Ocean acidification decreases plankton respiration: evidence from a mesocosm experiment

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Running title: Ocean acidification decreases respiration

Abstract

Anthropogenic carbon dioxide (CO_2) emissions are reducing the pH in the world's oceans. The plankton community is a key component driving biogeochemical fluxes, and the effect of increased CO_2 on plankton is critical for understanding the ramifications of ocean acidification on global carbon fluxes. We determined the plankton community composition and measured primary production, respiration rates and carbon export (defined here as carbon sinking out of a shallow, coastal area) during an ocean acidification experiment. Mesocosms (~55 m^3) were set up in the Baltic Sea with a gradient of CO_2 levels initially ranging from ambient (~240 µatm), used as control, to high CO_2 (up to ~1330 µatm). The phytoplankton community was dominated by dinoflagellates, diatoms, cyanobacteria and chlorophytes, and the zooplankton community by protozoans, heterotrophic dinoflagellates and cladocerans. The plankton community composition was relatively homogenous between treatments. Community respiration rates were lower at high CO_2 levels. The carbon-normalized respiration was approximately 40% lower in the high CO_2 environment compared with the controls during the latter phase of the experiment. We did not, however, detect any effect of increased CO_2 on primary production. This could be due to measurement uncertainty, as the measured total particular carbon (TPC) and combined results presented in this special issue suggest that the reduced respiration rate translated into higher net carbon fixation. The percent carbon derived from microscopy counts (both phyto- and zooplankton), of the measured total particular carbon (TPC) decreased from ~26% at *t0* to ~8% at *t31*, probably driven by a shift towards smaller plankton (<4 µm) not enumerated by microscopy. Our results suggest that reduced respiration lead to increased net carbon fixation at high CO_2. However, the increased primary production did not translate into increased carbon export, and did consequently not work as a negative feedback mechanism for increasing atmospheric CO_2 concentration.

1 Introduction

The ocean is a large sink of carbon dioxide (CO_2) and absorbs around 25 % of annual anthropogenic CO_2 emissions (Le Quéré et al., 2009). CO_2 is a weak acid when dissolved in water, and the increasing global atmospheric CO_2 concentration has reduced the average pH in the ocean by approximately 0.1 since the start of the Industrial Revolution (Orr, 2011). This pH reduction, with a concurrent increase in dissolved inorganic carbon, is called ocean acidification. Following the same trajectory, the pH could decline further by as much as 0.7 by 2300 (Zeebe et al., 2008).

The topic of ocean acidification has received a lot of attention over the past decade. There is a relatively good understanding of the rate of change and the effects on the ocean's carbon chemistry (Zeebe and Ridgwell, 2011). There are also a range of studies documenting the effects of decreasing pH on marine life, but the effect studied is often species or ecosystem specific and based on short term perturbation experiments (Riebesell and Tortell, 2011). There are still a lot of uncertainties as to what effect ocean acidification has on biological processes.

The key driving force in marine biogeochemical element cycling is the planktonic community that occupies the sunlit surface of the ocean. Primary producers use the energy from sunlight to take up CO_2 and fix carbon into organic compounds. Respiration is the opposite process where organic carbon is oxidized providing energy and releasing CO_2. This takes place at all trophic levels, from bacteria through to zooplankton, fish and marine mammals. At steady state, production and respiration are balanced. On a global scale, there is presently a surplus of organic matter being produced in the upper ocean through photosynthesis. The extra organic carbon is exported out of the surface layers to the deep ocean where it is sequestered for the foreseeable future, a process referred to as the biological carbon pump. (Volk and Hoffert, 1985; Siegenthaler and Sarmiento, 1993; Ducklow et al., 2001). In the case of coastal seas, part of the carbon is buried at the sea floor (Dunne et al., 2007).

The greater the difference between primary production and respiration, the more carbon can potentially be exported, and ocean acidification has the potential to affect this balance. Generally, more CO_2 stimulates photosynthetic carbon fixation, as CO_2 becomes more readily available for the key photosynthetic enzyme RubisCO (Falkowski and Raven, 2013), however, increased primary production at high CO_2 concentration is not always recorded (Sobrino et al., 2014) and the response is variable between different taxa (Mackey et al., 2015). In cases where additional carbon is fixed, it may be excreted as dissolved organic

carbon (DOC), providing carbon for bacterial growth, and also increasing bacterial respiration (Grossart et al., 2006; Piontek et al., 2010). Changes in pH might also directly affect both primary production (Spilling, 2007) and respiration (Smith and Raven, 1979).

The Baltic Sea is an almost landlocked sea with low alkalinity (Beldowski et al., 2010), and is thus particularly susceptible to variation in seawater pH. Because of the reduced water exchange with the North Atlantic and the large catchment area (population ~80 million), it is also subjected to a range of other environmental pressures, in particular increased nutrient inputs from human activities, i.e. eutrophication. Eutrophication has led to increased primary production and chlorophyll *a* (Chl *a*) biomass over the past decades in the Gulf of Finland (Raateoja et al., 2005), benefitting chrysophytes, chlorophytes and cyanobacteria, (Suikkanen et al., 2007). Dense blooms of diazotroph cyanobacteria are common in the summer, which further aggravates the eutrophication problem as nitrogen fixation introduces substantial amounts of new nitrogen into the system (Savchuk, 2005). The effect of ocean acidification on this type of system is largely unexplored. In order to investigate the effect of increased CO_2 (and lower pH) on primary production and total plankton respiration in the pelagic zone, we measured carbon fixation, oxygen consumption and export/sedimentation rates during a CO_2-manipulation study set up in the Gulf of Finland, Baltic Sea (further references within this special issue).

2 Materials and methods

2.1 Experimental set-up

Six pelagic mesocosms (approximately 55 m^3) were moored at Storfjärden, SW coast of Finland (59° 51.5' N; 23° 15.5' E) on 12 June 2012. The water depth at the mooring site is approximately 30 meters and the mesocosms extended from the surface down to 19 m depth. A more detailed description of the mesocosm bags and the experimental area can be found in Paul et al. (2015).

On *t-5* (17 June 2012, 5 days before the first CO_2 enrichment), the mesocosms were bubbled with compressed air to break down any existing pycnocline and ensure homogeneous water mass distribution. Different CO_2 concentrations in the bags were achieved by adding filtered (50 µm), CO_2-saturated seawater. This was done stepwise in four separate additions to reduce the shock of rapid change in pH on the plankton community. The first addition took place after sampling on *t0*, thus *t1* was the first day with a CO_2 treatment. The CO_2 enriched water was evenly distributed over the upper 17 m using a specially designed distribution device i.e.

'spider' (Riebesell et al., 2013). Two controls and four treatment mesocosms were used. Filtered water (with ambient CO_2 concentration) was added to the control mesocosms at the time when CO_2 was manipulated in the treatment mesocosms. The CO_2 fugacity gradient on *t4*, after the four additions, ranged from ambient (~240 µatm fCO_2) in the two control mesocosms (M1 and M5), up to ~1650 µatm fCO_2, but we used the average fCO_2 throughout the relevant part of this experiment (from *t1 – t31*) to denote the different treatments: 346 (M1), 348 (M5), 494 (M7), 868 (M6), 1075 (M3) and 1333 (M8) µatm fCO_2. On *t15*, additional CO_2–enriched seawater was added to the upper 7 m in the same manner as the initial enrichment to counteract outgassing of CO_2. The mesocosm bags were regularly cleaned to prevent wall growth. A more detailed description of the treatment and cleaning can be found in Paul et al (2015).

Mesocosm sampling was carried out every day (or every second day for some variables) in the morning. Two different depth-integrated water samples (0-10 m and 0-17 m) were taken using integrating water samplers (IWS, HYDRO-BIOS, Kiel). The water was collected into plastic carboys (10 L) and brought to the laboratory for sub-sampling and subsequent analysis of plankton community composition, carbon concentration and for respiration and primary production incubations. Sub-samples for primary production and respiration measurements were treated and stored minimizing the contact with the air in order to prevent any gas exchange.

