Effects of sea surface warming on the production and composition of dissolved organic matter during phytoplankton blooms: Results from a mesocosm study
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Effects of sea surface warming on the production and composition of dissolved organic matter during phytoplankton blooms:

Results from a mesocosm study

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Abstract

An experimental study was conducted to test the effects of projected sea surface warming (according to the IPPC scenarios) on the accumulation and composition of dissolved organic matter (DOM) during marine phytoplankton blooms in cold seas (<4°C). Eight mesocosms (~1400 L) were filled with natural seawater, and two replicate mesocosms each were incubated by raising temperature by +0, +2, +4, and +6°C, respectively. The enclosed water was initially fertilized with inorganic nutrients to induce the development of phytoplankton blooms, which were then dominated by diatoms. Over a four week period, dissolved combined carbohydrates (DCCHO) and dissolved amino acids (DAA) were determined as major components of freshly produced, labile to semi-labile DOM. In all mesocosms, the increase in DCCHO concentration occurred sharply after the peak of chlorophyll a concentration, when nutrients became depleted. Rising temperature resulted in an earlier, faster and higher accumulation of DCCHO, and of combined glucose predominantly. DCCHO yielded a maximum percentage of 35, 40, 49 and 59% of total polysaccharides in the +0, +2, +4, and +6°C treatments, respectively. Accumulation of DAA occurred more continuously and at an average rate of 0.79±0.20 nmol L\(^{-1}\) h\(^{-1}\), but was not affected by rising temperature. Due to the higher accumulation of DCCHO, the C:N ratio of DOM increased strongly during the course of the bloom, with higher ratios in the warmer treatments. Our study suggests that warming increases the extracellular release of carbohydrates from phytoplankton, and therefore may affect the bottom-up control of the microbial loop in cold seas in the future.
Introduction

The cycling of organic matter in the surface ocean is regulated by a complex interplay of production and loss processes. Loss of organic matter occurs primarily through heterotrophic activities, resulting in solubilisation and subsequent remineralisation of organic compounds, or through gravitational settling of particles to the deeper ocean. The balance between remineralisation and sinking processes thereby determines the efficiency of organic matter export to the deeper ocean. Particulate organic matter (POM) drives food web interactions at the higher trophic levels as well as export fluxes. The vast majority of organic matter in the ocean, however, is dissolved, comprising in general more than 90% of total organic carbon (TOC), equivalent to 662 Pg C (Hansell et al. 2009).

Marine dissolved organic matter (DOM) is produced primarily by extracellular release from phyto- and bacterioplankton cells (Fogg 1983, Ogawa et al. 2001), enzymatic solubilization of particles (Cho & Azam 1988, Smith et al. 1992), viral lysis (Fuhrmann 1999), and zooplankton grazing (Copping & Lorenzen 1980, Nagata 2000). Major loss processes for DOM are microbial uptake and remineralisation (Azam & Hodson 1977), and photochemical decomposition (Mopper et al. 1991). Another important loss of DOM is the aggregation of high-molecular weight components (HMW>1kDa) into gel particles, a physical process that transfers dissolved molecules into the particulate size spectrum (Leppard 1995, Kerner et al., 2003, Engel et al. 2004,Verdugo et al. 2004). Export of DOM from the ocean’s surface to the deeper ocean contributes significantly to the biological pump (Hopkinson and Vallino 2005).

When biological production and loss processes are decoupled, either temporarily or spatially, DOM accumulates. This is most evident in oceanic regions that experience annual phytoplankton blooms (Carlson 2002). The percentage of organic matter that is released by...
phytoplankton into the dissolved pool often increases when nutrient concentration declines (Myklestad et al. 1972, Biddanda and Benner 1997). Especially towards the end of nutrient-limited phytoplankton blooms, enhanced leakage of low-molecular weight compounds, and exudation of larger molecules therefore generate high DOM concentrations (Søndergard et al. 2000, Engel et al. 2004). Hansell and Carlson (1998) estimated that 1.2 Gt C year$^{-1}$ or 17% of global new production escapes rapid microbial utilization, accumulates in the surface waters and is available for export to the ocean’s interior.

The reactivity of organic matter is often described by its turnover time. Especially for DOM, which to a large extent is chemically uncharacterized, the times-scale of cycling have been used to differentiate between labile (hours to days), semi-labile (weeks to months) and refractory (months to years) components (Anderson and Williams 1999). The turnover of DOM is not solely a function of its chemical quality, but also depends of the abundance, species composition and activity of microorganisms that consume DOM, and of other factors such as temperature, light and nutrients (Hansell and Carlson, 1998). Therefore, turnover times of DOM vary greatly over time and space. A more direct indication of the reactivity and nutritious quality of organic matter can be obtained from molecular compound analysis (e.g., Lee and Cronin, 1984, Cowie and Hedges, 1992, Hernes et al., 1996). Carbohydrates (CHO) and amino acids (AA) are the major characterized components of organic matter in marine systems (e.g., Wakeham et al., 1997, Pakulski and Benner 1994, Benner 2002, Carlson 2002). While dissolved monomeric, i.e. free, amino acids and carbohydrates are rapidly taken up by organisms, in particular by heterotrophic bacteria, combined carbohydrates of high molecular weight (>1kDa), i.e. polysaccharides, and combined amino acids, e.g. proteins, represent the major fractions of the accumulating DOM during phytoplankton blooms (Søndergard et al. 2000).
Although knowledge on the temporal and spatial variability of polysaccharides and amino acids in the water column has greatly increased over the last decades, the mechanistic understanding of their environmental and biological control is still fragmentary. In general, the metabolic cycling of organic components is catalyzed by enzymes, whose specific requirements for activation energy determine the rate of the reaction. The optimum temperature and the sensitivity to changes in temperature vary between enzymes and so does the temperature effect on the various metabolic processes (Brown et al., 2004). As a consequence, the overall influence of rising temperature on a dynamic ecosystem and on the fluxes of organic matter therein is difficult to predict. Moreover, rates of metabolic processes are co-determined by many other factors, such as substrate quality, availability of co-enzymes and organism size.

