



Organic matter partitioning and stoichiometry in response to rising water temperature and copepod grazing

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ABSTRACT: Rising ocean temperature is expected to change the balance between production and degradation of organic matter due to different temperature sensitivities of auto- and heterotrophic processes. Copepods are the most prominent zooplankton group, and elevated temperature increases their growth and grazing rates. So far, it is unknown to what extent copepods affect the partitioning and stoichiometry of organic matter in a warmer surface ocean. We therefore conducted a mesocosm experiment with 3 copepod densities and 2 temperature scenarios to determine effects on the pools of dissolved and particulate organic matter and their C:N:P ratios. Here we show that particulate organic C (POC) concentrations decreased with increasing copepod abundance. This effect was more pronounced at elevated temperature, yielding a decrease in the POC to particulate nitrogen ratio (POC:PN) from 26 to 13 and in the POC:particulate organic phosphorus (POP) ratio from 567 to 257, from low to high copepod density. Dissolved organic carbon (DOC) accumulation was positively affected by temperature. However, increasing copepod abundance decreased the accumulation of DOC at elevated temperature. Copepod grazing and egestion enhanced the recycling of N and P, thereby increasing the availability of these nutrients for autotrophs. In concert with temperature-induced shifts in the phytoplankton community composition and size, changes in copepod abundance may therefore have contributed to altering the elemental composition of seston. Our findings suggest combined effects of zooplankton grazing and temperature on the composition and recycling of organic matter that should be taken into account when simulating biogeochemical cycles in a future ocean.

KEY WORDS: Climate change · Ocean warming · Phytoplankton spring bloom · Copepods · Mesocosm study · Organic matter · Transparent exopolymer particles · TEP · Nutrient recycling

INTRODUCTION

The ongoing global warming is mitigated by the ocean's ability to absorb and store high amounts of heat. Between the years 1955 and 2010, the average surface ocean temperature in the upper 700 m has increased by 0.18°C as the ocean has been taking up ~93% of the heat being added to the climate system (Levitus et al. 2012). This trend will continue as an

increase in Earth surface temperature between 0.3 to 4.8°C, depending on the CO₂ emissions scenario, is projected until the end of this century (IPCC 2013).

The warming trend is accompanied by increasing water column stratification (Prentice et al. 2001), decreasing the mixed layer depth and thereby changing nutrient supply and light availability for phytoplankton (Doney 2006). Apart from these indirect effects, temperature is the key factor for regulating metabolic

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processes. It has been suggested that metabolic rate influences all levels of biological organization from individuals to the biosphere (Brown et al. 2004). In general, autotrophic processes, such as phytoplankton growth, show a moderate sensitivity to temperature changes (Eppley 1972) and are generally thought to be more affected by the accompanied increase in water column stratification (Prentice et al. 2001). Compared to this, heterotrophic processes, such as bacterial growth (Pomeroy & Wiebe 2001) and metabolic rates of grazers like marine copepods (Ivleva 1980, Ikeda et al. 2001, Isla et al. 2008), are more sensitive to temperature changes. Hence, climate warming likely changes the balance between autotrophic production and heterotrophic removal processes in the pelagic food web with consequences for biogeochemical cycling of major elements, such as carbon (C), nitrogen (N) and phosphorus (P) (O'Connor et al. 2009, Riebesell et al. 2009, Finkel et al. 2010). The elemental composition of phytoplankton communities is crucial as it can alter the efficiency of the biological carbon pump, the process by which the photosynthetically fixed CO₂ is exported in particulate or dissolved organic matter (POM and DOM) from the surface to deeper parts of the ocean (Falkowski et al. 2000, Laws et al. 2000, Armstrong et al. 2002). Passive sinking of particulate carbon, vertical migration of zooplankton and down-mixing of DOM are the main pathways through which organic matter (OM) is transferred to the ocean's interior (Ducklow et al. 2001). Even small changes in the biological carbon pump, e.g. triggered by climatic changes, can have important feedbacks on the marine carbon cycle and atmospheric CO₂ concentration.

In temperate and high latitude oceans, the phytoplankton spring bloom is an important pulse for the development of biomass at the basis of the food web. The observation of a retardation in the phytoplankton spring bloom off Helgoland, North Sea, between 1962 and 2002, despite an increase in water temperature since 1962, led Wiltshire & Manly (2004) to hypothesize that the delay may be related to the activity of overwintering zooplankton (Wiltshire et al. 2008). Analyzing a set of mesocosm experiments, Gaedke et al. (2010) pointed out that biotic interactions, via grazing and the success of overwintering phyto- and zooplankton, may overrule direct climatic effects like temperature on spring phytoplankton bloom dynamics.

Some studies have detected negative effects of rising temperature on phytoplankton biomass (Keller et al. 1999, O'Connor et al. 2009, Lassen et al. 2010, Lewandowska & Sommer 2010) or, as a consequence

of an enhancement in ocean stratification, on primary production (Behrenfeld et al. 2006). Some of these studies related the negative effect of warming on phytoplankton biomass to increased abundances of zooplankton (Keller et al. 1999, O'Connor et al. 2009) or enhanced grazing pressure (Lewandowska & Sommer 2010). On the other hand, grazing of copepods can enhance the proportion of DOM in the water column by sloppy feeding, excretion, egestion and release from faecal pellets (Nagata 2000, Kragh et al. 2006) with consequences for the production of substrate for bacterial growth (Strom et al. 1997, Møller & Nielsen 2001).

DOM in the world's ocean comprises ~700 Pg C and harbours therein about 200 times more carbon than the marine biomass (Hansell et al. 2009, Sabine & Tanhua 2010). In the ocean, the largest characterized fractions of DOM are carbohydrates (CHO) (Bidanda & Benner 1997, Biersmith & Benner 1998). During phytoplankton blooms, CHO are released in high amounts by phytoplankton cells (Ittekkot et al. 1981, Mykkestad 1995). Primary production of DOM has been shown to increase with rising temperature (Morán et al. 2006). Recent mesocosm studies also found that rising temperature favours the partitioning between DOM and POM towards the DOM pool (Wohlers et al. 2009, Engel et al. 2011, Biermann et al. 2014). A shift from the POM pool towards the DOM pool would not only favour substrate availability for bacteria but also the potential for the formation of transparent exopolymer particles (TEP) that form from polysaccharide precursors (Passow 2002). TEP can abiotically spontaneously assemble from free DOM polymers (Chin et al. 1998) and are therefore a connection between the DOM and the POM pool (Engel et al. 2004, Verdugo et al. 2004). TEP can play a crucial role in aggregation processes during phytoplankton blooms (Logan et al. 1995). Moreover, Thornton & Thake (1998) observed a direct influence of temperature on the formation rate of particle aggregates, including phytoplankton cells, in the presence of acidic polysaccharides.

Concerning the stoichiometry of OM, grazing has also been recognized to be an important source of nutrient regeneration that can feed back to autotrophs (Ketchum 1962, Lehman 1980, Sterner 1986). Owing to stoichiometric properties of the phytoplankton and the grazing species, this consumer-driven nutrient recycling can differentially release N or P (Elser & Urabe 1999, Sterner & Elser 2002). Additionally, elemental stoichiometry of phytoplankton can change in response to a temperature change (Berges et al. 2002).

Several studies have addressed the effects of rising temperature on biogeochemical dynamics at the ecosystem level (Wohlers et al. 2009, Kim et al. 2011, Taucher et al. 2012) without, however, considering changes in grazer abundance. Therefore, the aims of this study were to assess the effect of a 6°C temperature elevation in combination with 3 different densities of copepods (1.5, 4, 10 ind. l⁻¹) on biogeochemical cycling, partitioning between DOM and POM, and organic matter stoichiometry during a winter-spring phytoplankton bloom. Our hypotheses were as follows:

- H1₀: Copepod density and temperature have no effect on phytoplankton bloom timing.
- H1_A: High copepod densities lead to a retardation of the phytoplankton bloom. This effect is more pronounced at elevated temperature, due to an enhancement in grazing activity.
- H2₀: Copepod density and temperature have no effect on POM or DOM accumulation.
- H2_A: The accumulation of POM decreases with increasing copepod density and with increasing grazing activity due to the warming, while DOM accumulation increases due to enhanced grazer activity and with increasing temperature.
- H3₀: Copepod density and temperature have no effect on the stoichiometry of OM.
- H3_A: Grazing differently recycles nutrients. Hence, higher densities of copepods or higher grazing activity at elevated temperature change the stoichiometry of OM.

