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Effects of increased atmospheric CO₂ on small and intermediate sized osmotrophs during a nutrient induced phytoplankton bloom

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Abstract

We report the transient population dynamic response of the osmotrophic community initiated by a nutrient pulse in mesocosms exposed to different pCO₂ levels as well as quantitative variations in phytoplankton and heterotrophic bacteria created by the differ-

- ⁵ ence in CO₂ exposure. Coastal seawater was enclosed in floating mesocosms (27 m³) and nutrients were supplied initially in order to stimulate growth of microbial organisms, including the coccolitophorid *Emiliania huxleyi*. The mesocosms were modified to achieve 350 μ atm (1×CO₂), 700 μ atm (2×CO₂) and 1050 μ atm (3×CO₂) CO₂ pressure. The temporal dynamics was related to the nutrient conditions in the enclosures.
- ¹⁰ Numerically small osmotrophs (picoeukaryotes and *Synechoccocus* sp.) dominated initially and towards the end of the experiment, whereas intermediate sized osmotrophs bloomed as the initial bloom of small sized osmotrophs ceased. Maximum concentrations of *E. huxleyi* were approximately 4.6×10^3 cells ml⁻¹ whereas other intermediate sized osmotrophs reached approximately twice as high concentrations. Osmotrophic
- ¹⁵ succession pattern did not change, and we were not able to detect differences with regard to presence or absence of specific osmotrophic taxa as a consequence of altered atmospheric CO_2 concentration. Quantitative effects on the microbial communities associated with the CO_2 treatment were, however, observed towards the end of the experiment.

20 **1** Introduction

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The pelagic food web is a complex and dynamic system where production is based largely on regenerated rather than new nutrients (Thingstad, 1998). In the pelagic zone nutrient limitation is believed to be a fundamental controlling factor for the community composition of osmotrophic microorganisms (organisms that feed on dissolved substrates) (Thingstad et al., 2005). Consequently, a change in inorganic nutrient availability is important for defining the primary productivity of the ocean and for regulating

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phytoplankton community composition and succession, Pinhassi et al. (2006). Such amendments can in turn change the bacterioplankton community structure as a response to the growth and decay of various phytoplankton species or groups, indicating that dissolved organic matter from different algae select for different bacteria (Pinhassi

et al., 2004; Grossart et al., 2005). Not only nutrients affect the osmotrophic community, however. Predation and lytic viruses are important mechanisms creating diversity and allowing for coexisting size classes of osmotrophs (Thingstad, 1998; Thingstad, 2000).

Phytoplankton and bacteria are key components of energy fluxes and nutrient cycling in the sea (Grossart et al., 2005). The major function of heterotrophic bacteria in interactions with phytoplankton is organic matter degradation (Cole et al., 1988; Smith et al., 1995; Grossart and Simon, 1998). Because heterotrophic bacteria are the major consumers of dissolved organic matter in the aquatic environment, limitation of bacterial growth by organic or inorganic nutrients can have important consequences in terms of biogeochemical C cycling (Pinbassi et al., 2006). Also, an important mechanism for

of biogeochemical C cycling (Pinhassi et al., 2006). Also, an important mechanism for the regulation of atmospheric CO₂ concentration is the fixation of CO₂ by marine phytoplankton and the subsequent export of the organically bound carbon to the deeper ocean (Engel et al., 2004).

The atmospheric CO_2 has increased from a pre-industrial level of 280 μ atm to the present level of 370 μ atm. Further increased atmospheric CO_2 concentration will lead to a rise in the CO_2 concentration in the surface ocean and consequently a shift in its chemical equilibrium (Brewer et al., 1997). Some phytoplankton species (diatoms and the haptophyte *Phaeocystis globosa*) seem to get their CO_2 requirement fulfilled at the present day levels, whereas others (like the haptophyte *Emiliania huxleyi*) may benefit,

in terms of increased primary production, from an increase in atmospheric CO₂ (Riebesell, 2004). On the other hand, an increase in atmospheric CO₂ may cause a decrease in biogenic calcification of organisms like *E. huxleyi*. The results from a mesocosm experiment in 2001 indicated that both average growth rates and calcification of *E. huxleyi* were sensitive to changes in pCO₂, whereas other nanoautotrophs and picoautotrophs



eukaryotes were not affected by altered CO₂ environments (Engel et al., 2005).

