



Warming, but not enhanced CO₂ concentration, quantitatively and qualitatively affects phytoplankton biomass

Carolin Paul*, Birte Matthiessen, Ulrich Sommer

GEOMAR Helmholtz-Centre for Ocean Research, Düsternbrooker Weg 20, 24105 Kiel, Germany

ABSTRACT: We investigated the impacts of predicted ocean acidification and future warming on the quantity and nutritional quality of a natural phytoplankton autumn bloom in a mesocosm experiment. Since the effects of CO₂-enrichment and temperature have usually been studied independently, we were also interested in the interactive effects of both aspects of climate change. Therefore, we used a factorial design with 2 temperature and 2 acidification levels in a mesocosm experiment with a Baltic Sea phytoplankton community. Our results show a significant time-dependent influence of warming on phytoplankton carbon, chlorophyll *a*, and particulate organic carbon. Phytoplankton carbon, for instance, decreased by more than half with increasing temperature at bloom time. Additionally, elemental carbon to phosphorus ratios (C:P) increased significantly, by approximately 5 to 8%, due to warming. Impacts of CO₂ or synergetic effects of warming and acidification could not be detected. We suggest that stronger grazing pressure induced by temperature was responsible for the significant decline in phytoplankton biomass. Our results suggest that the biological effects of warming on Baltic Sea phytoplankton are considerable and will likely have fundamental consequences for trophic transfer in the pelagic food web.

KEY WORDS: Phytoplankton biomass · Warming · Ocean acidification

INTRODUCTION

Ocean acidification, also known as ‘the other CO₂ problem’, is caused by an increasing uptake of CO₂ by surface water due to rising atmospheric CO₂ partial pressure. The uptake of CO₂ leads to increased aqueous CO₂, bicarbonate (HCO₃⁻), and hydrogen ion (H⁺) concentrations, while the concentration of carbonate ions (CO₃²⁻) declines. The increase of H⁺ ions has caused acidification of the surface water, with an overall decline of 0.1 pH units since the pre-industrial period (Caldeira & Wickett 2005) associated with a substantial decrease in carbonate ion concentration of 30% (Hoegh-Guldberg & Bruno 2010). As atmospheric CO₂ is predicted to rise from current values of approximately 390 ppm to values of 700 ppm by the end of the 21st century (IS92a scenario; Meehl et al. 2007), pH will decrease further by

0.3 to 0.4 U (Hama et al. 2012). Parallel to ocean acidification, sea-surface temperature has already increased by 0.6°C in the last 100 yr (Hoegh-Guldberg & Bruno 2010). A doubling of atmospheric CO₂ in the 21st century is predicted to accompany a rise in the estimated average global ocean surface temperature of 2 to 4.5°C (IPCC 2007, 2014).

So far, only a few studies have analyzed the combined effects of both factors on marine primary producers (Hare et al. 2007, Feng et al. 2008, 2009, Torstensson et al. 2012), although sea-surface pH and temperature will change in parallel in a future ‘greenhouse’ world. Instead, many studies have addressed the biological effects of either ocean acidification or warming in particular on phytoplankton species composition and biomass. These studies indicate that CO₂ can act as a stressor, in particular for calcifiers, but in cases where it is a limiting resource,

*Corresponding author: cpaul@geomar.de

it can also have a fertilizing effect. Altered survival, calcification, growth, development, and abundance have been observed across marine species in response to acidification (Kroeker et al. 2010, 2013). The magnitude of responses, however, varied significantly among species. Whereas growth of calcifying taxa was, on average, negatively affected by rising CO₂, growth of fleshy algae and diatoms increased (Kroeker et al. 2013). In natural Antarctic phytoplankton communities, Tortell et al. (2008) found an increase in growth of larger chain-forming diatoms, resulting in a species compositional shift from a dominance of small pennate diatoms (*Pseudo-nitzschia subcurvata*) to a dominance of large centric species (*Chaetoceros* spp.). Concordantly, in natural oceanic phytoplankton assemblages, Eggers et al. (2014) found a CO₂-induced increase of total phytoplankton biomass that was driven by a shift towards large-sized diatoms, especially *Chaetoceros* spp. and *Thalassiosira constricta*. In contrast to these observations, Schulz et al. (2013) observed no positive CO₂ effect on diatom biomass in a natural arctic plankton community. Instead, the pico-eukaryote biomass increased under enhanced CO₂. It should be noted, however, that the study was conducted in a post-bloom situation in which the initial diatom abundance was naturally very low (<0.5 μmol C l⁻¹).

Increasing CO₂ can also increase the efficiency of phytoplankton in the use of limiting nutrients to fix carbon. This, consequently, can result in higher elemental carbon to nitrogen (C:N) ratios (Tortell 2000). Increased C:N ratios with elevated CO₂ were also found by Eggers et al. (2014) in their experiment with natural oceanic phytoplankton communities. In natural phytoplankton assemblages from the Arctic, however, a significantly lower C:N ratio could be shown (Schulz et al. 2013). Similar to the study above (Eggers et al. 2014), higher elemental ratios of nitrogen to phosphorus (N:P) as well as carbon to phosphorus (C:P) were observed under high CO₂ for the arctic assemblage.

Increased water temperature is expected to change the distribution and abundance of phytoplankton communities as well as their phenology and productivity (Hoegh-Guldberg & Bruno 2010). Most studies that have tested warming on plankton communities have focused on the development of the spring bloom (see Lewandowska & Sommer 2010 and references therein), as this is one of the most important seasonal patterns in pelagic food webs. Mesocosm experiments with Baltic Sea spring phytoplankton showed a significant decrease in total phytoplankton biomass, as well as a shift towards picophytoplankton and

small nanophytoplankton (<5 μm) (Sommer & Lengfellner 2008, Lewandowska & Sommer 2010, Sommer & Lewandowska 2011). Both the decrease in biomass and the species shift were interpreted as footprints of more intensive grazing by copepods and ciliates under warming (Keller et al. 1999, Lewandowska & Sommer 2010). It was also observed that warming accelerated the occurrence of the phytoplankton bloom peak by approximately 1 d per degree Celsius (Sommer & Lengfellner 2008, Sommer & Lewandowska 2010). The altered growth rates observed by Eppley (1972) and Torstensson et al. (2012) indicate that increasing water temperature may also shift the competitive advantage between different algal species.

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

Addressing both factors, CO₂ and temperature, simultaneously, the meta-analysis by Kroeker et al. (2013) showed a strong trend towards lower growth rates and development at elevated temperature and CO₂, when all marine taxa are pooled together. The results also highlight a trend towards enhanced sensitivity to acidification with warming.

For phytoplankton in particular, contrasting and species-specific results have been found. The growth rate of the calcifier *Emiliania huxleyi* increased in response to higher temperature as well as to elevated CO₂, but an interaction effect among the parameters has not been found (Feng et al. 2008). In contrast, growth rates of the diatom *Navicula directa* increased by 43% under warming, but decreased by 5% under acidification (Torstensson et al. 2012). In bottle experiments with a natural North Atlantic spring bloom community, a trend of increased total chlorophyll *a* (chl *a*) was found under greenhouse conditions, i.e. increased temperature and CO₂, but no change was observed when just one of the parameters was manipulated (Feng et al. 2009). According to a bottle experiment by Hare et al. (2007), warming alone and in combination with acidification led to substantial increases in carbon fixation rates in a natural Bering Sea summer phytoplankton experiment. Further, a shift from diatom to nanophytoplankton dominance was de-

tected. However, mesocosm experiment studies based on natural plankton communities combining CO₂ and temperature are, in general, still scarce.