MATERIALS AND METHODS

Experimental set-up

The experiment in principle followed the mesocosm set-up explained in detail by Sommer et al. (2007). The indoor-mesocosm experiment started on January 8, 2009 (Day 0) and lasted until February 16, 2009 (Day 39). Twelve 1400 l mesocosms of 1 m depth were set up in climate chambers and simultaneously filled with seawater with a salinity of 17.6 from the Kiel Fjord, containing the natural winter/spring plankton community (phytoplankton, bacteria and protozoa). The detailed mesocosm set-up with species composition and phytoplankton biomass has been described by Sommer & Lewandowska (2011). Mesozooplankton was dominated by copepods (70% *Acartia* sp., 17% *Oithona similis*, 7% *Pseudocalanus* sp., 3% *Temora longicornis*, 3% *Centropages hamatus*), added from net catches. The water temperature

inside the mesocosms was ~3.5°C on the day of filling (7 January 2009). Then the temperature was slowly increased to 6.4 ± 1°C for the T+6 treatment or decreased to 2.8 ± 1°C for the T+0 treatment on the first sampling day (9 January 2009). The copepods were acclimated to higher temperatures in separate tanks (30 l) in the climate chambers and were then transferred into the mesocosms 1 d before the first sampling day. Copepod densities were 1.5 (low density, LD), 4 (intermediate density, ID) and 10 ind. l⁻¹ (high density, HD). Zooplankton was sampled weekly by vertical net hauls with a volume of 11.3 l each. The mesh size of the net was 64 µm with a 12 cm diameter mouth. Samples were fixed with Lugol's iodine and counted with a binocular microscope. Abundances of copepods did not change until the last phytoplankton peak (Day 20) (Sommer & Lewandowska 2011).

The temperature of ΔT = 0°C (T+0) was derived from the decadal (1993 to 2002) mean sea surface temperatures in Kiel Bight. The temperature over the whole experimental period was 2.5 ± 0.9°C and applied to 6 mesocosms. For the other 6 mesocosms, an initial temperature elevation of ΔT = 6°C (T+6) above the baseline was chosen according to the A1F1 scenario of the IPCC (Meehl et al. 2007). The temperature in the T+6 treatment was 8.2 ± 0.5°C. In accordance with the natural, seasonal water temperature increase during spring time, a slow increase in temperature of ~1°C over the whole experiment was programmed (Fig. 1). The temperatures in the T+0 treatments showed a higher variability for the experimental period, as 1 climate chamber with 3 T+0 mesocosms (Mesocosms 10, 11, 12) was on average 1°C cooler (Fig. 1). The light system simulated daily irradiance curves and a seasonal light pattern calculated from astronomic equations derived by Brock (1981), controlled by a computer programme (GHL, Prometheus). Two illuminating devices on top of each mesocosm were equipped with 12 full spectrum light tubes (10 solar tropic T5 Ultra, 4000 K, 2 solar nature T5 Ultra, 9000 K, JBL). The daily light dose was adjusted to an intermediate light of 57% of the natural surface irradiance, whereby the light dose increased linearly over the whole experiment, yielding on average 7.9 mol quanta m⁻² d⁻¹. In order to imitate early spring conditions, light and temperature were controlled by a computer program that started on the theoretical date of February 15. The experience of several previous mesocosm experiments showed that by this it was possible to shorten the lag-phase before the phytoplankton starts to grow, prevent growth on the inner mesocosm walls (Sommer & Lengfellner

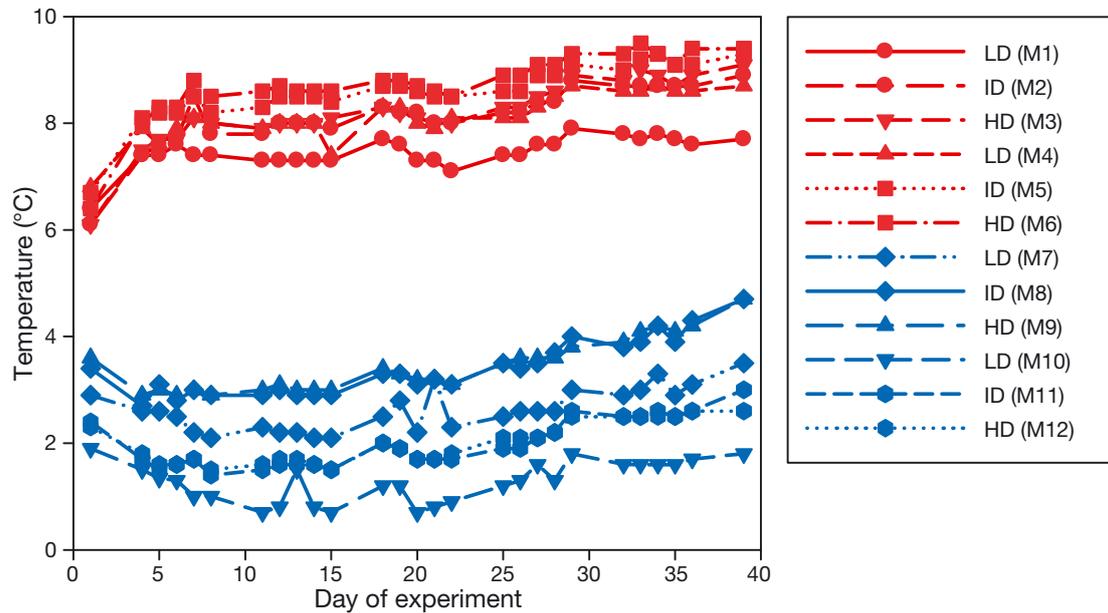


Fig. 1. Temperatures for all 12 mesocosms over the course of the experiment. T+0 treatments ($\Delta T = 0^\circ\text{C}$) are indicated by blue, T+6 ($\Delta T = 6^\circ\text{C}$) by red lines and symbols. LD = low, ID = intermediate and HD = high copepod density corresponding to 1.5, 4, and 10 ind. l^{-1} , M = mesocosm number

2008, Sommer & Lewandowska 2011), and make the results more comparable with each other. Filling the mesocosm in January had the advantage of starting in every mesocosm with comparable conditions, as the risk of transferring copepods entangled with chain-forming diatoms was minimised (Sommer & Lewandowska 2011). Initial nutrient concentrations (Day 1) were $14.3 \pm 0.22 \mu\text{mol l}^{-1}$ nitrate, $0.81 \pm 0.01 \mu\text{mol l}^{-1}$ nitrite, $2.56 \pm 0.12 \mu\text{mol l}^{-1}$ ammonium, $0.93 \pm 0.04 \mu\text{mol l}^{-1}$ phosphate and $30.3 \pm 0.7 \mu\text{mol l}^{-1}$ silicate. During the whole experiment, water inside the mesocosms was gently mixed with a propeller in order to keep cells and smaller particles in suspension, whereas larger particles and aggregates were able to settle out of the water column. Sampling took place at least 3 times a week. Additional sampling was conducted during bloom conditions. Samples were taken with a silicone hose from the middle of the mesocosm water body between 08:30 h and 09:00 h in the morning (1 to 2 h after lights on).

Analytical methods

Temperature, salinity and pH (NBS scale) were measured with a conductivity/pH probe (WTW) on each sampling day at 0.2 m depth. Inorganic nutrients (nitrate, nitrite, phosphate, silicate) were determined with an autoanalyser (AAII) according to Hansen & Koroleff (2007), and ammonium according to the

method of Holmes et al. (1999). Samples were analysed immediately or stored after $0.2 \mu\text{m}$ prefiltering (Filtropur, Sarstedt) overnight at -3°C . During bloom conditions (see Table 1), samples were filtered through $5.0 \mu\text{m}$ cellulose acetate filters. For biogenic silicate (BSi) determination, 50 to 250 ml of sample were filtered onto $0.65 \mu\text{m}$ cellulose acetate filters (Sartorius) and stored at -20°C until analysis according to Hansen & Koroleff (2007). For the determination of chlorophyll *a* (chl *a*), between 100 and 500 ml were filtered onto glass fibre filters (GF/F, Whatman) and stored overnight at -20°C . Pigment concentration was measured with a 10-AU Turner fluorometer after 90% acetone (v/v) extraction (Welschmeyer 1994). For particulate organic carbon (POC), particulate nitrogen (PN), and particulate organic phosphorous (POP), 100 to 500 ml of sample were filtered onto pre-combusted (500°C , 8 h) GF/F filters and stored at -20°C . For the determination of POC and PN, filters were dried at 60°C for 6 h prior to analysis, and C and N concentrations measured with an elemental analyser (EuroEA-3000, Eurovector) according to Sharp (1974). POP was determined photometrically after treatment with 1 dispensing spoon of Oxisolv[®] (Merck) (Hansen & Koroleff 2007). TEP concentration was determined colorimetrically (Passow & Alldredge 1995). Therefore, samples were prepared in duplicate by gentle filtration (~ 150 mbar) of 5 to 50 ml samples onto $0.4 \mu\text{m}$ polycarbonate filters (Whatman) and staining with Alcian Blue. The dye was calibrated ($f =$

