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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 $p\text{CO}_2$ levels. Differences in phytoplankton and heterotrophic bacteria abundances associated with the CO₂ treatment are also described. Coastal seawater was enclosed in floating mesocosms (27 m³) and nutrients were supplied initially in order to stimulate growth of microbial organisms, including the coccolithophorid *Emiliana huxleyi*. The mesocosms were modified to achieve 350 μatm ($1 \times \text{CO}_2$), 700 μatm ($2 \times \text{CO}_2$) and 1050 μatm ($3 \times \text{CO}_2$) CO₂ pressure. The temporal dynamics was related to nutrient conditions in the enclosures. Numerically small osmotrophs (picoeukaryotes and *Synechococcus* 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 $p\text{CO}_2$. Towards the end of the experiment we did, however, record significantly higher picoeukaryotic and lower *Synechococcus*-abundances in the higher CO₂ treatments. Slightly increased cell concentrations of *E. huxleyi* and other nanoeukaryotes were also recorded at elevated $p\text{CO}_2$ on certain days.

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 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).



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The partial pressure of CO₂ in the atmosphere ($p\text{CO}_2$) has increased from a pre-industrial level of 280 $\mu\text{g atm}$ to the present level of 370 $\mu\text{g atm}$. Further increased atmospheric CO₂ concentration will lead to a rise in the CO₂ 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 CO₂ requirement fulfilled at the present day levels, whereas others (like the haptophyte *Emiliana huxleyi*) may benefit, in terms of increased primary production, from an increase in seawater $p\text{CO}_2$ (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 $p\text{CO}_2$, whereas other nanoautotrophs and picoautotrophs eukaryotes were not affected by altered CO₂ environments (Delille et al., 2005; Engel et al., 2005).

Seawater mesocosms allow studies of $p\text{CO}_2$ 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 CO₂ Enrichment Studies (PeECE). The two first experiments had a maximum CO₂ 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 $p\text{CO}_2$ 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 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 May 11 with 27 m³ unfiltered, nutrient-poor, post-bloom fjord water. The atmospheric and seawater $p\text{CO}_2$ were manipulated to achieve levels of 1050 μatm simulating 2150 conditions (3 $\times\text{CO}_2$ mesocosms 1–3), to 700 μatm in a year 2100 scenario (2 $\times\text{CO}_2$ mesocosms 4–6) and to 350 μatm CO₂ as the present scenario (1 $\times\text{CO}_2$ mesocosms 7–9). To initiate the development of a bloom of the coccolithophore *Emiliana 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 $\mu\text{mol L}^{-1}$ NO₃ and 0.6 $\mu\text{mol L}^{-1}$ 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).

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 $\mu\text{l min}^{-1}$). 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 phycoerythrin). We followed the dynamics of five different autotrophic phytoplankton populations (*Synechococcus* sp., *Emiliana 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%.