Our study site, Kiel Fjord, western Baltic Sea, is known as a naturally CO₂-enriched area. Here, CO₂ concentrations fluctuate strongly and are elevated during much of the year (Thomsen et al. 2010, 2013). Due to high primary productivity caused by eutrophication in this area, amplified community respiration leads to a significant consumption of pO₂ and, at the same time, to a strong increase of CO₂ below the thermocline (HELCOM 2009, Thomsen et al. 2013). Typical winds from the southwest lead to upwelling events in which CO₂-enriched bottom water is brought to the surface of the otherwise seasonally stratified coastal waters (strong temperature and salinity gradients). During summer and autumn, temporal CO₂ maxima exceed >2300 ppm, leading to a pH < 7.5. Average CO₂ in summer and autumn exceeds 700 ppm (Thomsen et al. 2010). In this respect, the Kiel Fjord may be considered an analogue for the more acidic ecosystems of the future (Thomsen et al. 2010).

We tested the combination effects of warming and acidification on autumn phytoplankton biomass by crossing the factors temperature (9 and 15°C) and CO₂ (560 and 1400 ppm) to test the following hypotheses: (1) warming leads to a decrease in biomass and an earlier bloom; (2) rising CO₂ will increase phytoplankton biomass; (3) there is a synergetic effect of future warming and acidification on biomass; and (4) the quality of phytoplankton biomass, in terms of cellular stoichiometry, is influenced by rising temperature and CO₂.

MATERIALS AND METHODS

Experimental design

In order to address our hypotheses, 2 different temperature regimes (9 and 15°C) and 2 CO₂ levels (i.e. target values 560 and 1400 ppm CO₂) were full-factorially manipulated using natural Baltic Sea phytoplankton assemblages in indoor mesocosms, each with a volume of 1400 l and a surface area of approximately 1.54 m². Each treatment combination was replicated 3-fold (n = 3). The resulting setup of 12 mesocosms (designated M1 to M12) was installed in 4 temperature-controlled culture rooms.

Prior to experimental treatments mesocosms were filled with unfiltered natural seawater (salinity: 19.7) from the Kiel Bight, western Baltic Sea. The water contained the natural autumn plankton community,

including phytoplankton (photosynthetic bacteria and algae), bacteria, and protozoa. To minimize differences among the starting community compositions and densities between the mesocosms, prior to the actual filling, water from approximately 2 m depth was gently pumped into a mixing chamber by a rotary pump. From this chamber, water was simultaneously pumped into each of the mesocosms. Mesozooplankton from net catches (Kiel Bight) was added, mimicking natural densities, i.e. 20 ind. l⁻¹. Each mesocosm was covered by a PVC cover (polyvinylchloride, light-permeable) containing a sampling port which remained closed between sampling events. After filling (19 October 2012; hereafter, Day -3), all mesocosms had similar temperatures and CO₂ contents. The following 3 d were used for applying the temperature and CO₂ manipulations and reaching divergence between the treatment levels.

The temperature regimes were 9 and 15°C, representing 3°C above and below the actual water temperature of Kiel Bight on the filling day (Day -3). Temperature deviation in a mesocosm between Day 0 and Day 21 (last experimental day) was a maximum of ±0.3°C. Maximal temperature deviation between mesocosms in the same temperature treatment was 0.3°C (for the warm treatment) and 0.4°C (for the cold treatment). In order to obtain the targeted CO₂ levels, the headspace between cover and water surface received a flow of 30 to 60 l h⁻¹ of 2 different mixtures of air and CO₂ (560 and 1400 ppm CO₂). Due to incomplete CO₂ equilibration with the headspace, mean values between Experimental Days 0 to 21 in the water were 439 ppm (SD = 187) for low CO₂ and 1040 ppm (SD = 210) for high CO₂, with maxima of 686 and 1400 ppm during the experimental runtime. The average low CO₂ value was slightly higher than the mean present-day atmospheric level. However, as mentioned before, surface water in the Kiel Bight, on average, exceeds 700 ppm during summer and autumn (Thomsen et al. 2010). The high CO₂ level conformed to the IPCC prediction (Scenario IS92a, atmospheric CO₂: 788 ppm) for the year 2100, when surface seawater CO₂ in the Baltic Sea is predicted to reach 1400 ppm and higher (Thomsen et al. 2010, Melzner et al. 2013). To balance the natural drawdown of CO₂ by phytoplankton production, over the course of the experiment, CO₂-enriched water was added to the high CO₂ mesocosms 3 times (Days 7, 11, and 18). For this purpose the same amount of water was taken out of each mesocosm and consecutively filtered (0.2 µm pore size). The water was then CO₂-saturated by bubbling and transferred (with a measuring cylinder, beneath the water surface) back into the mesocosms.

The required volumes were calculated on the basis of DIC (dissolved inorganic carbon) and alkalinity (Tables S1 & S2 in the Supplement at www.int-res.com/articles/suppl/m528p039_supp.pdf).

Over the course of the experiment, light was supplied by computer-controlled light units (GHL Groß Hard- und Softwarelösungen; Lampunit HL3700 and ProfiluxII). Each light unit consisted of 5 HIBay-LED spotlights (purpose-built item of Econlux, 100 W each). Above each of the mesocosms 1 light unit was installed. Daily irradiance patterns were computer controlled (GHL, Prometheus) and stayed constant over the course of the experiment. The light–dark cycle was 11 h 50 min:12 h 10 min. Light supply and day length were aligned to the seasonal light patterns calculated in the astronomic model of Brock (1981), which conformed to 50% of solar irradiance of an approximated cloudless 21 September. Daily maximum light intensity was $252 \mu\text{mol m}^{-2} \text{s}^{-1}$, measured in the middle of the water column (0.7 m below the PVC cover).

An automatic and gently moving propeller reduced phytoplankton sedimentation, assured its homogeneous distribution, and simulated natural water movement. The experiment ended after 24 d, when the phytoplankton bloom was over. Additionally, wall growth of periphytic microalgae (patches of a thin biofilm in all mesocosms) and sedimentation (mainly material remaining from the bloom, which appeared long after the bloom peak in the final days of the experiment) became visible; if the runtimes had been longer, this would potentially have influenced the carbon balance and nutrient availability for phytoplankton.

Sampling and measurements

Water temperature, salinity, and pH were measured daily. For pH measurements the electrode was calibrated daily using standard pH buffers (pH 3, pH 7, pH 9; WTW). At least 1 h prior to measurements the electrode was placed in the climate room to adapt to the given temperature. Samples for phytoplankton biomass variables, i.e. relative fluorescence (as a control, data not shown here), particulate organic carbon (POC), chlorophyll *a* (chl *a*), and phytoplankton carbon (microscopy and flow cytometer) were taken 3 times a week (Monday, Wednesday, Friday), resulting in a total of 10 samplings over the course of the experiment. Samples for inorganic dissolved nutrients, particulate organic phosphorus (POP), particulate organic nitrogen (PON), and as such for determining ratios among the particulate

elements (i.e. C:N, C:P, N:P), were also taken 3 times a week.

