Micro- 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, thus some level of tolerance/adaptation may be expected. 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 microzooplankton (ciliates) and mesozooplankton to CO$_2$ elevations expected for this century. Besides the response to fCO$_2$ and associate changes in carbonate chemistry speciation, we also considered temperature and chlorophyll $a$ variations in our analyses. Shannon diversity of microzooplankton significantly decreased with fCO$_2$ and temperature with a greater dominance of smaller species. Small sized ciliates (*Myrionecta rubra*, *Balanion comatum*, *Strombidium* cf. *epidemum*, *Strobilidium* sp.) showed significant relations with one or more of the factors. The phototrophic *Myrionecta rubra* seemed to directly benefit from higher CO$_2$ concentrations and showed increased abundance in the pre-bloom phase. With respect to mesozooplankton, we neither detected significant effects for total abundance nor for Shannon diversity. The cladocera *Bosmina* occurred at distinctly higher abundance (more than twice as high compared to the control mesocosms) 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* with empty to embryo/resting egg bearing brood chambers, however, was significantly affected by all three factors. An indirect CO$_2$ effect via increased food availability stimulating *Bosmina* reproduction is suggested, but too low sampling frequency of this highly flexible organism probably entailed proving a significant relation with fCO$_2$. Filter-feeding cladocerans effectively transfer microbial loop carbon 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 enhanced and carbon transfer to higher trophic levels stimulated.

1 Introduction

Since the industrial revolution, anthropogenic CO\textsubscript{2} emissions have increased at an unprecedented rate and cause a concomitant increase of CO\textsubscript{2} concentration in the oceans. Thereby, ocean carbonate chemistry is altered with the main changes being reduced carbonate ion concentrations $[\text{CO}_3^{2-}]$ and increased proton concentrations $[\text{H}^+]$ leading to a pH decrease. This phenomenon is nowadays well recognized as ocean acidification (OA). Since the beginning of the industrial revolution, ocean pH has decreased by approx. 0.1 units 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 especially sensitive to CO\textsubscript{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 $f\text{CO}_2$ 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 (optimal) 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 (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 $f$CO$_2$/pH changes expected for this century and direct negative effects usually do not occur unless exposed to much higher $f$CO$_2$ 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 ($>30\,\mu$m), in terms of mesozooplankton by copepods and cirripedia larvae. 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).
The amount of carbon transferred to higher trophic levels depends on the mesozooplankton species composition (Hansen et al., 1994). In Storfärden, during late summer and autumn, the microbial loop is of particular importance when filter-feeding cladocerans mediate carbon transfer to higher trophic levels including fish (Koski et al., 1999, and references therein).

As part of the KOSMOS Tvärminne mesocosm experiment, we examined CO₂ effects on the enclosed micro- and mesozooplankton community. Between June and August 2012, an fCO₂ gradient was set up in approximately six 55 m³ mesocosms covering fCO₂ 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, micro- and mesozooplankton include heterotrophic proto- and/or metazoa ranging between 0.02–0.2 mm (20–200 µm) and 0.2–20 mm (200–20,000 µm) in size, respectively. In this study, we do not follow this classification strictly. The category “microzooplankton” (MiZP) comprises ciliates only, including some species that can be facultative autotrophs or obligate phototrophs (for instance *Myrionecta rubra*), whereas all metazoa independent of their body size were assigned to the category “mesozooplankton” (MZP). Temperature can have a general effect on 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). To consider possible impact of temperature variation and/or CO₂ driven chlorophyll a differences (Schulz et al., 2013), in addition to fCO₂, we also included temperature and chlorophyll a as explanatory variables in our statistical analyses.