Based on data from various oceanic provinces, Kirchman et al. (2009) recently reassessed the temperature effect on the ratio of bacterial to primary production. They suggested that the apparent temperature sensitivities of marine bacteria in waters <4°C are strongly co-determined by the availability of nutritious, labile DOC. Following this argument, the influence of global warming on microbial organic matter carbon cycling in cold seas may be rather small, if the amount of labile DOC was not to change.

During a mesocosm study, Wohlers et al. (2009) recently observed that increasing temperatures by +0, +2, +4, and +6°C resulted in a higher partitioning of carbon to the DOM pool, and in an overall decrease of the net biological draw-down of dissolved inorganic carbon (DIC). Here, we give detailed information on the compositional and quantitative changes of DOM produced during that mesocosm study. We focus particularly on the question how polysaccharides and amino acids, representing the major nutritious components of DOM, may respond to sea surface warming in the course of phytoplankton blooms in cold seas.
Method

Abbreviations and acronyms are defined in Table 1.

Mesocosm set-up

An indoor mesocosm study was conducted between 6\textsuperscript{th} January and 5\textsuperscript{th} February 2006. Eight mesocosms with an approx. volume of 1400 L each were set up in four temperature-controlled climate chambers (Sommer et al. 2007). Unfiltered seawater containing a natural winter/spring plankton community was pumped from 6 m depth in Kiel Fjord, Baltic Sea, and was simultaneously filled into the mesocosms. Mesozooplankton from net catches in Kiel Bight was added in natural densities of approximately 10 individuals L\textsuperscript{-1}.

Temperature of the four climate chambers was adjusted to 2.5, 4.5, 6.5 and 8.5°C, respectively. The lowest temperature of 2.5°C (\textit{in situ} treatment +0°C; mesocosms M7 and M8) was determined based on a 10-year meteorological database for Kiel Bight (1993-2002) for January and February. The elevated temperature regimes, hereafter named +2°C (M5 and M6), +4°C (M3 and M4), and +6°C (M1 and M2), were chosen according to the projected increase in global mean sea surface temperature of up to 6.4°C (high scenario A1FI) until the end of the 21\textsuperscript{st} century (summarised by Solomon et al. 2007). Light was supplied by light benches above each mesocosm, containing different types of light tubes in order to supply close-to-natural light conditions (T5, 10x JBL Solar Tropic [4,000 K], 2x JBL Solar Natur [9,000 K]). The light benches were controlled by separate computer units (ProfiLuxII). With these, a triangular light curve was created with a light: dark-cycle of 12h: 12h. At sunrise, the initial light intensity was 40 % of the total capacity, gradually increased up to 100 % at midday and then gradually decreased again to 40 % at sunset. The maximum light intensity at
midday was measured five times a week with a submersible 4π PAR sensor (LiCOR Inc, US) at a depth of approx. 10 cm and was on average 179 ± 24 µmol photons m⁻² s⁻¹. Temperature, salinity and pH were measured three times a week with a WTW conductivity and pH probe. Initial concentrations of dissolved inorganic phosphate (PO₄³⁻), nitrate (NO₃⁻), and silicate (Si(OH)₄) were approximately 0.9 µM, 8 µM, and 20.4 µM, respectively. Due to the unusually low NO₃⁻ concentration in that year, we added additional NO₃⁻ to a final concentration of 21.1 µM to ensure the development of a phytoplankton bloom.

After the addition of inorganic nitrate, we followed the build-up and decline of phytoplankton blooms in each mesocosm over a course of 30 days. During the experiment, the water body was gently mixed with a propeller attached to the side of the mesocosm. With this, cells and smaller particles were kept in suspension, whereas bigger particles and aggregates, forming at the end of the bloom, sank out of the water column.

**Sampling**

Water samples were taken daily by lowering a silicon tube into the middle of the mesocosm, closing it at the top, pulling the upper end out and filling a clean canister with the water by means of gravitation.

**Bulk chemical analysis**

A variety of chemical and biological analyses were performed throughout the experiment. Dissolved inorganic nutrients were measured with an autoanalyzer (AA II) on water samples filtered through 5.0 µm cellulose acetate following the protocol by Hansen and Koroleff (1983). Chlorophyll a (Chl a) was measured after extraction in 90 % acetone on a 10-AU Turner fluorometer from 50-250 ml water sample filtered onto glass fibre filters (GF/F, Whatman). Particulate organic carbon (POC) and particulate nitrogen (PN) were obtained from 100-500 ml filtered onto precombusted (5 h, 450°C) glass fibre filters (GF/F,
Whatman) and stored at -20°C. Filters were dried for 6 h at 60°C and analyzed on an elemental analyzer (EuroEA 3000, Eurovector). Dissolved organic carbon (DOC) and total dissolved nitrogen (TDN) were filtered through precombusted glass fibre filters and collected in 20 ml precombusted (12 h, 450°C) glass ampoules, and stored at -20°C. DOC and TDN were analyzed on a Shimadzu TOC$_{VCN}$ using the HTCO method. Values of TDN were corrected for nitrate, and ammonium, and thereafter referred to as DON.

