Biogeosciences Discuss., 9, 8173–8197, 2012
www.biogeosciences-discuss.net/9/8173/2012/
doi:10.5194/bgd-9-8173-2012

Biogeosciences Discussions

Effect of ocean acidification on the fatty acid composition of a natural plankton community

E. Leu[1,*], M. Daase[1], K. G. Schulz[2], A. Stuhr[2], and U. Riebesell[2]

[1]Norwegian Polar Institute, FRAM Centre, 9296 Tromsø, Norway
[2]Helmholtz Centre for Ocean Research Kiel (GEOMAR), 24105 Kiel, Germany
[*]now at: University of Hamburg, Institute for Hydrobiology and Fisheries, Grosse Elbstrasse 133, 22767 Hamburg, Germany

Received: 17 May 2012 – Accepted: 25 May 2012 – Published: 9 July 2012

Correspondence to: E. Leu (eva.leu@zmaw.de)

Published by Copernicus Publications on behalf of the European Geosciences Union.

Abstract

The effect of ocean acidification on the fatty acid composition of a natural plankton community in the Arctic was studied in a large-scale mesocosm experiment, carried out in Kongsfjorden (Svalbard, Norway) at 79° N. Nine mesocosms of ~ 50 cbm each were exposed to different pCO_2 levels (from natural background conditions to ~ 1420 µatm), yielding pH values (on the total scale) from ~ 8.3 to 7.5. Inorganic nutrients were added on day 13. The phytoplankton development during this 30 days experiment passed three distinct phases: (1) prior to the addition of inorganic nutrients, (2) first bloom after nutrient addition, and (3) second bloom after nutrient addition. The fatty acid composition of the natural plankton community was analysed and showed, in general, high percentages of polyunsaturated fatty acids (PUFAs): 44–60 % of total fatty acids. Positive correlations with pCO_2 were found for most PUFAs during phases 2 and/or 3, with the exception of 20 : 5n3 (eicosapentaenoic acid, EPA), an important diatom marker. There are strong indications for these correlations being mediated indirectly through taxonomic changes and the natural development of the communities in the mesocosms exposed to different pCO_2 levels. While diatoms increased during phase 3 mainly in the low and intermediate pCO_2 treatments, dinoflagellates were favoured by high CO_2 concentrations during the same time period. This is reflected in the development of group-specific fatty acid trophic markers. No indications were found for a generally detrimental effect of ocean acidification on the planktonic food quality in terms of essential fatty acids. The significant positive correlations between most PUFAs and pCO_2 reflected treatment-dependent differences in the community composition between the mesocosms rather than a direct positive effect of pCO_2 on specific fatty acids.

1 Introduction

Ocean acidification occurs as a consequence of increasing atmospheric CO_2 concentrations, and is thought to represent a major threat towards some groups of marine

BGD
9, 8173–8197, 2012

organisms. Due to the temperature-dependence of gas saturation in seawater, high latitudes are especially sensitive to anthropogenic carbon dioxide. Higher CO_2 solubility at lower temperatures results in naturally low buffering capacity, leading to lower carbonate ion saturation state (Broecker et al., 1979) and increase temporal pH variability (Steinacher et al., 2009). This potentially aggravates the threat of ocean acidification to organisms relying on carbonate or aragonite structures at high latitudes (see Lischka et al., 2011, and references therein). Little is known about the impact of ocean acidification on Arctic pelagic communities, and this study presents the first experimental approach to address this question in situ at a high Arctic location.

Storing metabolic energy in the form of lipids is one of the most wide-spread and important adaptive traits among organisms living at high latitudes, characterized by an extreme seasonality in environmental conditions and food supply. Hence, the transport of energy and biomass through the Arctic food web can be characterized as lipid-based. Most lipids consist mainly of fatty acids, hydrocarbon chains with varying numbers of double bonds. Among these fatty acids, some polyunsaturated fatty acids (PUFAs, with two or more double bonds) are essential metabolites. These are synthesized by algae only and have to be taken up via the diet by all other organisms. Both, in field observations (Pond et al., 1996; Jonasdottir et al., 2005) and experimental studies (Jonasdottir et al., 2009; Klein Breteler et al., 2005), they have been shown to be crucial for copepod egg production and hatching, as well as for proper development until reaching maturity. Also in fish, the essential role of PUFAs for successful recruitment and reproduction has been documented by, e.g., Watanabe et al. (1983) and Sargent et al. (1995). A comprehensive overview on the biochemical aspects of trophic interactions can be found in Muller-Navarra (2008). Moreover, the fatty acid composition of membrane lipids controls membrane fluidity, and is therefore intrinsically linked to the functioning of all membrane-bound physiological processes, like, e.g., photosynthesis or respiration. The fatty acid composition of major algae groups varies due to differences in the genetically determined biosynthetic pathways for fatty acid synthesis. Consequently, the fatty acid composition of a natural plankton community will reflect its

BGD
9, 8173–8197, 2012

taxonomic composition in a biomass-dependent way. Based on this knowledge, fatty acids can be used as fatty acid trophic markers (FATM) (Dalsgaard et al., 2003). In addition, the relative amount of PUFAs produced by algae will depend strongly on the algal physiological state, and, hence the environmental conditions. It has been shown that algal PUFA production is negatively affected by high irradiances (Thompson et al., 1990; Leu et al., 2006, 2010), low nutrient concentrations (Klein Breteler et al., 2005; Reitan et al., 1994), and high temperature (Thompson et al., 1992).

So far very few studies have examined the effect of ocean acidification on algal or planktonic fatty acid composition. All previous studies were carried out with unialgal cultures in the laboratory and yielded differing results: a first study was conducted by Tsuzuki et al. (1990), testing several species from different algal groups for changes in lipid class and fatty acid composition as a function of pCO_2. In four of seven species, no effects were found at all, and in general, no change in lipid class composition was observed between high and low CO_2 treatments. Only green algae showed a pCO_2 induced change in fatty acid composition. Carvalho and Malacta (2005) tried to optimize omega-3 fatty acid production in the prymnesiophyte *Pavlova lutheri*, and found an increase of total lipids under high pCO_2, but relatively less PUFAs. The biochemical response of three different species of prymnesiohytes to increased pCO_2 was studied by Fiorini et al. (2010), without finding a general decrease in food quality in terms of n3 fatty acids. Riebesell et al. (2000) reported a substantial decline of all major PUFAs in *Emiliania huxleyi* as a function of increasing pCO_2. Contrary to all these studies, Hoshida et al. (2005) described an accumulation of 20 : 5n3 (EPA) in *Nannochloropsis* as a response to elevated pCO_2. A first study was performed on indirect effects of ocean acidification on grazers (copepods), mediated by trophic transfer from algae grown under elevated CO_2 concentrations: the authors found strong negative effects of high pCO_2, both with respect to the algal content of essential PUFAs, but also in the copepods' fatty acid composition, development and egg production rates (Rossoll et al., 2012). To our knowledge, no previous attempts have been made to investigate the response of a natural community fatty acid composition to increased levels of pCO_2.