Seawater mesocosms allow studies of pCO_2 related impact on dynamics at a community level (Delille et al., 2005). Although not identical to the natural system they offer a good alternative that allow manipulation of complex ecosystems. We report result

- ⁵ from the third mesocosms experiment carried out by the project Pelagic Ecosystem CO_2 Enrichment Studies (PeECE). The two fist experiments had a maximum CO_2 concentrations corresponding to the atmospheric level expected in 2100 (710 μ atm). We here go a step further with maximum level of 1050 μ atm. The population dynamic in the osmotrophic community initiated by an initial nutrient pulse in mesocosms exposed to different pCO₂ levels as well as quantitative and qualitative variations in phytoplankton
- and heterotrophic bacteria created by the difference in CO_2 exposure were monitored by flow cytometry and are currently described.

2 Material and methods

- 2.1 Experimental design and sampling
- A mesocosm experiment was carried out at Marine Biological Station, University of Bergen, Norway between 11 May and 10 June 2005. Nine polyethylene enclosures (2 m diameter and 9.5 m deep, volume 27 m³) were mounted on floating frames, in a West-East line, and secured to a raft located in a small enclosed bay (Raunefjorden). The enclosures were filled on 11 May with 27 m³ unfiltered, nutrient-poor, post-bloom
 fiord water. The atmospheric and seawater pCO₂ were manipulated to achieve levels
- of $1050 \,\mu$ atm simulating 2150 conditions $(3 \times CO_2)$, to $700 \,\mu$ atm in a year 2100 scenario $(2 \times CO_2)$ and to $350 \,\mu$ atm CO_2 as the present scenario $(1 \times CO_2)$. To initiate the development of a bloom of the coccolithophore *Emiliania huxleyi* (Haptophyta) nitrate and phosphate were added on day 0 (16 May) of the experiment, in a ratio of 25:1 yielding initial concentrations of approximately $15 \,\mu$ mol L⁻¹ NO₃ and $0.6 \,\mu$ mol L⁻¹ PO₄
- (Egge, 1993; Egge and Jacobsen, 1997).



Samples for flow cytometric investigations were collected every second day for the first 6 days of the experiment and thereafter every day until the end of the investigation. For a full description of the experimental setup and sampling procedures, see Schulz et al. $(2007)^1$.

5 2.2 Flow cytometry (FCM)

All FCM analyses were performed with a FACSCalibur flow cytometer (Becton Dickinson) equipped with an air-cooled laser providing 15 mW at 488 nm and with standard filter set-up. The phytoplankton counts were obtained from fresh samples at high flow rate (average $104 \,\mu l \,min^{-1}$). The trigger was set on red fluorescence and the samples were analysed for 300 s. Discrimination between populations was based on dot plots of side scatter signal (SSC) and pigment autofluorescence (chlorophyll and phycoery-thrin). We followed the dynamics of five different autotrophic phytoplankton populations (*Synechococcus* sp., *Emiliania huxleyi*, two unknown groups of nanoeukaryotes (differing in FL3 signal and hence in chlorophyll content) and picoeukaryotes (Fig. 1a and b).

- ¹⁵ Samples for enumeration of heterotrophic bacteria samples were fixed with glutaraldehyde at a final concentration of 0.1% for 30 min at 4°C, frozen in liquid nitrogen and stored at -70° C until further analysis (Marie et al., 1999). Enumeration was performed for 60 s at an event rate between 100 and 1000 s^{-1} . Each sample was diluted at minimum two different dilutions from 10- to 200-fold in 0.2 μ m filtered seawater and stained with SYBR Green I (Molecular Probes Inc., Eugene, OR) for 10 min at 80°C in the dark (Marie et al., 1999). The flow cytometer instrumentation and the remain-
- in the dark (Marie et al., 1999). The flow cytometer instrumentation and the remaining methodology followed the recommendations of Marie et al. (1999). Detection and enumeration of bacteria was based on scatter plots of SSC signal versus green DNA dye (SYBR Green) fluorescence, and we followed the development of total bacteria ²⁵ (Fig. 1c).