Carbonate system

For measurements of total DIC, 10 ml samples were filled into a glass vial (Resteck) using a peristaltic pump with a flow rate of 6 ml min^{-1} . The intake tube of the pump contained a single-use syringe filter ($0.2 \mu\text{m}$, Sartorius). Filtered samples were poisoned with saturated HgCl_2 solution (20 μl), the vial was crimped with a headspace $<1\%$, and stored in the dark at 4°C . DIC was measured following Hansen et al. (2013) using a SRI-8610C (Torrence) gas chromatograph. For total alkalinity (TA), 25 ml samples were filtered (Whatman GF/F filter, $0.2 \mu\text{m}$) and titrated at 20°C with 0.05 M HCl solution (Dickson 1981, Dickson et al. 2003) in an automated titration device (Metrohm Swiss mode). The remaining carbonate parameter $p\text{CO}_2$ was calculated using $\text{CO}_2\text{-SYS}$ (Pierrot et al. 2006) and the constants supplied by Hansson (1973) and Mehrbach et al. (1973), which were refitted by Dickson & Millero (1987), and the KSO_4 dissociation constant from Dickson (1990).

Measures of phytoplankton biomass

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

Abundance of small phytoplankton ($<5 \mu\text{m}$) was assessed by a flow cytometer (FACScalibur, Becton Dickinson) immediately after sampling, distinguished according to cell size (spherical diameter, FSC value) and pigment fluorescence (chl *a* and phycoerythrin). Larger phytoplankton ($>5 \mu\text{m}$) were counted microscopically (>100 ind. for common taxa) from Lugol-fixed samples in Utermöhl chambers using an inverted microscope (Utermöhl 1958). Phytoplankton carbon was calculated by first converting cell abundances obtained from flow cytometry and microscopy to biovolume by multiplying cell numbers with linear measurements taking the nearest geometric standard (Hillebrand et al. 1999). Biovolume was then converted into carbon content according to

Menden-Deuer & Lessard (2000), i.e. $C = 0.288V^{0.811}$ for diatoms and $C = 0.216V^{0.939}$ for other phytoplankton (C is carbon content in pg, V is cell volume in μm^3). As $180 \mu\text{m}^3$ is the smallest cell size included in the analysis of Menden-Deuer & Lessard (2000), their non-linear models predict unrealistically high C for smaller algae. Therefore, conversion factors of $0.108 \text{ pg C } \mu\text{m}^{-3}$ for diatoms and $0.157 \text{ pg C } \mu\text{m}^{-3}$ for all other organisms were used for phytoplankton cells $<180 \mu\text{m}^3$ (Sommer et al. 2012b).

Particulate organic matter

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

Dissolved inorganic nutrients

For nitrate/nitrite ($\text{NO}_3^-/\text{NO}_2^-$), ammonium (NH_4^+), silicate (SiO_4^-), and phosphate (PO_4^-) 20 ml water was filtered through cellulose acetate filters (Sartorius) and immediately frozen at -20°C . Samples were measured following the protocols of Hansen & Koroleff (1999).

Growth rate

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

$$V = a / \{1 + [(a - b) / b] \times 2.71 \times (-c \times t)\}$$

where V indicates the measure of biomass, t is time, a is maximum biomass (i.e. carrying capacity), b is initial biomass, and c is growth rate. For this purpose, data from the first sampling day until the day after maximum biomass were used. Bloom time was defined as the time, i.e. the day, of highest biomass of each single mesocosm. From this, mean values of bloom time were calculated for the 4 treatments. For maximum biomass, the highest measured value (during bloom) of each mesocosm was taken, independent of the experimental day.

Data analysis

In order to test for treatment effects and to account for possible time dependence of the measured response variables (phytoplankton carbon, chl *a*, POC, C:N, C:P, N:P) a generalized least squares (gls) model (nlme package, R) with the factors time (continuous), temperature and CO_2 (both categorical), and the interactions $\text{CO}_2 \times$ temperature, time \times temperature, and time \times CO_2 was applied. Prior to analyses, the optimal variance-covariate structure was determined by using maximum-likelihood (ML) estimation. All model residuals were checked for normality and transformed if required. Potential heterogeneity of variances was tested using the Fligner test. Prior to conducting the gls we also tested the above-mentioned factors, including their interactions, using a linear mixed effect model; this model particularly allows an additional test of the effect 'mesocosm.ID' on all response variables using the nlme-package in R (Pinheiro et al. 2013). As no random mesocosm identification (ID) effect could be detected ($\text{SD} < 0.5$), we decided to apply the gls for consecutive analyses.

To account for resulting significant interactions among the manipulated factors and time a 2-way ANOVA with the factors temperature and CO_2 and their interaction was calculated on growth rate, maximum biomass, and on bloom peak time, for all measures of biomass. All statistical analyses were conducted using R, Version R386 3.1.0 (R Development Core Team, R Foundation for Statistical Computing).

All data shown here are openly accessible from the database PANGAEA (doi.10.1594/PANGAEA.840852).

RESULTS

The measured initial pH (Day -3) in all treatments was 7.8, with the exceptions of M11 (cold, low CO_2 ; pH 8.0) and M1 (warm, low CO_2 ; pH 7.63). Over the course of the experiment pH increased under low CO_2 conditions (grand mean over time course and replicate mesocosm: 7.97, $\text{SD} = 0.16$) and decreased under high CO_2 conditions (grand mean \pm SD over time course and replicate mesocosm: 7.61 ± 0.12) (Fig. 1A). The calculated $p\text{CO}_2$ increased in all acidified mesocosms up to sampling Day 7 (Fig. 1B). The decrease of $p\text{CO}_2$ from Day 9 on motivated us to add CO_2 -enriched water, which is reflected by the subsequent fluctuations in $p\text{CO}_2$ and pH (Fig. 1). Mean $p\text{CO}_2$ values (grand means \pm SD over time course and replicate mesocosms) were 439 ± 187 ppm for low CO_2 and 1040 ± 210 ppm for high CO_2 , respectively (Fig. 1B).

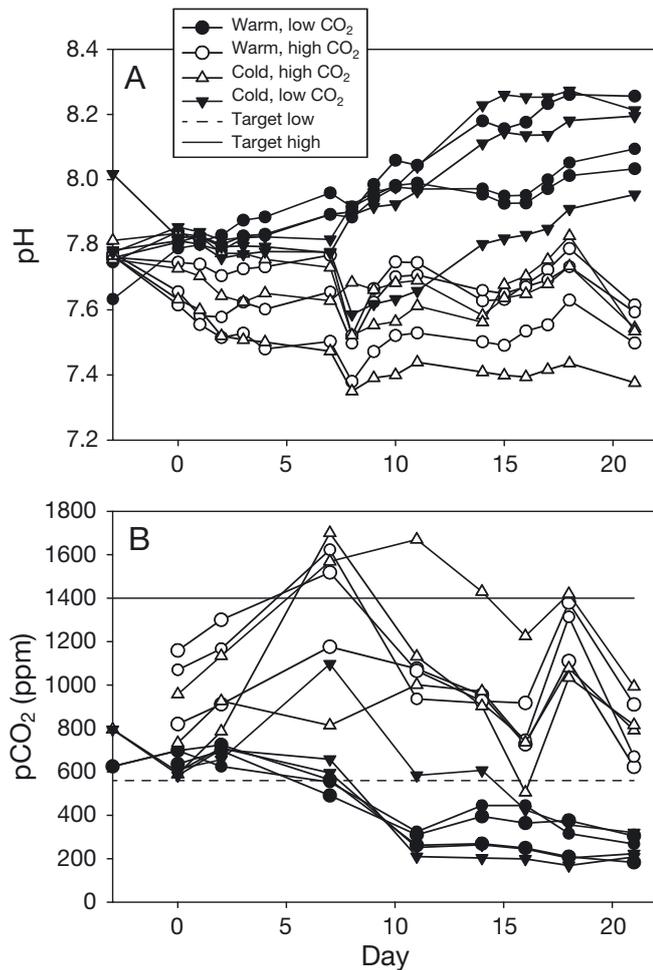


Fig. 1. Time course of (A) pH and (B) $p\text{CO}_2$ in ppm in each of the replicated mesocosms (3 replicates per treatment). Symbols for the treatment combinations as in key. Target lines in (B) represent target values for low CO_2 (560 ppm) and high CO_2 (1400 ppm)

