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

3

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24 ## Keywords
25 Fatty acids, *Acartia bifilosa*, *Eurytemora affinis*, plankton community, CO_2, ocean
26 acidification, Baltic Sea.

27

28 ## Abstract
29 Increasing atmospheric carbon dioxide (CO_2) is changing seawater chemistry towards reduced
30 pH, which consequently affects various properties of marine organisms. Coastal and brackish
31 water communities are expected to be less affected by ocean acidification (OA) as these
32 communities are typically adapted to high fluctuations in CO_2 and pH. Here we investigate the
33 response of a coastal brackish water plankton community to increasing CO_2 levels as projected
34 for the coming decades and the end of this century in terms of community and biochemical fatty
35 acid (FA) composition. A Baltic Sea plankton community was enclosed in a set of off-shore
36 mesocosms and subjected to a CO_2 gradient ranging from natural concentrations (~347 µatm
37 pCO_2) up to values projected for the year 2100 (~1333 µatm pCO_2). We show that the
38 phytoplankton community composition was resilient to CO_2 and did not diverge between the
39 treatments. Seston FA composition was influenced by community composition, which in turn

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40 was driven by silicate and phosphate limitation in the mesocosms, and showed no difference
41 between the CO_2 treatments. These results suggest that CO_2 effects are dampened in coastal
42 communities that already experience high natural fluctuations in pCO_2. Although this coastal
43 plankton community was tolerant to high pCO_2 levels, hypoxia and CO_2 uptake by the sea can
44 aggravate acidification and may lead to pH changes outside the currently experienced range for
45 coastal organisms.

46

47 **1 Introduction**

48

49 The steady increase of carbon dioxide (CO_2) due to anthropogenic emission since the beginning
50 of the industrial era has increase the atmospheric concentration (Boyd et al. 2014). The ocean
51 has a large carbon sink capacity, and increasing atmospheric CO_2 absorbed by the ocean is
52 changing the chemistry of the seawater, causing a decline in pH termed Ocean Acidification
53 (OA) (Boyd et al. 2014). OA has been shown to affect various biological processes of diverse
54 marine species (Doney et al. 2009; Kroeker et al. 2010). For instance OA can impact the
55 biochemical and elemental composition of organisms (Sato et al. 2003; Torstensson et al. 2013),
56 which can be transferred to higher trophic levels (Rossoll et al. 2012). OA can also drive
57 alterations in the community composition structure of primary producers (Hare et al. 2007;
58 Biswas et al. 2011; Schulz et al. 2013). Strong CO_2-effects may be particularly significant in
59 marine species that experience low natural fluctuations in CO_2 (Riebesell et al., in review). In
60 contrast, coastal and brackish-water environments encounter wide and frequent fluctuations in
61 pCO_2 (Hinga 2002; Rossoll et al. 2013), due to large fluxes of organic and inorganic carbon
62 from river runoff and lower alkalinity, and hence reduced buffer capacity (Melzner et al. 2013).
63 Consequently, it can be expected that coastal and brackish communities are more tolerant to
64 OA effects (Rossoll et al. 2013; Reusch & Boyd 2013) and adverse CO_2 effects in terms of the
65 biochemical composition of primary producers and variations in community composition may
66 be diminished.

67

68 Fatty acids (FA) are the main components of lipids in cell membranes. In particular
69 polyunsaturated fatty acids (PUFA) have important physiological roles in algae, which
70 synthesize them in high amounts. Heterotrophs at higher trophic levels cannot synthesize
71 certain FA *de novo*, especially PUFA, and have to acquire them from dietary sources (Izquierdo
72 et al. 2001). Diverse laboratory studies of monocultures showed that CO_2 alters the FA profile
73 of individual algal species (Sato et al. 2003; Fiorini et al. 2010; Torstensson et al. 2013;

