in mid-summer bloom 2013. Another study on a natural Arctic phytoplankton community (Leu et al. 2012) in contrast found significant effects of $pCO_2$ on fatty acids, in particular on PUFAs. However, these effects were indirectly caused by changes in community composition. Such a correlation can be excluded here.

Contrasting to community studies, various single-species studies (Hoshida et al. 2005; Rossoll et al. 2012; Bermudez et al. 2015) found positive and negative effects on PUFAs, which was interpreted as species-specific reactions to rising $pCO_2$. Such single-species effects, however, might be masked in communities as they in combination with physiological compensation within species could be outbalanced. This might explain the observed low response of fatty acids to $pCO_2$ in the present studies. Further, species habituation to higher $pCO_2$ levels in coastal areas with naturally strong fluctuating $CO_2$ concentrations, such as in Kiel Bight (Thomsen et al. 2010), might also explain the overall low response in fatty acids.

*Nutrients*

Nutrient availability is also known to affect fatty acid contents, as nutrient deplete situations have been observed to increase the contents of SFAs (Brett & Mueller-Navarra 1997) and total fatty acid by the need of carbon acquisition in form of lipids under suboptimal conditions (Thompson 1996; Malzahn et al. 2007; Steinhoff et al. 2014). As typical for the Baltic Sea and other seasonal stratified areas in summer, nitrogen was limited in both studies (Sørensen & Sahlsten 1987; Kratzer & Sørensen 2011,). Nitrogen limitation might have in total influenced fatty acids, but cannot be directly related to the observed treatment effects on fatty acids as dissolved inorganic nitrogen ($NO_3^-$, $NO_2^-$, $NH_4^+$) did not significantly differ between treatments (Fig. S3 a-d; Table 1, Table 2). Phosphate was available in all treatment of both studies during bloom, assuming low influence on fatty acid accumulation (Fig. S3 3,f).

## Conclusion

As one of the first studies we analyzed the fatty acids in two natural phytoplankton communities under combined future climate change scenarios. Contrasting to the general assumption (de Carvalho & Caramujo 2014), our results showed that the effects of climate change on fatty acids overall did not correlate with changes in the taxonomic composition of natural communities. However, the overall response of the taxonomical composition to climate change was possibly too weak for a visible correlation. Further, communities might in total outbalance most of the single-species effects, which in combination with physiological compensation within species might explain the observed low response of fatty acids to changing environmental conditions in natural communities. Nevertheless, warming might have the potential to

affect the fatty acid content, especially in terms of particular PUFAs, possibly leading to changes in the food quality for higher trophic levels. However, temperature effects seem to be complex and to variate strongly between studies, impeding general future predictions. Rising $pCO_2$ affected fatty acids in the phytoplankton communities in this study only minor, suggesting no change in food quality for higher trophic levels.

# Conclusions and outlook

Overall, the results of this study suggest that warming affects natural phytoplankton communities from the Baltic Sea stronger than rising $pCO_2$. Further, the results underline the importance of a combined analysis of different trophic levels in the plankton system, as warming showed the potential to change trophic relations in the pelagic system. This might lead to fundamental consequences for the biogeochemical cycles and the energy transfer to higher trophic systems, because the highly productive phytoplankton form the base of the food web in the oceans (Sommer et al. 2012b).

This thesis contains results of experimental studies testing the effects of simultaneously rising temperature and increasing $pCO_2$ on natural plankton communities with emphasis on phytoplankton under different seasonal bloom scenarios (chapters I-III). Hitherto, experiments based on communities under combined rising temperature and $pCO_2$ are still scarce and mainly considered the effects on the phytoplankton spring blooms. The responses of natural autumn and summer communities to combined future climate change in contrast, were unknown. Especially in natural seasonal stratified systems such as the Baltic Sea, the annual cycle of phytoplankton bloom events responds to various environmental factors (e.g. temperature, light and nutrient supply), which lead to the characteristic differences in phytoplankton species composition.

In chapter I, I showed that warming changes the temporal cycle of a phytoplankton bloom. Consistent with studies from the same geographical region investigating temperature effects on spring blooms (Sommer & Lengfellner 2008; Sommer & Lewandowska 2011), warming led to an earlier autumn bloom time. This indicates a possible mismatch in predator - prey relationships in seasonal bloom events, which would have fundamental consequences for the transfer of energy and organic matter between trophic levels. This is relevant as, especially in the Baltic Sea, the autumn bloom provides most of the energy for the overwintering zooplankton.

Further, my results (chapters I-II) largely confirm the expectation that warming has the potential to strengthen zooplankton grazing (Lewandowska et al. 2014) because rising temperature is known to more strongly enhance heterotrophic than autotrophic processes (O'Connor et al. 2009). In chapter II, I could even provide evidence for the patterns described in chapter I, showing that the decrease in phytoplankton biomass under warming was induced by enhanced top-down control of zooplankton

copepods. These results evidence that direct warming effects on phytoplankton, due to an overall enhanced metabolism (Brown et al. 2004), can be overruled by strong indirect effects of warming like enhanced grazing pressure. Such warming-induced intensified consumer control might strengthen the overall trophic cascade at all levels. Therefore, in natural communities, intensified top-down control on zooplankton grazers could potentially result in higher phytoplankton biomass (O'Connor et al. 2009). However, experiments including top-predators like fish are still rare and were also lacking in these experiments. Broader studies are needed to get deeper insights on the effects of warming on trophic cascades in aquatic ecosystems.

In chapter II, I was furthermore able to provide the first evidence that warming has the potential to switch a phytoplankton community from a bottom-up controlled system (via nutrient supply) to a top-down controlled one. Therefore, a previously suggested discrimination of responses to rising temperature between nutrient replete and deplete conditions (Lewandowska et al. 2014) is not necessarily clear-cut. Moreover, the reaction of nutrient deplete systems to temperature seems to depend strongly on the prevailing composition of the phytoplankton community. My results indicate that the key for the respective response to warming is not so much the nutrient concentration, but rather the proportion of phytoplankton species, which are edible for grazers, e.g. diatoms. This strongly impedes general predictions for the response of system like oligotrophic open oceans and seasonally stratified areas to global warming.

The chemical composition of phytoplankton in terms of stoichiometry and fatty acids were only marginally affected by warming (chapters I-III). Overall, phytoplankton community stoichiometry did not indicate changes in food quality for higher trophic levels (chapters I-II). The differences in the temperature response of fatty acid contents found in my study (chapter III) did not correlate with changes in the taxonomic composition of the phytoplankton communities, as assumed by Carvalho & Carmujo (2014). This leads me to the suggestion that under future climate change such relations might be less clear compared to ambient conditions. Additionally, the physiological responses of phytoplankton communities to environmental change and growth conditions seem to be highly variable and might be unpredictable by single-species effects. In that way, my results lead to the suggestion that experimental results on fatty acids in communities might be not necessarily transmittable to other

phytoplankton community studies. Moreover, it poses concerns on the further use of fatty acid composition and especially PUFA contents of communities to draw conclusions for higher trophic levels in response to climate change. However, subsequent community studies should test these conclusions in more detail.

The prospected doubling of $pCO_2$ levels by the year 2100 (IPCC 2014) has been shown to overall affect life in marine environments (Kroeker et al. 2012, 2013). For non-calcified phytoplankton species, rising $pCO_2$ is suggested to act as a fertilizer as the increased concentration in carbon ions might for instance reduce the energetic costs for their carbon concentrating mechanisms (CCM). Overall, I observed only minor indications for a direct response to rising $pCO_2$ in phytoplankton biomass and phytoplankton's chemical composition (chapter I-III). At least partly, I suggest that the history of exposure to the site-specific high variable $pCO_2$ levels in Kiel Bight might have already led to an adaptation to higher $pCO_2$ levels. Following Litchman et al. (2015), short generation times, high abundances and small sizes allows the phytoplankton to adapt to changing conditions evolutionary. Nevertheless, previous studies using natural phytoplankton communities of the Antarctic (Tortell et al. 2008) and the Northern Atlantic Ocean (Eggers et al. 2014) observed weak responses to rising $pCO_2$ up to 1000 µatm; such responses were mainly driven by changes in species composition. However, as my studies additionally simulated temperature increase and included higher trophic levels, my observations indicate that the warming-induced higher grazing pressure potentially has masked the generally hypothesized profits of the phytoplankton by $CO_2$. In this case it would explain the overall weak apparent response to $CO_2$ in phytoplankton biomass in chapter I and II. As I already suggested above, indirect temperature effects seem to be able to dominate and control the pelagic system more strongly under climate change. Unfortunately, with the experimental set up used here it is not possible to directly examine the interactive effects of warming and grazing. Future studies should test the effects of warming on natural phytoplankton communities by separately including and excluding zooplankton grazers.

Overall I like to point out that the results of this study corroborate the importance of future research on natural communities with focus on the combined analyses of different trophic levels under multiple climate change factors. Besides warming and

rising $pCO_2$ future climate change will go along with further factors like changes in light availability, which is suggested to influence the observed effects in an additional way. The magnitude and size of these interaction effects on ocean's plankton communities are to a large extent still unclear.

