1   **Alterations in microbial community composition with increasing fCO₂: a mesocosm study in the**

2   **eastern Baltic Sea**

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Abstract

Ocean acidification, resulting from the uptake of anthropogenic carbon dioxide (CO_2) by the ocean, is considered a major threat to marine ecosystems. Here we examined effects of ocean acidification on microbial community dynamics in the eastern Baltic Sea, during the summer of 2012 when inorganic nitrogen and phosphorus were strongly depleted. Large volume in situ mesocosms were employed to mimic present, future and far future CO_2 scenarios. All six groups of phytoplankton enumerated by flow cytometry (<20 μm cell diameter) showed distinct trends in net growth and abundance with CO_2 enrichment. The picoeukaryotic phytoplankton groups Pico-I and II displayed enhanced abundances, whilst Pico-III, *Synechococcus* and the nanoeukaryotic phytoplankton groups were negatively affected by elevated fugacity of CO_2 ($f$$CO_2$). Specifically, the numerically dominant eukaryote, Pico-I, demonstrated increases in gross growth rate with increasing $f$$CO_2$ sufficient to double its abundance. Dynamics of the prokaryote community closely followed trends in total algal biomass despite differential effects of $f$$CO_2$ on algal groups. Similarly, viral abundances corresponded to prokaryotic host population dynamics. Viral lysis and grazing were both important in controlling microbial abundances. Overall our results point to a shift, with increasing $f$$CO_2$, towards a more regenerative system with production dominated by small picoeukaryotic phytoplankton.

1 Introduction

Marine phytoplankton are responsible for approximately half of global primary production (Field et al., 1998), with shelf sea communities contributing an average 15-30 % (Kulinski and Pempkowiak, 2011). Since the industrial revolution atmospheric carbon dioxide (CO_2) concentrations have increased by nearly 40 % due to anthropogenic emissions, primarily caused by the burning of fossil fuels and deforestation (Doney et al., 2009). Atmospheric CO_2 dissolves in the oceans where it forms carbonic acid which reduces seawater pH, a process commonly termed, ocean acidification (OA).

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53   Currently, along with warming sea surface temperatures and changing light and nutrient conditions,

54   marine ecosystems face unprecedented decreases in ocean pH (Doney et al., 2009; Gruber, 2011).

55   Ocean acidification is considered one of the greatest current threats to marine ecosystems (Turley

56   and Boot, 2010) and has been shown to alter phytoplankton primary production with the direction

57   and magnitude of the responses dependent on community composition (eg. Hein and Sand-Jensen,

58   1997; Tortell et al., 2002; Leonardos and Geider, 2005; Engel et al., 2007; Feng et al., 2009; Eberlein

59   et al., 2017). Certain cyanobacteria, including diazotrophs, demonstrate stimulated growth under

60   conditions of elevated CO_2 (Qiu and Gao, 2002; Barcelos e Ramos et al., 2007; Hutchins, 2007;

61   Dutkiewicz et al., 2015). However, no consistent trends have been found for *Synechococcus* (Schulz

62   et al., 2017 and references therein). The responses of diatoms and coccolithophores also appear

63   more variable (Dutkiewicz et al., 2015 and references therein), although coccolithophore calcification

64   seems generally negatively impacted (Meyer and Riebesell, 2015; Riebesell et al., 2017). OA has also

65   been reported to increase the abundances of small-sized photoautotrophic eukaryotes in mesocosm

66   experiments (Engel et al., 2007; Meakin and Wyman, 2011; Brussaard et al., 2013; Schulz et al.,

67   2017).

68   Recently, data regarding the effects of OA on taxa-specific phytoplankton growth rates were

69   incorporated into a global ecosystem model. The results emphasized that elevated CO_2

70   concentrations can cause changes in community structure by altering the competitive fitness, and

71   thus competition between phytoplankton groups (Dutkiewicz et al., 2015). Moreover, OA was found

72   to have a greater impact on phytoplankton community size structure, function and biomass than

73   either warming or reduced nutrient supply (Dutkiewicz et al., 2015). Many OA studies have been

74   conducted using single-species under controlled laboratory conditions and therefore cannot account

75   for intrinsic community interactions that occur under natural conditions. Alternatively, larger-volume

76   mesocosm experiments allow for OA manipulation of natural communities and as such, are more

77   likely to capture and quantify the overall response of the natural ecosystems. To date, the majority of

78   these experiments started under replete nutrient conditions or received nutrient additions (Paul et

79   al., 2015 and references therein). Thus, little data is available for oligotrophic conditions, which are

80   present in ~75% of the world's oceans (Corno et al., 2007).

81   Whilst environmental factors such as temperature, light, nutrient and CO_2 concentrations regulate

82   gross primary production, loss factors determine the fate of this photosynthetically fixed carbon.

83   Grazing, sinking and viral lysis affect the cycling of elements in different manners, i.e. transferred to

84   higher trophic levels through grazing, carbon sequestration in deep waters and sediments, and

85   cellular content release by viral lysis (Wilhelm and Suttle, 1999; Brussaard et al., 2005). Released

86   detrital and dissolved organic matter (DOM) is quickly utilized by heterotrophic bacteria, thereby

87   stimulating activity within the microbial loop (Brussaard et al., 2008; Lønborg et al., 2013; Sheik et al.,

88   2014; Middelboe and Lyck, 2002). Consequently, bacteria may be affected indirectly by OA through

89   changes in the quality and/or quantity of DOM (Weinbauer et al., 2011). Viral lysis has been found to

90   be as important as microzooplankton grazing to the mortality of natural bacterio- and phytoplankton

91   (Weinbauer, 2004; Baudoux et al., 2006; Evans and Brussaard, 2012; Mojica et al., 2016). Thus far,

92   most studies examining the effects of OA on microzooplankton abundance and/or grazing have

93   found little or no direct effect (Suffrian et al., 2008; Rose et al., 2009; Aberle et al., 2013; Brussaard et

94   al., 2013; Niehoff et al., 2013). To our knowledge, no viral lysis rates have been reported for natural

95   phytoplankton communities under conditions of OA. A few studies have inferred rates based on

96   changes in viral abundances under enhanced CO_2, but the results are inconsistent (Larsen et al.,

97   2008; Brussaard et al., 2013). Therefore, the effect of OA on the relative share of these key loss

98   processes is still understudied for most ecosystems.

99   Here we report on the temporal dynamics of microbes (phytoplankton, prokaryotes and viruses)

100   under the influence of enhanced CO_2 concentrations in the low-salinity (around 5.7) Baltic Sea. Using

101   large mesocosms with in situ light and temperature conditions, the pelagic ecosystem was exposed

102   to a range of increasing CO_2 concentrations from ambient to future and far future concentrations.

103   The study was performed during the summer in the Baltic Sea near Tvärminne when conditions were

104   oligotrophic. Our data show, that over the 43 day long experiment, enhanced CO_2 concentrations

105 elicited distinct shifts in the microbial community, most notably an increase in the net growth of

106 small picoeukaryotic phytoplankton.

107

108 **2 Materials and Methods**

109 **2.1 Study site and experimental set-up**

110 The present study was conducted in the Tvärminne Storfjärden (59° 51.5' N, 23° 15.5' E) between 14

111 June and 7 August, 2012. Nine mesocosms, each enclosing ~55 m^3 of water, were moored in a square

112 arrangement at a site with a water depth of approximately 30 m. The mesocosms consisted of open

113 ended polyurethane bags 2 m in diameter and 18.5 m in length mounted onto floating frames

114 covered at each end with a 3 mm mesh. Initially, the mesocosms were kept open for 5 days to allow

115 for rinsing and water exchange while excluding large organisms from entering with the 3 mm mesh.

116 During this time, the bags were positioned such that the tops were submerged 0.5 m below the

117 water surface and the bottoms reached down to 17 m depth in the water column. Photosynthetically

118 active radiation (PAR) transparent plastic hoods (open on the side) prevented rain and bird droppings

119 from entering the mesocosms, which would affect salinity and nutrients, respectively. Five days

120 before the CO_2 treatment was to begin, the water column of the mesocosms was isolated from the

121 influence of the surrounding water. To do so, the 3 mm mesh was removed and sediment traps (2 m

122 long) were attached to close off the bottom of the mesocosms. The top ends of the bags were raised

123 and secured to the frame 1.5 m above the water surface to prevent water entering via wave action.

124 The mesocosms were then bubbled with compressed air for 3.5 min, to remove salinity gradients and

125 ensure that the water body was fully homogeneous.

126 The present manuscript includes results from six of the original mesocosms, due to the unfortunate

127 loss of three mesocosms which were compromised by leakage. The mean fugacity of CO_2 (fCO_2)

128 during the experiment, i.e. days 1-43, for the individual mesocosms were as follows: M1, 365 µatm;

129 M3, 1007 µatm; M5, 368 µatm; M6, 821 µatm; M7, 497 µatm; M8, 1231 µatm (Table 1). The gradient

130 of non-replicated fCO_2 of the present study (as opposed to a smaller number of replicated treatment

levels) was selected as a balance between the necessary, but manageable, number of mesocosms and minimizing the impact of the high potential for loss of mesocosms to successfully address the underlying questions of the study (Schulz et al., 2013). Moreover, it maximizes the potential of identifying a threshold fCO_2 level concentration, if present (by allowing for a larger number of treatment levels). Carbon dioxide manipulation was carried out in four steps and took place between days 0 to 4 until the target fCO_2 was reached. Initial fCO_2 was 240 µatm. For fCO_2 manipulations, 50 µm filtered natural seawater was saturated with CO_2 and then injected evenly throughout the depth of the mesocosms as described by Riebesell et al. (2013). Two mesocosms functioned as controls and were treated in a similar manner using only filtered seawater. On day 15, a supplementary fCO_2 addition was made to the top 7 m of mesocosms numbered 3, 6, and 8 to replace CO_2 lost due to outgassing (Paul et al., 2015; Spilling et al., 2016). Throughout this study we refer to fCO_2 which accounts for the non-ideal behavior of CO_2 gas and is considered the standard measurement required for gas exchange (Pfeil et al., 2012).

Initial nutrient concentrations were 0.05 µmol L^{-1}, 0.15 µmol L^{-1}, 6.2 µmol L^{-1} and 0.2 µmol L^{-1} for nitrate, phosphate, silicate and ammonium, respectively. Nutrient concentrations remained low for the duration of the experiment (Paul et al., 2015, this issue) and no nutrients were added. Salinity was relatively constant around 5.7. Temperature was more variable; on average temperature within the mesocosms (0-17 m) increased from ~8 °C to a maximum on day 15 of ~15 °C and then decreased again to ~8 °C by day 30. For further details of the experimental set-up, carbonate chemistry dynamics and nutrient concentrations throughout the experiment we refer to the general overview paper by Paul et al. (2015).