2 Methods

To study the effect of elevated fCO₂ on a natural plankton community in the Baltic Sea, nine KOSMOS offshore pelagic mesocosms (Kiel Off-Shore Mesocosms for future Ocean Simulation) 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 \( \text{CO}_2 \) 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 \( \text{CO}_2 \) saturated seawater to set up an initial gradient of \( f\text{CO}_2 \) 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. \( f\text{CO}_2 \) 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 \( \text{CO}_2 \) manipulations, and sampling procedures for various analyses see Paul et al. (2015). Sampling days were enumerated consecutively with \( t_{-3} \) indicating three days before \( \text{CO}_2 \) manipulation, \( t_0 \) as the day of the first \( \text{CO}_2 \) manipulation, and \( t_{1+X} \) as the days following the first \( \text{CO}_2 \) manipulation.
2.1 Microzooplankton 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 09:00 and 12:00 a.m. 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× 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. (2009) and Telesh et al. (2009). 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 CO₂ addition (t₋₃, t₋₂, t₋₁), at the day of the first CO₂ addition (t₀), and after the first CO₂ addition (t₃, t₁₀, t₁₇, t₂₄, t₃₁, t₃₈, t₄₅). After collection, the samples were brought back to the lab 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 microzooplankton 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, $f$CO$_2$, 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 $f$CO$_2$ 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 (R Development Core 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 micro-/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 microzooplankton, 14 species/groups were included to calculate $H$: *Balanion comatum*, *Strombidium cf. epidemicum*, *Mesodinium* sp., *Myrionecta rubra* ($\leq 10 \mu m$), *M. rubra* (11–20 µm), *M. rubra* (>20 µm), *Rimostrrombidium* sp., *Spathidium* sp., *Strobilidium* sp. (<20 µm), *Strobilidium* sp. (>20 µm), *Strombidium* sp., Tintinnids, cysts (*Strobilidium* sp., unidentified cysts), and ciliates sp. (*Euplotes* sp., *Lacrymaria* sp., *Strobilidium* sp., unidentified ciliates).

For the mesozooplankton, 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 Microzooplankton

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. epidemicum*, 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
ing 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/t25 and for M5/t23, these values were estimated as means of the preceding and following day. Chlorophyll a values were also missing for t41 and t43. A polynomial fit curve applied on phase III (according to temperature variations, three experimental phases were defined: phase I, II, III. Phase III lasted from t31 until t43; Paul et al., 2015) 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 fCO2, 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 fCO2 (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 Mesozoplankton

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 fCO2 occurred on t24, 20034.
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).

3 Results

3.1 Microzooplankton

3.1.1 Microzooplankton total abundance

Total abundance of microzooplankton 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 7080 (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 3580 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 (\(\leq 10\mu m, 11–20\mu m, > 20\mu m\)) 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 1740 cells L\(^{-1}\) among all mesocosms. Some striking difference, however, occurred between \(t_{25} \text{–} t_{35}\) when abundance in the three highest CO\(_2\) mesocosms was higher compared to the two controls and the lowest CO\(_2\) enriched mesocosm (mean: 4518 cells L\(^{-1}\) (SD 1082) and mean: 3459 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 2680–5800 cells L\(^{-1}\) on \(t_0\) and reaching very low numbers already on \(t_{7}/t_{9}\) (1080–280 cells L\(^{-1}\)). After \(t_{19}\), \(M. \ rubra \text{> 20 µm}\) occurred only exceptionally.

### 3.1.3 Abundance of other species/genera/groups

Other dominant groups/species that contributed to the total cell numbers of microzooplankton were *Mesodinium* sp., *Balanion comatum*, *Strombidium cf. epidemum*, *Rimostrombidium* sp., *Strobilidium* sp. (< 20 µm and > 20 µm), *Strombidium* sp., Tintinnids, *Spathidium* sp., cysts, and ciliates that could not be identified (Fig. 2b and c). Among those, *Strombidium cf. epidemum* was most dominant and showed three peaks, around \(t_9/t_{11}, t_{23}, \text{and} t_{37}\). Peak values ranged between 1160 cells L\(^{-1}\) and 4000 cells L\(^{-1}\) on \(t_9/t_{11}\) and showed some distinct difference between control and CO\(_2\) enriched mesocosm (mean: 1250 cells L\(^{-1}\) (SD 180) and mean: 2205 cells L\(^{-1}\) (SD 851), respectively). On \(t_{23}\) peak values ranged between 2300 cells L\(^{-1}\) and 3840 cells L\(^{-1}\), and between 1980 cells L\(^{-1}\) and 6,740 cells L\(^{-1}\) on \(t_{37}\). *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 of 1760 cells L\(^{-1}\) on \(t_7\), 1680 cells L\(^{-1}\) on \(t_0\), 3640 cells L\(^{-1}\) on \(t_{11}\), 1760 cells L\(^{-1}\) on \(t_0\), and 1080
cells L$^{-1}$ on $t_0$, respectively. 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 1360 cells L$^{-1}$ (SD 170) and 2400 cells L$^{-1}$ (SD 872) in the two controls and the CO$_2$ enriched mesocosms, respectively. Later in the experiment, these species/groups were not of importance anymore. *Mesodinium* sp. and *Strobilidium* sp. $> 20$ µm occurred always in relatively low cell numbers ($< 550$ cells L$^{-1}$ and $< 700$ cells L$^{-1}$, respectively). From $t_0$ onwards, cysts and unidentifiable ciliates never accounted for more than 700 cells L$^{-1}$ and 850 cells L$^{-1}$, respectively.