BGD
9, 8173–8197, 2012

In the framework of the large-scale mesocosm experiment in Ny-Ålesund (Svalbard) in summer 2010, we tested for the very first time the effect of ocean acidification on the fatty acid composition of a natural plankton community. The aim of this study was to describe how the fatty acid composition of a natural plankton community is affected by decreasing pH values, with a special emphasis on essential PUFAs. The study encompassed three phases dominated by different algal communities and characterized by a shortage of inorganic nutrients at the start of the experiment, and growth under nutrient replete conditions following artificial nutrient addition half way through the experiment.

2 Materials and methods

2.1 Study area

Kongsfjorden is a fjord on the western coast of Spitsbergen at a latitude of almost 79° N (Svalbard, Norway). It is 20 km long with a width ranging from 4 to 10 km and a maximum depth of 400 m. Kongsfjorden is influenced by both Atlantic and Arctic water masses, and receives a discharge of freshwater and sediments from the adjacent glaciers that varies seasonally, peaking in the summer. During winter, the inner part of the fjord will typically have a land-fast ice cover. The formation, thickness and break-up of this ice cover have a substantial interannual variation, linked to the climatic and hydrographic conditions. A detailed review of the physical environment of Kongsfjorden is given by Svendsen et al. (2002). The mesocosm experiment was carried out close to the settlement of Ny-Ålesund on the southern shore of Kongsfjorden at 78°56,2′ N und 11°53,6′ E.

BGD
9, 8173–8197, 2012

2.2 Experimental setup

A detailed description of the experimental setup, its deployment and technical features can be found in Riebesell et al. (2012). In brief, nine mesocosms consisting of 17 m long flexible plastic tubes fixed to a buoyancy and stability rack were deployed in Kongsfjorden for an experimental period of about 30 days. In each mesocosm, ~50 cbm of natural seawater were enclosed at the start of the experiment. When initially lowering the plastic tubes, a sieve with 3 mm mesh size was attached to the bottom of the tubes, thereby excluding organisms above this size from enclosure. The mesocosms were deployed at $t = -7$ days, and closed two days later ($t = -5$), in order to minimize differences in starting conditions between mesocosms which may arise from patchiness in plankton distributions during lowering of the mesocosm bags. CO_2 enrichment was achieved through addition of CO_2 saturated seawater to seven of nine mesocosms (the remaining two serving as control treatments) in five steps, between day −1 and day 4. The initial and final CO_2 concentrations and corresponding pH values for all nine mesocosms are provided in Table 1 (see also Bellerby et al., 2012).

On day 13, nitrate, phosphate and silicate were added to all mesocosms to increase concentrations by 5, 0.3 and 2.5 µmol l^{-1}, respectively, corresponding to approx. 50 % of the winter concentrations measured in Kongsfjorden (Leu et al. (2006)). Most parameters were sampled daily using a depth-integrated water sampler (IWS, Hydrobios), covering the upper 12 m of the water column. Samples for planktonic fatty acid composition were taken on days 4, 8, and on every other day between days 11 and 25.

2.3 Analyses

2.3.1 Fatty acid composition

For fatty acid analyses, filters of the various treatments were crushed by ultrasonification and extracted in dichloromethane : methanol (2 : 1, v/v) following the method after Folch et al. (1957). Prior to extraction, an internal standard was added (23 : 0). For

BGD
9, 8173–8197, 2012

gas liquid chromatography of fatty acids, methyl esters were prepared from aliquots of the extracted microalgae by transesterification with 3 % sulfuric acid in methanol for 4 h at 80 °C. After extraction with hexane, fatty acid methyl esters (FAME) were analyzed with a gas liquid chromatograph (Agilent 6890, Agilent GmbH, Waldbronn, Germany) on a capillary column (60 m × 0.25 mm I.D.; film thickness: 0.25 µm; liquid phase: DB-FFAP (Agilent) using temperature programming (53) (injection: splitless at 250 °C, detection: FID at 280 °C). FAMEs were identified by comparison with known standard mixtures. The total amount of lipids is referred to as the sum of total fatty acid methyl esters.

The following fatty acids were used as taxonomic marker fatty acids (modified after Dalsgaard et al., 2003; Ackman et al., 1968):

- Diatoms: 16 : 1n7 + 16 : 4n1 + 20 : 5n3
- Dinoflagellates: 18 : 4n3 + 18 : 5n3 + 22 : 6n3
- Chlorophytes: 18 : 2n6 + 18 : 3n3

Indicative for bacterial biomass are odd chain and branched fatty acids, e.g. i15 : 0 and a15 : 0.

2.3.2 Chlorophyll *a*

Chlorophyll *a* (Chl *a*) was determined fluorometrically on depth-integrated (0–12 m depth) water samples, filtered on GF/F filters, according to Welschmeyer (1994).

2.3.3 Statistical analyses

Linear regression analyses were used to investigate the relationship between proportions of different fatty acid markers (%) and CO_2 level (µatm) in the different mesocosms at different phases of the experiment. The duration of the experiment was divided into three phases with phase 1 starting on day 4 and lasting until day 12, phase 2 starting

BGD
9, 8173–8197, 2012

on day 13 until day 21 and phase 3 starting on day 22 and lasting to the end of the experiment. An average of the proportion of the different fatty acid markers and the CO_2 concentration in the different mesocosms was calculated for each phase. Principal component analysis (PCA) was used to obtain an overview of similarities in the fatty acid composition measured at the different days of the experiment. The PCA distributes the samples in an ordination space according to the fatty acid composition along orthogonal principal components that are extracted to explain as much of the variance as possible. The response variables are indicated by arrows in the direction of increased importance in separating the samples. The length of the arrows indicates the importance of the variables in explaining the variation on the samples (long arrow = high importance, short arrow = low importance). All statistical analyses were done using R (R Development Core Team, 2005).

