Effect of ocean acidification on the structure and fatty acid composition of a natural plankton community in the Baltic Sea

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Abstract

Increasing atmospheric carbon dioxide (CO_2) is changing seawater chemistry towards reduced pH, which consequently affects various properties of marine organisms. Coastal and brackish water communities are expected to be less affected by ocean acidification (OA) as these communities are typically adapted to high fluctuations in CO_2 and pH. Here we investigate the response of a coastal brackish water plankton community to increasing CO_2 levels as projected for the coming decades and the end of this century in terms of community and biochemical fatty acid (FA) composition. A Baltic Sea plankton community was enclosed in a set of off-shore mesocosms and subjected to a CO_2 gradient ranging from natural concentrations (~347 µatm pCO_2) up to values projected for the year 2100 (~1333 µatm pCO_2). We show that the phytoplankton community composition was resilient to CO_2 and did not diverge between the treatments. Seston FA composition was influenced by community composition, which in turn was driven by silicate and phosphate limitation in the mesocosms, and showed no difference between the CO_2 treatments. These results suggest that CO_2 effects are dampened in coastal

communities that already experience high natural fluctuations in pCO_2. Although this coastal plankton community was tolerant to high pCO_2 levels, hypoxia and CO_2 uptake by the sea can aggravate acidification and may lead to pH changes outside the currently experienced range for coastal organisms.

1 Introduction

The steady increase of carbon dioxide (CO_2) due to anthropogenic emission since the beginning of the industrial era has increased the atmospheric concentration (Boyd et al. 2014). The ocean has a large carbon sink capacity, and increasing atmospheric CO_2 absorbed by the ocean is changing the chemistry of the seawater, causing a decline in pH termed Ocean Acidification (OA) (Boyd et al. 2014). OA has been shown to affect various biological processes of diverse marine species (Doney et al. 2009; Kroeker et al. 2010). For instance OA can impact the biochemical and elemental composition of organisms (Sato et al. 2003; Torstensson et al. 2013), which can be transferred to higher trophic levels (Rossoll et al. 2012). OA can also drive alterations in the community composition structure of primary producers (Hare et al. 2007; Biswas et al. 2011; Schulz et al. 2013). Strong CO_2-effects may be particularly significant in marine species that experience low natural fluctuations in CO_2 (Riebesell 2004). In contrast, coastal and brackish-water environments encounter wide and frequent fluctuations in pCO_2 (Hinga 2002; Rossoll et al. 2013), due to large fluxes of organic and inorganic carbon from river runoff (Hinga 2002), seasonal processes (Melzner et al. 2013) and upwelling of CO_2 enriched water (Feely et al. 2009), all of which lead to wider pH variation in coastal systems compared to the open ocean (Hinga 2002). Consequently, it can be expected that coastal and brackish communities are more tolerant to OA effects (Rossoll et al. 2013; Reusch & Boyd 2013) and adverse CO_2 effect in terms of the biochemical composition of primary producers and variations in community composition may be diminished.

Fatty acids (FA) are the main components of lipids in cell membranes. In particular polyunsaturated fatty acids (PUFA) have important physiological roles in algae, which synthesize them in high amounts. Heterotrophs at higher trophic levels cannot synthesize certain FA *de novo*, especially PUFA, and have to acquire them from dietary sources (Izquierdo et al. 2001). Diverse laboratory studies of monocultures showed that CO_2 alters the FA profile of individual algal species (Sato et al. 2003; Fiorini et al. 2010; Torstensson et al. 2013; Bermúdez et al. 2015). A CO_2-driven change in algal food quality can be detrimental for

grazers, as has been shown in a laboratory study under elevated CO_2 levels (Rossoll et al. 2012). A strong decline of PUFA in a diatom, grown at high CO_2 affected the FA composition of copepods grazing on them and severely impaired their development and egg production rates. Furthermore, increasing seawater CO_2 can modify phytoplankton community composition by favoring certain taxa of primary producers (Graeme et al. 2005). In particular, small-sized cells benefit from high CO_2 (Hare et al. 2007; Biswas et al. 2011; Brussaard et al. 2013). This is ecologically relevant as taxonomic phytoplankton groups have contrasting FA profiles (Galloway & Winder 2015) and a change in community structure can affect higher trophic levels. For instance, a field study of two cladocerans having different phytoplankton composition as food source showed decreased egg production, lipid reserves, body size and abundance when fed with algae from an acidic lake (Locke & Sprules 2000).

The above observations suggest that changes in planktonic biochemical makeup and associated shifts in community composition of primary producers as a result of OA can affect the transfer of essential compounds to upper trophic levels. Laboratory studies have shown that algae subjected to long-term high CO_2 levels can restore their physiological optima through adaptive evolution (Lohbeck et al. 2012; Bermúdez et al. 2015) and that coastal communities are resilient to OA-driven changes in community composition and biomass (Nielsen et al. 2010; Rossoll et al. 2013). Therefore, it can be expected that organisms in these areas are adapted to high CO_2 fluctuations (Thomsen et al. 2010; Nielsen et al. 2010; Rossoll et al. 2013), hampering any CO_2-driven effects previously observed in plankton communities (Locke & Sprules 2000; Biswas et al. 2011).

