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Ciliate and mesozooplankton community response to increasing CO_2 levels in the Baltic Sea: insights from a large-scale mesocosm experiment

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Abstract.

Community approaches investigating ocean acidification (OA) effects suggest a high tolerance of micro- and mesozooplankton to carbonate chemistry changes expected to occur within this century. Plankton communities in the coastal areas of the Baltic Sea frequently experience pH variations partly exceeding projections for the near future both on a diurnal and seasonal basis. We conducted a large-scale mesocosm CO_2 enrichment experiment ($\sim$ 55 m^3) enclosing the natural plankton community in Tvärminne/ Storfjärden for eight weeks during June–August 2012 and studied community and species/ taxon response of ciliates and mesozooplankton to CO_2 elevations expected for this century. Besides the response to fCO_2, we also considered temperature and chlorophyll *a* variations in our analyses. Shannon diversity of ciliates significantly decreased with fCO_2 and temperature with a greater dominance of smaller species. The mixotrophic *Myrionecta rubra* seemed to indirectly and directly benefit from higher CO_2 concentrations in the post-bloom phase through increased occurrence of picoeukaryotes (most likely Cryptophytes) and Dinophyta at higher CO_2 levels. With respect to meszooplankton, we neither detected significant effects for total abundance nor for Shannon diversity. The cladocera *Bosmina* sp. occurred at distinctly higher abundance for a short time period during the second half of the experiment in three of the CO_2-enriched mesocosms except for the highest CO_2 level. The ratio of *Bosmina* sp. with empty to embryo/resting egg bearing brood chambers, however, was significantly affected by CO_2, temperature, and chlorophyll *a* . An indirect CO_2 effect via increased food availability (Cyanobacteria) stimulating *Bosmina* sp. reproduction can not be ruled out. Although increased regenerated primary production diminishes trophic transfer in general, the presence of organisms able to graze on bacteria such as cladocerans may positively impact organic matter transfer to higher trophic levels. Thus, under increasing OA in cladoceran dominated mesozooplankton communities, the importance of the microbial loop in the pelagic zone may be temporarily enhanced and carbon transfer to higher trophic levels stimulated.

1 Introduction

Since the industrial revolution, anthropogenic CO_2 emissions have increased at an unprecedented rate and cause a concomitant increase of CO_2 concentration in the surface oceans. Thereby, ocean carbonate chemistry is altered with the main changes being reduced carbonate ion concentrations [CO_3^{2-}] and increased proton concentrations [H^+] causing a pH decrease. This phenomenon is nowadays well recognized as ocean acidification (OA). Ocean pH has decreased by approx. 0.1 units already and projections suggest a further decrease of 0.14–0.43 units by the end of the century (IPCC, 2013). The Baltic Sea, one of the largest brackish water systems, is sensitive to CO_2 changes because it naturally has low alkalinity and thus carbonate buffer capacity. Models project a drop of 0.5 pH units for the Baltic Sea by the year 2100 (Hjalmarsson et al., 2008; Havenhand, 2012; Omstedt et al., 2012). Eutrophication specifically affects coastal areas and can add to the fCO_2 fluctuations by provoking low oxygen partial pressure due to increased degradation processes, respectively respiration. Therefore, diel and seasonal variations of carbonate chemistry parameters particularly of coastal areas of the Baltic Sea are already huge today and the amplitude of fluctuations has even increased since the beginning of the industrialization and concomitant eutrophication (Omstedt et al., 2009; Melzner et al., 2013; Jansson et al., 2013). Consequently, zooplankton in the coastal Baltic naturally experiences large pH fluctuations on a daily and seasonal basis and possibly are at least to some extent adapted to these highly variable abiotic conditions (Melzner et al., 2013; Almén et al., 2014).

Ocean acidification is suspected to have severe consequences for marine organisms and acts synergistically with the concurrent temperature increase due to greenhouse gas emissions (Riebesell et al., 2009). Until now, most attempts to test for sensitivities of marine organisms to OA were conducted as single species experiments under controlled laboratory conditions. Such an approach can not account for community interactions in natural environments, and thus application of results to natural environments is limited. Laboratory experiments suggest calcifying organisms to be most vulnerable to OA because the formation and preservation of calcareous structures is hindered (e.g. Riebesell et al., 2000; Hoegh-Guldberg et al., 2007; Lischka et al., 2011). Non-calcareous micro- and mesozooplankton is generally considered quite robust to elevated CO_2 concentrations. Effects on the microzooplankton level seem to be of more indirect nature through changes in primary production, phytoplankton community composition and stoichiometry (Suffrian et al., 2008; Feng et al., 2009; Rossoll et al., 2012). Mesozooplankton is often dominated by copepods (Longhurst, 1985) which are relatively insensitive to fCO_2/pH changes expected for this century and direct negative effects usually do not occur unless exposed to much higher fCO_2 levels projected only much later (Kurihara et al., 2004; IPCC, 2013). More recent evidence suggests, however, that nauplii stages may be the weak point in copepod's life cycles (Cripps et al., 2014). As for the microzooplankton, studies on copepods and cladocerans suggest CO_2 effects may be more indirectly mediated to the zooplankton level through CO_2 induced changes in the biochemical and/or stoichiometric composition of their food (Urabe et al., 2003; Rossoll et al., 2012).

Holistic approaches studying CO_2 effects on entire natural plankton communities including zooplankton are still rare. In a preceding similar mesocosm experiment, Aberle et al. (2013) and Niehoff et al. (2013) found no effects on Arctic micro- and mesozooplankton communities, neither with respect to abundance of

single species or total numbers nor with respects change in community diversity. In terms of ciliates, these communities were dominated by large-sized forms (> 30 μm), in terms of mesozooplankton by copepods and cirripedia larvae.

The Tvärminne/Storfärden area is an open archipelago on the eastern side of the Hanko peninsula on the south-west coast of Finland. Among microzooplankton, ciliates and heterotrophic dinoflagellates dominate in summer in Tvärminne/Storfärden, among mesozooplankton rotifers, copepods and cladocera (Kivi, 1986; Viitasalo, 1992; Koski et al., 1999). In the Tvärminne/Storfärden area during late summer and autumn, the microbial food web (MFW) is of particular importance when filter-feeding cladocerans mediate carbon transfer to higher trophic levels including fish (Koski et al., 1999, and references therein). Summer dynamics of the planktonic food web were described in more detail by Uitto et al. (1997). In general, omnivory dominates across all trophic groups, but the importance of herbivory and feeding on heterotrophs varies during summer. Earlier in summer, heterotrophic nanoflagellates (HNF) transfer carbon from picoplankton to ciliates, and ciliates constitute the link from nano- to metaozooplankton. Later in summer, HNF were largely bacteriovorous transferring bacterial carbon to ciliates and metazooplankton, when phytoplankton > 10 μm was grazed by metazooplankton and heterotrophic dinoflagellates. In July, < 10 μm phytoplankton increased and protists became the most important herbivores and the efficiency of the MFW in transferring bacterial carbon to metazooplankton was measured highest. However, the amount of carbon transferred to higher trophic levels depends also on the mesozooplankton species composition (Hansen et al., 1994). Elevated CO_2 concentrations can be beneficial for some phytoplankton groups, in particular picoeukaryotes. For micro- and mesozooplankton communities, so far no effects have been shown at least for CO_2 ranges projected to occur within this century (Aberle et al., 2013; Niehoff et al., 2013; Schulz et al., 2013).

As part of the KOSMOS Tvärminne mesocosm experiment, we examined CO_2 effects on the enclosed ciliate and mesozooplankton community. A map showing the study site and mesocosm moorings is included in Paul et al. (2015). Between June and August 2012, an fCO_2 gradient was set up in six approximately 55 m^3 mesocosms covering fCO_2 projections for this century or beyond (IPCC, 2013). Abundance and community composition was followed through enumeration of regularly taken water- and net samples. Per definition, mesozooplankton include metazoa ranging between 0.2–20 mm (200–20,000 μm) in size. In this study, we do not follow this classification strictly as we also included the smaller juvenile life stages (< 200 μm) to the category 'mesozooplankton' (MZP). In case of protozoa we focus on ciliates only. Other protozoa including Dinophyta and their response to CO_2 elevations are included in Bermúdez et al. (2016). Ciliates in our study include some species that can be facultative autotrophs or obligate mixotrophs (for instance *Myrionecta rubra*).

Temperature can have a general effect on microzooplankton (MiZP) abundance and community composition and governs the dynamics of crustacean species (for instance affects productivity of cladocerans) in late summer in our study area (Nanazato and Yasuno, 1985; Koski et al., 1999; Rose et al., 2009; Aberle et al., 2013). Furthermore, temperature changes towards a warming ocean are underway concurrently with ocean acidification with the potential to impact pelagic communities by providing suboptimal temperature

conditions for species (IPCC, 2013). To consider possible impact of temperature variation and/or CO_2 driven chlorophyll *a* differences (Schulz et al., 2013), we also included temperature and chlorophyll *a* as explanatory variables in our statistical analyses.

2 Methods

To study the effect of elevated fCO_2 on a natural plankton community in the Baltic Sea, nine KOSMOS offshore pelagic mesocosms (**K**iel **O**ff-**S**hore **M**esocosms for future **O**cean **S**imulation) were deployed and moored on 12 June 2012 until the middle of August in the Tvärminne/ Storfjärden archipelago area at the south-west coast of Finland at 59°51.5' N and 23°15.5' E. The water depth at the mooring site was approximately 30 m. The mesocosm bags extended down to 17 m and were closed with 2 m long sediment traps at the bottom of the bags to enclose an isolated water body with its natural plankton community. After deployment, the mesocosm bags were initially kept open and submerged ~0.5 m below the surface to allow for a free exchange of the water and plankton community in the bags with the surrounding water masses. Organisms > 3 mm such as fish and cnidaria were excluded by 3 mm nets at the top and bottom openings of the bags during the first five days. These nets were removed on t_{-7} (i.e. seven days before the first CO_2 addition on t_0), the sediment traps were attached to the bottom, and the top ends of the mesocosm bags pulled up to 1.5 m above the surface to isolate the enclosed pelagic community from the Baltic Sea. The final volumes of the mesocosms ranged between 53.1 and 55.1 m^3 (Paul et al., 2015). The nine mesocosms were enriched with different amounts of CO_2 saturated seawater to set up an initial gradient of fCO_2 from 240 μatm (ambient, control mesocosms) up to ~1650 μatm. Three mesocosms (M2, M4, M9) were lost during the course of the experiment due to leakage. fCO_2 values in the six remaining mesocosms averaged over the sampling period (t_1–t_{43}) were 365 μatm (M1 control), 368 μatm (M5, control), 497 μatm (M7), 821 μatm (M6), 1007 μatm (M3) and 1231 μatm (M8). CTD profiles and samples for dissolved inorganic nutrients (silicate, phosphate, nitrate, nitrite, ammonium) and carbonate chemistry system parameters (DIC, TA, pH_T) were either taken daily or every second day. For more technical details about the experimental set-up, the CO_2 manipulations, and sampling procedures for various analyses see Paul et al. (2015). Sampling days were enumerated consecutively with t_{-3} indicating three days before CO_2 manipulation, t_0 as the day of the first CO_2 manipulation, and t_{1+X} as the days following the first CO_2 manipulation.

2.1 Microzoolankton sampling

Water samples for the enumeration of ciliates were taken every second day with a depth-integrating sampler (0–17 m), IWS (HYDRO-BIOS, Kiel, Germany), between 9:00 and 12:00 am from six mesocosms. After careful mixing, 250 ml of seawater were filled into brown-glass bottles and preserved in acidic Lugol's iodine (1% final concentration). 50 ml of the sample were transferred to Utermöhl sedimentation chambers. After 24 h settling time, ciliates were counted with a Zeiss Axiovert 100 inverted microscope at 200 x magnification Utermöhl (1958). 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., 1995), and according to descriptions found at the planktonic ciliate project (http://ciliate.zooplankton.cn/). 138 samples were analyzed in total. Abundances were calculated as cells l^{-1}.

2.2 Mesozooplankton sampling

Mesozooplankton samples from six mesocosms were taken with an Apstein net of 17 cm diameter and 100 μm mesh size. Zooplankton were sampled between 08:00 and 11:00 am by towing the net vertically from 17 m depth to the mesocosm surface. In total, at eleven sampling days, vertical net hauls were done from the mesocosms: prior to the CO_2 addition (t_{-3}, t_{-2}, t_{-1}), at the day of the first CO_2 addition (t_0), and after the first CO_2 addition (t_3, t_{10}, t_{17}, t_{24}, t_{31}, t_{38}, t_{45}). After collection, the samples were brought back to the laboratory in the Tvärminne zoological station (University of Helsinki) and preserved in 70% ethanol. Zooplankton abundance was calculated assuming 100% filtering efficiency of the net. The samples were divided with a Folsom plankton splitter (1:2, 1:4, 1:8, 1:16, 1:32) and the aliquots of the samples were counted. Organisms were counted and determined under a stereo microscope (WILD M3B) to the lowest taxonomical level possible. Abundant species/taxa (> 30 individuals in an aliquot) were only counted from subsamples, while less abundant species/ taxa were counted from the whole sample. Juvenile bivalves did not distribute equally in the Folsom splitter due to their relatively large mass and were therefore counted from the whole sample. Copepods (*Acartia* spp., *Eurytemora* spp., *Temora* spp.) were identified according to different stages (adult females, adult males, copepodite stages CI–CV). Copepod nauplii were counted but not determined to species level. The counting of the cladoceran species (*Bosmina* spp., *Evadne* spp., *Podon* spp.) was distinguished according to organisms with empty or filled brood chambers, respectively (i.e. organisms that had empty brood chambers or bore embryos/resting eggs, respectively, in their brood chambers) and categorized as 'empty' or 'filled'. For data analyses, the ratio between the number of organisms with 'empty' to 'filled' individuals was calculated for each mesocosm and sampling day, i.e. a small ratio stands for a higher proportion of reproducing organisms in the population in a particular mesocosm at a particular sampling day. A total of 66 samples were analyzed. Abundances were calculated as individuals m^{-3}.