3 Results

3.1 General development of autotroph biomass (in terms of Chl *a*)

The development of fluorometrically determined Chl *a* concentrations in the nine mesocosms is shown in Fig. 1. The bloom development during the experimental period can be divided into three phases: phase 1 represents the phytoplankton development prior to the addition of inorganic nutrients (day 13). It was characterized by a nanoplankton community utilizing predominantly organic nutrients. After this bloom decreased, inorganic nutrients (nitrate, phosphate, silicate) were added corresponding to ~50 % of typical winter concentrations. This induced a second bloom, lasting from day 13 to 21, followed by a third bloom (day 22 to 30). The second bloom was dominated by picoplankton and rapidly controlled by microzooplankton and viruses, while the third bloom was dominated by nanophytoplankton (based on cell abundance), and by dinoflagellates in terms of biomass. It declined as a result of nutrient depletion (Brussaard et al., 2012). Interestingly, the pCO_2 levels in the different mesocosms yielded

BGD
9, 8173–8197, 2012

different effects in every bloom phase: during the first bloom, no treatment effect was observed. During the second bloom, CO_2 effects were found on growth, nutrient uptake rates and pigment biosynthesis (Schulz et al., 2012), with higher Chl *a* values in the high pCO_2 mesocosms. In the last bloom, the highest Chl *a* values were found in the intermediate and low pCO_2 treatments. Furthermore, diatoms, haptophytes and prasinophytes were more abundant under low/intermediate pCO_2 conditions, while dinoflagellates were favored by high CO_2 concentrations (Brussaard et al., 2012; Schulz et al., 2012). The temporal development of particulate carbon, nitrogen and phosphorus is described in detail by Schulz et al. (2012).

3.2 Planktonic fatty acid composition

The planktonic fatty acid composition was characterized by comparatively high values of PUFAs throughout the entire experimental period and within all treatments, ranging from 44 to 60 % of total fatty acids (Fig. 2, Table sI). On day 4, the PUFA content between the different mesocosms varied between 44 and 55 %, while in most mesocosms the lowest values were found on day 8 (46–50 %), with the exception of mesocosm 9 (44 % on day 4). After that, an increase was seen in almost all mesocosms, with the highest values in the three high pCO_2 treatments between days 19 and 25 (during the later part of phase 2, and phase 3). Among the n3 PUFAs, 20 : 5n3 (eicosapentaenoic acid, EPA) and 22 : 6n3 (docohexaenoic acid, DHA) were most abundant, each accounting for up to 20 % of total fatty acids (Fig. 2, Table sI). The percentages of EPA increased mainly during phase 3, whereas DHA rose most pronounced between days 8 and 18 (mainly phase 2). The C18 n3 PUFAs both declined during phase 3; the highest values of 18 : 4n3 were observed on day 8, followed by a continuous decline. 18 : 5n3 increased from initially 4–5 % up to almost 14 % throughout phases 1 and 2, followed by a decline during phase 3. The overall PUFA content (in % of total fatty acids) showed a significant positive correlation with pCO_2 in phases 2 and 3 (Fig. 3, Table 2). DHA was significantly negatively correlated with pCO_2 during phase 1, and strongly positively correlated during phase 3. The latter was also found for the dinoflagellate

BGD
9, 8173–8197, 2012

markers (18 : 4n3 + 18 : 5n3 + DHA). The diatom markers (16 : 1n7 + 16 : 4n1 + EPA) showed a significant negative correlation with pCO_2 in phases 2 and 3. For EPA alone, this correlation was only significant in phase 2. Of all PUFAs, 18 : 5n3 was the one depicting the greatest changes and treatment-dependent differences, with the highest values found in the highest pCO_2 treatment during phase 2 (Fig. 2).

The most important saturated fatty acid (SAFA) was 16 : 0, followed by 14 : 0, accounting for roughly 17 and 7 %, respectively, throughout the entire period and in all treatments (Fig. 2). In particular 16 : 0 showed almost no changes over time or differences between treatments. Relatively high (and differing) percentages of 18 : 0 were found in the first samples (day 4), followed by a clearly decreasing trend, and ending with 2–3 % for all treatments at the end of the investigated period. Amongst the monounsaturated fatty acids (MUFAs), 16 : 1n7 and 18 : 1n9 had the highest percentages (on average 5–7 % of total fatty acids, Fig. 2, Table sI), with the highest values of 18 : 1n9 found in the middle of the investigated period (between days 11 and 17), while 16 : 1n7 peaked in the beginning (day 4) and the end (days 23–25), particularly in the low and intermediate pCO_2 treatments.

The group-specific fatty acids, which can be used as taxonomic markers, followed the development and decline of the major phytoplankton groups (diatoms, dinoflagellates, chlorophytes/ prasinophytes): the diatom markers (16 : 1n7 + 16 : 4n1 + 20 : 5n3) were relatively high in all treatments on day 4 (21–27 %), decreased afterwards in all treatments to 12–16 % during the phase between the first and the second bloom (days 11–17), and increased in all treatments towards the end, particularly under low and intermediate pCO_2 conditions. The dinoflagellate markers (18 : 4n3 + 18 : 5n3 + 22 : 6n3) increased between day 8 and day 21 in the high pCO_2 treatments, while they decreased again after day 21 in the mesocosms treated with intermediate or low pCO_2 concentrations. The chlorophyte markers (18 : 2n6 + 18 : 3n3) increased from day 4 to 8, followed by a weakly decreasing trend towards the end of the experimental period in all treatments. Highest values were found under high pCO_2 conditions, however, the correlation with pCO_2 was only significant during phase 2.

BGD
9, 8173–8197, 2012

A principal component analysis (PCA) revealed that the temporal development of the blooms (i.e. taxonomic changes over time) was the major factor determining the extent of similarity in fatty acid composition between different samples (Fig. 4). Within those entities, samples from the mesocosms exposed to the highest pCO_2 concentrations were (partly) separated from the rest. This separation of the high pCO_2 samples from the rest was particularly clear during the later part of the experiment. With respect to dominating fatty acids, the first and last samples were characterized by relatively high percentages of diatom marker fatty acids, samples from the period between bloom phase 1 and 2 displayed increased values of chlorophyte markers, while dinoflagellate markers were most important in samples from the second bloom, as well as in the high pCO_2 samples of phase 3 (Fig. 4).