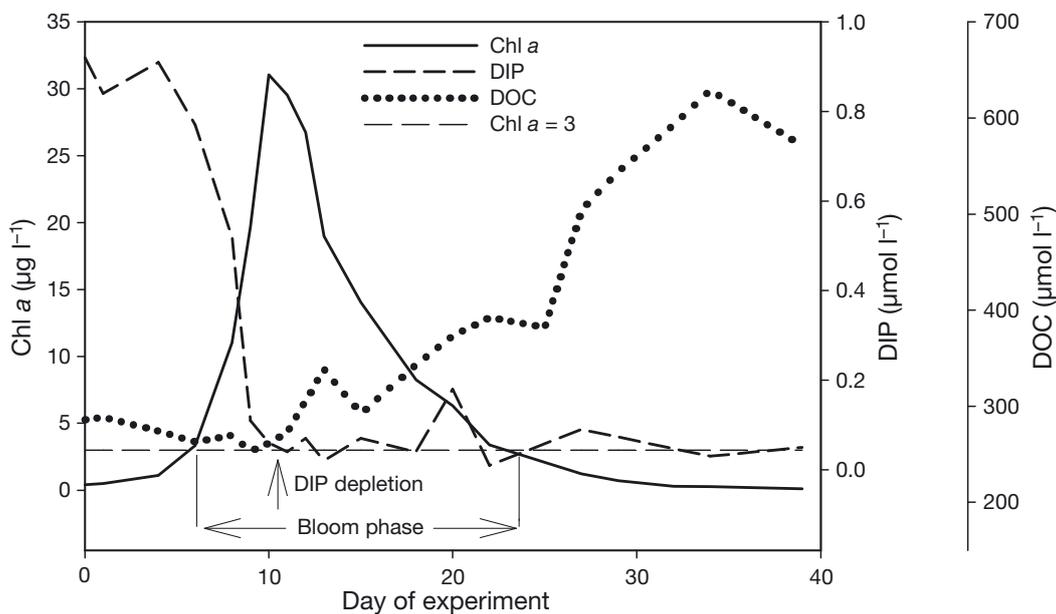


Fig. 2. Exemplary representation for the drawdown of dissolved inorganic phosphorus (DIP), the development of chl *a* and dissolved organic carbon (DOC) in Mesocosm 1. Also shown is the threshold chl *a* value ($\geq 3 \mu\text{g l}^{-1}$) for the definition of bloom conditions. The depletion of DIP (and also dissolved inorganic nitrogen, data not shown in this figure) mark the beginning of DOC and total dissolved carbohydrate (data not shown in this figure) accumulation

40.65) with the standard polysaccharide xanthan gum before and after the experiment. The filters were stored at -20°C until processing. The analysis was carried out by adding 80% H_2SO_4 to the filters and measuring supernatant absorption at 787 nm after 3 h with a spectrophotometer (Hitachi U-2000). Samples for dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were filtered through pre-combusted glass fibre filters and collected in 20 ml pre-combusted glass ampoules. Then, 200 μl 85% H_3PO_4 was added and the ampoules were stored at 6°C in the dark. DOC and TDN were analysed on a Shimadzu TOC_{VSH} using the HTCO method (Sharp et al. 1995). DON was calculated by subtracting DIN from TDN ($\text{DON} = \text{TDN} - \text{DIN}$, $\text{DIN} = \text{nitrate} + \text{nitrite} + \text{ammonium}$). For dissolved organic phosphorus (DOP), 40 ml of sample were filtered through pre-combusted glass fibre filters, collected in Nalgene bottles and immediately analysed with Oxisolv® according to the method of Hansen & Koroleff (2007). For determination of total dissolved carbohydrates (TCHO), including free and hydrolysable sugars, 15 ml of filtered sample were transferred into combusted glass vials, stored at -20°C , and then analysed using the 2,4,6-tripyridyl-s-triazine (TPTZ) spectrophotometric method (Mykkestad et al. 1997).

For analysis of fatty acids, samples were filtered onto precombusted GF/F filters (25 mm diameter) and extracted using a solvent mixture of chloro-

form:dichloromethane:methanol at a ratio of 1:1:1. The extracted lipids were methylated using toluene and methanol, and solubilised in hexane. Fatty acids were identified using a gas chromatograph (Trace GC-Ultra, Thermo Fisher Scientific) equipped with a TR-FAME-column (10 m, 0.1 mm i.d., 0.20 μm film) and a flame ionisation detector.

Calculations and statistics

We applied an operational threshold of $\geq 3 \mu\text{g chl } a \text{ l}^{-1}$ (~ 10 -fold increase from the start of the experiment; corresponding to POC ca. $>40 \mu\text{mol l}^{-1}$) for the definition of bloom conditions (Fig. 2). This yielded a bloom phase for T+0 ranging from Days 8 to 29, and for T+6 treatments from Days 6 to 22 (Table 1).

POM maxima (POC_{max} , PN_{max} , POP_{max} , BSi_{max}) were derived on the day of maximum accumulation of the respective POM component. To investigate the quality of POM, ratios were calculated for each measurement day for POC:PN, POC:POP, and POC:BSi and statistically compared on the day of POC_{max} . PN:POP was determined on the day of its peak concentration (\sim Day 16 for T+0 and \sim Day 9 for T+6). TEP concentration was also derived on the day of POC_{max} (TEP at POC_{max}), and in addition, TEP maximum concentration on Day 39 (Mesocosms 4 and 12 on Day 34) after dissolved inorganic phospho-

Table 1. Bloom phase defined by chl *a* $\geq 3 \mu\text{g l}^{-1}$ for each mesocosm in both temperature treatments T+6 ($\Delta T = 6^\circ\text{C}$) and T+0 ($\Delta T = 0^\circ\text{C}$). DIP = dissolved inorganic phosphorus, POC_{max} = maximum particulate organic carbon. LD = low, ID = intermediate and HD = high copepod density corresponding to 1.5, 4, and 10 ind. l^{-1} . Day = day of experiment

Meso-cosm no.	Temp.	Copepod density	Bloom phase (Day)	Onset of DIP depletion (Day)	POC_{max} (Day)
1	T+6	LD	6–22	11	15
2	T+6	ID	6–20	9	13
3	T+6	HD	6–18	10	13
4	T+6	LD	6–20	10	13
5	T+6	ID	6–18	9	11
6	T+6	HD	6–18	9	12
7	T+0	LD	10–27	14	20
8	T+0	ID	8–25	14	18
9	T+0	HD	8–22	13	16
10	T+0	LD	11–25	15	20
11	T+0	ID	10–25	14	18
12	T+0	HD	11–29	16	20

rus (DIP) depletion was added to the analysis. The days of POC_{max} and the days for the onset of DIP depletion are given in Table 1. The beginning of nutrient depletion in the T+0 treatment was on average on Day 14, and Day 10 in T+6, concomitant with the chl *a* peak in this treatment (on average Day 16 in T+0) (Fig. 2).

DOC and TCHO increased with the beginning of nutrient depletion. Therefore, fresh DOC and fresh TCHO were calculated by subtracting the concentration determined on the day after the onset of DIP depletion (Day 15 for T+0 and Day 11 for T+6) from the highest concentration after DIP depletion (Days 32 to 34). As DON concentrations showed a consistent increase over the whole experiment, fresh DON concentration was determined by subtracting the initial concentrations on Day 1 from the end concentrations on Day 39.

For the proportion of fresh accumulated DOC in total organic carbon (TOC), we calculated the maximum new accumulated DOC and divided it by fresh accumulated POC plus DOC ($\text{POC} + \text{DOC} = \text{TOC}$) (see Fig. 10). This was done for the bloom phase, indicated by chl *a* $\geq 3 \mu\text{g l}^{-1}$ (Table 1), and for the post-bloom phase.

DOC:DON ratios were calculated by dividing DOC by DON for each measurement day. The maximum

ratios after DIP depletion were then statistically compared. The same was done for DOC:DOP and DON:DOP.

Effects of temperature or copepod abundance were tested with general regression models using Statistica 6.0 (StatSoft), whereby temperature was defined as a categorical and copepod abundance as a continuous factor. In addition, a separate linear regression analysis was carried out for the cases in which a significant effect of copepod density was detected. A significance level of $p < 0.05$ was accepted.

