Title: Plankton responses to ocean acidification: The role of nutrient limitation**.**

Running head: Plankton responses to ocean acidification

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Abstract: *In situ* mesocosm experiments on the effect of ocean acidification (OA) are an important tool for investigating potential OA-induced changes in natural plankton communities. In this study we combined results from various in-situ mesocosm studies in two different ocean regions (Arctic and temperate waters) to reveal general patterns of plankton community shifts in response to OA and how these changes are modulated by inorganic nutrient availability. Overall, simulated OA caused an increase in phytoplankton standing stock, which was more pronounced in smaller-sized taxa. This effect on primary producers was channelled differently into heterotroph primary consumers depending on the inorganic nutrient availability. Under limiting conditions, bacteria and micro-heterotrophs benefited with inconsistent responses of larger heterotrophs. During nutrient replete periods, heterotrophs were in general negatively affected, although there was an increase of some mesozooplankton developmental stages (i.e. copepodites). We hypothesize that changes in phytoplankton size distribution and community composition could be responsible for these food web responses.

Introduction

Atmospheric CO_2 concentrations have increased by about 40% since the beginning of the industrial revolution, now reaching values of ca. 400ppmv (IPCC, 2013). This increase in atmospheric CO_2 is partly buffered by the oceans, accounting for the removal of at least one third of anthropogenic CO_2 emissions (Sabine *et al.*, 2004). However, oceanic CO_2 uptake is accompanied by profound changes in seawater chemistry. Dissolution of the anthropogenic CO_2 in seawater alters the marine carbonate system as CO_2 reacts with water to form carbonic acid. As a result, there is a decrease of pH, [CO_3^{2-}], and $CaCO_3$ saturation states, and an increase in [HCO_3^-] (Wolf-Gladrow *et al*., 1999).

All of these changes in carbonate chemistry can cause physiological responses in marine organisms. Individual responses have already been recorded in benthic and planktonic organisms (Doney *et al.*, 2009, Gattuso *et al.*, 1998, Riebesell *et al.*, 2000), in both calcifiers and non-calcifiers (Kroeker *et al.*, 2010). At a subcellular level, Bunse et al. (2016) showed differentiated gene expression as a

response to elevated pCO_2 with potential impacts on carbon cycling. Experimental and modelling studies on plankton species responses to increasing pCO_2 have shown changes in survival, calcification, growth, development, and abundance for a range of marine organisms. The magnitude of the response is variable across species and is often further modulated by other environmental factors, such as temperature or nutrient availability (e.g. Sett *et al.*, 2014, Taucher *et al.*, 2015). Phytoplankton shows a variable response to increasing pCO_2, both in abundance and photosynthetic rates, ranging from positive to negative (Gao *et al* 2014). Some copepod species suffer adverse effects with increasing pCO_2, particularly in early life-stages, showing up to a threefold increase in mortality rates, and a 35% decline in nauplii recruitment (Cripps *et al.*, 2014). Others show virtually no reaction at relevant pCO_2 or pH levels (Mayor *et al.*, 2012).

Community responses to increased pCO_2 have proven more difficult to assess and have been studied to a far lesser extent. In recent years, however, more studies have become available, particularly aiming at plankton community responses in indoor (Horn *et al.*, 2016, Paul *et al.*, 2015b, Sala *et al.*, 2016) and field experiments (Schulz *et al*., 2013, Bach *et al.* 2016). These studies showed that increased pCO_2 alters nutrient flow among different phytoplankton groups, and affects their gross growth rates differently.

Marine waters C:N and C:P ratios can be expected to increase as a direct effect of increased pCO_2, which will have direct consequences for the phytoplankton community, altering their own stoichiometry (van de Waal *et al.*, 2010). Primary consumers will excrete this excess carbon, and different pathways of C excretion have been already reported in laboratory experiments (Schoo *et al.*, 2013). In some cases, stoichiometric changes in primary producers propagate through the food-web, leading to negative effects on consumers (Malzahn *et al.*, 2010, Schoo *et al.*, 2012, Schoo *et al.*, 2013). Algae with high C:N and C:P ratios, and the resulting excretion of C, are often of inferior food quality for herbivorous consumers (Boersma *et al.*, 2008),.

In community studies, both micro- and mesozooplankton communities have shown variable responses to increased pCO_2 simulating 2100 IPCC prediction scenarios. Some studies showed tolerance (Niehoff *et al.*, 2013, Aberle *et al.*, 2013, Suffrian *et al.*, 2008), with no apparent change in grazing or body mass detected in copepod dominated communities, while other studies detected both changes in community size distribution (Lischka *et al.*, 2015) and stage-specific responses (Algueró-Muñiz et al., 2017).

It is clear from these previous studies that, as they involve complex food-web interactions, the responses of plankton communities to OA are very variable. Furthermore, differences in starting community composition and environmental characteristics of the experimental waters will affect responses in all these experiments, partially explaining the inter-experimental variability (Moreno de Castro et al., 2017). Nevertheless, if we are to come to a more general understanding of the effects of OA on planktonic communities, we need to compare the different studies, find commonalities in responses, and explain those patterns that are contradictory. Hence, in this study we amalgamate results of several OA community mesocosm experiments conducted at mid to high latitude sites. Geographical differences between studies allowed for the assessment of responses across a gradient of temperature regimes, nutrient availabilities and initial community compositions. By combining results, we test if there are common responses across experiments hidden by the intrinsic variability of individual study sites. Also, detection of common responses across experiments could point to the underlying overall mechanisms, and therefore help prediction of future responses of marine pelagic communities to OA.