The goal of the present study was to determine if an increase in CO_2 affects phytoplankton community composition and their FA composition, and if any effects are transferred to grazers of a natural plankton community in a coastal/brackish environment. A set of off-shore mesocosms, that enclosed a natural plankton assemblage of the Baltic Sea, were used as experimental units. The CO_2 levels ranged from current to projected next century values (Boyd et al. 2014, scenario A2). Algal FA were measured in total seston and in the copepods *Acartia bifilosa* and *Eurytemora affinis*, respectively, which were the dominant zooplankton during the experiment (Almén et al. 2015).

2 Material and Methods

2.1 Experimental set-up and CO_2 manipulation

Our study was conducted during an off-shore CO_2 mesocosm perturbation experiment off the Tvärminne Zoological Station at the entrance to the Gulf of Finland at 59° 51.5' N, 23° 15.5' E during late spring 2012. We used six enclosures with a length of 17 m containing ~55 m^3 of natural sea water (Paul et al. 2015). The mesocosms were set up and manipulated as described in detail by Paul et al. (2015) and Riebesell et al. (2013). Carbon dioxide enrichment was achieved in two phases through the addition of CO_2-saturated seawater to four out of six mesocosms. In phase 1, CO_2 was added in five steps between day 1 and day 5 to achieve values from ambient (~240 µatm) and up to ~1650 micro-atmospheres (µatm) fugacity of carbon dioxide (fCO_2). In phase 2 at day 15 CO_2 was again added in the upper 7 m to compensate for pronounced outgassing in the CO_2 enriched mesocosms. As described by Paul et al. (2015), dissolved inorganic carbon and total pH (on the total pH scale) were taken every sampling day to determine the carbonate system and determine fCO_2 in the mesocosms. Samples for nutrients were collected and analyzed as described by Paul et al. (2015). Samples for phytoplankton counts were taken every second day and for fatty acid concentrations every fourth day using a depth-integrated water sampler (Hydrobios, Kiel, Germany) covering the upper 15 m of the water column. Integrated zooplankton net tows were taken every seventh day as described by Almén et al. (2015).

2.2 Phytoplankton abundance and biomass calculation

Phytoplankton cell counts up to a cell size of 200 µm were carried out from 50 ml water samples, fixed with alkaline Lugol's iodine (1% final concentration) using the Utermöhl's (1958) method with an inverted microscope (ZEISS Axiovert 100). At 200 times magnification, cells larger than 12 µm were counted on half of the chamber area, while smaller cells were counted at 400 times magnification on two radial strips. The plankton was identified to genus or species level according to Tomas (1997), Hoppenrath et al. (2009) and Kraberg et al. (2010). Algal biovolume was calculated according to geometric shapes and converted to cellular organic carbon using taxon-specific conversion equations for phytoplankton (Menden-Deuer & Lessard 2000).

2.3 Fatty acid composition

For analysis of seston fatty acid (FA), 500 ml of seawater were filtered by a 100 µm size pore net and samples were collected in a pre-combusted (450°C, 6 h) Whatman GF/F (~0.7 µm nominal pore size) filters. For zooplankton gravid copepod females of *Acartia bifilosa* and *Eurytemora affinis* were picked with sterile tweezers under two stereomicroscopes (Nikon SMZ800, 25× magnification and Leica 25× magnification) and placed in pre-weighted tin cups. All samples were immediately stored at -80°C until analysis. FA were measured by gas chromatography as fatty acid methyl esters (FAMEs) following Klein Breteler et al. (1999). Lipids were extracted over night from the filters using 3 ml of a solvent mixture (dichloromethane:methanol:chloroform in 1:1:1 volume ratios). As internal standard, FAME C19:0 (Restek, Bad Homburg, Germany; c= 20 ng of component per sample) was added, and a C23:0 FA standard (c= 25.1 ng μl^{-1}) used as an esterification efficiency control (usually 80-85 %). Water-soluble fractions were removed by washing with 2.25 ml of KCl solution (c= 1 mol L^{-1}), and the remainder dried by addition of $NaSO_4$. The solvent was evaporated to dryness in a rotary film evaporator (100-150 mbar), re-dissolved in chloroform and transferred into a glass cocoon. The solvent was evaporated again (10-30 mbar), and esterification was performed overnight using 200 µl 1% H_2SO_4 (in CH_3OH) and 100 µl toluene at 50°C. Phases were split using 300 µl 5% sodium chloride solution, and FAMEs were separated using n-Hexane, transferred into a new cocoon, evaporated, and 100 µl (final volume) added. All solvents used were gas chromatography (GC) grade. FAME were analyzed by a Thermo GC Ultra gas chromatograph equipped with a non-polar column (RXI1-SIL-MS 0.32 µm, 30 m, company Restek) and Flame ionization detector. The column oven was initially set to 100°C, and heated to 220 °C at 2 °C min^{-1}. The carrier gas was helium at a constant flow of 2 ml min^{-1}. The flame ionization detector was set to 280 °C, with a gas flow of 350, 35 and 30 ml min^{-1} of synthetic air, hydrogen and helium, respectively. A 1 µl aliquot of the sample was injected. The system was calibrated with a 37-component FAME-mix (Supelco, Germany) and chromatograms were analyzed using Chrom-Card Trace-Focus GC software and the fatty acids were clustered according to their degree of saturation: saturated (SFA), monounsaturated (MUFA) and polyunsaturated (PUFA).