**Combined carbohydrates**

Hydrolysable combined carbohydrates in the molecular fraction >1kDa, i.e. polysaccharides, were collected daily from each mesocosm. For total polysaccharides (TCCHO) including particulate and dissolved components, two replicate samples of 20 ml were filled into combusted glass vials using 50 ml disposable syringes. For dissolved polysaccharides (DCCHO), two replicate samples were filtered through 0.45 µm syringe filters (GHP membrane, Acrodisk, Pall) before filling the sample into combusted glass vials. All samples were immediately frozen and stored at -20°C. Prior to filtration, all syringes and syringe filters were rinsed with several ml of Milli-Q water first and seawater sample thereafter. The samples were thawed immediately before analysis. Desalination of the samples was conducted by membrane dialysis (1kDa MWCO, Spectra Por) for 6 h at 0°C. Monomeric carbohydrates were yielded after acid hydrolysis of desalinated samples using 0.8M HCl final concentration for 20 h at 100°C, and neutralisation through acid evaporation ($\text{N}_2$). Carbohydrate monomers were determined by ion chromatography on a Dionex ICS 3000 system using high performance anion exchange chromatography (HPAEC) coupled with pulsed amperometric detection (PAD) following the protocol of Engel and Händel (2010). Separation of carbohydrates during chromatography was achieved using a Dionex CarboPac PA10 analytical column (2x250 mm) coupled with a Dionex CarboPac PA10 guard column (2 x 50mm). Column temperature was kept constant at 17°C during all analyses. To minimize
degradation of samples before analysis, the autosampler (Dionex AS50) was kept at 4°C. Carbohydrate analysis was performed by injection of 17.5 µl of sample, whereas standardization was carried out by injection of 10 µl, 12.5 µl, 15.0 µl and 17.5 µl of mixed sugar standard solution. The standard neutral, amino and acidic carbohydrates that were detected in this study are: fucose (Fuc), rhamnose (Rha), arabinose (Ara), galactosamine (Gal-AM), glucosamine (Glc-AM), galactose (Gal), glucose (Glc), mannose (Man), xylose (Xyl), galacturonic acid (Gal-URA), and glucuronic acid (Glc-URA). Due to co-elution the following carbohydrates were quantified together: 1. Ara and Glc-AM 2. Man and Xyl. All samples were analysed in duplicate, whereas the standard solutions for calibration were measured in triplicate. Milli-Q water was used as a blank to account for potential contamination during sample handling. Blanks were treated and analyzed in the same way as the samples. Blank concentrations were subtracted from sample concentration. Recovery of CHO was checked by analysing the standard solution after every second sample. The detection limit for this method was 10 nM with a standard deviation between replicate runs of <2%, indicating high precision. Concentrations of TCCHO and DCCHO after hydrolysis are given as monomer equivalents.

Dissolved amino acids

Dissolved free amino acids (DFAA) and combined amino acids (DCAA) were determined by HPLC after ortho-phthalaldehyde derivatization (Lindroth & Mopper 1979). Therefore, 20-mL samples were filtered through 0.45 µm syringe filters with low protein binding affinity (GHP membrane, Acrodisk, Pall Corporation) and stored at -20°C. Samples were thawed immediately before analysis. Dissolved combined amino acids (DCAA) were hydrolyzed with 6 N HCl at 155°C for 1 h before analysis. The following standard amino acids were used: aspartic acid (Asp), glutamic acid (Glu), histidine (His), serine (Ser), arginine (Arg), glycine (Gly), threonine (Thre), alanine (Ala), tyrosine (Tyr), valine (Val),
phenylalanine (Phe), isoleucine (Ileu), leucine (Leu). Alpha amino butric acid was used as an internal standard to account for losses during handling. For one mesocosm per temperature treatment, dissolved combined amino acids (DCAA) and dissolved free amino acids (DFAA) were determined separately. Concentrations of DCAA after hydrolysis are given as monomer equivalents.

Statistical treatment of data

Mean values were compared by means of a $t$-test. Significance of the correlation coefficient ($r^2$) against $H_0: \rho=0$, was tested by a Student-test according to Sachs (1974), with a degree of freedom, $df=n-2$. The influence of the temperature treatment on biological or chemical variables was determined by means of the analysis of variance (ANOVA). Significance level of each test was $p<0.05$.

Results

The general development of the phytoplankton bloom is described in more detail in Wohlers et al. (2009). Briefly, a bloom developed in each of the mesocosms after nutrient addition and was dominated by diatoms (in particular *Skeletonema costatum*, *Chaetoceros spp.* and *Thalassiosira spp.*). Bloom development was reflected after day 5 by an increase in Chl a concentration, and a rapid decline in dissolved inorganic nutrients. Rising water temperature led to an earlier onset of the bloom with an acceleration of 1 day/°C. Maximum and average Chl a concentrations, and primary production rates did not differ significantly between treatments. Accumulation of POM during the experiment ranged between 21 and 36 $\mu$mol L$^{-1}$ PN, 0.67-0.79 $\mu$mol L$^{-1}$ POP, and 220-393 $\mu$mol L$^{-1}$ POC with no significant effect of temperature. In addition, no temperature effect was observed for rates of nutrient draw-down. In contrast, the net drawdown of DIC decreased significantly with increasing
temperature, averaging 503±50, 485±58, 435±73, and 350±34 µmol C L\(^{-1}\) at +0, +2, +4, and +6°C, respectively. DOC accumulated in the course of the experiment, particularly after the onset of nutrient depletion. DOC accumulation ranged between 45 and 147 µmol L\(^{-1}\), with the lowest values of 45 and 74 µmol L\(^{-1}\) being observed in the +0°C mesocosms M7 and M8, respectively. After the onset of nutrient depletion, average DOC concentration in the mesocosms significantly increased with temperature (p<0.005). At this time, average DON concentration in all mesocosms was 8.39±1.64 µmol L\(^{-1}\) and did not respond to the temperature treatment. A higher net respiratory release of carbon was observed during the post-bloom phase in the mesocosms at +4°C and +6°C. Maximum rates of secondary production of free-living bacteria were higher in the warm mesocosms.