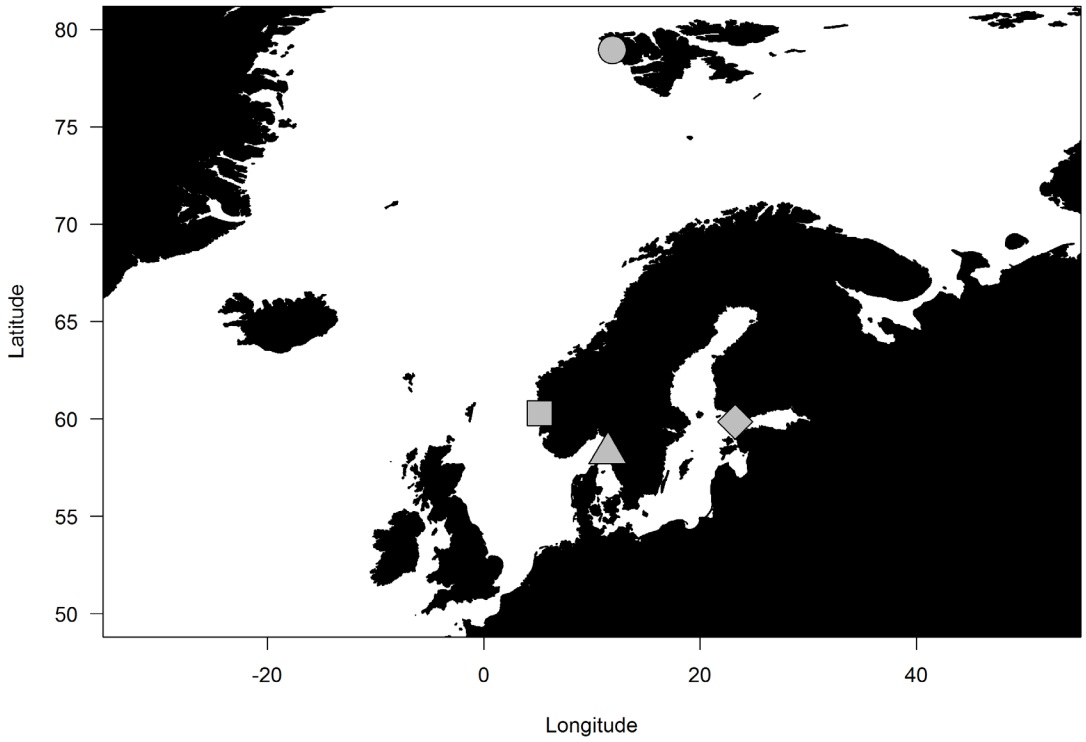

Figure 1. Location of the KOSMOS experiment: Kongsfjorden 2010 (●), Raunefjord 2011 (■), Tvärminne 2012 (♦), and Kristineberg 2013 (▲).

Materials and Methods

From 2010 to 2013, four Kiel Off-Shore Mesocosms for Future Ocean Simulations (KOSMOS) experiments were set up at different locations (Fig. 1). In 2010 nine mesocosms were deployed in Kongsfjorden on the west coast of Spitsbergen (Schulz *et al*., 2013), in 2011 in the Raunefjord near Bergen in Southern Norway (Endres *et al*., 2014), in 2012 in the Tvärminne Storfjärden (Paul *et al*., 2015a); finally in 2013 10 mesocosms were deployed in Kristineberg-Gullmar Fjord, ca. 100 km north of Gothenburg on the Swedish west coast (Bac*h et al*., 2016).

The natural seawater conditions in these areas range from arctic temperatures (< 5°C, Kongsfjorden 2010), to temperate (all other studies). Salinity conditions also cover a wide range from ca. 7 (Tvärminne 2012), to 34 ppt (Kongsfjorden 2010). The main environmental conditions are summarized in Table 1. Additionally, a summary of the manipulations made in each experiment can be found in Table 1 and the manipulations are visualized in figure 2. CO_2 manipulations were

achieved by distributing filtered, CO_2-saturated seawater equally into the mesocosm as described by Riebesell *et al.* (2013). Experimental setups were either gradient design (2010, 2011, and 2012), ranging from ambient conditions to 1.5-3 atm pCO_2, or treatment design (2013) where pCO_2 treatment averaged 0.76atm. Nutrient conditions also varied across experiments. In two occasions, nutrients were added mid experiment to mimic the natural phytoplankton pre-bloom conditions (2010, 2011), in 2013 nutrient concentrations were low throughout the experiment, while in 2014 nutrients were naturally depleted throughout the experiment with no nutrient additions. Further details on the experimental setups given in the overview papers for each experiment mentioned above and specified in Table 1.

For each experiment, a dataset was collated covering the main components of the plankton. Because of the differences in identification and techniques used in different years, some taxonomical detail was lost in the process.

Table 1. Summary of experimental set-ups and water characteristics for each KOSMOS experiment

	Kongsfjorden 2010	**Raunefjord 2011**	**Tvärminne 2012**	**Kristineberg 2013**
Reference	Schulz *et al*., 2013	Endres *et al*., 2014	Paul *et al*., 2015a	Bach *et al*., 2016
KOSMOS (N)	9	9	6	10
pCO_2 treatment	Gradient	Gradient	Gradient	Treatment
Mean pCO_2 (µatm)	ambient - 1000	ambient - 1600	ambient - 1121	ambient vs. ca. 760
Duration	30 days	34 days	45 days	113 days
Start month	June	May	June	March
Nutrient addition	Day 13	Day 14	-	-
Water column salinity	33.6 - 34.2	31.6 - 32.1	5.6 – 5.7	26.9 - 29.8
Water column temperature	2-5.5°C	6.8-10°C	8-16°C	1-16°C