2.4 Statistical analyses

The data was analyzed by a nested Mixed Effects ANOVA Model (LME) to determine the differences in taxa biomass (µgC ml^{-1}) and relative fatty acid content (% in the seston and zooplankton) between the CO_2 treatments (µatm fCO_2), with $fCO2$, silicate, inorganic nitrogen (nitrite + nitrate), phosphate, temperature and salinity as fixed effects, and sampling day and

mesocosm position as nested random variable (random distribution of CO_2 treatments among the mesocosm). Average mesocosm fCO_2 was calculated for the total duration of the sampling period plankton community composition (day 1 to 29) and for FA data analysis (day 1 to 25 for seston FA and day -1 to 33 for zooplankton FA). Linear regression models were used to determine the relation between PUFA and phytoplankton biomass. The similarity in the structure of the plankton community between the treatments was analyzed by Non Metrical Multidimensional Scaling (NMDS) with Bray distance, auto-transformation and 3 dimensions (k=3). This analysis distributes the samples in an ordination space according to the biomass of the different taxa in the community along orthogonal principal components using non Euclidean distances for ordination space, which makes it more robust to the presence of zero values (Clarke 1993). All statistical analyses were done using the R software environment 3.0.1 (R Development Core Team 2013).

3 Results

3.1 Plankton community composition

The initial algal community consisted of post-bloom species dominated by small-sized cells, with dinophyta being the most abundant phytoplankton group in all mesocosm throughout the experiment followed by heterokontophyta, euglenophyta, cholorophyta, cyanobacteria bigger than 5µm (usually filamentous) and small abundances of cryptophya (Fig. 1). Microzooplankton was present during the entire experimental period, particularly the choanoflagellate *Calliacantha natans* (Fig. 1). The plankton community was analyzed from day 1 to 29, which comprised two phases as described by Paul et al. (2015), with a Phase 1 (from day 1 to 15) where phytoplankton biomass gradually increased until day 10 when a bloom started and reached a peak around day 15 in all treatments; while in a Phase 2 (from day 17 to 29) the biomass began to decay from around day 19 up to day 29 (Fig. 1).

The more abundant taxa did not show differences in abundance between the CO_2 treatments on both phases (Fig. 2a, b). However, the biomass of some of the less abundant groups was affected by CO_2 within the different phases. In Phase 1, the nested mixed effects model analysis of the algal biomass showed that chlorophyta decrease significantly towards high CO_2 levels (Fig. 2a) (LME, F= 7.27, p= 0.01, df= 20). Nevertheless, there was a difference in the relative biomass of the more abundant plankton groups between Phases 1 and 2, with a decrease in dinophyta (37.2 ± 3.2 % to 28.3 ± 2.9 %) and heterokontophyta (19.1 ± 2.2 % to 14 ± 2.6) from phase 1

(Fig. 2c) to phase 2 (Fig. 2d), and an increase of euglenophyta (7.5 ± 1.4 % to 21 ± 2.7) and chlorophyta (14.0 ± 1.5 % to 19.1 ± 2.4) in the same period. An NMDS analysis of the entire phytoplankton community showed a rather homogeneous community composition between the different CO_2 treatments but variation among sampling days, especially at day 7, when the diatom *Melosira varians* was abundant during that particular sampling day (Fig. S1).

3.2 Seston fatty acid composition

The PUFA represented on average ~26 ± 4%, MUFA ~22 ± 3% and SFA ~52 ± 4% of the total FA content in the seston over the entire experimental period. The Mixed Effect Model (LME) analysis of relative PUFA content data showed no significant difference among the CO_2 treatments (LME, F_{45}= 0.0, p>0.05) (Fig. 3a PUFA). The MUFA and SFA did neither show any significant change in abundance in relation with CO_2 (LME, F_{45}= 0.0, p= 0.8, and F_{45}= 0.06, p= 0.79, respectively) (Fig. 3a MUFA, SFA). However, the FA composition of the seston showed that the relative PUFA content significantly decreased over time in all mesocosms (linear regression, R^2= 0.52, t= -7.64, p<0.0001, n=22) (Fig. 3b High CO_2 treatments, Low CO_2 treatments), while the MUFA and SFA increased, although the relation of both with time is weak (linear regression, R^2= 0.12, t= 2.88, p= 0.005 and R^2= 0.15, t= 3.26, p= 0.001, n=22 respectively) (Fig. S2). Regarding specific PUFA, 18:2n6c showed a significant correlation with CO_2 and Si, 16:3n4 with CO_2, P and Si; and 18:3n6 with CO_2 and N (Fig. S3).

Nevertheless, PUFA showed a positive relation with heterokontophyta (linear regression, R^2=0.58, p<0.001) and dinophyta (linear regression, R^2=0.41, p<0.001) biomass (Fig. 4a); and with silicate (LME, F= 22.8, p< 0.001, df= 35) and phosphate (LME, F= 9.3, p< 0.01, df= 35) abundance in the mesocosms (Fig. 4b). The PUFA 18:2n6c and 18:3n3 showed a positive effect of Silicate, while 20:5n3c and 22:6n3c showed a significant silicate and phosphate effect (Fig. S4).

3.3 Copepod fatty acids

The overall PUFA content of the copepod *A. bifilosa* represented ~12% (311 ± 175 ng FA mg dry wt.$^{-1}$) and in *E. affinis* ~16% (433 ± 597 ng FA mg dry wt. $^{-1}$) of the total FA.