2.3 Data analysis and statistics

To assure equally spaced data, some sampling days were excluded from statistical analyses. For the ciliate data this applied to t_{-3}, t_0, t_2 and t_4, and for the mesozoopankton this applied to t_{-3}, t_{-2}, t_{-1} and t_0. However, for demonstration purpose only, the data of these sampling days were included in the figures.

As explanatory variables, fCO_2, temperature and chlorophyll *a* were used to test for effects on different response variables (see below). Collinearity was checked prior to analyses. To account for the change in fCO_2 over time due to ingassing/outgassing as well as temperature and chlorophyll *a* changes over time, all explanatory variables were used as continuous variable for each *t*-day included in the analyses. All analyses

were carried out with R using the package nlme, mgcv, Hmisc and MASS. All plots were done in ggplot (Team, 2012).

The Shannon index (H) was calculated as a measure of diversity in each of the mesocosms and to estimate changes in the relative contribution of single species/groups in the whole ciliate/mesozooplankton community over time and in response to different abiotic parameters such as the fCO_2 levels. When all considered species/groups contribute equally to the community in terms of their abundances, H calculated on the natural logarithm becomes 2.3. The more a community is dominated by single species/group, the smaller the Shannon index gets. Calculations of H were performed in the vegan package of the R environment (Oksanen et al., 2012).

For the **ciliates**, 14 species/groups were included to calculate H: *Balanion comatum*, *Strombidium* cf. *epidemum*, *Mesodinium* sp., *Myrionecta rubra* ($\leqslant$ 10 μm), *M. rubra* (11–20 μm), *M. rubra* (> 20 μm), *Rimostrombidium* sp., *Spathidium* sp., *Strobilidium* spp. ($\leqslant$ 20 μm), *Strobilidium* spp. (> 20 μm), *Strombidium* sp., Tintinnids, cysts (*Strobilidium* sp., unidentified cysts), and ciliates sp. (*Euplotes* sp., *Lacrymaria* sp., *Strobilidium* sp., unidentified ciliates). *Lohmaniella* sp.could not be clearly separated from other Strobilids and was therefore included with Strobilidium spp. ($\leqslant$ 20 μm). Most of the Strobilids found, however, were probably *Lohmaniella* sp..

For the **meosozooplankton**, 17 species or taxonomic groups were included in the calculation of H: copepodite stages and larval stages of *Balanus* sp. (nauplii and cypris larvae) were summarized on the genus level (Copepoda: *Acartia* sp., *Eurytemora* sp., *Temora* sp., Harpacticoida sp., copepod nauplii; Cladocera: *Bosmina* sp., *Daphnia* sp., *Evadne* sp., *Podon* sp.; Rotifera: *Asplanchna* sp., *Keratella* sp., *Synchaeta* sp., Rotifera sp.; larvae of *Balanus* sp., juvenile bivalves, juvenile gastropods, and larvae of polychaets).

2.3.1 Ciliates

Statistical analyses were done on total cell numbers, the Shannon index H as well as the abundance of particular groups that showed distinct differences such as small size-class *Myrionecta rubra*, *Balanion comatum*, *Strombidium* cf. *epidemum*, and small *Strobilidium* sp.. Linear mixed effects modelling (LME) was applied on a Gaussian distribution to determine the effect of CO_2, temperature and chlorophyll *a*. Actually, count data should be modelled on a Poisson distribution, but model selection (s.b.) yielded in convergence problems in R for Poisson distribution. Therefore, we used a Gaussian distribution, which can also be applied on count data (Zuur et al., 2009). If preceding data exploration suggested interactions between the factors, respective interaction terms were included in the model. Model selection was based on the Akaike information criterion (AIC) by removing non-significant terms to find the simplest adequate model. However, missing values for chlorophyll *a* occurred for M3/t_{25} and for M5/t_{23}, these values were estimated as means of the preceding and following day. Chlorophyll *a* values were also missing for t_{41} and t_{43}. A polynomial fit curve applied on phase III (according to temperature variations, three experimental phases (I, II, III) were defined which are thoroughly introduced in Paul et al. (2015). Phase III lasted from t_{31} until t_{43}.) resulted in no meaningful values, therefore these values were estimated as phase III means.

The different response variables were modelled as a function of the daily change in fCO_2, temperature and chlorophyll *a* and if suggested with interaction terms as mentioned above. To account for the time dependency and the nested nature of the data, GLM models (generalized mixed effects) were applied on a Gaussian distribution using fCO_2 (values on a continuous scale for each sampling day) and sampling day nested in mesocosm as random intercept. In case of violation of the assumptions for linear models yielding to non trustworthy p-values, the GLM model was re-applied as a GA(M)M (generalized additive (mixed) model) and a smoother for sampling day included to prove the validity of the GLM outcome. In some cases, some residual patterns mostly due to sampling day still remained even after applying the GAMM. But GAMM is as much as can be done with current hard- and software, and therefore, for highly significant p-values, our results should still be reasonably robust, and p-values that are not highly significant should be seen with some caution (Zuur et al., 2009).

2.3.2 Mesozooplankton

The statistical approach with respect to MZP corresponded with description in section 2.2.1. Total abundance, the Shannon index *H* as well as total abundance of species that suggested distinct differences such as *Bosmina* and the ratio of *Bosmina* with empty to individuals with full brood chambers (i.e. either bearing embryos or resting eggs in their brood chambers) were analyzed statistically. Missing values for fCO_2 occurred on t_{24}, t_{38} and t_{45}, and for temperature, and chlorophyll *a* on t_{38} and t_{45}. Missing observations for t_{24} and t_{38} were estimated by building the mean of values measured at t_{23}/t_{25} and respectively t_{37}/t_{39}. t_{45} was the last sampling day and hence it was not possible to estimate a mean from the preceding and following day. Therefore missing values for t_{45} were estimated from a polynomial fit curve applied on phase III values (Paul et al., 2015).

2.3.3 Predator/prey relationships

Pearson correlation was used to investigate possible trophic relationships between ciliates and MZP, respectively, and bacteria, nano- and picoeukaryotes (total bacteria, low DNA bacteria, high DNA bacteria, Cyanobacteria, particle associated bacteria, *Synechococcus*, pico- and nanoeukaryotes), and phytoplankton groups (Prasinophytes, Cryptophytes, Chlorophytes, Cyanobacteria, Diatoms, Euglenophytes, auto- and heterotrophic dinoflagellates, and heterotrophic dinoflagellates excluding *Ebria* sp.). For these correlations, data from Crawfurd et al. (2016) and Paul et al. (2015) were used.

3 Results

3.1 Ciliates

3.1.1 Ciliate total abundance

Total abundance of ciliates at experiment start (t_0) varied between 78,120 cells l^{-1} (M5) and 52,360 cells l^{-1} (M3) and more or less continually decreased from the beginning over time until t_{17} when a plateau was

reached with low cell numbers between 7,080 (M8) and 10,940 (M3) until t_{33}. During the last five sampling days (t_{35}–t_{43}), total cell numbers were more variable again with some small ups and downs and reached minimum values between 900 cells l^{-1} (M6) and 3,580 cells l^{-1} (M8) on the last sampling day (Fig. 1).

3.1.2 Abundance of *Myrionecta rubra*

Myrionecta rubra was (by far) the most dominant ciliate species during the entire period (Fig. 2a). *M. rubra* occurred in three different size classes ($\leqslant$ 10 μm, 11–20 μm, > 20 μm) of which organisms of the smallest size range made up the highest numbers. On t_0 cell numbers of *M. rubra* of the smallest size class varied between 26,720 cells l^{-1} and 44,520 cells l^{-1}. Cell numbers stayed relatively high until t_{11}/t_{13} (16,600–37,400 cells l^{-1}) when they strongly declined to values below 10,000 cells l^{-1} on t_{17} and further decreased with some fluctuations until the end of the experiment to reach final values of between 130 cells l^{-1} and 1,740 cells l^{-1} among all mesocosms. Some striking difference, however, occurred between t_{25}–t_{35} when abundance in the three highest CO_2 mesocosms was higher compared to the two controls and the lowest CO_2 enriched mesocosm (mean: 4,518 cells l^{-1} (SD 1,082) and mean: 3,459 cells l^{-1} (SD 383), respectively). *M. rubra* of the medium size class also had maximum numbers on t_0 ranging from 17,600 cells l^{-1} to 25,680 cells l^{-1}. From the experiment start, numbers more or less continually decreased and reached minimum values of between 480 cells l^{-1} and 0 cells l^{-1} from t_{19} on. The largest *M. rubra* occurred only rarely but as in the other two size classes, highest numbers were found during the first few sampling days varying between 2,680–5,800 cells l^{-1} on t_0 and reaching very low numbers already on t_7/t_9 (1,080–280 cells l^{-1}). After t_{19}, *M. rubra* > 20 μm occurred only exceptionally.

3.1.3 Abundance of other species/genera/groups

Other dominant groups/species that contributed to the total cell numbers of ciliates were *Balanion comatum*, *Strombidium* cf. *epidemum*, *Strobilidium* sp. (< 20 μm and > 20 μm), *Mesodinium* sp., *Rimostrombidium* sp., *Strombidium* sp., Tintinnids, *Spathidium* sp., cysts, and ciliates that could not be identified (Fig. 2b, 2c). Among those, *Strombidium* cf. *epidemum* was most dominant and showed three peaks, around t_9/t_{11}, t_{23}, and t_{37}. On t_9/t_{11} some distinct difference occurred between control and CO_2 enriched mesocosm (mean: 1,250 cells l^{-1} (SD 180) and mean: 2,205 cells l^{-1} (SD 851), respectively). *Balanion comatum*, *Rimostrombidium* sp., *Strobilidium* sp. (< 20 μm), *Spathidium* sp., and tintinnids were of some importance during the first days of the experiment showing peaks in cell numbers between t_0 and t_{11}. Most interestingly, peak abundance of *Balanion comatum* diverged with CO_2 concentration with higher mean cell numbers in the control and lowest enriched mesocosm compared to the three high CO_2 mesocosms (mean: 1680 cells l^{-1} (SD 139) and mean: 880 cells l^{-1} (SD 223), respectively). Likewise, small *Strobilidium* sp. developed some CO_2 related difference with mean abundance of 1,360 cells l^{-1} (SD 170) and 2,400 cells l^{-1} (SD 872) in the two controls and the CO_2 enriched mesocosms, respectively. *Mesodinium* sp., *Strobilidium* sp. > 20 μm, cysts and unidentifiable ciliates occurred always in relatively low cell numbers (mostly < 850 cells l^{-1}).

3.1.4 Percent contribution of numerically dominant species/genera/groups to total cell numbers

Fig. 3a shows the percent contribution of dominant species/ genera/ groups to the total cell numbers over time for each of the mesocosms. For better clarity, *Myrionecta rubra* size classes, *Strobilidium* sp. size classes together with *Rimostrombidium* sp., *Strombidium* spp. and cysts together with ciliates sp. were combined. *M. rubra* dominated the ciliate community in all mesocosms most of the time. During the first days of the experiment, *M. rubra* contributed $\sim$ 90% to the total cell numbers in all mesocosms and stayed above 50% until t_{21}. Minimum contributions occurred on t_{37} when *M. rubra* had a share of only 6–24%. After t_{37}, *M. rubra* proportions ranged between 18% and 67%. The second most important group was *Strombidium* sp. and among this *Strombidium* cf. *epidemum*. *Strombidium* sp. had highest shares during the second half of the experiment varying between 58% and 69% during t_{35}–t_{39}. All remaining groups usually had contributions below 15%.

The Shannon diversity index H ranged from 0.58–1.66 over the whole period of time (Fig. 3b). In general, it showed a slightly increasing trend varying between 1.04 and 1.23 on t_{-3} and, respectively 1.30 and 1.66 on t_{43} and was generally lower during higher temperature phases (I + II) (Fig. 3c).

3.1.5 Statistical analyses ciliates

GAMM's determined significant synergistic effects for total abundance of small size class *Myrionecta rubra* in response to fCO_2*temperature (p = 0.024) and fCO_2*chlorophyll *a* (p= 0.004). Total abundance of *Balanion comatum* was affected by temperature and fCO_2 ($p_{temperature}$ = 0.022; p_{fCO_2} = 0.03), total abundance of *Strombidium* cf. *epidemum* by chlorophyll *a* (p = 0.002), that of *Strobilidium* sp. showed synergistic responses to the combination of the factors fCO_2*temperature and fCO_2*chlorophyll *a*, respectively (p = 0.0005 and p = 0.0002, respectively), and for the Shannon index H a synergistic effect between fCO_2*temperature was determined (p = 0.0008). Depiction of the statistical results of H showed a non-monotonic relationship with a slightly increasing trend at lower fCO_2 and a decreasing trend the more the fCO_2 increased, as well as a decreasing trend with temperature (Fig. 4a, 4b). Statistical results are shown in more detail in Table 1. Model validation showed some residual pattern in all cases, but most of the obtained p-values are highly significant and are therefore reasonably trustworthy (Zuur et al., 2009). Only with respect to *Balanion comatum*, p-values should be seen with some caution as they are not highly significant.

3.2 Mesozooplankton

3.2.1 Mesozooplankton total abundance

After a sharp initial decrease, total abundance of mesozooplankton increased continuously until peak abundances were reached between t_{24} and t_{31} (Fig. 5). M7, M6, and M3 (497–1007 μatm) had highest peak values ranging between 130,276 ind. m^{-3} and 162,082 ind. m^{-3}, whereas abundance in M1 and M8 were somewhat lower with 111,980 ind. m^{-3} and 90,975 ind. m^{-3}, respectively. In M5, no abundance peak occurred but zooplankton developed a plateau between t_{24} until t_{38} of around 70–74,000 ind. m^{-3}. Towards the end of the

experiment, zooplankton total abundance returned to about the initial values (29,325–44,824 ind. m^{-3} in M8 and M1, respectively).