RESULTS

Bloom development

Phytoplankton blooms, dominated by the diatom *Skeletonema costatum* (Sommer & Lewandowska 2011), developed in each mesocosm and were indicated by the onset of inorganic nutrient drawdown and the concomitant exponential increase in chl *a* concentrations (Figs. 3 & 4). Rising temperature accelerated the onset of the phytoplankton bloom, leading to ~ 1 d per $^\circ\text{C}$ temperature elevation earlier maximum chl *a* concentrations ($p = 0.0003$), while copepod abundance had no effect on bloom timing ($p = 0.74$). Bloom maxima were reached on Day 10 ± 0 for T+6 treatments and on Day 16 ± 1 for T+0 treatments. Chl *a* maximum concentrations and bloom duration were not significantly affected by temperature or copepod abundance (Fig. 3). Higher variabil-

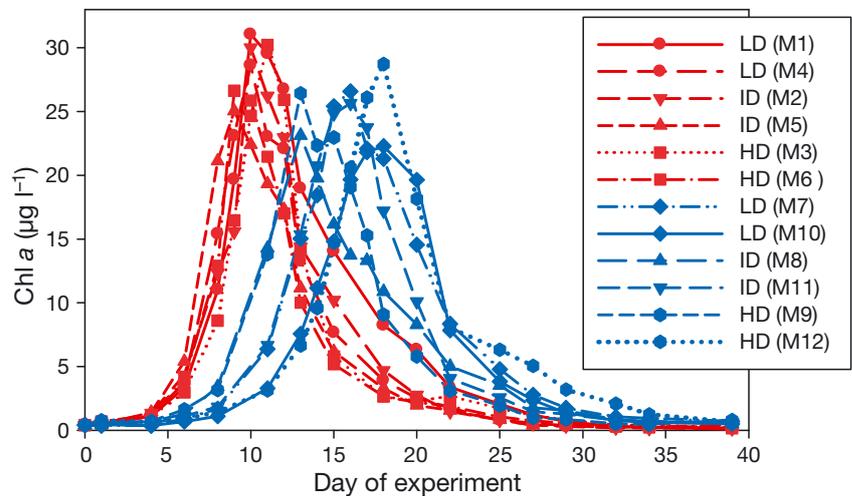


Fig. 3. Chl *a* concentrations for all 12 mesocosms over the course of the experiment. T+0 treatments ($\Delta T = 0^\circ\text{C}$) are indicated by blue, T+6 ($\Delta T = 6^\circ\text{C}$) by red lines and symbols. LD = low, ID = intermediate and HD = high copepod density corresponding to 1.5, 4, and 10 ind. l^{-1} , M = mesocosm number

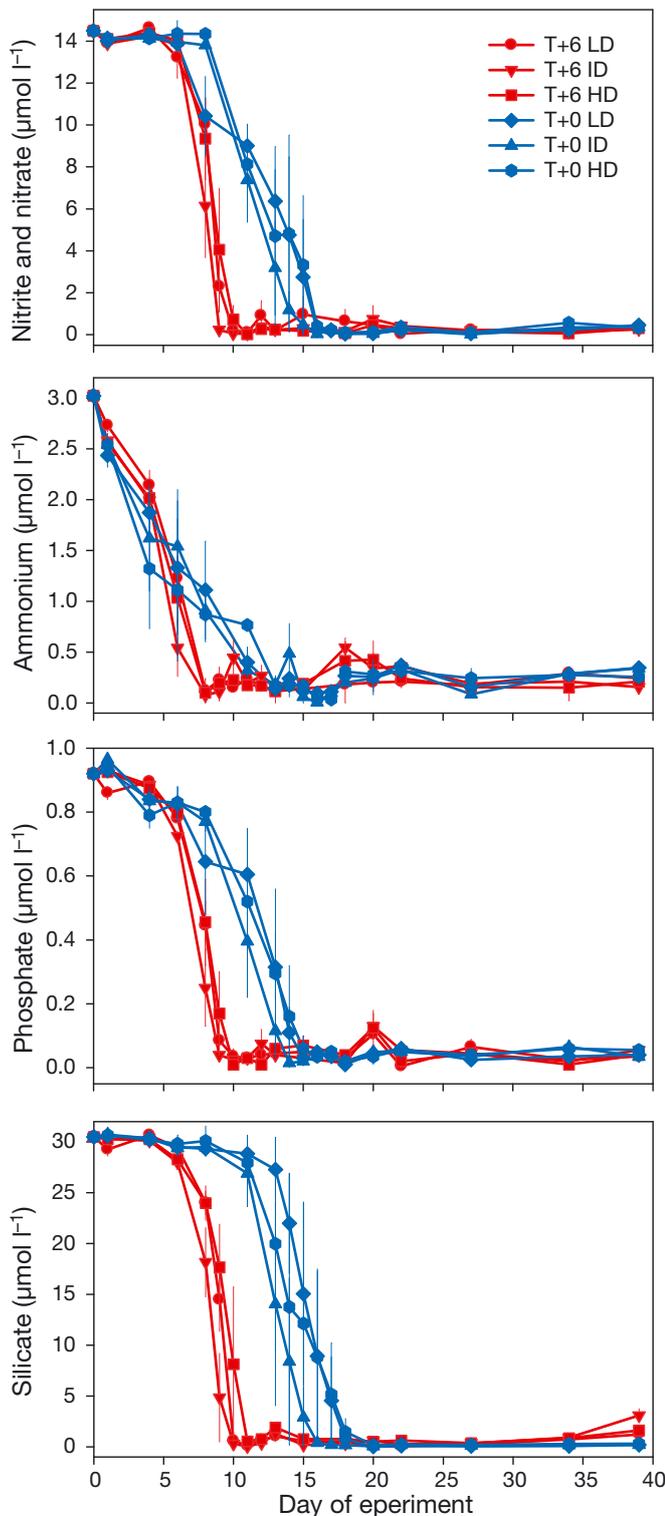


Fig. 4. Inorganic nutrient drawdown (nitrate+nitrite, ammonium, phosphate, silicate) averaged for the 2 replicates with the vertical bars indicating the range between the replicates. T+0 treatments ($\Delta T = 0^{\circ}\text{C}$) are indicated by blue, T+6 ($\Delta T = 6^{\circ}\text{C}$) by red lines and symbols. LD = low, ID = intermediate and HD = high copepod density corresponding to 1.5, 4, and 10 ind. l^{-1}

ity in bloom peak timing at T+0 (Fig. 3) can probably be explained by a greater variability in temperature (Fig. 1).

Effects of temperature and copepod abundance on POM accumulation and stoichiometry

Like chl *a* concentrations, the time point for the maximum accumulation of POC was earlier at T+6 (~5 d) compared to T+0, and not affected by copepod abundance (Table 1, Fig. 5). POC_{max} concentrations, which occurred 1 to 5 d after maximum chl *a*, decreased with increasing copepod abundance ($p = 0.02$) (Table 2, Fig. 5). A separate regression analysis revealed that copepod abundance affected POC accumulation more negatively at T+6 than at T+0 (HD – LD: $\Delta 152 \mu\text{mol l}^{-1}$ POC at T+6 vs. $\Delta 15 \mu\text{mol l}^{-1}$ POC at T+0, Tables 2 & 3, Fig. 7). Contrary to POC, maximum particulate nitrogen (PN_{max}) and particulate organic phosphorus (POP_{max}) accumulation were neither affected by temperature nor by copepod abundance (Table 2, Fig. 5). Hence, the POC:PN and POC:POP ratios (at the time point of POC_{max}) decreased with increasing copepod abundance ($p = 0.003$; $p = 0.006$) (Table 2, Fig. 6). The modulating effect of copepod abundance on POC:PN and POC:POP was also more pronounced at T+6 (Table 3, Fig. 7), with POC:PN decreasing from LD to HD from ~19 to 16 at T+0 and from ~26 to 13 at T+6. BSi_{max} was slightly negatively affected by copepod abundance ($p = 0.05$, Table 2). The separate linear regression analysis, however, showed that copepod abundance significantly negatively affected BSi_{max} at T+6 (Table 3). Consequently, POC:BSi ratios at POC_{max} were not significantly affected ($p = 0.48$), although POC:BSi at T+6 showed the tendency to decrease with increasing copepod abundance (Tables 2 & 3).

An analysis of fatty acid composition in seston, conducted for the ID treatment, revealed overall lower concentrations at elevated temperature, including those of essential fatty acids like EPA (eicosapentaenoic acid) and DHA (docosahexaenoic acid) (Fig. 8).

TEP concentration did not differ between treatments at POC_{max} , yielding $126 \pm 25 \mu\text{g gum xanthan equivalent (Xeq.) l}^{-1}$. In contrast, maximum TEP concentration after nutrient depletion responded positively to the warming ($p = 0.001$), showing almost 6 times higher concentrations at T+6, with $670 \pm 240 \mu\text{g Xeq. l}^{-1}$ compared to $120 \pm 50 \mu\text{g Xeq. l}^{-1}$ at T+0 (Fig. 9).