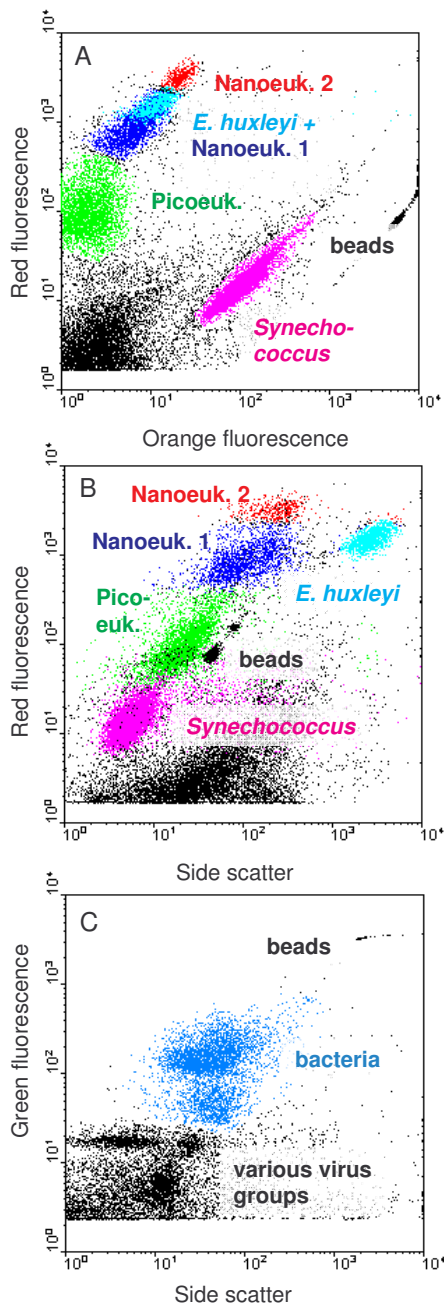


Fig. 1. Flow cytometric analysis of natural osmotrophic populations in the nine mesocosms during the third mesocosms experiment carried out by the project Pelagic Ecosystem CO₂ Enrichment Studies (PeECE III). Autotrophs were analysed from unstained samples (A and B) and heterotrophic bacteria from SYBRGreen DNA stained samples (C). (A) *Synechococcus* sp. and picoautotrophs were discriminated using a combination of red and orange fluorescence. (B) *Emiliana 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.

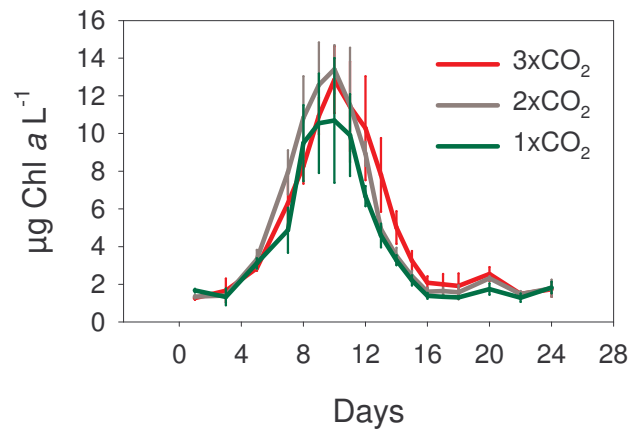


Fig. 2. Development of total chlorophyll-*a* in the mesocosms. Lines indicate average values for the three mesocosms in each treatment group (3×CO₂ (mesocosms 1–3), 2×CO₂ (mesocosms 4–6), 1×CO₂ (mesocosms 7–9), and error bars denote ±1 standard deviation. (Redrawn from Schultz et al., 2008).

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 (*Emiliana huxleyi*, Nanoeukaryotes 1 and 2, Fig. 3). Numerically the small osmotrophs dominated the phytoplankton community initially (Picoeukaryotes ≈1.3×10⁵ ml⁻¹ and *Synechococcus* ≈0.6×10⁵ 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 ≈0.1×10⁵ ml⁻¹ and *Synechococcus* to ≈0.1×10⁵ ml⁻¹). Both populations peaked again in the middle (days 15–16, Picoeukaryotes ≈0.7×10⁵ ml⁻¹ and *Synechococcus* ≈1.2×10⁵ ml⁻¹) and towards the end (days 23–25) of the experiment (Picoeukaryotes ≈0.7×10⁵ ml⁻¹ and *Synechococcus* ≈3.3×10⁵ ml⁻¹). The picoeukaryotes dominated the autotrophic small osmotroph community during the first of the three peaks (day 2) with cell concentrations around 1.8×10⁵ cells ml⁻¹, and an average picoeukaryote:

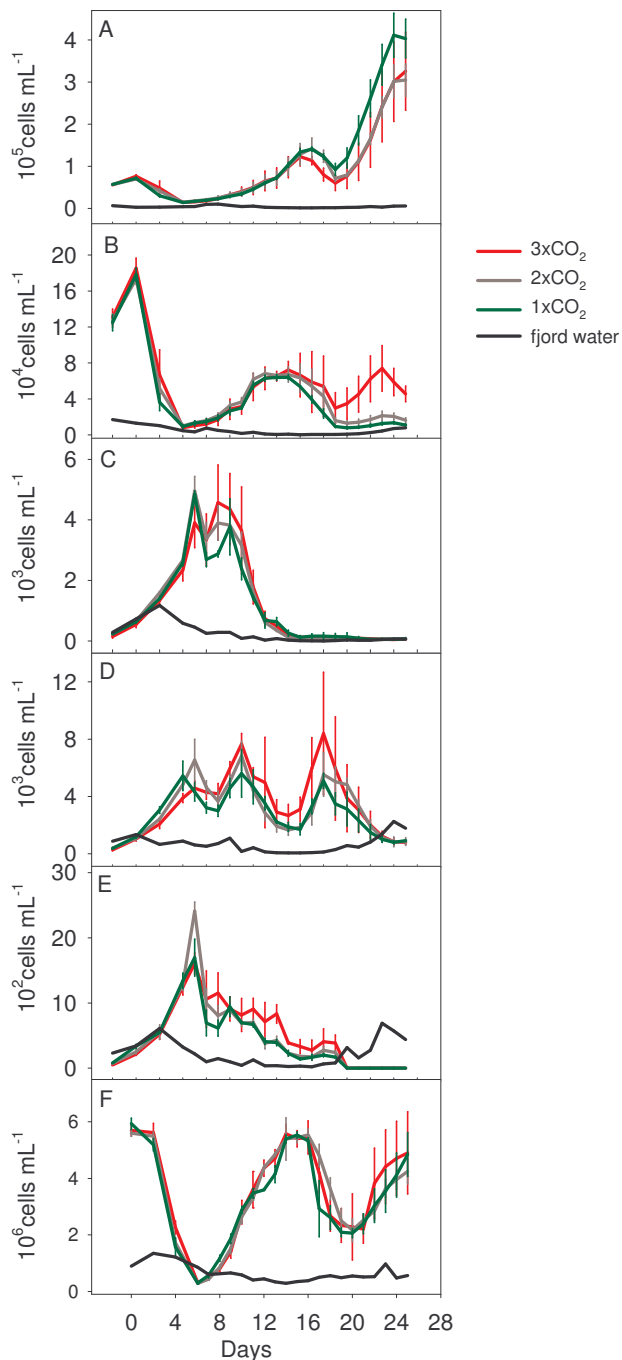


Fig. 3. Time series development of the six osmotrophic populations in the mesocosms as determined by flow cytometry. Lines indicate average values for the three mesocosms in each treatment group ($3\times\text{CO}_2$ (mesocosms 1–3), $2\times\text{CO}_2$ (mesocosms 4–6), $1\times\text{CO}_2$ (mesocosms 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) *Emiliana 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* $\approx 4.6\times 10^3$ cells mL^{-1} ; nanoeukaryotes 1 $\approx 5.2\times 10^3$ cells mL^{-1} ; nanoeukaryotes 2 $\approx 1.9\times 10^3$ 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 ($\approx 5.4\times 10^6$ 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

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 $p\text{CO}_2$ 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 CO₂ 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 $3\times\text{CO}_2$ ($\approx 4.6\times 10^3$ cells mL^{-1}) compared to the $1\times$ ($\approx 2.9\times 10^3$ cells mL^{-1}) and $2\times\text{CO}_2$ mesocosms ($\approx 3.9\times 10^3$ cells mL^{-1} ; Fig. 3c), but most days the differences were not statistically significant (Table 1). A similar trend of increasing abundances with increasing CO₂ 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 $1\times\text{CO}_2$ 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 CO₂ concentrations (Fig. 3b, Table 1).

One of the $3\times\text{CO}_2$ (mesocosm 3) and one of the $2\times\text{CO}_2$ (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 $3\times\text{CO}_2$ treatment group. This indicates that some other factor than seawater $p\text{CO}_2$ 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 $p\text{CO}_2$ 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×CO ₂ and 2×CO ₂		Between 3×CO ₂ and 1×CO ₂		Between 2×CO ₂ and 1×CO ₂	
	One tail	Two tail	One tail	Two tail	One tail	Two tail
<i>E. huxleyi</i>	–	–	–	–	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
<i>Synechococcus</i>	–	–	19, 21	–	21–25	24
Picoeukaryotes	23–25	25	21–25	24, 25	19–21	19
					24, 25	
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×CO₂ 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 CO₂ 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 CO₂ concentrations but a minute tendency (not statistically significant) of higher bacteria numbers in 3×CO₂ compared to the 1× and 2×CO₂ 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 re-

vealed 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 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 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