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# Supplement

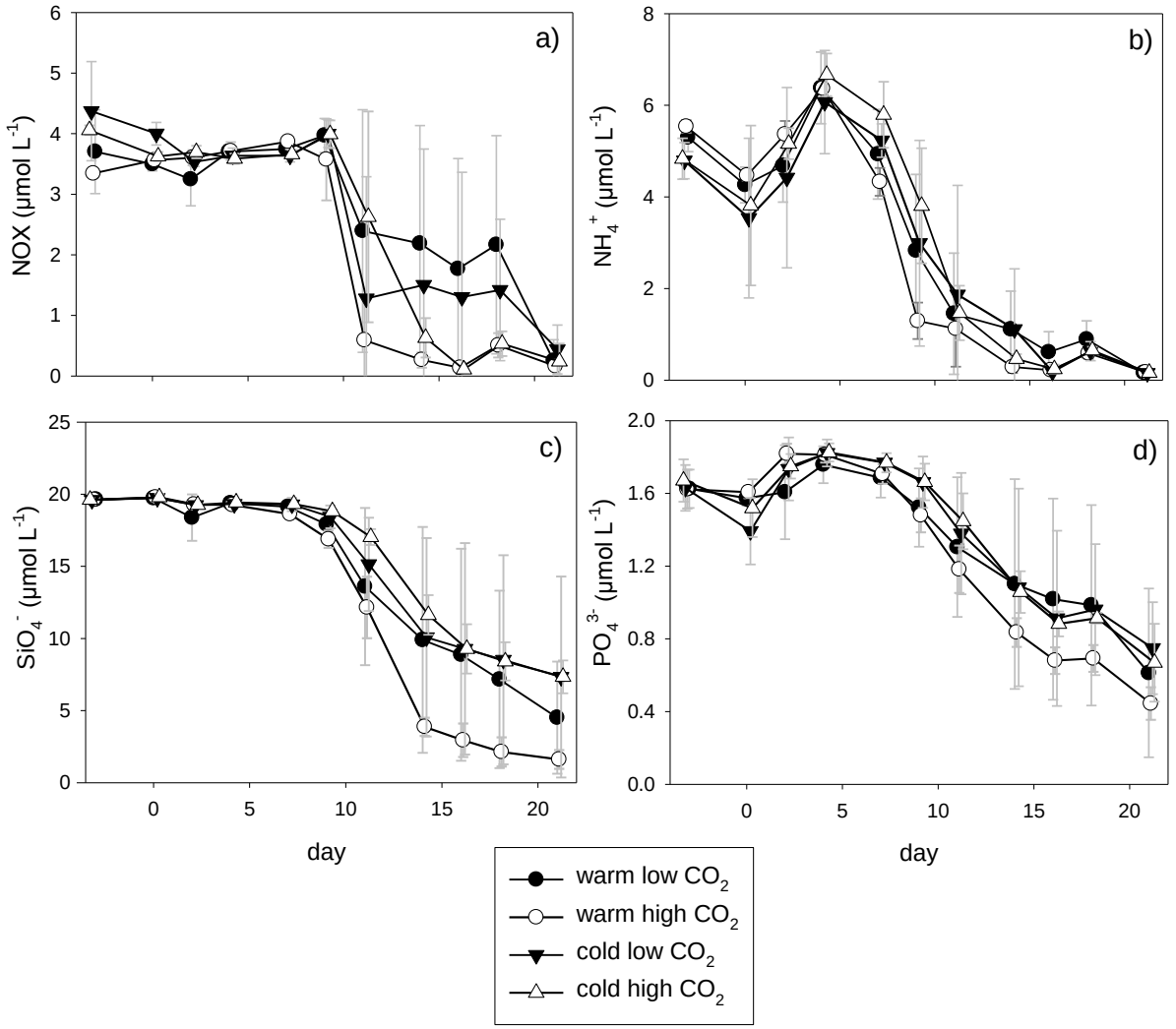
## Chapter I

**I - Table S1.** Measured DIC in µmol $kg^{-1}$ in the mesocosms M1- M12 from day -3 to 21.

| | Warm low $CO_2$ | | | Warm high $CO_2$ | | | Cold low $CO_2$ | | | Cold high $CO_2$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| day | M1 | M2 | M6 | M3 | M4 | M5 | M9 | M10 | M11 | M7 | M8 | M12 |
| -3 | 2051 | 2047 | | | | | | | 2048 | | | 2047 |
| 0 | 2035 | 2035 | 2025 | 2052 | 2079 | 2087 | 2047 | 2042 | 2045 | 2065 | 2091 | 2043 |
| 2 | 2007 | 2023 | 2019 | 2047 | 2072 | 2083 | 2038 | 2045 | 2046 | 2072 | 2092 | 2056 |
| 7 | 1995 | 1997 | 1981 | 2074 | 2107 | 2100 | 2090 | 2040 | 2030 | 2061 | 2127 | 2136 |
| 11 | 1927 | 1896 | 1921 | 2065 | 2051 | 2064 | 2028 | 1903 | 1929 | 2081 | 2134 | 2091 |
| 14 | 1969 | 1900 | 1954 | 2054 | 2049 | 2050 | 2032 | 1898 | 1935 | 2078 | 2117 | 2071 |
| 16 | 1969 | 1888 | 1943 | 2025 | 2028 | 2049 | 1994 | 1895 | 1925 | 2051 | 2101 | 2013 |
| 18 | 1927 | 1864 | 1951 | 2071 | 2089 | 2094 | 1977 | 1873 | 1901 | 2092 | 2120 | 2088 |
| 21 | 1903 | 1841 | 1922 | 2012 | 2020 | 2052 | 1964 | 1905 | 1915 | 2062 | 2084 | 2065 |

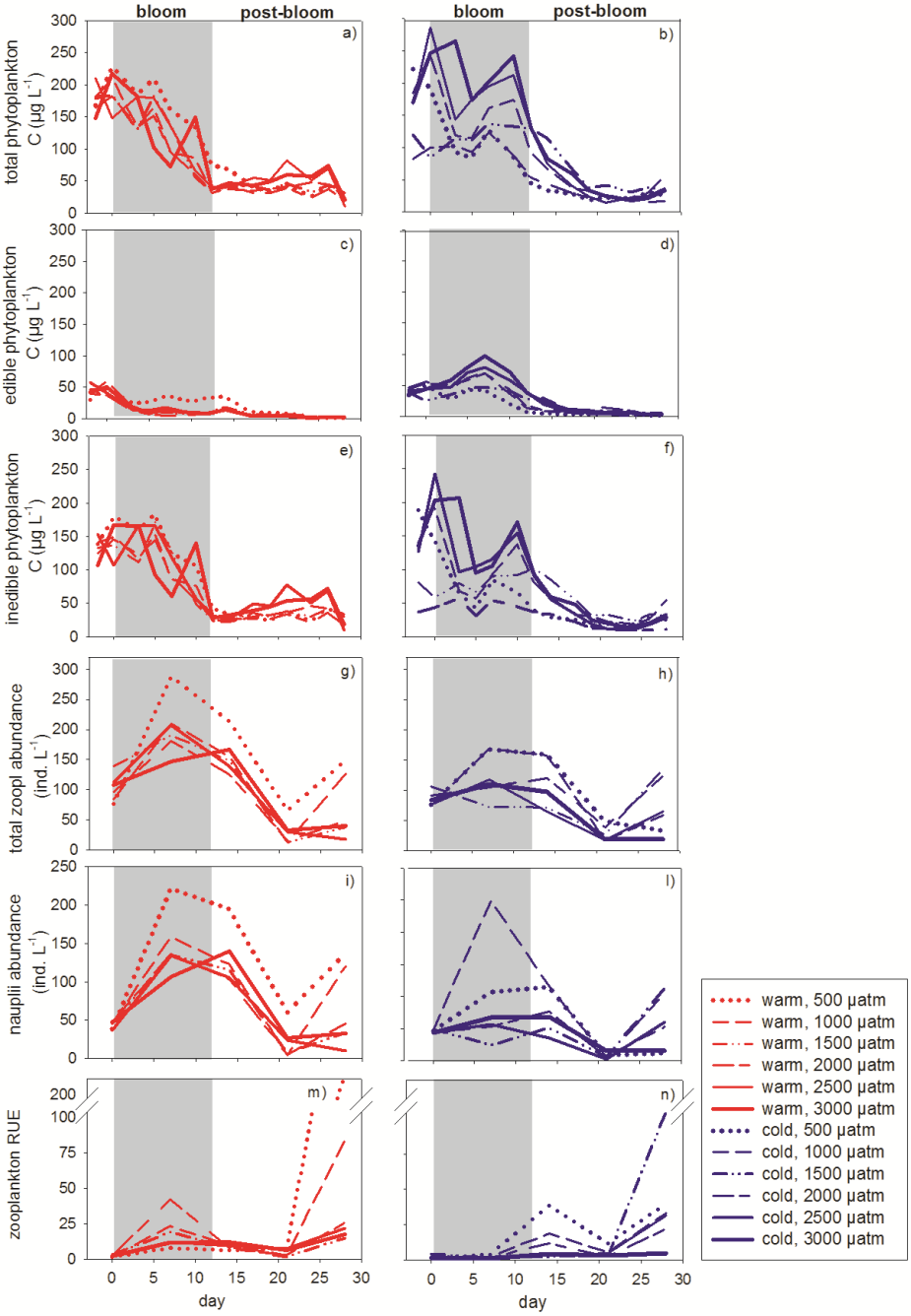
**I - Table S2.** Measured total alkalinity in µmol $kg^{-1}$ in the mesocosms M1- M12 from day -3 to 21.

| | Warm low $CO_2$ | | | Warm high $CO_2$ | | | Cold low $CO_2$ | | | Cold high $CO_2$ | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| day | M1 | M2 | M6 | M3 | M4 | M5 | M9 | M10 | M11 | M7 | M8 | M12 |
| -3 | 2100.4 | 2099.7 | | | | | | | 2096.9 | | | 2097.3 |
| 2 | 2087.3 | 2081.6 | 2083.3 | 2082.9 | 2085.5 | 2084.6 | 2088.4 | 2086.5 | 2085.7 | 2089.1 | 2087.1 | 2083.9 |
| 7 | 2092.4 | 2085.9 | 2087.4 | 2083.0 | 2084.9 | 2085.0 | 2087.3 | 2087.5 | 2088.0 | 2085.3 | 2086.4 | 2086.9 |
| 9 | 2089.2 | 2086.6 | 2086.3 | 2084.1 | 2091.3 | 2086.9 | 2089.3 | 2089.1 | 2089.9 | 2087.8 | 2085.5 | 2088.9 |
| 16 | 2093.0 | 2091.9 | 2082.1 | 2090.6 | 2090.8 | 2089.2 | 2084.2 | 2089.3 | 2091.5 | 2093.2 | 2092.1 | 2093.9 |
| 21 | 2095.9 | 2090.1 | 2080.9 | 2088.7 | 2085.8 | 2083.0 | 2092.7 | 2095.8 | 2092.5 | 2096.3 | 2096.2 | 2091.2 |

