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Effects of increased atmospheric CO_2 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 pCO_2 levels. Differences in phytoplankton and heterotrophic bacteria abundances associated with the CO_2 treatment are also described. Coastal seawater was enclosed in floating mesocosms ($27\,m^3$) 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 μatm ($1\times CO_2$), 700 μatm ($2\times CO_2$) and 1050 μatm ($3\times CO_2$) CO_2 pressure. The temporal dynamics was related to nutrient conditions in the enclosures. 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. The osmotrophic succession pattern did not change, and neither were we able to detect differences with regard to presence or absence of specific osmotrophic taxa as a consequence of altered pCO_2. Towards the end of the experiment we did, however, record significantly higher picoeukaryotic- and lower *Synechococcus*-abundances in the higher CO_2 treatments. Slightly increased cell concentrations of *E. huxleyi* and other nanoeukaryotes were also recorded at elevated pCO_2 on certain days.

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1 Introduction

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 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 (Pinhassi et al., 2006). Also, an important mechanism for the regulation of atmospheric CO_2 concentration is the fixation of CO_2 by marine phytoplankton and the subsequent export of the organically bound carbon to the deeper ocean (Engel et al., 2004).

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The partial pressure of CO_2 in the atmosphere (pCO_2) has increased from a pre-industrial level of 280 μg atm to the present level of 370 μg 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 seawater pCO_2 (Riebesell, 2004). On the other hand, such increase 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 pCO_2, whereas other nanoautotrophs and picoautotrophs eukaryotes were not affected by altered CO_2 environments (Delille et al., 2005; 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 results from the third mesocosms experiment carried out by the project Pelagic Ecosystem CO_2 Enrichment Studies (PeECE). The two first experiments had a maximum CO_2 concentration corresponding to the atmospheric level expected in 2100 (710 μatm). We here go a step further with a maximum level of 1050 μatm. The population dynamic in the osmotrophic community initiated by an initial nutrient pulse in mesocosms exposed to different pCO_2 levels as well as quantitative and qualitative variations in phytoplankton and heterotrophic bacteria 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 m^3) 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 May 11 with 27 m^3 unfiltered, nutrient-poor, post-bloom fjord water. The atmospheric and seawater pCO_2 were manipulated to achieve levels of 1050 μatm simulating 2150 conditions (3×CO_2 mesocosms 1–3), to 700 μatm in a year 2100 scenario (2×CO_2 mesocosms 4–6) and to 350 gμatm CO_2 as the present scenario (1×CO_2 mesocosms 7–9). 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 μmol L^{-1} NO_3 and 0.6 μmol L^{-1} PO_4 (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).

2.1 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 μl 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 phycoerythrin). We followed the dynamics of five different autotrophic phytoplankton populations (*Synechococcus* sp., *Emiliania huxleyi*, two unknown nanoeukaryotic populations (differing in FL3 signal and hence in chlorophyll content) and picoeukaryotes (Fig. 1a and b).

Samples for enumeration of heterotrophic bacteria 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 μm filtered seawater and stained with SYBR Green I (Molecular Probes Inc., Eugene, OR) for 10 min in the dark at room temperature (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 (Fig. 1c).

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).

2.2 Statistical analyses

In order to identify statistical significant differences in cell numbers at specific days we used Student's *t*-tests, according to Sokal and Rohlf (2001). The confidence level for all the analysis was set at 95%.