4 Discussion

The development of the phytoplankton community in the experiment can be separated into three successive bloom phases, characterized by different nutrient conditions and taxonomic compositions. While there was no detectable difference between the treatments during phase 1, the plankton communities in the different mesocosms developed increasingly different over time. The overall biomass response (in terms of Chl *a*) to the applied pCO_2 gradient was different for each bloom phase (for details see Schulz et al., 2012). Since changes in the community composition are reflected better in the relative fatty acid composition, we chose to mainly show these data. We did, however, also analyse the quantitative fatty acid data (see Tables sII and sIII, Figs. sI and sII). For standardizing them to a biomass measure we used particulate carbon instead of Chl *a*, since no distinction is possible between autotrophic and heterotrophic components of the plankton community in this size range. The quantitative fatty acid data showed a very similar development of the single fatty acids as the percentage data, and also a very similar pattern in their correlations with pCO_2. Slightly higher amounts of total

BGD
9, 8173–8197, 2012

fatty acids were found at higher CO_2 concentrations in all phases of the experiment, although these weak trends were never statistically significant (Fig. sII, Table sIII).

The overall high percentages of PUFAs in POM exceeded even the previously reported PUFA values during the early phase of a spring bloom in Kongsfjorden in 2003 (Leu et al. (2006)). This seems surprising given the low inorganic nutrient concentrations measured in the beginning of the experimental period, but can probably be explained by the fact that this situation was characterized by a dominance of nanophytoplankton, utilizing predominantly recycled nutrients (ammonia, DON), made available via grazing and the microbial loop. This implies that the phytoplankton community was not limited by nutrients. The low C : P and C : N ratios in particulate organic matter (POM) also confirm the absence of nutrient limitation during the first phase of the experiment (before nutrient addition), despite very low measurable amounts of free inorganic nutrients (Schulz et al., 2012). No direct effect of nutrient addition was seen on the overall percentage of PUFAs in the mesocosms exposed to low or intermediate pCO_2, while an increase was found in the high pCO_2 treatments (see Fig. 2). Previous studies about the impact of nutrient limitation on fatty acid composition have always shown a decrease in PUFAs as a consequence of poor nutrient supply (Roessler, 1988; Reitan et al., 1994), but those were laboratory studies with single species, neglecting the aspect of a phytoplankton community thriving mainly on (recycled) organic nutrients as found during phase 1 of this experiment. Mayzaud et al. (2012) reported similarly high values of DHA in POM from Kongsfjorden in late June 2007.

Our results provide no indications for an immediate negative effect of ocean acidification on essential fatty acids, neither with respect to the absolute nor the relative amounts (Tables sI, sII, Figs. 3 and sII). As sole exception the relative amount of EPA was significantly negatively correlated with pCO_2 during phase 2. This trend was also negative in phases 1 and 3, but not statistically significant. However, to which extent the positive correlation of high pCO_2 levels with the overall percentage of PUFAs were caused by a direct effect of CO_2 on specific fatty acids or their synthesis, cannot be deduced from a field study like this. The PCA rather points at taxonomic changes

BGD
9, 8173–8197, 2012

as the most important factor of change, thereby indicating an indirect effect on fatty acid composition, mediated by species-specific differences in the response to elevated pCO_2 levels. From an ecological point of view, however, it is remarkable that the overall community response to increased CO_2 concentrations was positive in this experiment. This may indicate that findings about detrimental effects of ocean acidification on single species in laboratory studies (as, for instance, Riebesell et al., 2000; Tsuzuki et al., 1990), and even their consequences for grazers (Rossoll et al., 2012) might be less relevant in a natural situation where other, more CO_2-tolerant species take over. But apart from the overall communities' PUFA content, a shift in taxonomic composition might well have an effect on the efficiency of trophic transfer, since different size-classes and taxonomic groups of phytoplankton are better or less suitable as food source for different grazers. These changes are, however, not related to the fatty acid composition per se.

With respect to the use of marker fatty acids for quantitative assessments of taxonomic composition we found reasonable agreement between our results and those obtained by CHEMTAX analysis (based upon a HPLC analysis of photosynthetic pigments), and by traditional microscopic analysis and species identification – at least for the later part of the experimental period. Microscopic analysis and fatty acid composition confirmed an increase of diatom biomass towards the end of the experiment, with their highest abundances found in the low and intermediate pCO_2 treatments. Similarly, the positive response of autotrophic dinoflagellates to high pCO_2 in the late phase of the experiment was found with all three methods (see Schulz et al., 2012; Brussaard et al., 2012). However, according to the HPLC and microscopic analyses the increase in autotrophic dinoflagellates started only after day 14 and continued until day 25 in all treatments. It decreased thereafter in the low CO_2 treatment, but stays high in intermediate and high CO_2. Microscopic analysis indicated furthermore that heterotrophic dinoflgellates were abundant throughout the experiment and did not differ much between treatments. The high levels of diatom marker fatty acids during phase 1, on the contrary, were not in accordance with the results from microscopic or HPLC analyses.

BGD
9, 8173–8197, 2012

Rather, they could be attributed to the numerous occurrences of cirripedia larvae in particular in the samples taken on day 4. These larvae are too small to be efficiently separated from the rest of the particulate organic matter collected on a GF/F-filter. As described by Niehoff et al. (2012), their abundance peaked around the day when the mesocosms were closed. Thereby, masses of these larval stages were entrapped in the enclosures and found in the water column during the early phase of the experiment. Later on, they sank out of the water column and supposedly started settling on the bottom or the walls of the mesocosms. A separate analysis of their fatty acid composition showed high values for typical membrane-specific fatty acids, such as 16 : 0, EPA and DHA (Table 3). As EPA represents at the same time the most important diatom marker, this explains the misleading finding of high percentages of diatom marker fatty acids. To our knowledge, this was the first time ever the fatty acid composition of cirriped nauplii was analysed specifically. Hence, there is no possibility to judge to which extent their specific fatty acid profile was related to their previous dietary uptake of algal fatty acids. We observed them during filtration only in the earliest samples taken for fatty acid analysis. Due to their relatively high biomass (compared to phytoplankton cells), their patchy occurrence in our samples probably also explains the striking variability in the fatty acid composition from different mesocosms on day 4 that exceeded by far the variability on any other sampling day. This was furthermore confirmed by a considerably higher content of total fatty acids in samples taken on day 4 (up to 250 µg FA per mg C vs. 70–160 µg FA per mg C on days without cirripedia "contamination", Table sII) that was similar to the total FA content of cirripeds analyzed separately (259 µg FA per mg C, Table 3).

5 Conclusions

We found no indications for a direct negative effect of ocean acidification on the nutritional quality of particulate matter in terms of its overall content in essential PUFAs. The applied pCO_2 gradient, however, did affect the taxonomic composition of the

BGD
9, 8173–8197, 2012

phytoplankton community in this experiment (particularly during the last phase), resulting in corresponding differences in fatty acid composition between pCO_2 treatments. Hence, the overall availability of essential PUFAs for higher trophic levels seems not to be affected negatively, although the specific fatty acid composition may change. It is likely, however, that other factors, most of all a change in average size distribution among phytoplankton due to taxonomic changes, will have a greater impact of the food web structure than the mere fatty acid composition of the community.