Settling particles were quantitatively collected in the sediment traps at the bottom end of the mesocosm units at 19 m water depth. Every 48 hours the accumulated material was vacuum pumped through a silicon tube to the sea surface and transferred into 5 L glass bottles for transportation to the laboratory. For a more detailed description of the sampling procedure and sample processing of the sediment see Boxhammer et al. (2016).

2.2 Phytoplankton community

Phytoplankton cells were counted in 50 mL sub-samples, which were fixed with acidic Lugol's iodine solution (1% final concentration) with an inverted microscope (ZEISS Axiovert 100) after Utermöhl (1958). The cells >20 µm were counted either from half of the chamber at 100-fold or on 3 to 4 stripes at 200-fold magnification. Filamentous cyanobacteria were counted in 50 µm length units. Cells 12 - 20 µm were counted at half of the chamber at 200-fold magnification, and cells 4-12 µm were counted at 400-fold magnification on two

radial strips. The phytoplankton counts of the smaller size classes (<20µm) stopped on *t29*, and these results have been used together with the *t31* results for larger (>20 µm) phytoplankton as the end date of the experiment. Further details can be found in Bermúdez et al. (2016)

Phytoplankton, heterotrophic dinoflagellates and protozoa were identified with the help of Tomas (1997); Hoppenrath et al. (2009) and Kraberg et al. (2010). Biovolumes of counted plankton cells were calculated according to Olenina et al. (2006) and converted to cellular organic carbon quotas by the equations of Menden-Deuer and Lessard (2000).

2.3 Microzooplankton community

Ciliates were enumerated from 50 ml sub-samples every second day with a Zeiss Axiovert 100 inverted microscope (Utermöhl 1958) at 200 x magnification. At high cell numbers (> 400 cells), half the bottom plate area was counted. If less than 400 cells were found in the first half of the bottom plate area, the entire chamber was counted. Rare species were counted on the whole bottom plate. Ciliates were identified to the lowest possible taxonomic level (genus/species) according to Setälä et al. (1992); Telesh et al. (2009) and to description plates found at the planktonic ciliate project (http://ciliate.zooplankton.cn/). For more details see Lischka et al. (2015) in this issue.

2.4 Mesozooplankton community

The term zooplankton includes here all metazoan species, i.e. organisms belonging strictly speaking to the micro- or mesozooplankton, respectively. Zooplankton samples were collected by net hauls from 17 m depth with an Apstein net of 17 cm diameter and 100 µm mesh size. After closing of the mesocosm bags, zooplankton samples were taken prior to the CO_2 addition on *t0* and at *t17* and *t31* (there were also other sampling days for zooplankton but these are not included here). Samples were preserved in 70% ethanol. Zooplankton was counted assuming 100% filtering efficiency of the net. The samples were divided with a Folsom plankton splitter (1:2, 1:4, 1:8, 1:16, and 1:32) and aliquots were counted using a WILD M3B stereomicroscope. Abundant species/taxa were enumerated from sub-samples (> 30 individuals in an aliquot) while less abundant and rare species/taxa were counted from the whole sample. For more details on mesozooplankton collection, processing and species determination, see Lischka et al. (2015). Carbon biomass (CB) in µmol C L^{-1} was calculated using the displacement volume (DV) and the equation of Wiebe (1988):

$$(\text{Log DV} + 1.429)/0.82 = \log \text{CB} \quad (1)$$

2.5 Total particulate carbon

Samples for total particulate carbon (TPC) measurements were sub-sampled from 10 L carboys and filtered onto GF/F filters (Whatman, nominal pore size of 0.7 µm, diameter = 25 mm) under reduced vacuum (< 200 mbar). Sampling for TPC occurred every 2nd day from *t-3* until the end of the experiment. Filters were stored in glass petri dishes at -20°C directly after filtration until preparation of samples for analyses. Petri dishes and filters were combusted at 450°C for 6 hours before use.

Samples were analyzed for total particulate carbon (organic + inorganic) as no acidifying step was made to remove particulate inorganic carbon. Filters were dried at 60°C and packed into tin capsules and stored in a desiccator until analysis on an elemental analyzer (EuroEA) as described by (Sharp, 1974).

The particles collected from the sediment traps were allowed to settle down in the sampling flasks at in-situ temperature before separation of supernatant and the dense particle suspension at the bottom. TPC content of the supernatant was analysed from 10–50 mL sub-samples as described above for water column measurements. The dense particle suspension was concentrated by centrifugation, then freeze-dried and ground to a very fine powder of homogeneous composition. From this material, small sub-samples of 1–2 mg were transferred into tin capsules and TPC content was analysed analogue to the supernatant and water column samples. Vertical carbon flux was calculated from the two measurements and is given as the daily amount of TPC (mmol) collected in the sediment traps per square meter of mesocosm surface area (3.142 m^2).

2.6 Dissolved inorganic carbon

Samples for dissolved inorganic carbon (DIC) were gently pressure-filtered (Saarstedt Filtropur 0.2 µm) before measurements to remove all particulates. DIC concentrations were determined by infrared absorption (LICOR LI-7000 on an AIRICA system, Marianda). Four (2 mL) replicates were measured, and the final DIC concentration was calculated from the mean of the three most consistent samples.

2.7 Plankton community respiration

Samples for respiration rate measurements were subsampled from the depth integrated sample from the entire water column (0–17 m). Oxygen was measured using a fiber optical dipping probe (PreSens, Fibox 3), which was calibrated against anoxic (0% O_2, obtained by

adding sodium dithionite) and air saturated water (obtained by bubbling sampled water with air for 5 minutes followed by 15 minutes of stirring with a magnetic stirrer). The final O_2 concentration was calculated using the Fibox 3 software including temperature compensation. We filled three replicate 120 mL O_2 bottles (without headspace) for each mesocosm. After the initial O_2 determination, the bottles were put in a dark, temperature controlled room, set to the ambient water temperature at the surface. The O_2 concentration was determined again after an incubation period of 48 hours, and the oxygen consumption (i.e. respiration rate) was calculated from the difference between the O_2 concentration before and after the incubation period. Respiration rates were measured every day *t-3* to *t31*, with the exception of days: *t2* and *t14* because of technical problems.

2.8 Primary production

Primary production was measured using radio labeled $NaH^{14}CO_3$ (Steeman-Nielsen, 1952) from the 0-10 m depth integrated sample. The rational for using the upper (0-10 m) part of the mesocosm was the low light penetration depth, and 0-10 m was representative of the euphotic zone. The water was gently filled into 12 small (8 mL) scintillation vials per mesocosm and 10 µl of ^{14}C bicarbonate solution (DHI Lab; 20 µCi mL^{-1}), was added. The vials were filled completely and after adding the cap there was only a very small (2-3 mm) air bubble remaining corresponding to ~0.1% of total volume.

Duplicate samples for each mesocosm were incubated just below the surface and at 2, 4, 6, 8 and 10 m depths for 24 h on small incubation platforms moored next to the mesocosms (Fig. S1). In addition, a dark incubation (vials covered with aluminium foil) was incubated at the same location at 11 m depth.

After incubation, 3 mL of the sample was removed from each vial and acidified with 100 µl 1 mol L^{-1} HCl, and left without a lid for 24 h to ensure removal of remaining inorganic ^{14}C. Four mL of scintillation cocktail (Instagel Plus, Perkin Elmer) was added, and the radioactivity was determined using a scintillation counter (Wallac 1414, Perkin Elmer). Primary production was calculated knowing the ^{14}C incorporation (with dark values subtracted) and the fraction of the ^{14}C addition to the total inorganic carbon pool according to Gargas (1975). The primary production incubations were set up at the same time as the respiration incubations, but here we missed measurements for two periods: *t1- t3* and *t6- t8*, due to loss of the incubation platform.

2.9 Data treatment

The average of the three respiration bottles was used to calculate the respiration rate. There were two days without measurements: *t*2 and *t*14 and for these days we estimated the respiration rate by using the average of the day before and after this day. TPC was measured only every second day, therefore for the days without TPC measurements we normalized respiration to average TPC from the day before and the day after the respiration measurement.