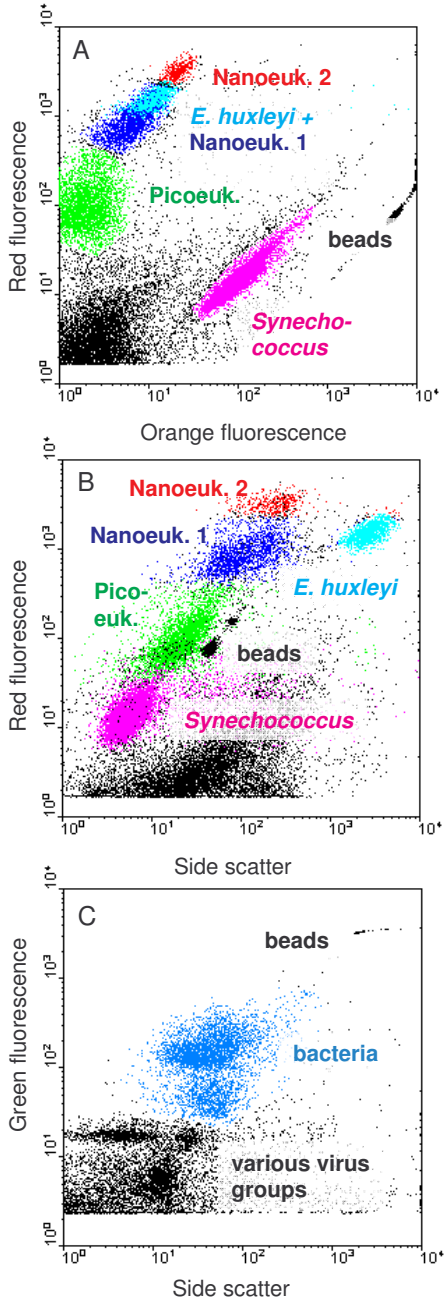

Fig. 1. Flow cytometric analysis of natural osmotrophic populations in the nine mesocosms during the third mesocosms experiment carried out by the project Pelagic Ecosystem CO_2 Enrichment Studies (PeECE III). Autrotrophs were analysed from unstained samples (A and B) and heterotrophic bacteria from SYBRGreen DNA stained samples (C). **(A)** *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.

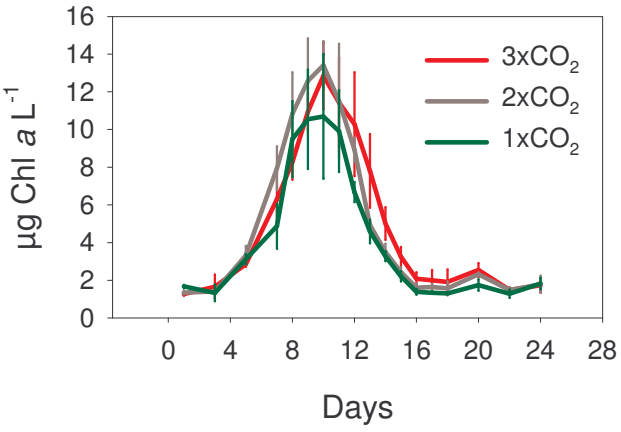

Fig. 2. Development of total chlorophyll-*a* in the mesocosms. Lines indicate average values for the three mesocosms in each treatment group ($3\times CO_2$ (mesocosms 1–3), $2\times CO_2$ (mesocosms 4–6), $1\times CO_2$ (mesocosms 7–9), and error bars denote ± 1 standard deviation. (Redrawn from Schultz et al., 2008).

3 Results

3.1 Dynamics of osmotrophs

The nutrients added at day 0 caused an increase in algal biomass (chlorophyll-*a* concentration) from approximately 2 μg chl-*a* l^{-1} to maximum values between 16 and 20 μg chl-*a* l^{-1} on day 9–10 (Fig. 2, Schultz et al., 2008). Towards the end of the experiment a second, and much smaller, peak (3–4 μg Chl-*a* l^{-1}) was observed. The major part of the two chl-*a* peaks consisted of diatoms and dinoflagellates, respectively (large osmotrophs) (Schultz et al., 2008; 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 intermediate sized osmotrophs (*Emiliania huxleyi*, Nanoeukaryotes 1 and 2, Fig. 3). Numerically the small osmotrophs dominated the phytoplankton community initially (Picoeukaryotes $\approx 1.3\times 10^5$ ml^{-1} and *Synechococcus* $\approx 0.6\times 10^5$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 $\approx 0.1\times 10^5$ ml^{-1} and *Synechococcus* to $\approx 0.1\times 10^5$ ml^{-1}). Both populations peaked again in the middle (days 15–16, Picoeukaryotes $\approx 0.7\times 10^5$ ml^{-1} and *Synechococcus* $\approx 1.2\times 10^5$ml^{-1}) and towards the end (days 23–25) of the experiment (Picoeukaryotes $\approx 0.7\times 10^5$ ml^{-1} and *Synechococcus* $\approx 3.3\times 10^5$ ml^{-1}). The picoeukaryotes dominated the autotrophic small osmotroph community during the first of the three peaks (day 2) with cell concentrations around 1.8×10^5 cells ml^{-1}, and an average picoeukaryote:

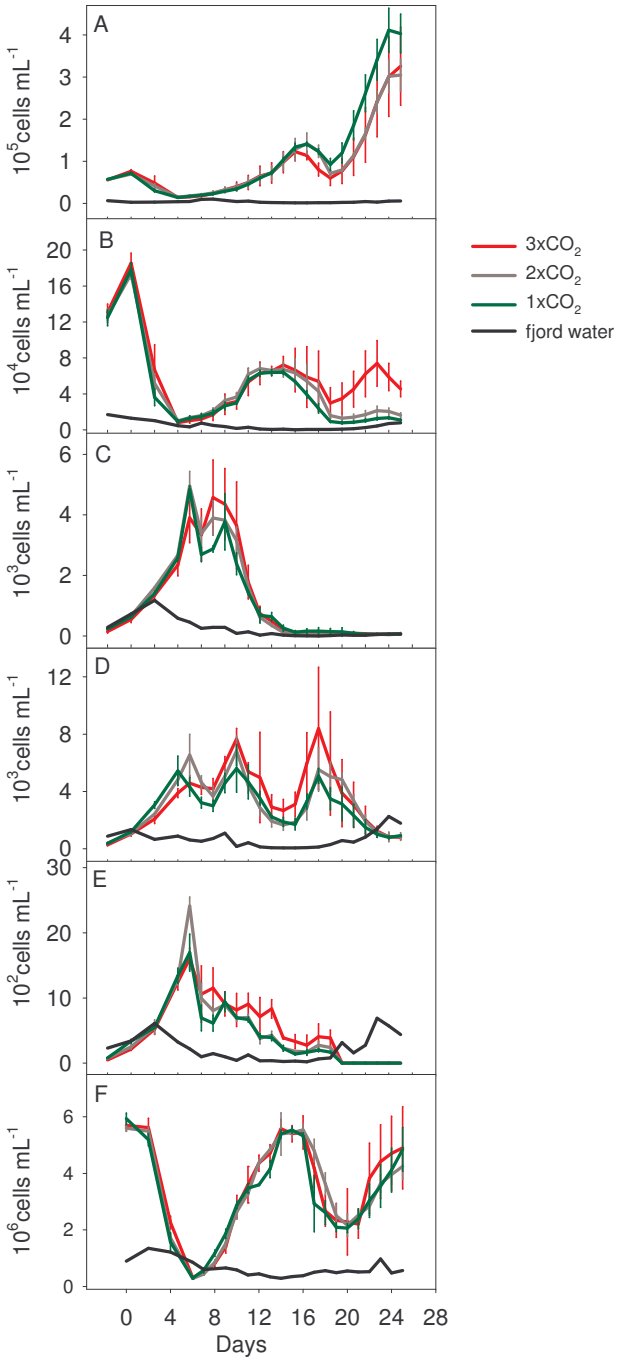

Fig. 3. Time series development of the six osmotrophic populations in the mesocosms as determined by flow cytometry. Lines indicate average values for the three mesocosms in each treatment group ($3\times CO_2$ (mesocosms 1–3), $2\times CO_2$ (mesocosms 4–6), $1\times CO_2$ (mesocosms 7-9). Error bars 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.

Synechococcus ratio of 2.5:1. The 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* $\approx 4.6\times10^3$ cells ml^{-1}; nanoeukaryotes $1\approx 5.2\times10^3$ cells ml^{-1}; nanoeukaryotes $2\approx 1.9\times10^3$ cells ml^{-1}; Fig. 3c, d, e). Nanoeukaryotes 1 peaked twice after this with maximum cell concentrations around 7×10^3 and 8×10^3 cells ml^{-1} at day 11 and 18, respectively.

Heterotrophic bacteria showed a dynamic similar to that of small autotrophic osmotrophs with high initial concentrations (ca. 7.7×10^6 cells ml^{-1}), a rapid decrease that was followed by a new peak ($\approx 5.4\times10^6$ cells ml^{-1}) culminating at day 15, and new maximum the last day of the experiment ($\approx 4.6\times10^6$ cells ml^{-1} day 25, Fig. 3f).

3.2 CO_2 effects

Although statistically significant treatment effects in Chl-*a* concentrations were observed some days only (Fig. 2; Schultz et al., 2008) there was a tendency of higher concentrations at the two highest pCO_2 concentrations (Fig. 2).