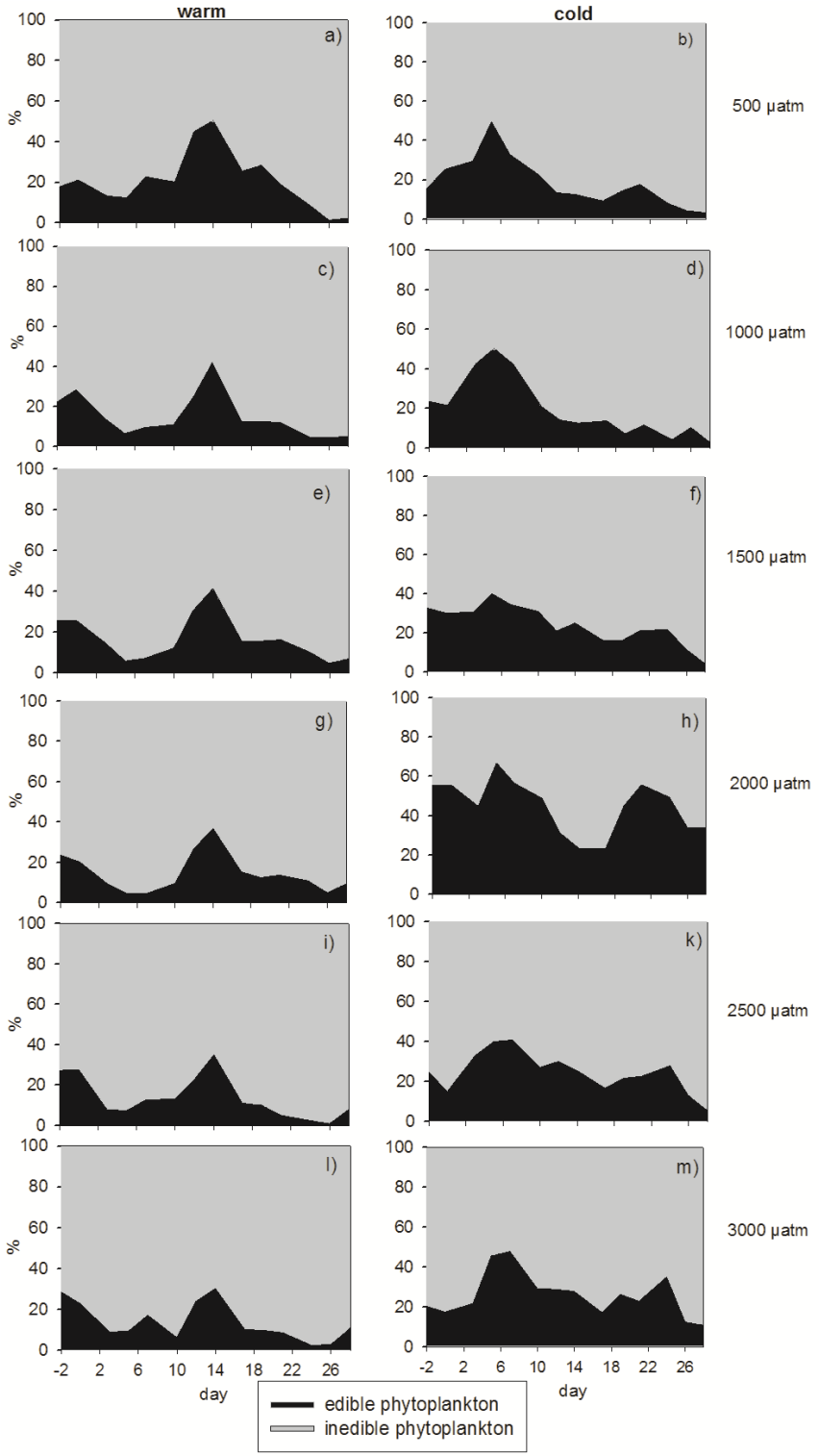



**I - Fig. S1.** Time course of the dissolved inorganic nutrients a) nitrate/nitrite ($NO_3^-/NO_2^-$ (NOX), µmol $L^{-1}$), b) ammonium ($NH_4^+$, µmol $L^{-1}$), c) silicate ($SiO_4^-$, µmol $L^{-1}$), d) phosphate ($PO_4^{3-}$, µmol $L^{-1}$). Vertical error bars denote standard error from triplicate samples. For symbol attribution to treatment combination see legend. The peaks of the phytoplankton blooms occurred between day 12 and 18.

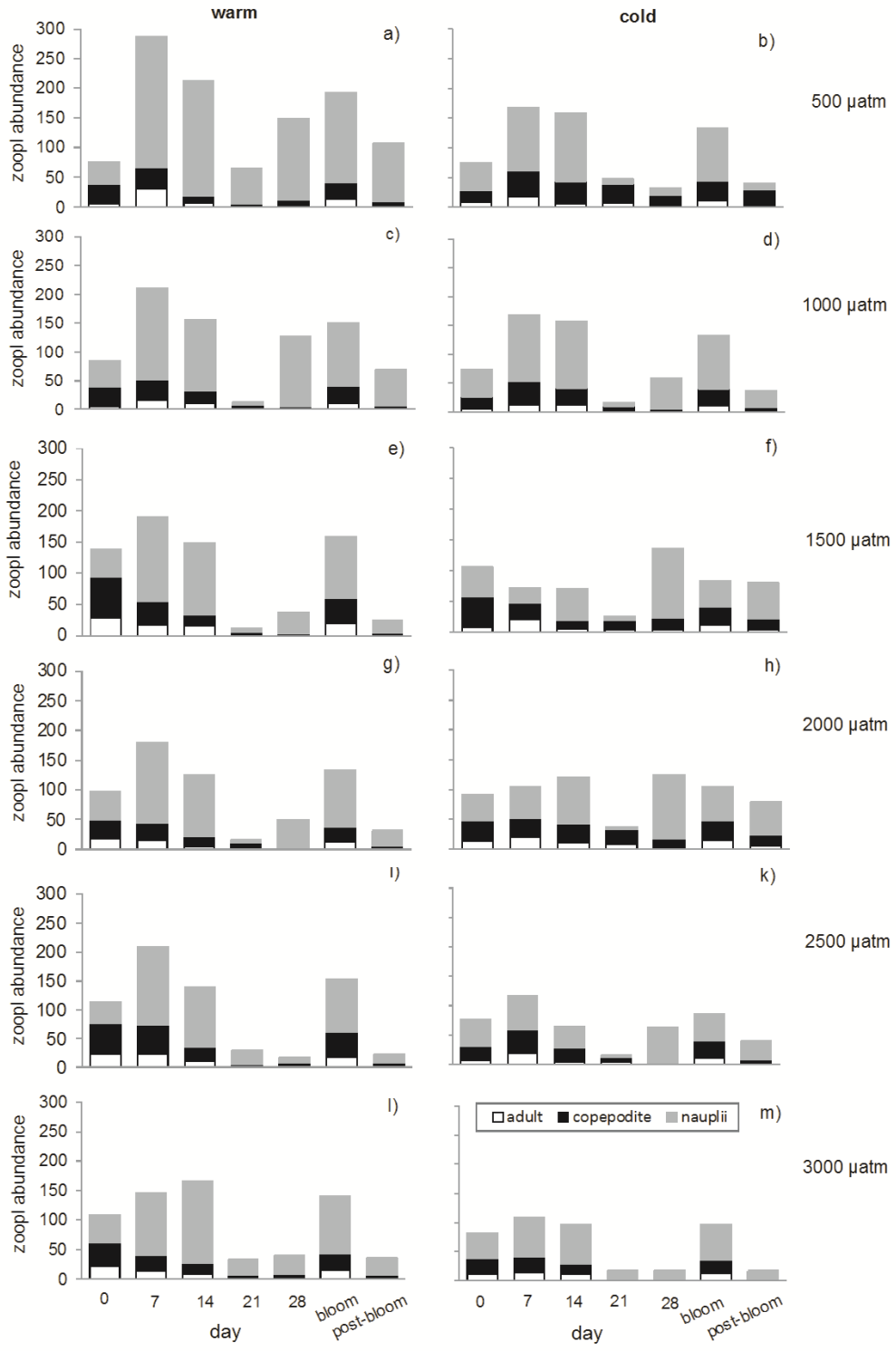
## Chapter II



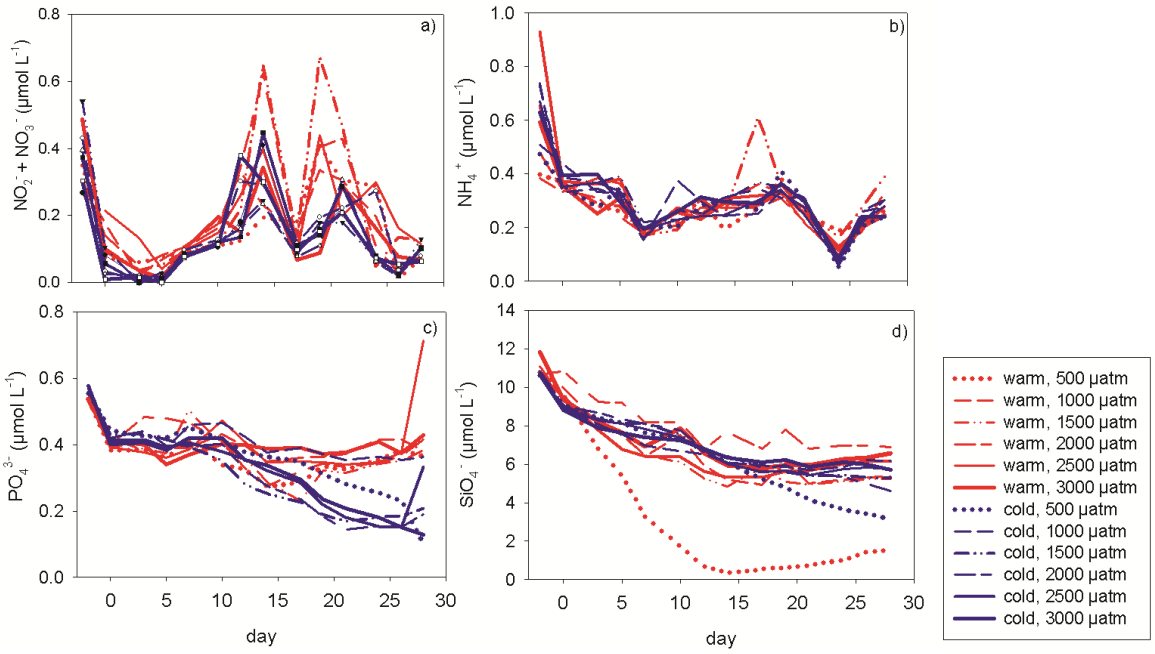

**II - Fig. S1.** Time-course, here in detail separated after temperature treatments, of: a-b) total phytoplankton C, c-d) edible phytoplankton C, e-f) inedible phytoplankton C, g-h) total zooplankton abundance, i-l) nauplii abundance, m-n) zooplankton resource use efficiency (zooplankton RUE). For symbol attribution to treatment combination (temperature treatment, $pCO_2$ target value in µatm) see legend.