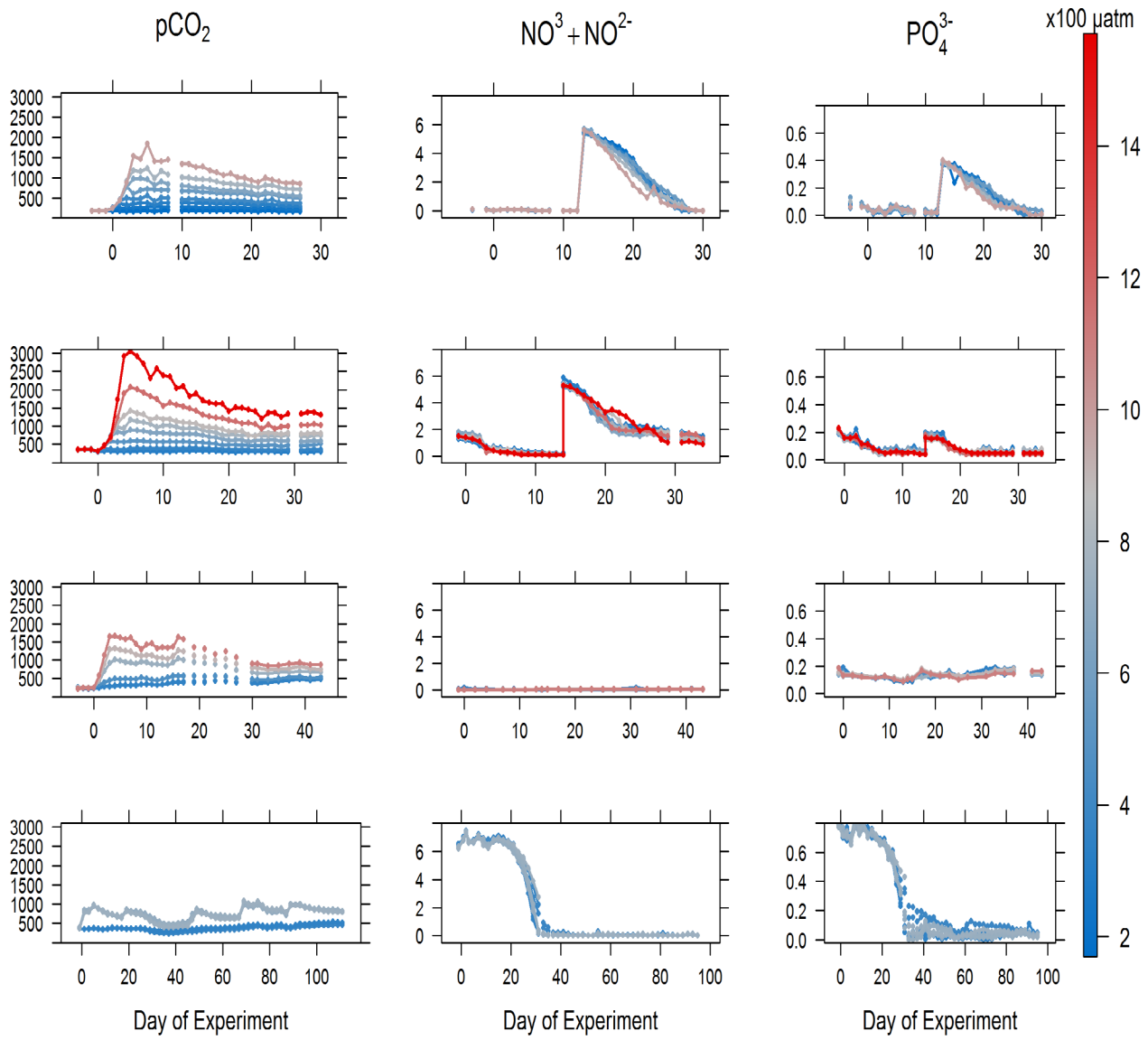

Figure 2. pCO_2 (left), nitrogen (middle), and phosphate (right) concentrations in each experiment (Kongsfjorden, Raunefjord, Tvärminne, Kristineberg) in order from top to bottom. The colour scale indicates mean values of pCO2 (x100 µatm) per mesocosm. Experimental treatment was applied on day 0.

Phytoplankton was the most frequently sampled biological parameter across all experiments, typically with a frequency of every 1-2 days. Flow cytometry data (cells < 20µm) and taxonomic composition of the phytoplankton community was available for all the experiments. Flow cytometry (FCM) counts were clustered depending on their fluorescence and scatter in different groups for each experiment (Bac*h et al.*, 2016. Brussaard *et al.*, 2013, Crawfurd *et al.*, 2016). Average cell size of the different clusters was determined by serial gravity filtration (Brussaard et al. 2013; Crawfurd et al. 2016).

In order to have comparable data for all experiments, HPLC chlorophyll a and phytoplankton group partitioning by CHEMTAX data was used as a proxy of autotroph community taxonomic composition. The CHEMTAX matrix factorisation program (Mackey *et al.*, 1996) was used in each experiment to convert the concentrations of marker pigments to Chl *a* proportion in each taxonomic group. Although using chlorophyll as a proxy of either phytoplankton biomass or abundance is not without problems (Alvarez-Fernandez & Riegman, 2014, Behrenfeld *et al*., 2005), especially when environmental conditions vary, here we consider changes in chlorophyll per group (under the same nutrient and temperature conditions but different OA treatment) to represent changes in the standing stocks of the respective taxonomic group.

Micro- and mesozooplankton were sampled ca. once a week in each experiment (Hildebrandt *et al.*, 2016, Lischka *et al.*, 2015, Niehoff *et al.*, 2013, Aberle *et al.*, 2013, Algueró-Muñiz *et al*., 2017), with the exception of Raunefjorden 2011 where microzooplankton data were not available. Mesozooplankton composition and identification accuracy also differed between experiments. Therefore, in order to standardize data on heterotrophs across experiments, we aggregated different groups based on the available information. Ciliates were classified according to size into two groups: small (~ <30µm) and large (~ >30µm). Copepod data was split into 4 groups: nauplii, copepodites, female and male copepod abundances. This gives an indication of both the size distribution of the copepod community (based on the relative abundance of adults vs. earlier life stages), and the reproductive potential (based on the female and male abundances). Copepods were the dominant mesozooplankton group in all experiments except for Tvärminne-2013. In order to account for other groups, total mesozooplankton catches (including developmental stages) were also analysed. Total heterotrophic bacterial abundance was estimated using flow cytometry methods in all experiments (Brussaard et al., 2013; Endres et al., 2014; Crawfurd et al., 2016; Bach et al., 2016), and also included in our analyses.