**Concentration and composition of polysaccharides**

Initial total polysaccharide (TCCHO) concentration was 1.60±0.19 µmol L\(^{-1}\), with 1.23±0.19 µmol L\(^{-1}\) being comprised of dissolved polysaccharides (DCCHO). Following biological production, polysaccharide concentration increased in all mesocosms to maximum values of 51 µmol L\(^{-1}\) for TCCHO (+2°C M6) and 24 µmol L\(^{-1}\) for DCCHO (+4°C M4). In all mesocosms, TCCHO increased before DCCHO, indicating a general direction of carbohydrate partitioning from the particulate into the dissolved phase (Fig. 1).

Temperature affected the temporal development of polysaccharide concentration significantly, leading to an earlier exponential increase of TCCHO (p<0.05), and of DCCHO (p<0.01), and to earlier peak concentrations in DCCHO (p<0.001). For DCCHO, the timing of increase in concentration was accelerated by 1.6 days/°C (Fig. 2a). The time span between the increase in TCCHO and in DCCHO narrowed with increasing temperature by 0.87 days/°C (p<0.05), indicating a faster partitioning of carbohydrates from the particulate into the dissolved pool at higher temperatures (Fig. 2b). Concentration of DCCHO declined after 3
weeks in the warmest treatment (+6°C), but continued to increase in the colder mesocosms until the end of the experiment.

Temperature also affected the absolute amount of total and of dissolved polysaccharides (ANOVA, p<0.005). The maximum yield, defined as the difference between the maximum and the initial concentration of a component: \[\text{Yield}_{\text{max}} = [C_{\text{max}}] - [C_{\text{initial}}]\], significantly increased with temperature for DCCHO (p<0.005) (Fig.2c). In contrast, a maximum yield of TCCHO was observed in the coldest mesocosms, and decreased with increasing temperature (p<0.005)(Fig.2d). This points towards a decline in the yield of particulate polysaccharides with rising temperature. Over the course of the experiment, the average ratio of [DCCHO]: [TCCHO] was 35, 40, 49 and 59% in the +0, +2, +4, and +6°C treatments, respectively. During the post-bloom phase, polysaccharides in the warmer mesocosm (+6°C and +4°C) were almost completely contained in the dissolved phase (Fig.1). On few individual days, concentrations of DCCHO slightly exceeded those of TCCHO, which can be explained by analytical and sample variability.

In all mesocosms, molar composition of total polysaccharides showed a clear dominance of Glucose (Glc) (Table 2), which is derived as a primary product from algal photosynthesis. Reflecting algal productivity, mole percentages of Glc in polysaccharides increased sharply around the Chl a peak, and dominated carbohydrate composition by >80% during the bloom and post-bloom phase. Percentages of Glc were generally higher in TCCHO than in DCCHO (Fig. 3, Table 2), reflecting the production of Glc by algal photosynthesis and its preferential removal from the dissolved phase. The increase of mole percentages of Glc in DCCHO followed the increase in TCCHO much sooner in the warmer treatments than in the colder, illustrating again the more rapid partitioning of polysaccharides into DOM (Fig.3).
To compare the efficiency in Glc production between the temperature treatments, Glc concentration was related to Chl a. Maximum Chl a concentration varied between 40 and 68 µg L\(^{-1}\), but Chl a variability was not related to the temperature treatment (Wohlers et al. 2009). Specifically, Chl a concentrations in the +4°C treatment were similar to those in the +0°C treatment. The onset of Chl a increase, however, was earlier at higher temperatures with an acceleration of 1 day/°C (Wohlers et al. 2009). The ratio of total Glc to Chl a ([TGlc]:[Chl a]; µmol:µg) differed significantly between the temperature treatments (ANOVA, p<0.005), but was more similar among the warmer treatments (+4°C and +6°C). During the first 10 days, [TGlc]:[Chl a] decreased in all mesocosms (Fig. 4), as a consequence of Chl a synthesis preceding new Glc production. After the onset of the bloom, [TGlc]:[Chl a] increased steadily in all mesocosms, but earlier in the warmer mesocosms. The temporal rate of change, i.e. \(\Delta([TGlc]:[Chl a]):\Delta(t)\), was determined by linear regression for five days of exponential increase (with p<0.005 for each regression), and yielded values of 0.10±0.032, 0.16±0.002, 0.22±0.005, 0.20±0.32 µmol TGlc µg\(^{-1}\) Chl a d\(^{-1}\) for the +0, +2, +4, and +6°C treatment, respectively. Significant differences between the rates of change in Chl a specific TGlc, however, were only determined between the +0°C and the +4°C treatment (t-test, p=0.05, n=10). During the post-bloom phase, phytoplankton cells settled to the bottom of the mesocosms, and Chl a concentration decreased. At this time, ratios of [TGlc]:[Chl a] were more than five times higher in the warmer treatments (+4°C and +6°C) compared to the +0°C treatment, primarily due to the higher abundance of dissolved Glc.