Growth and biomass of phytoplankton

Time course

All measures of biomass (i.e. phytoplankton carbon, chl *a*, POC) were affected by experimental time due to the build-up of blooms, and significantly decreased in response to the warming treatment. The latter effect, however, depended on the time of the experiment (significant interaction term time \times temperature; Table 1, Fig. 2A–C). In other words, warming negatively affected phytoplankton biomass during the bloom, but not at times of low biomass in the post-bloom situation. Maximum values of phytoplankton carbon (but not chl *a* or POC) were marginally significantly lower in the warm treatments compared to the cold ones (Table 2, Fig. 3). CO_2 did not affect phytoplankton biomass as a main or inter-

Table 1. Results of generalized least-squares model (gls) testing for the effects of temperature (T), CO_2 , time, as well as the interactions T \times CO_2 , time \times T, and time \times CO_2 on phytoplankton carbon, chl *a*, particulate organic carbon (POC), C:N, C:P, and N:P. Significant results are in **bold**; * $p < 0.05$, *** $p < 0.001$

Response variable Factor	df	<i>t</i> -value	<i>p</i>
log phytoplankton C ($\mu\text{g l}^{-1}$)			
T	132	0.996	0.321
CO_2	132	-0.916	0.361
Time	132	9.504	<0.001***
T \times CO_2	132	-0.005	0.996
Time \times T	132	-3.694	<0.001***
Time \times CO_2	132	-0.899	0.371
log chl <i>a</i> (mg m^{-3})			
T	120	1.197	0.234
CO_2	120	0.436	0.663
Time	120	4.856	<0.001***
T \times CO_2	120	0.005	0.996
Time \times T	120	-2.002	0.047*
Time \times CO_2	120	-0.673	0.502
log POC ($\mu\text{g l}^{-1}$)			
T	119	1.843	0.068
CO_2	119	1.453	0.149
Time	119	5.699	<0.001***
T \times CO_2	119	-1.428	0.156
Time \times T	119	-3.354	0.001***
Time \times CO_2	119	-1.876	0.063
C:N			
T	118	1.034	0.303
CO_2	118	0.359	0.720
Time	118	2.100	0.038*
T \times CO_2	118	-1.316	0.191
Time \times T	118	-0.126	0.900
Time \times CO_2	118	0.086	0.931
C:P			
T	119	2.190	0.031*
CO_2	119	-0.179	0.858
Time	119	-0.249	0.804
T \times CO_2	119	0.480	0.632
Time \times T	119	-2.197	0.030*
Time \times CO_2	119	0.741	0.460
N:P			
T	118	0.140	0.900
CO_2	118	0.969	0.335
Time	118	-1.290	0.200
T \times CO_2	118	0.135	0.892
Time \times T	118	-0.321	0.749
Time \times CO_2	118	-0.283	0.778

action effect with temperature or time (Tables 1 & 2, Figs. 2A–C & 3).

The bloom time of phytoplankton carbon met our expectation that warming would lead to a significantly earlier biomass peak by 2 to 3 d (Table 2, Fig. 2A). Phytoplankton carbon started below $10 \mu\text{g l}^{-1}$ (Fig. 2A). Highest values were reached under cold

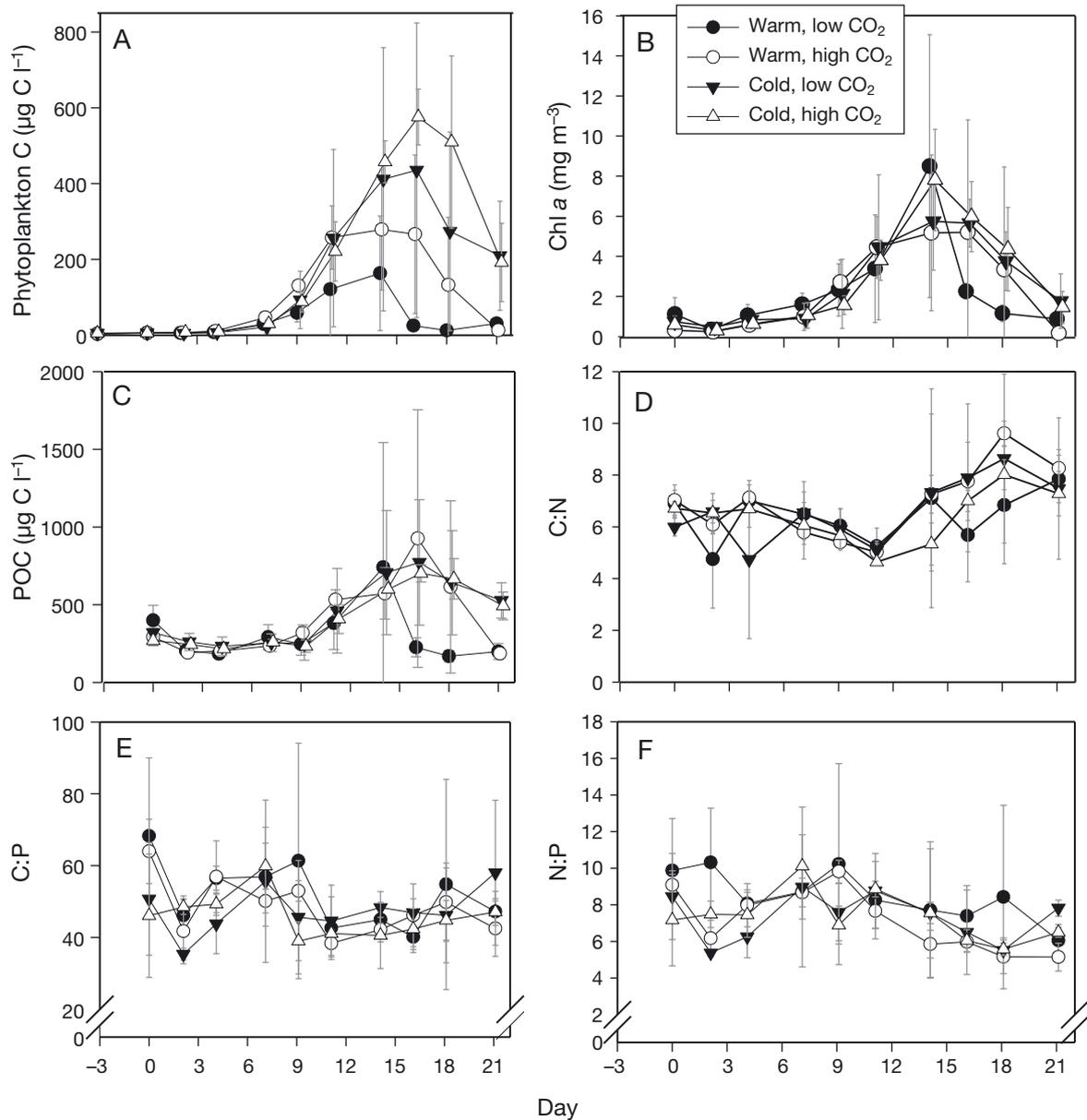


Fig. 2. Time course of (A) phytoplankton carbon ($\mu\text{g l}^{-1}$), (B) chlorophyll a (chl a, mg m^{-3}), (C) particulate organic carbon (POC, $\mu\text{g l}^{-1}$), (D) C:N, (E) C:P, and (F) N:P. Vertical error bars denote standard errors from triplicate samples. Symbols for the treatment combinations as in key

conditions on Days 16 to 18, and in the warm mesocosms, between Days 11 and 14. The other measures of phytoplankton biomass (i.e. chl a, POC) did not show an altered timing of bloom in response to the manipulated factors (Table 2, Fig. 2B,C). Chl a concentration started below 1 mg m^{-3} and reached peaks between Days 11 and 18 (Fig. 2B). POC tended to increase earlier under warm conditions (Fig. 2C). The highest values, however, were reached between Days 14 and 18 for all treatments.