3.2.2 Community composition

The mesozooplankton community was dominated by five taxonomic groups, i.e. cladocera (*Bosmina* sp., *Daphnia* sp., *Evadne* sp., *Podon* sp.), copepoda (*Acartia* sp., *Eurytemora* sp., *Temora* sp., copepod nauplii, Harpacticoida, Cyclopoida, Copepoda sp.), crustacea (*Balanus* sp., inlcuding nauplii and cyprid larvae), mollusca (juvenile Bivalvia and Gastropoda) and rotifera (*Asplanchna* sp., *Keratella* sp., *Synchaeta* sp., Rotifera sp.). The group 'others' comprises larvae of Bryozoans (cyphonautes), juvenile Polychaeta, and unidentifiable organisms (Fig. 6). Among these groups, cladocerans and copepods dominated the zooplankton community during the entire experimental period. Cladocerans contributed mostly between 50% and 95% to the total abundance. Copepods had their highest share half way through the experiment when they constituted 74–84% (t_{17}) of the whole community. Rotifera were a major part of the zooplankton only during the first days of the experiment with about 11% to 42% between t_{-1} and t_3. Among the group mollusca, gastropods always had a smaller share than bivalves with usually below 2% (max. 5%) contribution to the total abundance of this group. Juvenile bivalves mainly occurred from the start until day t_{10} and had maximum contributions of 17–45% to the total zooplankton community between t_{-2} and t_0. The group 'crustacea' comprises mainly larvae of *Balanus* sp. (nauplii and cyprids). Only very rarely a mysid was found and specimen of this order were also included in the group crustacea. The main occurrence of 'crustacea' was from t_{-1} until t_{10} contributing between 10% and 2% to the total zooplankton community during this time. The group 'others' always contributed less than 0.5% to the total abundance.

In all mesocosms, the Shannon diversity index was highest at the beginning of the experiment (T_3: 1.78–1.89) and decreased continuously with time reaching lowest values on the last sampling day (T_{45}: 0.23–0.5) indicating that towards the second half of the experiment and at the end, the dominance of single species/ groups increased.

3.2.3 Copepoda

Eurytemora sp. was the dominant copepod species in the zooplankton community over the entire period. *Acartia* sp. occurred regularly but in much lower abundances. *Temora* sp. occurred only in very low numbers mainly during the first part of the experiment (Fig.7a). The abundances of *Eurytemora* sp. were relatively low at the beginning (82–2,496 ind. m^{-3}). Peak abundances were reached around day t_{17} and t_{24} (19,192–32,297 ind. m^{-3}) and then declined. During the course of the experiment, *Acartia* sp. varied in numbers between 117 ind. m^{-3} and 4624 ind. m^{-3} and did not show clear abundance peaks in most of the mesocosms. *Temora* sp. was present during the whole time (though not always in all mesocosms) but always in low abundances ranging between 330 ind. m^{-3} and 3 ind. m^{-3} among all mesocosms. Copepod nauplii occurred during the entire experiment duration with peak abundance between t_{10} and t_{24} (9,003– 33,555 ind. m^{-3}).

The three copepod species were determined to copepodite stages (CI–CV) and adult females and males (Fig.7b). *Eurytemora* sp. copepodites CI–CV were present in high proportions almost during the whole period of time with up to > 90%. Adult females and males had their minimum during the abundance peak of this species (t_{17}–t_{31}) but occurred during the entire study period indicating more or less continuous reproduction in all mesocosms. At the beginning and towards the end of the study, most of *Acartia* sp. were in the copepodite stage CI–CV. Adult females and males occurred during the whole period of time and had maximum proportions half way through the experiment (t_{17}, t_{24}). During this time, reproduction took place indicated by the following increase in copepodite stages during the second half of the study. The stage distribution of *Temora* sp. was similar to *Acartia* sp. with a peak of copepodite stages CI–CV during the first and the last sampling days. Most of the time, however, adult females and males dominated.

3.2.4 Cladocera

Four species of cladocera were found in the mesocosms: *Bosmina* sp., *Podon* sp., *Evadne* sp. and *Daphnia* sp. *Daphnia* sp. occurred only rarely in very low abundances (< 0.5% contribution to total cladocera, abundance range: 2.6–12.8 ind. m^{-3}). *Evadne* sp. had maximum abundances on t_3/t_{10} (184 ind. m^{-3}–3,893 ind. m^{-3}) and contributed up to 38% to this group during the first days of the experiment but decreased noticeably in importance later. *Podon* sp. dominated among the cladocerans at the beginning of the experiment accounting for more than 80% of the total abundance until day t_{10} (max. numbers: 43,688–15,272 ind. m^{-3}). By day t_{17} *Bosmina* sp. reached more than a 90% share until termination of the experiment. Peak abundance of *Bosmina* sp. occurred between t_{24}–t_{38} and was substantially higher in the medium range CO_2 mesocosms M7 (497 μatm), M6 (821 μatm) and M3 (1007 μatm) (138,394 ind. m^{-3}, 114,169 ind. m^{-3}, 127,080 ind. m^{-3}, respectively) compared to the two controls M1, M5 and the highest CO_2 mesocosm (M8, 1231 μatm) (72,020 ind. m^{-3}, 58,107 ind. m^{-3}, 63,182 ind. m^{-3}, respectively) (Fig. 8a, only *Bosmina* sp. is shown).

The counting of the two dominant cladoceran species *Podon* sp. and *Bosmina* sp. was divided into organisms with empty brood chambers and organisms bearing embryos/ resting eggs in their brood chambers to inspect for a possible direct or indirect effect of CO_2 on asexual/ sexual reproduction and subsequently a ratio was calculated, s.a. Mostly, the percent contribution of organisms with filled brood chambers varied between 40% and 10% in all mesocosms among the study period. Only during the very first days, *Bosmina* sp. with filled chambers had contributions of up to 67% (not shown). The ratio of *Bosmina* brood chambers varied during peak occurrence (t_{24}–t_{31}) between 3.47 (M8) and 17.18 (M7) (Fig. 8b). During times of high *Podon* sp. abundances, the share of this organism with full brood chambers varied roughly between about 25% and 50%. *Podon* actively reproduced during the first days of the experiment indicated by a low ratio of organisms with empty/ full brood chambers (0.79–2.77), whereas lowest reproductive activity occurred on t_{17}/t_{24} (5.09–33.10) (not shown).

3.2.5 Statistical analyses mesozooplankton

For total abundance of mesozooplankton we determined no significant relationship with fCO_2 or any of the other explanatory variables (temperature, chlorophyll *a*) (Table 1).

The cladocera *Bosmina* sp. showed distinct abundance peaks in M7, M6, and M3 with approx. 110–130 ind. 10^3 m^{-3} higher numbers between t_{24} and t_{31} compared to the two control mesocosms and M8. The GLM model revealed neither a significant relation of the total abundance of *Bosmina* sp. with fCO_2 nor temperature. Chlorophyll *a* concentration was determined to significantly affect the *Bosmina* occurrence but model validation showed heterogeneity of the residuals mostly due to experiment day. Running the GAMM model with a smoother on experiment day did not confirm this result.

GAMM analysis on the ratio between *Bosmina* with empty brood chambers to organisms with full brood chambers yielded in significance of all three main terms as well as in a significant interaction term between fCO_2 and chlorophyll *a* (p = 0.01). Some minor residual structure remained after GAMM on the *Bosmina* ratio that should be kept in mind with respect to resulting p-values (Zuur et al., 2009).

According to a GAMM applied on the Shannon diversity index *H*, neither of the factors significantly affected MZP species diversity.

3.2.6 Predator/prey relationships

Pearson correlation coefficients larger than $\pm$ 0.7 are listed in Table 2 and shown in the supplementary material (Fig. S1−S2). *Myrionecta rubra* and *Bosmina* sp. turned out to be of particular importance in this study. Therefore, in the following, we focus on correlations of these two species with particular phytoplankton and bacteria groups, respectively. *M. rubra* positively correlated with Cryptophytes and heterotrophic Dinoflagellates, whereas the species negatively correlated with Cyanobacteria and low DNA bacteria. Pearson correlation for the different size classes of *M. rubra* were very similar when determined for all fCO_2 levels (0.8; 1.0; 0.9) or low (0.8; 0.9; 0.8) and high (0.8; 1.0; 0.9) levels separate, respectively. *Bosmina* sp. showed a strong positive correlation with Cyanobacteria (0.7). Fig. 9 depicts the succession of the two species in relation to the mentioned potential prey organisms during the course of the experiment.

4 Discussion

4.1 Ciliates

4.1.1 Ciliate succession

The ciliate abundance and species succession in our experiment corresponded well with description by Kivi (1986) on annual succession of protozooplankton in Tvärminne/Storfjärden. In May, shortly after the chlorophyll maximum, this author observed the highest protozoan biomass whereas a minimum was found in June/July two weeks after the spring bloom (mostly ciliates and heterotrophic dinoflagellates). Dominant ciliates during the summer month were *Lohmaniella* spp. or small *Strombidium* spp. (35 μm). *Myrionecta*

rubra was always present with maximum abundance in late spring. *Lohmaniella* spp. also occurred in the present study but was classified with *Strobilidium* spp. ($\leqslant$ 20 μm) due to difficulties with clear identification. However, most of the Strobiliids $\leqslant$ 20 μm probably belonged to *Lohmaniella* spp. In our study, the ciliate community was dominated by the primarily photoautotrophic ciliate *M. rubra* (=*Mesodinium rubrum*) Lohmann (1908); Jankowski (1976) (Mesodiniidae, Litostomatea) most of the time (Lindholm, 1985). Only towards the end of our experiment, heterotrophic ciliates became more important in the ciliate community when small Strombidiids such as *Strombidium* cf. *epidemum* occurred with similar abundances as *M. rubra*. *M. rubra* is also a common species in the Baltic Sea with maximum reported densities of 26,600 cells l^{-1} in the Arkona Basin usually above the thermocline and associated with the euphotic layer (Setälä and Kivi, 2003). Maximum total ciliate densities in the entrance of the Gulf of Finland varied between 10–50,000 cells l^{-1} in 1988 and 1990, respectively, and hence are in the same range as in our study, and also consisted of the same typical species/groups (Setälä and Kivi, 2003).

4.1.2 Changes in ciliates species diversity

Previous studies on sensitivities of MiZP communities towards ocean acidification are inconsistent. For example Rose et al. (2009) report on significant changes in MiZP abundance and community composition in the open North Atlantic Ocean between their single factor (only temperature) and two factor (temperature and CO_2) experiments and conclude that a combination of direct and indirect (bottom-up) effects were responsible for observed changes. Mesocosm studies off the coast of Norway and in the Arctic revealed no effect of different CO_2 concentrations on the MiZP community neither with respect to abundance nor community composition (Suffrian et al., 2008; Nielsen et al., 2010; Aberle et al., 2013). In the latter study, positive effects on the autotrophic biomass with higher and lower CO_2 concentrations were found for dinoflagellates and respectively prasinophytes and haptophytes but these effects did not translate to the MiZP level (Schulz et al., 2013).

We found no significant relation between ciliate total abundance and fCO_2 concentration, but total abundance was significantly affected by temperature. Moreover, there seemed to be a trend with respect to species diversity H towards a higher dominance of single species with increasing temperature and fCO_2, respectively. Most likely, small species/genus are responsible for this change in diversity. During the first days of the experiment (t_5, t_5–t_9, and t_7–t_{13}, respectively) small species such as *Balanion comatum*, *Strombidium* cf. *epidemum*, and *Strobilidium* sp. (< 20 μm) show some distinct differences in abundance between the three higher and lower fCO_2 mesocosms. While *B. comatum* occurs at higher abundance in the control mesocosms and the lowest CO_2 enrichment level (M7, 497 μatm), *S.* cf. *epidemum* and *Strobilidium* sp. have higher abundances in the three high CO_2 mesocosms. Later in the experiment, between t_{19} and t_{31}, the small size class *Myrionecta rubra* for example occurred in much higher numbers in the mesocosms with the three highest fCO_2 concentrations. For the mentioned species, significant relations were determined for all factors included in our analyses, except for *Balanion comatum* that showed no significant response to chlorophyll *a* and *Strombidium* cf. *epidemum* that only showed a significant relation with chlorophyll *a*. Rose et al. (2009)

also report on increased dominance of smaller taxa (mostly *Lohmaniella* sp. among ciliates) during the course of their experiment, but dependent on a combination of different factors, i.e. temperature, CO_2 and changes in the top-down control. Finally, they conclude on a more general effect of temperature on MiZP abundance and community composition. A relationship between temperature and Shannon diversity H on ciliate communities and on heterotrophic ciliates, respectively, was also shown by Setälä and Kivi (2003) and Aberle et al. (2007). In contrast to our present study, Aberle et al. found H to increase with higher temperature and it was larger ciliates (mostly *Strobilidium* species) that caused the community shift. Like Rose et al. (2009), the temperature effect determined in the present study, is most likely of more general nature related to the natural succession of ciliates during the summer season.

Although some of the species mentioned above significantly correlated with chlorophyll *a* concentrations (*Strobilidium* sp., *Strombidium* sp.), chlorphyll *a* had no significant effect on species diversity H. Most likely this is due to the occurrence of species with different (heterotrophic/autotrophic) food preferences during the course of the experiment. Species diversity was lowest during phase I and II and this was due to the dominance of the mixotroph *Myrionecta rubra*. Later in the experiment when chlorophyll *a* concentrations had decreased, *M. rubra* still occurred with lower cell numbers but also other ciliates like the mixotrophic *Strombidium* sp. increased in abundance and as a consequence H increased. Members of the genus *Strombidium* feed on a variety of organisms including bacteria, nano- and dinoflagellates (Fenchel and Jonsson, 1988; Ichinotsuka et al., 2006; Stoecker et al., 2009). Furthermore, this experiment was conducted during the post-bloom phase. Possibly, if our experiment also covered the phytoplankton peak-bloom phase and H was determined over the whole duration from the peak- to the post-bloom phase, the relationship between H and chlorophyll *a* was more pronounced.