The FA did not show a CO_2-related effect in *A. bifilosa* (LME, F= 0.62, p= 0.4374, df= 26) (Fig. 5a), or *E. affinis* (F= 0.13, p= 0.71, df= 26) (Fig. 5b). Nevertheless the relative PUFA content in *A. bifilosa* and *E. affinis* showed a significant decrease over time in all high and low

242 CO_2 treatments (linear regression, *A. bifilosa*; R^2= 0.22, t= -3.288, p= 0.002 *E. affinis;* R^2= 0.47,
243 t= -5.51, p< 0.0001) (Fig. 5c) and neither did any specific PUFA in *A. bifilosa* (Fig. S5) or *E.*
244 *affinis* (Fig. S6); while there is a decrease in MUFA and an increase in SFA in both copepod
245 species (Fig. S7). Furthermore, the relative FA content in *E. affinis* varied over time following
246 the changes in the seston FA, this relation was significant but weak for PUFA MUFA and SFA
247 (Fig. S8), while in *A. bifilosa* this change appeared only in the MUFA (Fig. S8).
248
249 ## 4. Discussion
250
251 ### 4.1 Community composition
252 The plankton community composition in the present experiment did change over time and
253 showed little differences in relation to the different CO_2 treatments. The observed absence of a
254 strong CO_2 effect on the community composition in the present study is in line with the
255 observations in the western Baltic Sea (Thomsen et al., 2010; Nielsen et al., 2010; Rossoll et
256 al., 2013). In these studies the authors suggested that the plankton community is adapted to OA
257 due to the recurrent large seasonal and daily variance of pH and CO_2 experienced by the
258 communities in this productive low-salinity region (Thomsen et al. 2010; Nielsen et al. 2010;
259 Rossoll et al. 2013; Almén et al. 2014). Our study region, a coastal zone in the western Gulf of
260 Finland in the northern Baltic Sea, is a brackish environment with low salinity (~5.7 ‰), a high
261 fresh water runoff (~111 km^3 $year^{-1}$) (Savchuk 2005) and a strong inter- and intra-seasonal pH
262 variability, sometimes reaching extreme values of 9.2 and 7.4 with an average of 8.1 (Brutemark
263 et al. 2011). Therefore, it seems that the plankton community in our study area, which
264 experiences high natural pH fluctuations, is composed of species and genotypes that are less
265 pH/CO_2 sensitive (Nielsen et al. 2010; Lohbeck et al. 2012; Melzner et al. 2013; Rossoll et al.
266 2013) which allows them to cope with the CO_2 range applied in the current field experiment.
267
268 Chlorophytes were the only group that showed a significant response to the CO_2 treatment,
269 although their contribution to total biomass was low. Chlorophytes decreased at elevated fCO_2,
270 which is contrasting to laboratory studies showing that several species in this group benefit
271 from high CO_2 and can increase their growth rates (Tsuzuki et al. 1990; Yang & Gao 2003).
272
273 ### 4.2 Seston FAs
274 The relative PUFA content of seston showed a significant decrease over time, which can be
275 attributed to nutrient depletion in the mesocosms, particularly silicate and phosphate

concentrations, which caused a decrease in dinophyta and heterokontophyta abundances. These two groups of microalgae have been identified as rich in PUFA content (Galloway & Winder 2015) and their decrease in the mesocosms explains the concomitant decrease in PUFA. Silicate is required by heterokontophyta for the formation of new frustules during cell division, and when limited, cell division stops (Flynn & Martin-Jézéquel 2000). Phosphorus is required for the production of PUFA-rich membrane phospholipids during cell division and growth (Guschina & Harwood 2009). Nutrient limitation, which causes reduced cell division rates, results in a lower production of phospholipid and increased production of storage lipid, primarily triacylglycerols (Guschina & Harwood 2009). Triacylglycerols are rich in SFA and MUFA; therefore the increase in triacylglycerols with nutrient limitation typically resulted in decreased proportions of PUFA in most algae (Guschina & Harwood 2009). This is consistent with our observations in the mesocosms, where the relative PUFA content of seston followed the phosphate concentration. From this perspective one may expect that any CO_2 effect in algal PUFA will occur when cells are actively growing since nutrient limitation (silicate and phosphorus) will have more profound consequences in the physiology of the cell than an excess of CO_2.

The absence of a PUFA response to CO_2 differs with a report of an Arctic plankton community showing an increase of PUFA at high CO_2 levels during part of a mesocosm experiment experiencing nutrient additions (Leu et al. 2013). This was attributed to a change in the plankton community composition due to a rise in abundance of dinoflagellates at high CO_2 (Leu et al. 2013). Our results show a decrease in PUFA due to a decline in dinoflagellates. The different PUFA trend between these experiments can be attributed to the specific plankton community composition and their related FA profiles alongside with phosphate and silicate limitation in our study, which causes a reduction of the biomass of some PUFA-rich taxa. Species composition of a natural plankton assemblage determines its food quality properties as distinct algal taxonomic groups have different FA composition profiles (Galloway & Winder 2015). A CO_2-driven change in the Arctic plankton community composition (Leu et al. 2013) promoted the presence of species rich in PUFA. In our study the absence of a CO_2 response in taxa composition and the apparent influence of phosphate and silicate limitation in the algal FA composition resulted in a rather homogeneous PUFA concentration between CO_2 treatments.