Collective sampling was performed every morning using depth integrated water samplers (IWS, HYDRO-BIOS, Kiel). These sampling devices were gently lowered through the water column collecting ~5 L of water gradually between 0-10 m (top) or 0-17 m (whole water column). Water was collected from all mesocosms and the surrounding water. Subsamples were obtained for enumeration of phytoplankton, prokaryotes and viruses. Samples for viral lysis and grazing experiments were taken

157 from 5 m depth using a gentle vacuum-driven pump system. Samples were protected against sunlight

158 and warming by thick black plastic bags containing wet ice. Samples were processed at in situ

159 temperature (representative of 5 m depth) under dim light and handled using nitrile gloves. As viral

160 lysis and grazing rates were determined from samples taken from 5 m depth, samples for microbial

161 abundances reported here were taken from the top 10 m integrated samples.

162 The experimental period has been divided into four phases based on major physical and biological

163 changes (Paul et al., 2015): Phase 0 before CO_2 addition (days -5 to 0), Phase I (days 1-16), Phase II

164 (days 17-30) and Phase III (days 31-43). Throughout this manuscript the data are presented using

165 three colors (blue, grey and red), representing low (mesocosms M1 and M5) intermediate (M6 and

166 M7) and high (M3 and M8) fCO$_2$ levels (Table 1).

167

168 **2.2 Microbial abundances**

169 Microbes were enumerated using a Becton Dickinson FACSCalibur flow cytometer (FCM) equipped

170 with a 488 nm argon laser. The samples were stored on wet ice and in the dark until counting. The

171 photoautotrophic cells (<20 μm) were counted directly using fresh seawater and were discriminated

172 by their autofluorescent pigments. Six phytoplankton clusters were differentiated based on the

173 bivariant plots of either chlorophyll (red autofluorescence) or phycoerythrin (orange

174 autofluorescence, for *Synechococcus* and Pico-III) against side scatter. The size of the different

175 phytoplankton clusters was determined by gentle filtration through 25 mm diameter polycarbonate

176 filters (Whatman) with a range of pore sizes (12, 10, 8, 5, 3, 2, 1, and 0.8 μm) according to Veldhuis

177 and Kraay (2004). Average cell sizes for the different phytoplankton groups were 1, 1, 3, 2.9, 5.2, and

178 8.8 μm diameter for the prokaryotic cyanobacteria *Synechococcus* spp. (SYN), picoeukaryotic

179 phytoplankton I, II and III (Pico-I-III), and nanoeukaryotic phytoplankton I, and II (Nano-I, II),

180 respectively. Pico-III was discriminated from Pico-II (comparable average cell size) by a higher orange

181 autofluorescence signature, potentially representing small-sized cryptophytes (Klaveness, 1989);

182 alternatively large single cells or microcolonies of *Synechococcus* (Haverkamp et al., 2009). The

183 cyanobacterial species *Prochlorococcus* spp. were not observed during this experiment. Counts were

184 converted to cellular carbon by assuming a spherical shape equivalent to the average cell diameters

185 determined from size fractionations and applying conversion factors of 237 fg C μm^{-3} (Worden et al.,

186 2004) and 196.5 fg C μm^{-3} (Garrison et al., 2000) for pico- and nano-sized plankton, respectively.

187 Microbial net growth and loss rates were derived from exponential regressions of changes in the cell

188 abundances over time.

189 Abundances of prokaryotes and viruses were determined from 0.5 % glutaraldehyde fixed, flash

190 frozen (-80 °C) samples according to Marie et al. (1999) and Brussaard (2004), respectively. The

191 prokaryotes include heterotrophic bacteria, archaea and unicellular cyanobacteria, the latter

192 accounting for maximal 10 % of the total abundance in our samples, as indicated by their

193 autofluorescence. Briefly, thawed samples were diluted with sterile autoclaved Tris-EDTA buffer (10

194 mM Tris-HCl and 1 mM EDTA, pH 8.2; Mojica et al., 2014) and stained with the green fluorescent

195 nucleic acid-specific dye SYBR-Green I (Invitrogen Inc.) to a final concentration of the commercial

196 stock of 1.0×10^{-4} (for prokaryotes) or 0.5×10^{-4} (for viruses). Virus samples were stained at 80 °C for

197 10 min and then allowed to cool for 5 min at room temperature in the dark. Prokaryotes were

198 stained for 15 min at room temperature in the dark (Brussaard, 2004). Prokaryotes and viruses were

199 discriminated in bivariate scatter plots of green fluorescence versus side scatter. Final counts were

200 corrected for blanks prepared and analyzed in a similar manner as the samples. Two groups of

201 prokaryotes were identified by their stained nucleic acid fluorescence, referred here on as low (LNA)

202 and high (HNA) fluorescence prokaryotes.

203

2.3 Viral lysis and grazing

205 Microzooplankton grazing and viral lysis of phytoplankton was determined using the modified

206 dilution assay, based on reducing grazing and viral lysis mortality pressure in a serial manner allowing

207 for increased phytoplankton growth (over the incubation period) with dilution (Mojica et al., 2016).

208 Briefly, two dilution series were created in clear 1.2 L polycarbonate bottles by gently mixing 200 μm

209   sieved whole seawater with either 0.45 μm filtered seawater (i.e. microzooplankton grazers

210   removed) or 30 KDa filtered seawater (i.e. grazers and viruses removed) to final dilutions of 20, 40,

211   70 and 100 %. The 0.45 μm filtrate was produced by gravity filtration of 200 μm mesh sieved

212   seawater through a 0.45 μm Sartopore capsule filter. The 30 KDa ultrafiltrate was produced by

213   tangential flow filtration of 200 μm pre-sieved seawater using a 30 kDa Vivaflow 200 PES membrane

214   tangential flow cartridge (Vivascience). All treatments were performed in triplicate. Bottles were

215   suspended next to the mesocosms in small cages at 5 m depth for 24 hours. Subsamples were taken

216   at 0 and 24 h, and phytoplankton abundances of the grazing series (0.45 μm diluent) were

217   enumerated by flow cytometry. Due to time constraint, the majority of the samples of the 30 kDa

218   series were fixed with 1 % (final concentration) formaldehyde : hexamine solution (18 % v/v : 10 %

219   w/v), for 30 min at 4 °C, flash frozen in liquid nitrogen and stored at −80 °C until flow cytometry

220   analysis in the home laboratory. Fixation had no significant effect (student's t-tests, p-value >0.05) as

221   tested periodically against fresh samples. The modified dilution assay was only run for Mesocosms 1

222   (low fCO$_2$) and 3 (high fCO$_2$) due to the logistics of handling times. Experiments were performed until

223   day 31. Grazing rates and the combined rate of grazing and viral lysis were estimated from the slope

224   of a regression of phytoplankton apparent growth versus dilution of the 0.45 μm and 30 kDa series,

225   respectively. A significant difference between the two regression coefficients (as tested by analysis of

226   covariance) indicated a significant viral lysis rate. Phytoplankton gross growth rate, in the absence of

227   grazing and viral lysis, was derived from the y-intercept of the 30 kDa series regression. Similarly,

228   significant differences between mesocosms M1 and M3 (low and high fCO$_2$) were determined

229   through analysis of covariance of the dilution series for the two mesocosms. A significance threshold

230   of 0.05 was used and significance is denoted throughout the manuscript by an asterisk (*).

231   Occasionally, the regression of apparent growth rate versus fraction of natural water resulted in a

232   positive slope (thus no reduction in mortality with dilution). In addition, very low phytoplankton

233   abundances can also prohibit statistical significance of results. Under such conditions dilution

234   experiments were deemed unsuccessful (see for limitations of the modified dilution method,

235   Baudoux et al., 2006; Kimmance and Brussaard, 2010; Stoecker et al., 2015).

236   Viral lysis of prokaryotes was determined according to the viral production assay (Wilhelm et al.,

237   2002; Winget et al., 2005). After reduction of the natural virus concentration, new virus production

238   by the natural bacterial community is sampled and tracked over time (24 h). Briefly, free viruses were

239   reduced from a 300 ml sample of whole water by re-circulation over a 0.2 µm pore size polyether

240   sulfone membrane (PES) tangential flow filter (Vivaflow 50, Vivascience) at a filtrate expulsion rate of

241   40 ml min^{-1}. The concentrated sample was then reconstituted to the original volume using virus-free

242   seawater. This process was repeated a total of three times to gradually wash away viruses. After the

243   final reconstitution, 50 ml aliquots were distributed into six polycarbonate tubes. Mitomycin C

244   (Sigma-Aldrich) (final concentration, 1 µg ml^{-1}, maintained at 4 °C), which induces lysogenic bacteria

245   (Weinbauer and Suttle, 1996) was added to a second series of triplicate samples for each mesocosm.

246   A third series of incubations with 0.2 µm filtered samples was used as a control for viral loss (e.g.

247   viruses adhering to the tube walls) and showed no significant loss of free viruses during the

248   incubations. At the start of the experiment, 1 ml subsamples were immediately removed from each

249   tube and fixed as previously described for viral and bacterial abundance. The samples were dark

250   incubated at in situ temperature and 1 ml subsamples were taken at 3 h, 6 h, 9 h, 12 h and 24 h.

251   Virus production was determined from linear regression of viral abundance over time. Viral

252   production due to induction of lysogeny was calculated as the difference between production in the

253   unamended samples and production of samples to which mitomycin C was added. Although

254   mortality experiments were initially planned to be employed for mesocosms 1, 2, and 3 representing

255   low, mid and high fCO$_2$ conditions, mesocosm 2 was compromised due to leakage. Additionally, due

256   to logistical reasons assays were only performed until day 21.

257   To determine grazing rates on prokaryotes, fluorescently labeled bacteria (FLBs) were prepared from

258   enriched natural bacterial assemblages (originating from the North Sea) labeled with 5-([4,6-

259   Dichlorotriazin-2-yl]amino) fluorescein (DTAF, 36565 Sigma-Aldrich 40 µg ml^{-1}) according to Sherr et

260 al. (1993). Frozen ampoules of FLB (1-5 % of total bacterial abundance) were added to triplicate 1 L

261 incubation bottles containing whole water gently passed through 200 μm mesh. Twenty ml samples

262 were taken immediately after addition (0 h) and the headspace was removed by gently squeezing air

263 from the bottle. The 1 L bottles were incubated on a slow turning wheel (1 rpm) at in situ light and

264 temperature conditions (representative of 5 m depth) for 24 h. Sampling was repeated after 24 h. All

265 samples were fixed to a 1 % final concentration of gluteraldehyde (0.2 μm filtered; 25 % EM-grade),

266 stained (in the dark for 30 min at 4 °C) with 4',6-Diamidino-2-Phenylindole, Dihydrochloride (DAPI)

267 solution (0.2 μm filtered; Acrodisc ®25 mm Syringe filters, PALL Life Sciences; 2 μg ml^{-1} final

268 concentration; Sherr et al., 1993) and filtered onto 25 mm, 0.2 μm black polycarbonate filters (GE

269 Healthcare life sciences). Filters were then mounted on microscopic slides and stored at -20 °C until

270 analysis. FLBs present on a ~0.75 mm^2 area were counted using a Zeiss Axioplan 2 microscope.