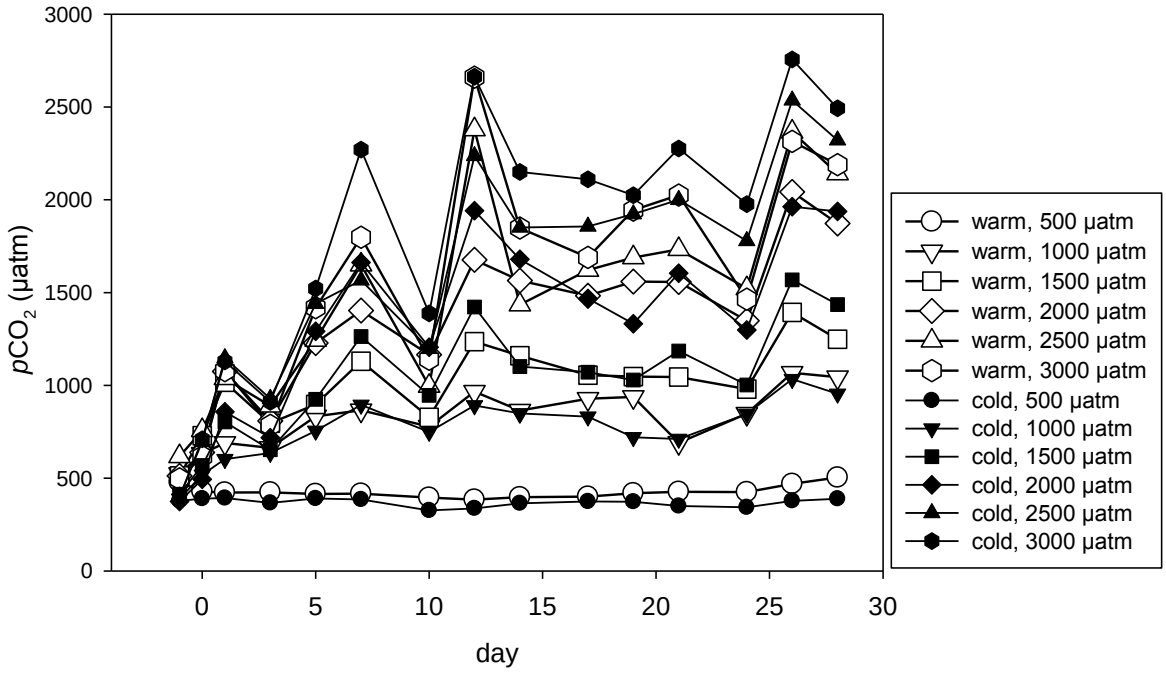

**II - Fig. S2.** Percentage (%) of edible and inedible phytoplankton carbon on total phytoplankton carbon for the treatment combinations (temperature treatment, $pCO_2$ target value): a) warm, 500 µatm; b) cold, 500 µatm; c) warm, 1000 µatm; d) cold, 1000µatm; e) warm, 1500 µatm; f) cold, 1500 µatm; g) warm, 2000 µatm; h) cold, 2000 µatm; i) warm, 2500 µatm; k) cold, 2500 µatm; l) warm, 3000 µatm; m) cold, 3000 µatm. Edible phytoplankton: black; inedible phytoplankton: grey color.



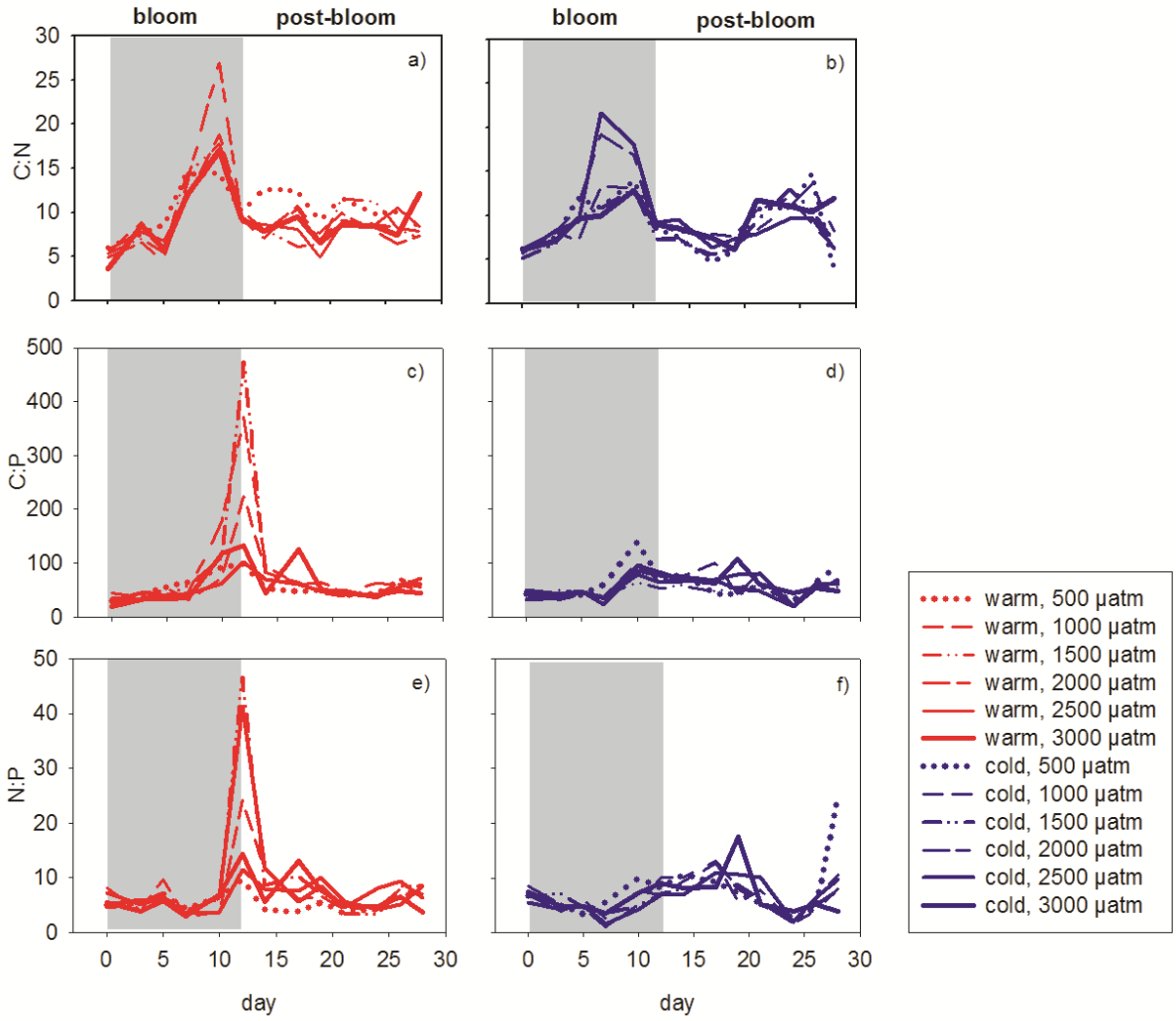

**II - Fig. S3.** Zooplankton abundance (adult, copepodite, nauplii stage) separated for each sample day, and the average abundance during phytoplankton bloom and post-bloom period for the treatment combinations (temperature, $pCO_2$ target value): a) warm, 500 µatm; b) cold, 500 µatm; c) warm, 1000 µatm; d) cold, 1000µatm; e) warm, 1500 µatm; f) cold, 1500 µatm; g) warm, 2000 µatm; h) cold, 2000 µatm; i) warm, 2500 µatm; k) cold, 2500 µatm; l) warm, 3000 µatm; m) cold, 3000 µatm. For symbol attribution to zooplankton stage see legend.




**II - Fig. S4.** Time-course of nutrient concentrations (µmol $L^{-1}$) of: a) nitrate ($NO_3^-$) and nitrite ($NO_2^-$); b) ammonium ($NH_4^+$); c) phosphorus ($PO_4^{3-}$); d) silicate ($SiO_4^-$). For symbol attribution to treatment combination (temperature treatment, $pCO_2$ target value in µatm) see legend.




**II - Fig. S5.** Time course of $pCO_2$ (µatm) for each of the replicated mesoscosms. For symbol attribution to treatment combination (temperature treatment, $pCO_2$ target value in µatm) see legend.




**II - Fig. S6**. Time-course, here in detail separated after temperature treatments of: a-b) C:N (mol:mol), c-d) C:P (mol:mol), e-f) N:P (mol:mol). For symbol attribution to treatment combination (temperature treatment, $pCO_2$ target value in µatm) see legend.