Percentages of other carbohydrates decreased relative to Glc in the course of the bloom. Quantitatively, the acidic sugar Glc-URA was the second most important carbohydrate with yields of 1-18%. The individual molar percentages of the neutral sugars Fuc, Rha, Gal, and Man/Xyl ranged between 1-12%. The deoxysugars Fuc and Rha were generally more enriched in DCCHO than in TCCHO, underlining the more refractory
character of DOM when compared to POM. Percentages of deoxysugars were ~30% of DCCHO, initially, and increased in each mesocosm before the peak of the bloom, to maximum values of 38-44%, and decreased thereafter to minimum values of 1.8-9.5%. The amino sugar Glc-AM comprised about 1.5-3.6% of carbohydrates. Glc-AM had generally higher percentages in TCCHO than in DCCHO, indicating that particulate Glc-AM was either less solubilised than the other carbohydrates, or dissolved Glc-AM more rapidly incorporated into particles. The acidic sugar Gal-URA was below the detection limit in most samples and contributed < 2% to total carbohydrates.

Acidic polysaccharides represent a characteristic fraction of transparent exopolymer particles (TEP), which are determined by staining with the acidic-sugars-specific dye Alcian Blue (Alldredge et al. 1993). TEP production during this mesocosm study was relatively low, yielding an overall average concentration of 193±131 µg Xeq. L⁻¹ (Wohlers et al. 2009). TEP concentration sharply increased in the +6°C mesocosm (M1 and M2) after day 20. In these mesocosms, TEP concentration was significantly correlated to the mole percentage of Glc-URA (p<0.001, data not shown).

**Dissolved amino acids (DAA)**

In all mesocosms, total dissolved amino acids (DAA), including dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA), started with an average concentration of 1.72±0.67 µmol L⁻¹, and increased continuously at an average rate of about 0.79±0.20 nmol L⁻¹ h⁻¹ (n=99, r²=0.15, p<0.001) (Fig. 5) until the end of the study. The overall average concentration of DAA was 2.23±0.79 µmol L⁻¹ (n=99). DFAA were determined separately for one mesocosm (+6°C M2, +4°C M4, +2°C M5, +0°C M7) per temperature treatment. There, DFAA concentrations ranged between 80 and 360 nmol L⁻¹ (n=81) and comprised between 5 and 20% of DAA. Concentrations of DFAA in the
mesocosms fluctuated over time, but did not exhibit significant differences between the temperature treatments (ANOVA, p=0.30).

In general, the molecular composition of DAA was quite similar in all mesocosms and not significantly related to the temperature treatment (ANOVA, p>0.05). DAA mole composition in all mesocosms was dominated by neutral compounds, with Gly and Ala having the highest contributions of 15% and 14%, respectively, followed by the hydroxilic amino acid Ser (~13%) and the acidic amino acid Glu (~10%). The lowest contribution was due to the aromatic amino acid Phe.

The mole composition of amino acids reflects the diagenetic state of organic matter, which can be estimated by the degradation index (DI) of Dauwe and Middelburg (1998, see also Dauwe et al. 1999). For the calculation of DI from DAA in this study, mole percentages of amino acid were standardized using averages, and standard deviations as given in Dauwe et al. (1999) and multiplied with factor coefficients as determined by these authors after Principal Component Analysis (PCA) of 28 marine samples of different diagenetic state. The DI represents the cumulative variation of amino acids relative to the average composition of the 28-sample dataset weighed by the factor coefficient of the first axis. Lower values indicate more degraded, higher values rather fresh organic material. DI values typically range between +2 and -2. During this study, DI values were generally >0, reflecting the freshness of organic matter produced during the phytoplankton bloom (Fig. 6). A significant change in DI over the duration of the experiment was observed for the two mesocosms of the +6°C treatment solely. Here, a linear decline of DI indicated the proceeding degradation of organic matter (p<0.001). During the post-bloom phase, i.e. after day 17, DI values of the +6°C and +4°C treatments were significantly lower than those of the +0°C treatment (p<0.001), indicating that DAA were more degraded at higher temperatures.

*Contribution of CHO and AA to carbon and nitrogen cycling*
Averaged over all observations, TCCHO contributed 39±28% to TOC (calculated as sum of POC and DOC)\(n=194\), DCCHO 24±23% \(n=194\) to DOC, and particulate polysaccharides (PCCHO, calculated by subtracting DCCHO from TCCHO), comprised 57±44% of POC \(n=181\). Polysaccharide production contributed largely to the build-up of DOC during the course of the bloom, yielding a non-DCCHO background DOC concentration of about 120 µmol L\(^{-1}\) (data not shown). Besides polysaccharides, dissolved free carbohydrates (DFCHO) are an important carbohydrate source for heterotrophic organisms. DFCHO were not determined during this study, and are thus not included in the estimation of fresh DOC and DON. However, we can assume that DFCHO were quantitatively negligible for DOC accumulation compared to the observed high concentrations of DCCHO. In contrast to DCCHO, DFCHO are low molecular weight compounds, consisting of mono- and dimeric carbohydrates that can be taken up by the heterotrophic cell without prior enzymatic cleavage. Therefore, DFCHO are generally turned over rapidly in the presence of heterotrophic microbes, and concentrations of DFCHO are often <100nM (Rich et al., 1996; Skoog et al., 2002).

Before the onset of the phytoplankton bloom, the average molar C:N ratio of DCCHO was 24±7 in all mesocosms. Due to the high amount of Glc released into DOM, the C:N ratio of DCCHO clearly increased during and after the bloom, yielding maximum ratios of 450-500 on individual days in the +6°C and +4°C mesocosms. The overall average C:N ratio of DCCHO was 122±125 \(n=194\). Temperature significantly affected the C:N ratio of DCCHO (ANOVA p<0.001), leading to higher values in the warmer mesocosms (+4°C and +6°C).