The cumulative respiration was calculated by adding the total oxygen consumption for each day. When evaluating the data, there were two clear periods emerging from the experiment: the initial period *t0 – t16* (Phase I) and period from *t17 – t31* (Phase II) when the effect of the CO_2 addition was more evident. This division was also seen in e.g. Chl *a* and temperature (Paul et al. 2015). Using the respiration data from Phase II we calculated the average respiration for each treatment by linear regression. From the linear regression, the standard error (SE) from the residuals and the coefficient of determination (R^2) were calculated, in addition to a statistical test comparing the linear regression with a flat line, using Sigma Plot software.

The areal primary production was calculated based on a simple linear model of the production measurements from the different depths (Fig S2). The cumulative primary production was carried out similar to respiration, but as the two missing periods were >1 day, we did not estimate missing values, and the final cumulative production is therefore a slight underestimate (missing 6 days of production). We normalized the production data to the TPC in the euphotic zone, defined by the areal production model (Fig S2).

From the two different phases of the experiment (Phases I and II; *t0 – t16* and *t17 – t31* respectively) we calculated the average for the different parameters and SE, with 9 and 7 sampling points during Phase I and II respectively.

3 Results

3.1 Phytoplankton community composition

The phytoplankton community in the mesocosms was dominated by dinoflagellates, cyanobacteria, diatoms, chrysophytes and chlorophytes at the start of the experiment (Fig 1). The two latter groups consisted almost exclusively of small cells (<20 µm). There was an initial increase in phytoplankton biomass from an average of 3 µmol C L^{-1} to a maximum of ~4.1 µmol C L^{-1} in the two controls (M1 and M5), but at the end of Phase I (*t0–t16*) the biomass had declined and at *t17* it ranged between 3.2 to 3.5 µmol C L^{-1}. During Phase I, large (>20µm) diatoms decreased in abundance and euglenophytes increased from a

negligible group initially (0.5% of the biomass) to constituting 15-25% of the autotrophic biomass at *t17*. It was, however, the small (<20 µm) phytoplankton cells (small diatoms, chrysophytes and chlorophytes) that made up the majority (70-80%) of the counted autotroph biomass during Phase I.

During Phase II (*t17–t31*), there was a decline in phytoplankton biomass to 0.5-1 µmol C L^{-1} and at *t31* dinoflagellates had become the dominating group in all treatments except at the highest CO_2 level. Cyanobacteria and chlorophytes were also abundant and the dominating groups in the highest CO_2. There was no consistent difference between phytoplankton communities in the different CO_2 treatments, but dinoflagellate abundance was lower in the highest CO_2 treatment (M8), and consequently the total phytoplankton biomass was lower in this treatment at *t31*. The relative increase of large dinoflagellates decreased the contribution of the smaller autotroph size class (4-20µm) to 40-60% of the counted phytoplankton biomass at *t31*.

3.2 Zooplankton community composition

Protozoans, ciliates and heterotrophic dinoflagellates dominated the microzooplankton and constituted a major part (2.8 µmol C L^{-1}) of the whole zooplankton community at the start of the experiment (Fig. 2). Protozoans, dominated by the choanoflagellate *Calliacantha natans,* decreased from the initial high concentrations during Phase I, in particular in the M1 control bag. The photosynthesizing, *Myrionecta rubra* (syn. *Mesodinium rubrum*) made up approximately half of the ciliate biomass at *t0*, but both this species and the total biomass of ciliates decreased during Phase I. The biomass of heterotrophic dinoflagellates was relatively stable throughout Phase I, but started to decrease during Phase II.

The mesozooplankton community was initially dominated by copepods, cladocerans and rotifers (Fig. 2). The average initial biomass was 0.05 µmol C L^{-1} and increased to 0.13 µmol C L^{-1} at *t17*. During Phase I, copepods became the dominating group with >50% of the mesozooplankton biomass. In Phase II of the experiment, mesozooplankton biomass increased and was on average 0.27 µmol C L^{-1} at *t31*. This was caused by an increase in cladocerans, mainly *Bosmina* sp., whereas copepod biomass was more constant over the course of the experiment. The population peak of *Bosmina* sp. had slightly different timing in the different mesocosms but was higher in the mesocosms with added CO_2, except for the highest CO_2 addition (M8).

3.3 Total particulate carbon and export of carbon

Average TPC was 22.5 µmol C L^{-1} at the beginning of the experiment and after an initial increase to 32 µmol C L^{-1} it decreased to 19.2 µmol C L^{-1} at *t17* (Fig 3). In the beginning of Phase II it was relatively stable and with no clear effect of CO_2 treatment, but at the end of the study period (*t31*) there was more TPC in the higher CO_2 treatments, and the increase in TPC during Phase II was highest in the CO_2 additions (Table 1). At *t31* the average TPC was 19.9 µmol C L^{-1}, ranging from 18.9 ± 0.6 (SE) µmol C L^{-1} in the controls to 22.1 µmol C L^{-1} in the highest CO_2 treatment.

The carbon accounted for by biologically active organisms counted in the microscope (phytoplankton and zooplankton) was initially 26% of the TPC. At *t17* and *t31* this percentage decreased to ~20% and ~8% respectively.

The export of carbon, defined here as carbon settling out of the mesocosms, decreased during the experiment and there was no effect of CO_2 concentration. The average export of TPC was in the range of 6.1 – 7.4 mmol C m^{-2} d^{-1} during Phase I (Table 1). This decreased to 2.5 – 3.3 mmol C m^{-2} d^{-1} during Phase II.

3.4 Primary production and respiration

There was no clear effect of CO_2 addition on primary production (Fig. 4). There were relatively large daily variations in depth-integrated primary production depending on the light environment, and days with clear skies and more light increased carbon fixation. One of the control bags (M1) had clearly lower primary production from the very start of the experiment, and this was evident even before the initiation of the CO_2 addition (Fig 4). The average production during the whole experiment was 3.67 ± 0.42 (SE) mmol C m^{-2} d^{-1} in M1, and for all other bags 10.5 ± 0.67 (SE) mmol C m^{-2} d^{-1}. Production on clear, sunny days was (except for M1) approximately 25 mmol C m^{-2} d^{-1}. The general pattern in areal primary production was similar to TPC-normalized production (Table 1). Cumulative production values in mol C m^{-2} are presented in the supplementary material (Fig S3).

The respiration rate was higher in the ambient than the high CO_2 treatments (Fig 5). In one of the two controls (M1), the respiration rate was clearly higher compared to all other treatments from the beginning of the experiment. The respiration rate in the other control (M5) increased approximately two weeks later than the CO_2 treatments. After *t17*, the mesocosm with highest CO_2 concentration (average of 1333 µatm fCO_2) started to have lower cumulative respiration compared to those with intermediate CO_2 levels (494-1075 µatm fCO_2). After another week (~*t27*), differences between the intermediate CO_2 treatments became apparent. At the end of Phase II (*t20–t31*), there was a 40% difference in respiration rate between the

lowest and highest fCO_2 treatments (slope -0.0002; p = 0.02; R^2 = 0.77; Fig. 6). The volumetric respiration during Phase II was 7.6 and 7.1 µmol O_2 L^{-1} d^{-1} for the two controls, and 4.7 - 5.7 µmol O_2 L^{-1} d^{-1} for the CO_2 treatment mesocosms. Outside the mesocosms, at ambient CO_2 concentration (average of 343 µatm fCO_2 but with larger variability than inside the mesocosms), the carbon normalized respiration rate was lower than inside the mesocosms and the cumulative, carbon-normalized respiration was approximately half of that measured in the control bags at the end of the experiment (Fig 5). The general pattern of lower respiration rates at high CO_2 concentration was the same without normalization to TPC (Table 1, Fig S4).

4 Discussion

4.1 Plankton community

The particulate and dissolved standing stocks during this experiment are presented in Paul et al. (2015). In the initial Phase I of the experiment the Chl *a* concentration was relatively high (~2 µg Chl *a* L^{-1}), but started to decrease during Phase II, and reached ~1 µg Chl *a* L^{-1} at *t31* in all of the treatments. During this transition there was a shift in the plankton community with decreasing phytoplankton and microzooplankton, and increasing abundance of mesozooplankton, primarily cladocerans (Figs 1 and 2).