When comparing abundances of the six individual populations of small and intermediate sized osmotrophs (Fig. 3) we did not observe any effect of the CO_2 treatment from day 0 to day 7. As the bloom of *E. huxleyi* progressed (e.g. on day 9) somewhat higher cell concentrations were found in the $3\times CO_2$ ($\approx 4.6\times10^3$ cells ml^{-1}) compared to the $1\times$($\approx 2.9\times10^3$ cells ml^{-1}) and $2\times CO_2$ mesocosms ($\approx 3.9\times10^3$ cells ml^{-1}; Fig. 3c), but most days the differences were not statistically significant (Table 1). A similar trend of increasing abundances with increasing CO_2 level was detected in nanoeukaryotes 1 and nanoeukaryote 2 from day 8 onwards (Fig. 3d and e) but with statistically significant differences certain days only (Table 1). The most apparent differences between treatment groups were found in small autotrophic osmotroph abundances towards the end of the experiment (Fig. 3a and b). *Synechococcus* concentrations were higher in $1\times CO_2$than in the other mesocosms from around day 19 onwards (Fig. 3a, Table 1) whereas the picoeukaryotes were found at highest numbers in the mesocosms at higher CO_2 concentrations (Fig. 3b, Table 1)

One of the $3\times CO_2$ (mesocosm 3) and one of the $2\times CO_2$ (mesocosm 6) had a salinity structure somewhat different from the rest (Schultz et al., 2008), and the largest variability between mesocosms were recorded within the $3\times CO_2$ treatment group. This indicates that some other factor than seawater pCO_2 may have influenced the development of osmotrophs in these two units. One could therefore argue that the observed differences within the microbial communities associated with different pCO_2 levels could have been caused by other factors than the treatment itself. However,

Table 1. List of days at which osmotrophic cell concentrations were significantly different between treatment groups ($p<0.05$). One- and two tailed T-test were applied.

Population	Between $3\times CO_2$ and $2\times CO_2$		Between $3\times CO_2$ and $1\times CO_2$		Between $2\times CO_2$ and $1\times CO_2$	
	One tail	Two tail	One tail	Two tail	One tail	Two tail
E. huxleyi	–	–	–	–	11	–
Nanoeukaryotes 1	10	–	8, 9, 10, 16	8	8	8
Nanoeukaryotes 2	14, 15	14, 15	9, 14, 15	14, 15	16, 19	16, 19
Synechococcus	–	–	19, 21	–	21-25	24
Picoeukaryotes	23–25	25	21–25	24, 25	19–21 24, 25	19
Heterotrophic bacteria	–	–	–	–	–	–

a closer inspection of each of the time series subjected to a given treatment (see supplementary information) reveals that for all osmotrophic populations the data obtained from mesocosm #3 draws the average values for treatment $3\times CO_2$ closer to the average values of the other two treatments rather than the opposite. The average value displayed in Fig. 3 is thus more likely an underestimation than an overestimation of possible CO_2 treatment effects. Moreover, lack of significant differences (Table 1) when statistically tested, in spite of a relatively large difference between averages (Fig. 3), may in several instances be due to the large variance caused by mesocosms #3 and #6.

The heterotrophic bacteria were not affected much by changes in CO_2 concentrations but a minute tendency (not statistically significant) of higher bacteria numbers in $3\times CO_2$ compared to the $1\times$ and $2\times CO_2$ mesocosms was found the last few days of the experiment (Fig. 3f).

4 Discussion

4.1 Dynamics of osmotrophic populations

Based on the inorganic nutrient environment, phosphate availability, and the dominating phytoplankton succeeding the initial nutrient manipulation, Tanaka et al. (2008) divided the experimental period into five different, and partly overlapping, 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 10–16) Si and P depletion took place (N still replete) and by the end of phase 4 (days 13–20) Si, P and N were all depleted. In phase 5 (days 21–24) Si, P and N were still depleted but the situation 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; Schultz et al., 2008). The flow cytometry results presented here revealed a much more varied dynamic among the various osmotrophic populations: The initial nutrient pulse resulted in a community shift from small sized (=picoplankton: heterotrophic bacteria, *Synechococcus* and picoeukaryotes) to intermediate (*Emiliania 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 defence specialists 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 are thought to outcompete 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 brought about by the initial nutrient pulse.