4.1.3 *Myrionecta rubra*

Increased abundances of the mixotrophic ciliate *Myrionecta rubra* ($\leqslant 10\ \mu$m) in the high CO_2 mesocosms coincided well with increased chlorophyll *a* concentrations at high CO_2 levels during phases II and III attributed for up to 90% to picophytoplankton ($\leqslant 2\ \mu$m). The relative contribution of the 2–20 μm size fraction to total chlorophyll *a* was estimated as about 20% (Paul et al., 2015). Blooms of *M. rubra* can contribute significantly to chlorophyll *a* values and primary production in estuaries, fjords and upwelling areas. *M. rubra* robs plastids from Cryptophytes (Lindholm, 1985; Gustafson Jr et al., 2000, and references therein). Cryptophytes were among the main contributors to total chlorophyll *a* in particular during phase I (Paul et al., 2015). Moreover, small picoeukaryotes (PICO III) of approx. 2.9 μm cell diameter most likely representing Cryptophytes had highest abundances during phases II and III and showed a distinct negative correlation with fCO_2 (Crawfurd et al., 2016). Cryptophyte biomass decreased from t_3 to t_{17} (Paul et al., 2015) as did the total abundance of *M. rubra*, but the small size-class cells remained and during phase II developed a distinct difference in abundance between the higher and lower CO_2 mesocosms. Growth and photosynthetic performance of *M. rubra* is ultimately dependent on the availability of Cryptophytes, but the ciliate can sustain long periods without feeding by functioning as a phototroph and has the ability to control cryptophyte plastids' division and syn-

thesize chlorophyll (Johnson and Stoecker, 2005; Johnson et al., 2006). Photosynthetic performance of *M. rubra* may have been stimulated by elevated CO_2 concentrations and thus this ciliate may be 'co-responsible' for the CO_2 driven total chlorophyll *a* differences observed during phases II and III. Consequently, higher cell numbers of small sized *M. rubra* at elevated CO_2 may be a combination of indirect and direct CO_2 effects through 1) availability of Cryptophytes in particular during phase I, and 2) through a CO_2–mediated higher photosynthetic rate of *M. rubra* supporting its own growth. Losses of PICO III during phase II were largely due to microzooplankton grazing (Crawfurd et al., 2016). In further support of our assumption are the strong positive Pearson correlations between *M. rubra* and Cryptophytes and Dinophyta suggesting a high grazing pressure of *M. rubra*. During phase II, Dinophyta showed a significant decrease in relative biomass with increasing CO_2 consistently with the CO_2 stimulated increase of small *M. rubra* (Bermúdez et al., 2016). Overall, a CO_2 effect on *M. rubra* was only visible during the post-bloom phase, when cell numbers were rather low compared to initial numbers. However, possibly, differences were established already before but we were not able to see that because we only looked at abundances but not at processes.

4.2 Mesozooplankton

4.2.1 Mesozooplankton succession

The MZP community enclosed in the mesocosms reflected fairly well the natural succession of MZP in Tvärminne/Storfjärden where rotifers, cladocerans and calanoid copepods comprise the major zooplankton taxa (Kivi, 1986; Viitasalo, 1992; Koski et al., 1999). Usually rotifers numerically dominate in spring/early summer (*Synachaeta* sp.) and reach a second peak in mid-summer/autumn (*Keratella* sp.). The calanoid copepods *Acartia bifilosa* and *Eurytemora affinis* show two abundance peaks, in mid-June and mid-September, respectively, and *Temora longicornis* occurs only at low numbers year-round. Cladocerans peak in summer (August/September) with *Bosmina longispina maritima* clearly dominating among *Podon* spp. and *Evadne nordmanni*. Highest MZP biomass is build up in summer (August/September) (Kivi, 1986; Viitasalo, 1992; Koski et al., 1999).

The species composition in the mesocosms resembled well natural conditions and were dominated by the most common and successful genus/species known for the Gulf of Finland and the Tvärminne region such as *Acartia bifilosa*, *Eurytemora affinis*, *Bosmina longispina maritima*. Due to the rather late start of our mesocosm experiment after the spring phytoplankton bloom, the usual peak of *Synchaeta* sp. in spring/early summer – also one of the most successful species (i.e. *Synchaeta baltica*, Viitasalo (1992)) – was barely visible during the first days, later rotifers still occurred until termination but were not of great importance anymore.

Total population densities known for mesozooplankton in the Tvärminne area more or less coincide with abundances found in the mesocosms and range from median values between $\sim$ 22,000– $\sim$ 40,000 ind m^{-3} with occasional peak abundance for *Acartia bifilosa* and *Bosmina* sp. of up to 45,000 and 82,000 ind. m^{-3}, respectively. Average peak abundance of *Acartia bifilosa* and *Bosmina* sp. during a period from 1967–1984

was $\sim$ 10,000 ind. m^{-3} and $\sim$ 20,000 ind. m^{-3}, respectively (Viitasalo et al., 1995; Viitasalo, 1992). Between t_{24} and t_{31}, however, some exceptional high numbers (> 150,000 ind. m^{-3}) occurred in the mesocosms mainly attributed to extremely high occurrence of *Bosmina* sp.. Even higher densities exceeding 1,000,000 ind. m^{-3} during bloms of blue-green algae are known for *B. fatalis* in an eutrophic lake in Japan (Hanazato and Yasuno, 1987). The MZP community in the surrounding water did not entirely correspond with the mescosms over the course of the experiment. Whereas the dominance of particular species corresponded quite well until t_3, it diverged progressively after t_{10} when in the surrounding water the occurrence of colonies of blue-green algae (*Aphanizomenon*) and rotifera where higher than in the mesocosms, and the abundance of copepods and cladocerans comparatively lower (S. Lischka, pers. obs.). Most likely, this is a result of isolation of the mesocosm bags from surrounding water mass exchange and incoming plankton communities and selective advantage of single species in the mesocosms.

4.2.2 Copepods

This study is one of the first to follow MZP community development subjected to ocean acidification scenarios projected for this century in a close-to natural holistic plankton community (IPCC, 2013; Riebesell et al., 2008, 2013b). Previous study using the same mesocosm set-up investigated effects on an Arctic MZP community and found no significant difference neither in total abundance or abundance of single taxa nor in species diversity (Niehoff et al., 2013; Riebesell et al., 2013a).

Copepods comprised one of the two dominant taxonomic groups in the present study and the mesocosm approach allowed to investigate CO_2 effects on the succession of all different life stages from eggs to reproducing adults. While copepods are thought to be rather robust against ocean acidification with negative effects occurring usually not until pCO_2 levels far beyond projections for end of this century (Kurihara et al., 2004; Mayor et al., 2007; Weydmann et al., 2012; McConville et al., 2013; Almén et al., 2016), more recent studies give evidence that copepods' sensitivity may be highly stage dependent and thus so far mostly underestimated due to the fact that most studies done to-date considered only adult stage copepods (Cripps et al., 2014). Over the CO_2 range projected for this century, we found no distinct abundance differences for neither of the species. The permanent occurrence of adult males and females together with copepodite stages and nauplii suggest more or less continuous reproduction. Concurrent lab experiments investigating the effect of CO_2 on reproductive success of *Eurytemora affinis* are in agreement with the observations from the mesocosms (Almén et al., 2016, this issue). Incubated *Acartia bifilosa* showed fCO_2 unaffected egg production, but slight negative effects on egg hatching and development were found and adult females were smaller in the two highest CO_2 mesocosms (Vehmaa et al., 2015, this issue). Our results are also in line with Niehoff et al. (2013) who do not describe any apparent CO_2 effect on an Arctic MZP community including copepods. Copepods in the study region naturally experience fCO_2, pH and also temperature fluctuations of more than 0.5 pH units and 5°C temperature during daily vertical migrations which is more than the predicted climate change for the year 2100. I.e. these copepods are probably well adapted to short-term physico-chemical changes (Lewis et al., 2013; Almén et al., 2014).

4.2.3 Cladocera – OA effect on *Bosmina* spp. through increased food availability?

Most conspiciuous differences found in mesozooplankton abundance are due to the cladoceran *Bosmina* sp. between t_{24} and t_{31}. In three of the four CO_2 enriched mesocosms (497 μatm, 821 μatm, 1007 μatm) peak numbers were twice or even more than twice as high compared to the control and the highest CO_2 mesocosms, though a significant relation with fCO_2 could not be proved. Nevertheless, this striking difference may possibly point to an indirect CO_2 effect through higher food availability under high CO_2.

Cladocerans are highly reproductive at times of favourable environmental conditions. The life-span of *Bosmina* spp. varies between 20–25 days, age of first reproduction is between 4–7 days (food dependent) and populations can increase twofold within 5–10 days (Purasjoki, 1958; Kankaala and Wulff, 1981; Hanazato and Yasuno, 1987; Biswas et al., 2014). Population dynamics of *Bosmina longirostris* are highly food-sensitive with food quantity and quality having a significant effect on growth, net reproductive rate and rate of population increase to shorten life time to up to 10 days (Kankaala and Wulff, 1981; Hanazato and Yasuno, 1987; Urabe, 1991). Cladocerans are opportunistic feeders that graze on nano- and microplankton, bacteria (including Cyanobacteria), and detritus (Purasjoki, 1958; Nanazato and Yasuno, 1985; Work and Havens, 2003; Kluijver et al., 2012). *Bosmina* tolerates low pH in acidic lakes well (Uimonen-Simola and Tolonen, 1987).

The above mentioned population increase of *Bosmina* in the mesocosms coincides with significant CO_2 mediated differences during phase II in Cyanobacteria during the respective days and may have represented favourable food conditions for this species enhancing asexual reproduction in particular in the elevated CO_2 mesocosms (Paul et al., 2015). The highly positive correlation between Cyanobacteria and *Bosmina* sp. supports this assumption. Only M8, the mesocosm with the highest CO_2 concentration, diverged from this trend. Peak abundance in all mesocosms occurred only on one sampling day, i.e. did not stay high for a longer period but was low at the preceding sampling day and had dropped already at the following sampling day. Possibly, the drop in population size that occurred earlier than to be expected from *Bosmina*'s lifespan of around 20 days was due to high mortality and/or change to sexual reproduction producing resting eggs. Therefore, a possible explanation why *Bosmina* in M8 did not follow the trend observed in the other CO_2-elevated mesocosms may be that due to the rather low possible sampling frequency (every seven days) the actual abundance peak was missed (Riebesell et al., 2013a). Reason for mortality could be in response to the overall drop in available food during phases II and III and/or stress response due to extreme densities or reproductive rates of *Bosmina* itself. It is known, that *Bosmina* sp. can die earlier when they have higher reproductive rates and switch to sexual reproduction producing resting eggs, respectively, at too high population densities (so called "crowding phenomenon") (Purasjoki, 1958; Acharya et al., 2005). In Kankaala (1983), *Bosmina* started sexual reproduction at around 4,500 ind. m^{-3} which is about 1–2 orders of magnitude less than observed peak numbers in the mesocosms.

The significant results we found for the ratio of *Bosmina* with empty and full brood chambers strongly suggest that organisms in the high CO_2 mesocosms had higher reproductive activities during the time of actual peak abundance. In particular, *Bosmina* in M8 and M3 (two highest CO_2 levels) had continuously

low brood chamber ratios (i.e. large proportion of actively reproducing organisms in the population) from t_{10} onwards (with the ratio in M8 mostly even lower than in M3). This supports our assumption that we may have missed to sample the abundance peak of *Bosmina* in M8 possibly obstructing to prove a significant indirect fCO_2 effect on *Bosmina* abundance through increased food availability.

4.2.4 Predator/prey relationships

We have some evidence for fCO_2 stimulated predator/prey relationships between *Myrionecta rubra*/Cryptophytes and *Bosmina* sp./Cyanobacteria, though the mixotrophic ciliate *M. rubra* may also have benefitted directly from elevated fCO_2 concentrations (see above). With respect to *Balanion comatum*, *Strombidium* cf. *epidemum*, *Strobilidium* sp., the fCO_2 related abundance differences during particular phases of the experiment can not be explained through enhanced predator/prey relationships.

Although our results show no direct significant CO_2 effect on *Bosmina* abundance, we can not rule out that growth and reproduction was stimulated from increased Cyanobacteria availability at elevated CO_2 mostly during phases II and III or from increased heterotrophic bacterial production (Hornick et al., 2016). This would point to an indirect CO_2 effect that was masked as a consequence of too low sampling frequency not allowing to adequately capture the population dynamics of this short-lived and highly adjustable genus. For the study region, microbial loop has been shown to be of particular importance during late summer and autumn when most of the secondary production including fish is fueled by carbon channeled from the microbial loop to crustacean zooplankton (Uitto et al., 1997; Koski et al., 1999). In the present study, heterotrophic bacterial production and biovolume was strongly linked to phytoplankton dynamics and suggested several indirect responses to fCO_2 (Hornick et al., 2016). Enhanced bacterial grazing, and thus stimulated microbial loop, was assumed in relation to higher fCO_2 (Crawfurd et al., 2016). This was mostly reflected in relatively high rates of cell-specific bacterial protein production of particle-associated heterotrophic bacteria throughout the entire experiment, though they only contributed a minor fraction to the overall heterotrophic bacterial biovolume (Hornick et al., 2016). Filter-feeding cladocerans directly feed on bacteria and flagellates and effectively transfer carbon from the microbial loop to higher trophic levels. In the eastern and western Gulf of Finland as well as in the southern Baltic Sea, *Bosmina longispina* can be the dominant prey for herring (*Clupea harengus*), sprat (*Sprattus sprattus*) and three-spined stickleback (*Gasterosteus aculeatus*) (Casini et al., 2004; Peltonen et al., 2004). Larger herring feed more on Mysids during autumn that in turn can effectively prey on cladocerans including *Bosmina* sp. (Rudstam et al., 1992). Increased bacterial production (Hornick et al., 2016) may have provided optimal feeding conditions to favor reproduction of *Bosmina* sp. at elevated fCO_2, but, unfortunately our data can not provide a sufficient proof.