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74 Bermúdez et al. 2015). A CO_2-driven change in algal food quality can be detrimental for
75 grazers, as has been shown in a laboratory study under elevated CO_2 levels (Rossoll et al. 2012).
76 A strong decline of PUFA in a diatom, grown at high CO_2 affected the FA composition of
77 copepods grazing on them and severely impaired their development and egg production rates.
78 Furthermore, increasing seawater CO_2 can modify phytoplankton community composition by
79 favoring certain taxa of primary producers (Graeme et al. 2005). In particular, small-sized cells
80 benefit from high CO_2 (Hare et al. 2007; Biswas et al. 2011; Brussaard et al. 2013). This is
81 ecologically relevant as taxonomic phytoplankton groups have contrasting FA profiles
82 (Galloway & Winder 2015) and a change in community structure can affect higher trophic
83 levels. For instance, a field study of two cladocerans having different phytoplankton
84 composition as food source showed decreased egg production, lipid reserves, body size and
85 abundance when fed with algae from an acidic lake (Locke & Sprules 2000).
86
87 The above observations suggest that changes in planktonic biochemical makeup and associated
88 shifts in community composition of primary producers as a result of OA can affect the transfer
89 of essential compounds to upper trophic levels. However, organisms and communities from
90 coastal/brackish environments may show a high tolerance to elevated pCO_2 levels due to
91 adaptation (Thomsen et al. 2010; Nielsen et al. 2010; Rossoll et al. 2013). In coastal/brackish
92 systems variation in CO_2 is more frequent and severe due to river runoff (Hinga 2002), reduced
93 buffer capacity (Feely et al. 2004), seasonal processes (Melzner et al. 2013)and upwelling of
94 CO_2 enriched water (Feely et al. 2009), all of which lead to wider pH variation in coastal
95 systems compared to the open ocean (Hinga 2002). Laboratory studies have shown that algae
96 subjected to long-term high CO_2 levels can restore their physiological optima through adaptive
97 evolution (Lohbeck et al. 2012; Bermúdez et al. 2015) and that coastal communities are resilient
98 to OA-driven changes in community composition and biomass (Nielsen et al. 2010; Rossoll et
99 al. 2013). Therefore, it can be expected that organisms in these areas are adapted to high CO_2
100 fluctuations, hampering any CO_2-driven effects previously observed in plankton communities
101 (Locke & Sprules 2000; Biswas et al. 2011).
102
103 The goal of the present study was to determine if an increase in CO_2 affects phytoplankton
104 community composition and their FA composition, and if any effects are transferred to grazers
105 of a natural plankton community in a coastal/brackish environment. A set of off-shore
106 mesocosms, that enclosed a natural plankton assemblage of the Baltic Sea, were used as
107 experimental units. The CO_2 levels ranged from current to projected next century values (Boyd

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108 et al. 2014, scenario A2). Algal FA were measured in total seston and in the copepods *Acartia*
109 *bifilosa* and *Eurytemora affinis*, respectively, which are dominant zooplankton in this
110 ecosystem (Almén et al. 2015).
111
112 **2 Material and Methods**
113
114 **2.1 Experimental set-up and CO_2 manipulation**
115 Our study was conducted during an off-shore CO_2 mesocosm perturbation experiment off the
116 Tvärminne Zoological Station at the entrance to the Gulf of Finland at 59° 51.5' N, 23° 15.5'
117 E during late spring 2012. We used six enclosures with a length of 17 m containing ~55 m^3 of
118 natural sea water (Paul et al. 2015). The mesocosms were set up and manipulated as described
119 in detail by Paul et al. (2015) and Riebesell et al. (2013). Carbon dioxide enrichment was
120 achieved in two phases through the addition of CO_2-saturated seawater to four out of six
121 mesocosms. In phase 1, CO_2 was added in five steps between day 1 and day 5 to achieve values
122 from ambient (~240 µatm) and up to ~1650 µatm fCO_2. In phase 2 at day 15 CO_2 was again
123 added in the upper 7 m to compensate for pronounced outgassing in the CO_2 enriched
124 mesocosms. Samples for phytoplankton counts were taken every second day and for fatty acid
125 concentrations every fourth day using a depth-integrated water sampler (Hydrobios, Kiel,
126 Germany) covering the upper 15 m of the water column. Integrated zooplankton net tows were
127 taken every seventh day.
128
129 **2.2 Phytoplankton abundance and biomass calculation**
130 Phytoplankton cell counts up to a cell size of 200 µm were carried out from 50 ml water
131 samples, fixed with alkaline Lugol's iodine (1% final concentration) using the Utermöhl's
132 (1958) method with an inverted microscope (ZEISS Axiovert 100). At 200 times magnification,
133 cells larger than 12 µm were counted on half of the chamber area, while smaller cells were
134 counted at 400 times magnification on two radial strips. The plankton was identified to genus
135 or species level according to Tomas (1997), Hoppenrath et al. (2009) and Kraberg et al. (2010).
136 Algal biovolume was calculated according to geometric shapes and converted to cellular
137 organic carbon using taxon-specific conversion equations for phytoplankton (Menden-Deuer &
138 Lessard 2000).
139
140
141

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142 **2.3 Fatty acid composition**