A more careful examination is needed to explain how similar sized populations within each of the osmotrophic groups (small and intermediate/big) can co-exist. By inspecting the defence group (intermediate and big osmotrophs) it appears that when silicate got exhausted (phase 2) 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 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 3. The coccolithophorid experienced a viral attack, however (Larsen et al., 2007) giving room for Nanoeukaryotes 1 and 2, which retained with oscillations until phase 5. Our analyses did not allow for species designation of Nanoeukaryotes 1 and 2, but

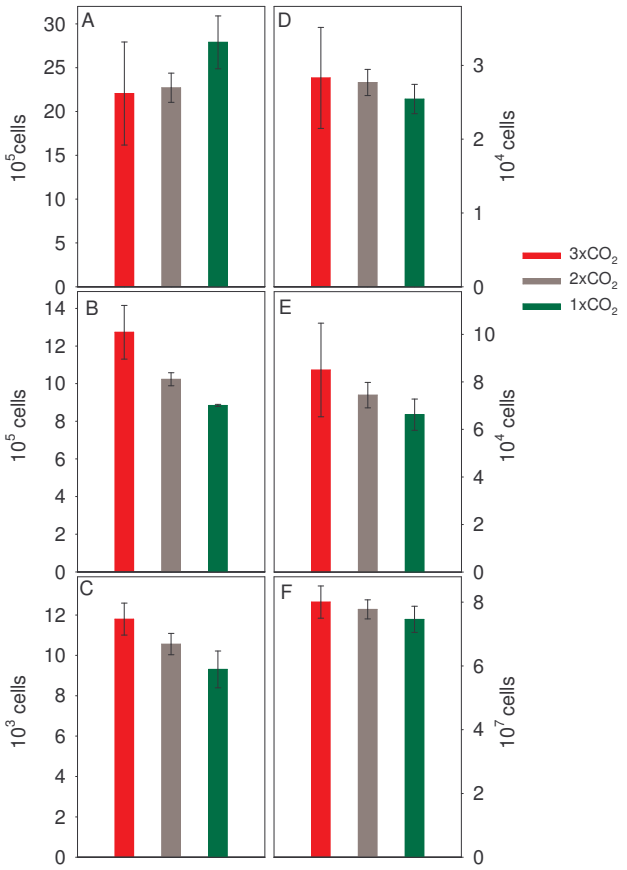

Fig. 4. Total cell number of the six osmotrophic populations during the entire experiment. Each bar denotes average total cell number for the three mesocosms of the treatment group ($3\times CO_2$ (mesocosms 1–3), $2\times CO_2$ (mesocosms 4–6), $1\times CO_2$ (mesocosms 7–9). Error bars denote ± 1 standard deviation. **(A)** *Synechococcus*, **(B)** Picoeukaryotes, **(C)** *Emiliania huxleyi*, **(D)** Nanoeukaryotes 1, **(E)** Nanoeukaryotes 2, **(F)** Heterotrophic bacteria.

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 viruses, closely related to viruses within the Phycodnavirideae, were present (Larsen et al., 2007). It may therefore well be that the different peaks represent 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 1 and 4 (and towards the end of phase 5). 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 al. (2008) concludes that bacterial growth was not limited by the availability of labile DOC whereas mineral nutrients were depleted from phase 4. The latter explanation thus seem more plausible and can explain why the picoeukaryotes dominated the small sized autotrophic community in the beginning of the experiment (phase 1) whereas *Synechococcus* took on the lead role in phase 5. The bacterio-, cyanophages and algal virus dynamic demonstrated in Larsen et al. (2007) suggests that viruses played an essential role for the dynamics within each of the three populations of small osmotrophs (Thingstad, 2000).

It has already been mentioned that the initial nutrient addition was followed by a noticeable decrease in abundance of competition specialists (small sized osmotrophs: heterophic bacteria, *Synechococcus* and picoeukaryotes). However, when comparing the concentration of these three populations with the corresponding populations 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 CO_2 levels killed off possible predators and/or released DOM, which they could have benefited from if they were nutrient limited in the fjord water 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 only speculate that these were the mechanisms leading to the high initial concentration of small osmotrophs.

4.2 CO_2 effects on the osmotrophic community

The current study did not reveal omotrophic successional shifts that can be traced back to altered CO_2 concentrations. Nor were we able to detect introduction or removal of specific osmotrophic taxa as a result of the CO_2 manipulation. We did, however, observe some differences in population abundances between the three treatment groups ($1\times CO_2$, $2\times CO_2$ and $3\times CO_2$). Our results may thus possibly support previous observations indicating that increased seawater pCO_2 can affect relative abundances within the phytoplankton community (Tortell et al., 2002; Grossart et al., 2006; Engel et al., 2008). The differences were most obvious in phase 4 and 5, with elevated picoeukaryote- and reduced *Synechococcus* concentrations at the highest CO_2 level. Similar differences between treatment groups were not as evident for the remaining osmotrophs, but a trend of

higher cell numbers with increasing CO_2 for all populations except *Synechococcus* emerged when calculating total 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 (Egge et al., 2007), and less available phosphate, expressed by increased alkaline phosphate activity (Tanaka et al., 2008), in the second half of the experiment.