4.3 Copepod fatty acids

Our results showed that the PUFA concentration of the dominating copepod species, *A. bifilosa* and *E. affinis* did not vary between the different CO_2 treatments. However, the PUFA decrease in both copepods over the experimental period reflects the decline in the PUFA content of the seston. This observation is consistent with other studies showing that copepods strongly rely on their diet as source of FA and that their composition, especially PUFA, mirrors the algae they graze on (Ishida et al. 1998; Caramujo et al. 2007; Rossoll et al. 2012).

Several studies have shown a limited direct CO_2 effect in the copepods FA of some species, like the genus *Acartia*, which is rather insensitive to projected high CO_2 exposure up to 5000 µatm CO_2 (Kurihara et al. 2004; Kurihara & Ishimatsu 2008). Copepods experience widely varying pH conditions on a daily basis during their vertical migration, shown in the same area as the current study (Almén et al. 2014), which may explain their tolerance to pH variations. Several studies have demonstrated that food quality of the available prey in terms of PUFA content can affect egg production, hatching success and nauplii survival in copepods (Jónasdóttir 1994; Caramujo et al. 2007; Jónasdóttir et al. 2009). Indirect adverse CO_2 effects through the diet of primary consumers have been reported in laboratory and field experiments (Rossoll et al. 2012; Locke & Sprules 2000). However, the absence of a CO_2-driven change in the community composition of primary producers and the homogeneous algal FA composition due to phosphate and silicate limitations masked any noticeable CO_2-related effects in the algae FA profile that could have affected the copepods during our experiment.

5 Conclusions

Considering that the Baltic Sea is a coastal sea with a natural frequent and wide pH variability (Omstedt et al. 2009), it can be expected that the effects of OA on plankton communities will be rather small within the range of predicted values for this century (Havenhand 2012). A reduced OA sensitivity in systems experiencing high CO_2 fluctuations is supported by our results and other studies using communities from the Baltic (Thomsen et al. 2010; Nielsen et al. 2010; Rossoll et al. 2013). However, in coastal upwelling areas undergoing an increase in hypoxic events, it is likely that elevated CO_2 values as presently experienced by coastal organisms and projected by the end of the century (Melzner et al. 2013) will be more recurrent in the future (Feely et al. 2004), with the potential to affect various properties of plankton communities.

342 Nonetheless, it is clear that the plankton community response to OA and concomitant effects
343 on its food quality for higher trophic levels will strongly depend on the sensitivity of primary
344 producers and on how OA affects the species composition of plankton assemblages (Leu et al.
345 2013; Rossoll et al. 2013). This result is important as any change in primary producers in terms
346 of FA, particularly essential biomolecules such as PUFA, may scale up in food webs since FAs
347 are incorporated into the lipids of larval fish (Fraser et al. 1989; Izquierdo et al. 2001).
348 Considering that fish is a critical natural resource (FAO, 2010), adverse OA effects on food
349 quality can reach up to human populations who rely on fisheries as an important food source
350 (Sargent et al. 1997; Arts et al. 2001).
351

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Figures and Figure Legends

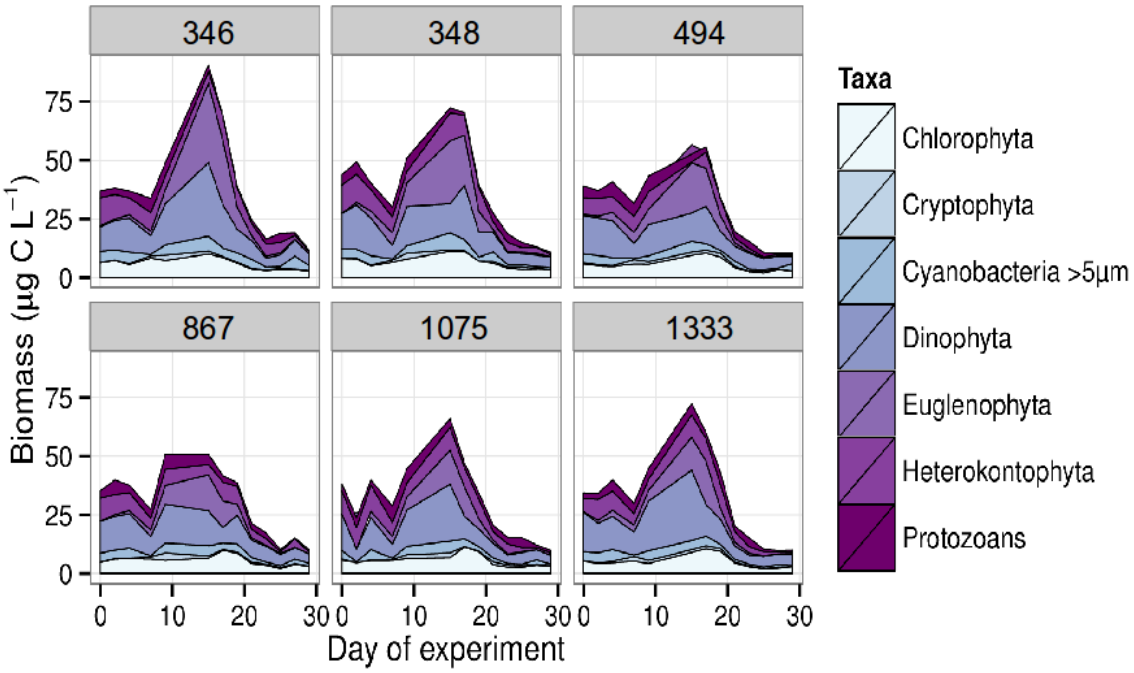

Figure 1. Calculated biomass after cell counts of the main plankton taxonomic groups in the different CO_2 treatments between day 1 and 29. Each treatment is labeled with the average fCO_2 level of the entire experimental period (top).