143 For analysis of algal fatty acid (FA), 500 ml of seawater were filtered by using pre-combusted
144 (450°C, 6 h) Whatman GF/F (~0.7 µm pore size) filters. For zooplankton gravid copepod
145 females of *Acartia bifilosa* and *Eurytemora affinis* were picked with sterile tweezers under two
146 stereomicroscopes (Nikon SMZ800, 25× magnification and Leica 25× magnification) and
147 placed in pre-weighted tin cups. All samples were immediately stored at -80°C until analysis.
148 FA were measured by gas chromatography as fatty acid methyl esters (FAMEs) following
149 Breteler et al. (1999). Lipids were extracted over night from the filters using 3 ml of a solvent
150 mixture (dichloromethane:methanol:chloroform in 1:1:1 volume ratios). As internal standard,
151 FAME C19:0 (Restek, Bad Homburg, Germany; c= 20.0 ng component^{-1}µl^{-1}) was added, and
152 a C23:0 FA standard (c= 25.1 ng µl^{-1}) used as an esterification efficiency control (usually 80-
153 85 %). Water-soluble fractions were removed by washing with 2.25 ml of KCl solution (c= 1
154 mol L^{-1}), and the remainder dried by addition of $NaSO_4$. The solvent was evaporated to dryness
155 in a rotary film evaporator (100-150 mbar), re-dissolved in chloroform and transferred into a
156 glass cocoon. The solvent was evaporated again (10-30 mbar), and esterification was performed
157 overnight using 200 µl 1% H_2SO_4 (in CH_3OH) and 100 µl toluene at 50°C. Phases were split
158 using 300 µl 5% sodium chloride solution, and FAMEs were separated using n-Hexane,
159 transferred into a new cocoon, evaporated, and 100 µl (final volume) added. All solvents used
160 were gas chromatography (GC) grade. FAME were analyzed by a Thermo GC Ultra gas
161 chromatograph equipped with a non-polar column (RXI1-SIL-MS 0.32 µm, 30 m, company
162 Restek) and Flame ionization detector. The column oven was initially set to 100°C, and heated
163 to 220 °C at 2 °C min^{-1}. The carrier gas was helium at a constant flow of 2 ml min^{-1}. The flame
164 ionization detector was set to 280 °C, with a gas flow of 350, 35 and 30 ml min^{-1} of synthetic
165 air, hydrogen and helium, respectively. A 1 µl aliquot of the sample was injected. The system
166 was calibrated with a 37-component FAME-mix (Supelco, Germany) and chromatograms were
167 analyzed using Chrom-Card Trace-Focus GC software (Breteler & Schouten, 1999) and the
168 fatty acids were clustered according to their degree of saturation: saturated (SFA),
169 monounsaturated (MUFA) and polyunsaturated (PUFA).
170

171 **2.4 Statistical analyses**

172 The data was analyzed by a nested Mixed Effects ANOVA Model (LME) to determine the
173 differences in taxa biomass (µgC ml^{-1}) and relative fatty acid content (% in the seston and
174 zooplankton) between the CO_2 treatments (µatm fCO_2), with *f*CO2, silicate, inorganic nitrogen
175 (nitrite + nitrate), phosphate, temperature and salinity as fixed effects, and sampling day and

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176 mesocosm position as nested random variable (random distribution of CO_2 treatments among
177 the mesocosm). Average mesocosm fCO_2 was calculated for the total duration of the sampling
178 period plankton community composition (day 1 to 29) and for FA data analysis (day 1 to 25 for
179 seston FA and day -1 to 33 for zooplankton FA). Linear regression models were used to
180 determine the relation between PUFA and phytoplankton biomass. The similarity in the
181 structure of the plankton community between the treatments was analyzed by Non Metrical
182 Multidimensional Scaling (NMDS) with Bray distance, auto-transformation and 3 dimensions
183 (k=3). This analysis distributes the samples in an ordination space according to the biomass of
184 the different taxa in the community along orthogonal principal components using non Euclidean
185 distances for ordination space, which makes it more robust to the presence of zero values
186 (Clarke 1993). All statistical analyses were done using the R software environment 3.0.1 (R
187 Development Core Team 2013).
188

189 **3 Results**

190

191 **3.1 Plankton community composition**

192 The initial algal community consisted of post-bloom species dominated by small-sized cells,
193 with dinophyta being the most abundant phytoplankton group in all mesocosm throughout the
194 experiment followed by heterokontophyta, euglenophyta, cholorophyta, cyanobacteria bigger
195 than 5µm (usually filamentous) and small abundances of cryptophya (Fig. 1).
196 Microzooplankton was present during the entire experimental period, particularly the
197 choanoflagellate *Calliacantha natans* (Fig. 1). The plankton community was analyzed from day
198 1 to 29, which comprised two phases as described by Paul et al. (2015), with a Phase 1 (from
199 day 1 to 15) where phytoplankton biomass gradually increased until day 10 when a bloom
200 started and reached a peak around day 15 in all treatments; while in a Phase 2 (from day 17 to
201 29) the biomass began to decay from around day 19 up to day 29 (Fig. 1).
202

203 The more abundant taxa did not show differences in abundance between the CO_2 treatments on
204 both phases (Fig. 2a, b). However, the biomass of some of the less abundant groups was affected
205 by CO_2 within the different phases. In Phase 1, the nested mixed effects model analysis of the
206 algal biomass showed that chlorophyta decrease significantly towards high CO_2 levels (Fig. 2a)
207 (LME, $F= 7.27$, $p= 0.01$, $df= 20$). Nevertheless, there was a difference in the relative biomass
208 of the more abundant plankton groups between Phases 1 and 2, with a decrease in dinophyta
209 (37.2 ± 3.2 % to 28.3 ± 2.9 %) and heterokontophyta (19.1 ± 2.2 % to 14 ± 2.6) (Fig. 2c) and