145 Analyses

146 A two-step approach was taken in the analyses. First, periods when community development was
147 different between treatments were detected via multivariate ordinations on the FCM phytoplankton
148 datasets. Second, a meta-analysis approach was taken for each parameter of autotrophs standing
149 stock (CHEMTAX) and heterotroph abundances per period. This way the focus of the second step
150 was on periods where community-scale changes were expected, based on detected changes in FCM
151 counts.

152 Multivariate analyses: A multivariate analysis was run on FCM phytoplankton data of each
153 experiment. This choice was based on the higher frequency sampling for FCM. A non-metric
154 multidimensional scaling technique (NMDS) was applied to identify any potentially different
155 temporal patterns in the data depending on pCO_2 treatment (Cox & Cox, 2000). For this purpose,
156 data from all mesocosms were used. NMDS is a rank-based technique, so it avoids potential
157 problems caused by abundance magnitude differences among groups. Each point in the NMDS
158 space represents a sampling day in each mesocosm, therefore each line represents the development
159 in time of each mesocosm community. Inspection of NMDS plots allowed identification of
160 divergence periods between treatments (i.e. periods in which the community development was
161 different between mesocosms), and which size-classes were more abundant in each treatment per
162 period.

Meta-analysis: A meta-analysis approach was taken in order to detect any consistent effects of pCO_2 on plankton communities across experiments. In order to standardize treatments and controls across experiments, mesocosms with average pCO_2 values under 450 µatm were considered as control, and mesocosms between average 600 and 1000 µatm were considered treatment. This way the potential problems of extreme pCO_2 values (up to 3000µatm) being present in some experiments was avoided, while keeping a balanced number of replicates per experiment (a minimum of 2 replicates each of control and treatment in Tvärminne and a maximum of 5 replicates each in Kristineberg). Furthermore, the pCO_2 values considered as treatment are well in the range of IPCC end of the 21st century predictions.