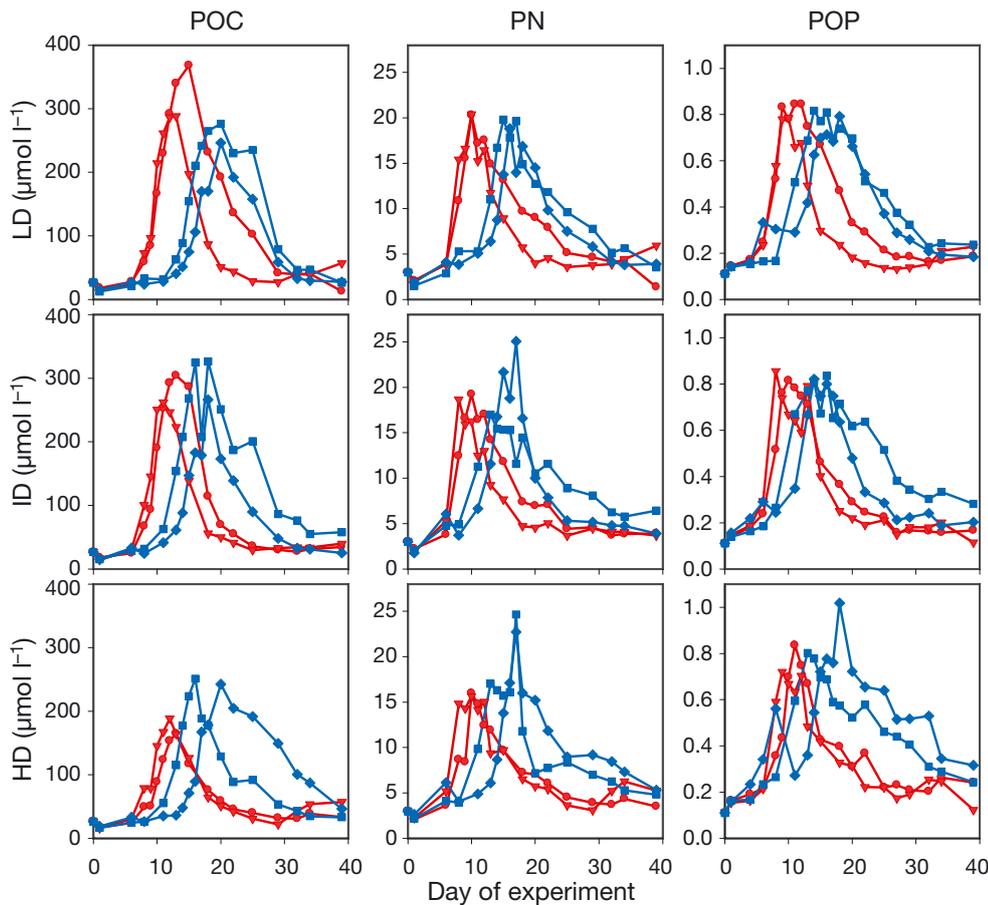


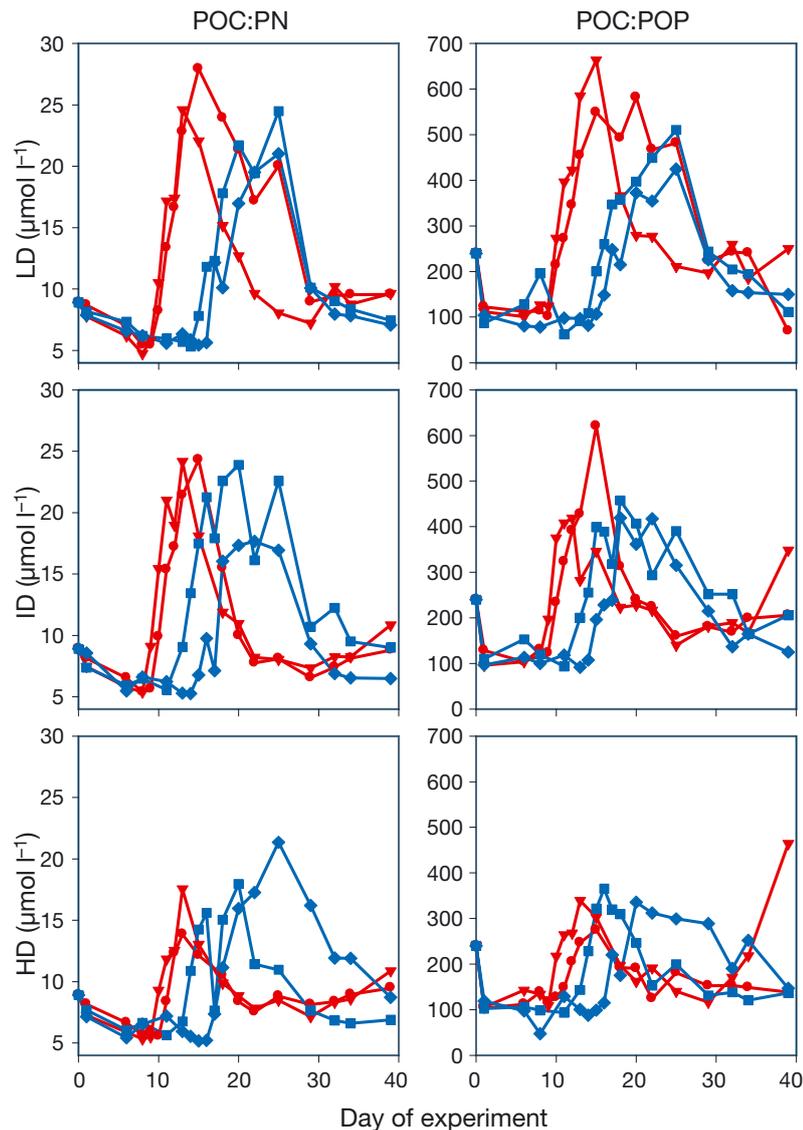
Fig. 5. Particulate organic carbon (POC), particulate nitrogen (PN), and particulate organic phosphorus (POP) concentrations at low (LD), intermediate (ID), and high (HD) copepod density corresponding to 1.5, 4, and 10 ind. l⁻¹ for each mesocosm. T+0 treatments ($\Delta T = 0^\circ\text{C}$) are indicated by blue, T+6 ($\Delta T = 6^\circ\text{C}$) by red lines and symbols

Table 2. Maximum or mean concentrations (in $\mu\text{mol l}^{-1}$) and stoichiometry of particulate and dissolved organic matter (mean points and range of 2 replicates) for the 2 temperature treatments T+0 ($\Delta T = 0^\circ\text{C}$) and T+6 ($\Delta T = 6^\circ\text{C}$). POC = particulate organic carbon, DOC = dissolved organic carbon, TCHO = total dissolved carbohydrates, PN = particulate nitrogen, DON = dissolved organic nitrogen, POP = particulate organic phosphorus, DOP = dissolved organic phosphorus, BSi = biogenic silicate. LD = low, ID = intermediate and HD = high copepod density corresponding to 1.5, 4, and 10 ind. l⁻¹

	T+0			T+6		
	LD	ID	HD	LD	ID	HD
Carbon						
POC _{max}	261 ± 15	296 ± 30	247 ± 4	328 ± 40	283 ± 21	177 ± 12
Fresh DOC	171 ± 44	221 ± 74	190 ± 10	287 ± 69	266 ± 19	113 ± 44
Fresh TCHO	132 ± 56	181 ± 72	138 ± 11	196 ± 49	165 ± 7	69 ± 5
Nitrogen						
PN _{max}	19.3 ± 0.5	21.0 ± 4.0	19.9 ± 2.9	20.3 ± 0.0	18.9 ± 0.3	15.8 ± 0.2
Fresh DON	5.76 ± 1.34	5.60 ± 0.81	5.08 ± 0.60	1.56 ± 1.56	4.48 ± 0.11	3.40 ± 0.59
Phosphorus						
POP _{max}	0.80 ± 0.01	0.83 ± 0.01	0.91 ± 0.11	0.81 ± 0.03	0.84 ± 0.02	0.78 ± 0.06
DOP _{mean}	0.25 ± 0.00	0.24 ± 0.00	0.24 ± 0.02	0.25 ± 0.00	0.24 ± 0.02	0.24 ± 0.01
Silica						
BSi _{max}	30.3 ± 5.2	33.1 ± 5.7	24.5 ± 1.5	27.2 ± 0.4	27.2 ± 0.3	22.2 ± 0.0
Stoichiometry						
POC:PN at POC _{max}	19.3 ± 2.4	19.3 ± 3.3	15.8 ± 0.2	26.3 ± 1.7	21.2 ± 0.2	13.2 ± 0.7
DOC:DON _{max}	33.4 ± 3.5	35.3 ± 6.3	35.1 ± 0.9	40.8 ± 3.5	36.4 ± 0.4	26.6 ± 1.2
POC:POP at POC _{max}	385 ± 12	438 ± 19	351 ± 15	567 ± 18	418 ± 9	257 ± 11
DOC:DOP _{max}	1967 ± 115	2341 ± 222	3192 ± 1132	2590 ± 720	3227 ± 795	1960 ± 80
PN:POP _{max}	26.0 ± 0.3	28.2 ± 5.3	26.3 ± 3.7	26.4 ± 0.3	24.2 ± 0.1	24.7 ± 0.4
DON:DOP _{max}	73.1 ± 2.4	74.6 ± 5.8	66.2 ± 10.8	75.0 ± 9.9	97.3 ± 21.8	75.3 ± 5.1
POC:BSi at POC _{max}	13.3 ± 1.7	10.7 ± 0.7	13.4 ± 2.0	15.4 ± 1.9	12.1 ± 3.3	11.2 ± 0.9