Growth rate

In most of the cases the fit of the S-curve was sufficient to calculate growth rates from the start of the experiment to the peaks. There was no significant temperature or CO_2 effect on growth rates of all biomass measures (Table 2). In general phytoplankton showed 2 to 3 doublings d^{-1} during the growth phase (Fig. 4). Phytoplankton carbon and POC, however, showed a slight trend towards faster growth under

Table 2. Results of 2-way ANOVAs for the effects of temperature (T), CO₂, as well as the interaction T × CO₂ on phytoplankton carbon, chl *a*, and particulate organic carbon (POC) according to bloom time, maximum values, and growth rates per day. Significant results are in **bold**; *p < 0.05

Response variable	Factor	df	MS	F	p
Bloom time					
Phytoplankton C	T	1.8	18.8	6.42	0.035*
	CO ₂	1.8	0.08	0.03	0.871
	T × CO ₂	1.8	0.75	0.26	0.626
Chl <i>a</i>	T	1.8	4.08	0.92	0.364
	CO ₂	1.8	0.75	0.17	0.691
	T × CO ₂	1.8	2.08	0.47	0.511
POC	T	1.8	16.33	3.06	0.118
	CO ₂	1.8	5.33	1.00	0.347
	T × CO ₂	1.8	8.33	1.56	0.247
Maximum values					
Phytoplankton C (µg l ⁻¹)	T	1.8	21428	5.14	0.053
	CO ₂	1.8	96380	2.31	0.167
	T × CO ₂	1.8	4916	0.12	0.740
Chl <i>a</i> (mg m ⁻³)	T	1.8	22.3	1.78	0.219
	CO ₂	1.8	10.6	0.85	0.384
	T × CO ₂	1.8	2.49	0.20	0.667
POC (µg l ⁻¹)	T	1.8	215548	0.87	0.377
	CO ₂	1.8	92681	0.37	0.557
	T × CO ₂	1.8	211421	0.86	0.382
Growth rate					
Phytoplankton C (d ⁻¹)	T	1.8	2.06	0.65	0.451
	CO ₂	1.8	2.67	0.84	0.395
	T × CO ₂	1.8	0.76	0.24	0.643
Chl <i>a</i> (d ⁻¹)	T	1.8	1.04	2.18	0.184
	CO ₂	1.8	0.006	0.01	0.916
	T × CO ₂	1.8	1.04	2.18	0.183
POC (d ⁻¹)	T	1.8	0.007	2.11	0.207
	CO ₂	1.8	0.010	3.26	0.131
	T × CO ₂	1.8	0.0005	0.16	0.711

warming conditions (Fig. 4A,C), but standard deviations were high. An interaction effect between temperature and CO₂ was not found (Table 1).

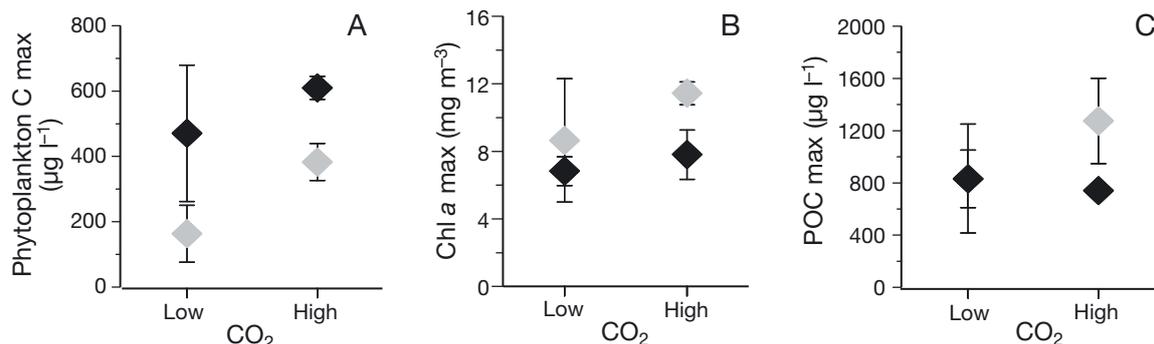


Fig. 3. Maximum values of (A) phytoplankton carbon (µg l⁻¹), (B) chlorophyll *a* (chl *a*, mg m⁻³), and (C) particulate organic carbon (POC, µg l⁻¹). Vertical error bars denote standard errors from triplicate samples. Warm mesocosms: grey symbols; cold mesocosms: black symbols

Chemical composition of phytoplankton

The C:N ratios increased significantly over the course of the experiment, independent of the manipulated factors (Table 1, Fig. 2D). The C:P ratios were significantly higher at higher temperatures, but again the effect depended on experimental time (significant interaction time × temperature; Table 1, Fig. 2E). N:P ratios were not affected by the manipulated factors or time (Table 1, Fig. 2F).

Dissolved inorganic nutrients

The average initial nitrate/nitrite and ammonium concentrations were 3.7 and 4 µmol l⁻¹, respectively. The average initial silicate concentration was 19 µmol l⁻¹. Phosphate concentration was initially 1.5 µmol l⁻¹. Dissolved nutrient concentrations started to decline at the onset of the blooms. Whereas nitrate/nitrite and ammonium were depleted in all treatments by the end of the experiments, phosphate was still available at termination. Silicate was depleted at the end only in the warm and high-CO₂ treatment. In the other treatment combinations silicate was still available. Temporal developments of all measured dissolved inorganic nutrients are shown in Fig. S1 in the Supplement at www.int-res.com/articles/suppl/m528p039_supp.pdf.

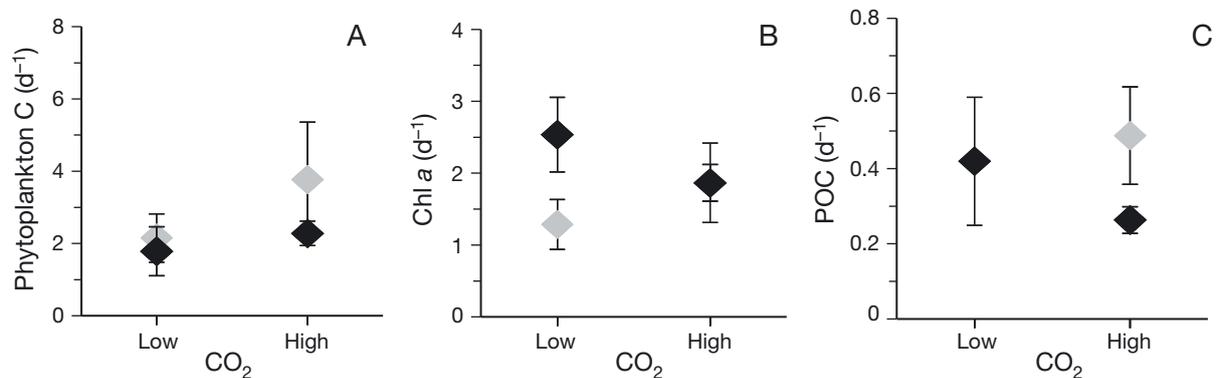


Fig. 4. Growth rate calculated from the biomass parameters: (A) phytoplankton carbon (d^{-1}), (B) chlorophyll *a* ($\text{chl } a$, d^{-1}), (C) particulate organic carbon (POC, d^{-1}). Vertical error bars denote standard errors from triplicate samples. Warm mesocosms: grey symbols; cold mesocosms: black symbols

DISCUSSION

The influence of multiple stressors related to global change, such as increasing temperatures and CO_2 concentration, was hypothesized to synergistically affect phytoplankton biomass. In our study, testing both factors on a Baltic Sea autumn bloom, however, we could show that only warming, but not CO_2 , significantly affected phytoplankton biomass, bloom time, and biochemical composition. In particular, warming led to an earlier bloom and overall decline of phytoplankton biomass, but to higher C:P ratios.