Supplementary material related to this article is available online at: http://www.biogeosciences-discuss.net/9/8173/2012/bgd-9-8173-2012-supplement.pdf.

Acknowledgements. This work is a contribution to the "European Project on Ocean Acidification" (EPOCA) which received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement no. 211384. We gratefully acknowledge the logistical support of Greenpeace International for its assistance with the transport of the mesocosm facility from Kiel to Ny-Ålesund and back to Kiel. We also thank the captains and crews of M/V *ESPERANZA* of Greenpeace and R/V *Viking Explorer* of the University Centre in Svalbard (UNIS) for assistance during mesocosm transport and during deployment and recovery in Kongsfjorden. We thank the staff of the French-German Arctic Research Base at Ny-Ålesund, in particular Marcus Schumacher, for on-site logistical support. We are grateful to Martin Graeve for his support of the fatty acid analysis, and to Barbara Niehoff for the collection and sorting of cirripedia nauplii. Financial support for E. Leu and M. Daase was provided by the Norwegian Research Council through project grant no. 184860 (MERCLIM).

References

Ackman, R. G., Tocher, C. S., and McLachlan, J.: Marine phytoplankter fatty acids, J. Fish. Res. Board Can., 25, 1603–1620, 1968.

BGD
9, 8173–8197, 2012

Bellerby, R. G. J., Silyakova, A., Nondal, G., Slagstad, D., Czerny, J., De Lange, T., and Ludwig, A.: Marine carbonate system evolution during the EPOCA Arctic pelagic ecosystem experiment in the context of simulated future Arctic ocean acidification, Biogeosciences, in prep., 2012.

Broecker, W. S., Takahashi, T., Simpson, H. J., and Peng, T. H.: Fate of fossil fuel carbon dioxide and the global carbon budget, Science, 26, 409–418, 1979.

Brussaard, C. P. D., Noordeloos, A. A. M., Witte, H., Collenteur, M., Schulz, K. G., Bellerby, R. G. J., Ludwig, A., Czerny, J., and Riebesell, U.: Arctic microbial community dynamics influenced by elevated CO_2 levels, Biogeosciences, in prep., 2012.

Carvalho, A. P. and Malcata, F. X.: Optimization of omega-3 fatty acid production by microalgae: crossover effects of CO_2 and light intensity under batch and continuous cultivation modes, Mar. Biotechnol., 7, 381–388, 2005.

Dalsgaard, J., St John, M., Kattner, G., Muller-Navarra, D., and Hagen, W.: Fatty acid trophic markers in the pelagic marine environment, Adv. Mar. Biol., 46, 225–340, 2003.

Fiorini, S., Gattuso, J.-P., van Rijswijk, P., and Middelburg, J.: Coccolithophores lipid and carbon isotope composition and their variability related to changes in seawater carbonate chemistry, J. Exp. Mar. Biol. Ecol., 394, 74–85, doi:10.1016/j.jembe.2010.07.020, 2010.

Folch, J., Lees, M., and Sloane Stanley, G. H.: A simple method for isolation and purification of total lipids from animal tissue, J. Biol. Chem., 226, 497–509, 1957.

Hoshida, H., Ohira, T., Minematsu, A., Akada, R., and Nishizawa, Y.: Accumulation of eicosapentaenoic acid in Nannochloropsis sp in response to elevated CO_2 concentrations, J. Appl. Phycol., 17, 29–34, doi:10.1007/s10811-005-5512-9, 2005.

Jonasdottir, S. H., Trung, N. H., Hansen, F., and Gartner, S.: Egg production and hatching success in the calanoid copepods Calanus helgolandicus and Calanus finmarchicus in the North Sea from March to September 2001, J. Plankton Res., 27, 1239–1259, 2005.

Jonasdottir, S. H., Visser, A. W., and Jespersen, C.: Assessing the role of food quality in the production and hatching of *Temora longicornis* eggs, Mar. Ecol.-Prog. Ser., 382, 139–150, 2009.

Klein Breteler, W. C. M., Schogt, N., and Rampen, S.: Effect of diatom nutrient limitation on copepod development: role of essential lipids, Mar. Ecol.-Prog. Ser., 291, 125–133, 2005.

Leu, E., Falk-Petersen, S., Kwasniewski, S., Wulff, A., Edvardsen, K., and Hessen, D. O.: Fatty acid dynamics during the spring bloom in a high Arctic fjord: importance of abiotic factors versus community changes, Can. J. Fish. Aquat. Sci., 63, 2760–2779, 2006.

BGD
9, 8173–8197, 2012

Leu, E., Wiktor, J., Søreide, J. E., Berge, J., and Falk-Petersen, S.: Increased irradiance reduces food quality of sea ice algae, Mar. Ecol.-Prog. Ser., 411, 49–60, 2010.
Lischka, S., Büdenbender, J., Boxhammer, T., and Riebesell, U.: Impact of ocean acidification and elevated temperatures on early juveniles of the polar shelled pteropod *Limacina helicina*: mortality, shell degradation, and shell growth, Biogeosciences, 8, 919–932, doi:10.5194/bg-8-919-2011, 2011.
Mayzaud, P., Boutoute, M., and Gasparini, S.: Differential response of fatty acid composition in the different lipid classes from naturally occurring particulate organic matter during spring and summer in a high Arctic fjord (Kongsfjorden, Svalbard), submitted, 2012.
Muller-Navarra, D. C.: Food web paradigms: the biochemical view on trophic interactions, Int. Rev. Hydrobiol., 93, 489–505, doi:10.1002/iroh.200711046, 2008.
Niehoff, B., Wolf, T., Czerny, J., and Boxhammer, T.: Mesozooplankton community development at elevated CO_2 concentrations: results from a mesocosm experiment in a high Arctic fjord, Biogeosciences, this volume, 2012.
Pond, D., Harris, R., Head, R., and Harbour, D.: Environmental and nutritional factors determining seasonal variability in the fecundity and egg viability of Calanus helgolandicus in coastal waters off Plymouth, UK, Mar. Ecol.-Prog. Ser., 143, 45–63, 1996.
Reitan, K. I., Rainuzzo, J. R., and Olsen, Y.: Effect of nutrient limitation on fatty acid and lipid content of marine microalgae, J. Phycol., 30, 972–979, 1994.
Riebesell, U., Revill, A. T., Holdsworth, D. G., and Volkman, J. K.: The effects of varying CO_2 concentration on lipid composition and carbon isotope fractionation in *Emiliania huxleyi*, Geochim. Cosmochim. Ac., 64, 4179–4192, doi:10.1016/s0016-7037(00)00474-9, 2000.
Riebesell, U., v. Bröckel, K., Büdenbender, J., Czerny, J., Deckelnick, M., Hoffmann, D., Lenz, U., Ludwig, A., and Schulz, K. G.: A mobile, free-floating mesocosm platform for in situ experimentation, Biogeosciences, in prep., 2012.
Roessler, P. G.: Effects of silicon deficiency on lipid composition and metabolism in the diatom Cyclotella Cryptica, J. Phycol., 24, 394–400, 1988.
Rossoll, D., Bermudez, R., Hauss, H., Schulz, K. G., Riebesell, U., Sommer, U., and Winder, M.: Ocean acidification-induced food quality deterioration constrains trophic transfer, PLoS ONE, 7, e34737, doi:10.1371/journal.pone.0034737, 2012.
Sargent, J. R., Bell, J. G., Bell, M. V., Henderson, R. J., and Tocher, D. R.: Dietary origins and functions of long-chain (n-3) polyunsaturated fatty acids in marine fish, J. Mar. Biotechnol., 3, 26–28, 1995.