A more recent publication by Wikner and Andersson (2012) states that increased microbial heterotrophy decreases trophic transfer efficiency of biomass to higher trophic levels. This work investigated the influence of increased river discharge through increased precipitation on phytoplankton biomass production and finds a shift in the carbon flow towards microbial heterotrophy. This shift was mainly due to an increase in freshwater and riverine organic carbon supply on phytoplankton growth despite a concomitant increase in nutrients. As

already mentioned above, during phase III, increasing importance of production of the microbial food web related to higher fCO_2 was found in the present study (Paul et al., 2015; Crawfurd et al., 2016; Hornick et al., 2016) concomitant with the abundance peak of the cladoceran *Bosmina* sp.. In plankton communities comprising species able to effectively graze on bacteria such as *Bosmina* sp., trophic transfer to higher trophic levels may not be necessarily decreased but could still be enhanced.

The results described for *M. rubra* and *Bosmina* should be robust also if biomass estimates were considered. The significant response of *M. rubra* to CO_2 was determined for cells of the same size class (all mostly 10 μm), i.e. biomass-based results would scale proportionally with cell numbers. In case of *Bosmina* the significant CO_2 relation was found for the ratio of embryo-bearing to non-embryo bearing organisms which is an abundance-/biomass-independent measure. As regards a possible indirect CO_2 effect that we suggested for *Bosmina* sp., abundance increased more than two-fold in the mentioned mesocosms and consisted of different-sized individuals. I.e. a respective increase in biomass would probably be smaller as compared to the abundance increase, but certainly still existent.

5 Conclusions

This study describes for the first time fCO_2 related effects on the zooplankton community level in a close to natural plankton community. Some ciliate species as well as the species diversity of ciliates responded to elevated fCO_2 levels. On the mesozooplankton level, significant fCO_2 effects were only found for the ratio of empty to full brood chambers of the cladocera *Bosmina* sp. but an indirect effect on *Bosmina* abundance via food seems likely. Although for the ciliates, in particular the mixotroph *Myrionecta rubra*, the magnitude of change in abundance was rather minor as effects were observed only in the post-bloom phase, and for the cladoceran *Bosmina* sp. a fCO_2 effect could only be carefully assumed, our study has shown that ocean acidification effects can potentially translate up from the primary production level to higher trophic levels. Certainly, this is not a general consequence but is probably highly dependent on the species composition of a pelagic community, i.e. the presence of species that have the ability to quickly respond to changes in food availability and composition with increased reproduction or cell division, respectively, such as the highly flexible cladocerans or the mixotroph ciliate *Myrionecta rubra*.

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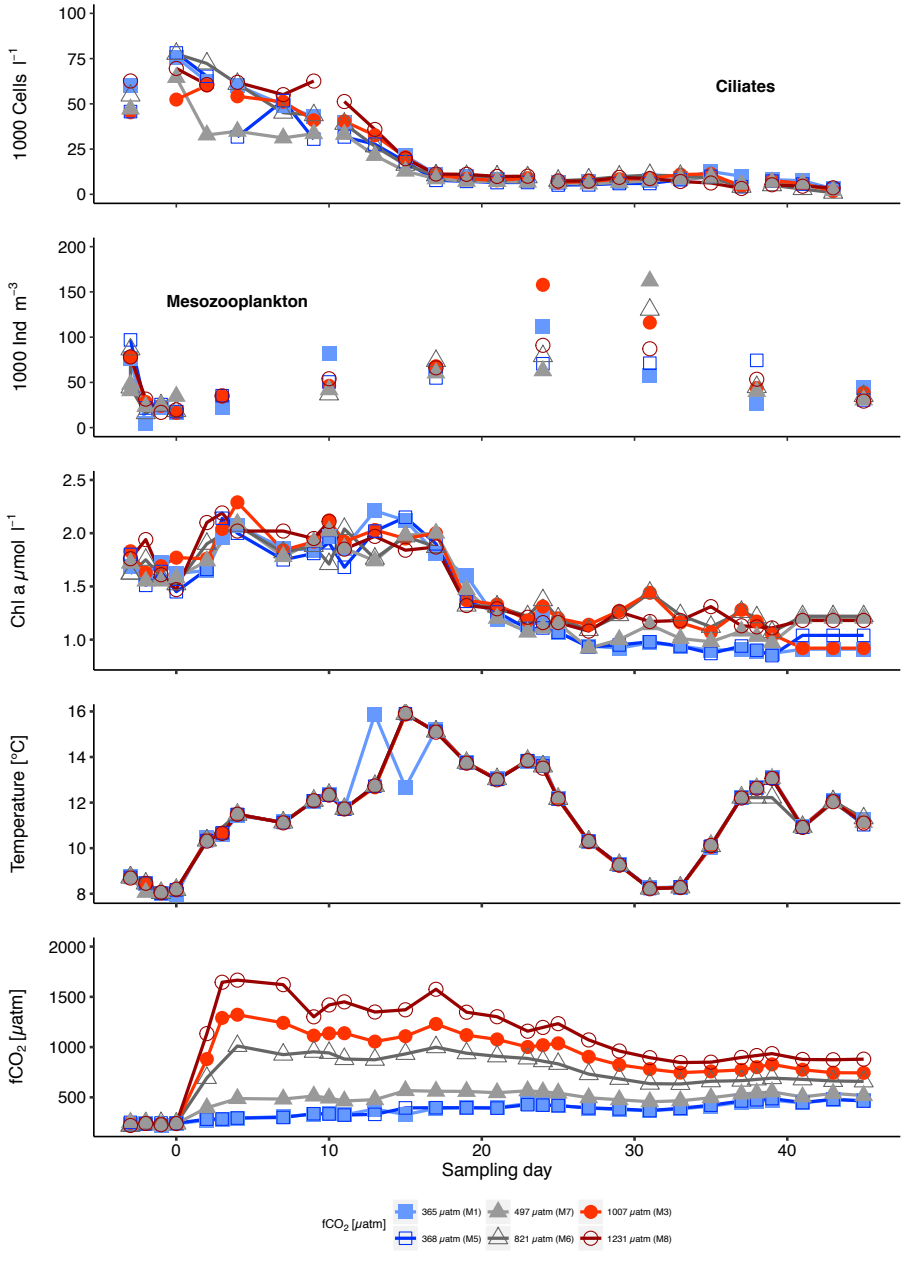

Figure 1. Total cell numbers of ciliates and total abundance of mesozooplankton during the course of the experiment as well as chlorophyll *a* succession, temperature and fCO_2 development. According to temperature variations and the first CO_2 manipulation, different experimental phases were defined: Phase 0 = t_{-5} to t_0, Phase I = t_1 to t_{16}, Phase II = t_{17} to t_{30}, Phase III = t_{31} to t_{43}. Note there is one missing value in M1 on t_{13}.

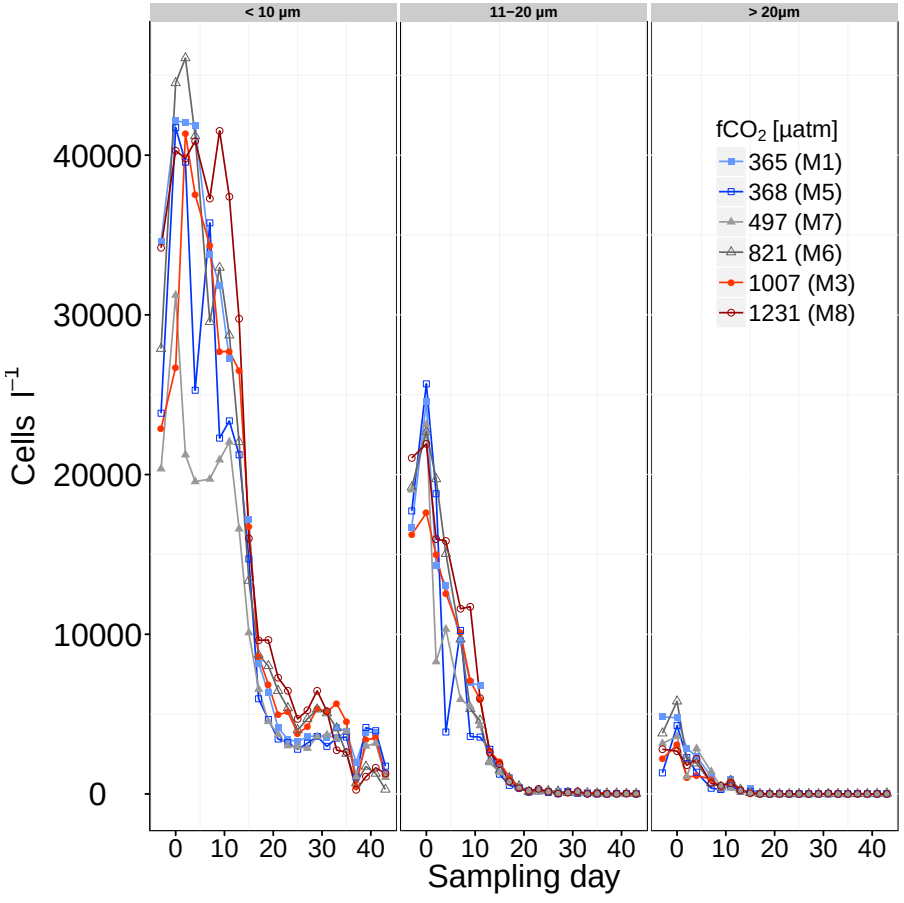

Figure 2a. Abundance of different size classes of *Myrionecta rubra*. Note there is one missing value in M1 on t_{13}.

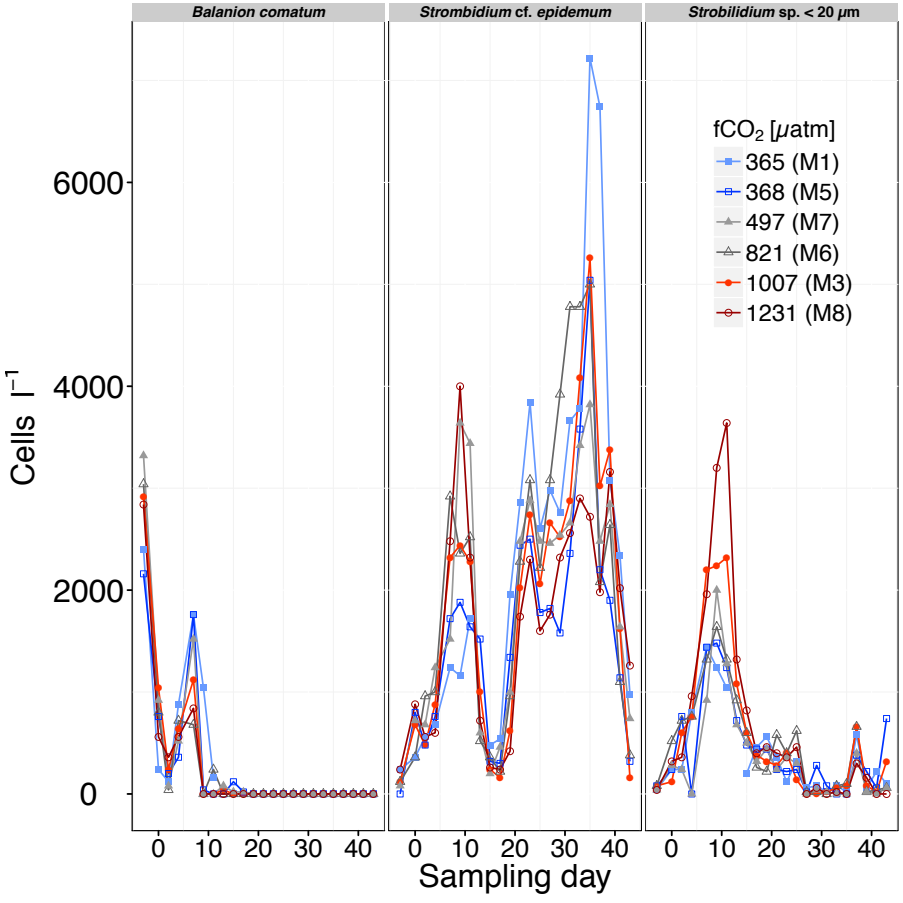

Figure 2b. Abundance of other ciliate species/genera/groups. Note there is one missing value in M1 on t_{13}.

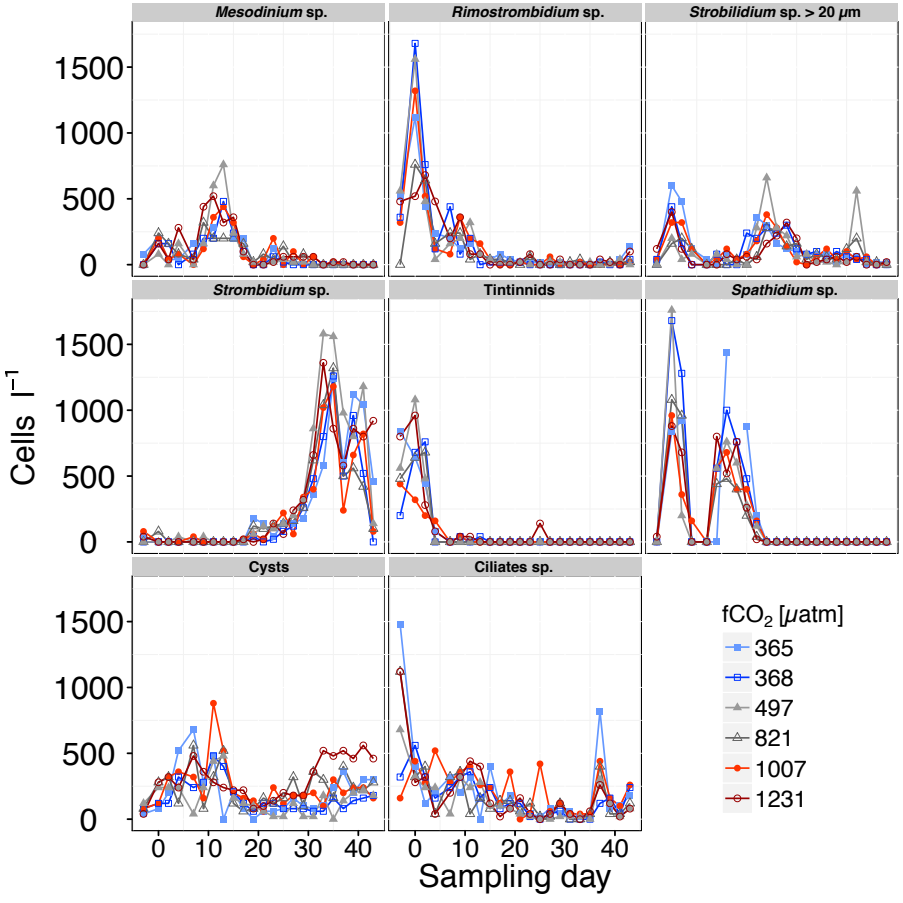

Figure 2c. Abundance of other ciliate species/genera/groups. Note there is one missing value in M1 on t_{13}.