The phytoplankton community composition was dominated by common species in the area (Hällfors, 2004). In the latter part (Phase II), the relative dominance by dinoflagellates was mainly due to reduction in biomass of the other groups, with the exception of the highest CO_2 concentration where also the dinoflagellates decreased in abundance. Dinoflagellates are generally favored in low turbulence (Margalef, 1978; Smayda and Reynolds, 2001), and were probably benefitting from the relative stable conditions within the mesocosms. Blooms of filamentous cyanobacteria do occur in the area, but did not develop within the mesocosms. The relatively low temperature (mostly <15°C; Paul et al., 2015) could be a reason for that (Kanoshina et al., 2003).

Protozoans, ciliates and heterotrophic dinoflagellates dominated the microzooplankton, and *Myrionecta rubra* initially made up a large proportion of the ciliates. *M. rubra* can be regarded as mixotropic and would also have contributed to the carbon fixation (Johnson et al., 2006). Copepods and cladocerans initially dominated the mesozooplankton, and during Phase II, cladocerans became the dominant mesozooplankton group. Cladocerans are typically predominant in freshwater but in the brackish Baltic Sea they can be common, in particular when stability in the water column is high (Viitasalo et al., 1995).

The combined phyto- and zooplankton carbon derived from microscope counts decreased during the experiment. TPC did not decrease to the same extent, and the percentage microscope-derived carbon of TPC decreased from 26% at *t0* to only ~8% of the measured TPC at *t31*. These numbers are not directly comparable, as detritus, i.e. non-living carbon particles, are included in TPC. However, any large aggregates sink rapidly and are not expected to have contributed much to the TPC. The reduction of microscopy-derived carbon to TPC indicate rather increasing importance of smaller size classes (<4 µm), not enumerated by the microscope counts. This conclusion is also supported by flow cytometer data from this experiment (Crawfurd et al., 2016), increasing uptake of PO_4 by the <3 µm fraction (Nausch et al., 2016) and the increasing proportion of the smallest (<2 µm) size class of Chl *a* (Paul et al., 2015).

4.2. Primary production and respiration

Primary production and respiration rates were comparable to values obtained under similar conditions in the area (Kivi et al., 1993). There are relatively few records of respiration, but the measured respiration rates in the control bags were similar to the average respiration rate obtained for a range of coastal waters of 7.4 ± 0.54 mmol O_2 m^{-3} d^{-1} (n = 323) (Robinson and Williams, 2005). The incubation period we used for primary production measurements (24 h) provides production rates close to net production (Marra, 2009).

The higher respiration and lower production in the M1 control bag was probably connected, i.e. higher respiration lead to lower net carbon fixation, however, the reason for the M1 bag being very different from the very start is not clear. Most of the other variables were similar in the M1 bag compared to the rest (Paul et al., 2015), but there was some indication of difference in community. In particular, protozoans were lower in the M1 bag compared with the rest of the mesocosms throughout the experiment. However, judging from the development in carbon pools (Paul et al., 2015) and fluxes in the system (Spilling et al., 2016), the NPP measurements for the M1 bag must be an underestimate. Bacterial production during Phase II was highest in the ambient CO_2, in particular in M1 (Hornick et al., 2016), and could partly be the reason for the elevated respiration rate in this mesocosm bag.

Having the respiration incubation at a fixed temperature might have caused a slight bias as there was varying thermal stratification throughout the experiment and the temperature was not even throughout the mesocosm bags. A better approach would have been to have respiration incubations in temperatures above and below the thermocline, but logistical constrains prevented us from doing this.

Another factor that could have influenced our incubations is UV light, which is a known inhibitor of primary production (Vincent and Roy, 1993), and elevated CO_2 concentration may increase the sensitivity to UV light (Sobrino et al., 2009). Additionally, UV light reduces the release of DOC by phytoplankton, in particular at high CO_2 concentration (Sobrino et al., 2014), but also cause photochemical mineralization of dissolved organic matter (DOM) (Vahatalo and Jarvinen, 2007). Both DOC release and DOM break down may have implications for bacterial production and nutrient cycling. The mesocosm bags were made in a material absorbing UV light (thermoplastic polyurethane) whereas our primary production incubations were done in glass vials (transmitting some UV light) moored outside the mesocosm bags. The difference in UV transmittance could have produced a bias in the primary production measurements. However, the DOM concentration in the Baltic Sea is very high compared with most other oceans and coastal seas (Hoikkala et al., 2015). Most of this is terrestrial derived, refractory DOM, which effectively absorbs in the UV region, and typically the depth at which 1% of UVB remains is <50 cm (Piazena and Häder, 1994). UVA penetrates a little deeper and may have affected slightly the incubation platform moored at 2 m depth, but we do not believe that UV light caused major inhibition of our primary production measurements or affected phytoplankton DOC production.

4.3. Effect of CO_2 on the balance between respiration and carbon fixation

Increased CO_2 concentration has increased carbon fixation in some studies (Egge et al., 2009; Engel et al., 2013). This was not observed in this study, but the higher Chl *a*, TPC and DOC in the high CO_2 treatments at the end of the experiment (Paul et al., 2015) could have been caused by the lower respiration rate in the highest CO_2 enriched mesocosms, rather than increased primary production. Bacterial production was higher in the low CO_2 after *t20* during this experiment (Hornick et al., 2016), which fits with the higher respiration rate at ambient CO_2 concentration. The biomass of the smallest plankton size fraction (<4 µm, not counted by microscope) increased in relative importance with CO_2 addition in the latter part of the experiment, in particular two groups of pico-eukaryotes (Crawfurd et al., 2016), and seems to have benefitted most by elevated CO_2 concentration, similar to findings in the Arctic (Brussaard et al., 2013). Temporal changes in bacterial abundances followed largely that of phytoplankton biomass, and there were significant increases in viral lysis rates in the high CO_2 treatment (Crawfurd et al., 2016). This was most likely a consequence of higher

abundances of pico-eukaryotes and pointing towards a more productive but regenerative system (Crawfurd et al., 2016).

This study is, to our knowledge, the first one describing reduced respiration rates with ocean acidification on a plankton community scale. There are relatively few measurements of community respiration in ocean acidification experiments, and existing studies have revealed no specific responses in respiration (Egge et al., 2009; Tanaka et al., 2013; Mercado et al., 2014). Some of these studies have been relatively short (<2 weeks) compared to the current study. Our results revealed a CO_2 effect only two weeks into the experiment, suggesting that potential effects may have been present but remained below the detection limits in previous studies.

The effect of increasing CO_2 concentration on respiration has mostly been documented for single species. For example, the copepod *Centropages tenuiremis* (Li and Gao, 2012) and the diatom *Phaeodactylum tricornutum* (Wu et al., 2010) exhibited increased respiration rates in a high CO_2 environment (≥1000 µatm fCO_2), contrary to our findings. However, these types of studies have revealed different responses even when comparing different populations of the same species (Thor and Oliva, 2015), and any interpolation from single-species, laboratory-studies should be carried out with great caution. The larger-scale mesocosm approach taken here has the advantage that the whole plankton community and possible interacting effects between different components of the food web are included.

For primary producers in aquatic environment, changes in carbonate chemistry speciation affects the availability of the sole substrate, i.e. CO_2, at the site of photosynthetic carbon fixation. At present, marine waters typically have a pH of 8 or above, and most of the carbon is in the form of bicarbonate (HCO_3^-). Many phytoplankton groups have developed carbon concentrating mechanisms (CCMs) as a way to increase substrate availability at the site of carbon fixation (Singh et al., 2014), reducing the cost of growth (Raven, 1991). For phytoplankton with CCMs, increased CO_2 availability would suppress the CCM, freeing resources for growth, in particular under light limiting conditions (Beardall and Giordano, 2002). There are examples of experiments with ocean acidification that has indicated downregulation of CCM (Hopkinson et al., 2010) and photosynthetic apparatus (Sobrino et al., 2014), which could reduce respiration in phytoplankton.