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210 an increase of euglenophyta (7.5 ± 1.4 % to 21 ± 2.7) and chlorophyta (14.0 ± 1.5 % to 19.1 ±
211 2.4) (Fig. 2d). An NMDS analysis of the entire phytoplankton community showed a rather
212 homogeneous community composition between the different CO_2 treatments but variation
213 among sampling days, especially at day 7, when the diatom *Melosira varians* was abundant
214 during that particular sampling day (Fig. S1).
215
216 **3.2 Seston fatty acid composition**
217 The PUFA represented on average ~26 ± 4%, MUFA ~22 ± 3% and SFA ~52 ± 4% of the total
218 FA content in the seston over the entire experimental period. The Mixed Effect Model (LME)
219 analysis of relative PUFA content data showed no significant difference among the CO_2
220 treatments (LME, F_{45}= 0.0, p>0.05) (Fig. 3a PUFA). The MUFA and SFA did neither show any
221 significant change in abundance in relation with CO_2 (LME, F_{45}= 0.0, p= 0.8, and F_{45}= 0.06, p=
222 0.79, respectively) (Fig. 3a MUFA, SFA). However, the FA composition of the seston showed
223 that the relative PUFA content significantly decreased over time in all mesocosms (linear
224 regression, R^2= 0.52, t= -7.64, p<0.0001, n=22) (Fig. 3b High CO_2 treatments, Low CO_2
225 treatments), while the MUFA and SFA increased, although the relation of both with time is
226 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
227 respectively) (Fig. S2).
228
229 Nevertheless, PUFA showed a positive relation with heterokontophyta (linear regression,
230 R^2=0.58, p<0.001) and dinophyta (linear regression, R^2=0.41, p<0.001) biomass (Fig. 4a); and
231 with silicate (LME, F= 22.8, p< 0.001, df= 35) and phosphate (LME, F= 9.3, p< 0.01, df= 35)
232 abundance in the mesocosms (Fig. 4b).
233
234 **3.3 Copepod fatty acids**
235 The overall PUFA content of the copepod *A. bifilosa* represented ~12% (311 ± 175 ng FA mg
236 dry wt.$^{-1}$) and in *E. affinis* ~16% (433 ± 597 ng FA mg dry wt. $^{-1}$) of the total FA.
237
238 The FA did not show a CO_2-related effect in *A. bifilosa* (LME, F= 0.62, p= 0.4374, df= 26)
239 (Fig. 5a), or *E. affinis* (F= 0.13, p= 0.71, df= 26) (Fig. 5b). Nevertheless the relative PUFA
240 content in *A. bifilosa* and *E. affinis* showed a significant decrease over time in all high and low
241 CO_2 treatments (linear regression, *A. bifilosa*; R^2= 0.22, t= -3.288, p= 0.002 *E. affinis;* R^2= 0.47,
242 t= -5.51, p< 0.0001) (Fig. 5c), while MUFA and SFA increased in both species (Fig. S3).
243 Furthermore, the relative FA content in *E. affinis* varied over time following the changes in the

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244 seston FA, this relation was significant but weak for PUFA MUFA and SFA (Fig. S4), while in
245 *A. bifilosa* this change appeared only in the MUFA (Fig. S4).
246
247 **4. Discussion**
248
249 **4.1 Community composition**
250 The plankton community composition in the present experiment did change over time and
251 showed little differences in relation to the different CO_2 treatments. The observed absence of a
252 strong CO_2 effect on the community composition in the present study is in line with the
253 observations in the western Baltic Sea (Thomsen et al., 2010; Nielsen et al., 2010; Rossoll et
254 al., 2013). In these studies the authors suggested that the plankton community is adapted to OA
255 due to the recurrent large seasonal and daily variance of pH and CO_2 experienced by the
256 communities in this productive low-salinity region (Thomsen et al. 2010; Nielsen et al. 2010;
257 Rossoll et al. 2013; Almén et al. 2014). Our study region, a coastal zone in the western Gulf of
258 Finland in the northern Baltic Sea, is a brackish environment with low salinity (~5.7 ‰), a high
259 fresh water runoff (~111 km^3 $year^{-1}$) (Savchuk 2005) and a strong inter- and intra-seasonal pH
260 variability, sometimes reaching extreme values of 9.2 and 7.4 with an average of 8.1 (Brutemark
261 et al. 2011). Therefore, it seems that the plankton community in our study area, which
262 experiences high natural pH fluctuations, is composed of species and genotypes that are less
263 pH/CO_2 sensitive (Nielsen et al. 2010; Lohbeck et al. 2012; Melzner et al. 2013; Rossoll et al.
264 2013) which allows them to cope with the CO_2 range applied in the current field experiment.
265
266 Chlorophytes were the only group that showed a significant response to the CO_2 treatment,
267 although their contribution to total biomass was low. Chlorophytes decreased at elevated fCO_2,
268 which is contrasting to laboratory studies showing that several species in this group benefit
269 from high CO_2 and can increase their growth rates (Tsuzuki et al. 1990; Yang & Gao 2003).
270
271 **4.2 Seston FAs**
272 The relative PUFA content of seston showed a significant decrease over time, which can be
273 attributed to nutrient depletion in the mesocosms, particularly silicate and phosphate
274 concentrations, which caused a decrease in dinophyta and heterokontophyta abundances. These
275 two groups of microalgae have been identified as a rich in PUFA content (Galloway & Winder
276 2015) and their decrease in the mesocosms explains the concomitant decrease in PUFA. Silicate
277 is required by heterokontophyta for the formation of new frustules during cell division, and