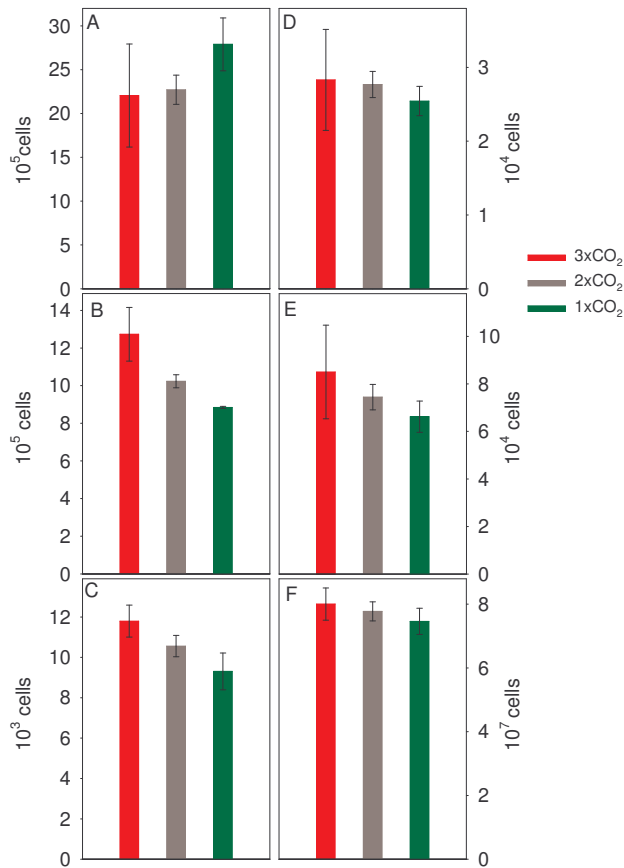


Fig. 4. Total cell number of the six osmotrophic populations during the entire experiment. Each bar denotes average total cell number for the three mesocosms of the treatment group (3×CO₂ (mesocosms 1–3), 2×CO₂ (mesocosms 4–6), 1×CO₂ (mesocosms 7–9)). Error bars denote ±1 standard deviation. (A) *Synechococcus*, (B) Picoeukaryotes, (C) *Emiliana huxleyi*, (D) Nanoeukaryotes 1, (E) Nanoeukaryotes 2, (F) Heterotrophic bacteria.

several *Chrysochromulina* (Prymnesiophyceae) and *Pyramimonas* (Prasinophyceae) species are common nanoeukaryotes in our coastal waters (Thronsen et al., 2003), and species within these genera have proven susceptible to virus within the Phycodnaviridae family (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 Phycodnaviridae, 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 (heterotrophic bacteria, *Synechococcus* and picoeukaryotes) in phase 1 and 4 (and towards the end of phase 5). Such within-community similarities suggest a com-

mon 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: heterotrophic 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 mesocosms 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 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 CO₂ effects on the osmotrophic community

The current study did not reveal osmotrophic successional shifts that can be traced back to altered CO₂ concentrations. Nor were we able to detect introduction or removal of specific osmotrophic taxa as a result of the CO₂ manipulation. We did, however, observe some differences in population abundances between the three treatment groups (1×CO₂, 2×CO₂ and 3×CO₂). Our results may thus possibly support previous observations indicating that increased seawater pCO₂ 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 CO₂ level. Similar differences between treatment groups were not as evident for the remaining osmotrophs, but a trend of

higher cell numbers with increasing CO₂ 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 CO₂ 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 CO₂ 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 *p*CO₂ will promote growth of calcifying primary producers. Our results do not necessarily support this conclusion as all intermediate autotrophic osmotrophs (including the non calcifiers) seemed to experience a similar, and small, increase in abundance as CO₂ increased. One aspect that could interfere with our interpretation of possible CO₂ 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. huxleyi*, and one presumably infecting 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.