**II - Table S1.** Results of generalized least squares models (gls) testing for the effects of temperature (T), $pCO_2$, time as well as the interaction of temperature and $pCO_2$ (T x $CO_2$), time and temperature (time x T) and time and $pCO_2$ (time x $CO_2$) over the course of time on: total phytoplankton C, edible phytoplankton C, inedible phytoplankton C, total zooplankton abundance, nauplii abundance, zooplankton resource use efficiency (zooplankton RUE), C:N, C:P, N:P. Significant results are in **bold**. *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$.

| Response variable | factor | df residual | t-value | *p* |
|---|---|---|---|---|
| Total phytoplankton C ($\mu g\ L^{-1}$) | T | 160 | 2.629 | **<0.01**** |
| | $CO_2$ | 160 | 3.102 | **<0,01**** |
| | time | 160 | -3.970 | **<0,001***** |
| | T x $CO_2$ | 160 | -3.084 | **<0.01**** |
| | time x T | 160 | -1.739 | 0.072 |
| | time x $CO_2$ | 160 | -1.840 | **0.056*** |
| | time x T x $CO_2$ | 160 | 2.145 | **0.027*** |
| (Log) edible phytoplankton C C ($\mu g\ L^{-1}$) | T | 160 | -0.142 | 0.886 |
| | $CO_2$ | 160 | 1.396 | 0.164 |
| | time | 160 | -9.618 | **<0.001***** |
| | T x $CO_2$ | 160 | -2.099 | **0.037*** |
| | time x T | 160 | 1.466 | 0.144 |
| | time x $CO_2$ | 160 | 1.861 | 0.064 |
| | time x T x $CO_2$ | 160 | -1.034 | 0.302 |
| (Log) inedible phytoplankton C ($\mu g\ L^{-1}$) | T | 160 | 1.921 | **0.056*** |
| | $CO_2$ | 160 | 1.853 | 0.065 |
| | time | 160 | -3.536 | **<0.001***** |
| | T x $CO_2$ | 160 | -1.702 | 0.090 |
| | time x T | 160 | -1.344 | 0.180 |
| | time x $CO_2$ | 160 | -1.696 | 0.091 |
| | time x T x $CO_2$ | 160 | 1.854 | 0.065 |
| (Sqrt) total zooplankton abundance (ind. $L^{-1}$) | T | 52 | 0.349 | 0.750 |
| | $CO_2$ | 52 | -0.510 | 0.612 |
| | time | 52 | -0.818 | 0.416 |
| | T x $CO_2$ | 52 | 0.522 | 0.603 |
| | time x T | 52 | 0.292 | 0.771 |
| | time x $CO_2$ | 52 | -0.151 | 0.880 |
| | time x T x $CO_2$ | 52 | -0.840 | 0.404 |
| Nauplii abundance (ind. $L^{-1}$) | T | 52 | 0.529 | 0.555 |
| | $CO_2$ | 52 | -0.797 | 0.428 |
| | time | 52 | -0.578 | 0.559 |
| | T x $CO_2$ | 52 | 0.230 | 0.818 |
| | time x T | 52 | 0.567 | 0.573 |
| | time x $CO_2$ | 52 | 0.377 | 0.707 |
| | time x T x $CO_2$ | 52 | -0.917 | 0.363 |
| (Sqrt) adult abundance (ind. $L^{-1}$) | T | 52 | 0.175 | 0.861 |
| | $CO_2$ | 52 | 0.607 | 0.546 |
| | time | 52 | -1.897 | 0.063 |
| | T x $CO_2$ | 52 | 0.578 | 0.565 |
| | time x T | 52 | -0.309 | 0.758 |
| | time x $CO_2$ | 52 | -0.727 | 0.470 |
| | time x T x $CO_2$ | 52 | -0.546 | 0.587 |
| Copepodite abundance (ind. $L^{-1}$) | T | 52 | 0.220 | 0.826 |
| | $CO_2$ | 52 | -0.261 | 0.794 |
| | time | 52 | -0.922 | 0.361 |
| | T x $CO_2$ | 52 | 0.816 | 0.418 |
| | time x T | 52 | -1.747 | 0.086 |
| | time x $CO_2$ | 52 | -1.341 | 0.185 |
| | time x T x $CO_2$ | 52 | 0.449 | 0.654 |

| Response variable | factor | df residual | t-value | *p* |
|---|---|---|---|---|
| (Log) zooplankton RUE | T | 52 | -0.036 | 0.971 |
| | $CO_2$ | 52 | -0.527 | 0.599 |
| | time | 52 | 3.263 | **<0.01**** |
| | T x $CO_2$ | 52 | 1.128 | 0.264 |
| | time x T | 52 | -0.198 | 0.843 |
| | time x $CO_2$ | 52 | -1.044 | 0.301 |
| | time x T x $CO_2$ | 52 | -0.353 | 0.725 |
| C:N | T | 148 | 0.245 | 0.806 |
| | $CO_2$ | 148 | 0.392 | 0.695 |
| | time | 148 | -0.413 | 0.679 |
| | T x $CO_2$ | 148 | -0.625 | 0.532 |
| | time x T | 148 | -0.420 | 0.674 |
| | time x $CO_2$ | 148 | 0.302 | 0.762 |
| | time x T x $CO_2$ | 148 | -0.122 | 0.902 |
| C:P | T | 148 | 0.542 | 0.588 |
| | $CO_2$ | 148 | 0.253 | 0.800 |
| | time | 148 | 0.307 | 0.758 |
| | T x $CO_2$ | 148 | -0.489 | 0.624 |
| | time x T | 148 | -0.215 | 0.830 |
| | time x $CO_2$ | 148 | -0.157 | 0.874 |
| | time x T x $CO_2$ | 148 | 0.349 | 0.727 |
| N:P | T | 148 | 0.327 | 0.743 |
| | $CO_2$ | 148 | -0.025 | 0.979 |
| | time | 148 | 0.543 | 0.587 |
| | T x $CO_2$ | 148 | -0.035 | 0.971 |
| | time x T | 148 | -0.464 | 0.642 |
| | time x $CO_2$ | 148 | -0.337 | 0.736 |
| | time x T x $CO_2$ | 148 | 0.392 | 0.694 |

**II - Table S2.** Results of generalized least squares models (gls) testing for the effects of temperature (T), $pCO_2$ as well as the interaction of temperature and $pCO_2$ (T x $CO_2$) during bloom and post-bloom on: time-point of the bloom, total phytoplankton carbon C, edible phytoplankton C, inedible phytoplankton C, total zooplankton abundance, nauplii abundance, zooplankton resource use efficiency (zooplankton RUE), $NO_3^-$+$NO_2^-$, $NH_4^+$, $PO_4^{3-}$, $SiO_4^-$, C:N, C:P, N:P. Significant results are in **bold**. *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$.

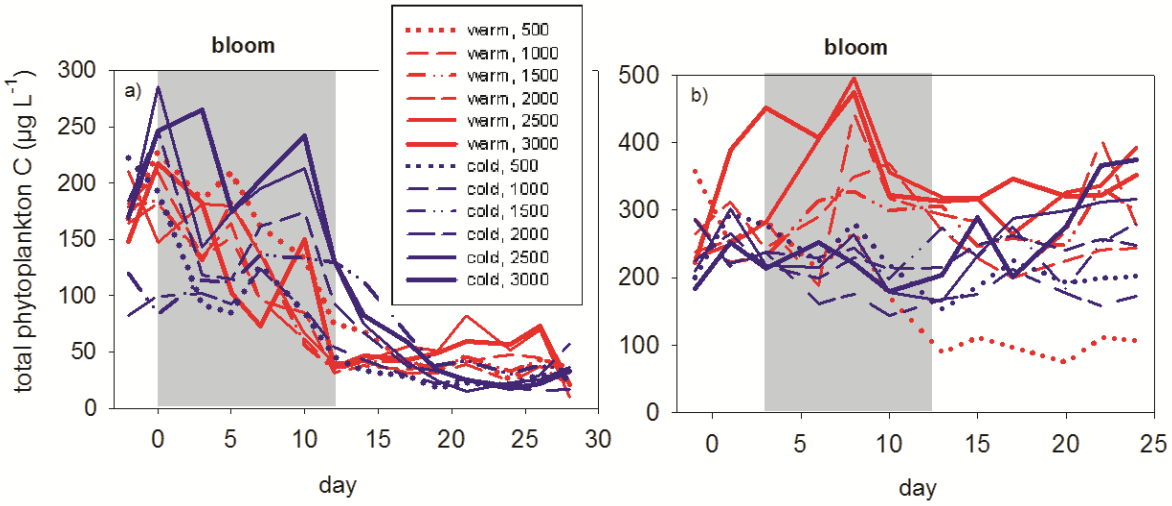
| Response variable | factor | df residual | t-value | *p* |
|---|---|---|---|---|
| **Bloom** | | | | |
| Time-point of the bloom | T | 8 | -1.349 | 0.214 |
| | $CO_2$ | 8 | -1.249 | 0.246 |
| | T x $CO_2$ | 8 | 0.177 | 0.864 |
| Total phytoplankton C | T | 8 | 1.617 | 0.144 |
| (µg C $L^{-1}$) | $CO_2$ | 8 | 2.463 | **0.039*** |
| | T x $CO_2$ | 8 | -2.267 | **0.053*** |
| Edible phytoplankton C | T | 8 | 0.209 | 0.839 |
| (µg $L^{-1}$) | $CO_2$ | 8 | 4.287 | **<0.01**** |
| | T x $CO_2$ | 8 | -4.282 | **<0.01**** |
| Inedible phytoplankton C | T | 8 | 2.797 | 0.109 |
| (µg $L^{-1}$) | $CO_2$ | 8 | 1.869 | 0.098 |
| | T x $CO_2$ | 8 | -1.646 | 0.138 |
| (Log) total zooplankton | T | 8 | 1.553 | **0.003**** |
| abundance (ind. $L^{-1}$) | $CO_2$ | 8 | -2.385 | **0.044*** |
| | T x $CO_2$ | 8 | 0.666 | 0.523 |
| Nauplii abundance (ind. $L^{-1}$) | T | 8 | 4.591 | **0.001**** |
| | $CO_2$ | 8 | -3.118 | **0.012*** |
| | T x $CO_2$ | 8 | -0.242 | 0.698 |
| Adult abundance (ind. $L^{-1}$) | T | 8 | 0.741 | 0.480 |
| | $CO_2$ | 8 | 0.614 | 0.555 |
| | T x $CO_2$ | 8 | -0.041 | 0.968 |
| Copepodite abundance | T | 8 | -0.793 | 0.451 |
| (ind. $L^{-1}$) | $CO_2$ | 8 | -1.093 | 0.305 |
| | T x $CO_2$ | 8 | 1.313 | 0.225 |
| (Log) RUE | T | 8 | -1.159 | 0.279 |
| | $CO_2$ | 8 | -3.358 | **0.010**** |
| | T x $CO_2$ | 8 | 2.774 | **0.024*** |
| $NO_2^-$ + $NO_3^-$ (µmol $L^{-1}$) | T | 8 | 0.881 | 0.403 |
| | $CO_2$ | 8 | -0.058 | 0.955 |
| | T x $CO_2$ | 8 | 0.389 | 0.707 |
| $NH_4^+$ (µmol $L^{-1}$) | T | 8 | -0.116 | 0.909 |
| | $CO_2$ | 8 | 0.660 | 0.527 |
| | T x $CO_2$ | 8 | -0.588 | 0.510 |
| $PO_4^{3-}$ (µmol $L^{-1}$) | T | 8 | -1.541 | 0.161 |
| | $CO_2$ | 8 | -1.365 | 0.205 |
| | T x $CO_2$ | 8 | 1.502 | 0.171 |
| $SiO_4^-$ (µmol $L^{-1}$) | T | 8 | -1.936 | 0.089 |
| | $CO_2$ | 8 | 0.025 | 0.980 |
| | T x $CO_2$ | 8 | 1.509 | 0.169 |
| (Log) C:N | T | 8 | 0.663 | 0.525 |
| | $CO_2$ | 8 | 0.703 | 0.501 |
| | T x $CO_2$ | 8 | -0.843 | 0.423 |
| (Log) C:P | T | 8 | 1.137 | 0.288 |
| | $CO_2$ | 8 | -0.357 | 0.730 |
| | T x $CO_2$ | 8 | -0.133 | 0.897 |
| (Log) N:P | T | 8 | 1.099 | 0.303 |
| | $CO_2$ | 8 | -0.388 | 0.707 |
| | T x $CO_2$ | 8 | -0.054 | 0.957 |