Growth and biomass of phytoplankton

One reason for the absence of the CO_2 effect might be pre-adaptation of Baltic Sea phytoplankton communities to increased CO_2 levels because of pronounced natural short-term and seasonal fluctuations in CO_2 concentrations. Natural conditions in Kiel Fjord vary by ca. 0.7 pH units, and $p\text{CO}_2$ can reach short-term peak values of 4000 ppm in summer (Thomsen et al. 2010) as a consequence of upwelling of respiration-dominated deep water. Adaptation to a wide $p\text{CO}_2$ range for coastal phytoplankton in natural acidified waters has already been suggested as an explanation of the weak responsiveness of phytoplankton to CO_2 -enrichment (Feely et al. [2008]) for the Pacific coast; Rossoll et al. [2013] for a summer Baltic Sea bloom). In fact, evolutionary adaptation via genotypic selection was shown for the calcifier *Emiliania huxleyi* after 500 generations (Lohbeck et al. 2012). The coastal Baltic Sea short-term variability by far exceeds the atmospheric signal of 700 ppm $p\text{CO}_2$ (IPCC 2014) for the end of the 21st century. Therefore, future mean conditions may not have a dramatic influence

on diatom-dominated autumn blooms. However, impacts of future maximal values, which will probably exceed present-day values, cannot be excluded. Another possible reason for the absence of a main and interaction effect of CO_2 on phytoplankton growth and biomass might be that a potential positive fertilizing effect of CO_2 , in particular on diatom biomass, remained below the level of detection, because biomass was generally kept low by the presence of grazers. Zooplankton grazing in our system might potentially have masked enhanced phytoplankton growth due to increased CO_2 concentrations. This may be supported by a CO_2 -fertilizing effect in the phytoplankton carbon data (time course, maximum phytoplankton carbon, growth rate; see Figs. 2A, 3A, 4A). Here, under both temperature treatments, biomass and growth rate tended to be higher, on average, under high CO_2 concentrations. In fact, in studies that found a positive effect of CO_2 on phytoplankton community growth or biomass, mesograzers were excluded prior to experimental treatments (Tortell et al. 2008, Eggers et al. 2014, Feng et al. 2009). A thorough test for the suggested grazer effect would be a factorial experimental design manipulating CO_2 in the absence and presence of grazers.

The earlier onset of phytoplankton blooms due to higher temperatures can be explained by the fact that temperature is a major environmental factor controlling the metabolic rates of organisms and thus the initiation of biological processes in nearly all living species (Brown et al. 2004). Although sampling every other day, instead of daily, potentially decreases the strength of our results, our findings were consistent with those of previous studies in which earlier bloom times followed an increase in temperature (Sommer & Lengfellner 2008, Lewandowska & Sommer 2010).

The time-dependent biomass decrease in phytoplankton due to increased temperature, i.e. a temperature effect during bloom, largely matches the assumption that temperature has the potential to strengthen grazing because it is known to more strongly enhance heterotrophic than autotrophic processes (O'Connor et al. 2009). Our results also match those of the majority of studies published so far from the same geographical region that have investigated the temperature effects on spring blooms (Lewandowska & Sommer 2010, Sommer & Lewandowska 2011, Sommer et al. 2012a). Under spring conditions, and likewise the autumn conditions presented in this study, nutrient conditions were sufficient to ensure favorable growth conditions. At the same time, the elevated temperature increased copepod grazing rates and changed the bottom-up to top-down control of the phytoplankton biomass (Keller et al. 1999, Lewandowska & Sommer 2010, Sommer et al. 2012a). Indeed, in our experiment, the development of copepods (Garzke 2014, PANGAEA accession number, doi:10.1594/PANGAEA.842399) was faster at warmer temperatures. The metabolic demands of heterotrophs and hence feeding rates are known to rise with temperature (Brown et al. 2004, O'Connor et al. 2009), which underpins the suggested increased grazing pressure and, in that way, the observed lower phytoplankton biomass. However, as mentioned before, it was unfortunately not possible to verify this effect with the present experimental design.

Changes in species composition during bloom as a possible reason for the observed changes in biomass can be excluded. The most dominant taxa (diatoms and cryptophytes, representing on average 83 and 10.5% of the total biovolume, respectively) and the species with the highest cell abundance (*Skeletonema marinoi*, *Teleaulax acuta*) showed no significant response to temperature or CO₂ (Sommer et al. in press, PANGAEA accession number, doi:10.1594/PANGAEA.840845). Only pico-plankton, e.g. picochlorophytes and pico-cyanobacteria, showed a significantly higher abundance due to warming, but their contribution to total biomass was very low (<1%).

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

In contrast, increasing biomass with warming was found for 2 experimental early summer blooms (Taucher et al. 2012, Lewandowska et al. 2014), as well as for a Baltic Sea long-term field study (1979–2011) by Suikkanen et al. (2013). In summer conditions, nutrient concentrations are naturally low. Lewandowska et al. (2014) suggest that under such conditions phytoplankton are mainly controlled by the rate of nutrient delivery via reduced mixing and not by grazing. Warming under such conditions has a positive effect on phytoplankton biomass. Suikkanen et al. (2013) also suggested that warming was the key environmental factor explaining the general increase in total phytoplankton biomass in northern summer Baltic Sea communities in recent decades.

To the best of our knowledge, our study is one of the first to compare 3 different biomass parameters in response to manipulated climate change. Here we proved a time-dependent temperature effect for all 3 proxies (chl *a*, phytoplankton carbon, POC), but with unequal strengths. The inevitable shortcomings of the various parameters should be borne in mind when attempting to explain these differences. Actual phytoplankton might have different cell volume to carbon relationships, as shown in the data base of Menden-Deuer & Lessard (2000), and these may have affected our measure of microscopically derived phytoplankton carbon. POC contains considerable non-phytoplankton carbon (detritus, bacteria, heterotrophic protists), and the chl *a* content of biomass is subject to taxonomic and physiological variability (Moline & Prezelin 2000), i.e. this might have led to an underestimation of diatom biomass.

Chemical composition of phytoplankton

We did not find a significant CO₂ effect or an interaction effect of warming and acidification on the C:N:P ratios. This may be due to the pre-adaptation of phytoplankton to high CO₂ levels in the Baltic Sea and might explain the difference compared to studies of oceanic phytoplankton communities (Tortell 2000, Eggers et al. 2014) which found significantly increased C:N ratios in response to increased CO₂.