271 Grazing (μ d^{-1}) was measured according to $N_{T24} = N_{T0} * e^{-\mu t}$, where N_{T24} and N_{T0} are the number of FLBs

272 present at 24 h and 0 h, respectively.

273

274 **2.4 Statistics**

275 Non-metric multidimensional scaling (NMDS) was used to follow microbial community development

276 in each mesocosm over the experimental period. NMDS is an ordination technique which represents

277 the dissimilarities obtained from an abundance data matrix in a *2*-dimensional space (Legendre and

278 Legendre, 1998). In this case, the data matrix was comprised of abundance data for each

279 phytoplankton group in each mesocosm for every day of sampling. The treatment effect was

280 assessed by analysis of similarity (ANOSIM; Clarke, 1993) and inspection of the NMDS biplot. ANOSIM

281 compares the mean of ranked dissimilarities of mesocosms between fCO$_2$ treatments (low: 1, 5, 7;

282 high: 6, 3, 8) to the mean of ranked dissimilarities within treatments per phase. The NMDS plots

283 allowed divergence periods in the development and community composition between treatments to

284 be visually assessed (period 1 from day 3-13 and period 2 from days 16-24). Net growth rates of each

285 of the different microbial groups were calculated for these identified divergence periods.

286   Relationships between net growth rates and peak cell abundances with $f\mathrm{CO_2}$ were evaluated by

287   linear regression against the average $f\mathrm{CO_2}$ per mesocosm during each period or peak day. A

288   generalized linear model was used to test the relationship between prokaryote abundance and

289   carbon biomass with an ARMA correlation structure of order 3 to account for temporal

290   autocorrelation. The model fulfilled all assumptions such as homoscedasticity and avoiding

291   autocorrelation of the residuals (Zuur et al., 2007). A significance threshold of $p \leq 0.05$ was used and

292   significance is denoted by an asterisk (*). All analyses were performed using the statistical software

293   program R, using packages nlme (Pinheiro et al., 2017) and vegan (Oksanen et al., 2017) (R core

294   Team, 2017). Where average of low and high mesocosm abundance data are reported, values

295   represent the average of mesocosms 1, 5, 7 (mean $f\mathrm{CO_2}$ 365-497 µatm) and 6, 3, 8 (821-1231 µatm),

296   respectively.

297

298   **3 Results**

299   **3.1 Total phytoplankton dynamics in response to CO_2 enrichment**

300   During Phase 0, low variability in phytoplankton abundances in the different mesocosms (1.5 ± 0.05 x

301   10^5 ml^{-1}) indicated good replicability of initial conditions prior to CO_2 manipulation (Fig. 1). This was

302   further supported by the high similarity between microbial communities in the different mesocosms

303   as indicated by the tight clustering of points in the NMDS plot during this period (Fig. 2). During

304   Phase 0, the phytoplankton community (<20 µm) was dominated by pico-sized autotrophs, with the

305   prokaryotic cyanobacteria *Synechococcus* (SYN) and Pico-I accounting for 69 % and 27 % of total

306   phytoplankton abundance, respectively. After CO_2 addition, there were two primary peaks in

307   phytoplankton, which occurred on day 4 in Phase I and day 24 in Phase II (Fig. 1a). The phytoplankton

308   community became significantly different over time in the different treatments (ANOSIM, p=0.01,

309   Fig. 2). Two periods were identified based on their divergence (Fig.2), the first (NMDS-based period

310   1) followed the initial peak in abundance (days 3-13) with highest abundances occurring in the

311   elevated CO_2 mesocosms (Fig. 1a). During the second period (NMDS-based period 2, days 16-24),

312   abundances were higher in the low fCO$_2$ mesocosms (Fig. 1a). In general the NMDS plot shows that

313   throughout the experiment, mesocosm M1 followed the same basic trajectory as mesocosms M5 and

314   M7, whilst mesocosm M3 followed M6 and M8 (Fig. 2). Thus, the two mesocosms (representing high

315   and low fCO$_2$ treatments) deviated from each other during Phase I and were clearly separated during

316   Phases II and III (Fig. 2).

317   Phytoplankton abundances in the surrounding water started to differ from the mesocosms during

318   Phase 0 (on average 44 % lower) which was primarily due to lower abundances of SYN. This effect

319   was seen from day -1, prior to CO$_2$ addition but following bubbling with compressed air (day -5). On

320   day 15, a deep mixing event occurred as a result of storm conditions (with consequent alterations in

321   temperature and salinity) and as a result phytoplankton abundances in the surrounding open water

322   diverged more strongly from the mesocosms but remained similar in their dynamics (Fig. 3).

323   Microbial abundances in the 0-17 m samples were slightly lower but showed very similar dynamics to

324   those in the 0-10 m samples (Fig. S1).

325

326   **3.1.1 *Synechococcus***

327   The prokaryotic cyanobacteria *Synechococcus* (SYN) accounted for the majority of total abundance,

328   i.e. 74 % averaged across all mesocosms over the experimental period. Abundances of SYN showed

329   distinct variability between the different CO$_2$ treatments, starting on day 7, with the low CO$_2$

330   mesocosms exhibiting nearly 20 % lower abundances between days 11-15 as compared to high fCO$_2$

331   mesocosms (Fig. 3a). SYN net growth rates during days 3-13 (NMDS-based period 1) were positively

332   correlated with CO$_2$ (p=0.10, R^2=0.53; Table 2, Fig. S2a). One explanation for higher net growth rates

333   at elevated CO$_2$ could be the significantly (p<0.05) higher grazing rate in the low fCO$_2$ mesocosm M1

334   (0.56 d^{-1}) compared to the high fCO$_2$ M3 (0.27 d^{-1}) as measured on day 10 (Fig. 4a). After day 16, SYN

335   abundances increased in all mesocosms and during this period (days 16-24) net growth rates had a

336   significant negative correlation to fCO$_2$ (p=0.05, R^2= 0.63; Figs. 3a, Table 2 and Fig. S3a).

337   Consequently, the net increase in SYN abundances during this period was on average 20 % higher at

338 low fCO$_2$ compared to high fCO$_2$. This corresponded to higher total loss rates in high fCO$_2$ treatments

339 measured on day 17 (0.33 vs 0.17 d^{-1} for M3 and M1, respectively; Fig. 4a). The higher net growth

340 most likely led to the peak in SYN abundance observed on day 24 (max. 4.7 x 10^5 ml^{-1}), which was

341 negatively correlated with fCO$_2$ (p=0.01, R^2=0.80; Table 3, Fig. S4a). After this period (days 24-28),

342 SYN abundances declined at comparable rates in the different mesocosms, irrespective of fCO$_2$ (Fig.

343 3a). Abundances in the low fCO$_2$ mesocosms remained higher into Phase III (Fig. 3a). SYN abundances

344 in the surrounding water were generally lower than in the mesocosms, with the exception of days

345 17-21.

346

347 **3.1.2 Picoeukaryotes**

348 In contrast to the prokaryotic photoautotrophs, the eukaryotic phytoplankton community showed a

349 strong positive response to elevated fCO$_2$ (Fig. 1b). Pico-I was the numerically dominant group of

350 eukaryotic phytoplankton, accounting for an average 21-26 % of total phytoplankton abundances.

351 Net growth rates leading up to the first peak in abundance (from day 1 to 5) had a strong positive

352 correlation with fCO$_2$ (p<0.01, R^2=0.90; Fig. 3b, Table 3, Fig. S5a). Accordingly, the peak on day 5

353 (max. 1.1 x 10^5 ml^{-1}; Fig. 3b) was also correlated positively with fCO$_2$ (p=0.01, R^2=0.81; Table 3, Fig.

354 S4b). During Phase I, from days 3-13 (i.e. NMDS-based period 1), net growth rates of Pico-I remained

355 positively correlated to CO$_2$ concentration (p=0.01, R^2=0.80; Table 2, Fig. S2b). However, during this

356 period there was also a decline in abundance (days 5-9; p<0.01, R^2=0.89; Table 3, Fig. S5b) with 23 %

357 more cells lost in the low fCO$_2$ mesocosms. Accordingly, following this period, gross growth rate was

358 significantly higher in the high fCO$_2$ mesocosm M3 as compared to the low fCO$_2$ mesocosm M1 (day

359 10, p<0.05; Fig. 4b). Pico-I abundances in the surrounding open water started to deviate from the

360 mesocosms after day 10, and were on average around half that of the low fCO$_2$ mesocosms (Fig. 3b).

361 Following a brief increase (occurring between days 11-13) correlated to fCO$_2$ (p<0.01, R^2=0.94; Table

362 3, Fig. S4c), abundances declined sharply between days 13-16 (Fig. 3b), coinciding with a significantly

363 higher total mortality rate in the high fCO$_2$ mesocosm M3 (day 13; Fig. 4b). Viral lysis was a

14

364 substantial loss factor relative to grazing, for this group, comprising an average 45 % and 70 % of

365 total losses in M1 and M3, respectively (Table S1). During NMDS-based period 2, net growth rates of

366 Pico-I were significantly higher at high fCO$_2$ (p=0.05, R^2=0.64; Table 2, Fig S3b). By day 21,

367 abundances in the high fCO$_2$ mesocosms were (on average) ~2-fold higher than at low fCO$_2$

368 (maximum abundances 8.7 x 10^4 ml^{-1} and 5.9 x 10^4 ml^{-1} for high and low fCO$_2$ mesocosms; p=0.01,

369 R^2=0.84; Table 3, Fig. S4d). Standing stock of Pico-I remained high in the elevated fCO$_2$ mesocosms

370 for the remainder of the experiment (7.9 x 10^4 vs 4.3 x 10^4 ml^{-1} on average for high and low fCO$_2$

371 mesocosms, respectively; Fig. 3b). Additionally, gross growth rates during this final period were

372 relatively low (0.14 and 0.16 d^{-1} in M1 and M3, respectively) and comparable to total loss rates

373 (averaging 0.13 and 0.10 d^{-1} over days 25-31, for M1 and M3, respectively; Fig. 4b).

374 Another pico-eukaryote group, Pico-II, slowly increased in abundance until day 13, when it increased

375 more rapidly (Fig. 3c). Gross growth rates measured during Phase I were high (0.69 and 0.72 d^{-1} on

376 average in the low and high fCO$_2$ mesocosms M1 and M3, respectively; Fig. 4c), and comparable to

377 loss processes (0.46 and 0.58 d^{-1}), indicative of a relatively high turnover rate of production. Overall

378 net growth rates during days 3-13 (NMDS-based period 1) did not correlate to CO$_2$ (p=0.52, R^2=0.11;

379 Table 2, Fig. S2c). However, during periods of rapid increases in net growth, abundances were

380 positively correlated to CO$_2$ concentration (days 12-17; p=0.01, R^2=0.82; Table 3, Fig. S5c).