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

Figure 3b and c show 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 microzooplankton 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. It started with relatively low contributions during the first days of the experiment and increased depending on the mesocosm from $t_{19}/t_{21}$ on to proportions of 17–36 %. Maximum contributions varied 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. 4). 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 $t_{43}$. Overall, $H$ showed a non-monotonic relationship with a slightly increasing trend at lower $fCO_2$ and a decreasing trend the more the $fCO_2$ increased, and as well as a decreasing trend with temperature.

### 3.1.5 Statistical analyses microzooplankton

GAMM’s determined significant effects for total abundance, small size class *Myrionecta rubra, Balanion comatum, Strombidium cf. epidemum, Strobilidium* sp., and the Shannon index $H$ in response to one or more of the included explanatory variables. Detailed statistical results are shown 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

Total abundance of mesozooplankton caught in the net samples on $t_{-2}$ varied between 4841 ind. m$^{-3}$ in M1 and 31471 ind. m$^{-3}$ in M8 (Fig. 5). During the course of the experiment abundances increased in all mesocosms continuously until peak abundances were reached between $t_{24}$ and $t_{31}$. 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., including 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 usually between 50 and 95% to the total abundance. Low abundances of cladocerans occurred only at the beginning (t_−2, t_−1) and on t_17 when they only had a share of between 8 and 28% of the whole community. Copepods had a relatively low share early in the experiment and at the end (∼10–20%), but halfway through the experiment copepods 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, later on they almost disappeared in all mesocosms (<1%) except for M8 where on t_45 2% of the whole community were rotifera. Among the group mollusca, gastropods always had a smaller share than bivalves with usually below 2% contribution to the total abundance of this group, only in a few cases (M1 and M5 on t_10 and t_24) gastropods had a share of 5% and at the last day in M8 of 14% of the mollusca 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. By day t_10 their abundances decreased to 7–0.6% of the total community. 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 with highest numbers at the beginning of the experiment. 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 (T₃: 1.78–1.89) and decreased continuously with time reaching lowest values on the last sampling day (T₄₅: 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 until day t₁₀ ranging between 82 ind. m⁻³ and 2496 ind. m⁻³. Peak abundances were reached around day t₁₇ and t₂₄ with between 19 192 ind. m⁻³ and 32 297 ind. m⁻³. After this, the numbers of *Eurytemora* sp. sharply 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 but always in low abundances ranging between 330 ind. m⁻³ and 3 ind. m⁻³ among all mesocosms. In M1, M5, and M7, *Temora* sp. was absent on day t₃₈ and t₄₅, respectively. Copepod nauplii occurred during the entire experiment duration with peak abundance between t₁₀ and t₂₄ (9003–33 555 ind. m⁻³). Outside this period, nauplii abundance ranged between 17 ind. m⁻³ and 2700 ind. m⁻³.

The three copepod species were determined to copepodite stages (Cl–CV) and adult females and males (Fig.7b). *Eurytemora* sp. copepodites Cl–CV were present in high proportions almost during the whole period of time with up to > 90 %, except on t₃ and t₁₀ they had a lower share in all mesocosms (≤ 50 %). Adult females and males had their minimum during the abundance peak of this species (t₁₇–t₃₁) but occurred during the entire study period indicating more or less continuous reproduction in all meso-
cosms. 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*$_{24}$) when the share of copepodite stages was negligible. 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 and overall *Temora* sp. was not of great importance in the copepod community.