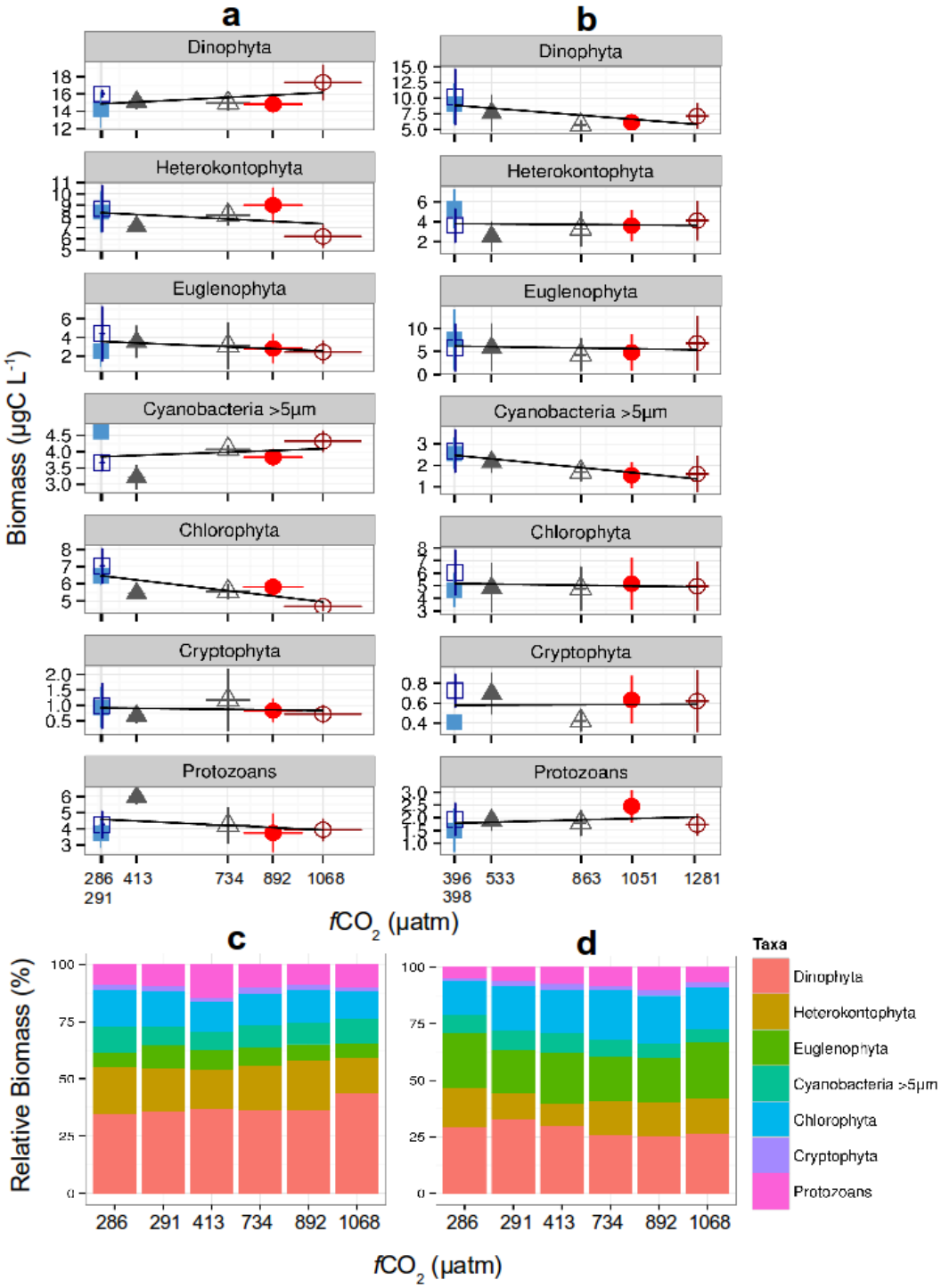

Figure 2. The top panels show the mean of the calculated biomass of each plankton taxon in a) Phase 1, between the days 0 to 15; and b) Phase 2, between days 15 to 29, in the CO_2 gradient treatments. The bottom panels show the relative biomass of the different plankton groups between c) Phases 1 and d) Phase 2. The x-axes show the measured average fCO_2 in each phase, error bars show standard error in a and b (n=5 for a; n=5 for b).