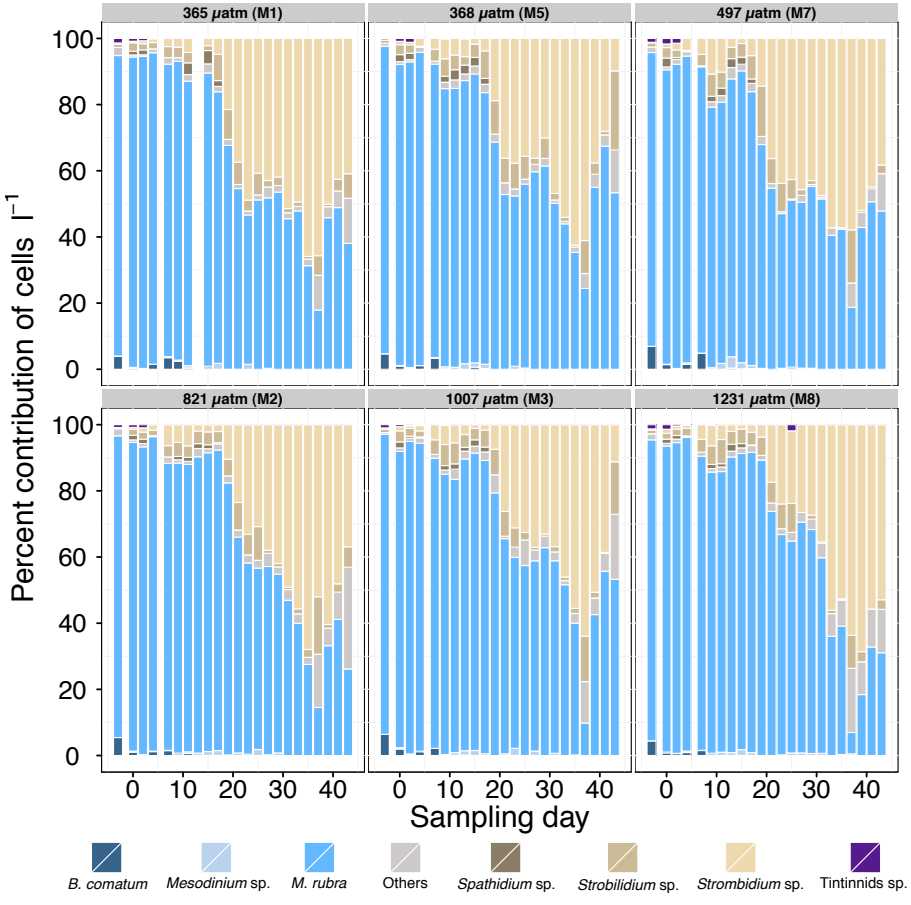

Figure 3a. Percent contribution of abundance of major taxonomic species/genera/groups to the ciliate community. *B. comatum* = *Balanion comatum*,*M. rubra* = *Myrionecta rubra*. Note there is one missing value in M1 on t_{13}.

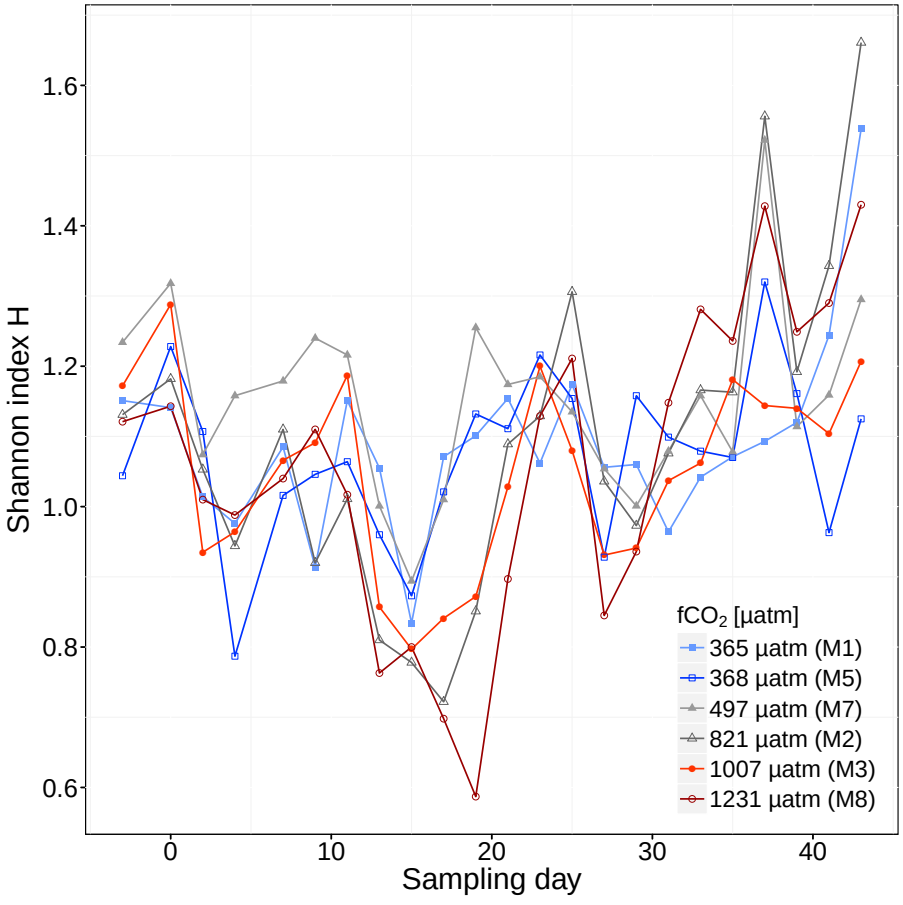

Figure 3b. Ciliates, daily change of the Shannon diversity index *H* at the different fCO_2 levels in the mesocosms.

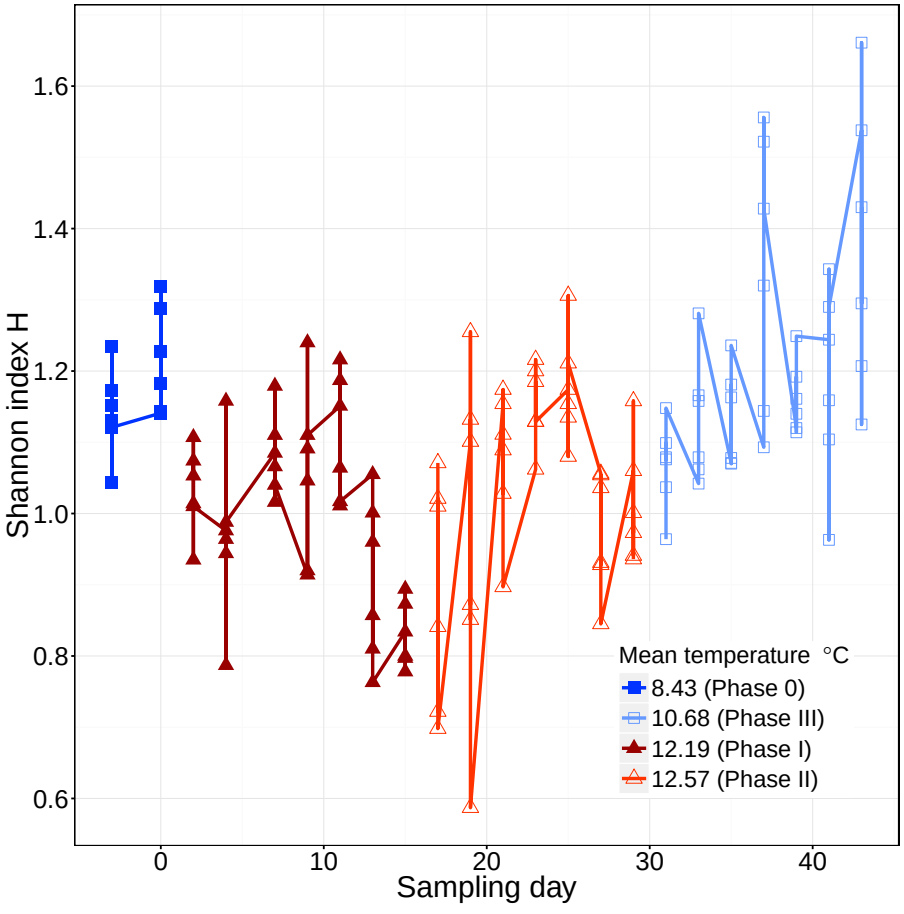

Figure 3c. Ciliates, daily change of the Shannon diversity index *H* during the 4 different temperature phases defined. Colour legend gives mean temperature during Phase 0 (12.57 °C), Phase I (8.43 °C), Phase II (10.68 °C), and Phase III (12.19 °C).

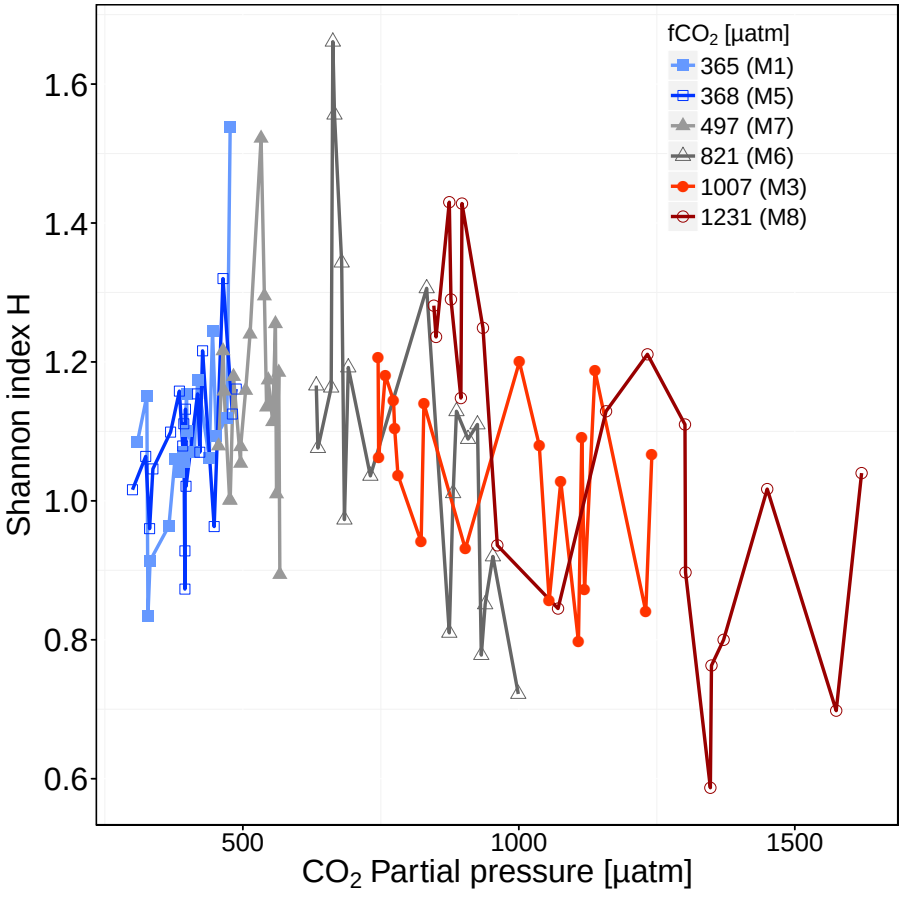

Figure 4a. Ciliates, graphical depiction of statistical results for Shannon diversity index *H* as a function of fCO_2: H is shown in relation to the daily change of fCO_2. Symbols and colours identify the mean fCO_2 for each mesocosm.

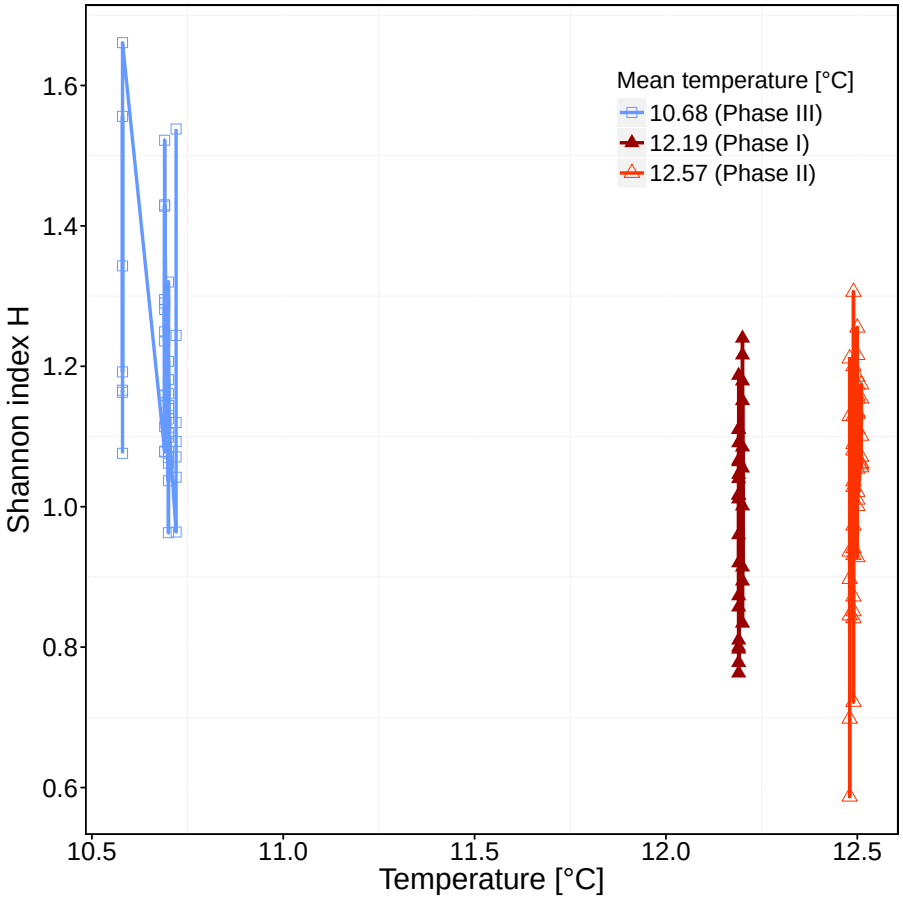

Figure 4b. Ciliates, graphical depiction of statistical results for Shannon diversity index *H* as a function of temperature. For better visibility, *H* is plotted against the mean phase (I, II, III) temperature of each mesocosm. Symbols and colours identify mean phase temperature across all mesocosms.