It has previously been documented that some phytoplankton species (*E. huxleyi*, *G. oceanica*) increase photosynthetic carbon fixation rates with increasing in CO_2 concentrations (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 seawater pCO_2 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 which influence the marine microbial systems profoundly (reviewed by Brussaard, 2004). Larsen et al. (2007) showed that one virus which infect *E. huxelyi*, and one assumingly infecting some other nanoeukaryote, occurred in higher numbers in mesocosms with the lowest CO_2 level. This is obviously an additional reason for lower *E. huxleyi*- and nanoeukaryotes 1 and 2 concentrations in these very same enclosures.

The only osmotrophic population with higher biomasses (this study) and production (Egge et al., 2007) in the lower CO_2 treatments was *Synechococcus*. Engel et al. (2005) report that average abundances of *Synechococcus* in a similar mesocosm experiment in 2001 were not affected by the CO_2 concentrations, but a closer inspection of the dynamics of osmotrophs (presented by Rochelle-Newall, 2004, Fig. 2) reveal that also in that case densest *Synechococcus* populations occurred within the enclosures exposed to the lowest CO_2 concentration. In both experiments higher *Synechococcus* abundances at lower CO_2 levels were visible only towards the end when inorganic N and P were depleted and osmotrophic production dependent on remineralised nutrients. When not combined with a simultaneous increase in temperature, Fu et al. (2007) unveiled only a modest (and not significant) increase in growth rates of *Synechococcus* when increasing CO_2. Although CO_2 did not exceed 750 ppm in their experiment, this may indicate that at the present day temperatures and CO_2 level *Synechococcus* has CO_2 requirement fulfilled. Moreover, direct competition experiments have demonstrated that low CO_2concentrations favour the growth of cyanobacteria over other phytoplankton species in freshwater systems (Shapiro, 1973), and freshwater *Synechococcus* has proved to 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 CO_2 concentrating mechanisms (CCMs). The observed *Synechococcus* dominance in phase 5 could thus be a combined 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 limited picoeukaryotic growth. The fact that picoeukaryotic abundance increased considerably when CO_2 concentration was raised to 1050 μatm (Fig. 3) indicates that this could have been true. Prasinophytes (the marine 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 increases at the expense of cyanobacteria when CO_2 increases. $2\times CO_2$ equals the highest CO_2 level tested in 2001, and in neither experiment this CO_2 concentration resulted in elevated picoeukaryotic abundances (Fig. 3 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 CO_2 environment and link the indirect effect of changes in pCO_2 on bacterial activities to phytoplankton dynamics. In the 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 4, in these enclosures.

4.3 Concluding remarks

The osmotrophic community within our mesocosms may have experienced three perturbing events: A potentially effect of the filling and/or bubbling procedures, CO_2 manipulations, and nutrient addition. 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 specialists/intermediate sized phytoplankton 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. Effects of the CO_2 manipulations were not quite as obvious. This may be 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 units. Nevertheless, our results seem to substantiate previous works suggesting that CO_2 variations influence

the relative taxonomic composition of marine phytoplankton (Tortell et al., 2002; Grossart et al., 2006; Engel et al., 2008). Differences in population abundances between treatment groups were most noticeable towards the end of the experiment when nutrients were limiting (Tanaka et al., 2008), net production zero or below (i.e. based on regenerated nutrients; Egge et al., 2007), and small and intermediate sized osmotrophs had increased their importance relatively to the diatoms (this study; Riebesell et al.,2007; Schultz et al., 2008). A number of CCM variants, differing in manner of operation and efficiency, are found among different phytoplankton, and nutrient availability is also known to play a significant role in modulating CCMs (reviewed by Giordano et al., 2005). From our results alone it is therefore difficult to judge whether increase in atmospheric CO_2 might have a greater effect when production is based on regenerated nutrients, or whether our observations possibly 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 CO_2 levels (John et al., 2007). An observed a shift from diatoms to nanophytoplankton when Hare et al., 2007 incubated phytoplankton communities at elevated pCO_2 support the latter explanation. In any case, our experiment do indicate, as previously suggested (Tortell, 2000), that the competitive balances between microbial taxa may be altered when seawater pCO_2 changes. Proven synergetic effects (Fu et al., 2007; Hare et al., 2007) implies greater alterations when/if increased pCO_2 is accompanied (as predicted) by elevated seawater temperatures.

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