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All concentrations were calculated from measured instrument flow rate, based on volumetric measurements, and all data files analyzed using EcoFlow (version 1.0.5, available from the authors).

3 Results

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5 3.1 Osmotrophic dynamics

The nutrients added at day 0 caused an increase in algal biomass (chlorophyll-*a* concentration) from approximately $2 \mu g \operatorname{chl} a I^{-1}$ to maximum values between 16 and $20 \mu g \operatorname{chl} a I^{-1}$ on day 9–10 (Fig. 2, Schulz et al., 2007¹). Towards the end of the experiment a second, and much smaller, peak (3–4 $\mu g \operatorname{chl} a I^{-1}$) was observed. The major part of the two chl-*a* peaks consisted of diatoms and dinoflagellates, respectively (large osmotrophs) (Schulz et al., 2007¹; Riebesell et al., 2007).

Cell numbers were 7 (Nanoeukaryotes 2) to 74 (Synechococcus) times higher during the blooms within the mesocosms than in the reference fjord water (Fig. 3), and a transient population dynamic response to the nutrient addition was evident within small (Synechococcus, Picoeukaryotes, Heterotrophic bacteria) and intermedi-15 ate sized osmotrophs (Emiliania huxleyi, Nanoeukaryotes 1 and 2, Fig. 3). Numerically the small osmotrophs dominated the phytoplankton community initially (Picoeukaryotes $\approx 1.3 \times 10^5 \text{ ml}^{-1}$ and *Synechococcus* $\approx 0.6 \times 10^5 \text{ ml}^{-1}$; Fig. 3a and b). Their abundance increased until day 2 after which they decreased during the bloom of the intermediate sized osmotrophs (Picoeukaryotes reduced to $\approx 0.1 \times 10^5 \text{ ml}^{-1}$ and Svne-20 *chococcus* to $\approx 0.1 \times 10^5 \text{ ml}^{-1}$). Both groups peaked again in the middle (days 15–16, Picoeukaryotes $\approx 0.7 \times 10^5 \text{ ml}^{-1}$ and Synechococcus $\approx 1.2 \times 10^5 \text{ ml}^{-1}$) and towards the end (days 23–25) of the experiment (Picoeukaryotes $\approx 0.7 \times 10^5$ ml⁻¹ and Synechococ $cus \approx 3.3 \times 10^5 \text{ ml}^{-1}$). The picoeukaryotes dominated the autotrophic small osmotroph community during the first of the three peaks (day 2) with cell concentrations around 25 1.8×10⁵ cells ml⁻¹, and an average picoeukaryote: *Synechococcus* ratio of 2.5:1. The

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last peak (day 23–25) was dominated by *Synechococcus*, which was then found in concentrations of 3.4×10^5 cells ml⁻¹, with an average picoeukaryotes: *Synechococcus* ratio of 1: 11 (at day 24).

The abundance of all three intermediate sized osmotrophs increased from the onset of the experiment with blooms culminating on day 6–7 (*E. huxleyi* \approx 4.6 x 10³ cells ml⁻¹; nanoeukaryotes 1 \approx 5.2×10³ cells ml⁻¹; nanoeukaryotes 2 \approx 1.9×10³ cells ml⁻¹, Fig. 3c, d, e). Nanoeukaryotes 1 peaked twice after this with maximum cell concentrations around 7×10³ and 8×10³ cells ml⁻¹ at day 11 and 18, respectively.

Heterotrophic bacteria showed a dynamic similar to that of small autotrophic os-¹⁰ motrophs with high initial concentrations (ca. 7.7×10^6 cells ml⁻¹), a rapid decrease that was followed by a new peak ($\approx 5.4 \times 10^6$ cells ml⁻¹) culminating at day 15, and new maximum the last day of the experiment ($\approx 4.6 \times 10^6$ cells ml⁻¹ day 25, Fig. 3f).