381 Accordingly, the peak in abundances of Pico-II on day 17 displayed a distinct positive correlation with

382 fCO$_2$ (p<0.01, R^2=0.93; Table 3, Fig. S4e), with maximum abundances of 4.6 x 10^3 ml^{-1} and 3.4 x 10^3

383 ml^{-1} for the high and low fCO$_2$ mecosoms, respectively (Fig. 3c). In M8 (the highest fCO$_2$ mesocosm),

384 abundances increased for an extra day with the peak occurring on day 18, resulting in an average 23

385 % higher abundances. During the decline of the Pico-II peak (days 16-24), net growth rates were

386 negatively correlated with fCO$_2$ (p=0.10, R^2=0.52; Table 2, Fig S3c). Moreover, the rate of decline was

387 faster for the high fCO$_2$ mesocosms during days 18-21 (p<0.01, R^2=0.85). The Pico-II abundances in

388 the surrounding water were comparable to the mesocosms during Phases 0 and I, lower during

389 Phase II and higher during Phase III (Fig. 3c).

390 Pico-III exhibited a short initial increase in abundances in the low fCO$_2$ treatments, resulting in nearly

391 2-fold higher abundances at low fCO$_2$ by day 3 compared to the high fCO$_2$ treatment (Fig. 3d). After

392 this initial period, net growth rates of this group had a significant positive correlation with fCO$_2$ (days

393 3-13; p=0.04, R^2=0.67; Table 2, Fig. S2d). In general, during Phase I gross growth (p<0.01, days 1, 3,

394 10; Fig. 4d) and total mortality (p<0.05, days 1, 6, 10; Fig. 4d) were significantly higher in the low fCO$_2$

395 mesocosm M1, as compared to the high fCO$_2$ mesocosm M3 resulting in low net growth rates. During

396 Phase II (days 16-24, NMDS-based period 2) the opposite occurred; i.e. net growth rates were

397 negatively correlated with fCO$_2$ (p<0.01, R^2=0.86; Table 2, Fig S.3d). Maximum Pico-III abundances

398 (day 24: 4.2 x 10^3 and 8.3 x 10^3 ml^{-1} for high and low fCO$_2$) had a strong negative correlation with fCO$_2$

399 (p<0.01, R^2=0.91; Table 3, Fig. S4f). Pico-III abundances remained noticeably higher in the low fCO$_2$

400 mesocosms during Phases II and III (on average 80 %; Fig. 3d). Unfortunately, almost half of the

401 mortality assays in this second half of the experiment failed (see Materials and Methods), but the

402 successful assays suggest that losses were minor (<0.15 d^{-1}; Fig. 4d) and primarily due to grazing, as

403 no significant viral lysis was detected (Table S1).

404

405 **3.1.3 Nanoeukaryotes**

406 Nano-I showed maximum abundances (4.3 ± 0.4 x 10^2 ml^{-1}) on day 6 (except M1 which peaked on day

407 5), independent of fCO$_2$ (p=0.23, R^2=0.33; Fig. 3e). There was, however, a negative correlation of net

408 growth rate with fCO$_2$ during days 3-13 (NMDS-based period 1; p=0.01, R^2=0.79; Table 2, Fig. S2e). A

409 second major peak in abundance of Nano-I occurred on day 17, with markedly higher numbers in the

410 low fCO$_2$ mesocosms (4.1 x 10^2 ml^{-1} as compared to 2.4 x 10^2 ml^{-1} in high fCO$_2$ mesocosms; p=0.04,

411 R^2=0.67; Fig. 3e, Table 3 and Fig. S4g). Total loss rates in the high fCO$_2$ mesocosm M3 on days 6 and

412 10 were 2.3-fold higher compared to the low fCO$_2$ mescososm M1 (Fig. 4e), which may help to

413 explain this discrepancy in total abundance between low and high fCO$_2$ mesocosms. Viral lysis made

414 up to 98 % of total losses in the high fCO$_2$ mesocosm M3 during this period, whilst in M1 viral lysis

415 was only detected on day 13 (Table S1). Peak abundances (around 5.0 x 10^2 ml^{-1}) were much lower

416   compared to those in the surrounding waters (max ~2.4 x 10^3 ml^{-1}; Figs. 3e and S6a). During Phase II,

417   Nano-I abundances in the surrounding waters displayed rather erratic dynamics compared to those

418   of the mesocosms, but converged during certain periods (e.g. days 19-22). No significant relationship

419   was found between net loss rates and fCO$_2$ for the second NMDS-based period (p=0.26, R^2=0.30;

420   Table 2, Fig S.3e). At the end of Phase II, abundances were similar in all mesocosms but diverged

421   again during Phase III (days 31-39) due primarily to a negative effect of CO$_2$ on Nano-I abundances, as

422   depicted in the average 36 % reduction in Nano-I.

423   The temporal dynamics of Nano-II, the least abundant phytoplankton group analysed in our study,

424   displayed the largest variability (Fig. 3f), perhaps due to the spread of this cluster in flow cytographs

425   (which may indicate that this group represents several different phytoplankton species). No

426   significant relationship was found between net growth rate and fCO$_2$ for this group for the two

427   NMDS-based periods (Table 2, Figs S2f and S3f) nor with the peak in abundances on day 17 (p=0.13,

428   R^2=0.46; Fig. S4h). Moreover, no consistent trend was detected in mortality rates (Fig. 4f). Similar to

429   Nano-I, abundances in the surrounding water were often higher than in the mesocosms (max 3.5 x

430   10^2 ml^{-1} vs 1.1 x 10^4 ml^{-1}, respectively; Figs. 3f and S6b).

431

432   **3.1.4 Algal carbon biomass**

433   The mean combined biomass of Pico-I and Pico-II showed a strong positive correlation with fCO$_2$

434   throughout the experiment (p<0.05, R^2=0.95; Fig. 5a), an effect already noticeable by day 2. Their

435   biomass in the high fCO$_2$ mesocosms was, on average 11 % higher than in the low fCO$_2$ mesocosms

436   between days 10-20 and 20 % higher between days 20-39. Conversely, the remaining algal groups

437   showed an average 10 % reduction in carbon biomass at enhanced fCO$_2$ (days 3-39, the sum of SYN,

438   Pico-III, Nano-I and II ; p<0.01; Fig. 5b). The most notable response was found for the biomass of

439   Pico-III, which showed an immediate negative response to CO$_2$ addition (Fig. S7a) and remained, on

440   average, 29 % lower throughout the study period (days 2-39). For Nano-I and II the lower carbon

441   biomass only became apparent during the end of Phase I and beginning of Phase II (days 14-20; Fig.

442   S7b). Due to its small cell size, the numerically dominant SYN accounted for an average of 40 % of

443   total carbon biomass.

444

445   **3.2 Prokaryote and virus population dynamics**

446   Prokaryote abundance in the mesocosms was positively related to total algal biomass independent of

447   treatment ($p<0.05$, R^2=0.33; Fig. 8) and generally followed total algal biomass (Fig. S7c). The initial

448   increase in total prokaryote abundances occurred during the first few days following the closure of

449   the mesocosms (Fig. 6a). This was primarily due to increases in the HNA-prokaryote group (Fig. 6b)

450   which displayed higher net growth rates (0.22 d^{-1}) compared to the LNA-prokaryotes (0.14 d^{-1} on days

451   -3 to 3; Fig. 6c). A similar, albeit somewhat lower, increase was also recorded in the surrounding

452   waters (Fig. 6a). The decline of the first peak in prokaryote abundances coincided with the decay in

453   phytoplankton abundance/biomass (Figs. 1a and S7c). Concurrently the share of viral lysis increased,

454   representing 37-39 % of total mortality on day 11 (Fig. 7b). No measurable rates of lysogeny were

455   found for the prokaryotic community during the experimental period (all phases). From days 10 to 15

456   prokaryote dynamics (total, HNA and LNA) became noticeably affected by CO_2 concentration with a

457   significant positive correlation between net growth and fCO$_2$ during Phase I (days 3-13 NMDS-based

458   period 1; Table 2, Fig. S2 g and h). In the higher fCO$_2$ mesocosms, the decline in prokaryote

459   abundance occurring between days 13 and 16 (Fig. 6a) was largely (70 %) due to decreasing HNA-

460   prokaryote numbers (Fig. 6b). The grazing was 1.6-fold higher in the high fCO$_2$ mesocosm M3

461   compared to M1 (0.36 ± 0.13 and 0.14 ± 0.08 d^{-1} on day 14; Fig. 7a). At the same time, virus

462   abundance increased in the high fCO$_2$ mesocosms (Fig. 6d).

463   During Phase II, prokaryote abundances increased steadily until day 24 (for both HNA and LNA),

464   corresponding to increased algal biomass (Figs. 6 and S7c) and lowered grazing rates (Fig. 7a).

465   Specifically, during days 16-24 (NMDS-based period 2), the HNA-prokaryotes showed an average 10

466   % higher abundances in the low, as compared to the high fCO$_2$ mesocosms (Fig. 6b). However, a

467   significant negative correlation of net growth rates and fCO$_2$ was only found for LNA (Table 2, Fig S3g

468 and h). No significant differences in loss rates between M1 and M3 were found during Phase II

469 (p=0.22, 0.46 days 18 and 21 respectively; Fig. 7). Halfway through Phase II (day 24), the prokaryote

470 abundance in the surrounding water leveled off (Fig. 6a). Prokaryote abundance ultimately declined

471 during days 28-35 (Fig. 6a), whereby the net growth of LNA was again negatively correlated with

472 enhanced CO_2 (p=0.02, R^2=0.76; Table 2, Fig S3g). Unfortunately, no experimental data on grazing

473 and lysis of prokaryotes is present after day 25. However, viral abundances increased steadily at 2.2 x

474 10^6 d^{-1}, concomitant with a decline in prokaryote abundance (Fig. 6a and d). There was no significant

475 correlation between viral abundances and fCO$_2$ during Phases II and III (p=0.36, R^2=0.21).

476

477 **4 Discussion**

478 In most experimental mesocosm studies, nutrients have been added to stimulate phytoplankton

479 growth (Schulz et al., 2017) therefore little data exists for oligotrophic phytoplankton communities.

480 In this study, we describe the impact of increased fCO$_2$ on the brackish Baltic Sea microbial

481 community during summer (nutrient depleted; Paul et al., 2015). Small-sized phytoplankton

482 numerically dominated the autotrophic community, in particular SYN and Pico-I (both about 1 µm

483 cell diameter). Our results demonstrate variable effects of fCO$_2$ manipulation on temporal

484 phytoplankton dynamics, dependent on phytoplankton group. In particular, Pico-I and Pico-II showed

485 significant positive responses, whilst the abundances of Pico-III, SYN and Nano-I were negatively

486 influenced by elevated fCO$_2$. The impact of OA on the different groups was, at times, a direct

487 consequence of alterations in gross growth rate, whilst overall phytoplankton population dynamics

488 could be explained by the combination of growth and losses. OA effects on community composition

489 in these systems may have consequences on both the food web and biogeochemical cycling.