Instead, warming led to greater carbon accumulation per unit phosphorus (increased C:P ratio), i.e. higher temperatures seem to allow phytoplankton to yield a higher C-based biomass per unit P. Reasons could be physiological, as C accumulation might have been faster than P accumulation under warming conditions, due to the metabolic stimulation of

carbon uptake processes. Another explanation could be temperature-induced higher POC content, as shown by De Senerpont Domis et al. (2014); this, however, was not found in our experiment. An increase in POC was potentially masked by high grazing pressure in warm treatments. On the other hand, the POP content did not differ between treatments (gls; $t \geq -1.3$; $p \leq 0.16$), indicating that P uptake processes were not stimulated by temperature. De Senerpont Domis et al. (2014) also explained the observed higher C:P ratios by a better nutrient use efficiency, which enabled the fixation of carbon under higher temperatures. A possible reason for this might be the phenotypic adaptation of the entire community. They also suggested that colder temperatures contributed to lower C:P ratios by reallocation of nutrients to cellular compounds such as RNA and proteins. However, as we did not measure such values, we cannot substantiate this suggestion.

In contrast to the results we observed, the particulate matter C:P decreased in 3 of 4 experiments with Baltic Sea phytoplankton spring communities (Wohlers-Zöllner et al. 2012). There it was suggested that the turnover dynamics of organic phosphorus compounds shifted with warming (Wohlers-Zöllner et al. 2012). The faster replenishment of the POP pool was explained by the temperature stimulation of phosphorus cycling. In our study, particulate C:P ratios were generally low and clearly below Redfield ratio. However, dissolved inorganic phosphorus (DIP) concentrations were high throughout the experiment (0.4 to 1.68 $\mu\text{mol l}^{-1}$); hence, phosphorus was not limiting. Therefore, a potential stimulation of phosphorus cycling through warming did not have consequences for our autumn bloom.

Potential consequences of our results for the planktonic food web

Based on our results we suggest that in terms of food quality the food web will be marginally affected by warming and/or acidification. Furthermore, the increasing C:P ratios in response to warming are probably not deleterious for zooplankton feeding, because even the highest ratios were clearly lower than the usual C:P ratios in copepods (Sommer & Stibor 2002), the dominant group of marine mesozooplankton. Thus, potential mineral nutrient limitation for higher trophic levels due to stoichiometrically imbalanced food (Sterner & Hessen 1994, Elser et al. 2001) can be excluded for all treatment combinations in our study.

Conclusions

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

Acknowledgements. The study was funded by the program BIOACID, a project of the German Ministry of Education and Research (BMBF). The authors thank all members of the BIOACID indoor mesocosm group, as well as their student helpers, for cooperation during the experiment. We especially thank J. Garzke and J. Steffen for their assistance in sampling, sample preparation, and chlorophyll measurement. J. Garzke and H. Horn are also acknowledged for sharing their insights on zooplankton development. T. Hansen is gratefully acknowledged for general technical support. C. Meyer and B. Gardeler are acknowledged for processing DIC and nutrient data.

LITERATURE CITED

- Brock TD (1981) Calculating solar radiation for ecological studies. *Ecol Model* 14:1–19
- Brown JH, Gillooly JF, Allen AP, Savage VM, West GB (2004) Toward a metabolic theory of ecology. *Ecology* 85: 1771–1789
- Caldeira K, Wickett ME (2005) Ocean model predictions of chemistry changes from carbon dioxide emissions to the atmosphere and ocean. *J Geophys Res* 110:C09S04, doi: 10.1029/2004JC002671
- De Senerpont Domis LN, Van de Waal DB, Helmsing NR, Van Donk E, Mooij WM (2014) Community stoichiometry

- in a changing world: combined effects of warming and eutrophication on phytoplankton dynamics. *Ecology* 95: 1485–1495
- Dickson AG (1981) An exact definition of total alkalinity and procedure for the estimation of alkalinity and total inorganic carbon from titration data. *Deep-Sea Res* 28: 609–623
- Dickson AG (1990) Standard potential of the reaction: $\text{AgCl(s)} + 1/2\text{H}_2\text{(s)} + \text{HCl(aq)}$ and the standard acidity constant of the ion HSO_4^- in synthetic sea water from 273.15 to 318.15 K. *J Chem Thermodyn* 22:113–127
- Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociations of carbonic acid in seawater media. *Deep-Sea Res* 34:1733–1741
- Dickson AG, Afghan JD, Anderson GC (2003) Reference materials for oceanic CO_2 analysis: a method for the certification of total alkalinity. *Mar Chem* 80:185–197
- Eggers SL, Lewandowska AM, Barcelos E, Ramos J, Blanco-Ameiheras S, Gallo F, Matthiessen B (2014) Community composition has greater impact on the functioning of marine phytoplankton communities than ocean acidification. *Glob Chang Biol* 20:713–723
- Elser JJ, Hayakawa K, Urabe J (2001) Nutrient limitation reduces food quality for zooplankton: *Daphnia* response to seston phosphorus enrichment. *Ecology* 82:898–903
- Eppley RW (1972) Temperature and phytoplankton growth in the sea. *US Fish Wildl Serv Fish Bull* 70:1063–1085
- Feely RA, Sabine CL, Hernandez-Ayon JM, Ianson D, Hales B (2008) Evidence for upwelling of corrosive ‘acidified’ water onto the continental shelf. *Science* 320:1490–1492
- Feng Y, Warner ME, Zhang Y, Sun J, Fu FX, Rose JM, Hutchins DA (2008) Interactive effects of increased $p\text{CO}_2$, temperature and irradiance on the marine coccolithophore *Emiliania huxleyi* (Prymnesiophyceae). *Eur J Phycol* 43:87–98
- Feng Y, Hare CE, Leblanc K, Rose JM and others (2009) Effects of increased $p\text{CO}_2$ and temperature on the North Atlantic spring bloom. I. The phytoplankton community and biogeochemical response. *Mar Ecol Prog Ser* 388:13–25
- Garzke J (2014) Global change effects on zooplankton body size: a range of experimental approaches. PhD dissertation, Christian-Albrechts-Universität, Kiel. http://macau.uni-kiel.de/receive/dissertation_diss_00015716
- Hama T, Kawashima S, Shimotori K, Satoh Y and others (2012) Effects of ocean acidification on coastal phytoplankton composition and accompanying organic nitrogen production. *J Oceanogr* 68:183–194
- Hansen HP, Koroleff F (1999) Determination of nutrients. In: Grasshoff K, Kremling K, Ehrhardt M (eds) *Methods of seawater analysis*, 3rd edn. Wiley VCH, Weinheim, p 159–228
- Hansen T, Gardeler B, Matthiessen B (2013) Highly precise quantitative measurements of total dissolved inorganic carbon from small amounts of seawater using a common gas chromatographic system: an alternative method compared to established detection systems. *Biogeosciences Discuss* 10:4439–4460
- Hansson I (1973) A new set of acidity constants for carbonic acid and boric acid in seawater. *Deep-Sea Res* 20: 661–678
- Hare CE, Leblanc K, DiTullio GR, Kudela RM and others (2007) Consequence of increased temperature and CO_2 for phytoplankton community structure in the Bering Sea. *Mar Ecol Prog Ser* 352:9–16
- Havenhand JN (2012) How will ocean acidification affect Baltic Sea ecosystems? An assessment of plausible impacts on key functional groups. *Ambio* 41:637–644
- HELCOM (2009) Eutrophication in the Baltic Sea — an integrated thematic assessment of the effects of nutrient enrichment and eutrophication in the Baltic Sea region. Baltic Sea Environ Proc No. 115B. Helsinki Commission, Helsinki
- Hillebrand H, Dürselen CD, Kirschtel D, Pollinger U, Zohari T (1999) Biovolume calculation for pelagic and benthic microalgae. *J Phycol* 35:403–424
- Hoegh-Guldberg O, Bruno JF (2010) The impact of climate change on the world’s marine ecosystems. *Science* 328: 1523–1528
- IPCC (Intergovernmental Panel on Climate Change) (2007) *Climate change 2007: the physical science basis. Contribution of Working Group I to the 4th assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge
- IPCC (2014) *Climate change 2014: impacts, adaptation and vulnerability. IPCC Working Group II contribution to the 5th assessment report of the International Panel on Climate Change*. Cambridge University Press, Cambridge
- Jeffrey SW, Humphrey GF (1975) New spectrophotometric equation for determining chlorophyll *a*, *b*, *c*1 and *c*2. *Biochem Physiol Pflanz* 167:194–204
- Keller AA, Oviatt CA, Walker HA, Hawk JD (1999) Predicted impacts of elevated temperature on the magnitude of the winter–spring phytoplankton bloom in temperate coastal waters: a mesocosm study. *Limnol Oceanogr* 44:344–356
- Kroeker KJ, Kordas RL, Crim RN, Singh GG (2010) Meta-analysis reveals negative yet variable effects of ocean acidification on marine organisms. *Ecol Lett* 13: 1419–1434
- Kroeker KJ, Kordas RL, Crim RN, Hendriks IE and others (2013) Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob Change Biol* 19:1884–1896
- Lewandowska A, Sommer U (2010) Climate change and the spring bloom: a mesocosm study on the influence of light and temperature on phytoplankton and mesozooplankton. *Mar Ecol Prog Ser* 405:101–111
- Lewandowska AM, Boyce DG, Hofmann M, Matthiessen B, Sommer U, Worm B (2014) Effects of sea surface warming on marine plankton. *Ecol Lett* 17:614–623
- Lohbeck K, Riebesell U, Reusch TBH (2012) Adaptive evolution of a key phytoplankton species to ocean acidification. *Nat Geosci* 5:346–351
- Meehl GA, Stocker TF, Collins WD, Friedlingstein P and others (2007) Global climate projections. In: Solomon S, Qin D, Manning M, Chen Z and others (eds) *Climate change 2007: the physical science basis. Contribution of Working Group I to the 4th assessment report of the Intergovernmental Panel on Climate Change*. Cambridge University Press, Cambridge, p 747–845
- Mehrbach C, Culbertson CH, Hawley JE, Pytkowicz RM (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnol Oceanogr* 18:897–907
- Melzner F, Thomsen J, Koeve W, Oschlies A and others (2013) Future ocean acidification will be amplified by hypoxia in coastal habitats. *Mar Biol* 160:1875–1888
- Menden-Deuer S, Lessard EJ (2000) Carbon to volume relationships for dinoflagellates, diatoms, and other protist plankton. *Limnol Oceanogr* 45:569–579