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278 when limited, cell division stops (Flynn & Martin-Jézéquel 2000). Phosphorus is required for
279 the production of PUFA-rich membrane phospholipids during cell division and growth
280 (Guschina & Harwood 2009). Nutrient limitation, which causes reduced cell division rates,
281 results in a lower production of phospholipid and increased production of storage lipid,
282 primarily triacylglycerols (Guschina & Harwood 2009). Triacylglycerols tends to be rich in
283 SFA and MUFA; therefore the increase in triacylglycerols with nutrient limitation typically
284 resulted in decreased proportions of PUFA in most algae (Guschina & Harwood 2009). This is
285 consistent with our observations in the mesocosms, where the relative PUFA content of seston
286 followed the phosphate concentration. From this perspective one may expect that any CO_2
287 effect in algal PUFA will occur when cells are actively growing since nutrient limitation
288 (silicate and phosphorus) will have more profound consequences in the cell physiology than an
289 excess of CO_2.
290
291 The absence of a PUFA response to CO_2 differs with a report of an Arctic plankton community
292 showing an increase of PUFA at high CO_2 levels during part of a mesocosm experiment
293 experiencing nutrient additions (Leu et al. 2013). This was attributed to a change in the plankton
294 community composition due to a rise in abundance of dinoflagellates at high CO_2 (Leu et al.
295 2013). Our results show a decrease in PUFA due to a decline in dinoflagellates. The different
296 PUFA trend between these experiments can be attributed to the specific plankton community
297 composition and their related FA profiles alongside with phosphate and silicate limitation in
298 our study, which causes a reduction of the biomass of some PUFA-rich taxa. Species
299 composition of a natural plankton assemblage determines its food quality properties as distinct
300 algal taxonomic groups have different FA composition profiles (Galloway & Winder 2015). A
301 CO_2-driven change in the Arctic plankton community composition (Leu et al. 2013) promoted
302 the presence of species rich in PUFA. In our study the absence of a CO_2 response in taxa
303 composition and the apparent influence of phosphate and silicate limitation in the algal FA
304 composition resulted in a rather homogeneous PUFA concentration between CO_2 treatments.
305

306 **4.3 Copepod fatty acids**

307 Our results showed that the PUFA concentration of the dominating copepod species, *A. bifilosa*
308 and *E. affinis* did not vary between the different CO_2 treatments. However, the PUFA decrease
309 in both copepods over the experimental period reflects the decline in the PUFA content of the
310 seston. This observation is consistent with other studies showing that copepods strongly rely on

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311 their diet as source of FA and that their composition, especially PUFA, mirrors the algae they
312 graze on (Ishida et al. 1998; Caramujo et al. 2007; Rossoll et al. 2012).
313
314 Several studies have shown a limited direct CO_2 effect in the copepods FA of some species,
315 like the genus *Acartia*, which is rather insensitive to projected high CO_2 exposure up to 5000
316 µatm CO_2 (Kurihara et al. 2004; Kurihara & Ishimatsu 2008). Copepods experience widely
317 varying pH conditions on a daily basis due during their vertical migration, shown in the same
318 area as the current study (Almén et al. 2014), which may explain their tolerance to pH
319 variations. Several studies have demonstrated that food quality of the available prey in terms of
320 PUFA content can affect egg production, hatching success and nauplii survival in copepods
321 (Jónasdóttir 1994; Caramujo et al. 2007; Jónasdóttir et al. 2009). Indirect adverse CO_2 effects
322 through the diet of primary consumers have been reported in laboratory and field experiments
323 (Rossoll et al. 2012; Locke & Sprules 2000). However, the absence of a CO_2-driven change in
324 the community composition of primary producers and the homogeneous algal FA composition
325 due to phosphate and silicate limitations masked any noticeable CO_2-related effects in the algae
326 FA profile that could have affected the copepods during our experiment.