490

491 **Comparison with surrounding waters**

492 During Phase 0, the microbial assemblage showed good replicability between all mesocosms,

493 however they had already began to deviate from the community in the surrounding waters. This was

most likely a consequence of water movement altering the physical conditions and biological composition of the surrounding water body. The dynamic nature of water movement in this region has been shown to alter the entire phytoplankton community several times over within a few months, due to fluctuations in nutrient supply, advection, replacement/mixing of water masses and water temperature (Lips and Lips, 2010). Alternatively, effects of enclosure and the techniques (bubbling) used to ensure a homogenous water column may have stimulated SYN within the mesocosms, which has been found to occur in several mesocosm experiments (Paulino et al., 2008; Gazeau et al., 2017). By Phases II and III, the microbial abundances within the mesocosms were distinctly different from the surrounding waters, with generally fewer SYN and Pico-I, and more Nano-I and Nano-II. Our statistical analysis shows that during this time, there was little similarity between the surrounding waters and mesocosms regardless of the CO_2 treatment level. Thus, the deviations during this time were most likely due to an upwelling event in the archipelago (days 17-30; Paul et al., 2015). Cold, nutrient-rich deep water has been shown to upwell during summer, with profound positive influence on ecosystem productivity (Nômmann et al., 1991; Lehman and Myrberg, 2008). A relaxation from nutrient limitation in vertically stratified waters disproportionately favours larger-sized phytoplankton, due to their higher nutrient requirements and lower capacity to compete at low concentrations dictated by their lower surface to volume ratio (Raven, 1998; Veldhuis et al., 2005). Inside the mesocosms, which were isolated from upwelled nutrients, picoeukaryotes dominated similar to a stratified water column. Following this upwelling event, the pH of the surrounding waters dropped from 8.3 to 7.8, a level comparable to the highest CO_2 treatment (M8) on day 32 (Paul et al., 2015). Suggesting that other factors contributed to the observed differences between mesocosms and surrounding water, than can be accounted for by CO_2 concentration alone e.g. nutrients. Alternatively, the magnitude and source of mortality occurring in the surrounding water may have been altered, compared to within the mesocosms, after such an upwelling event. Although the grazer community in the surrounding waters was not studied during this campaign, it is likely that the grazing community was completely restructured during the upwelling event (Uitto et

520   al., 1997). It is nonetheless noteworthy that the phytoplankton groups with distinct responses to CO_2

521   enrichment (either positive or negative) in the low (ambient) fCO_2 mesocosms diverged from those in

522   the surrounding water before the upwelling event occurred.

523

524   **Phytoplankton dynamics**

525   *Synechococcus* showed significantly lower net growth rates and peak abundances at higher fCO_2.

526   Both in laboratory and mesoscosm experiments, *Synechococcus* has been reported to have diverse

527   responses to CO_2, with approximately equal accounts of positive (Lu et al., 2006; Schulz et al., 2017),

528   negative (Paulino et al., 2008; Hopkins et al., 2010; Traving et al., 2014,) and insignificant changes (Fu

529   et al., 2007; Lu et al., 2006) in net growth rate with fCO_2. This variable response is probably due, at

530   least in part, to the broad physiological and genetic diversity of this species. In the Gulf of Finland

531   alone, 46 different strains of *Synechococcus* were isolated in July 2004 (Haverkamp et al. 2009).

532   Direct effects on physiology have been implied from laboratory studies. One isolate, a phycoerythrin

533   rich strain of *Synechococcus* WH7803 (Traving et al., 2014) elicited a negative physiological effect on

534   the growth rate from increased CO_2. This was most likely a consequence of higher sensitivity to the

535   lower pH (Traving et al., 2014), and the cellular cost of maintaining pH homeostasis or conversely a

536   direct effect on protein export. Additionally, Lu et al. (2006) reported increased growth rates in a

537   cultured phycocyanin rich but not a phycoerythrin rich strain of *Synechococcus*, suggesting that

538   pigments may play some part in defining the direct physiological response within *Synechococcus*. In

539   addition, within natural communities (Paulino et al., 2008; Hopkins et al., 2010; Schulz et al., 2017)

540   variability can also arise from indirect effects such a altering competition with other picoplankton

541   (Paulino et al., 2008). The delay and dampened effect of fCO_2 on SYN abundances within our study

542   was more likely due to indirect effects arising from alterations in food web dynamics than to direct

543   impacts on the physiology of this species. Specifically, significant differences in grazing rates of SYN

544   between M1 and M3 (days 10 and 17, no significant lysis detected) could be responsible for the

545   differing dynamics between the mesocosms at the end of Phase I and beginning of Phase II.

546   The gross growth rates of Pico-I were significantly higher ($p<0.05$) at high fCO$_2$ compared to the low

547   CO$_2$ concentrations during the first 10 days of Phase I. Moreover, no differences were detected in the

548   measured loss rates, demonstrating that increases in Pico-I were the due to increases in growth

549   alone. The stimulation of Pico-I by elevated fCO$_2$ may be due to a stronger reliance on diffusive CO$_2$

550   entry compared to larger cells. Model simulations reveal that whilst near-cell CO$_2$/pH conditions are

551   close to those of the bulk water for cells <5 μm in diameter, they diverge as cell diameters increase

552   (Flynn et al., 2012). This is due to the size-dependent thickness of the diffusive boundary layer, which

553   determines the diffusional transport across the boundary layer and to the cell surface (Wolf-Gladrow

554   and Riebesell, 1997; Flynn et al., 2012). It is suggested that larger cells may be more able to cope

555   with fCO$_2$ variability as their carbon acquisition is more geared towards handling low CO$_2$

556   concentrations in their diffusive boundary layer, e.g. by means of active carbon acquisition and

557   bicarbonate utilization (Wolf-Gladrow and Riebesell, 1997; Flynn et al., 2012). Moreover, as the Baltic

558   Sea experiences particularly large seasonal fluctuations in pH and fCO$_2$ (Jansson et al., 2013) due to

559   the low buffering capacity of the waters, phytoplankton here are expected to have a higher degree of

560   physiological plasticity. Our results agree with previous mesocosm studies, which reported enhanced

561   abundances of picoeukaryotic phytoplankton (Brussaard et al., 2013; Davidson et al, 2016; Schulz et

562   al., 2017), and particularly the prasinophyte *Micromonas pusilla* at higher fCO$_2$ (Engel et al., 2007;

563   Meakin and Wyman, 2011). Furthermore, Schaum et al. (2012) found that 16 ecotypes of

564   *Ostreococus tauri* (another prasinophyte similar in size to Pico-I) increased in growth rate by 1.4-1.7

565   fold at 1,000 compared to 400 μatm fCO$_2$. All ecotypes increased their photosynthetic rates and

566   those with most plasticity (those most able to vary their photosynthetic rate in response to changes

567   in fCO$_2$) were more likely to increase in frequency within the community. It is possible that Pico-I cells

568   are adapted to a highly variable carbonate system regime and are able to increase their

569   photosynthetic rate when additional CO$_2$ is available. This ability would allow them to out-compete

570   other phytoplankton (e.g. nanoeukaryotes in this study) in an environment when nutrients are

571   scarce.

572   The net growth rates and peak abundances of Pico-II were also positively affected by fCO$_2$. Gross

573   growth rates were significantly higher at high fCO$_2$ on only two occasions (days 10 and 20) and were

574   accompanied by high total mortality rates. Pigment analysis suggests that both Pico-I and Pico-II are

575   chlorophytes (Paul et al., 2015) and as such may share a common evolutionary history (Schulz et al.,

576   2017); thus Pico-II may be stimulated by fCO$_2$ in a similar manner to Pico I. Chlorophytes are found in

577   high numbers at this site throughout the year (Kuosa, 1991), suggesting the ecological relevance of

578   Pico-I and Pico-II in this ecosystem. In addition, Pico-II bloomed exactly when Pico-I declined which

579   may suggest potential competitive exclusion.

580   Pico-III showed the most distinct and immediate response to CO$_2$ addition. The significant reduction

581   in gross growth rates observed during Phase I suggests a direct negative effect of CO$_2$ on the

582   physiology of these cells. For this group, the lower gross growth rates were matched by lower total

583   mortality rates with increased fCO$_2$. Although the mean cell size of Pico-III and Pico-II were

584   comparable (2.9 and 2.5 µm, respectively), they showed opposing responses to fCO$_2$ enrichment

585   (lower Pico-III abundances at high fCO$_2$). These differences may arise from taxonomic differences

586   between the two groups. Pico-III displayed relatively high phycoerythrin orange autofluorescence,

587   likely representing small-sized cryptophytes (Klaveness, 1989), although rod-shaped *Synechococcus*

588   up to 2.9 µm in length (isolated from this region; Haverkamp et al., 2009) or *Synechococcus*

589   microcolonies (often only two cells in the Baltic; Motwani and Gorokhove, 2013) cannot be excluded.

590   In agreement with Pico-III response to CO$_2$ enrichment, Hopkins et al. (2010) reported reduced

591   abundances of small cryptophytes under increased CO$_2$ in a mesocosm study in a Norwegian fjord

592   near Bergen.

593   Lastly, the two nanoeukaryotic phytoplankton groups also displayed a negative response to fCO$_2$

594   enrichment, whereby Nano-II was the least defined, most likely due to a high taxonomic diversity in

595   this group. Nano-I started to display lower abundances at high fCO$_2$ during Phase I (after day 10),

596   which was likely the result of greater differences between gross growth and total mortality

597   (compared to low fCO$_2$). Alternatively, enhanced nutrient competition due to increased abundances

598 of SYN and Pico-I (and later on also Pico-II) at elevated fCO$_2$ may also have contributed to the

599 dampened response of Nano-I in the high fCO$_2$ mesocosms. The overall decline in Nano-I, during

600 Phase II, and sustained low abundances during Phase III may well have been the result of grazing by

601 the increased mesozooplankton abundances during Phase II (Lischka et al., 2017).