### 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*$_{10}$ ranging between 184 ind. m$^{-3}$ and 3893 ind. m$^{-3}$, respectively, 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$^{-3}$) when *Bosmina* sp. started to increase in abundance. By day *t*$_{17}$ *Bosmina* clearly dominated among cladocerans reaching more than a 90% share until termination of the experiment (Fig. 8a and b). Peak abundance of *Bosmina* sp. occurred at different time points in the different mesocosms and was distinctly higher at 497 µatm (M7, 138 394 ind. m$^{-3}$, *t*$_{31}$), 821 µatm (M6, 114 169 ind. m$^{-3}$, *t*$_{38}$), and 1007 µatm (M3, 127 080 ind. m$^{-3}$, *t*$_{24}$) as compared to the two controls (M1, 72 020 ind. m$^{-3}$, *t*$_{24}$ and M5, 58 107 ind. m$^{-3}$, *t*$_{38}$, respectively) and the mesocosm with the highest fCO$_2$ concentration of 1231 µatm (M8, 63 182 ind. m$^{-3}$, *t*$_{31}$).
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. (Fig. 9a and b). 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 and 17.18. During the remaining days values fall between 0.15 and 8.44, except for M1 on $t_{-3}$ when no organisms with filled brood chambers were counted. *Podon* sp. occurred mainly during the first part of the experiment until $t_{17}$. During that time, the share of organisms with full brood chambers varied roughly between about 25 and 50%. Later, the importance of *Podon* sp. was negligible, but among the few organisms that still occurred were both animals with empty and with full brood chambers. *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).

### 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 significant interaction term between $f$CO$_2$ and chlorophyll $a$. 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.

4 Discussion

4.1 Microzooplankton

The MiZP 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 $\mu$m). *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. ($\leq 20 \mu m$) due to difficulties with clear identification. In our study, the MiZP 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 MiZP 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).

### 4.1.1 Changes in microzooplankton 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). While we found no significant relation between microzooplankton total abundance and fCO$_2$ concentration, total abundance was significantly affected by temperature and the microzooplankton community seemed to change 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 fCO₂ 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, CO₂ 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.

4.1.2 May Myrionecta rubra benefit from OA?

For the present study, a positive CO₂ effect on community chlorophyll a is described with significantly higher concentrations between day t₂₁ and t₃₉ (phase II and III) attributed for up to 90% to picophytoplankton (⩽ 2 µm). The relative contribution of the 2–20 µm size fraction to total chlorophyll a was estimated as about 20% (Paul et al., 2015). This period of increased chlorophyll a concentrations under high CO₂ also coincides well with increased abundances of the probably predominantly photoautotrophic ciliate Myrionecta rubra (⩽ 10 µm) in the high CO₂ mesocosms. Blooms of M. rubra can cause red tides and are characterized by high uptake rates of inorganic nutrients and they can contribute significantly to chlorophyll a values and primary production in estuaries, fjords and upwelling areas. M. rubra robs plastids from cryptophytes. In the absence of cryptophytes, they sustain a larger cell volume but exposure to cryptophytes stimulates incorporation and cell division of M. rubra resulting in a decreased average cell but increased population size (hence biomass) (Lindholm, 1985; Gustafson Jr
Cryptophytes were among the main contributors to total chlorophyll $a$ in particular during phase I and showed a significant negative effect of CO$_2$ during the first phase of the experiment (Paul et al., 2015). Moreover, small picophytoplankton 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., 2015). Cryptophyte biomass decreased from $t_3$ to $t_{17}$ as did the total abundance of *M. rubra*, but whereas the 11–20 µm size class of *M. rubra* almost disappeared by that time or even earlier for the $>20$ µm size class, the small size-class cells remained and 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 synthesize 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* may be a combination of indirect and direct CO$_2$ effects through 1) availability of cryptophytes in phase I, II and III maybe being partly responsible for the negative CO$_2$ effect on cryptophyte biomass and abundances reported by Crawfurd et al. (2015) and Paul et al. (2015), and 2) through a CO$_2$–mediated higher photosynthetic rate of *M. rubra* supporting its own growth. We have no (strong) support, however, that the negative CO$_2$ effect on cryptophyte abundance during phase I is related to higher grazing pressure of *M. rubra* resulting in an abundance increase. A certain affinity of *Mesodinium rubrum* (synonymous *Myrionecta rubra*) to low pH is supported by a microcosm study with a coastal plankton community that revealed clearly higher abundance at the lowest pH level (6.0) Nielsen et al. (2010). During this phase, *M. rubra* abundances showed much variability between sampling days. Some higher abundances in the small and medium size class *M. rubra* especially in M6 and M8 between $t_2$ and $t_{11}$, however, may point
in this direction. During phase II and III, however, *M. rubra* may have benefitted from CO₂ stimulated photosynthetic activities and controlled cryptophyte abundances, and hence, decreased cryptophyte numbers would represent an indirect CO₂ effect through *M. rubra* (Crawfurd et al., 2015).