327 **5 Conclusions**
328
329 Considering that the Baltic Sea is a coastal sea with a natural frequent and wide pH variability
330 (Omstedt et al. 2009), it can be expected that the effects of OA on plankton communities will
331 be rather small within the range of predicted values for this century (Havenhand 2012). A
332 reduced OA sensitivity in systems experiencing high CO_2 fluctuations is supported by our
333 results and other studies using communities from the Baltic (Thomsen et al. 2010; Nielsen et
334 al. 2010; Rossoll et al. 2013). However, in coastal upwelling areas undergoing an increase in
335 hypoxic events, it is likely that elevated CO_2 values as presently experienced by coastal
336 organisms and projected by the end of the century (Melzner et al. 2013) will be more recurrent
337 in the future (Feely et al. 2004), with the potential to affect various properties of plankton
338 communities.
339
340 Nonetheless, it is clear that the plankton community response to OA and concomitant effects
341 on its food quality for higher trophic levels will strongly depend on the sensitivity of primary
342 producers and on how OA affects the species composition of plankton assemblages (Leu et al.
343 2013; Rossoll et al. 2013). This result is important as any change in primary producers in terms

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344 of FA, particularly essential biomolecules such as PUFA, may scale up in food webs since FAs
345 are incorporated into the lipids of larval fish (Fraser et al. 1989; Izquierdo et al. 2001).
346 Considering that fish is a critical natural resource (FAO, 2010), adverse OA effects on food
347 quality can reach up to human populations who rely on fisheries as an important food source
348 (Sargent et al. 1997; Arts et al. 2001).

349

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358

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492 **Figures and Figure Legends**

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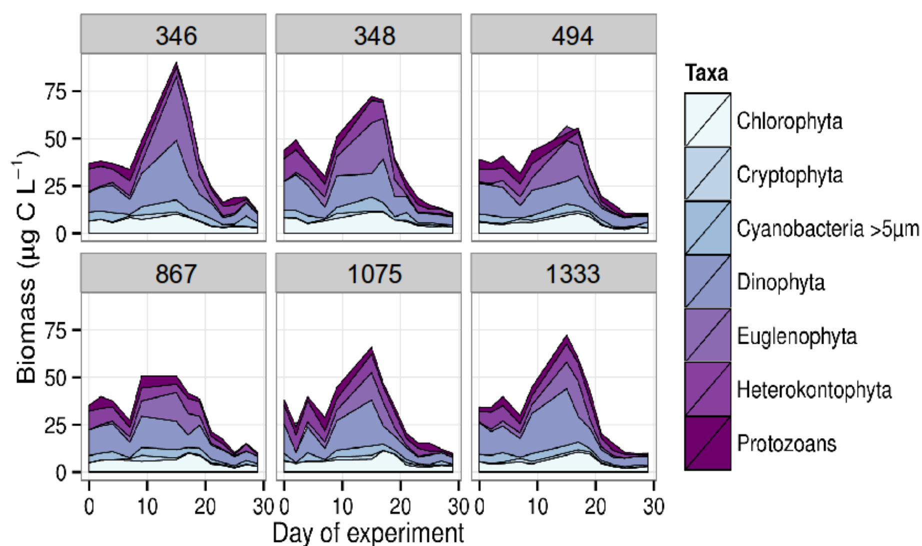

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499 **Figure 1.** Calculated biomass after cell counts of the main plankton taxonomic groups in the
500 different CO_2 treatments between day 1 and 29. Each treatment is labeled with the average fCO_2
501 level of the entire experimental period (top).