602

603 **Microbial loop**

604 The strong association of prokaryote abundance with algal biomass, present throughout the

605 experiment, suggests that the effect of CO$_2$ was an indirect consequence of alterations in the

606 availability of phytoplankton carbon. Others have reported a tight coupling of autotrophic and

607 heterotrophic communities at this location, with an estimated 35 % of the total net primary

608 production being utilized directly by bacteria or heterotrophic flagellates (Kuosa and Kivi, 1989),

609 suggesting a highly efficient microbial loop in this ecosystem. In addition to phytoplankton exudation,

610 viral lysis may also contribute to the dissolved organic carbon pool (Wilhelm and Suttle, 1999;

611 Brussaard et al., 2005; Lønborg et al., 2013). We calculated that viral lysis of phytoplankton between

612 days 9 and 13 resulted in the release of 1.3 and 13.1 ng C ml^{-1} for M1 and M3, respectively. Assuming

613 a bacterial growth efficiency of 30 % and cellular carbon conversion of 7 fg C cell^{-1} (Hornick et al.,

614 2017), we estimate that the organic carbon required to support bacterial dynamics during this period

615 (taking into account the net growth and loss rates) was 2.9 and 11.5 ng C ml^{-1} in low and high fCO$_2$

616 mesocosms M1 and M3, respectively. These results suggest that viral lysis of phytoplankton was an

617 important source of organic carbon for the bacterial community. Our results are consistent with

618 bacterial-phytoplankton coupling during this eastern Baltic Sea mesocosm study (Hornick et al.,

619 2017), and agree with earlier work on summer carbon flow in the northern Baltic Sea showing that

620 prokaryotic growth was largely supported by recycled carbon (Uitto et al., 1997). The average net

621 growth rates of the prokaryotes during the first period of increase in Phases 0 and I (0.2 d^{-1}) were

622 comparable to rates reported for this region (Kuosa, 1991). In order to sustain the concomitant daily

623 mortality (between 0.3-0.5 d^{-1}) measured during our study, prokaryotic gross growth rates must have

624   been close to one doubling a day (0.5-0.7 d^{-1}). During Phase I, grazing was the dominant loss factor of

625   the prokaryotic community although there was also evidence that viral lysis was occurring.

626   Bermúdez et al. (2016) reported the highest biomass of protozoans around day 15. This was

627   predominantly the heterotrophic choanoflagellate *Calliacantha natans,* which selectively feeds on

628   particles <1 µm in diameter (Marchant and Scott, 1993; Hornick et al., 2017). Indeed, an earlier study

629   in this area showed that heterotrophic nanoflagellates were the dominant grazers of bacteria,

630   responsible for ingestion of approximately 53 % of bacterial production compared to only 11 % being

631   grazing by ciliates (Uitto et al., 1997). During the first half of Phase II, grazing was reduced and likely

632   contributed to the steady increase in prokaryote abundances. Specifically, a negative relationship

633   between the abundances of HNA-prokaryotes and fCO$_2$ was detected and corresponded to reduced

634   bacterial production and respiration at higher fCO$_2$ (Hornick et al., 2017; Spilling et al., 2016).

635   Although CO$_2$ enrichment may not directly affect bacterial growth, co-occurring global rise in

636   temperature can increase enzyme activities, affecting bacterial production and respiration rates

637   (Piontek et al., 2009; Wohlers et al., 2009; Wohlers-Zöllner et al., 2011). Enhanced bacterial re-

638   mineralization of organic matter may stimulate autotrophic production by the small-sized

639   phytoplankton (Riebesell et al., 2009; Riebesell and Tortell, 2011; Engel et al., 2013), intensifying the

640   selection of small cell size.

641   Mean viral abundances were higher under CO$_2$ enrichment towards the end of Phase I and into Phase

642   II which is expected under conditions of increased phytoplankton and prokaryote biomass. The

643   estimated average viral burst size, obtained from this increase in total viral abundance and

644   concurrent decline in bacterial abundances, was about 30 which is comparable to published values

645   (Parada et al, 2006; Wommack and Colwell, 2000). Viral lysis rates of prokaryotes were measured

646   until day 25 and indicated that during days 18-25 an average 10-15 % of the total prokaryote

647   population was lysed per day. Moreover, the concurrent steady increase in viral abundances during

648   Phase III indicates that viral lysis of the prokaryotes remained important. Thus, the combined impact

649   of increased viral mortality together with reduced production (Hornick et al., 2017) ultimately led to

650   the decline in prokaryote abundance (this study). Lysogeny did not appear to be an important life

651   strategy of viruses during our campaign. Direct effects of higher fCO$_2$ on viruses are not expected, as

652   marine virus isolates are quite stable (both in terms of particle decay and loss of infectivity) over the

653   range of pH of the present study (Danovaro et al., 2011; Mojica and Brussaard, 2014). The few

654   studies which have inferred viral lysis rates based on changes in viral abundances show reduced

655   abundances of algal viruses (e.g. *Emiliania huxleyi* virus) under enhanced CO$_2$ (Larsen et al., 2008)

656   while mesocosm results by Brussaard et al. (2013) indicated a stronger impact of viruses on bacterial

657   abundance dynamics with CO$_2$ enrichment.

658

659   **5 Conclusions**

660   Due to the low buffering capacity of the Baltic Sea and the paucity of data regarding OA impact in

661   nutrient-limited waters, the results presented here are pertinent to increasing our understanding of

662   how projected rises in fCO$_2$ will affect the microbial communities in this region. Our study provides

663   evidence that cell size, taxonomy and sensitivity to loss can all play a role in the outcome of CO$_2$

664   enrichment. Physiological constraints of cell size favour nutrient uptake by small cells under

665   conditions of reduced nutrients and our results show that these effects can be further exacerbated

666   by OA. Gross growth rates along with the complementary mortality rates allowed for a more

667   comprehensive understanding of the phytoplankton population dynamics and thus perception of

668   how microbial food web dynamics can influence the response of the autotrophic and heterotrophic

669   components of the community. Our results further suggest that alterations in CO$_2$ concentrations are

670   expected to affect prokaryote communities (mainly) indirectly through alterations in phytoplankton

671   biomass, productivity and viral lysis. Overall, the combination of growth and losses (grazing and viral

672   lysis) could explain microbial population dynamics observed in this study. It is noteworthy to

673   mention, a recent study in the oligotrophic northeast Atlantic Ocean reported a shift from grazing-

674   dominated to viral lysis-dominated phytoplankton community with strengthening of vertical

675   stratification (shoaling the mixed layer depth and enhancing nutrient limitation) (Mojica et al., 2016).

676   Thus, we highly recommend that future research on OA combine mesocosm studies focusing on

677   changes in microbial community composition and activity with experiments aimed at understanding

678   the effects of OA on food web dynamics, i.e. partitioning mortality between grazing and viral lysis

679   (Brussaard et al., 2008).

680

681   **Author Contribution**

682   Design and overall coordination of research by CB. Organization and performance of analyses in the

683   field by KC. Data analysis by KC, CB, and SA-F. Design and coordination of the overall KOSMOS

684   mesocosm project by UR. All authors contributed to the writing of the paper (KC, KM and CB are lead

685   authors).

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Table 1. *f*CO$_2$ concentrations (µatm) averaged over the duration of the experiment (following CO$_2$ addition) and subsequent classification as low, intermediate or high. Mesocosms sampled for mortality assays are denoted by an asterisk. The symbols and colours are used throughout this manuscript and corresponding articles in this issue.

Mesocosm	M1*	M5	M7	M6	M3*	M8
CO$_2$ Level	LOW	LOW	INTERMEDIATE	INTERMEDIATE	HIGH	HIGH
Mean fCO$_2$ (µatm) days 1-43	365	368	497	821	1007	1231
Symbol						

1 **Table 2.** The fit (R^2) and significance (*p*-value) of linear regressions applied to assess the relationship
2 between net growth rate and temporally averaged $f\text{CO}_2$ for the different microbial groups
3 distinguished by flow cytometry. The results presented are for two periods distinguished from NMDS
4 analysis; NMDS-based period 1 (days 3-13) and 2 (days 16-24). A significance level of p≤0.05 was
5 taken and significant results are shown in bold.

6

Phytoplankton Group	NMDS period 1 (days 3-13)		NMDS period 2 (days 16-24)	
	p	R^2	*p*	R^2
SYN	*0.10*	*0.53*	***0.05***	***0.63***
Pico-I	***0.01***	***0.80***	***0.05***	***0.64***
Pico-II	*0.52*	*0.11*	*0.10*	*0.52*
Pico-III	***0.04***	***0.67***	***<0.01***	***0.91***
Nano-I	***0.01***	***0.79***	*0.26*	*0.30*
Nano-II	*0.20*	*0.36*	*0.06*	*0.61*
HNA	***0.05***	***0.64***	*0.89*	*0.00*
LNA	***<0.01***	***0.95***	***0.02***	***0.76***

7

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Table 3. The fit (R^2) and significance (*p*-value) of linear regressions used to relate peak abundances and net growth rate with temporally averaged $f\mathrm{CO_2}$ for the different microbial groups distinguished by flow cytometry during specific periods of interest. A significance level of $p \le 0.05$ was taken and significant results are shown in bold.

	Peak abundance		Net growth rate	
	p	*R2*	*p*	*R2*
SYN day 24	**0.01**	**0.80**		
Pico-I day 5	**0.01**	**0.81**		
Pico-I day 13	**<0.01**	**0.94**		
Pico-I day 21	**0.01**	**0.84**		
Pico-II day 17	**<0.01**	**0.93**		
Pico-III day 24	**<0.01**	**0.91**		
Nano-I day 17	**0.04**	**0.67**		
Pico-I days 1-5			**<0.01**	**0.90**
Pico-I days 5-9			**<0.01**	**0.89**
Pico-II days 12-17			**0.01**	**0.82**

1 **Figure captions**

2

3 **Fig. 1. (a)** Time-series plot of depth-integrated (0.3–10 m) total phytoplankton abundance (< 20 μm)
4 and **(b)** total eukaryotic phytoplankton abundance for each mesoscosm and the surrounding waters
5 (Baltic). Dotted lines indicate the end of Phase I and end of Phase II. Colours and symbols represent
6 the different mesocosms and are consistent throughout the manuscript. Mean fCO$_2$ during the
7 experiment (days 1-43) were: M1, 365 μatm; M3, 1007 μatm; M5, 368 μatm; M6, 821 μatm; M7,
8 497 μatm; M8, 1231 μatm.

9

10 **Fig. 2.** Non-metric multidimensional scaling (NMDS) ordination plot of microbial community
11 development in each mesocosm and surrounding waters (Baltic) over the experimental period.
12 Phases are indicated by different open symbols. Days of experiment (DoE) when communities
13 separate (3,13,16 and 24) are indicated by different closed symbols. Phytoplankton groups are
14 denoted as: SYN (Syn), Pico-I (P-I), Pico-II (P-II), Pico-III (P-III), Nano-I (N-I), Nano-II (N-II), Low NA
15 prokaryotes (LNA) and High NA prokaryotes (HNA).

16

17

18 **Fig. 3.** Time-series plot of depth-integrated (0.3–10 m) abundances of **(a)** *Synechococcus* (SYN) **(b)**
19 picoeukaryotes I (Pico-I) **(C)** picoeukaryotes II (Pico-II) **(d)** picoeukaryotes III (Pico-III) **(e)**
20 nanoeukaryotes I (Nano-I) and **(f)** nanoeukaryotes II (Nano-II) distinguished by flow cytometric
21 analysis of the microbial community in each mesocosm and the surrounding waters (Baltic). Dotted
22 lines indicate the end of Phase I and end of Phase II, grey areas indicate NMDS-based periods 1 and 2
23 where net growth rates were analysed.