Table 3. Regression analysis according to the model $y = ax + b$, separated for the 2 temperature treatments T+0 ($\Delta T = 0^\circ\text{C}$) and T+6 ($\Delta T = 6^\circ\text{C}$) with x being the copepod abundance. See Table 2 for definition of abbreviations. *significant $p < 0.05$

	a	SE of a	b	SE of b	F	p	r^2
T+0:							
POC _{max}	-2.90	3.73	283	23.4	0.610	0.479	0.13
POC:PN at POC _{max}	-0.450	0.334	20.5	2.10	1.82	0.249	0.31
POC:POP at POC _{max}	-5.96	4.64	422	29.10	1.65	0.268	0.30
BSi _{max}	-0.829	0.697	33.6	4.38	1.41	0.300	0.26
POC:BSi _{max}	0.090	0.281	12.0	1.78	0.103	0.765	0.03
T+6:							
POC _{max}	-17.8	3.75	355	23.6	22.5	0.0090*	0.85
POC:PN at POC _{max}	-1.50	0.165	28.0	1.04	82.6	0.0008*	0.95
POC:POP at POC _{max}	-34.7	4.174	593	26.2	69.0	0.0011*	0.95
BSi _{max}	-0.642	0.100	28.8	0.626	41.4	0.0030*	0.91
POC:BSi _{max}	-0.425	0.345	15.09	2.17	1.51	0.286	0.27



Effects of temperature and copepod abundance on DOM accumulation and stoichiometry

DOC started to increase with the onset of nutrient depletion around Day 10 for T+6 and Day 14 for T+0 (Figs. 2 & 9). The newly accumulated TOC was comprised of ~35% fresh DOC at T+0 and 29% at T+6 during the bloom phase, while the average post-bloom contribution was ~40% at T+0 and ~69% at T+6, showing a temperature effect ($p = 0.02$) on the accumulation of DOC (Fig. 10).

Similar to DOC, TCHO concentration started to increase with the onset of nutrient depletion (Fig. 9). Between 61 and 94% of the newly accumulated DOC was comprised of TCHO, without significant differences in response to temperature or copepod abundance. A separate linear regression analysis for T+6 revealed that copepod abundance negatively affected the accumulation of fresh DOC_{max} and fresh TCHO_{max} in these treatments ($p = 0.04$ and $p = 0.02$, respectively).

DON and DOP dynamics were completely different from those of DOC and TCHO. DON increased throughout the experiment from an initial 10.4 ± 2.1 to $14.8 \pm 0.7 \mu\text{mol l}^{-1}$ at the end of the experiment. In contrast, DOP concentrations stayed at the same level of $0.25 \pm 0.02 \mu\text{mol l}^{-1}$ on average for the whole time. Relatively low DON and DOP and high DOC concentrations led to strong increases in DOC:DON and DOC:DOP ratios (Table 2).

Fig. 6. Particulate organic carbon to particulate nitrogen (POC:PN) and to particulate organic phosphorus (POC:POP) (mol/mol) ratios at low (LD), intermediate (ID), and high (HD) copepod density corresponding to 1.5, 4, and 10 ind. l^{-1} for each mesocosm. T+0 treatments ($\Delta T = 0^\circ\text{C}$) are indicated by blue, T+6 ($\Delta T = 6^\circ\text{C}$) by red lines and symbols

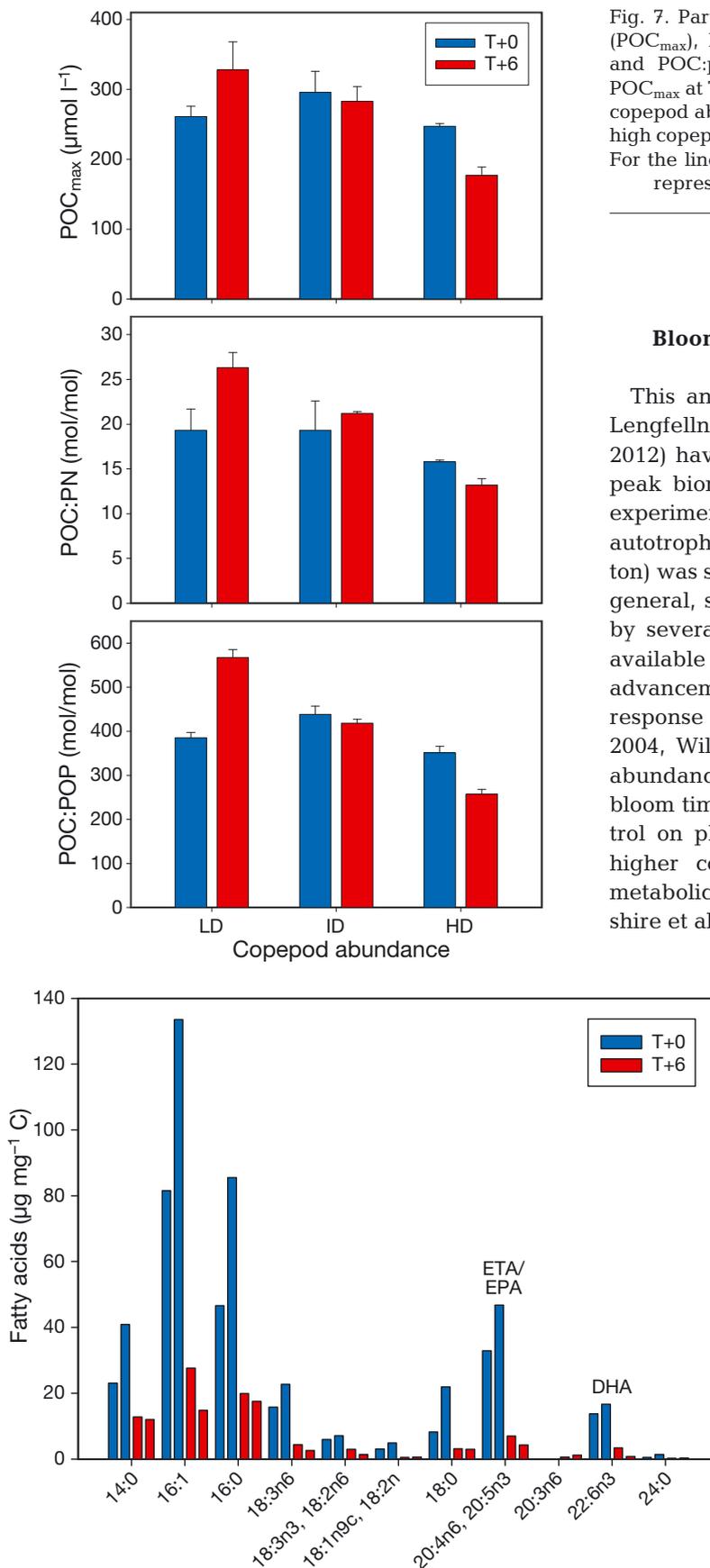


Fig. 7. Particulate organic carbon maximum concentration (POC_{max}), POC:particulate nitrogen (PN) ratio at POC_{max}, and POC:particulate organic phosphorus (POP) ratio at POC_{max} at T+0 ($\Delta T = 0^\circ\text{C}$) and T+6 ($\Delta T = 6^\circ\text{C}$) in response to copepod abundance. LD = low, ID = intermediate and HD = high copepod density corresponding to 1.5, 4, and 10 ind. l⁻¹. For the linear regression equations see Table 3. Error bars represent mean points and range of two replicates

DISCUSSION

Bloom timing and changes in the POM pool

This and previous mesocosm studies (Sommer & Lengfellner 2008, Wohlers et al. 2009, Winder et al. 2012) have shown that the timing of phytoplankton peak biomass was accelerated by 1 to 2 d per $^\circ\text{C}$ experimental temperature elevation, indicating that autotrophic production (growth rates of phytoplankton) was stimulated by the experimental warming. In general, spring bloom timing in the ocean can vary by several weeks and the marine time series data available so far has not provided evidence for an advancement in diatom spring bloom timing in response to ocean warming (Edwards & Richardson 2004, Wiltshire et al. 2008). In this study, copepod abundance had no effect on the phytoplankton bloom timing, although an enhanced top-down control on phytoplankton was expected due to either higher copepod density or accelerated copepod metabolic rates at warmer temperature (H1) (Wiltshire et al. 2008, Gaedke et al. 2010).