| Response variable | factor | df residual | t-value | *p* |
|---|---|---|---|---|
| **Post-bloom** | | | | |
| (Log) total phytoplankton C | T | 8 | 0.397 | 0.701 |
| (µg $L^{-1}$) | $CO_2$ | 8 | 0.466 | 0.653 |
| | T x $CO_2$ | 8 | 0.214 | 0.835 |
| (Log) edible phytoplankton C | T | 8 | 2.876 | **0.020*** |
| (µg $L^{-1}$) | $CO_2$ | 8 | 3.096 | **0.014*** |
| | T x $CO_2$ | 8 | -3.250 | **0.011*** |
| (Log) inedible phytoplankton C | T | 8 | -0.098 | 0.923 |
| (µg $L^{-1}$) | $CO_2$ | 8 | -0.142 | 0.890 |
| | T x $CO_2$ | 8 | 0.816 | 0.437 |
| (Log) total zooplankton | T | 8 | 0.595 | 0.568 |
| abundance (ind. $L^{-1}$) | $CO_2$ | 8 | -0.862 | 0.413 |
| | T x $CO_2$ | 8 | -0.746 | 0.476 |
| Nauplii abundance | T | 8 | 2.027 | 0.077 |
| (ind. $L^{-1}$) | $CO_2$ | 8 | 0.142 | 0.890 |
| | T x $CO_2$ | 8 | -1.926 | 0.090 |
| (Log) adult abundance | T | 8 | -1.146 | 0.194 |
| (ind. $L^{-1}$) | $CO_2$ | 8 | -1.507 | 0.170 |
| | T x $CO_2$ | 8 | 1.013 | 0.340 |
| (Log) copepodite abundance | T | 8 | -2.220 | **0.057*** |
| (ind. $L^{-1}$) | $CO_2$ | 8 | -2.591 | **0.032*** |
| | T x $CO_2$ | 8 | 1.644 | 0.138 |
| Zooplankton RUE | T | 8 | 0.577 | 0.579 |
| | $CO_2$ | 8 | -1.835 | 0.103 |
| | T x $CO_2$ | 8 | -0.365 | 0.724 |
| (Log) C:N | T | 8 | 0.663 | 0.525 |
| | $CO_2$ | 8 | 0.703 | 0.501 |
| | T x $CO_2$ | 8 | -0.843 | 0.423 |
| (Log) C:P | T | 8 | -0.553 | 0.594 |
| | $CO_2$ | 8 | 0.550 | 0.597 |
| | T x $CO_2$ | 8 | 0.171 | 0.867 |
| (Log) N:P | T | 8 | -2.229 | **0.056*** |
| | $CO_2$ | 8 | -0.755 | 0.471 |
| | T x $CO_2$ | 8 | 1.558 | 0.157 |

**II - Table S3.** Results of generalized least squares models (gls) testing for the effect of $pCO_2$ under high and low temperature separately: total phytoplankton C at bloom, edible phytoplankton C at bloom, edible phytoplankton C at postbloom, zooplankton resource use efficiency (zooplankton RUE) at bloom. Significant results are in **bold**. *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$.

| Response variable | factor | df residual | t-value | *p* |
|---|---|---|---|---|
| Phytoplankton C bloom (µg $L^{-1}$) | $pCO_2$ warm | 4 | -1.232 | 0.285 |
| | $pCO_2$ cold | 4 | 1.993 | 0.117 |
| Edible phytoplankton C bloom | $pCO_2$ warm | 4 | -1.798 | 0.146 |
| (µg $L^{-1}$) | $pCO_2$ cold | 4 | 4.222 | **0.013*** |
| (Log) edible phytoplankton C | $pCO_2$ warm | 4 | -2.390 | 0.075 |
| post-bloom (µg $L^{-1}$) | $pCO_2$ cold | 4 | 2.443 | 0.071 |
| (Log) zooplankton RUE bloom | $pCO_2$ warm | 4 | 0.930 | 0.728 |
| | $pCO_2$ cold | 4 | -2.841 | **0.040*** |