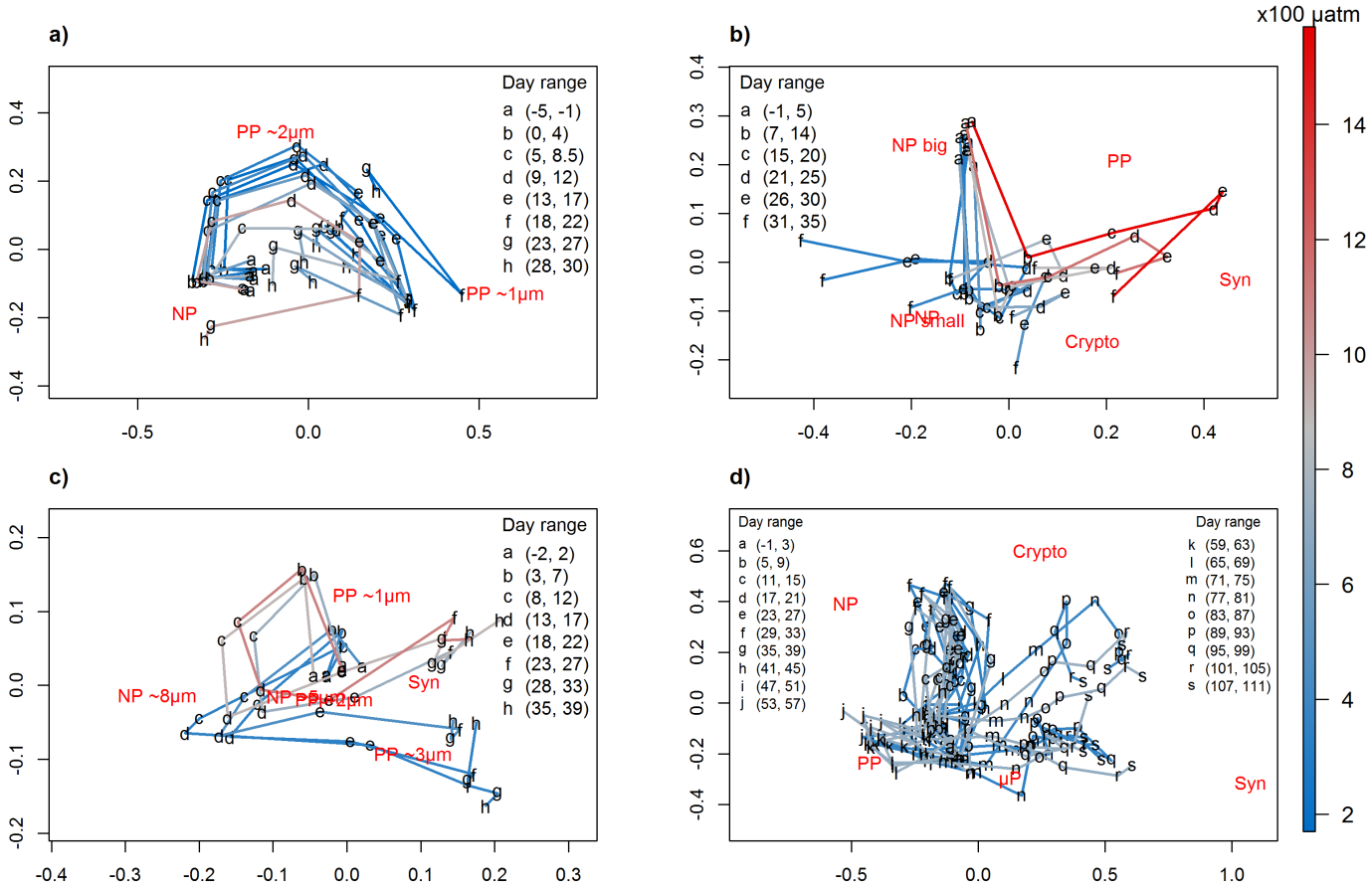

Figure 3: Non-metric multidimensional scaling plot for each experiment: a) Kongsfjorden, b) Raunefjord, c) Tvärminne, and d) Kristineberg. Experimental days are grouped in each letter (see legend) and each color line represents one mesocosm experiment with the color indicating the CO_2 level (see legend). Autotrophic biological groups are represented on red text as PP – picoeukaryotes, Syn – *Synechococcus*. NP – nanophytoplankton, µP – microphytoplankton, and Crypto – Cryptophyceae,

172 Parameters considered for this meta-analyses include the chlorophyll average value of each

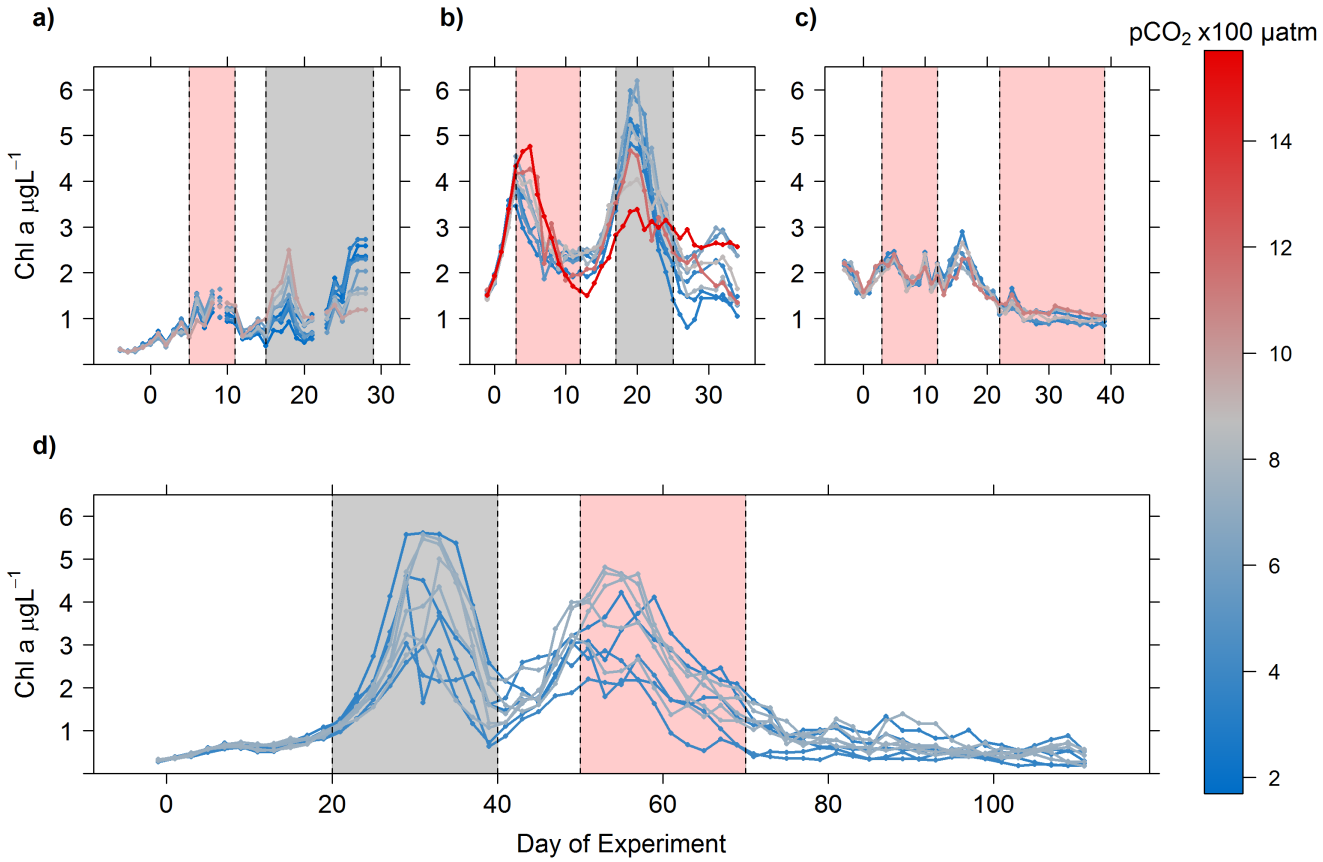

Figure 4. Chlorophyll *a* concentration for a) Kongsfjorden 2010, b) Raunefjord 2011, c) Tvärminne 2012, and d) Kristineberg 2013. Shaded areas indicate the selected period for the assessment of pCO_2 effects in the meta-analysis; with colour indicating the type of limitation red= N-deplete, grey =N-replete. See definitions in text

173 CHEMTAX group and the average abundance for bacteria and each zooplankton group per period.

174 By this approach an overall effect of pCO_2 on different parameters per experiment ln(RR) was

175 calculated as suggested by Kroeker *et al.* (2010):

176 $$\ln(RR) = \ln(X_E) - \ln(X_C),$$

177 where X_E and X_C are the parameter mean values in the experimental and control treatments,

178 respectively.

179 A random effects model was then used to calculate the overall effect on each parameter across

180 experiments. This model was weighted depending on the variance of log-transformed response per

experiment, so experiments with high replication and low response variance are weighted more heavily. Variance of the log response per experiment was calculated as

;

where S^2 represents variance, n is the number of mesocosms, and X is the parameter mean value per experimental treatment (E) and control (C).