3.2 CO₂ effects

Chl-a concentrations did not vary greatly between the different treatments, but at the peak of the bloom (day 9–10) there was a tendency of higher chl-a concentrations in 15 the 2x and 3x compared to the 1xCO₂ mesocosm, and from day 13 onwards higher in the $3 \times CO_2$ than in the rest of the mesocosms (Fig. 2; Schulz et al., 2007¹). From day 0 to day 8 we did not observe any effect of the CO₂ treatment in any of the six groups of small and intermediate sized omotrophs (Fig. 3). As the bloom of E. huxleyi proceeded (day 9), however, somewhat higher E. huxleyi concentrations were registered in the $3 \times CO_2$ ($\approx 4.6 \times 10^3$ cells ml⁻¹) compared to the 1x ($\approx 2.9 \times 10^3$ cells ml⁻¹) and $2 \times CO_2$ mesocosms ($\approx 3.9 \times 10^3$ cells ml⁻¹; Fig. 3c). A similar trend was also detected in nanoeukaryotes 1 and nanoeukaryote 2 from day 8 onwards (Fig. 3d, e). Towards the end of the experiment a more conspicuous CO₂ effect was observed within the small autotrophic osmotrophs (Fig. 3a, b); Synechococcus abundances were notably 25 higher in the 1×CO₂ than in the other mesocosms from day 19 onwards whereas the picoeukaryotes were found at highest numbers in the mesocosms with highest CO₂ con-

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centrations (3×CO₂). The heterotrophic bacteria were not affected much by changes in CO₂ concentrations but a minute tendency of higher bacteria numbers in 3×CO₂ compared to the 1× and 2×CO₂ mesocosms was registered the last few days of the experiment (Fig. 3f).

5 4 Discussion

4.1 Osmotrophic population dynamic

As described in Tanaka et al. (2007) the inorganic nutrient environment that succeeded the initial nutrient manipulation can be divided into five different phases. Phase 1 (days 0–6) was characterized by no nutrient depletion and during phase 2 (days 7–11) the silicate (Si) got exhausted (phosphate (P) and nitrate (N) still being replete). In phase 3 (days 12–16) Si and P depletion took place (N still replete) and by the end of phase 4 (days 17–20) Si, P and N was all depleted. Phase 5 (days 21–24) was characterized by some re-suspension of N and by an increase in P turnover time.

- The Chl-*a* data exposed only one major (and one minor) peak during the course of the above described phases (in phase 2 and phase 5 respectively), and pigment analyses revealed that diatoms accounted for most of the chlorophyll during the main bloom (Riebesell et al., 2007; Schulz et al., 2007¹). The flow cytometry results presented here revealed a much more varied dynamic among the various osmotrophic groups: The initial nutrient pulse resulted in a community shift from small sized (picoplank-
- ton: heterotrophic bacteria, *Synechococcus* and picoeukaryotes) to intermediate (*E. huxleyi* and other eukaryotic nanoflagellates) in addition to the big sized (diatoms) osmotrophs. On a competition to defence specialist axis (Thingstad et al., 2005) intermediate/big osmotrophs represent the latter characterized by features (e.g. size, silicate scale) making them less vulnerable for grazing (Thingstad, 1998; Hamm, 2000; Hamm
 et al., 2003) and/or infection (Raven and Waite, 2004), whereas the small osmotrophs
- et al., 2003) and/or infection (Raven and Waite, 2004), whereas the small osmotrophs are thought to out-compete bigger ones when nutrients are low (Kuenen et al., 1977;



Smith and Kalff, 1982; Bratbak and Thingstad, 1985; Thingstad et al., 2005). The observed shift thus represents a change from competition specialists, which dominated the mesocosm water before nutrient addition, to defence specialists taking advantage of the nutrient replete conditions that was created after the initial nutrient pulse.