The only osmotrophic population with higher biomasses (this study) and production (Egge et al., 2007) in the lower CO₂ 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 CO₂ concentrations, but a closer inspection of the dynamics of osmotrophs (presented by Rochelle-Newall, 2004, Fig. 2) reveal that also in that case dense *Synechococcus* populations occurred within the enclosures exposed to the lowest CO₂ concentration. In both experiments higher *Synechococcus* abundances at lower CO₂ 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 CO₂. Although CO₂ did not exceed 750 ppm in their experiment, this may indicate that at the present day temperatures and CO₂ level *Synechococcus* has CO₂ requirement fulfilled. Moreover, direct competition experiments have demonstrated that low CO₂ concentrations 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 CO₂ 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 CO₂ concentration was raised to 1050 μ atm (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 CO₂ increases. 2 \times CO₂ equals the highest CO₂ level tested in 2001, and in neither experiment this CO₂ 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 CO₂ environment and link the indirect effect of changes in *p*CO₂ on bacterial activities to phytoplankton dynamics. In the current experiment the effect, if any, was a slight tendency of higher concentration in 3 \times CO₂ mesocosms than in 1 \times and 2 \times CO₂, 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, CO₂ 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 CO₂ 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 CO₂ 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 CO₂ 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 CO₂ levels (John et al., 2007). An observed shift from diatoms to nanophytoplankton when Hare et al., 2007 incubated phytoplankton communities at elevated pCO₂ 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 pCO₂ changes. Proven synergetic effects (Fu et al., 2007; Hare et al., 2007) implies greater alterations when/if increased pCO₂ is accompanied (as predicted) by elevated seawater temperatures.

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References

- Badger, M. R. and Price, G. D.: CO₂ concentrating mechanisms in cyanobacteria: molecular components, their diversity and evolution, *J. Exp. Bot.*, 54, 609–622, 2003.
- Bratbak, G. and Thingstad, T. F.: Phytoplankton-bacteria interactions: an apparent paradox? Analysis of a model system with both competition and commensalism, *Mar. Ecol.- Prog. Ser.*, 25, 23–30, 1985.
- Brewer, P. G., Goyet, C., and Friederich, G.: Direct observation of the oceanic CO₂ increase revisited. *P. Natl. Acad. Sci. USA*, 94, 8308–8313, 1997.
- Brussaard, C. P. D.: Viral Control of Phytoplankton Populations — a Review, *J. Eukaryot. Microbiol.*, 51, 125–138, 2004.
- Burkhardt, S., Riebesell, U., and Zondervan, I.: Effects of growth rate, CO₂ concentration, and cell size on the stable carbon