The intracellular pH can be highly variable between different cellular compartments and organelles, but in the cytosol the pH is normally close to neutral (pH ~7.0), and is to a large extent independent of the external pH (Roos and Boron, 1981). In plants, animals and also bacteria, there is a complex set of pH regulatory mechanisms that is fundamentally controlled

by physiological processes such as membrane transport of H^+ or OH^- and intracellular metabolism (Smith and Raven, 1979; Kurkdjian and Guern, 1989). Internal pH regulation can be a considerable part of baseline respiration (Pörtner et al., 2000). With ocean acidification, the external pH becomes closer to the intracellular pH, and this might reduce the metabolic cost (respiration) related to internal pH regulation. Teira et al. (2012) studied the effect of elevated CO_2 concentration on two bacterial cultures and found reduced respiration in one of the two in a high CO_2 environment (1000 µatm CO_2), and they suggesting reduced metabolic cost for internal pH regulation as a possible mechanism. However, the other strain did not have any change in respiration rate and more studies of the effect of changed external pH on membrane transport are needed (Taylor et al., 2012). There might additionally be considerable difference between marine organisms depending on e.g. size, metabolic activity and growth rates, which directly affect pH in the diffusive boundary layer surrounding the organism (Flynn et al., 2012).

Judging from the importance of the smallest size class in this study, bacterial and picophytoplankton community (Crawfurd et al., 2016) and bacterial production (Hornick et al., 2016), the decreased respiration at higher CO_2 concentration was probably mostly due to reduced picoplankton respiration. The underlying mechanisms behind the reduced respiration are unclear and this is an underexplored research avenue that deserves further study.

4.4. Interacting effects and community composition

Our measurements outside the mesocosm bags demonstrate that plankton physiology and community composition can have a big impact on both primary production and respiration. The plankton community was relatively uniform across all mesocosm bags. Unfortunately, we do not have any community data from outside the mesocosm bags, but the amplitude of Chl *a* dynamics was different, with an upwelling event leading to a doubling of the Chl *a* concentration (~5 µg Chl *a* L^{-1}) around *t17* (Paul et al., 2015). This suggests a different availability of inorganic nutrients and different plankton community as other environmental variables such as light and temperature were similar both inside and outside the mesocosm bags, except that UV light was absent inside the mesocosm bags. The carbon-normalized respiration rate outside the mesocosm bags (with ambient fCO_2) was approximately half of the respiration rates in the controls with the same average fCO_2, and also absolute respiration was clearly lower during Phase II, when nitrate was depleted inside the bags and plankton biomass was decreasing. However, the fCO_2 was more variable outside the mesocosm bags

529 compared with the control bags (although their averages were similar), and the fCO_2
530 increased throughout Phase II outside the bags to approximately 700 µatm by *t31* (Paul et al.
531 2015). This could have influenced the carbon normalized respiration, which started to deviate
532 outside the bags during Phase II, but it could also have been interacting effects of different
533 environmental changes (different nutrient dynamics) leading to this lower respiration rate. An
534 often overlooked aspect is the importance of the plankton community composition, which can
535 be more important than changes in external factors (Verity and Smetacek, 1996; Eggers et al.,
536 2014).
537 Bacterial production (Grossart et al., 2006) and bacterial degradation of polysaccharides
538 (Piontek et al., 2010) have been demonstrated to increase under elevated CO_2 concentration,
539 contrary to the findings during this experiment (Hornick et al., 2016). All of these responses
540 are to a large extent dependent on the plankton community composition. For example, the
541 increased bacterial production observed in a mesocosm study in a Norwegian fjord was
542 probably a response to increased carbon availability produced by phytoplankton (Grossart et
543 al., 2006). DOC production by phytoplankton is determined by the physiological state and the
544 composition of the community (Thornton, 2014); in particular diatoms have been intensively
545 studied in this respect and are known to be important DOC producers (Hoagland et al., 1993).
546 Shifts in the phytoplankton community may alter the DOC production (Spilling et al., 2014),
547 and any shifts in the plankton community composition, caused by ocean acidification, may
548 have greater effects on ecosystem functioning than any direct effect of increasing fCO_2 /
549 decreasing pH (Eggers et al., 2014).
550 It is evident that there were other variables that influence the physiology of the plankton
551 community as a whole outside the mesocosms. Changes in community composition and
552 nutrient availability seem the most plausible reasons. A better understanding of how different
553 physical, chemical and biological factors interact with each other is needed in order to
554 improve our understanding of how marine ecosystems change under the influence of a range
555 of environmental pressures.
556
557 **4.5. Potential implications for carbon cycling**
558 A lot of attention during past decades has been directed to understanding the biological
559 carbon pump, as it is a key mechanism for sequestering atmospheric CO_2. The potential
560 export is ultimately determined by gross primary production minus total community
561 respiration. Even small changes in the production or loss term of this equation have the
562 potential to greatly affect biogeochemical cycling of carbon.

563 The exported carbon decreased during the experiment. Part of this decrease was probably due
564 to sinking of existing organic material at the start of the experiment and can be seen as the
565 reduction in TPC. However, this also coincided with the shift towards increased dominance
566 of picoplankton. Size is a key parameter determining sinking speed, and picoplankton is very
567 inefficient in transporting carbon out of the euphotic layer (Michaels and Silver, 1988). The
568 shift towards smaller size classes was likely also contributing to the reduction in exported
569 carbon.
570 The 40% reduction in respiration with increasing fCO_2 found in our study could have great
571 implications for net export of carbon in the future ocean. There is, however, uncertainty in the
572 results, in particular that the measured net carbon fixation under increased CO_2 was not
573 higher than in the controls. In the case of reduced respiration, an increase in net primary
574 production can be expected, as loss rates are reduced. That the measured carbon fixation was
575 not evidently different between treatments could be due to similar reduction in GPP, as
576 indicated by carbon flux estimates (Spilling et al., 2016). Alternatively, the measurement
577 uncertainty in our small scale incubations (8 mL), involving several pipetting steps, was
578 likely higher than the respiration measurements, which could have prevented us from picking
579 up any CO_2 effect on primary production. Another complicating factor is what the ^{14}C
580 method is actually measuring (Sakshaug et al., 1997; Falkowski and Raven, 2013). The
581 consensus seems to be somewhere between gross and net production, but leaning towards net
582 production with long incubation times (Marra, 2009).
583 There was evidence of a positive CO_2 effect on the amount of Chl *a*, TPC and DOC pools
584 (Paul et al., 2015), suggesting that the reduced respiration does translate into higher net
585 carbon fixation. This effect was seen from the latter part of Phase II and the trend continued
586 after *t31* (these variables were sampled until *t43*). This increased net carbon fixation did not,
587 however, affect carbon export as there was no detectable difference in the sinking flux
588 measurements (Table 1 and Paul et al. 2015). The results suggest that the increased carbon
589 fixation ended up in the smallest size fraction of TPC not being exported and/or into the
590 dissolved organic carbon pool. Further support for this conclusion is presented in Paul et al.
591 (2015), Crawfurd et al. (2016) and Lischka et al. (2015).
592 In conclusion, this study suggests that elevated CO_2 reduced respiration which in turn
593 increased net carbon fixation. However, the increased primary production did not translate
594 into increased carbon export, and did consequently not work as a negative feedback
595 mechanism for increasing atmospheric CO_2 concentration.
596

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Table 1. Average net primary production (NPP), total respiration (TR) and exported total particulate carbon (Exp_{TPC}) in mmol C m^{-2} d^{-1} ± SE during Phase I and Phase II of the experiment. The pool of total particulate carbon (TPC) is the average during the two periods in mmol C m^{-2} ± SE. The standard error was calculated throughout the period: Phase I, n = 9 and Phase II, n = 7. NPP and TR was corrected for the missing measuring days during Phase I. TR was measured as O_2 consumption and for comparison with carbon fixation we used a respiratory quotient (RQ) of 1.