- ⁵ But how can the co-existence within each of the two groups (small and intermediate/big) be explained? By looking more closely into the defence group (intermediate and big osmotrophs) it is evident that when silicate was exhausted (phase II) and thus limiting for further diatom growth, this gave room for the nanoeukaryotes (including *E. huxleyi*). *Emiliania huxleyi* has a high P-affinity (Riegmann et al., 2000) and ability to preduce entrupted for utilization of pheepherup from errors substrates (Kuenzler
- to produce enzymes for utilization of phosphorus from organic substrates (Kuenzler and Perras, 1965). It could therefore potentially have a competitive advantage to other nanoeukaryotes as phosphate became depleted in phase III. The coccolithophorid experienced a viral attack, however (Larsen et al., 2007) giving room for Nanoeukaryotes 1 and 2 which retained with oscillations until phase V. Our analyses did not allow for
- ¹⁵ species designation of Nanoeukaryotes 1 and 2, but several *Chrysochromulina* (Prymnesiophyceae) and *Pyramimonas* (Prasinophycea) species are common nanoeukaryotes in our coastal waters (Throndsen et al., 2003), and species within these genera have proven susceptible to virus within the Phycodnaviridae familiy (Suttle and Chan, 1995; Sandaa et al., 2001). Studies of the viral community showed that CeV and two other closely related to virus within the Phycodnaviridaea ware propert (Largen et al., 2003).
- other closely related to viruses within the Phycodnavirideae were present (Larsen et al., 2007). It may therefore well be that the different peaks contains different species with one species taking over when others are infected and killed. The observed oscillating development within the intermediate osmotrophs thus demonstrate how the "killing the winner mechanism" also apply for algae and algal viruses (Thingstad and Lignell, 1997; Thingstad, 2000).

We observed a simultaneous decrease of all small osmotrophs (heterophic bacteria, *Synechococcus* and picoeukaryotes) in phase I and IV (and towards the end of phase V). Such within-community similarities suggest a common size-selective predator (heterotrophic flagellates) as the major loss mechanism for the competition group (Fenchel,



1980; Fenchel, 1987; Thingstad et al., 2005). The coexistence within the group needs further explanations though and two theoretical ones come to mind: 1) growth rate limitation of heterotrophic bacteria by bioavailable organic carbon (Thingstad et al., 2007) and 2) differences in the ability to use organic nitrogen sources. Tanaka et

- 5 al. (2007) concludes that bacterial growth was not limited by the availability of labile DOC whereas mineral nutrients were depleted from phase four. The latter explanation thus seem more plausible and can also explain why the picoeukaryotes dominated the small sized autotrophic community in the beginning of the experiment (phase I) whereas Synechococcus took on the lead role in phase V. The bacterio-, cyanophages
- and algal virus dynamic demonstrated in Larsen et al. (2007) suggests viruses played 10 an essential role in the population dynamics within each of the three groups of small osmotrophs (Thingstad, 2000).

It has already been mentioned that the initial nutrient addition was followed by a noticeably decrease in abundance of competition specialists (small sized osmotrophs:

- heterophic bacteria, Synechococcus and picoeukaryotes). However, when comparing 15 the concentration of these three groups with what we observed in the reference seawater it is evident that some mechanism prior to nutrient addition caused them to increase substantially. One possible explanation is that filling the mecosoms and/or bubbling the water to achieve the desired CO₂ levels killed off possible predators and/or released
- DOM, which they could have benefited from if they were nutrient limited in the fjord wa-20 ter prior to the experiment. The plankton community contains species that are fragile and therefore may be sensitive to the filling/bubbling procedure, but as neither DOM nor predator abundances were measured before and after onset of filling/bubbling the mesocosms we can no more than speculate that these were the mechanisms leading to the high initial concentration of small osmotrophs. 25

4.2 CO₂ effects on the osmotrophic community

The current study did not reveal omotrophic successional shifts that can be traced back to the altered CO₂ concentrations. Nor were we able to detect introduction or



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removal of specific osmotrophic taxa as a result of the CO_2 manipulation. Our results do, however, in agreement with previous observations (Tortell et al., 2002; Grossart et al., 2006; Engel et al., 2007) indicate that an increase in atmospheric CO_2 may affect the relative abundance of the various osmotrophs in the marine pelagic environment.