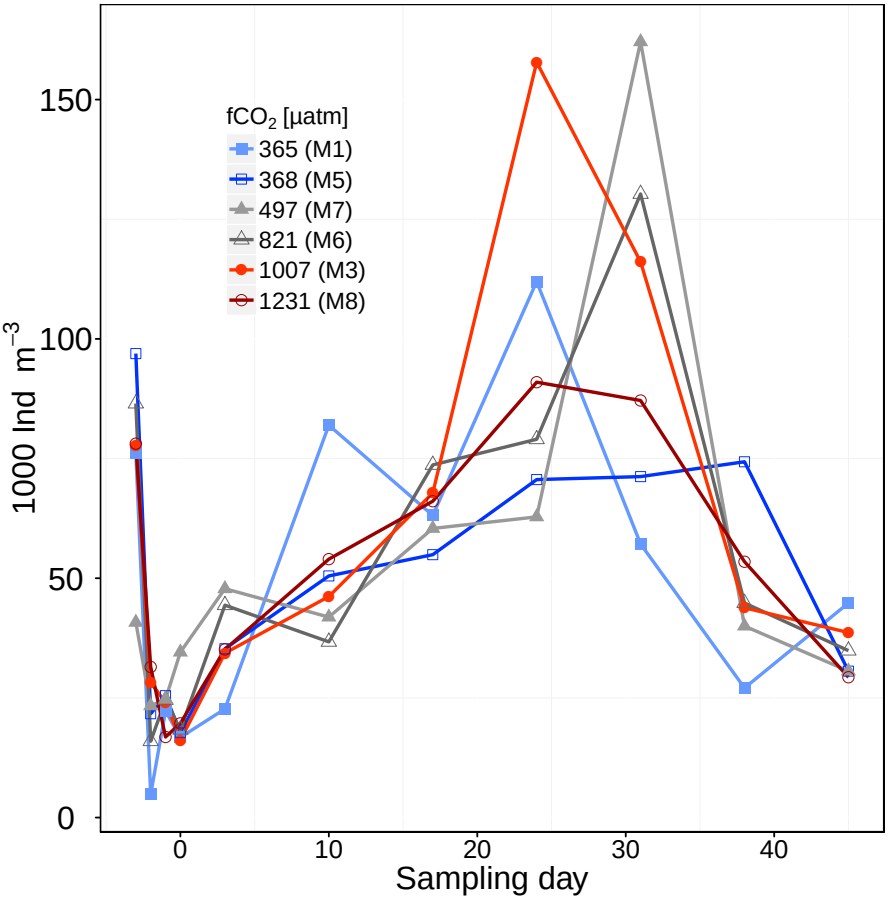

Figure 5. Mesozooplankton total abundance. According to temperature variations and the first CO_2 manipulation, different experimental phases were defined: Phase 0 = t_{-5} to t_0, Phase I = t_1 to t_{16}, Phase II = t_{17} to t_{30}, Phase III = t_{31} to t_{43}.

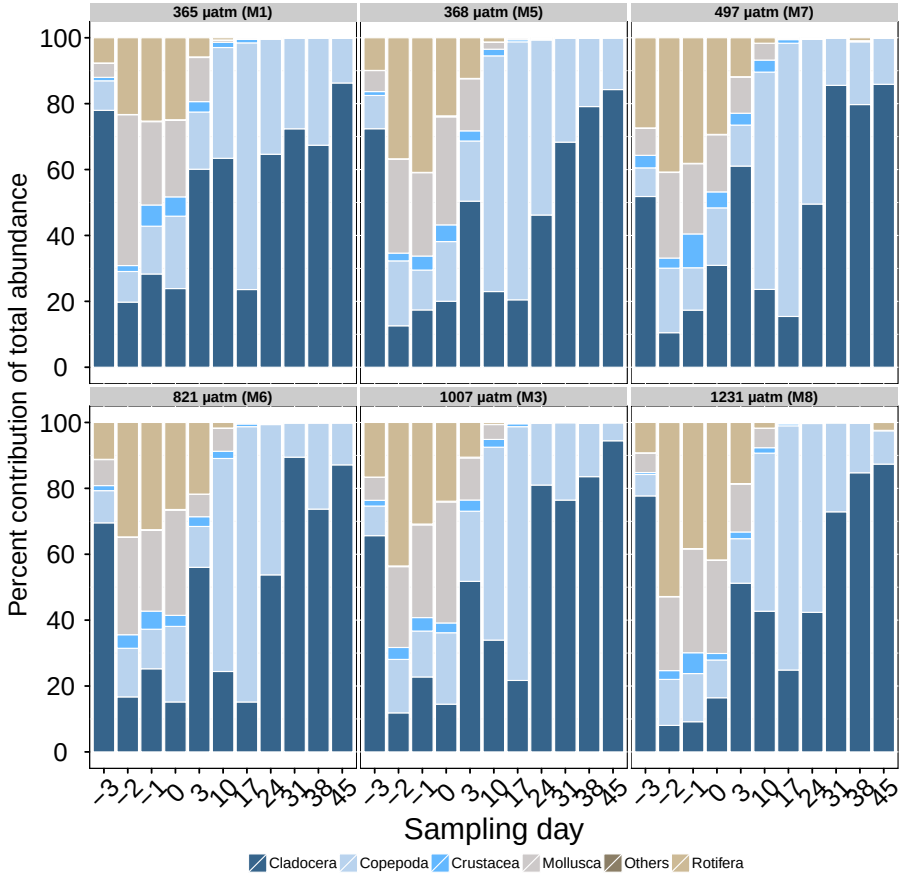

Figure 6. Percent contribution of mesozooplankton main taxonomic groups.

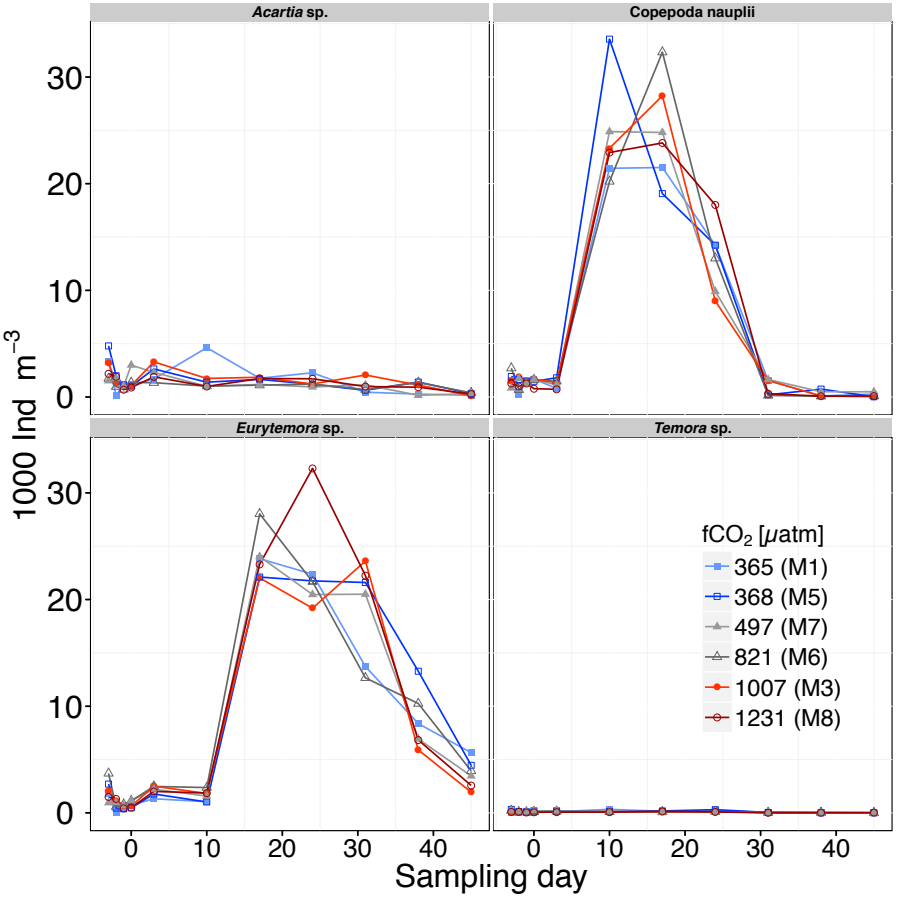

Figure 7a. Abundance of the dominant copepods species *Acartia* sp., *Eurytemora* sp., *Temora* sp., and copepod nauplii.

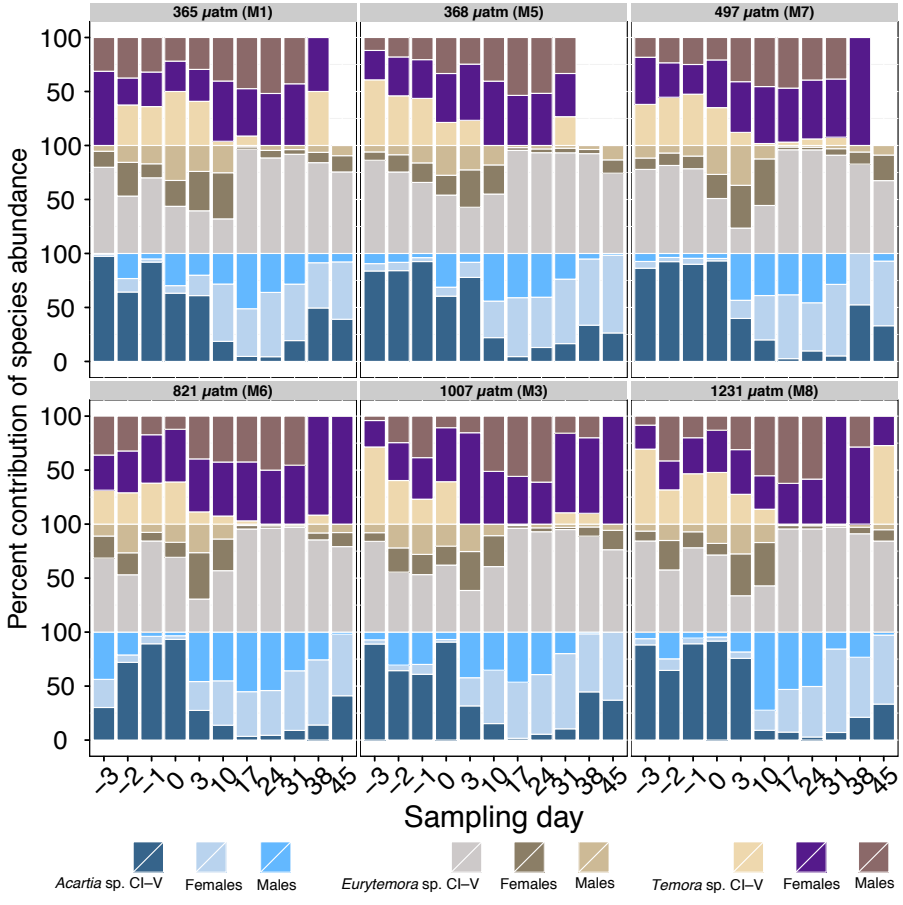

Figure 7b. Percent contribution of different stages of dominant copepods.

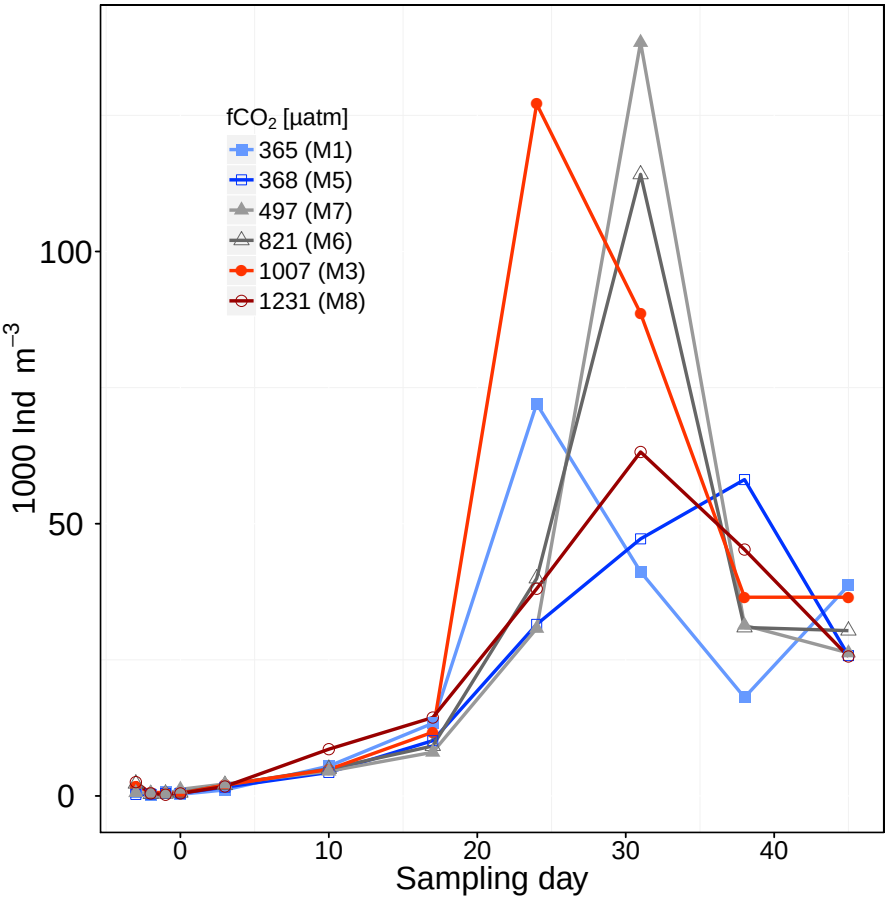

Figure 8a. Total abundance of the most dominant cladoceran species *Bosmina* sp..