For TCCHO, the C:N ratio was generally lower than for DCCHO, and yielded an overall average value of 91±77 \(n=194\). This can be explained by the higher mole percentages of amino sugars in TCCHO than in DCCHO (Table 2).
TCCHO was 8.7±1.1 and increased in the course of the phytoplankton bloom to maximum values of 300-360.

Amino acids contributed on average 5.4±2.4% to DOC and 35±15% to DON. The average C:N ratio of DAA during this study was 3.2±0.26 (n=186). This ratio did not change significantly over time and was not affected by the temperature treatment (ANOVA, p>0.05).

During this mesocosm study, DCCHO and DAA were determined as major components of freshly produced, labile to semi-labile DOM. In order to identify the role of polysaccharides and amino acids in DOM dynamics, we calculated the total sum of carbon and nitrogen in the identified molecular components of DCCHO and DAA, and refer to this as iDOC and iDON.

In all mesocosms, new DOM was produced shortly after the depletion of nitrate (Fig. 7). Initially, iDOC contributed on average 11.7±3.6% to DOC, and increased to maximum values at the onset of the post-bloom phase. Maximum percentages of iDOC of about 90% DOC were observed in the +4°C treatments. The average concentration of iDOC was significantly different between the temperature treatments (ANOVA, p<0.001), yielding 61, 122, 175, and 132 µmol C L⁻¹ in the +0, +2, +4, and +6°C treatment, respectively. Due to the much higher accumulation of DCCHO than of DAA, molar C:N ratios of freshly produced DOM* increased strongly during the experiment and yielded maximum ratios of 39, 65, 87, and 74 for the +0, +2, +4 and +6°C treatments, respectively. This induced an increase in C:N ratios of total DOC from an average initial value of 6.3±1.0 to maximum values of 28, 32, 41, and 29 for the +0, +2, +4 and +6°C treatments, respectively.
Discussion

This study investigated how an increase in sea surface water temperature of boreal seas, such as those projected in the context of global warming until the year 2100, may affect the production and composition of labile DOM during phytoplankton blooms. The scenario chosen for this experimental study can be described as a high light, high nutrient, new production system, such as is typical for phytoplankton spring blooms at higher latitudes.

*Temperature effects on polysaccharide production and release*

Phytoplankton growth and productivity are co-determined by light, nutrients and temperature (Verity 1981, Geider 1987, Schofield et al. 1998). Temperature is considered to affect the synergy between electron transport and carbon fixation in algal photosynthesis (Verity 1981). The photosynthetic response to temperature depends on the amount of PAR, and is different between sub-saturating and saturating light levels (Davison, 1991). Carbohydrates are the primary product of photosynthesis and play a central role in cell growth and metabolism. Investigations of temperature effects on the release of organic carbon or carbohydrates from the cell are scarce, and limited to phytoplankton culture studies conducted under nutrient replete conditions (Verity 1981, Wolfstein and Staal 2002, Claquin et al. 2008). Carbohydrates are often exuded from phytoplankton cells in form of high molecular weight polysaccharides (>1kDa) (Myklestad et al. 1972, Biddanda and Benner, 1994) supposedly due to an imbalance of nutrient acquisition and primary production (Wood and Van Walen 1990, Engel et al. 2004). Dissolved polysaccharides can aggregate in the extracellular medium to form TEP. Claquin et al. (2008) reported that TEP formation in nutrient replete phytoplankton cultures increased with temperature until an optimum of about 22 – 25°C.

During this study, we observed that total combined Glc (TGlc) sharply increased together with Chl a concentration in all temperature treatments. Higher temperatures led to an
earlier onset of the bloom development and of polysaccharides production, and to a faster and more intense partitioning of polysaccharides into DOM. During the rise of the bloom, the rate increase of Chl a-normalised TGlc concentration was higher in the warmer treatments, suggesting a more efficient photosynthesis of the phytoplankton community. This interpretation agrees with earlier observation from phytoplankton cultures showing that the Chl a specific maximum primary production rates increased with temperature under high light condition (Verity, 1981, Schofield et al. 1998). Schofield et al. (1998) suggested that a more efficient photosynthesis at higher temperatures could be attributed to the higher activity of enzymes involved in the dark reactions, yielding a higher production of carbohydrates per unit Chl a. On the other hand, carbohydrates are the main substrates for cellular respiration, which has been shown to increase with temperature in both autotrophic (Verity, 1982), and heterotrophic plankton (Hoppe et al. 2008). Wohlers et al. (2009) reported that the community respiration rate during this mesocosm study also increased with temperatures. Higher respiration of carbohydrates by the microbial community was likely the major loss for carbohydrates in this study and may explain the lower yield of TC CHO in the warmer mesocosms.

The faster and higher partitioning of carbohydrates into DOM during our study suggests that temperature not only affected the rate of processes involved in carbohydrate production and respiration, but also those of organic matter release. A potential explanation may be given in line with the ‘overflow-hypothesis’ that considers exudation as a release of excess photosynthetic products (Wood and VanValen 1990, Schartau et al. 2007). Because the rate of nutrient draw down was similar in all mesocosms, a faster accumulation of carbohydrates at higher temperatures could increase the intracellular amount of excess organic carbon, and therefore the exudation rates of polysaccharides.
In general, the percentage extracellular release (PER) of polysaccharides from phytoplankton in marine systems varies strongly between phytoplankton species and environmental conditions (see Carlson, 2002 for review). Up to 75% of primary production can be released by the phytoplankton cell as DOC (Baines and Pace 1991, Wolfstein et al. 2002), with the largest fraction in form of polysaccharides. Studying the extracellular carbon release by phytoplankton in the Southern Ocean, Moran et al. (2006) showed that a warming by 2°C resulted in a ~54% increase in PER during short-term incubations. Our results strongly support the assumption that phytoplankton exudation is temperature sensitive. Here, a temperature rise from 2°C to 8°C clearly accelerated and increased the release of polysaccharides into DOM, and enabled an earlier, faster and higher supply of labile DOM to the heterotrophic microbial community.