- ⁵ This is most clearly expressed by the small autotrophic osmotrophs in phase IV and V, with lowered *Synechococcus* and elevated picoeukaryotes abundances, at the highest CO₂ level. Similar effects of varying CO₂ concentrations was not as evident for the remaining osmotrophs, but the trend of higher cell numbers with increasing CO₂ for all groups, except for *Synechococcus*, emerged more clearly when calculating total
- ¹⁰ cell numbers for the entire experimental period for the autotrophic osmotrophs (Fig. 4). Higher abundances of primary producers at the highest CO_2 level as the experiment progressed is in agreement with a somewhat higher total primary production in the second half of the experiment (Egge et al., 2007²).
- It has previously been documented that some phytoplankton species (*E. huxleyi*, *G. oceanica*) benefit, in terms of increased photosynthetic carbon fixation rates, from an increase in CO₂ concentrations compared to the present day level (Riebesell et al., 2000; Rost et al., 2003) whereas others do not (*P. pouchetii*, several diatom species; Burkhardt et al., 1999, 2001; Rost et al., 2003). Riebesell (2004) conclude from this that the current increase in atmpospheric CO₂ will promote growth of calcifying primary producers. Our results do not necessarily support this conclusion as all intermediate
- autotrophic osmotrophs (including the non calcifyers) seemed to experience a similar (and small) increase in abundance as CO_2 increased. One aspect that could interfere with our interpretation of possible CO_2 effect on the osmotrophs is the phytoplankton-virus interactions that have a profound influence on the marine microbial systems (re-
- viewed by Brussaard, 2004). Larsen et al. (2007) showed that one virus which infect *E. huxelyi* and one that assumingly infect some other nanoeukaryote, occurred in higher numbers in mesocosms with the lowest CO₂ level. This is obviously an additional reason for lower *E. huxleyi* and nanoeukaryotes 1 and 2 concentrations in these very same enclosures.

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The only group with higher biomass (this study) and production (Egge et al., 2007^2) at 1×CO₂ than at 2× and 3×CO₂ was Synechococcus. Engel et al., 2005, report that average abundances of Synechococcus in a similar mesocosm experiment in 2001 was not affected by the CO₂ concentrations, but a closer inspection of the osmotrophic dynamic (presented by Rochelle-Newall, 2004, Fig. 2) reveal that also in that case the 5 densest Synechococcus population occurred within the enclosure exposed to the lowest CO₂ concentration. In both experiments this is a result only visible towards the end when inorganic N and P are depleted and osmotrophic production depends on remineralised nutrients. Direct competition experiments have demonstrated that low CO₂

- concentrations favour the growth of cyanobacteria over other phytoplankton species in 10 freshwater systems (Shapiro, 1973), and that freshwater Synechococcus compete well for dissolved inorganic carbon (Williams and Turpin, 1987). Cyanobacteria in general (Badger and Price, 2003), and more specifically marine Synechococcus (Hassidim et al., 1997), have demonstrated effective photosynthetic CO₂ concentrating mechanisms
- (CCMs). The observed Synechococcus dominance in phase V could thus be a com-15 bined effect of its superiority over picoeukaryotes in competition for dissolved organic nitrogen (as discussed above) and for dissolved inorganic carbon (DIC). In order for the latter to be the case, however, DIC must have been limiting. The fact that picoeukaryotic abundance increased considerably when CO₂ concentration was raised
- to $1050 \,\mu$ atm (Fig. 3) indicates that this could have been true. Prasinophytes (the ma-20 rine counterpart to green algae, frequently represented by Micromonas pusilla) are often dominating the picoeukaryotic communities in coastal and nutrient rich environments (Not et al., 2005). Our results may thus illustrate that comparable to fresh water green algae (Shapiro, 1973), this group increase on behalf of cyanobacteria when CO₂ increases. 2×CO₂ equals the highest CO₂ level tested in 2001, and in neither exper-25
- iment this CO₂ concentration resulted in elevated picoeukaryotic abundances (Fig. 3

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²Egge, J. K., Thingstad T. F., Engel, A., and Riebesell, U.: Primary production during nutrient-induced blooms at elevated CO₂ concentrations, Biogeosciences Discuss., submitted, 2007.

this study, and Fig. 2 in Rochelle-Newall, 2004).

Grossart et al. (2006) were not able to detect significant changes in heterotrophic bacterial abundance as a result of a variable CO₂ environment and link the indirect effect of changes in pCO2 on bacterial activities to phytoplankton dynamics. In the ⁵ current experiment the effect, if any, was a slight tendency of higher concentration in $3 \times CO_2$ mesocosms than in $1 \times$ and $2 \times CO_2$, and only detectable towards the end of the experiment. This might have been a secondary effect of more nanoeukaryotic cells being terminated, releasing higher amounts of DOM in phase IV, in these enclosures.