BGD
9, 8173–8197, 2012

Schulz, K. G., Bellerby, R. G. J., Brussaard, C. P. D., Büdenbender, J., Czerny, J., Fischer, M., Koch-Klavsen, S., Krug, S., Lischka, S., Ludwig, A., M., M., Nondal, G., Silyakova, A., Stuhr, A., and Riebesell, U.: Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide, Biogeosciences, in prep., 2012.

Steinacher, M., Joos, F., Frölicher, T. L., Plattner, G.-K., and Doney, S. C.: Imminent ocean acidification in the Arctic projected with the NCAR global coupled carbon cycle-climate model, Biogeosciences, 6, 515–533, doi:10.5194/bg-6-515-2009, 2009.

Svendsen, H., Beszczynska-Moeller, A., Hagen, J. O., Lefauconnier, B., Tverberg, V., Gerland, S., Oerbaek, J. B., Bischof, K., Papucci, C., Zajaczkowski, M., Azzolini, R., Bruland, O., Wiencke, C., Winther, J. G., and Dallmann, W.: The physical environment of Kongsfjorden–Krossfjorden, an Arctic fjord system in Svalbard, Polar Res., 21, 133–166, 2002.

Thompson, P. A., Harrison, P. J., and Whyte, J. N. C.: Influence of irradiance on the fatty acid composition of phytoplankton, J. Phycol., 26, 278–288, 1990.

Thompson, P. A., Guo, M. X., Harrison, P. J., and Whyte, J. N. C.: Effects of variation in temperature on the fatty acid composition of 8 species of marine phytoplankton, J. Phycol., 28, 488–497, 1992.

Tsuzuki, M., Ohnuma, E., Sato, N., Takaku, T., and Kawaguchi, A.: Effects of CO_2 concentration during growth on fatty acid composition in microalgae, Plant Physiol., 93, 851–856, doi:10.1104/pp.93.3.851, 1990.

Watanabe, T., Tamiya, T., Oka, A., Hirata, M., Kitajima, C., and Fujita, S.: Improvement of dietary value of live foods for fish larvae by feeding them on Omega-3 highly unsaturated fatty-acids and fat soluble vitamins, B. Jpn. Soc. Sci. Fish., 49, 471–479, 1983.

Welschmeyer, N. A.: Fluorometric analysis of chlorophyll *a* in the presence of chlorophyll *b* and pheopigments, Limnol. Oceanogr., 39, 1985–1992, 1994.

BGD
9, 8173–8197, 2012

Table 1. pH and pCO_2 levels in the nine mesocosms at the start and the end of the experimental period.

	Initial (t8–t9)		Final (t26–t27)	
Mesocosm #	pH	pCO_2 (µatm)	pH	pCO_2 (µatm)
3	8.32	185	8.36	165
7	8.31	185	8.37	160
2	8.18	270	8.25	220
4	8.05	375	8.15	290
8	7.96	480	8.07	365
1	7.81	685	7.94	500
6	7.74	820	7.90	555
5	7.64	1050	7.80	715
9	7.51	1420	7.73	855
Fjord	8.41	145	8.32	180

BGD
9, 8173–8197, 2012

Table 2. Linear regression analyses of fatty acid markers (%) against CO_2 concentration (µatm) in different phases of the experiment. Proportion of different fatty acids markers and CO_2 concentrations were averaged over the time period of the three phases: Phase 1 = day −4 to 12, Phase 2 = day 13 to 21, Phase 3 = day 22 to end. Entries in the table give the estimates for the intercept and the slope, the R^2 value and the statistical significant level given as p values and indicated as *** $p < 0.001$, ** $0.001 < p < 0.01$, *$0.01 < p < 0.05$.

Phase	Fatty Acid	Intercept	Slope	P	Sign.	R^2
1	PUFA	51.31	−0.002	0.144		0.18
2	PUFA	52.01	0.003	0.035	*	0.42
3	PUFA	48.81	0.011	0.004	**	0.68
1	Diatom	17.89	0.000	0.755		−0.13
2	Diatom	17.69	−0.004	0.013	*	0.56
3	Diatom	28.84	−0.014	0.020	*	0.50
1	Dinoflagellates	30.36	−0.002	0.098	.	0.25
2	Dinoflagellates	33.62	0.003	0.059	.	0.34
3	Dinoflagellates	24.33	0.016	0.009	**	0.60
1	Chlorophyte	6.06	0.000	0.138		0.18
2	Chlorophyte	4.40	0.002	0.000	***	0.86
3	Chlorophyte	4.14	0.002	0.183		0.13
1	18 : 5n3	5.68	0.000	0.079	.	0.29
2	18 : 5n3	7.89	0.003	0.032	*	0.43
3	18 : 5n3	4.35	0.008	0.016	*	0.53
1	18 : 4n3	8.40	0.001	0.258		0.06
2	18 : 4n3	6.13	0.002	0.027	*	0.46
3	18 : 4n3	5.13	0.002	0.057	.	0.34
1	20 : 5n3	12.25	0.000	0.381		−0.02
2	20 : 5n3	12.39	−0.002	0.030	*	0.44
3	20 : 5n3	16.08	−0.005	0.087	.	0.27
1	22 : 6n3	16.28	−0.003	0.019	*	0.51
2	22 : 6n3	19.60	−0.002	0.139		0.18
3	22 : 6n3	14.85	0.006	0.004	**	0.67
1	18 : 1n9	4.483	0.001	0.008	**	0.60
2	18 : 1n9	4.433	0.002	0.130		0.20
3	18 : 1n9	3.714	0.000	0.930		−0.14
1	Haptophytes	14.080	0.001	0.434		−0.04
2	Haptophytes	14.021	0.005	0.011	*	0.58
3	Haptophytes	9.479	0.010	0.020	*	0.50

BGD
9, 8173–8197, 2012

Table 3. Fatty acid (FA) composition (%) and content (standardized to µg FA mg^{-1} C) of *cirripedia nauplii*. Values are averages of three replicates and standard deviation (SD). Each sample was comprised of 120 individuals.