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- isotope fractionation in marine phytoplankton, *Geochim. Cosmochim. Ac.*, 63, 3729–3741, 1999.
- Burkhardt, S., Amoroso, G., Riebesell, U., and Sultemeyer, D.: CO₂ and HCO₃⁻ uptake in marine diatoms acclimated to different CO₂ concentrations, *Limnol. Oceanogr.*, 46, 1378–1391, 2001.
- Cole, J. J., Findlay, S., and Pace, M. L.: Bacterial production in fresh and saltwater ecosystems: a cross-system overview, *Mar. Ecol.- Prog. Ser.*, 43, 1–10, 1988.
- Delille, B., Harlay, J., Zondervan, I., Jacquet, S., Chou, L., Wollast, R., Bellerby, R. G. J., Frankignoulle, M., Borges, A. V., Riebesell, U., and Gattuso, J. P.: Response of primary production and calcification to changes of pCO₂ during experimental blooms of the coccolithophorid *Emiliania huxleyi*, *Global Biogeochem. Cy.*, 19, GB2023, doi:10.1029/2004GB002318, 2005.
- Egge, J. K.: Nutrient control of phytoplankton growth: Effects of macronutrient composition (N, P, Si) on species succession, *Dr.s. thesis*, University of Bergen, Norway. 40pp., 1993.
- Egge, J. K., and Jacobsen, A.: Influence of silicate on particulate carbon production in phytoplankton, *Mar. Ecol.-Prog. Ser.*, 147, 219–230, 1997.
- Egge, J. K., Thingstad T. F., Engel, A., and Riebesell, U.: Primary production during nutrient-induced blooms at elevated CO₂ concentrations, *Biogeosciences Discuss.*, 4, 3913–3936, 2007, <http://www.biogeosciences-discuss.net/4/3913/2007/>.
- Engel, A., Delille, B., Jacquet, S., Riebesell, U., Rochelle-Newall, E., Terbruggen, A., and Zondervan, I.: Transparent exopolymer particles and dissolved organic carbon production by *Emiliania huxleyi* exposed to different CO₂ concentrations: A mesocosm experiment, *Aquat. Microb. Ecol.*, 34, 93–104, 2004.
- Engel, A., Zondervan, I., Aerts, K., Beaufort, L., Benthien, A., Chou, L., Delille, B., Gattuso, J. P., Harlay, J., Heemann, C., Hoffmann, L., Jacquet, S., Nejstgaard, J., Pizay, M. D., Rochelle-Newall, E., Schneider, U., Terbruggen, A., and Riebesell, U.: Testing the direct effect of CO₂ concentration on a bloom of the coccolithophorid *Emiliania huxleyi* in mesocosm experiments, *Limnol. Oceanogr.*, 50, 493–507, 2005.
- Engel, A., Schulz, K. G., Riebesell, U., Bellerby, R., Delille, B., Schartau, M.: Effects of CO₂ on particle size distribution and phytoplankton abundance during a mesocosm bloom experiment (PeECE II), *Biogeosciences*, 5, 509–521, 2008, <http://www.biogeosciences.net/5/509/2008/>.
- Fenchel, T.: Relation between particle size selection and clearance in suspension-feeding ciliates, *Limnol. Oceanogr.*, 25, 733–738, 1980.
- Fenchel, T.: Ecology – Potentials and Limitations, *Excellence in Ecology Series*, Vol. 1. Ecology Institute, 43–54, 1987.
- Fu, F. X., Warner, M. E., Zhang, Y. H., Feng, Y. Y., and Hutchins, D. A.: Effects of increased temperature and co₂ on photosynthesis, growth, and elemental ratios in marine *Synechococcus* and *Prochlorococcus* (Cyanobacteria), *J. Phycol.*, 43, 485–496, 2007.
- Giordano, M., Beardall, J., and Raven, J. A.: CO₂ concentrating mechanisms in algae: Mechanisms, environmental modulations, and evolution, *Annu. Rev. Plant. Biol.*, 56, 99–131, 2005.
- Grossart, H.P., and Simon, M.: Bacterial colonization and microbial decomposition of limnetic aggregates (lake snow), *Aquat. Microb. Ecol.*, 15, 127–140, 1998.
- Grossart, H. P., Levold, F., Allgaier, M., Simon, M., and Brinkhoff, T.: Marine diatom species harbour distinct bacterial communi-