5 Concluding remarks

- ¹⁰ The osmotrophic community within our mesocosms may have experienced three perturbing events: Nutrient addition, a potentially effect of the filling and/or bubbling procedures, and CO₂ manipulations. By contributing significantly to the early success of the small sized osmotrophs, the bubbling/filling did perhaps influence the onset of the observed community composition shifts. However, the bloom of defence special-¹⁵ ists/intermediate sized phytoplankton that can be foreseen as a consequence of elevated nutrient concentrations (Thingstad et al., 2005) was apparently not disturbed by this. A series of community composition shifts succeeded the initial nutrient amendment and as such this seemed, not surprisingly to be the single one parameter affecting the microbial community most profoundly. The effect of the CO₂ manipulations
- ²⁰ was not quite as obvious, probably because short time experiments like the current do not provide sufficient time to create differences detectable as successional shifts and introduction or removal of certain taxonomic groups. Nevertheless, our results do substantiate previous works suggesting that CO₂ variations influence the relative taxonomic composition of marine phytoplankton (Tortell et al., 2002; Grossart et al., 2006;
- Engel et al., 2007). These differences were most noticeable towards the end of the experiment when nutrients were limiting (Tanaka et al., 2007), net production zero or below (i.e. based on regenerated nutrients; Egge et al., 2007²), and small and interme-



diate sized osmotrophs had increased their importance relatively to the diatoms (this study; Riebesell et al., 2007; Schulz et al., 2007¹). A number of CCM variants, differing in manner of operation and efficiency, are found among different phytoplankton groups, and nutrient availability is also known to play a significant role in modulating

- ⁵ CCMs (reviewed by Giordano et al., 2005). It is therefore difficult to judge whether our observations suggest that increase in atmospheric CO₂ will have a greater effect when production is based on regenerated nutrients, or whether they rather reflect that small and intermediate sized osmotrophs are not equipped with carbon concentration mechanisms as efficient as the diatoms and therefore benefit more from increased CO₂ levels than the latter (John et al., 2007). The experiment do, however, illustrate as
- $_{10}$ CO₂ levels than the latter (John et al., 2007). The experiment do, however, illustrate as previously suggested (Tortell, 2000), that the competitive balances between microbial taxa may be altered when atmospheric CO₂ changes.

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Fig. 1. Flow cytometric analysis of natural osmotrophic populations in the nine mesocosms during the third mesocosms experiment carried out by the project Pelagic Ecosystem CO_2 Enrichment Studies (PeECE III). Autrotrophs were analysed from unstained samples (**A** und **B**) and heterotrophic bacteria from SYBRGreen DNA stained samples (**C**). (B) *Synechococcus* sp. and picoautotrophs were discriminated using a combination of red and orange fluorescence. (B) *Emiliania huxleyi*, nanoeukaryotes 1 and nanoeukaryotes 2 were discriminated using a combination of red fluorescence and side scatter signal. (C) Heterotrophic bacteria were discriminated on the basis of green fluorescence versus side scatter signal.

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Fig. 2. Development of total chlorophyll-*a* in the mesocosms. Lines indicate average values for the three mesocosms in each treatment group $(3 \times CO_2, 2 \times CO_2, 1 \times CO_2)$, and error bars denote ±1 standard deviation. (Redrawn from Schulz et al., 2007¹).



Fig. 3. Time series development of the six osmotrophic populations in the mesocosms as determined by flow cytometry. Lines indicate average values for the three mesocosms in each treatment group $(3 \times CO_2, 2 \times CO_2, 1 \times CO_2)$. Error bars denote ±1 standard deviation. Abundance in the reference fjord water adjacent to the mesocosms is denoted with a single line (black). (A) *Synechococcus*, (B) Picoeukaryotes, (C) *Emiliania huxleyi*, (D) Nanoeukaryotes 1, (E) Nanoeukaryotes 2, (F) Heterotrophic bacteria.



Fig. 4. Total cell number of the six osmotrophic populations during the entire experiment. Each bar denotes average total cell number for the three mesocosms of the treatment group $(3 \times CO_2, 2 \times CO_2, 1 \times CO_2)$. Error bars denote ±1 standard deviation. (A) Synechococcus, (B) Picoeukaryotes, (C) Emiliania huxleyi, (D) Nanoeukaryotes 1, (E) Nanoeukaryotes 2, (F) Heterotrophic bacteria.

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