Phase I (*t0-t16*)						
CO_2 treatment (µatm *f*CO_2)	**346**	**348**	**494**	**868**	**1075**	**1333**
NPP	4.8±0.8	11.4±2.1	14.9±3.6	12.3±2.3	11.3±2.4	14.5±2.7
TR	107±9	82±7	81±6	80±8	75±8	74±8
Exp_{TPC}	6.6±0.10	5.6±0.04	5.4±0.07	6.0±0.07	5.6±0.06	6.0±0.05
TPC	410±25	385±25	402±31	415±33	408±27	424±38
Phase II (*t17-t31*)						
CO_2 treatment (µatm *f*CO_2)	**346**	**348**	**494**	**868**	**1075**	**1333**
NPP	3.8±0.6	11.2±1.9	10.8±2.0	14.3±2.8	10.4±2.1	12.0±2.5
TR	140±7	127±5	103±3	103±4	101±5	86±4
Exp_{TPC}	3.3±0.08	2.6±0.06	2.5±0.08	2.6±0.06	2.8±0.07	2.9±0.06
TPC	301±11	313±11	305±16	316±7	317±5	326±10

Figure legends

Fig 1. The main phytoplankton groups at the start of the experiment, *t*0, and *t*17 (upper panel) and *t*31 (lower panel). The initial (*t0*) was the average of all mesocosm bags. A more detailed description of the temporal development in the phytoplankton community can be found in Bermúdez et al. (2016).

Fig 2. The main micro- and mesozooplankton groups at the start of the experiment, *t*0, and *t*17 (upper panel) and *t*31 (lower panel). The initial (*t0*) was the average of all mesocosm bags. A more detailed description of the temporal development in the phytoplankton community can be found in Lischka et al. (2015).

Fig 3. The development of total particulate carbon (TPC) during the experiment.

Fig. 4. The cumulative primary production in the different fCO_2 treatments normalized to total particulate carbon (TPC) in the euphotic zone. The fCO_2 (µatm) were the average measured over the duration of the experiment. The two lowest fCO_2 treatments (346 and 348 µatm) were controls without any CO_2 addition. The two phases of the experiment is indicated by the horizontal bars on top.

Fig. 5. The cumulative respiration in the different fCO_2 treatments normalized to total particulate carbon (TPC). The fCO_2 (µatm) were the average measured over the duration of the experiment. The two lowest fCO_2 treatments (346 and 348 µatm) were controls without any CO_2 addition. The two phases of the experiment is indicated by the horizontal bars on top.

Fig 6. The respiration rate, normalized to total particulate carbon (TPC), in the different fCO_2 treatments during the latter half of the experiment (*t20 – t31*). Respiration was estimated by linear regression from the data presented in Fig. 4 from the time when an effect of increased CO_2 concentration was first observed. The error bars represent standard error (SE) of the residuals from the linear regression. The solid line represents the linear regression (slope -0.0002; $p = 0.02$; $R^2 = 0.77$) and dotted lines the 95% confidence intervals.

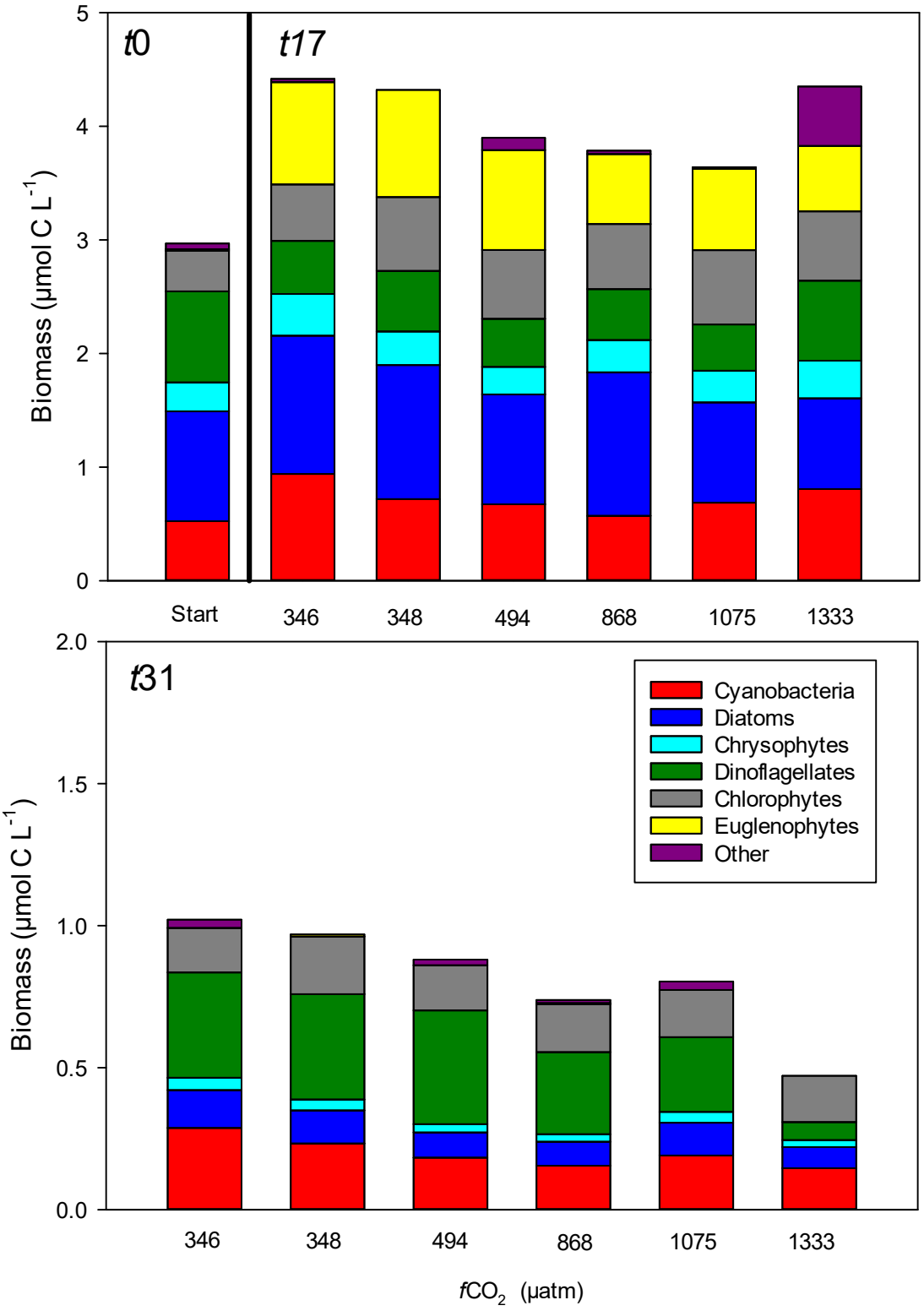
t0
t17
Biomass (µmol C L-1)
0
1
2
3
4
5
Start
346
348
494
868
1075
1333
t31
Biomass (µmol C L-1)
0.0
0.5
1.0
1.5
2.0
346
348
494
868
1075
1333
fCO2 (µatm)
Cyanobacteria
Diatoms
Chrysophytes
Dinoflagellates
Chlorophytes
Euglenophytes
Other