### 4.2 Mesozooplankton

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. Usually rotifers numerically dominate in spring/early summer (*Synchaeta* 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*) – was barely visible during the first days, later rotifers still occurred until termination but were not of great importance anymore (Viitasalo, 1992).

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 ~22 000–~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., respectively, during a period from 20047
1967–1984 was ∼ 10 000 ind. m$^{-3}$ and respectively ∼ 20 000 ind. m$^{-3}$ (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 blooms 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 mesocosms 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., 2012). 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.

### 4.2.1 Copepods

Up to date, there are only very few large CO$_2$ enrichment mesocom studies with an as far as possible holistic plankton community approach (Riebesell et al., 2008, 2013b). Therefore, this study is still one of the first to follow MZP community development subjected to ocean acidification scenarios projected for this century in a close-to natural whole plankton community. 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. This Arctic MZP community was dominated by meroplanktonic larvae and copepods played a minor role, and thus differed in species type composition compared to the Baltic community enclosed in our mesocosms (Niehoff et al., 2013; Riebesell et al., 2013a). In general, on the mesozooplankton level, calcifiers seem to be more sensitive to CO$_2$ increases than crustaceans (Kurihara, 2008; Kroeker et al., 2013), i.e. copepods which dominate
zooplankton communities in boreal and higher latitude regions. 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., 2015), 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. Mortality of the nauplii stage *Acartia tonsa* for example increased threefold already at $CO_2$ concentrations expected for the end of this century (Cripps et al., 2014). These authors highlight the importance of a holistic life-stage approach in order to provide meaningful data for climate change projections.

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. The $CO_2$ scenario chosen in the present study covered the range projected for this century (IPCC, 2013). Over this range, 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., 2015, 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. Therefore, results from our study are completely in line with earlier studies describing copepods rather robust against $CO_2$ changes at least in the range projected for the end of this century. 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).

4.2.2 Mollusks

Mollusks enclosed in the mesocosms comprised for more than 90 % of juvenile bivalves of the species *Macoma balthica* and occurred during the first ten days of the experiment. Calcifiers are among the most vulnerable organisms to ocean acidification, and within this investigation bivalve larvae were therefore subjected to a more detailed study on their occurrence and length distribution over time. The main findings from this study suggest reduced settling rates and a developmental delay with increasing $f$CO$_2$. For more details see Jansson et al. (2015, this issue).

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

Most conspicuous 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 $f$CO$_2$ could not be proved. Nevertheless, this striking difference may possibly point to an indirect CO$_2$ effect through higher food availability under high CO$_2$.

In the inner parts of the Baltic proper, the endemic *Bosmina longispina maritima* is most abundant among the genus *Bosmina*. Its life cycle includes many parthenogenetic generations in the summer, sexual reproduction occurs only during late summer and autumn from which fertilized resting eggs result that overwinter in the sediment and develop in spring (Kankaala and Wulff, 1981, and references therein). Cladocerans are highly reproductive at times of favourable environmental conditions. The lifespan
Bosmina spp. varies between 20–25 days, age of first reproduction is between 4–7 days (food dependent), they can bear several developmental stages in the brood pouch of the mother and at favourable conditions, 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 concentrations having a significant effect on growth, net reproductive rate and rate of population increase. For example at high food concentration more broods occur and more eggs are produced (Kankaala and Wulff, 1981; Urabe, 1991), whereat not only food quantity but also quality influences life time and together were shown to shorten it to up to 10 days. (Hanazato and Yasuno, 1987). Bosmina is also very quick in taking advantage of favourable environmental conditions and has a high plasticity to customize its life cycle and growth patterns. For example, to avoid predation they can allocate energy to somatic growth instead of early reproduction to attain larger size and escape prey size spectrum of copepods (Kankaala, 1983; Jankowski, 2004).

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) and has two modes of feeding: small-particle filtering and large-particle grasping (DeMott, 1982a, b; Bleiwas and Stokes, 1985).