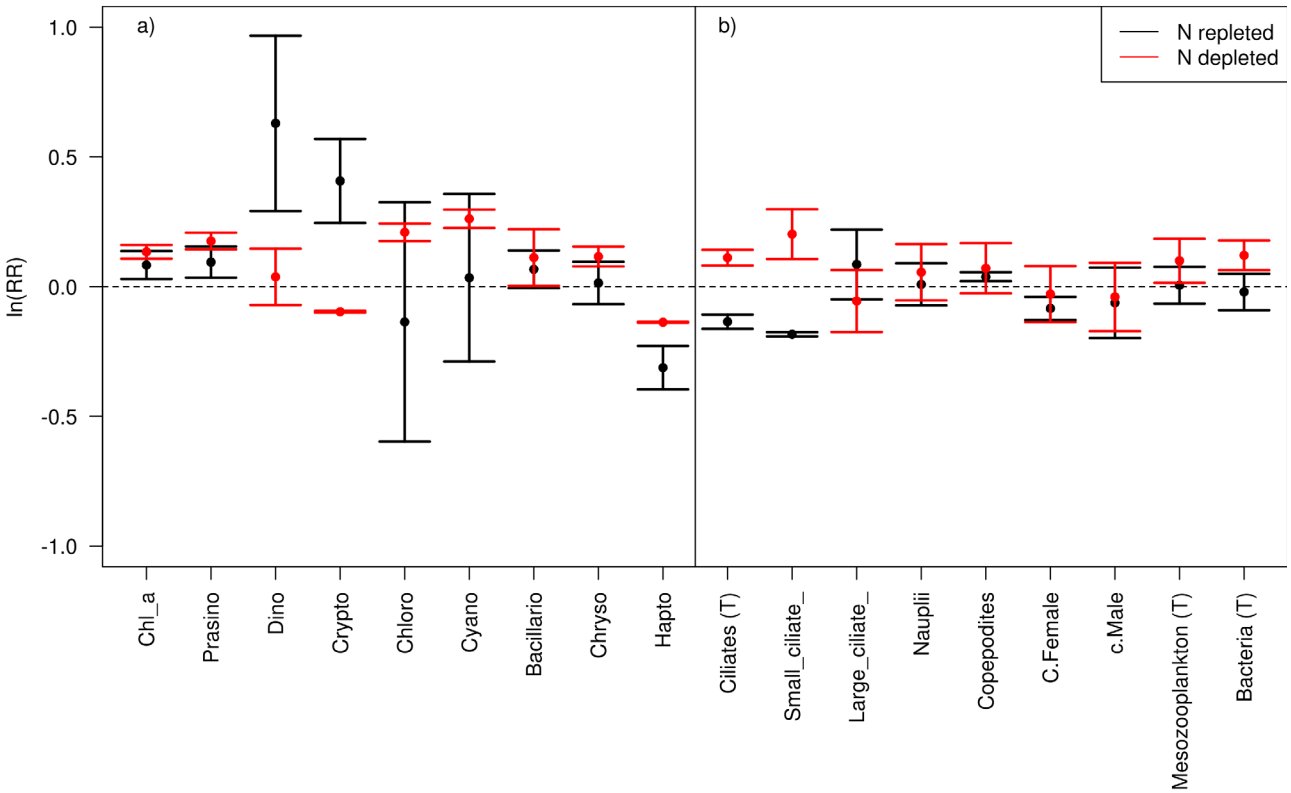

Figure 5. Overall effect of pCO_2 increase across experiments in autotroph (a) and heterotroph (b) plankton parameters under N-dep (red) and N-rep (black) periods. The results are shown as the estimate of the random effect model (point) and the standard error of the estimate (bars). Effects can be considered statistically significant when the estimate error does not cross the zero line (dashed). (T) stands for total catch.

Results

189

190 Flow cytometry data analysi

191 NMDS spaces (Fig.3) represent the temporal development of each mesocosm phytoplankton
192 community (FCM) in a 2-dimensional space. The FCM groups relative abundances for each period
193 can also be inferred from their position in the NMDS space.

194 Except for Kristineberg 2013, all NMDS analyses showed different temporal trajectories in the
195 mesocosms depending on pCO_2 treatment (Fig. 3). This was particularly clear for mesocosms with
196 average pCO_2 over 1000 µatm (Fig. 3a-c). In the case of the Kristineberg 2013 experiment we

observed no clear pattern, with the high pCO_2 and low pCO_2 treatments intertwining in the NMDS space (Fig. 3d).

In Kongsfjorden (Fig. 3a) the highest pCO_2 mesocosms (mean pCO_2 ~ 1000µatm) showed the clearest different development from the others (Fig. 3a). The other studies showed an apparent gradient in response at the beginning of the experiment (days 0 to 12), becoming more similar after day 12 (nutrient addition), and separating again towards the end of the experiment (after day 23).

During the Raunefjord experiment (Fig. 3b), the highest pCO_2 mesocosm (mean pCO_2 1600 µatm) clearly separated from the others from the beginning. The two following mesocosms (mean pCO_2 1189, and 900µatm) also showed a different development from the rest. This trend became more apparent after nutrient addition (day 14).

In Tvärminne (Fig. 3c), two different trends of the small phytoplankton community could be detected (Fig. 3c). The three highest pCO_2 treatments (average pCO_2 756, 920, and 1120µatm) showed one pattern, while the three lowest showed a different one. These two patterns converged during the middle of the experiment (days 13-17).

In all cases, the difference between the low and high pCO_2 was due to abundance differences of the smaller-sized phytoplankton (<3µm). This can be clearly seen in Raunefjord (Fig. 3b). The positioning of picoeukaryotes to the right-hand side of the plot indicates that the development towards that side of NMDS space is related to a predominance of picoeukaryotes. In a similar way, the higher position of the high pCO_2 treatments in the Tvärminne NMDS (Fig. 3c) indicates the predominance of picoeukaryotes, while sample times closer to the top-left show a predominance of *Synechococcus*.