However, copepod abundance did affect POC_{max} negatively (H2). A regression analysis for the 2 temperature treatments also revealed a pronounced significant negative effect of temperature on POC accumulation. This result is in line with those of Sommer & Lewandowska (2011), who also found phytoplankton (carbon) biomass to decrease with warming and higher copepod density. Other mesocosm experiments have also detected negative effects of elevated

Fig. 8. Fatty acid composition of seston in the ID mesocosms (nos. 8, 11 [T+0, $\Delta T = 0^\circ\text{C}$], 2, 5 [T+6, $\Delta T = 6^\circ\text{C}$]) at the time point of phytoplankton maximum biomass. The fatty acid EPA is essential for egg production, DHA for hatching success

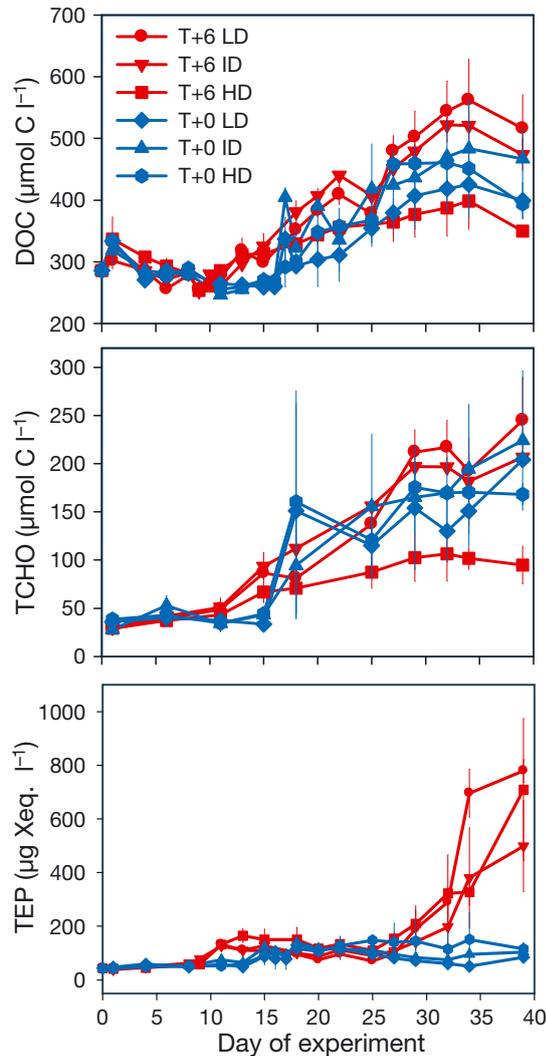


Fig. 9. Dissolved organic carbon (DOC), total dissolved carbohydrates (TCHO) and transparent exopolymer particles (TEP) averaged for the 2 replicates with the vertical bars indicating the range between the replicates. T+0 treatments ($\Delta T = 0^\circ\text{C}$) are indicated by blue, T+6 ($\Delta T = 6^\circ\text{C}$) by red lines and symbols. LD = low, ID = intermediate and HD = high copepod density corresponding to 1.5, 4, and 10 ind. l^{-1} . Xeq = gum xanthan equivalent

temperature on phytoplankton biomass (Keller et al. 1999, O'Connor et al. 2009, Lassen et al. 2010, Lewandowska & Sommer 2010) and a general reduction in phytoplankton cell size (Lewandowska & Sommer 2010). Although, reduced phytoplankton (carbon) biomass and POC accumulation in seston at higher copepod density and temperature point to an enhancement in copepod grazing pressure, PN and POP were not affected by copepod grazing or temperature in this study. Hence, the stoichiometry of POM changed (H3), namely POC:PN and POC:POP decreased with increasing copepod density. In this

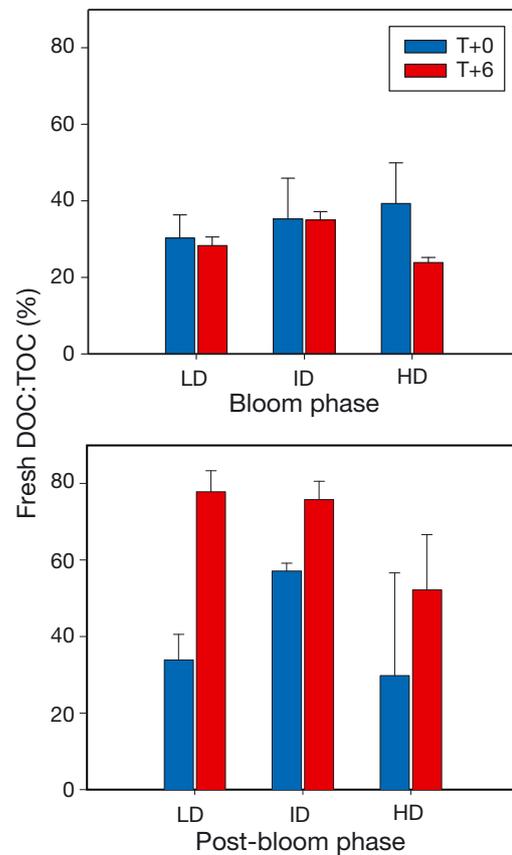


Fig. 10. Proportion of fresh dissolved organic carbon (DOC) in total organic carbon (TOC) during the bloom phase and during the post-bloom phase at T+0 ($\Delta T = 0^\circ\text{C}$ blue bars) and T+6 ($\Delta T = 6^\circ\text{C}$ red bars). LD = low, ID = intermediate and HD = high copepod density corresponding to 1.5, 4, and 10 ind. l^{-1}

study, the phytoplankton assemblage shifted to decreased abundance of diatoms and to higher proportions of small flagellates at T+6 and HD (Sommer & Lewandowska 2011). This, and the general reduction in phytoplankton cell size that was mainly driven by the negative response of the diatoms *Thalassiosira nordenskiöldii*, *T. rotula* and *S. costatum*, as well as the positive response of small flagellates to temperature and zooplankton density (Sommer & Lewandowska 2011), may explain the observed differences in POM stoichiometry. It has been shown that distinct differences in elemental stoichiometry exist between functional groups of phytoplankton (Ho et al. 2003, Quigg et al. 2003). Shifts in the community structure of phytoplankton may therefore have altered the elemental composition of POM (Finkel et al. 2010). The C:N and C:P ratios of diatoms have been found to be lower than those of dinoflagellates (Quigg et al. 2003). This result is, however, contradictory to our study, as high grazing activity and temperature

tended to reduce the proportions of diatoms, increase the proportion of dinoflagellates and lead to low C:N and C:P ratios of POM.

It is known that consumers, via egestion and excretion, can be important drivers for nutrient regeneration. This can feed back to autotrophs by increasing the availability of nutrients (Ketchum 1962, Lehman 1980, Sterner 1986, Elser & Urabe 1999). The combination of grazing and nutrient regeneration can have a modifying effect on autotroph stoichiometry. In principle, low grazing pressure (low nutrient recycling) results in high autotroph biomass of low quality in terms of C:nutrient ratios, while high grazing pressure (high nutrient recycling) leads to low autotroph biomass with low C:nutrient ratios (Sterner & Elser 2002, Hessen et al. 2013). Following the principles of ecological stoichiometry theory, low C:nutrient ratios of POM at high(er) copepod density suggest that the copepod grazers were limited by C (energy), and therefore liberally recycled N and P.

Limitation of an element or biochemical constituent in copepods is commonly assessed by means of reproductive success, e.g. egg production or hatching success (Kjørboe 1989, Kleppel & Burkart 1995, Irigoien et al. 2005, Mayor et al. 2009). Although previous studies report positive correlations between copepod egg production rates and food availability (Hirst & Bunker 2003, Irigoien et al. 2005), and carbon limitation has been predicted with stoichiometric models (Mayor et al. 2009), food limitation in terms of carbon seems unlikely for this study due to the high POC concentrations during phytoplankton bloom conditions. However, the quality of the food, e.g. in terms of elemental stoichiometry (Kjørboe 1989, Augustin & Boersma 2006) and biochemical composition, such as fatty acids (Kleppel & Burkart 1995, Pond et al. 1996, Anderson & Pond 2000), is also decisive for the reproductive success of copepods. An analysis of the fatty acid composition of seston during this study showed generally lower concentrations at elevated temperature and, specifically, lowered concentrations of essential fatty acids like EPA and DHA, which might suggest a lower food quality of seston for copepods at elevated temperature. However, egg production rates or other variables that could elucidate the reproductive success of copepods were not assessed in this study. The effect of decreasing seston C:nutrient ratios with increasing copepod density was more pronounced at elevated temperature, and may suggest elevated C limitation in these treatments. The underlying mechanism might be that higher grazing activity and respiration of copepods at elevated temperature increased the copepod carbon

demand, thereby aggravating C limitation, with the result that more nutrients were recycled at elevated temperature and high copepod density.