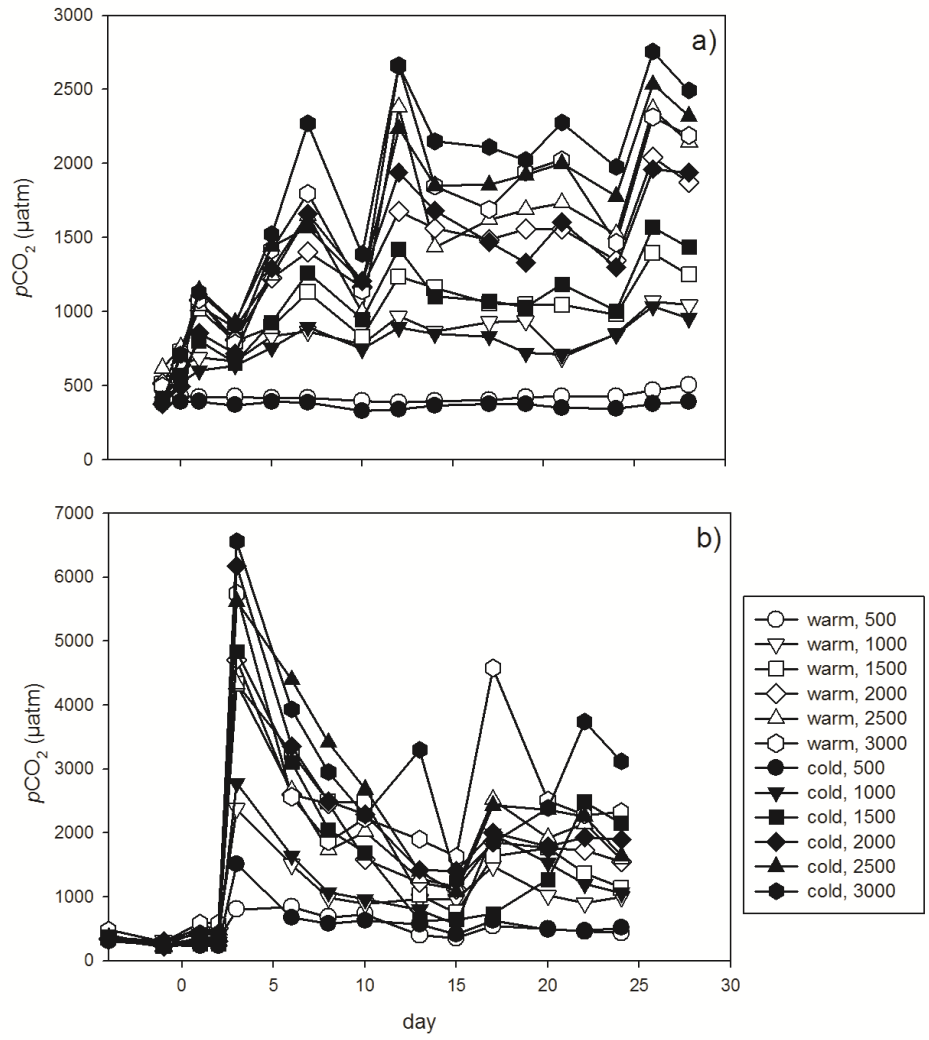
## Chapter III



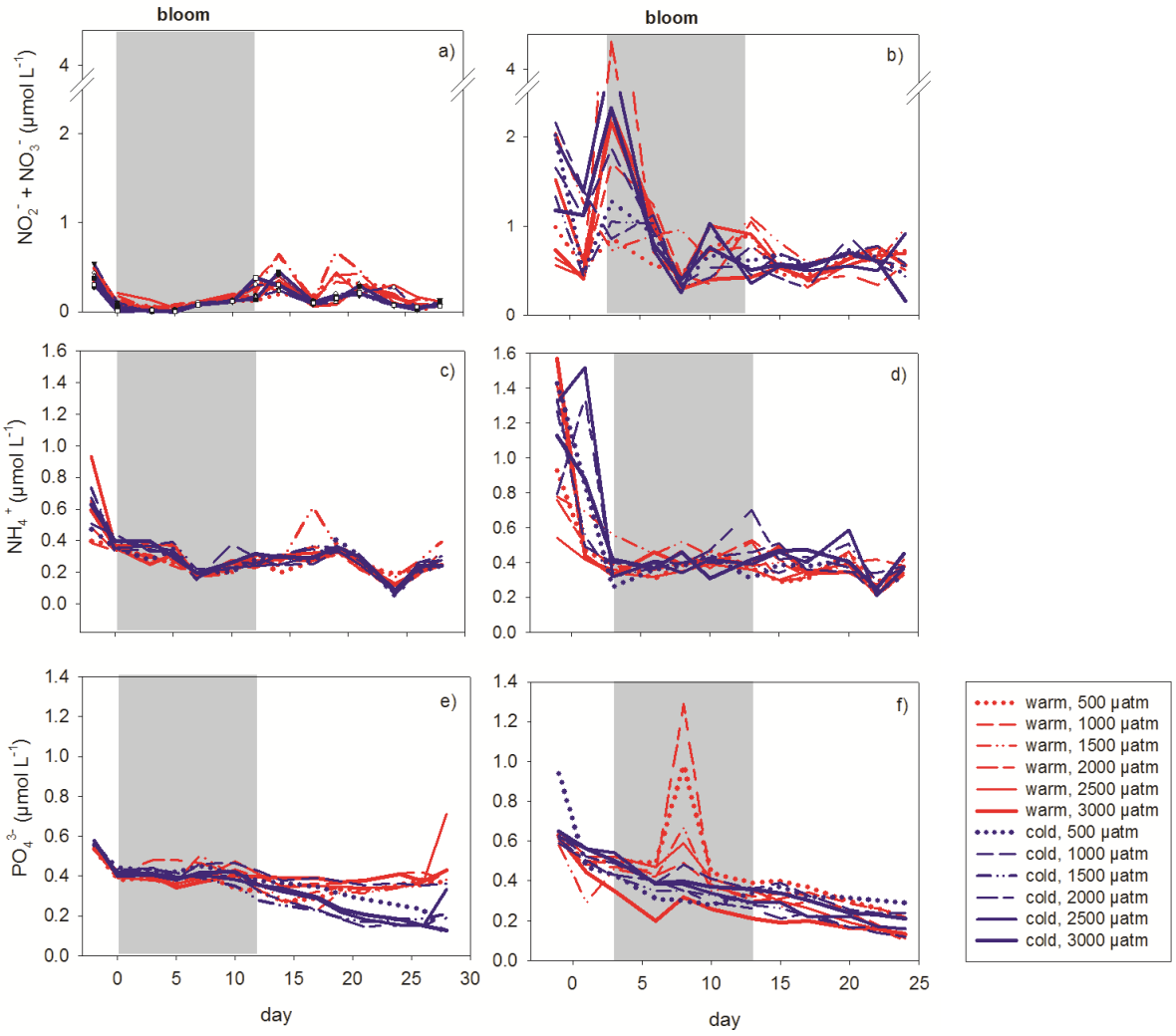

**III - Fig. S1.** Phytoplankton Carbon content (µg C $L^{-1}$) over the experimental course of time: a) mid-summer bloom 2013, b) late-summer bloom 2014. For symbol attribution to treatment combination (temperature, $pCO_2$ target value) see legend. The time-point of phytoplankton bloom is marked in grey colour.

**III - Table S1.** Results of generalized least squares models (gls) testing for the effects of temperature (T), $pCO_2$ as well as the interaction of temperature and $pCO_2$ (T x $CO_2$) during bloom for total phytoplankton C (µg C $L^{-1}$). Additionally, the effects of $pCO_2$ under high and low temperature were tested separately. Significant results are in **bold**. *$p \leq 0.05$, **$p < 0.01$, ***$p < 0.001$.

| Response variable | factor | df residual | t-value | *p* |
|---|---|---|---|---|
| **Mid-summer bloom 2013** | | | | |
| Total phytoplankton C | T | 8 | 1.617 | 0.144 |
| (µg C $L^{-1}$) | $CO_2$ | 8 | 2.463 | **0.039*** |
| | T x $CO_2$ | 8 | -2.267 | **0.053*** |
| Single $pCO_2$ effects of | $pCO_2$ warm | 4 | -1.232 | 0.285 |
| total phytoplankton C | $pCO_2$ cold | 4 | 1.993 | 0.117 |
| **Late-summer bloom 2014** | | | | |
| Total phytoplankton C | T | 8 | -1.035 | 0.331 |
| (µg C $L^{-1}$) | $CO_2$ | 8 | -0.807 | 0.443 |
| | T x $CO_2$ | 8 | 4.585 | **0.002*** |
| Single $pCO_2$ effects of | $pCO_2$ warm | 4 | 5.087 | **0.007*** |
| total phytoplankton C | $pCO_2$ cold | 4 | -0.929 | 0.406 |




**III - Fig. S2.** Time course of calculated $pCO_2$ values: a) mid-summer bloom 2013, b) late-summer bloom 2014. For symbol attribution to treatment combination (temperature, $pCO_2$ target value in µatm) see legend.




**III - Fig. S3.** Time-course of nutrient concentrations (µmol $L^{-1}$) of: a-b) nitrate ($NO_3^-$) and nitrite ($NO_2^-$); c-d) ammonium ($NH_4^+$); e-f) phosphorus ($PO_4^{3-}$). Diagrams on the left sight represent results of mid-summer bloom 2013, diagrams on the right sight represent results of late-summer bloom 2014. For symbol attribution to treatment combination (temperature, $pCO_2$ target value) see legend. The time-point of phytoplankton bloom is marked in grey colour.

**III - Table S2**. Results of generalized least squares models (gls) testing for the effects of temperature (T), target $pCO_2$ and the interaction of temperature and $pCO_2$ (T x $CO_2$) during mid-summer bloom period 2013 of: time-point of bloom, total fatty acid content (TFA, ng µg $C^{-1}$), the percentages (%) of MUFA on TFA, % PUFA on TFA, % SFA on TFA, total PUFA content (ng µg $C^{-1}$), the content of EPA (ng µg $C^{-1}$), DHA (ng µg $C^{-1}$), linolenic acid (ng µg $C^{-1}$), ARA (ng µg $C^{-1}$) and 18:1n9 (ng µg $C^{-1}$). Significant results are highlighted. *p ≤ 0.05, **p < 0.01, ***p < 0.001

| Response variable | factor | df residual | t-value | *p* |
|---|---|---|---|---|
| Time-point of the phytopl. | T | 8 | -1.349 | 0.214 |
| bloom | $CO_2$ | 8 | -1.249 | 0.246 |
| | T x $CO_2$ | 8 | 0.177 | 0.864 |
| % cyanobacteria | T | 8 | 3.079 | **0.015*** |
| on total phytopl. C | $CO_2$ | 8 | -1.740 | 0.120 |
| | T x $CO_2$ | 8 | 0.081 | 0.938 |
| % diatoms | T | 8 | -0.992 | 0.350 |
| on total phytopl. C | $CO_2$ | 8 | 1.050 | 0.324 |
| | T x $CO_2$ | 8 | -1.408 | 0.197 |
| % flagellates | T | 8 | -1.197 | 0.266 |
| on total phytopl. C | $CO_2$ | 8 | -0.749 | 0.475 |
| | T x $CO_2$ | 8 | 0.216 | 0.835 |
| % small phytopl. | T | 8 | 0.122 | 0.906 |
| on total phytopl. C | $CO_2$ | 8 | -0.021 | 0.984 |
| | T x $CO_2$ | 8 | 1.275 | 0.238 |
| TFA | T | 8 | -1.684 | 0.131 |
| (ng µg $C^{-1}$) | $CO_2$ | 8 | 0.077 | 0.941 |
| | T x $CO_2$ | 8 | 2.439 | **0.041*** |
| % MUFA on TFA | T | 8 | 0.521 | 0.617 |
| | $CO_2$ | 8 | 0.160 | 0.877 |
| | T x $CO_2$ | 8 | 0.470 | 0.651 |
| % PUFA on TFA | T | 8 | -1.124 | 0.259 |
| | $CO_2$ | 8 | -0.290 | 0.779 |
| | T x $CO_2$ | 8 | -0.683 | 0.514 |
| % SFA on TFA | T | 8 | 0.249 | 0.810 |
| | $CO_2$ | 8 | 0.746 | 0.477 |
| | T x $CO_2$ | 8 | -0.277 | 0.789 |
| Total PUFA | T | 8 | -1.366 | 0.209 |
| (ng µg $C^{-1}$) | $CO_2$ | 8 | 0.306 | 0.767 |
| | T x $CO_2$ | 8 | 1.360 | 0.211 |
| EPA (ng µg $C^{-1}$) | T | 8 | -1.268 | 0.241 |
| | $CO_2$ | 8 | -0.426 | 0.682 |
| | T x $CO_2$ | 8 | 1.842 | 0.103 |

| Response variable | factor | df residual | t-value | *p* |
|---|---|---|---|---|
| DHA (ng µg $C^{-1}$) | T | 8 | -0.070 | 0.946 |
| | $CO_2$ | 8 | -0.515 | 0.621 |
| | T x $CO_2$ | 8 | -0.195 | 0.850 |
| Linolenic acid | T | 8 | -0.754 | 0.473 |
| (ng µg $C^{-1}$) | $CO_2$ | 8 | -0.044 | 0.966 |
| | T x $CO_2$ | 8 | 1.258 | 0.244 |
| ARA (ng µg $C^{-1}$) | T | 8 | -1.444 | 0.187 |
| | $CO_2$ | 8 | -0.218 | 0.833 |
| | T x $CO_2$ | 8 | 2.480 | **0.038*** |
| 18:1n9 (ng µg $C^{-1}$) | T | 8 | -1.031 | 0.333 |
| | $CO_2$ | 8 | 0.003 | 0.997 |
| | T x $CO_2$ | 8 | 1.713 | 0.125 |
| $NO_2^-$ + $NO_3^-$ | T | 8 | 0.881 | 0.403 |
| (µmol $L^{-1}$) | $CO_2$ | 8 | -0.058 | 0.955 |
| | T x $CO_2$ | 8 | 0.389 | 0.707 |
| $NH_4^+$ (µmol $L^{-1}$) | T | 8 | -0.116 | 0.909 |
| | $CO_2$ | 8 | 0.660 | 0.527 |
| | T x $CO_2$ | 8 | -0.588 | 0.510 |
| $PO_4^{3-}$ (µmol $L^{-1}$) | T | 8 | -1.541 | 0.161 |
| | $CO_2$ | 8 | -1.365 | 0.205 |
| | T x $CO_2$ | 8 | 1.502 | 0.171 |
| $SiO_4^-$ (µmol $L^{-1}$) | T | 8 | -1.936 | 0.089 |
| | $CO_2$ | 8 | 0.025 | 0.980 |
| | T x $CO_2$ | 8 | 1.509 | 0.169 |