24

25

26 **Fig. 4.** Total mortality rates (i.e., grazing and lysis, solid bars) and gross growth rates (striped bars) d^{-1}
27 of the different phytoplankton groups in mesocosms M1 (blue) and M3 (red) on the day indicated:
28 **(a)** *Synechococcus* (SYN) **(b)** picoeukaryotes I (Pico-I) **(C)** picoeukaryotes II (Pico-II) **(d)** picoeukaryotes
29 III (Pico-III) **(e)** nanoeukaryotes I (Nano-I) and **(f)** nanoeukaryotes II (Nano-II). Significant (p ≤0.05)
30 differences between mesocosms are indicated by an asterisk above the relevant bar (either total loss
31 or gross growth). A colored zero indicates that a rate of zero was measured in the mesocosm of the
32 corresponding colour and the absence of a bar, or zero indicates a failed experiment. Dotted lines
33 indicate the end of Phase I and end of Phase II.

34

35

36 **Fig. 5.** Time-series plot of the mean phytoplankton carbon biomass in high fCO$_2$ (M3,6,8; red) and low
37 fCO$_2$ (M1,5,7; blue) mesocosms of **(a)** Pico I and II combined and **(b)** SYN, Pico III, Nano I and II

1 combined. Error bars represent one standard deviation from the mean. Carbon biomass calculated

2 assuming a spherical diameter equivalent to the mean average cell diameters for each group and

3 conversion factors of 237 fg C μm^{-3} (Worden et al.2004) and 196.5 fg C μm^{-3} (Garrison et al. 2000) for

4 pico- and nano-sized plankton, respectively. Dotted lines indicate the end of Phase I and end of Phase

5 II.

6

7 **Fig. 6.** Time series plot of depth-integrated (0.3–10 m) abundances of **(a)** Total prokaryotes **(b)** High

8 fluorescent nucleic acid prokaryote population (HNA) **(c)** Low fluorescent nucleic acid prokaryote

9 population (LNA) and **(d)** Total virus. Dotted lines indicate the end of Phase I and end of Phase II, grey

10 areas indicate NMDS-based periods where net growth rates were analysed.

11

12 **Fig. 7.** Prokaryote mortality rates: **(a)** Total grazing (d^{-1}) and **(b)** viral lysis rates as % of prokaryote

13 standing stock, in mesocosms M1 (low fCO$_2$, blue) and M3 (high fCO$_2$, red). Grazing rates were

14 determined from fluorescently labelled prey, viral lysis rates from viral production assays. Error bars

15 represent one standard deviation of triplicate assays. Significant ($p \leq 0.05$) differences between

16 mesocosms are indicated by an asterisk. Dotted lines indicate the end of Phase I.

17

18 **Fig. 8.** Correlation between total carbon biomass ($\mu mol\ L^{-1}$) and total prokaryote abundance in low

19 fCO$_2$ mesocosms (M1, 5 and 7; blue) and high fCO$_2$ mesocosms (M3,6, 8; red) throughout the

20 experiment (days -2 to 39).