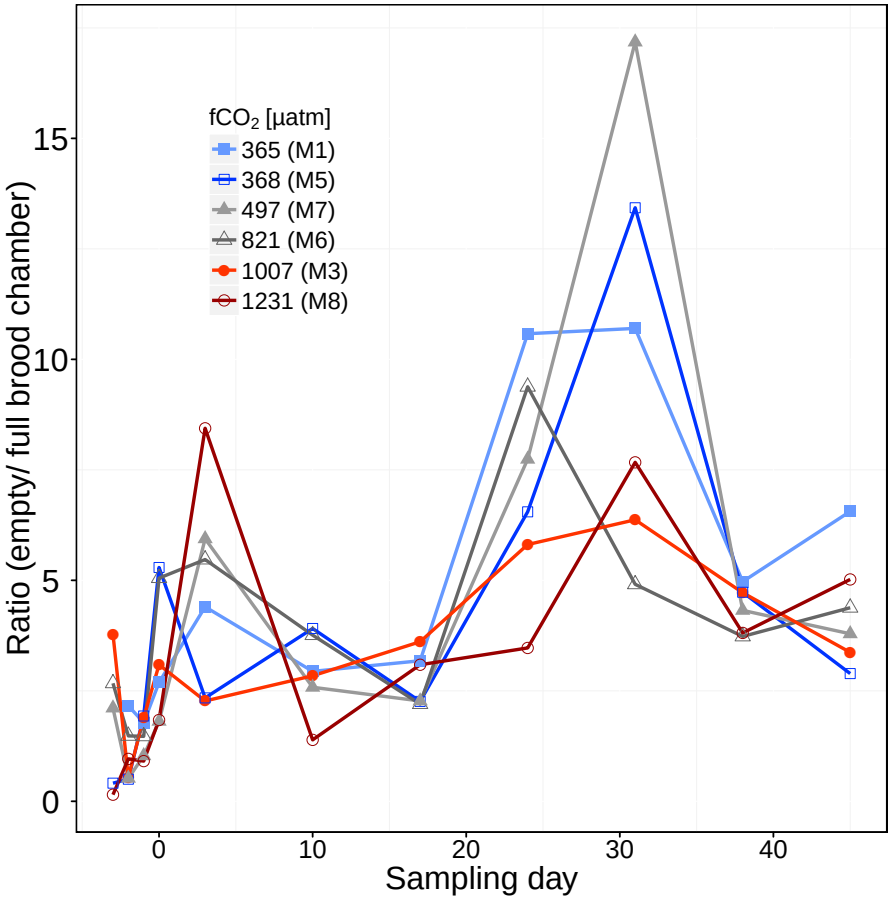

Figure 8b. Ratio of *Bosmina* with empty to full brood chambers. Note: Figure shows all data, but statistics were done on data from t_3–t_{45} only to assure equally spaced data.

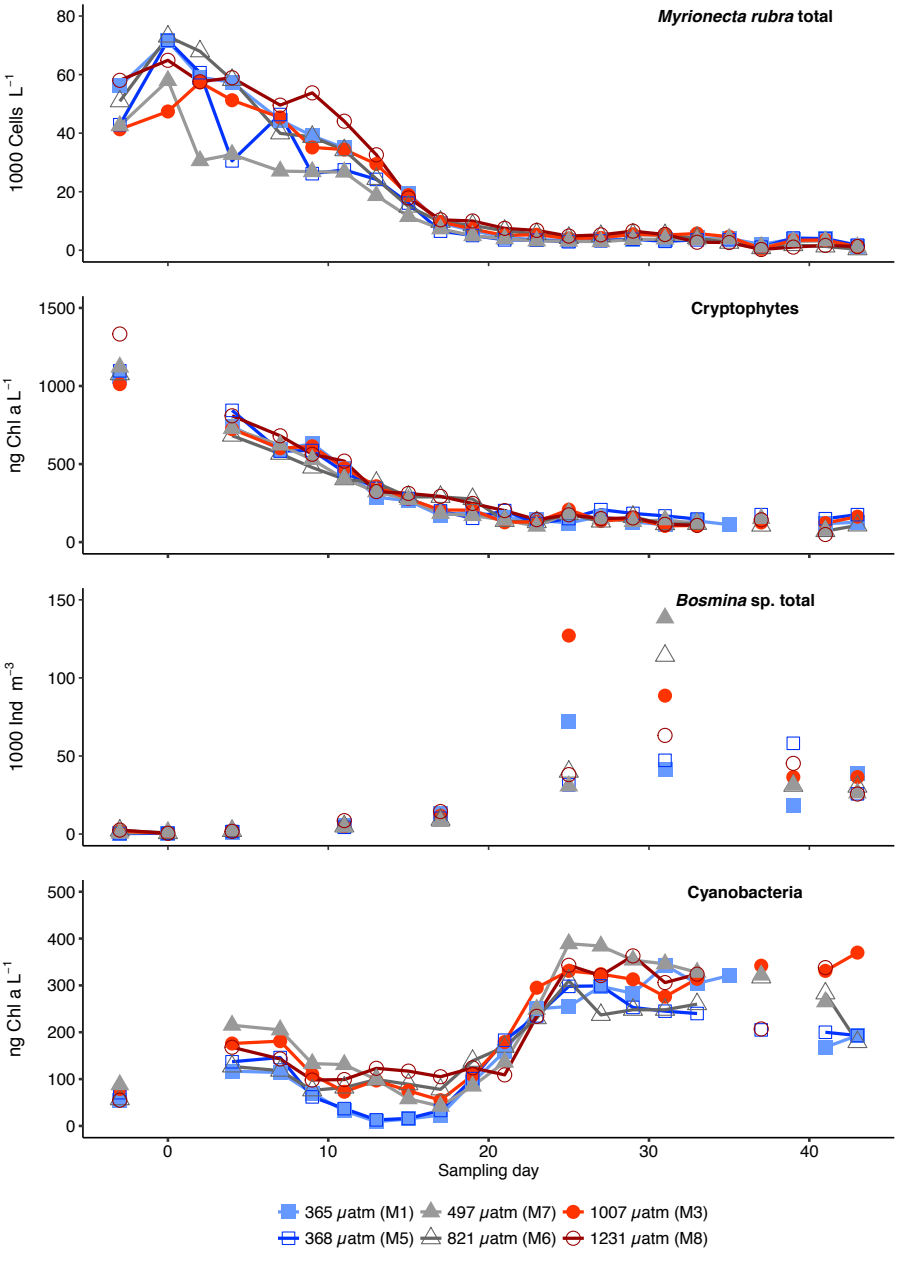

Figure 9. Succession of total cell numbers of *Myrionecta rubra*, total biomass of Cryptophytes, total abundance of *Bosmina* sp. and total biomass of Cyanobacteria during the course of the experiment. According to temperature variations and the first CO_2 manipulation, different experimental phases were defined: Phase 0 = t_{-5} to t_0, Phase I = t_1 to t_{16}, Phase II = t_{17} to t_{30}, Phase III = t_{31} to t_{43}. Note there is one missing value in M1 on t_{13}.

Table 1. Statistics summary table of retained fixed effects of the GLM's and GAMM's. Significant p-values are indicated in bold (Temp: temperature).

	Explanatory variable	DF	t	p-value	Model
Ciliates					
Ciliates total abundance	Temp	1	-3.506	**0.0007**	GAMM
Myrionecta rubra, $\leqslant$ 10 μm	Temp	1	2.376	**0.019**	GAMM
Myrionecta rubra, $\leqslant$ 10 μm	fCO_2 * Temp	1	-2.298	**0.024**	GAMM
Myrionecta rubra, $\leqslant$ 10 μm	fCO_2 * Chl *a*	1	2.936	**0.004**	GAMM
Balanion comatum	Temp	1	2.320	**0.022**	GAMM
Balanion comatum	fCO_2	1	-2.210	**0.030**	GAMM
Strombidium cf. *epidemum*	Chl *a*	1	-3.229	**0.002**	GAMM
Strobilidium sp., < 20 μm	Temp	1	2.811	**0.006**	GAMM
Strobilidium sp., < 20 μm	Chl *a*	1	-4.603	**< 0.00001**	GAMM
Strobilidium sp., < 20 μm	fCO_2 * Temp	1	-3.600	**0.0005**	GAMM
Strobilidium sp., < 20 μm	fCO_2 * Chl *a*	1	3.926	**0.0002**	GAMM
Shannon index *H*	Temp	1	3.652	**0.0004**	GAMM
Shannon index *H*	fCO_2	1	2.824	**0.006**	GAMM
Shannon index *H*	fCO_2 * Temp	1	-3.454	**0.0008**	GAMM
Mesozooplankton					
MZP total abundance	Temp	31	-1.155	0.257	GLM
MZP total abundance	fCO_2	31	-0.025	0.980	GLM
MZP total abundance	Chl *a*	31	0.550	0.586	GLM
MZP total abundance	fCO_2 * Temp	31	0.947	0.351	GLM
MZP total abundance	fCO_2 * Chl *a*	31	-1.081	0.288	GLM
Bosmina sp.	Chlor *a*	1	0.76	0.453	GAMM
Bosmina sp. ratio empty/full brood chambers	Temp	1	-3.572	**0.001**	GAMM
Bosmina sp. ratio empty/full brood chambers	fCO_2	1	-2.684	**0.011**	GAMM
Bosmina sp. ratio empty/full brood chambers	Chl *a*	1	-3.980	**0.0004**	GAMM
Bosmina sp. ratio empty/full brood chambers	fCO_2 * Chl *a*	1	2.738	**0.01**	GAMM
Shannon index *H*	Chl *a*	1	-0.555	0.582	GAMM

Table 2. Pearson correlation for various predator/ prey relationships. Listed are only correlations $\geqslant 0.7$. The pairwise correlation plots for all group combinations and the Pearson correlation coefficients can be seen from supplemental material (Fig. S2−S1). het Dino.: heterotrophic dinoflagellates, excl.: excluded. For *Myrionecta rubra* Pearson correlation was determined combined for all fCO_2 levels and also separate for low (365 μatm, 368 μatm, 497 μatm) and high (821 μatm, 1007 μatm, 1231 μatm) fCO_2 levels. [1]data from Paul et al. (2015), [2]Crawfurd et al. (2016), [3]data from A. Stuhr (unpublished), [4]this study.

Predator/Prey	Pearson correlation	fCO_2 levels	Method
Ciliates/Bacteria, Phytoplankton groups			
Myrionecta rubra < 10 μm/Cyanobacteria	-0.7	high	CHEMTAX[1]
Myrionecta rubra < 10 μm/low DNA bacteria	-0.7/ -0.7/ -0.7	all/ low/ high	Flowcytometry[2]
Myrionecta rubra < 10 μm/Picoeukaryotes III	-0.7/ -0.7	low/ high	Flowcytometry[2]
Myrionecta rubra < 10 μm/Synechococcus	-0.7	high	Flowcytometry[2]
Myrionecta rubra < 10 μm/Cryptophytes	0.8/ 0.8/ 0.8	all/ low/ high	CHEMTAX[1]
Myrionecta rubra 10–20 μm/Cryptophytes	1.0/ 0.9/ 1.0	all/ low/ high	CHEMTAX[1]
Myrionecta rubra > 20 μm/Cryptophytes	0.9/ 0.8/ 0.9	all/ low/ high	CHEMTAX[1]
Myrionecta rubra < 10 μm/het. Dino.	0.8	all	Microscopy[3]
Myrionecta rubra 10–20 μm/het. Dino.	0.7	all	Microscopy[3]
Myrionecta rubra < 10 μm/het. Dino. (*Ebria* sp. excl.)	0.8	all	Microscopy[3]
Myrionecta rubra 10–20 μm/het. Dino. (*Ebria* sp. excl.)	0.7	all	Microscopy[3]
Myrionecta rubra > 20 μm/het. Dino. (*Ebria* sp. excl.)	0.7	all	Microscopy[3]
Balanion comatum/Cryptophytes	0.8	all	CHEMTAX[1]
Mesodinium sp./Euglenophytes	0.7	all	CHEMTAX[1]
Rimostrombidium sp./Cryptophytes	0.8	all	CHEMTAX[1]
Tintinnids sp./Cryptophytes	0.7	all	CHEMTAX[1]
Spathidium sp./Euglenophytes	0.7	all	CHEMTAX[1]
Mesozooplankton/Bacteria, Phytoplankton groups, Ciliates			
Podon sp./Cryptophytes	0.9	all	CHEMTAX[1]
Bosmina sp./Cyanobacteria	0.7	all	CHEMTAX[1]
Podon sp./het. Dino.	0.7	all	Microscopy[3]
Podon sp./het. Dino. (*Ebria* sp. excl.)	0.7	all	Microscopy[3]
Eurytemora sp./Picoeukaryotes II	0.7	all	Flowcytometry[2]
Eurytemora sp./Cryptophytes	-0.7	all	CHEMTAX[1]
Copepod nauplii/Euglenophytes	0.7	all	CHEMTAX[1]
Copepod nauplii/Nanoeukaryotes II	0.8	all	Flowcytometry[2]
Podon sp./*Balanion comatum*	0.8	all	Microscopy[4]