Temperature effect on amino acid production and release

Amino acids represent the largest identifiable pool of DON and typically range between 0.15 to 4.20 µmol L⁻¹ in marine systems (Bronk 2002, Kaiser and Benner 2009). DAA are the major resource for the growth of heterotrophic microbes (Rosenstock and Simon 1993, Carlson 2002, Bronk 2002, Simon and Rosenstock, 2007) and have been shown to supply ~50% of the bacterial nitrogen demand in coastal and estuarine systems (Keil and Kirchman 1993, Middelboe et al. 1995). In addition, some phytoplankton species have been shown to possess ectoenzymes that cleave NH₄⁺ from amino acids for subsequent uptake by the autotrophic cell (Palenik and Morel 1990).

DFAA are released from organisms by diffusive leakage, cell break-up by grazing or result from enzymatic cleavage of DCAA (Smith et al. 1992). During this study no significant differences were observed in DFAA concentration over time or between temperature
treatments. This suggests that the overall balance of production and loss processes for DFAA was similar in all mesocosms, and may rather be related to inorganic and particulate organic nitrogen cycling, which did not display temperature sensitivities during this study either. However, DFAA are particularly labile components of marine DOM. Because DFAA are small enough to be directly taken up by microbes, their turn-over times range within a few hours, which is smaller than the sampling interval applied during this study. Therefore, differences between mesocosms with respect to DFAA production and consumption rates could not be resolved here.

During this study the identifiable DAA represented on average 35±15% of DON. This value is higher than observed in field studies, where DAA percentages of DON were reported to vary between 1 and 13% (Bronk 2002), and emphasizes the fresh nature of DOM in our mesocosms. Concentrations of DAA increased continuously during the bloom, due to the accumulation of DCAA. Processes of DAA production were not determined directly during this study. However, we assume that active exudation from phytoplankton was likely not the major source for DCAA, because this would constitute the loss of a limiting resource as nitrogen depletion was observed in the course of the study. Grossart et al. (2007) did not observe an accumulation of DCAA during mesocosm experiments with phytoplankton cultures and bacterial assemblages, despite an increase in DFAA. Another source of DCAA is the release from microbes during zooplankton grazing, and viral lysis (Bronk 2002). Because the accumulation of DCAA during this study did not differ significantly between the treatments, processes responsible for DCAA production either did not respond to rising temperature, or loss processes, e.g. bacterial consumption of DCAA, responded likewise. A close coupling between DCAA production and subsequent consumption by phyto- and bacterioplankton has been observed previously in other studies (Kawasaki and Benner 2006, Grossart and Simon 2007).
We did notice a significant diagenetic change in amino acid composition over time in the mesocosms with the warmest treatment (+6°C), which points to an intense microbial processing of DAA. This may partly be due to the longer duration of the post-bloom period in the +6°C treatment, giving the heterotrophic community more time to evolve. Thingstad et al. (2008) suggested that more nutrients get recycled in mature microbial food-webs, having a higher abundance of microzooplankton. As a consequence of a higher availability of regenerated nutrients, bacteria are then better able to degrade freshly produced DOM. The decline of DCCHO concentration in the +6°C mesocosms towards the end of the study likely reflected such an intensified microbial degradation of fresh DOM.

Consequences for carbon and nitrogen cycling

Marine microbes, and heterotrophic bacteria in particular, are the major sink of fresh DOM in the ocean. It has been suggested that marine bacteria are sensitive to global warming, but that individual processes such as bacterial respiration (BR) and bacterial biomass production (BBP) may respond differently to temperature (Vazquez-Dominguez et al. 2007, Kritzberg et al. 2010). The response of bacterial growth efficiencies (BGE) to temperature, with BGE= BBP/ (BBP+BR), is co-determined by the quality of the organic resources (delGiorgio and Cole, 1998). Consumption of carbon-rich substrates, such as carbohydrates, may enhance bacterial respiration and thus lower BGE, while nitrogen-rich compounds, such as amino acids, stimulate cell growth and increase BGE (Kroer 1993).

During this study, we observed that rising seawater temperature in a range of 2°-8°C selectively enhanced the release of polysaccharides during phytoplankton blooms that are terminated by nutrient depletion. The higher release of polysaccharides induced a rapid increase in the C:N ratio of fresh DOM. Warming also increased community respiration,
resulting in a reduced net draw-down of DIC over the course of the bloom (Wohlers et al. 2009). The stimulation of bacterial respiration has also been observed earlier in other experimental studies that simulated effects of sea surface warming on bacterioplankton communities (Vazquez-Dominguez et al. 2007, Hoppe et al. 2008, Kritzberg et al. 2010).

We therefore conclude that warming leads to a selective accumulation of carbon rich components of DOM, as a result of a faster extracellular release of carbohydrates by phytoplankton. This, in turn, could result in accelerated microbial respiration and hence carbon dioxide liberation. This scenario may be specifically applicable to boreal areas with present day temperatures of <4°C and at times when phytoplankton blooms are terminated by nutrient limitation. Along with the enhanced release of DOC from phytoplankton cells, one may expect a reduction in the C:N ratio of sinking particles, as was indeed observed during this mesocosm experiment (Piontek et al. 2009).