$\underline{pCO_2 \text{ effects}}$

Divergence periods in each experiment occurred under two different nutrient conditions (Fig. 4). These periods were characterised by either periods of low nutrient concentrations (hereafter N-

222 deplete, Fig. 2), or periods of relatively high N and P concentration (>2µmol $L^{-1,}$ and ca. 0.2µmol L^{-1} respectively), which can be considered as nutrient replete (herein N-replete, Fig. 2 and 4).

224 The results of the meta-analysis (Fig. 5) showed some general responses to OA independent of the
225 nutrient environment, while others remained specific to the nutrient regime. Independently of the
226 nutrient environment there were higher Chl *a* concentrations at high pCO_2, indicating an increased
227 standing stock of autotroph plankton. CHEMTAX results showed that the Prasinophyceae standing
228 stock was consistently higher under high pCO_2 and the Haptophyceae standing stock was
229 consistently higher under low pCO_2. Under nitrogen limiting conditions Chlorophyceae,
230 Cyanophyceae, Chrysophyceae, and Baccilariophyceae also showed increased standing stocks (Fig.
231 5a). In contrast, the responses in N-replete periods were mostly statistically non-significant. Only
232 Dino- and Cryptophyceae standing stocks showed a positive response when sufficient nitrogen was
233 present (Fig. 5a).

234 There was no consistent pCO_2 effect under both nutrient conditions when looking at heterotrophic
235 groups (Fig. 5b). Bacterial abundances were higher with pCO_2 treatment under N-dep (Fig. 5b),
236 while no significant effect was found under N-rep (Fig. 5b). Ciliates also showed higher abundances
237 under high pCO_2 when nutrients were depleted, but lower abundances when nutrients were
238 available. This change was driven mostly by small ciliates, as larger ciliates did not show a
239 statistically significant response. Copepod developmental stages showed no statistically significant
240 response to pCO_2 treatment under nutrient depletion, but there was higher abundance of copepodites
241 at high pCO_2 in N-rep periods (Fig. 5b). Copepod females had lower abundances at high pCO_2 in N-
242 replete periods (Fig. 5b), while no statistically significant response was detected under N-deplete
243 conditions (Fig. 5b). A consistent positive effect of total mesozooplankton abundance was detected
244 under N-dep (Fig. 5b).

Discussion

In our study, we detected patterns in the responses of plankton communities that only became visible through the combination of the results from different mesocosm experiments, and including the relationships with nutrient availability. Whereas some responses were consistent over all experiments, others were not. By distinguishing between nutrient replete and deplete experimental phases we were able to detect the mechanisms that govern CO_2-related responses of the plankton community.

The combined results of the NMDS analyses of all mesocosm experiment results reveal overall patterns of phytoplankton response to OA at the community level. An increased dominance of smaller-sized phytoplankton (picoeukaryotes <3µm) was previously reported in high pCO_2 treatments in these and other experiments (Brussaard *et al.*, 2013; Bermudez *et al.*, 2016; Crawfurd *et al.*, 2016, Bach et al. 2017., Sala et al. 2016) independently of the initial community composition. In our results, this pattern became particularly clear in those NMDS ordinations which included mesocosms with average pCO_2 over 1000µatm (Fig. 3). In the Kristineberg 2013 experiment, for example, such high levels were never reached (Fig. 2) so that the NMDS pattern differentiating different pCO_2 treatments was less clear. Furthermore, the Kristineberg experiment was considerably longer than the others, and also showed large within treatment variability. Hence, it is not surprising that there were less clear responses to pCO_2 in the Kristineberg (Fig. 3d) experiment, especially since NMDS plots represent variability within and between treatments and also in time in the same 2-dimensional space. However, experimental duration alone did not drive the results of the NMDS, as analysing different subsets of the data using time periods of 35 days yielded qualitatively comparable results (not shown).

Our meta-analysis showed two important aspects that had not been detected when analysing the studies separately. First, we see a decreasing responsiveness in higher trophic levels of the food web (Fig 5). Variable responses were observed in the microzooplankton, and no overall responses could

be detected in the mesozooplankton. Hence, microzooplankton seems to be more affected by high pCO_2 than higher trophic levels. More importantly, however, is how crucial it is to differentiate between different periods of nutrient availability. If we had not carried out this differentiation, we would have come to the conclusion that most of the taxonomic groups under consideration did not respond consistently to changes in CO_2 availability. Our discussion would have concentrated on only three variables that showed a consistent response: chlorophyll *a*, Haptophyceae and Prasinophyceae. So, we would like to stress the importance of nutrient availability to explain responses to additional stressors.

Autotrophic standing stocks (chlorophyll *a*) were consistently higher at high pCO_2, the same was the case for the Prasinophyceae. Haptophyceae were the only class being consistently negatively affected by high pCO_2 across experiments (Fig.5a). The latter is not difficult to explain with existing literature data. Other mesocosm studies showed a diminished abundance of calcifying algae, and coccololitophores (*Haptophyceae*) showed a strong negative response during the Raunefjord experiments (Bermudez *et al.*, 2016). Furtermore, Phaeocystis sp. (also a dominant Haptophyte) has also been found to react negatively to increasing pCO_2 (Hoogstraten et al. 2012). As for the Prasinophyceae, the consistent increase detected at high pCO_2 is well in line with the FCM analyses, and published studies (Schulz et al., 2013; Bermudez et al., 2016, Bach et al., 2017). Finding this response across different communities, environments and nutrient availabilities suggests strong mechanisms affecting the phytoplankton size-structure response to OA. This pCO_2-induced increase of smaller-sized phytoplankton could be the result of a combination of their competitive advantage for inorganic nutrients caused by their larger surface to volume ratios (Riegman et al., 1993), as well as differential grazing pressure on different phytoplankton size classes.