**III - Table S3.** Results of generalized least squares models (gls) testing for the effects of temperature (T), target $pCO_2$ and the interaction of temperature and $pCO_2$ (T x $CO_2$) during late-summer bloom period 2014 of: time-point of bloom, total fatty acid content (TFA, ng µg $C^{-1}$), the percentages (%) of MUFA on TFA, % PUFA on TFA, % SFA on TFA, total PUFA content (ng µg $C^{-1}$), the content of EPA (ng µg $C^{-1}$), DHA (ng µg $C^{-1}$), linolenic acid (ng µg $C^{-1}$), ARA (ng µg $C^{-1}$) and 18:1n9 (ng µg $C^{-1}$). Significant results are highlighted. *p ≤ 0.05, **p < 0.01, ***p < 0.001

| Response variable | factor | df residual | t-value | *p* |
|---|---|---|---|---|
| Time-point of the | T | 8 | -0.794 | 0.450 |
| bloom | $CO_2$ | 8 | 0.115 | 0.911 |
| | T x $CO_2$ | 8 | 0.306 | 0.768 |
| % cyanobacteria | T | 8 | -0.282 | 0.785 |
| on total phytopl. C | $CO_2$ | 8 | 3.744 | **<0.01**** |
| | T x $CO_2$ | 8 | 0.387 | 0.709 |
| % diatoms | T | 8 | -1.855 | 0.101 |
| on total phytopl. C | $CO_2$ | 8 | 0.307 | 0.766 |
| | T x $CO_2$ | 8 | -1.365 | 0.210 |
| % flagellates | T | 8 | -4.170 | **<0.01**** |
| on total phytopl. C | $CO_2$ | 8 | -5.123 | **<0.001***** |
| | T x $CO_2$ | 8 | 1.915 | 0.092 |
| % small phytopl. | T | 8 | 3.551 | **<0.01**** |
| on total phytopl. C | $CO_2$ | 8 | 0.958 | 0.366 |
| | T x $CO_2$ | 8 | -1.558 | 0.158 |
| TFA | T | 8 | -1.912 | 0.092 |
| (ng µg $C^{-1}$) | $CO_2$ | 8 | -0.931 | 0.379 |
| | T x $CO_2$ | 8 | 0.977 | 0.357 |
| % MUFA on TFA | T | 8 | -0.926 | 0.382 |
| | $CO_2$ | 8 | -0.127 | 0.902 |
| | T x $CO_2$ | 8 | 0.847 | 0.422 |
| % PUFA on TFA | T | 8 | 0.786 | 0.455 |
| | $CO_2$ | 8 | 0.967 | 0.362 |
| | T x $CO_2$ | 8 | -0.686 | 0.512 |
| % SFA on TFA | T | 8 | 0.106 | 0.918 |
| | $CO_2$ | 8 | -0.667 | 0.524 |
| | T x $CO_2$ | 8 | -0.122 | 0.906 |
| Total PUFA | T | 8 | -1.494 | 0.173 |
| (ng µg $C^{-1}$) | $CO_2$ | 8 | -0.038 | 0.971 |
| | T x $CO_2$ | 8 | 0.388 | 0.708 |
| EPA (ng µg $C^{-1}$) | T | 8 | -5.267 | **<0.001***** |
| | $CO_2$ | 8 | 0.581 | 0.577 |
| | T x $CO_2$ | 8 | -0.419 | 0.686 |

| Response variable | factor | df residual | t | p |
|---|---|---|---|---|
| DHA (ng µg $C^{-1}$) | T | 8 | -3.013 | **0.017*** |
| | $CO_2$ | 8 | -0.740 | 0.481 |
| | T x $CO_2$ | 8 | -0.627 | 0.548 |
| Linolenic acid | T | 8 | 5.604 | **<0.001***** |
| (ng µg $C^{-1}$) | $CO_2$ | 8 | 2.553 | **0.034*** |
| | T x $CO_2$ | 8 | -1.281 | 0.236 |
| ARA (ng µg $C^{-1}$) | T | 8 | 5.701 | <0.001*** |
| | $CO_2$ | 8 | -1.349 | 0.214 |
| | T x $CO_2$ | 8 | 2.073 | 0.072 |
| 18:1n9 (ng µg $C^{-1}$) | T | 8 | -3.226 | **0.012*** |
| | $CO_2$ | 8 | -0.369 | 0.722 |
| | T x $CO_2$ | 8 | -0.308 | 0.766 |
| $NO_2^-$ + $NO_3^-$ | T | 8 | 0.623 | 0.550 |
| (µmol $L^{-1}$) | $CO_2$ | 8 | 0.765 | 0.467 |
| | T x $CO_2$ | 8 | -0.143 | 0.890 |
| $NH_4^+$ (µmol $L^{-1}$) | T | 8 | 0.008 | 0.994 |
| | $CO_2$ | 8 | 0.588 | 0.573 |
| | T x $CO_2$ | 8 | 0.244 | 0.814 |
| $PO_4^{3-}$ (µmol $L^{-1}$) | T | 8 | 2.296 | **0.051*** |
| | $CO_2$ | 8 | 0.373 | 0.719 |
| | T x $CO_2$ | 8 | -1.625 | 0.143 |
| $SiO_4^-$ (µmol $L^{-1}$) | T | 8 | 1.222 | 0.257 |
| | $CO_2$ | 8 | 0.342 | 0.741 |
| | T x $CO_2$ | 8 | -1.608 | 0.147 |

# Curriculum Vitae

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| Sept. 2011 - April 2012 | Wissenschaftliche Assistentin<br>Universität Rostock |
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**Publikationen:** Effects of increased $CO_2$ concentration on nutrient limited coastal summer plankton depend on temperature
C. Paul, U. Sommer, J. Garzke, M. Moustaka-Gouni, A. Paul, B. Matthiessen
accepted by *Journal of Limnology and Oceanography (L&O)*

Warming but not enhanced $CO_2$ concentration quantitatively and qualitatively affects phytoplankton biomass
C. Paul, B. Matthiessen, U. Sommer
*Mar Ecol Prog Ser, Vol.528: 39-51, 2015 (doi: 10.3354/meps11264)*

Warming and Ocean Acidification Effects on Phytoplankton - from species shifts to size shifts within species in a mesocosm
U. Sommer, C. Paul, M. Moustaka-Gouni
*PLOS ONE, May 2015, doi: 10.1371/journal.pone.0125239*

# Description of the individual scientific contribution to the multiple author papers

The chapters of this thesis are (i) published (chapter I), (ii) accepted (chapter II) or (iii) at pending submission (chapter III) in / by different scientific journals with multiple authorships. This list clarifies my contribution on each publication.

**Chapter I**

**Warming but not enhanced $CO_2$ concentration quantitatively and qualitatively affects phytoplankton biomass**

Authors: Carolin Paul, Birte Matthiessen, Ulrich Sommer

Published in: *Marine Ecology and Progress Series* (2015) 528: 39-51

Contributions: CP, BM and US discussed the ideas for the study; CP conducted the experiment; CP and US conducted the data analyses; CP conducted the statistics; CP, BM and US discussed the results; CP wrote the manuscript

**Chapter II**

**Effects of increased $CO_2$ concentration on nutrient limited coastal summer plankton depend on temperature**

Authors: Carolin Paul, Ulrich Sommer, Jessica Garzke, Maria Moustaka-Gouni, Birte Matthiessen

Accepted by: *Journal of Limnology and Oceanography*

Contributions: CP, JG and US discussed the ideas for the study; CP and JG conducted the experiment; CP, US, JG and MM-G conducted the data analyses; CP conducted the statistics; CP, JG, BM and US discussed the results; CP wrote the manuscript

**Chapter III**

**Climate change effects on phytoplankton fatty acids**

Authors: Carolin Paul, Birte Matthiessen, Juan-Carlos Molinero, Ulrich Sommer

Pending submission

Contributions: CP and US discussed the ideas for the studies; CP conducted the experiments; CP and US conducted the data analyses; CP and J-CM conducted the statistics; CP, BM, J-CM and US discussed the results; CP wrote the manuscript

# Eidesstattliche Erklärung

Hiermit erkläre ich, dass die vorliegende Dissertation – abgesehen von der Beratung meiner Betreuer – selbstständig von mir angefertigt wurde und nach Form und Inhalt meiner eigenen Arbeit entstammt. Sie wurde keiner anderen Institution im Rahmen eines Prüfungsverfahrens vorgelegt und ist meine bisher erste und einzige Promotionsarbeit. Die Promotion erfolgt im Fach Biologie. Des Weiteren erkläre ich hiermit, dass Zuhörer bei der Disputation zugelassen sind.

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Ort, Datum Carolin Paul