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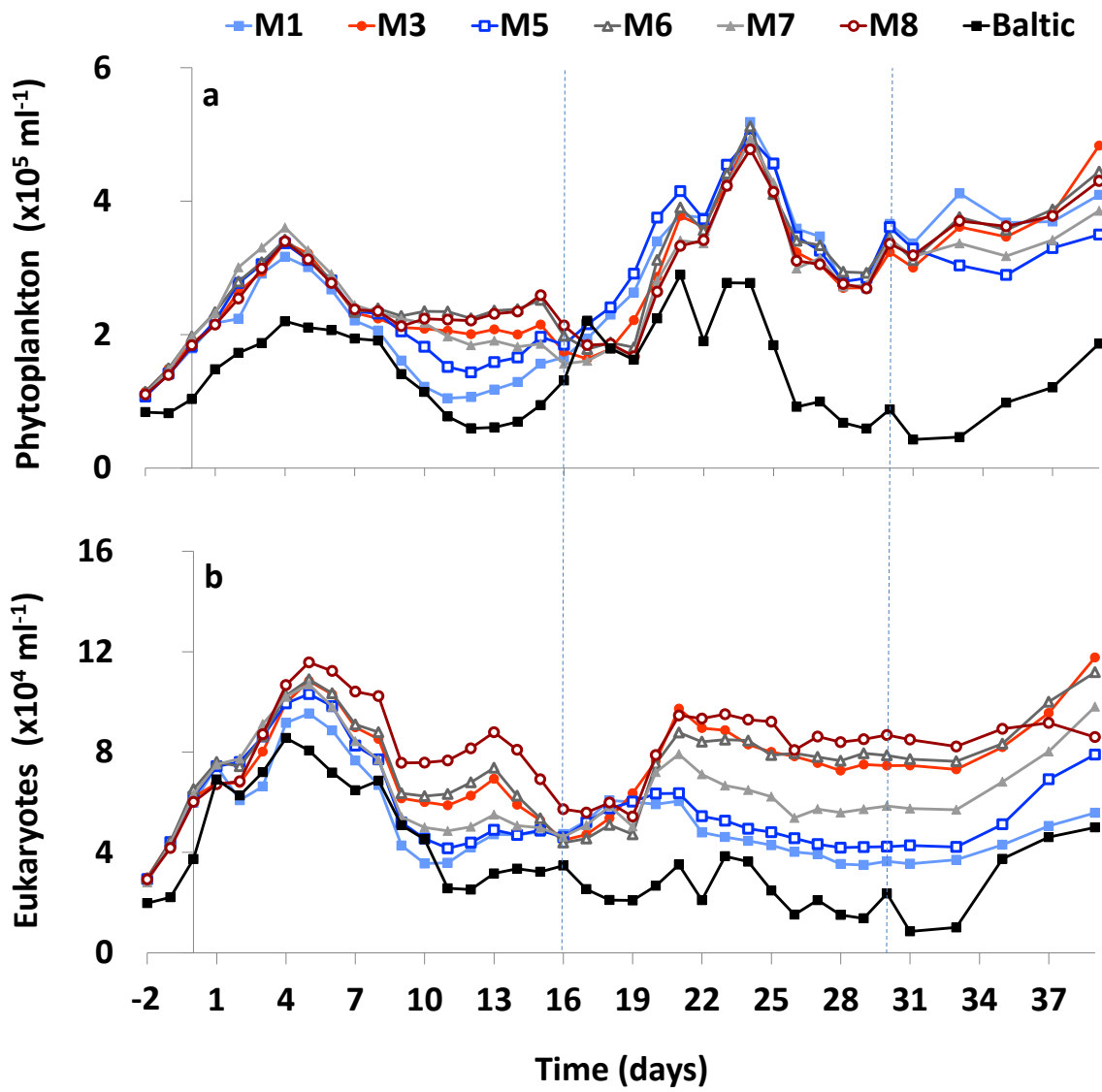Figure 1.
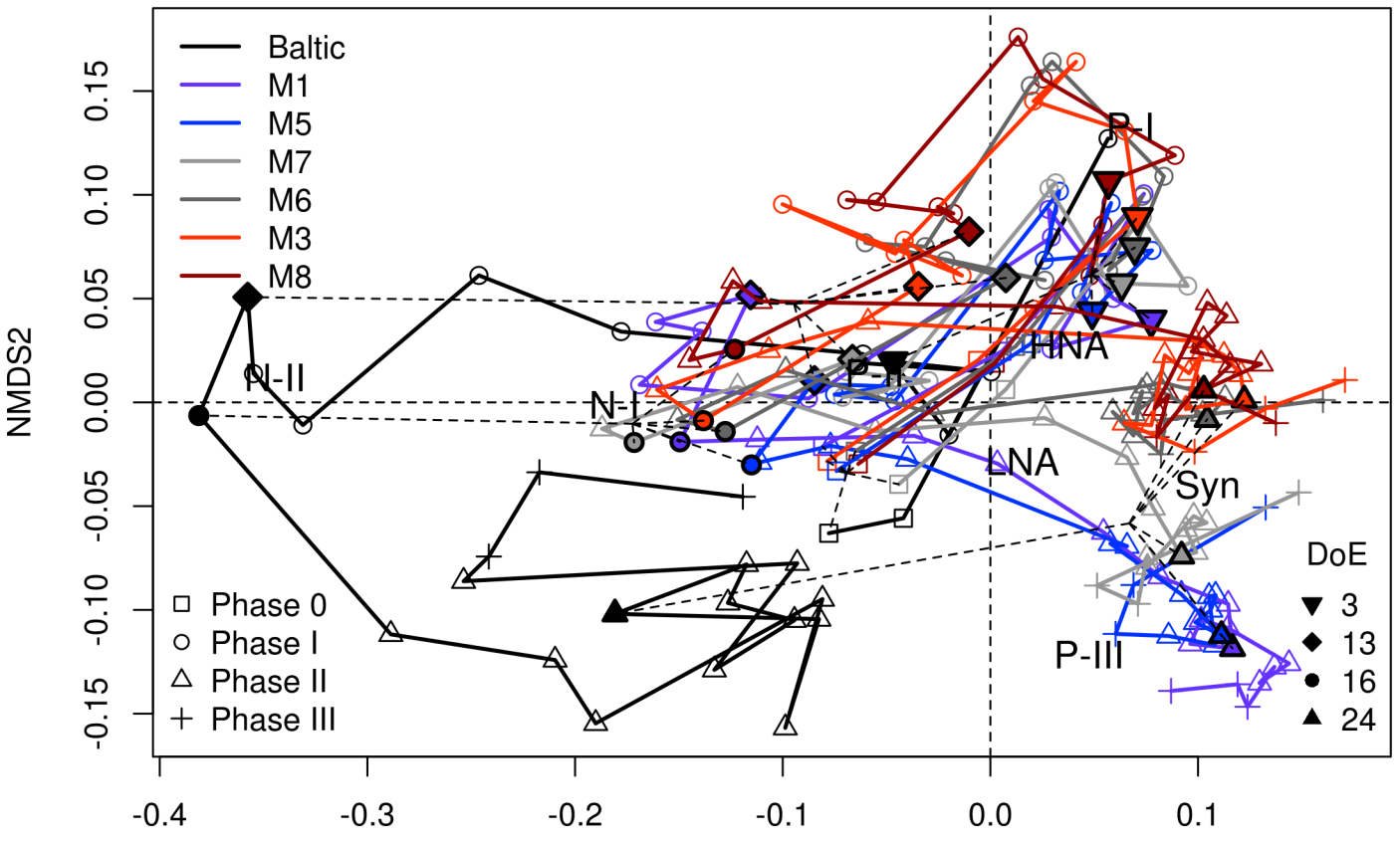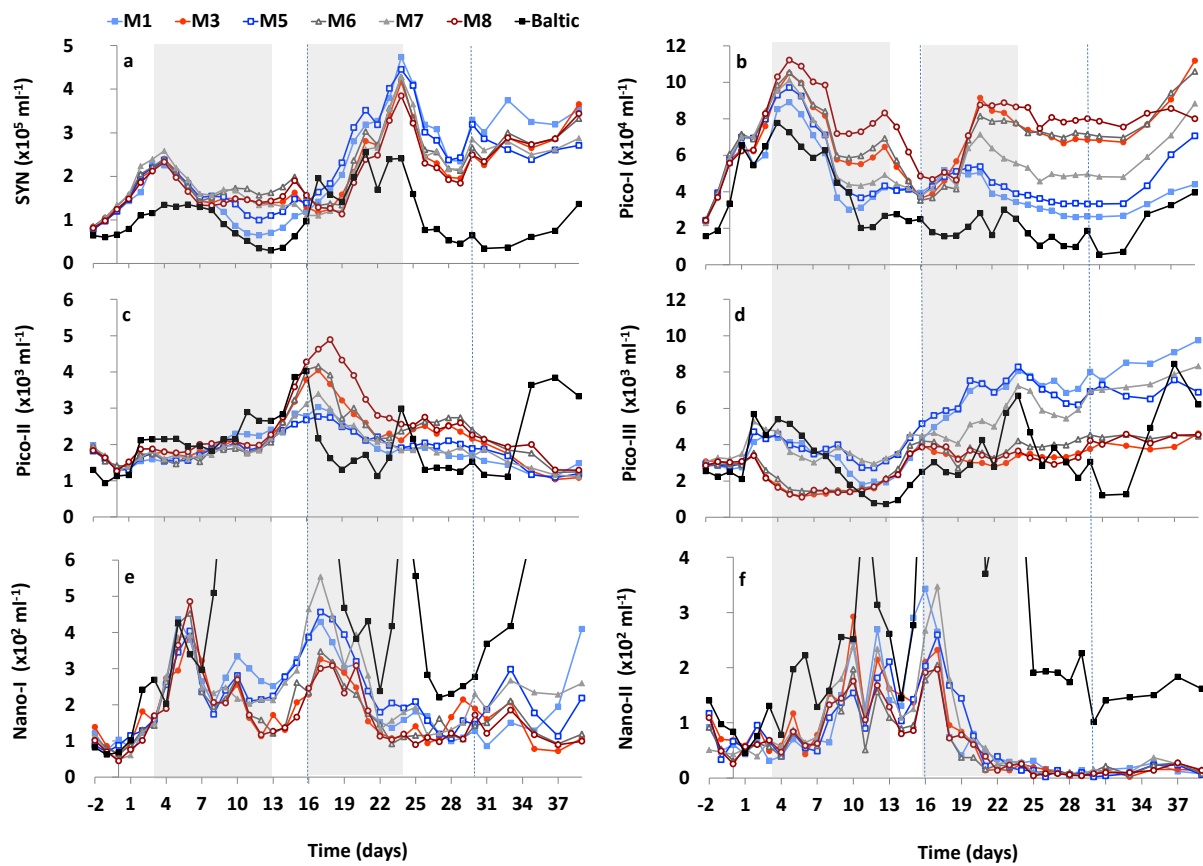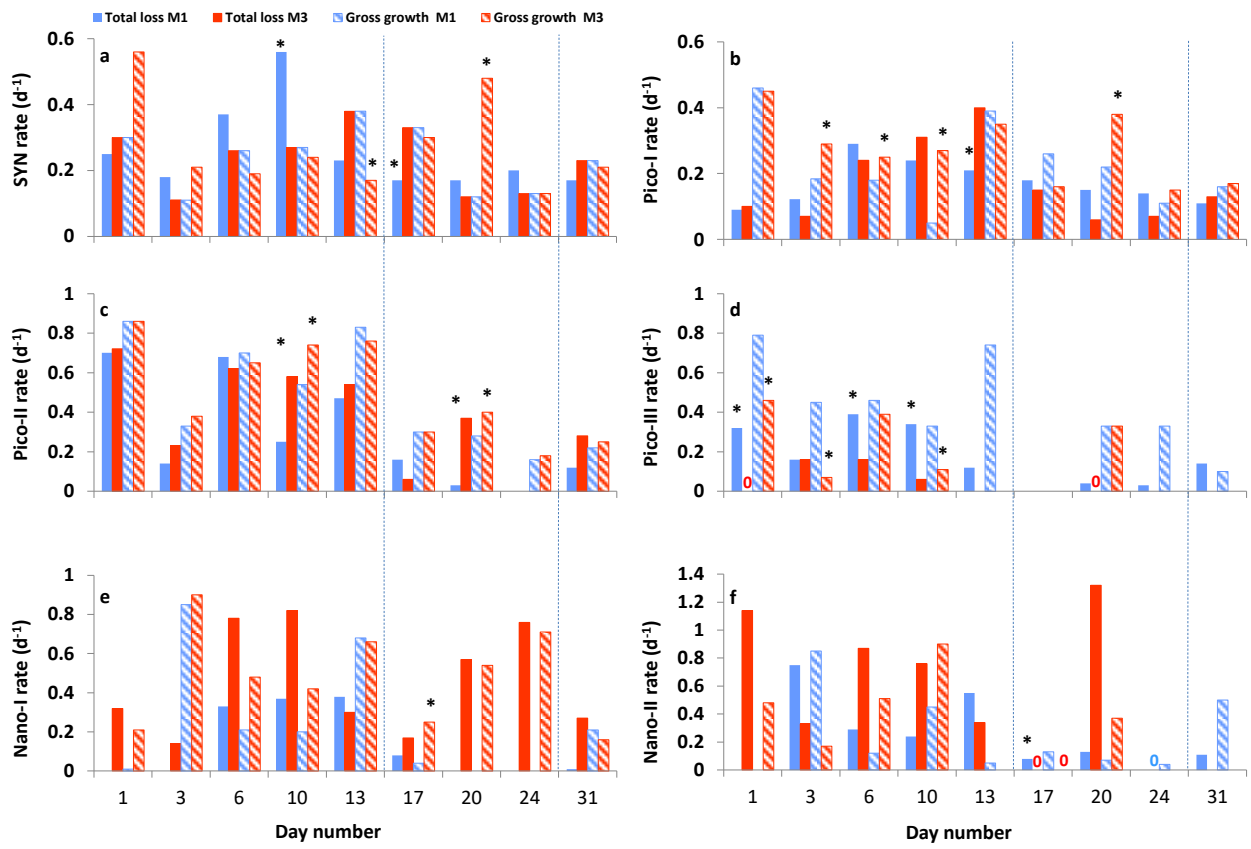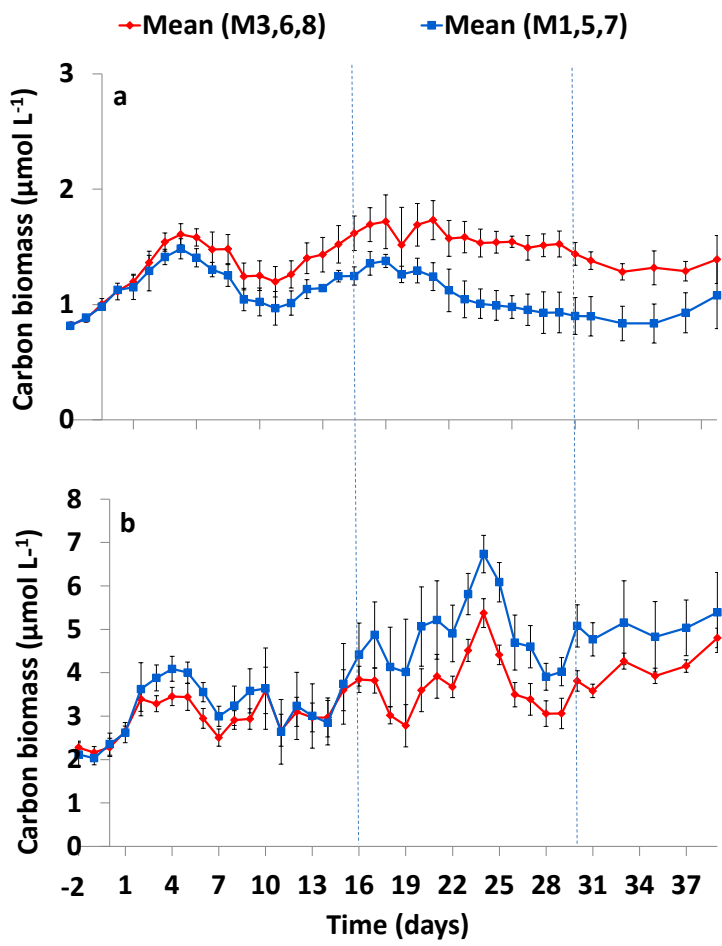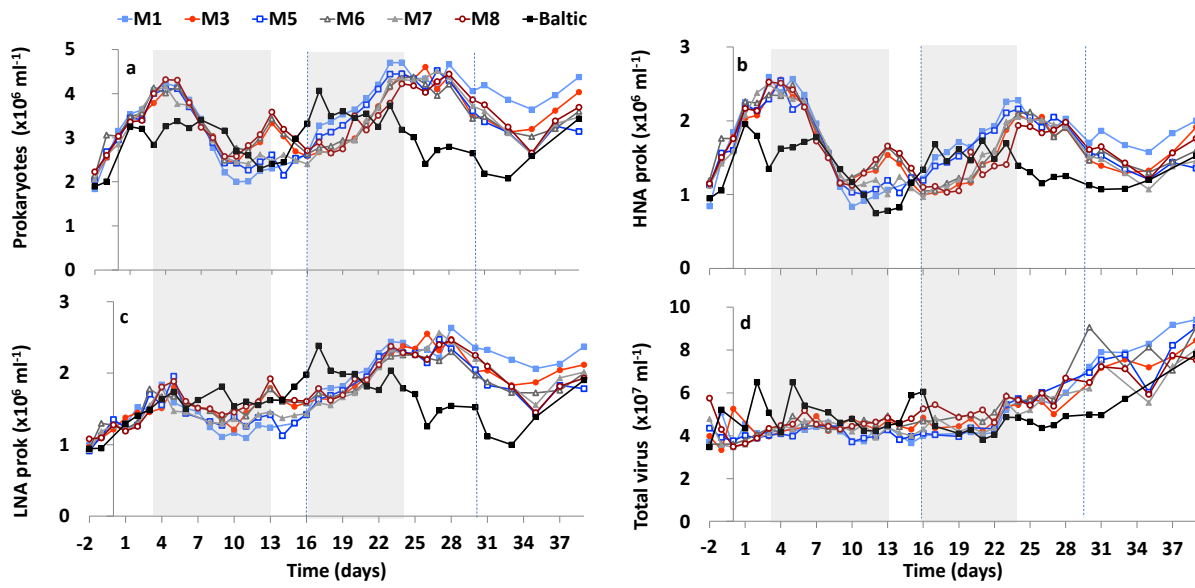
Figure 2.

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