The reactions of the remaining plankton groups to pCO_2 depended to a considerable extent on the availability of nutrients. During N-replete periods, Cryptophyceae, and Dinophyceae showed a

295 positive response to an increase in pCO_2; heterotroph (i.e. ciliate) abundances showed a negative
296 response, while bacterial abundances were not affected. In N-dep periods, all autotroph groups apart
297 from Dinophyceae, Cryptophyceae (and of course Haptophyceae) showed a positive response to
298 pCO_2, as did small heterotroph (i.e. ciliate and bacteria) abundances.
299 For the remaining discussion we will try to explain these differential responses under different
300 nutrient conditions, and then consider the potential effects of the changes in community
301 composition on the flow of matter and energy through the planktonic food web. These deliberations
302 are out of necessity (as no rates are available) somewhat theoretical, but they might yield
303 hypotheses that can be tested in future studies. The main differences between N-replete and N-
304 depleted situations are the abundances of ciliates as grazers and of dinoflagellates and cryptophytes
305 as primary producers. Given the fact that the responses are dependent on the nutrient conditions we
306 do not need to try to find an explanation in a direct response to pH, as pH values were not different
307 between nutrient treatments (see original studies). Hence, the explanation of the differential
308 responses needs to emerge from food web effects.
309 Under N-depleted conditions, we observed an increase in ciliate grazers, in bacteria and a decrease
310 in cryptophytes, and no response of dinoflagellates (as opposed to N-replete conditions). Bunse et
311 al. (2016) indicated how bacterial gene expression responses to high pCO_2 could also be dependant
312 on nutrient availability, potentially affecting their growth efficiency. Furthermore, N depletion,
313 together with an excess of carbon, causes an increase in the production of polysaccharides by
314 phytoplankton, particularly the production of transparent exopolymer particles (TEP) (Engel *et al.*,
315 2014, MacGilchrist *et al.*, 2014). TEPs are not constrained by stoichiometric ratios and are rich in
316 carbon (Passow, 2002). Bacteria could benefit from these and other exudates. Lignell (1990) already
317 showed that bacterial productivity was significantly correlated with primary productivity, whereas
318 its correlation with algal biomass was weak. In fact, bacterial production relies strongly on
319 phytoplankton exudates in marine coastal waters (Fouilland *et al.*, 2014). These higher bacterial

biomasses could have led to higher ciliate abundances, and, especially to an increase in small-sized, bacterivore ciliates. At the same time, we see a negative response of two groups (cryptophytes and dinoflagellates) that, in general, can switch between autotrophic and heterotrophic feeding modes. One potential explanation for this negative response could be that under nutrient limitation, these groups rely more on heterotrophic feeding and, as they are competitors of the ciliates for bacterial prey (Jones 2000, Tarangkoon, 2010), will suffer under conditions of higher ciliate abundance (see also theory on the effects of enrichment in an intraguild predation system, Shchekinova et al., 2014).

When inorganic nutrients are replete, phytoplankton may produce less exudates, with as a result lower bacterial production. At times of low bacterial abundance some small-sized ciliate species could be negatively affected by low bacterial standing stocks thus reducing the number of bacteria predators. At the same time, dinoflagellates and cryptophytes will strongly rely on autotrophy, and hence benefit from the fertilization effects on photosynthesis of additional CO_2. These are potential explanations of what has happened but they are to a certain extent speculative. The most critical uncertainty is the mixotrophs feeding mode. Unfortunately, the feeding modes of these organisms was not considered during the experiments. Moreover, other important factors (i.e. viral lysis), have not been considered although they could have important effects on the observed food web interactions. Viral lysis can be one of the main losses for bacterial communities (Fuhrman *et al.*, 2015). Viral lysis of phytoplankton has previously been reported as an important source of organic matter for heterotrophic bacteria (Hornick et al. 2016), and it responded positively to pCO_2 in some of these experiments (Crawfurd et al. 2016, Brussaard et al. 2013). The relative importance of grazing and viral lysis under OA could have important implications on the energy flow through the microbial foodweb (Fuhrman *et al.*, 2015). Therefore pCO_2 effects on viral lysis could be partly responsible for some of the community effects presented in this study.

344 The line of reasoning presented here opens up a whole new venue of potential research: that
345 depending on the nutrient conditions OA could lead to a changes in mixotrophic and heterotrophic
346 microorganisms. So, how would these changes under nutrient limited OA conditions affect higher
347 trophic levels? In contrast to adult copepods, copepodites feed relatively more on the pico-size
348 range (Brucet *et al*., 2008). Therefore, they can be expected to benefit from the higher abundances
349 of picoautotrophs under OA. In contrast, carbonate system changes caused by increased pCO_2 will
350 affect the C/N/P stoichiometry of phytoplankton (Schoo *et al.*, 2013), and therefore their nutritional
351 value. Ciliate prey, on the contrary, will trophically upgrade poor stoichiometric autotrophic food
352 quality for copepod and higher trophic levels (Golz *et al.*, 2015). Hence, from this study it becomes
353 clear that nutrient conditions play an important role in how plankton communities react to OA.
354 Whether different responses are triggered by differences in absolute nutrient concentrations or
355 stoichiometric changes, identical communities will reorganise differently under different nutrient
356 scenarios. We suggest complex changes in food-web interactions, caused by taxon-specific
357 mixoautotroph OA responses, as a plausible explanation for the observed differences in both
358 mixoautotroph and heterotroph responses. Further research is needed to assert the actual role of
359 predation/competition feedbacks between micro-heterotrophs and mixoautotrophs, and the relative
360 importance of viral lysis and grazing, as this could bring new light to the mechanisms causing the
361 reorganization of plankton communities under OA.
362
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368

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