The above mentioned population increase of Bosmina in the mesocosms coincides with significant CO₂ mediated differences during phase III in chlorophyll a, chlorophytes and particulate organic matter during the respective days and probably represented favourable food conditions for this species enhancing asexual reproduction in particular in the elevated CO₂ mesocosms (Paul et al., 2015). Only M8, the mesocosm with the highest CO₂ concentration, diverged from this trend. The stoichiometry of food organisms can affect growth in cladocerans, for example, growth of Daphnia pulicaria was reduced due to reduced C : P ratios of high CO₂ cultivated food algae (Urabe et al., 2003). In the present study the average C : P ratio of particulate organic matter did not vary with fCO₂, however this can only be seen as an estimate for the food that was
effectively ingested by *Bosmina* (Paul et al., 2015). 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. Most likely, 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 4500 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 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.
5 Conclusions

Our study gives evidence for direct and/or indirect CO$_2$ effects on the micro- and mesozooplankton community. For the MiZP community composition we determined significant changes with a shift towards smaller species/genus with increasing CO$_2$ levels. The phototrophic ciliate *Myrionecta rubra*, as well as *Balanion comatum, Strombidium cf. epidemum, Strombidium* sp. and the short-lived cladocera *Bosmina* seemed to benefit from increased CO$_2$ concentrations, the first one probably directly, the others rather indirectly. Although our results show no direct significant relation with abundance, we assume *Bosmina* growth and reproduction was stimulated from increased food availability at elevated CO$_2$ mostly during phases II and III (higher post-bloom chlorophyll a and particulate organic matter). This may 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. Filter-feeding cladocerans directly feed on bacteria and flagellates and effectively transfer carbon from the microbial loop to higher trophic levels. Contrary, in copepod dominated communities, the carbon transfer from microbial loop is comparatively low because an intermediate trophic level is needed (heterotrophic flagellates, ciliates) (Koski et al., 1999, and references therein). Therefore, we conclude, under increasing ocean acidification in cladoceran dominated MZP communities, the importance of trophic transfer from the microbial loop to higher trophic levels becomes more efficient.

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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).