Fig 1

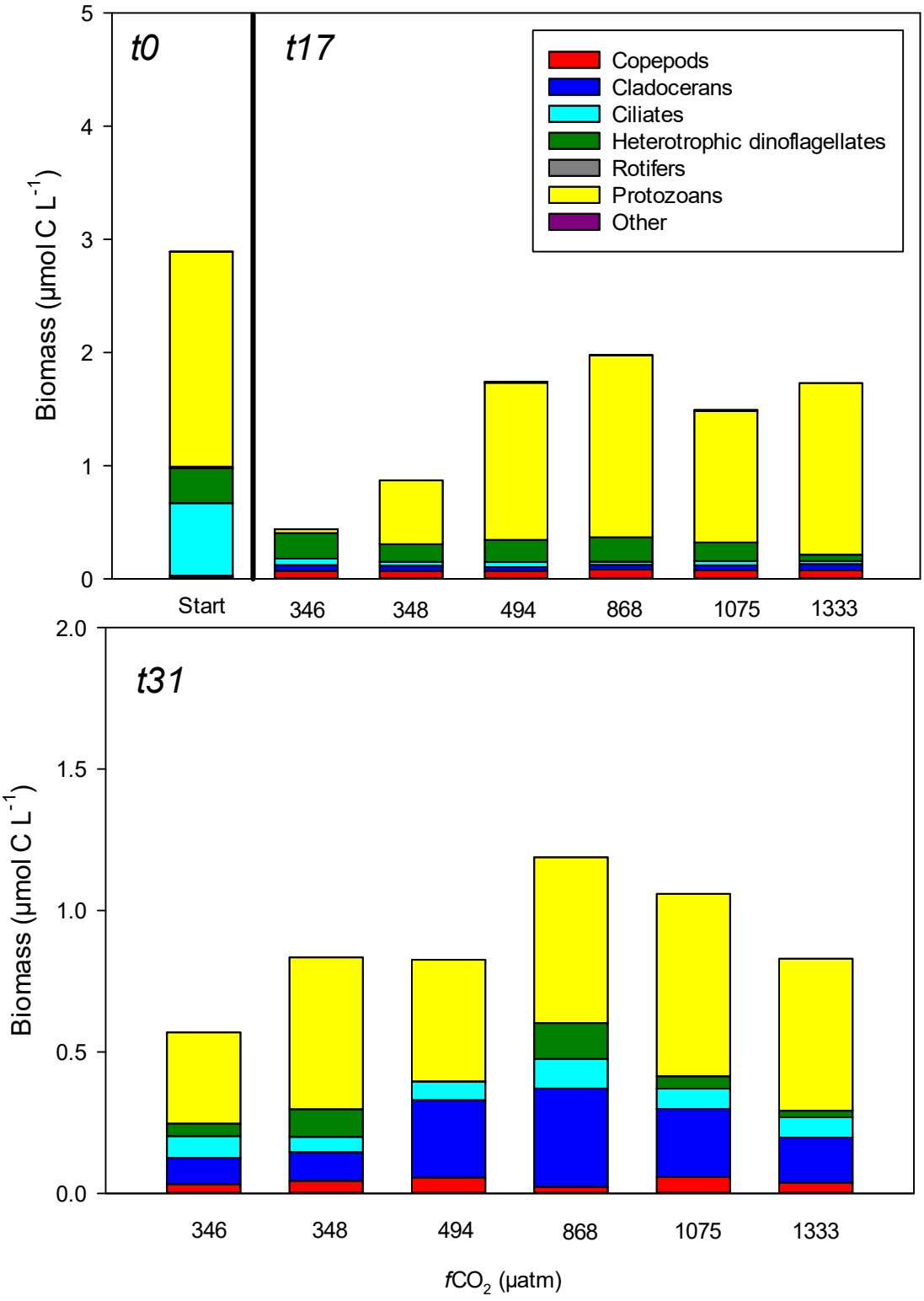
t0
t17
t31
Copepods
Cladocerans
Ciliates
Heterotrophic dinoflagellates
Rotifers
Protozoans
Other
Biomass (µmol C L-1)
Start
346
348
494
868
1075
1333
fCO2 (µatm)

Fig 2

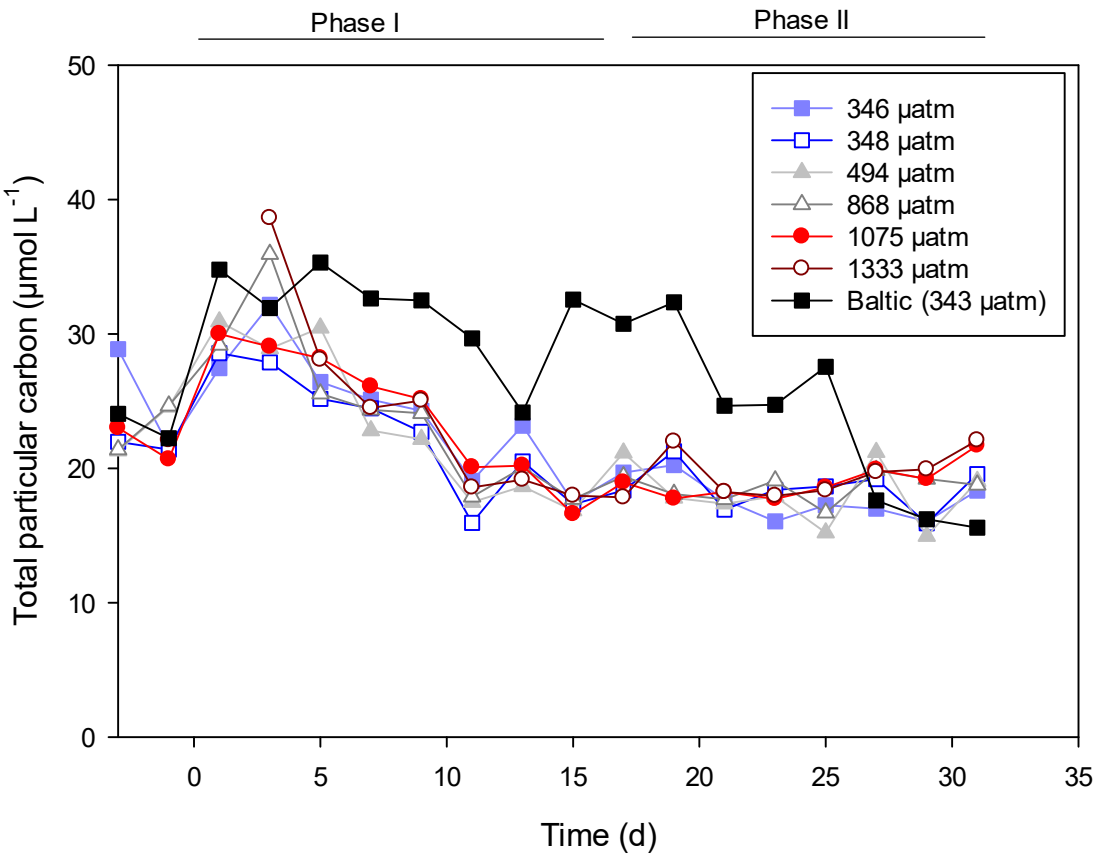
Phase I
Phase II
346 µatm
348 µatm
494 µatm
868 µatm
1075 µatm
1333 µatm
Baltic (343 µatm)
Total particular carbon (µmol L-1)
Time (d)
0
10
20
30
40
50
0
5
10
15
20
25
30
35

Fig 3.

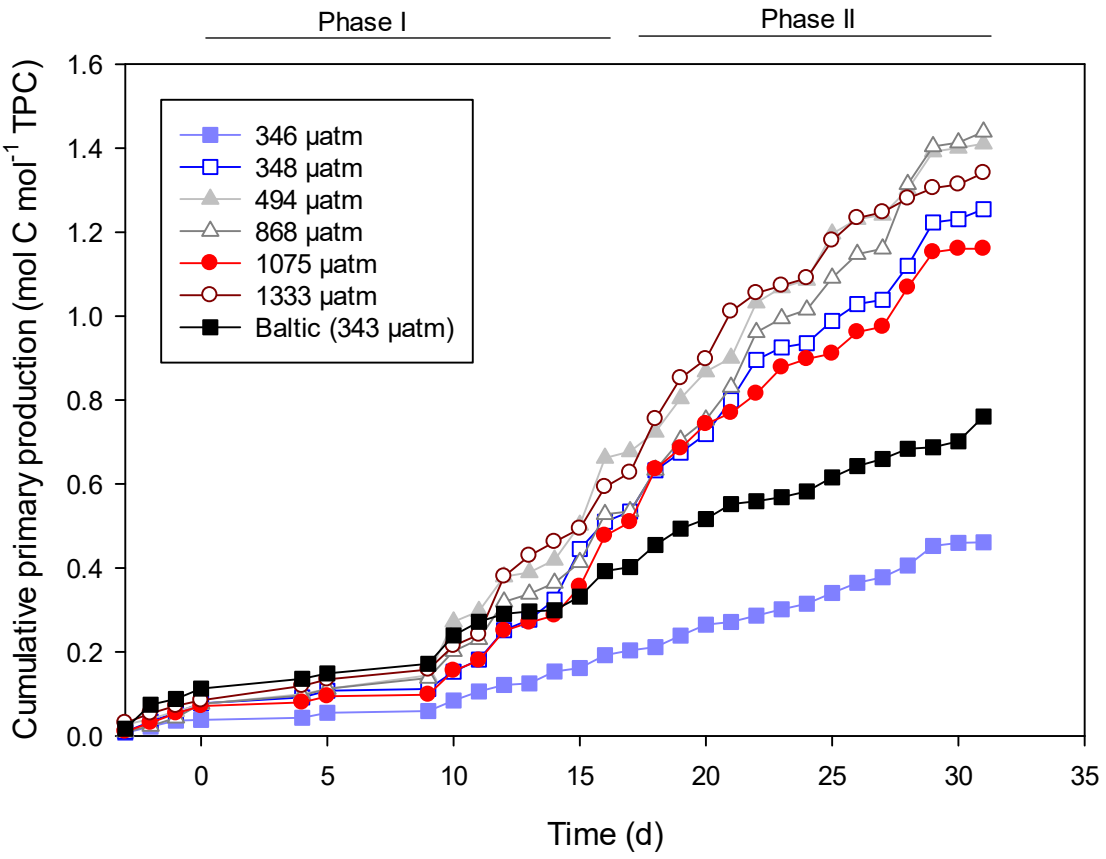
Phase I
Phase II
346 µatm
348 µatm
494 µatm
868 µatm
1075 µatm
1333 µatm
Baltic (343 µatm)
Cumulative primary production (mol C mol-1 TPC)
Time (d)
0.0
0.2
0.4
0.6
0.8
1.0
1.2
1.4
1.6
0
5
10
15
20
25
30
35