Highest particulate C:N and C:P ratios were observed shortly after nutrient (N, P, Si) exhaustion. That carbon and nitrogen dynamics can decouple after nitrate depletion during phytoplankton blooms has been observed before and attributed to excess carbon fixation (Engel et al. 2002, Wetz & Wheeler 2003). Excess carbon fixation or carbon overconsumption describes the process in which DIC relative to inorganic N (or P) is taken up by phytoplankton in excess of the Redfield ratio (Sambrotto et al. 1993, Toggweiler 1993). Engel et al. (2002) traced increased POC:PN back to an enhanced channelling of carbon into the formation of TEP from the DOM pool. It has been suggested that higher temperatures enhance excess DIC consumption, leading to increased C:N of POM and DOM (Taucher et al. 2012). As a possible explanation, the authors suggested an enhancement of the channelling of excess carbon into TEP. In our study, higher POC:PN ratios were observed during the bloom and were thus not directly related to TEP formation, as TEP formation began to increase at T+6 during the last days of the experiment (Day 25 until end). DOC can abiotically form polymer particles (Chin et al. 1998, Kerner et al. 2003). This mechanism was probably also valid during our study, but only at elevated temperature. This result is in line with the observed temperature effect on TEP formation in a previous mesocosm experiment. Although, TEP were also found to increase in high concentrations concomitant with a phytoplankton bloom, a stimulating effect of temperature on POC:PN was not observed (Biermann et al. 2014).

PN:POP was not significantly affected by temperature, but a declining trend with increasing copepod abundance was observed at T+6, hinting at a preferential remineralisation of P. In general, P is remineralised faster than N and C (Shaffer et al. 1999, Loh & Bauer 2000). Higher abundance of bacteria may enhance remineralisation. Indeed, total bacteria numbers (TBN) and bacterial secondary production were positively affected by temperature, as their peak was earlier at T+6 compared to T+0 and almost concomitant with the phytoplankton peak (Wohlers-Zöllner et al. 2012). A tighter temporary coupling between phytoplankton and bacterial maximal abundance with warming has previously been reported (Hoppe et al. 2008, von Scheibner et al. 2014). Although this result may imply higher nutrient remineralisation at T+6 compared with T+0, for which TBN were low during the phytoplankton

bloom, a competition for nutrients between phytoplankton and bacteria could also be possible, leading to earlier nutrient deficiency for phytoplankton. Bacteria biomass was, however, only 1% of phytoplankton biomass at T+6 and less than that at T+0 at the time of the phytoplankton biomass peak, indicating a minor importance of nutrient competition between bacteria and phytoplankton.

Changes in the DOM pool

The accumulation of DOC and TCHO after nutrient depletion was positively affected by the temperature elevation. A more pronounced production of dissolved organic matter at higher temperatures has previously been found in short-term incubations (Morán et al. 2006) and mesocosm studies (Wohlers et al. 2009, Engel et al. 2011, Taucher et al. 2012). DOC increased only after the depletion of inorganic nutrients. It is often observed that extracellular release increases at the end of phytoplankton blooms in response to nutrient depletion (Nagata 2000, Engel et al. 2002). While, in general, a surplus of carbon in organisms can be used for fitness-promoting purposes, or be disposed of through enhanced metabolic activity and respiration (Hessen & Anderson 2008), an alternative for phytoplankton is to proceed with photosynthesis after nutrient exhaustion and exude the accumulating photosynthetic products that can no longer be used for cell growth (Fogg 1966, Bjørnsen 1988). Extracellular release by phytoplankton can comprise up to 80–90% polysaccharides (Myklestad 1995). As TCHO simultaneously increased with DOC, and DOC consisted on average of 70% TCHO, an algal origin of this DOC is very likely.

Contrary to DOC, DON showed an increasing trend that continued over the whole experimental period. This effect has been observed before in mesocosm studies (Engel et al. 2011, Taucher et al. 2012). No trend was observed for DOP at all. Neither DON nor DOP showed a response to increased temperature or copepod abundance. This can probably be attributed to the fact that inorganic N and P can be severely limiting nutrients for phytoplankton, while inorganic C can generally be only rate limiting. In marine ecosystems, inorganic C is rarely a limiting resource, as many phytoplankton species can take up both CO_2 and HCO_3^- (Kaplan & Reinhold 1999, Martin & Tortell 2008). DOP is the fraction of DOM that is preferentially mineralised (Clark et al. 1998) and organic P is easily hydrolysed back to DIP at the alkaline pH of seawater (Lalli & Parsons 1997). Small mol-

ecules (amino acids, urea) of DON and DOP can be utilised by phytoplankton and bacteria (Bronk 2002, Karl & Björkman 2002). Dissolved amino acids (DAA) are the main component of characterised DON. Engel et al. (2011) found a contribution of dissolved free amino acids (DFAA) of up to 20% of DAA and pointed out that DFAA are small enough to be taken up by microbes with turnover times of a few hours. Hence, DON and DOP were probably quickly recycled and an accumulation was therefore, at least for DOP, not detectable. In contrast, DOC accumulated in the water column until Day 34, indicating that bacteria were temporarily unable to balance the increase in DOC. This can be attributed to either nutrient limitation (Thingstad et al. 1997) or a refractory nature of the fresh DOC (Fry et al. 1996).

DOM did not increase due to higher grazer abundance as shown by other studies (Nagata 2000, Kragh et al. 2006) (H2), but rather DOC decreased with increasing copepod abundance at T+6. We detected a small increase in ammonium at T+6 in HD and ID but not LD treatments around Days 18 to 20 (Fig. 4), supporting the hypothesis of enhanced nutrient recycling at higher copepod abundance. Hence, nutrient deficiency was likely higher at LD. This may have contributed to enhanced nutrient stress and subsequent DOC release by phytoplankton.

Owing to relatively high DOC concentrations right from the start of this study, ratios for DOC:DON and DOC:DOP were at the upper end of or higher than literature values. A summary of surface and deep ocean DOM gives a range of 9 to 18 for DOC:DON and 180 to 570 for DOC:DOP (Benner 2002). After nitrate depletion, DOM with C:N \geq 16 accumulated during a phytoplankton bloom dominated by the diatom *Chaetoceros* sp. (Wetz & Wheeler 2003). Kähler & Koeve (2001) calculated a DOC:DON of 23.5 for surface water (5 m depth) along a transect in the North Atlantic, a similarly high ratio to those observed in our study, at least under bloom conditions. In this study, DOC increased after nutrient depletion. As the DOC accumulation was more pronounced at elevated temperature and lower copepod densities, DOC:DON ratios decreased with increasing copepod abundance at T+6 (H3).

Consequences for a future ocean

Changes in the balance of POM and DOM can affect the efficiency of the biological carbon pump that transports CO_2 bound in organic carbon compounds to greater ocean depth where it is sequest-

ered on time scales of hundreds of years. This and previous studies showed that a temperature increase shifts the partitioning between particulate and dissolved organic carbon towards the DOM pool (Engel et al. 2011, Taucher et al. 2012, Wohlers-Zöllner et al. 2012), potentially enhancing TEP formation (Wohlers-Zöllner et al. 2012). According to our results the accumulated DOC consisted mainly of TCHO, a substrate that is readily recycled by bacteria. Hence, for a warmer future ocean, we can expect an earlier and probably also more pronounced 'fuelling' of the microbial loop with DOC. In contrast, copepods counteract the DOC release by phytoplankton cells by prolonging nutrient availability to phytoplankton through remineralisation of primary produced organic matter. While copepod abundance could thereby also affect bacterial abundance and production, our study did not reveal such a correlation between bacteria and copepods. Our results further indicate that the 'excess inorganic carbon consumption' by phytoplankton that is often favoured by nutrient stress and suspected to increase due to sea-surface warming (Taucher et al. 2012) or elevated CO₂ concentrations (Riebesell et al. 2007), could be moderated by the abundance and activity of grazers.

This study further suggests that shifts in the community and size distribution of phytoplankton in response to higher grazing activity and warming can be responsible for the changes in elemental composition of seston or POM. Hence, the future development of grazer abundance and activity is of major importance when considering climate change responses on the stoichiometry of OM. Ocean warming effects on zooplankton include changes in the distribution of individual species, in the timing of life-cycle events, and in the community structure and abundance (Richardson 2008). As heterotrophic processes in relation to primary production are expected to increase with rising temperature (López-Urrutia et al. 2006), top-down effects on the phytoplankton community will likely become stronger. So far, little is known about the modulating effects of copepods and ocean warming on the elemental composition of organic matter. Extrapolation of our results to open ocean ecosystems should be approached with caution considering the limitations of mesocosm experiments. In natural ecosystems organisms experience more gradual changes in temperature than in our simplified system, which gives them more time to adapt or migrate. However, the general shape of the phytoplankton response to rising temperature and the underlying mechanisms could be successfully tested with our approach, advancing our understand-

ing of the impact of warming on elemental cycling in the ocean.

More research is clearly needed to better account for effects of grazing on nutrient recycling and phytoplankton community composition in models that simulate organic matter cycling and carbon sequestration in a future warmer ocean.

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