Fatty acids	composition (%)		SD	content (µg FA mg^{-1} C)		SD
14 : 0	5.76	±	1.22	15.10	±	2.20
14 : 1	0.34	±	0.12	0.89	±	0.21
a-15 : 0	0.55	±	0.38	1.44	±	0.69
15 : 0	0.66	±	0.13	1.73	±	0.24
16 : 0	20.15	±	2.95	52.64	±	5.34
16 : 1n7	10.19	±	2.69	26.76	±	4.87
16 : 1n5	0.41	±	0.27	1.10	±	0.50
16 : 2n4	0.09	±	0.02	0.23	±	0.03
16 : 3n4	0.47	±	0.11	1.22	±	0.19
16 : 4n1	0.98	±	0.27	2.58	±	0.49
18 : 0	5.99	±	1.40	15.42	±	2.54
18 : 1n9 c+t	5.91	±	1.11	15.48	±	2.01
18 : 1n7	6.43	±	0.79	16.81	±	1.43
18 : 2n6 cis	1.53	±	0.10	3.99	±	0.18
18 : 3(n6)	0.28	±	0.09	0.73	±	0.16
18 : 3n3	1.03	±	0.13	2.69	±	0.24
18 : 4n3	3.79	±	0.87	9.94	±	1.58
20 : 1n9	2.35	±	0.36	6.14	±	0.66
20 : 0	1.60	±	0.24	4.18	±	0.44
20 : 4n6	0.33	±	0.04	0.87	±	0.07
20 : 5n3	18.05	±	3.65	47.22	±	6.61
22 : 0	0.36	±	0.09	0.95	±	0.16
22 : 1n11	0.93	±	0.16	2.44	±	0.30
22 : 1n9	0.93	±	0.25	2.43	±	0.46
22 : 5n3	0.30	±	0.02	0.79	±	0.04
24 : 1n11	0.25	±	0.11	0.65	±	0.20
22 : 6n3	9.36	±	2.47	24.41	±	4.48
Total FA				258.80	±	22.76
PUFA	36.21	±	7.20	94.66	±	13.04
MUFA	27.74	±	5.63	72.68	±	10.19
SAFA	35.08	±	3.65	91.45	±	6.60

BGD
9, 8173–8197, 2012

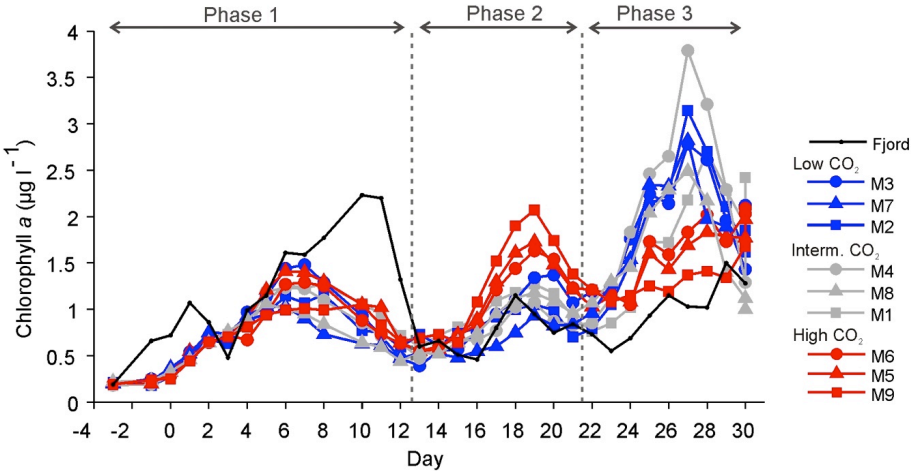

Fig. 1. Chlorophyll *a* concentration ($\mu g l^{-1}$) in the different mesocosms (M1–M9) and in the fjord during the experiment. Data from mesocosms with low CO_2 concentration (~ 180–250 µatm), intermediate CO_2 concentration (~ 340–600 µatm) and high CO_2 concentration (~ 650–1100 µatm) are marked in blue, grey, and red, respectively. The different phases of the experiment are indicated (Phase 1 = day −4 to 12, Phase 2 = day 13 to 21, Phase 3 = day 22 to 30).