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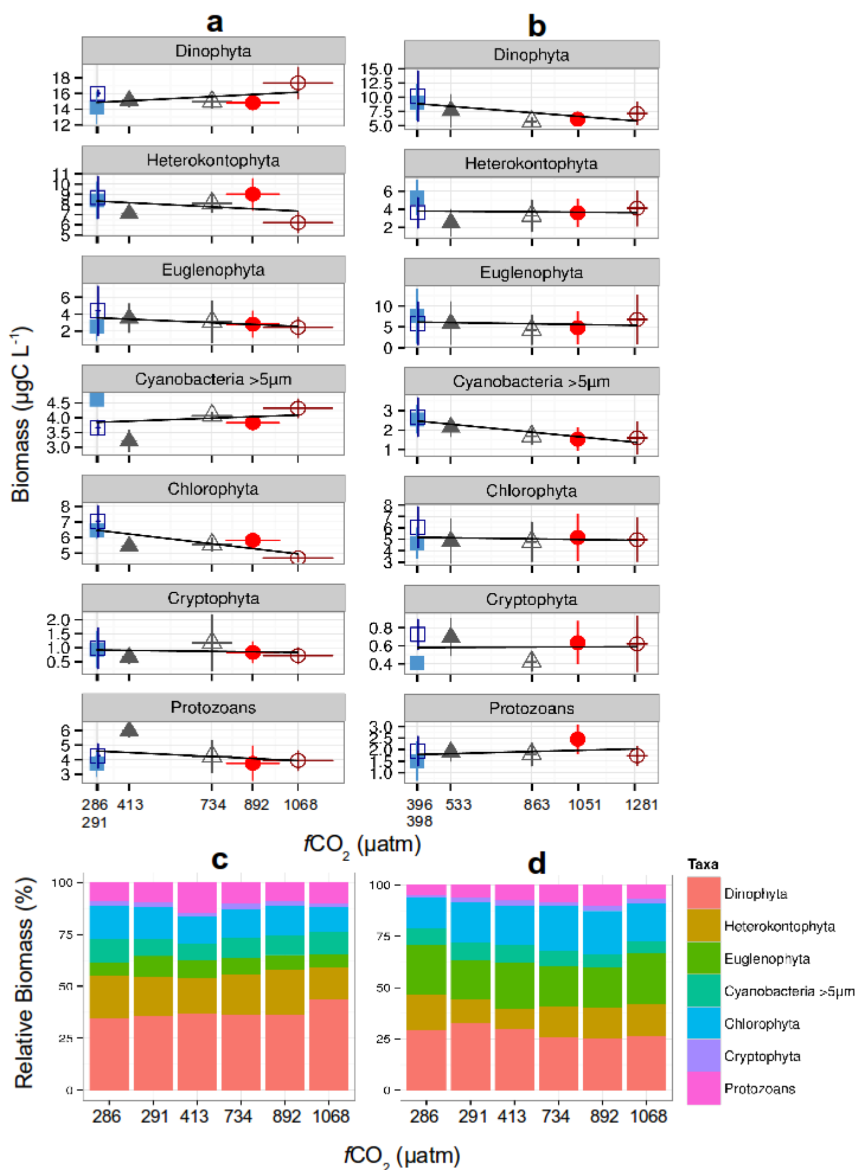

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513 **Figure 2.** The top panels show the mean of the calculated biomass of each plankton taxon in a)
514 Phase 1, between the days 0 to 15; and b) Phase 2, between days 15 to 29, in the CO_2 gradient
515 treatments. The bottom panels show the relative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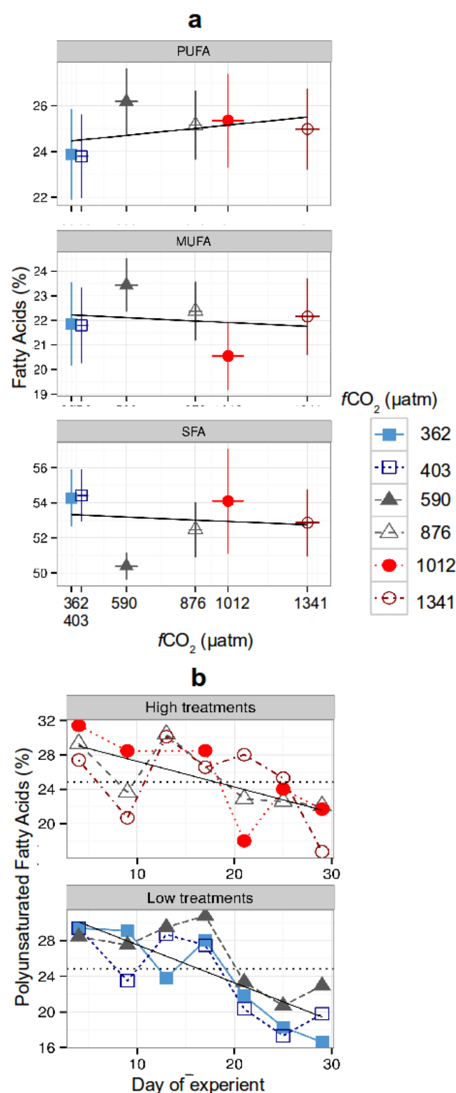