- ties, *Environ. Microbiol.*, 7, 860–873, 2005.
- Grossart, H. P., Allgaier, M., Passow, U., and Riebesell, U.: Testing the effect of CO₂ concentration on the dynamics of marine heterotrophic bacterioplankton, *Limnol Oceanogr.*, 51, 1–11, 2006.
- Hamm, C. E.: Architecture, ecology and biogeochemistry of phaeocystis colonies, *J. Sea Res.*, 43, 307–315, 2000.
- Hamm, C. E. M., Merkel, R., Springer, O., Jurkojc, P., Maier, C., Prechtel, K., and Smetacek, V.: Architecture and material properties of diatom shells provide effective mechanical protection, *Nature*, 421, 841–843, 2003.
- Hare, C. E., Leblanc, K., DiTullio, G. R., Kudela, R. M., Zhang, Y., Lee, P. A., Riseman, S., and Hutchins, D. A.: Consequences of increased temperature and CO₂ for phytoplankton community structure in the Bering Sea, *Mar. Ecol.-Prog. Ser.*, 352, 9–16, 2007.
- Hassidim, M., Keren, N., Ohad, I., Reinhold, L., and Kaplan, A.: Acclimation of *Synechococcus* strain WH7803 to ambient CO₂ concentration and to elevated light intensity, *J. Phycol.*, 33, 811–817, 1997.
- John, D. E., Wang, Z. A., Liu, X., Byrne, R. H., Corredor, J. E., López, J. M., Cabrera, A., Bronk, D., Tabita, F.R., and Paul, J. H.: Phytoplankton carbon fixation gene (RuBisCO) transcripts and air-sea CO₂ flux in the Mississippi River Plume, *The ISME Journal*, 1, 517–531, 2007.
- Kuenen, J. G., Boonstra, J., Scroder, H. G. J., and Veldkamp, H.: Competition for inorganic substrates among chemoorganotrophic and chemolithotrophic bacteria, *Microbial Ecol.*, 3, 119–130, 1977.
- Kuenzler, E. J. and Perras, J. P.: Phosphatases of marine algae, *Biol. Bull.*, 128, 271–284, 1965.
- Larsen, J. B., Larsen, A., Thyraug, R., Bratbak, G., and Sandaa, R.-A.: Response of marine viral populations to a nutrient induced phytoplankton bloom at different pCO₂ levels, *Biogeosciences*, 5, 523–533, 2008, <http://www.biogeosciences.net/5/523/2008/>.
- Marie, D., Brussaard, C. P. D., Partensky, F., and Vaulot, D.: Enumeration of phytoplankton, bacteria, viruses in marine samples, in: *Current Protocols in Cytometry*, edited by: Robinson, J. P., Darzynkiewicz, Z., Dean, P. N., Orfao, A., and 4 others, John Wiley & Sons, Chichester, 11.11.1–11.11.15, 1999.
- Not, F., Massana, R., Latasa, M., Marie, D., Colson, C., Eikrem, W., Pedros-Alio, C., Vaulot, D., and Simon, N.: Late summer community composition and abundance of photosynthetic picoeukaryotes in Norwegian and Barents Seas, *Limnol. Oceanogr.*, 50, 1677–1686, 2005.
- Pinhassi, J., Sala, M. M., Havskum, H., Peters, F., Guadayol, O., Malits, A., and Marrase, C. L.: Changes in bacterioplankton composition under different phytoplankton regimens, *Appl. Environ. Microb.*, 70, 6753–6766, 2004.
- Pinhassi, J., Gomez-Consarnau, L., Alonso-Saez, L., Sala, M. M., Vidal, M., Pedros-Alio, C., and Gasol, J. M.: Seasonal changes in bacterioplankton nutrient limitation and their effects on bacterial community composition in the NW mediterranean sea, *Aquat. Microb. Ecol.*, 44, 241–252, 2006.
- Raven, J. and Waite, A.: The evolution of silification in diatoms: inescapable sinking and sinking as escape, *New Phytol.*, 162, 45–65, 2004.
- Riebesell, U.: Effects of CO₂ enrichment on marine phytoplankton, *J. Oceanogr.*, 60, 719–729, 2004.
- Riebesell, U., Revill, A. T., Holdsworth, D. G., and Volkman, J. K.: The effects of varying CO₂ concentration on lipid composition and carbon isotope fractionation in *Emiliania huxleyi*, *Geochimica Et Cosmochimica Acta*, 64, 4179–4192, 2000.
- Riebesell, U., Schultz, K. G., Bellerby, R. G. J., Botros, M., Fritsche, P., Meyerhöfer, M., Neill, C., Nondal, G., Oschlies, A., Wohlers, J., and Zöllner, E.: Enhanced biological carbon consumption in high CO₂ ocean, *Nature*, 450, 545–548, doi:10.1038/nature06267, 2007.
- Riegman, R., Stolte, W., Noordeloos, A. A. M., and Slezak, D.: Nutrient uptake and alkaline phosphatase (EC 3:1:3:1) activity of *Emiliania huxleyi* (Prymnesiophyceae) during growth under N and P limitation in continuous cultures, *J. Phycol.*, 36, 87–96, 2000.
- Rochelle-Newall, E., Delille, B., Frankignoulle, M., Gattuso, J.-P., Jacquet, S., Riebesell, U., Terbruggen, A., and Zondervan, I.: Chromophoric dissolved organic matter in experimental mesocosms maintained under different pCO₂ levels, *Mar. Ecol.-Prog. Ser.*, 272, 25–31, 2004.
- Rost, B., Riebesell, U., Burkhardt, S., and Sultemeyer, D.: Carbon acquisition of bloom-forming marine phytoplankton, *Limnol. Oceanogr.*, 48, 55–67, 2003.
- Sandaa, R.-A., Heldal, M., Castberg, T., Thyraug, R., and Bratbak, G.: Isolation and characterization of two marine viruses infecting *Chrysochromulina ericina* and *Pyramimonas orientalis*, *Virology*, 290, 272–280, 2001.
- Schulz, K. G. and Riebesell, U.: Build-up and decline of organic matter during PeECE III, *Biogeosciences*, 5, 707–718, 2008, <http://www.biogeosciences.net/5/707/2008/>.
- Shapiro J.: Blue-green algae: Why they become dominant, *Science*, 179, 382–384, 1973.
- Smith, R. E. H. and Kalf, J.: Size-dependent phosphorus uptake kinetics and cell quota in phytoplankton, *J. Phycol.*, 18, 275–284, 1982.
- Smith, D. C., Steward, G. F., Long, R. A., and Azam, F.: Bacterial mediation of carbon fluxes during a diatom bloom in a mesocosm, *Deep-Sea Res. Pt II*, 42, 75–97, 1995.
- Sokal, R.R. and Rohlf, F.J.: *Biometry, the principles and practice of statistics in biological research* 3rd Ed. 7th Printing. W. H. Freeman and Company, 887 pp., 2001.
- Suttle, C. and Chan, A.: Viruses infecting the marine prymnesiophyte *Chrysochromulina* spp.: isolation, preliminary characterization and natural abundance. *Mar. Ecol.-Prog. Ser.*, 118, 275–282, 1995.
- Tanaka, T., Thingstad, T. F., Løvdaal, T., Grossart, H. P., Larsen A., Schultz, K., Riebesell, U.: Availability of phosphate for phytoplankton and bacteria and of glucose for bacteria at different pCO₂ levels in a mesocosm study, *Biogeosciences*, 5, 669–678, 2008, <http://www.biogeosciences.net/5/669/2008/>.
- Thingstad, T. F.: A theoretical approach to structuring mechanisms in the pelagic food web, *Hydrobiologia*, 363, 59–72, 1998.
- Thingstad, T. F.: Elements of a theory for the mechanisms controlling abundance, diversity, and biogeochemical role of lytic bacterial viruses in aquatic systems, *Limnol. Oceanogr.*, 45, 1320–1328, 2000.
- Thingstad, T. F. and Lignell, R.: Theoretical models for the control of bacterial growth rate, abundance, diversity and carbon demand, *Aquat. Microb. Ecol.*, 13, 19–27, 1997.