BGD
9, 8173–8197, 2012

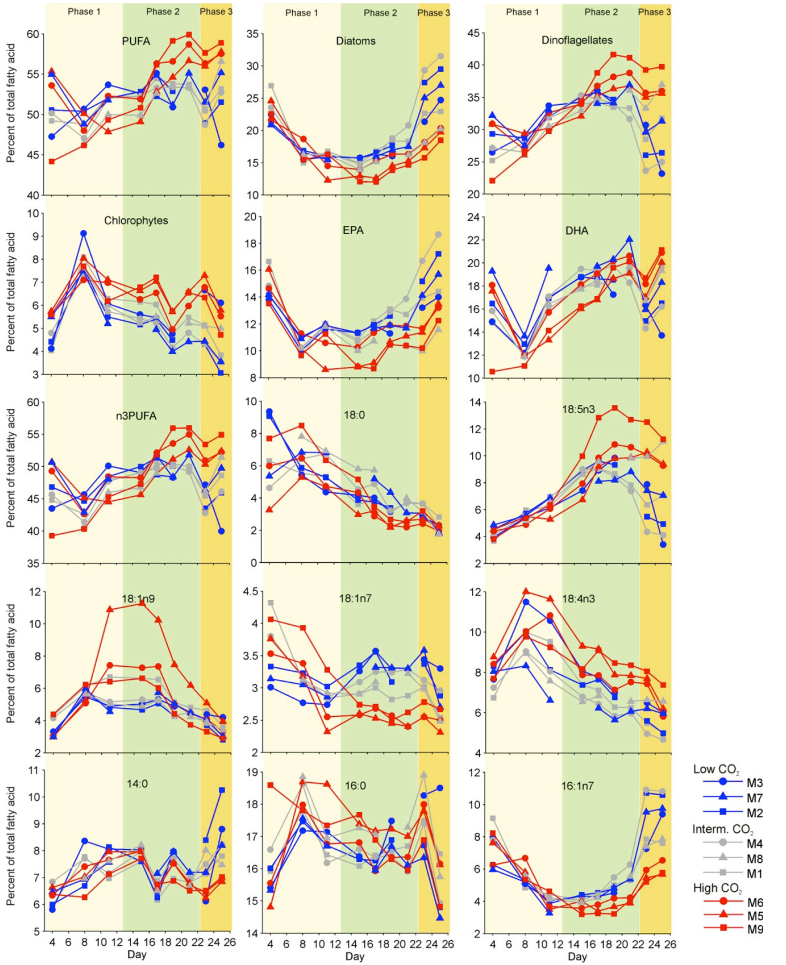

Fig. 2. Temporal development of selected fatty acids (in % of total fatty acids) during the experiment. Color coding and experimental phases as in Fig. 1.

BGD
9, 8173–8197, 2012

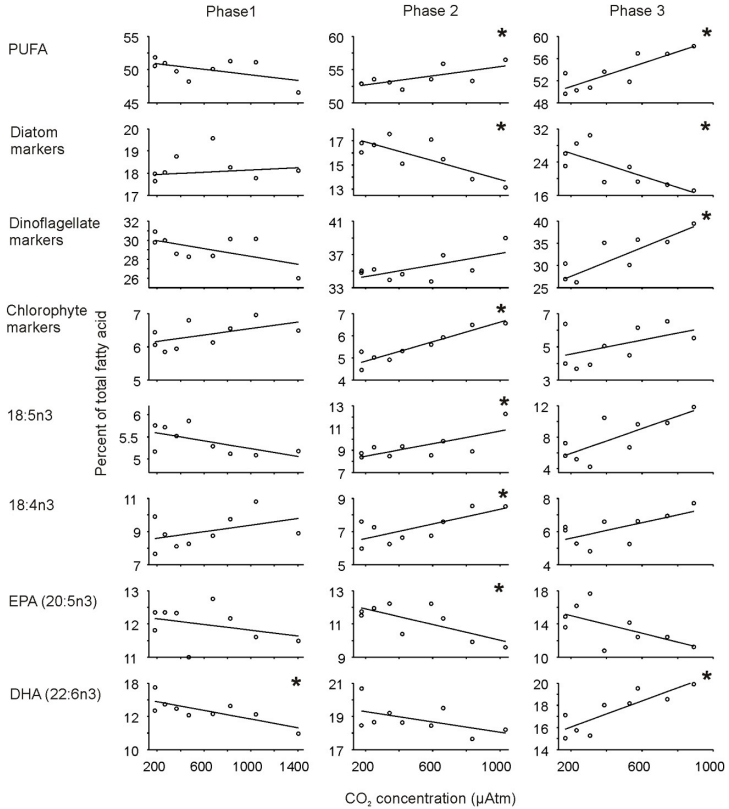

Fig. 3. Relationship between proportions of different fatty acid markers (%) and CO_2 concentration (µatm) at different phases of the experiment. Proportion of different fatty acids markers and CO_2 concentrations were averaged over the time period of the three phases: Phase 1 = day −4 to 12, Phase 2 = day 13 to 21, Phase 3 = day 22 to end. A linear regression lines is fitted to the each data set. Asterisks (*) mark those relationships for which the linear regression analysis was statistically significant ($p < 0.05$, see Table 2).

BGD
9, 8173–8197, 2012

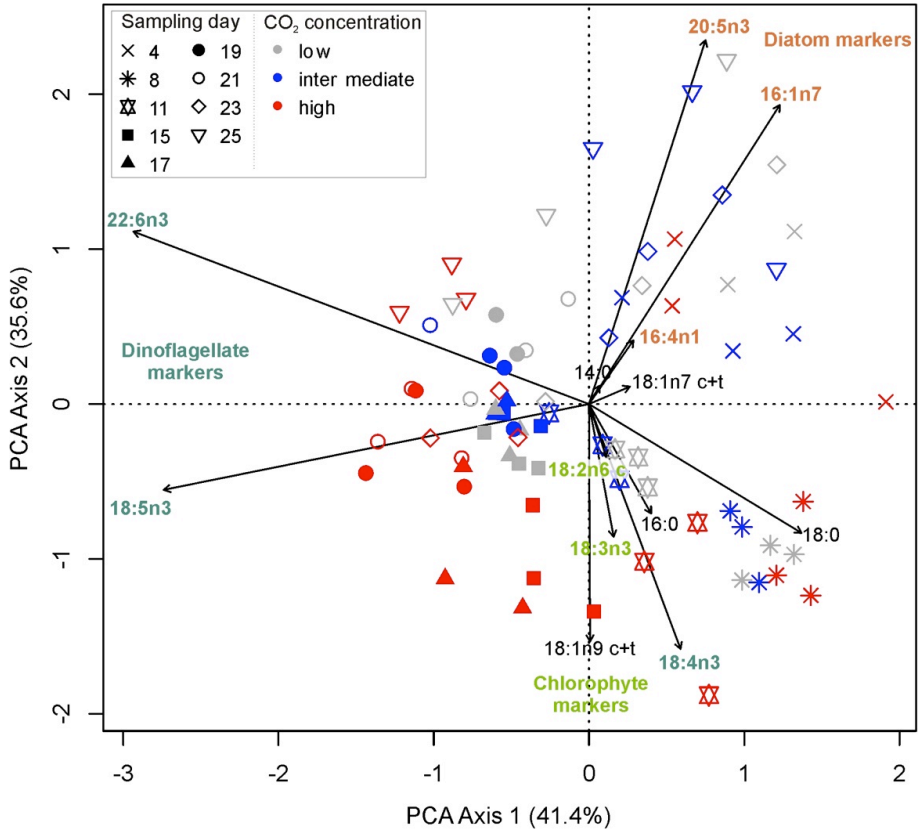

Fig. 4. Biplot of principal component analysis (PCA) based on fatty acid composition in each mesocosm on each sampling day. Individual samples are color coded according to pCO_2 concentration as in Fig. 1. Symbols refer to sampling days. The 1st axis explains 41.4 % of the variation, the 2nd axis 35.6 %.

BGD
9, 8173–8197, 2012