In response to an increased availability of labile carbon, and hence of energy, marine bacteria are better capable to utilizing inorganic nitrogen resources (Kirchman et al., 1990; Goldman and Dennett, 1991, Kirchman, 2000, Thingstad et al., 2008). A higher supply with fresh DOC during warming may thus enhance the resource competition between heterotrophic bacteria and phytoplankton, and may even exacerbate nutrient limitation of phytoplankton. A presently inadequate supply of labile DOC has been suggested to co-limit marine bacteria in polar seas, in addition to temperature (Kirchman et al. 2009). If our experimental findings are applicable to polar microbial systems, the predicted increase in sea surface temperature by 2-6°C (IPCC 2007) may alleviate carbon limitation of polar bacteria through an enhanced availability of dissolved carbohydrates. This process may become particularly important as the bacterial carbon demand also increases with temperature (Pomeroy and Wiebe 2001).
Applying a pelagic food-web model, Laws et al. (2000) identified temperature as a major control of the ratio of new, respectively export, production to regenerated production (e/f-ratio) in various oceanic provinces; in warmer seas, a larger fraction of primary production is supported by regenerated nutrients and does not contribute to export fluxes. The regeneration of nutrients in pelagic food-webs is primarily controlled by the microbial loop, i.e. the cycling of elements and energy through processes of extracellular release of primary production, subsequent bacterial utilization of DOM, and grazing by heterotrophic nanoflagellates (Azam et al. 1983). If the bottom-up control of the microbial loop in the ocean changes in response to global warming, such as suggested from our mesocosm study, nutrient regeneration in the future surface ocean may increase, potentially reducing e/f ratios.

However, warming is only one factor in the complex of environmental changes in the ocean that are expected to proceed rapidly in the wake of climate change. Differences in stratification depth and intensity may occur and will alter the supply of nutrients to the surface ocean, as well as the light regime experienced by the phytoplankton communities. Uptake of anthropogenic CO$_2$ by the surface ocean has induced the carbonation as well as the acidification of seawater. In a recent study, Piontek et al. (2010) demonstrated that ocean acidification accelerates the decomposition of polysaccharides by bacterial extracellular enzymes. Thus, future changes in the ocean may not only enhance the supply of labile DOM, in case of warming, but also accelerate its microbial metabolism, in the case of acidification, with the overall result of higher CO$_2$ remineralisation.
Acknowledgements

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Table and Figure legends

**Table 1**: Abbreviations and acronyms.

**Table 2**: Mean carbohydrate composition (% Mol) of total polysaccharides (TCCHO) and of dissolved polysaccharides (DCCHO) during phytoplankton blooms in 8 mesocosms (M1-M8) subjected to different temperature treatments. Mean values (\(\bar{x}\)) and standard deviations (SD) were calculated from 30 observations, n.d.: not detectable

**Figure 1**: Concentrations of dissolved (grey bars) and total (black bars) combined carbohydrates (CCHO) >1kDa, i.e. polysaccharides, in the course of phytoplankton blooms in 8 mesocosms under different temperature treatment.

**Figure 2a-d**: Temperature effects on the onset of DCCHO production (a), the time-lag between the onset of TCCHO and of DCCHO production (b), and the maximum yield of DCCHO (c) and TCCHO (d) during the mesocosm blooms.

**Figure 3**: Molar percentages of Glucose (Glc, %) in TCCHO (open circles) and DCCHO (solid circles) in the course of the phytoplankton blooms as indicated by changes in chlorophyll a (Chl a, solid line) concentration. Data exemplified for two replicate mesocosms of the warmest (+6°C) and coldest (+0°C) treatment.

**Figure 4**: Total glucose (TGlc) normalised to Chl a in the course of the phytoplankton blooms at +0°C (open circles), +2°C (light-gray circles), +4°C (dark-grey circles), and +6°C (black circles).
Figure 5: Increase of dissolved amino acids (DAA) concentrations, including dissolved free amino acids (DFAA) and dissolved combined amino acids (DCAA), in the course of the mesocosm experiment (p<0.001, n=99); data from all mesocosms averaged per temperature treatment. Symbols: +0°C (open circles), +2°C (light-gray circles), +4°C (dark-grey circles), and +6°C (black circles).

Figure 6: Decline in the degradation index (DI) of dissolved amino acids (DAA) in the +6°C treatment (M1 and M2) in the course of the mesocosm experiment; (p<0.001, n=47).

Figure 7a-d: Changes in the ratios of freshly produced and identified DOC (iDOC) to total DOC (iDOC:DOC, open circles), and of nitrate concentrations (µmol L⁻¹) (dotted line, right scale) in the course of the mesocosm experiments; data from all mesocosms averaged per temperature treatment.

Table 1: Abbreviations and acronyms.

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<th>AAS</th>
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<th>Glucosamine</th>
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Table 2: Mean sugar composition (% Mol) of total polysaccharides (TCCHO) and of dissolved polysaccharides (DCCHO) during phytoplankton blooms in 8 mesocosms (M1-M8) subjected to different temperature treatments. Mean values (\( \bar{x} \)) and standard deviations (SD) were calculated from 30 observations for each mesocosm; n.d.: not detectable.

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Figure 2a-d

(a) Temperature (°C) vs. Day of DCCHO increase

(b) Temperature (°C) vs. Timing of DCCHO (days)

(c) Temperature (°C) vs. Maximum yield DCCHO [µmol L⁻¹]

(d) Temperature (°C) vs. Maximum yield TCCHO [µmol L⁻¹]
Figure 3
Figure 4

Day 0 5 10 15 20 25 30 35

Glucose [µmol µg⁻¹ Chl a]
Figure 7

Graphs showing changes in DOC and nitrate concentrations over time at different temperatures:

- +6°C
- +4°C
- +2°C
- +0°C