- Moline MA, Prezelin BB (2000) Optical fractionation of chlorophyll and primary production for coastal waters of the Southern Ocean. *Polar Biol* 23:129–136
- Nausch G, Bachor A, Petanati T, Voß J, von Weber M (2011) Nutrients in the German coastal waters of the Baltic Sea and adjacent areas. *Meeresumwelt aktuell: Nord-und Ostsee* 2011:1–15
- O'Connor MI, Piehler MF, Leech DM, Anton A, Bruno JF (2009) Warming and resource availability shift food web structure and metabolism. *PLoS Biol* 7:e1000178
- Pierrot D, Lewis E, Wallace DWR (2006) MS Excel program developed for CO₂ system calculations: ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Centre, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN
- Pinheiro J, Bates D, DebRoy S, Sarkar D, R Core Team (2013) nlme: linear and nonlinear mixed effects models. R package version 3.1-107.4. <http://CRAN.R-project.org/package=nlme>
- Rossoll D, Sommer U, Winder M (2013) Community interactions dampen acidification effects in a coastal plankton system. *Mar Ecol Prog Ser* 486:37–46
- Schulz KG, Bellerby RGJ, Brussaard CPD, Büdenbender J and others (2013) Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide. *Biogeosciences* 10:161–180
- Sommer U, Lengfellner K (2008) Climate change and the timing, magnitude, and composition of the phytoplankton spring bloom. *Glob Change Biol* 14:1199–1208
- Sommer U, Lewandowska A (2011) Climate change and the phytoplankton spring bloom: warming and overwintering zooplankton have similar effects on phytoplankton. *Glob Change Biol* 17:154–162
- Sommer U, Stibor H (2002) Copepoda–Cladocera–Tunicata: the role of three major mesozooplankton groups in pelagic food webs. *Ecol Res* 17:161–174
- Sommer U, Aberle N, Lengfellner K, Lewandowska A (2012a) The Baltic Sea spring phytoplankton bloom in a changing climate: an experimental approach. *Mar Biol* 159:2479–2490
- Sommer U, Lengfellner K, Lewandowska A (2012b) Experimental induction of a coastal spring bloom early in the year by intermittent high-light episodes. *Mar Ecol Prog Ser* 446:61–71
- Sommer U, Paul C, Moustaka-Gouni M (in press) Warming and ocean acidification effects on phytoplankton — from species shifts to size shifts within species in a mesocosm experiment. *PLoS ONE*
- Sterner W, Hessen DO (1994) Stoichiometric relationships among producers, consumers and nutrient cycling in pelagic ecosystems. *Annu Rev Ecol Syst* 25:1–29
- Suikkanen S, Pulina S, Engström-Öst J, Lehtinen S, Brutemark A (2013) Climate change and eutrophication induced shifts in northern summer plankton communities. *PLoS ONE* 8:e66475
- Taucher J, Schulz KG, Dittmar T, Sommer U, Oschlies A, Riebesell U (2012) Enhanced carbon overconsumption in response to increasing temperatures during a mesocosm experiment. *Biogeosciences* 9:3531–3545
- Thomsen J, Gutowska MA, Saphorster J, Heinemann A and others (2010) Calcifying invertebrates succeed in a naturally CO₂-rich coastal habitat but are threatened by high levels of future acidification. *Biogeosciences* 7:3879–3891
- Thomsen J, Casties I, Pansch C, Körtzinger A, Melzner A (2013) Food availability outweighs ocean acidification effects in juvenile *Mytilus edulis*: laboratory and field experiments. *Glob Change Biol* 19:1017–1027
- Torstensson A, Chierici M, Wulff A (2012) The influence of increased temperature and carbon dioxide levels on the benthic/sea ice diatom *Navicula directa*. *Polar Biol* 35:205–214
- Tortell PD (2000) Evolutionary and ecological perspectives on carbon acquisition in phytoplankton. *Limnol Oceanogr* 45:744–750
- Tortell PD, Payne CD, Li Y, Trimborn S and others (2008) CO₂ sensitivity of Southern Ocean phytoplankton. *Geophys Res Lett* 35:L04605, doi:10.1029/2007GL032583
- Utermöhl H (1958) Zur Vervollkommnung der quantitativen Phytoplankton-Methodik. *Mitt Int Ver Theor Angew Limnol* 9:263–272
- Wohlers-Zöllner J, Biermann A, Engel A, Dörge P, Lewandowska AM, von Schreiber M, Riebesell U (2012) Effects of rising temperature on pelagic biogeochemistry in mesocosm systems: a comparative analysis of the AQUASHIFT Kiel experiments. *Mar Biol* 159:2503–2518

Editorial responsibility: Antonio Bode,
A Coruña, Spain

Submitted: March 18, 2014; Accepted: February 28, 2015
Proofs received from author(s): May 11, 2015