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522 **Figure 3.** a) Relative polyunsaturated (PUFA), monounsaturated (MUFA), and saturated (SFA)
523 fatty acids content in the seston as a function of fCO_2 between day 1 and 29. The x-axes show
524 the mean fCO_2 measured during the sampling period,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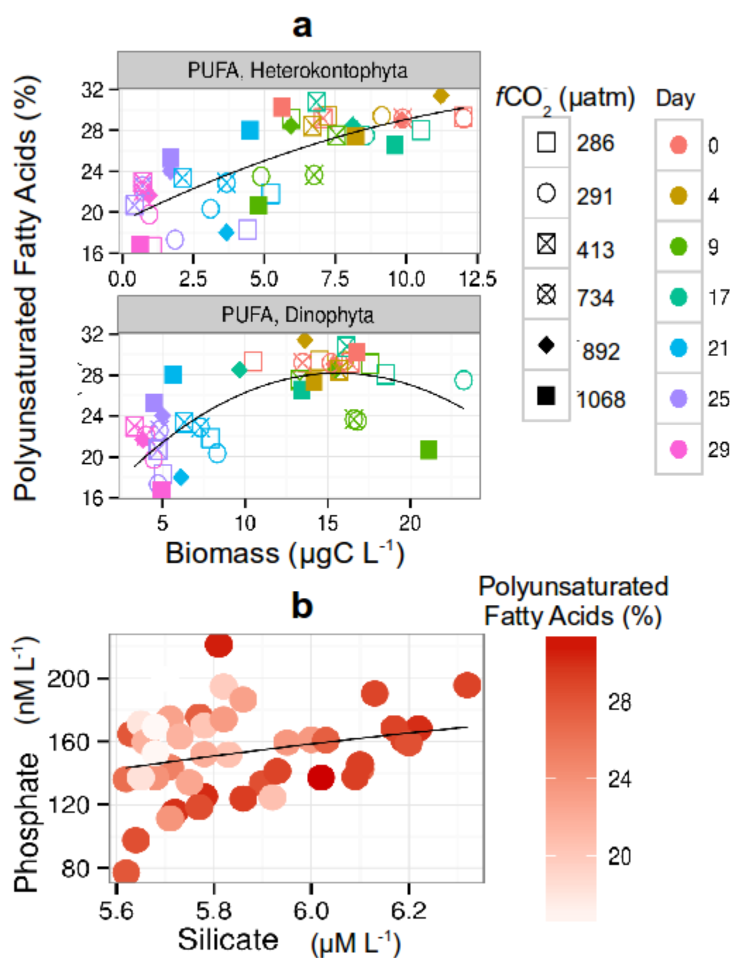

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533 **Figure 4.** a) Relation between sestonic relative polyunsaturated fatty acids (PUFA) with
534 heterokontophyta (PUFA, heterokontophyta) and dinophyta (PUFA, dinophyta) biomass. b)
535 Relation between relative sestonic PUFA content with silicate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

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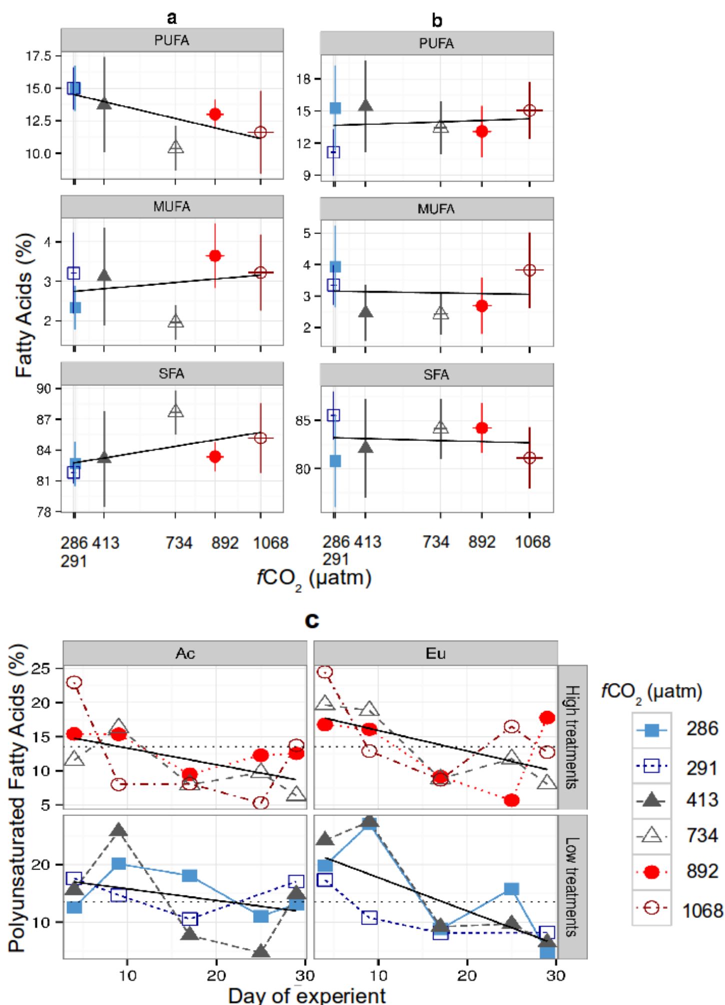

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553 **Figure 5.** a) and b) show the relative polyunsaturated (PUFA), monounsaturated (MUFA), and
554 saturated (SFA) fatty acids content in the copepods *Acartia bifilosa* and *Eurytemora affinis*,
555 respectively, under the fCO_2 gradient treatments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