Fig 4

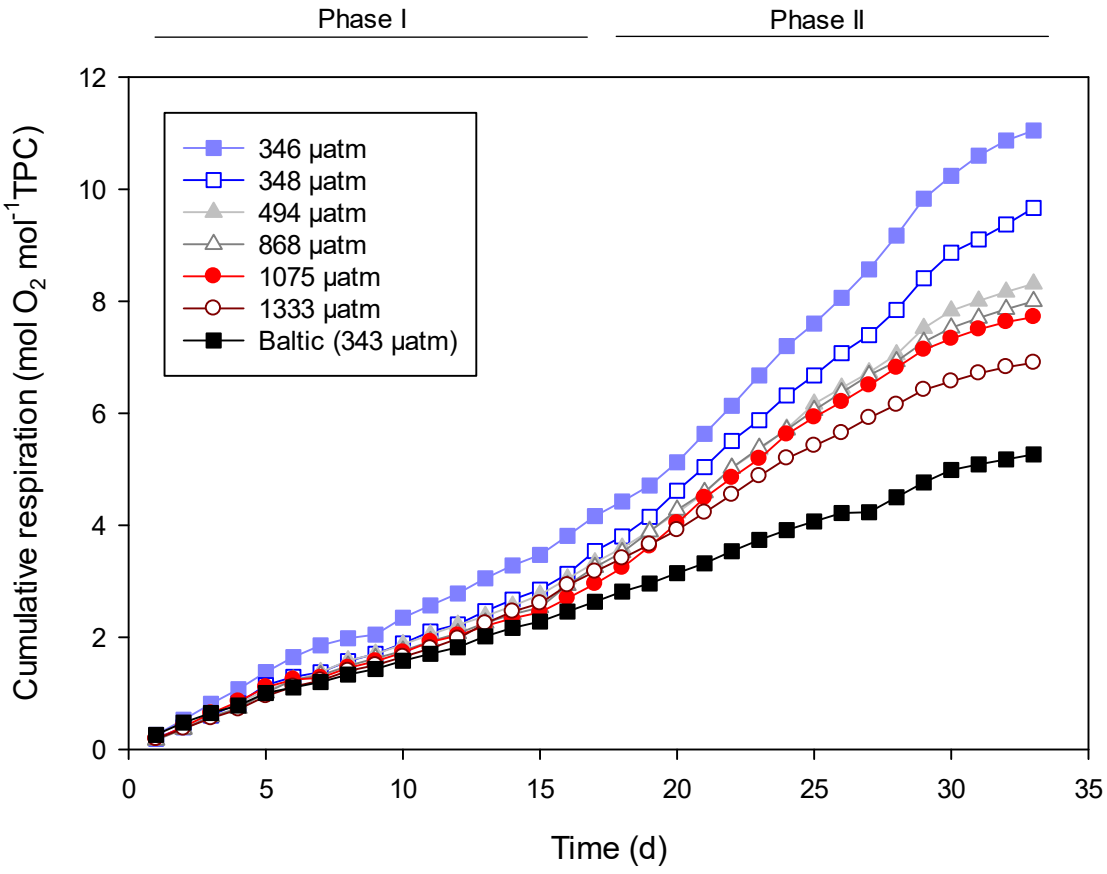
Phase I
Phase II
346 µatm
348 µatm
494 µatm
868 µatm
1075 µatm
1333 µatm
Baltic (343 µatm)
Cumulative respiration (mol O2 mol-1TPC)
Time (d)
0
2
4
6
8
10
12
0
5
10
15
20
25
30
35

Fig 5

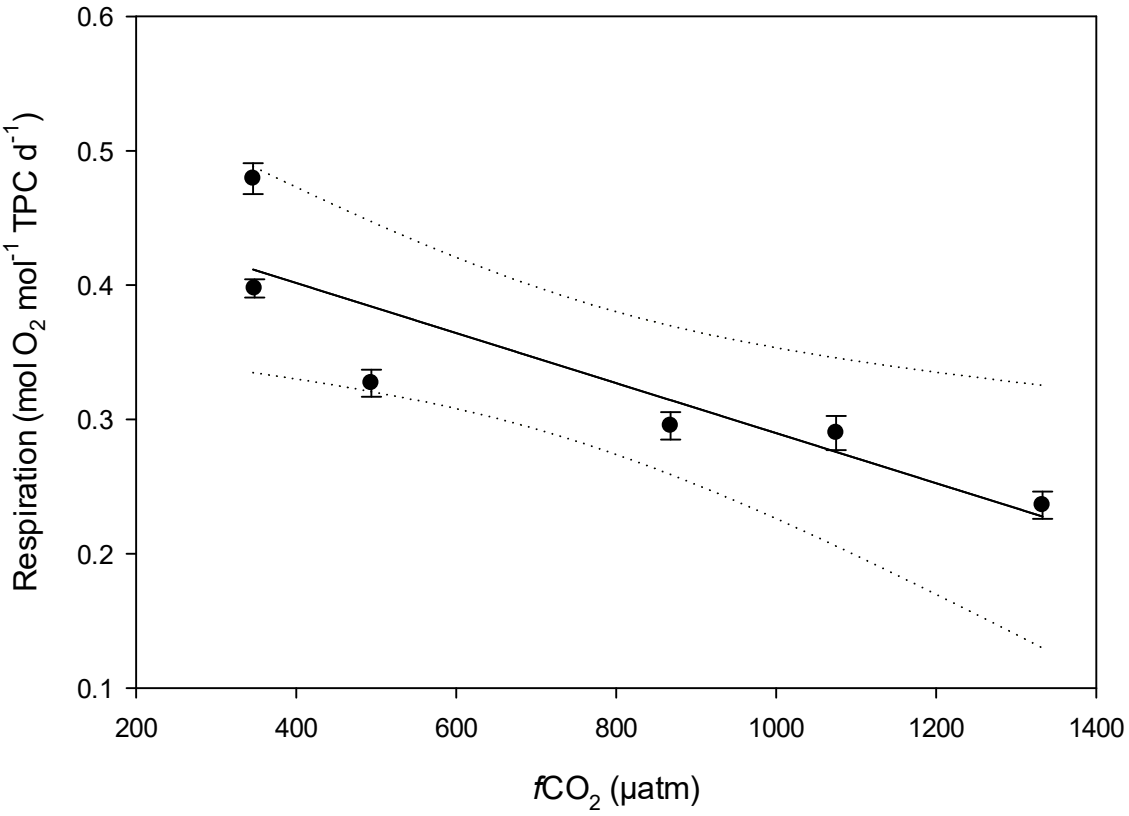
0.6
0.5
0.4
0.3
0.2
0.1
200
400
600
800
1000
1200
1400
Respiration (mol O2 mol-1 TPC d-1)
fCO2 (µatm)

Fig 6