- Thingstad, T. F., Havskum, H., Zweifel, U. L., Berdalet, E., Sala, M.M., Peters, F., Alcaraz, M., Scharek, R., Perez, M., Jacquet, S., Flaten, G. A. F., Dolan, J.R., Marrasé, C., Rassoulzadegan, F., Hagstrøm, A., and Vaulot, D.: Ability of a “minimum” microbial food web model to reproduce response patterns observed in mesocosms manipulated with N and P, glucose, and Si, *J. Marine Syst.*, 64, 15–34, 2007.
- Thingstad, T. F., Ovreas, L., Egge, J. K., Lovdal, T., and Heldal, M.: Use of non-limiting substrates to increase size; a generic strategy to simultaneously optimize uptake and minimize predation in pelagic osmotrophs?, *Ecol. Lett.*, 8, 675–682, 2005.
- Thronsen J., Hasle G. R., and Tangen K.: *Norsk Kystplankton Flora*, Oslo Almater Forlag AS, Norway, 2003.
- Tortell, P. D.: Evolutionary and ecological perspectives on carbon acquisition in phytoplankton, *Limnol Oceanogr.*, 45, 744–750, 2000.
- Tortell, P. D., Rau G. H., and Morell, F. M., M.: Inorganic carbon acquisition in coastal pacific phytoplankton communities, *Limnol. Oceanogr.*, 45, 1485–1500, 2000.
- Tortell, P. D., DiTullio, G. R., Sigman, D. M., and Morel, F. M. M.: CO₂ effects on taxonomic composition and nutrient utilization in an equatorial pacific phytoplankton assemblage, *Mar. Ecol.–Prog. Ser.*, 236, 37–43, 2002.
- Williams, T. G., and Turpin, D. H.: Photosynthesis kinetics determine the outcome of competition for dissolved inorganic carbon by freshwater microalgae: implications for acidified lakes, *Oecologia (Berlin)*, 73, 307–311, 1987.