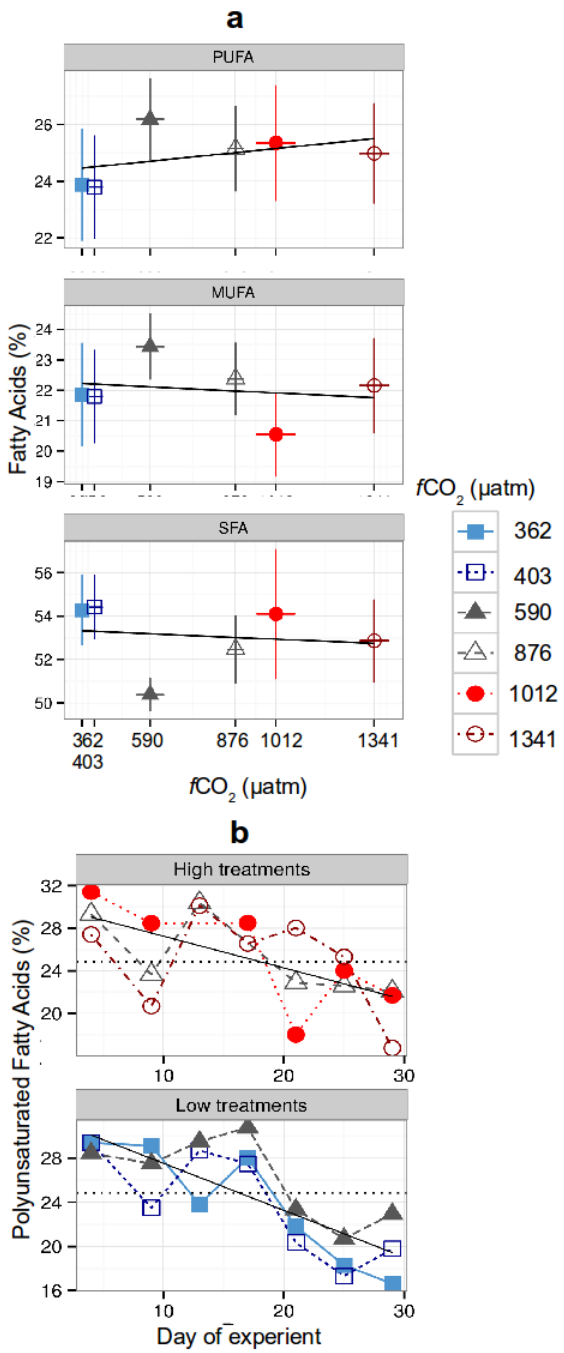

Figure 3. a) Relative polyunsaturated (PUFA), monounsaturated (MUFA), and saturated (SFA) fatty acids content in the seston as a function of fCO_2 between day 1 and 29. The x-axes show the mean fCO_2 measured during the sampling period, bars shows standard error. b) Relative PUFA composition of the seston showed over time in the 876, 1012 and 1314 µatm fCO_2 levels (high CO_2 treatments) and the 362, 403 and 590 µatm fCO_2 levels (low CO_2 treatments). Horizontal dashed line indicates the position of the overall mean PUFA value.