<table>
<thead>
<tr>
<th>Explanatory variable</th>
<th>DF</th>
<th>$t$</th>
<th>$p$-value</th>
<th>Model</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Microzooplankton</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MiZP total abundance</td>
<td>Temp</td>
<td>1</td>
<td>-3.506</td>
<td>0.0007</td>
</tr>
<tr>
<td><em>Myrionecta rubra</em>, ≤ 10 µm</td>
<td>Temp</td>
<td>1</td>
<td>2.376</td>
<td>0.019</td>
</tr>
<tr>
<td><em>Myrionecta rubra</em>, ≤ 10 µm</td>
<td>$\text{fCO}_2 \cdot \text{Temp}$</td>
<td>1</td>
<td>-2.298</td>
<td>0.024</td>
</tr>
<tr>
<td><em>Myrionecta rubra</em>, ≤ 10 µm</td>
<td>$\text{fCO}_2 \cdot \text{Chl} \ a$</td>
<td>1</td>
<td>2.936</td>
<td>0.004</td>
</tr>
<tr>
<td><em>Balanion comatum</em></td>
<td>Temp</td>
<td>1</td>
<td>2.320</td>
<td>0.022</td>
</tr>
<tr>
<td><em>Balanion comatum</em></td>
<td>$\text{fCO}_2$</td>
<td>1</td>
<td>-2.210</td>
<td>0.030</td>
</tr>
<tr>
<td><em>Strombidium cf. epidemum</em></td>
<td>Chl a</td>
<td>1</td>
<td>-3.229</td>
<td>0.002</td>
</tr>
<tr>
<td><em>Strobilidium sp.</em>, &lt; 20 µm</td>
<td>Temp</td>
<td>1</td>
<td>2.811</td>
<td>0.006</td>
</tr>
<tr>
<td><em>Strobilidium sp.</em>, &lt; 20 µm</td>
<td>Chl a</td>
<td>1</td>
<td>-4.603</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td><em>Strobilidium sp.</em>, &lt; 20 µm</td>
<td>$\text{fCO}_2 \cdot \text{Temp}$</td>
<td>1</td>
<td>-3.600</td>
<td>0.0005</td>
</tr>
<tr>
<td><em>Strobilidium sp.</em>, &lt; 20 µm</td>
<td>$\text{fCO}_2 \cdot \text{Chl} \ a$</td>
<td>1</td>
<td>3.926</td>
<td>0.0002</td>
</tr>
<tr>
<td>Shannon index $H$</td>
<td>Temp</td>
<td>1</td>
<td>3.652</td>
<td>0.0004</td>
</tr>
<tr>
<td>Shannon index $H$</td>
<td>$\text{fCO}_2$</td>
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<td>2.824</td>
<td>0.006</td>
</tr>
<tr>
<td>Shannon index $H$</td>
<td>$\text{fCO}_2 \cdot \text{Temp}$</td>
<td>1</td>
<td>-3.454</td>
<td>0.0008</td>
</tr>
<tr>
<td><strong>Mesozooplankton</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>MZP total abundance</td>
<td>Temp</td>
<td>31</td>
<td>-1.155</td>
<td>0.257</td>
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<tr>
<td>MZP total abundance</td>
<td>$\text{fCO}_2$</td>
<td>31</td>
<td>-0.025</td>
<td>0.980</td>
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<tr>
<td>MZP total abundance</td>
<td>Chl a</td>
<td>31</td>
<td>0.550</td>
<td>0.586</td>
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<tr>
<td>MZP total abundance</td>
<td>$\text{fCO}_2 \cdot \text{Temp}$</td>
<td>31</td>
<td>0.947</td>
<td>0.351</td>
</tr>
<tr>
<td>MZP total abundance</td>
<td>$\text{fCO}_2 \cdot \text{Chl} \ a$</td>
<td>31</td>
<td>-1.081</td>
<td>0.288</td>
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<tr>
<td><em>Bosmina</em> sp.</td>
<td>Chl a</td>
<td>1</td>
<td>0.76</td>
<td>0.453</td>
</tr>
<tr>
<td><em>Bosmina</em> sp. ratio empty/full brood chambers</td>
<td>Temp</td>
<td>1</td>
<td>-3.572</td>
<td>0.001</td>
</tr>
<tr>
<td><em>Bosmina</em> sp. ratio empty/full brood chambers</td>
<td>$\text{fCO}_2$</td>
<td>1</td>
<td>-2.684</td>
<td>0.011</td>
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<tr>
<td><em>Bosmina</em> sp. ratio empty/full brood chambers</td>
<td>Chl a</td>
<td>1</td>
<td>-3.980</td>
<td>0.0004</td>
</tr>
<tr>
<td><em>Bosmina</em> sp. ratio empty/full brood chambers</td>
<td>$\text{fCO}_2 \cdot \text{Chl} \ a$</td>
<td>1</td>
<td>2.738</td>
<td>0.01</td>
</tr>
<tr>
<td>Shannon index $H$</td>
<td>Chl a</td>
<td>1</td>
<td>-0.555</td>
<td>0.582</td>
</tr>
</tbody>
</table>
Figure 1. Total abundance of microzooplankton during the course of the experiment. Note there is one missing value in M1 on $t_{13}$. 
Figure 2. (a) Abundance of different size classes of *Myrionecta rubra*. (b) Abundance of other microzooplankton species/genera/groups. (c) Abundance of other microzooplankton species/genera/groups. Note there is one missing value in M1 on $t_{13}$ in each of the subfigures.
Figure 3. Percent contribution of major taxonomic species/genera/groups to the microzooplankton community. Note there is one missing value in M1 on t_{13}.
Figure 4. (a) Microzooplankton, Shannon diversity index $H$ in relation to the daily change of $f$CO$_2$. Symbols and colours identify the mean $f$CO$_2$ for each mesocosm. (b) Microzooplankton, Shannon diversity index $H$. 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.
Figure 6. Percent contribution of mesozooplankton main taxonomic groups.
Figure 7. (a) Abundance of the dominant copepods species Acartia sp., Eurytemora sp., Temora sp., and copepod nauplii. (b) Percent contribution of different stages of dominant copepods. CI–V: copepodite stages, F: females, M: males.
Figure 8. (a) Abundance of cladoceran species. (b) Percent contribution of different cladoceran species to the total abundance of cladocera.
Figure 9. (a) 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. (b) Ratio of *Podon* with empty to full brood chamber. Note 1: Ratio on $t_3$ was huge and therefore values not shown here to obtain reasonable scaled y-axis. Note 2: occurrence of missing values means no individuals with full brood chambers were present, hence, no ratio could be calculated.