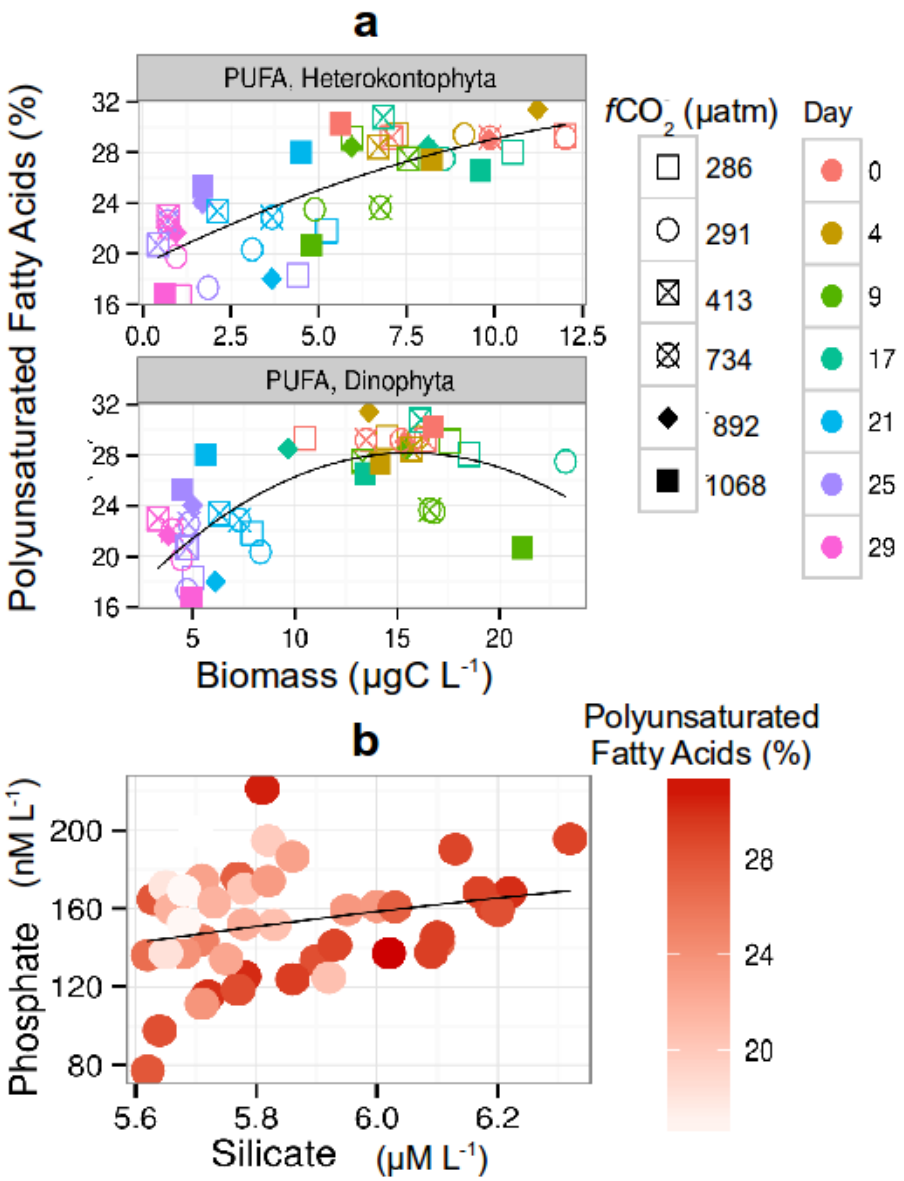

Figure 4. a) Relation between sestonic relative polyunsaturated fatty acids (PUFA) with heterokontophyta (PUFA, heterokontophyta) and dinophyta (PUFA, dinophyta) biomass. b) Relation between relative sestonic PUFA content with silicate and phosphate abundance in the mesocosms.

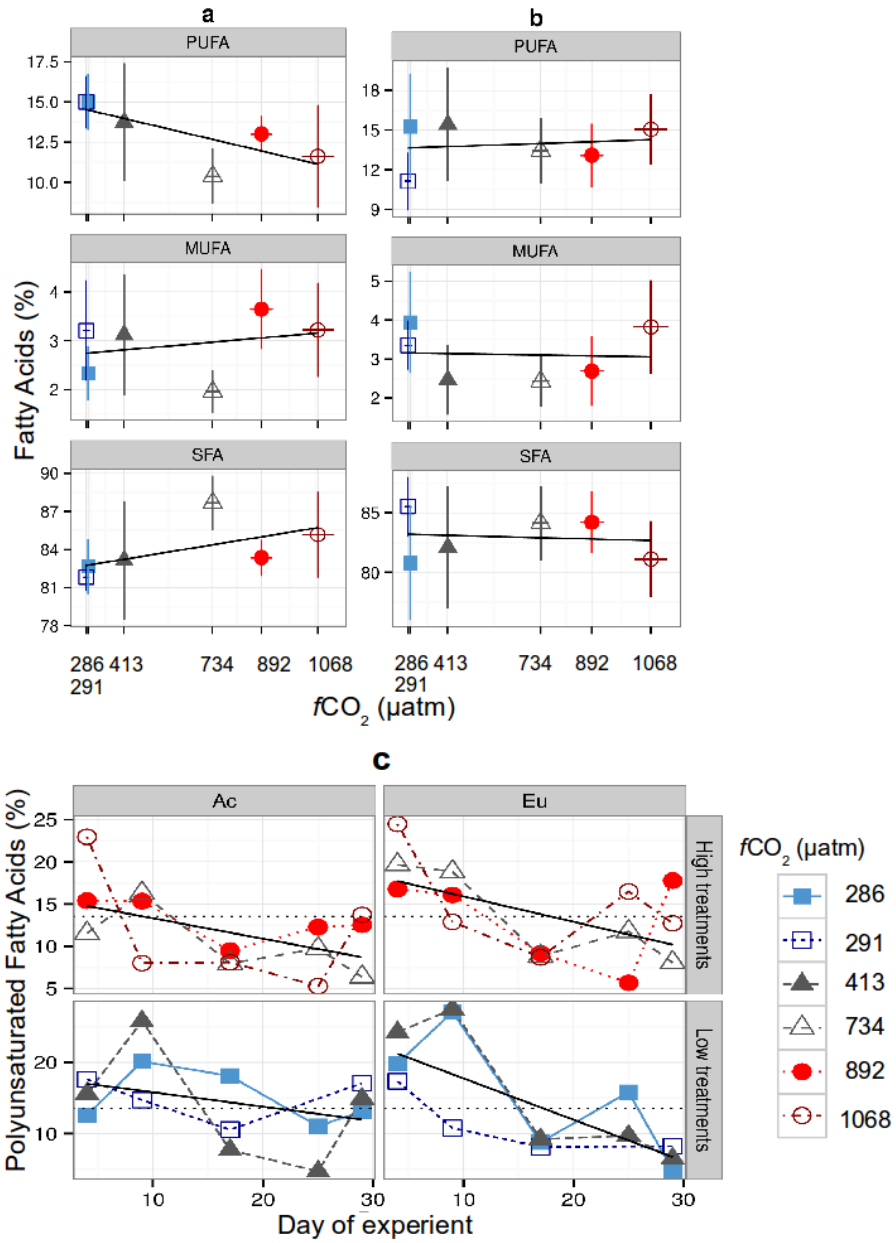

Figure 5. a) and b) show the relative polyunsaturated (PUFA), monounsaturated (MUFA), and saturated (SFA) fatty acids content in the copepods *Acartia bifilosa* and *Eurytemora affinis*, respectively, under the fCO_2 gradient treatments between day 1 to 29. The x-axes show the mean fCO_2 measured during the sampling period, bars shows standard error. c) Relative PUFA composition of *Acartia bifilosa* (Ac) and *Eurytemora affinis* (Eu) over time in the 876, 1012 and 1314 µatm fCO_2 levels (high CO_2 treatments) and the 362, 403 and 590 µatm fCO_2 levels (low CO_2 treatments). Horizontal dashed line indicates the position of the overall mean PUFA value.