# Survival and settling of larval *Macoma balthica* in a large-scale mesocosm experiment at different *f*$CO_2$ levels

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## Abstract

Anthropogenic carbon dioxide ($CO_2$) emissions are causing severe changes in the global inorganic carbon balance of the oceans. Associated ocean acidification is expected to impose a major threat to marine ecosystems worldwide, and it is also expected to be amplified in the Baltic Sea where the system is already exposed to relatively large natural seasonal and diel pH fluctuations. We studied the responses of larvae of the benthic key-species *Macoma balthica* to a range of future $CO_2$-scenarios using six ~ 55 m3 mesocosms encompassing the entire pelagic community. The mesocosms were deployed in the northern Baltic Sea in June 2012. We focused on the survival, growth and subsequent settlement process of *Macoma balthica* when exposed to different levels of future $CO_2$. The size and time to settlement of *M. balthica* increased along the $CO_2$ gradient, suggesting a developmental delay. With on-going climate change, both the frequency and extent of regularly occurring high $CO_2$ conditions is likely to increase, and a permanent pH decrease will likely occur. The strong impact of increasing $CO_2$ levels on early-stage bivalves is alarming as these stages are crucial for sustaining viable populations, and a failure in their recruitment would ultimately lead to negative effects on the population.

## 1 Introduction

Anthropogenic $CO_2$-emissions are causing severe changes in the oceans (Feely et al., 2004). Future ocean acidification (OA), which includes changes in the inorganic carbon balance of the seawater coupled with a decrease in pH, is occurring at a rate faster than experienced in the geological past (Hönisch et al., 2012), and is expected to impose a major threat to marine ecosystems worldwide (Orr et al., 2005; Fabry et al., 2008). The sea surface pH is estimated to decrease by 0.4 units in the global open oceans by the year 2100 (Caldeira and Wickett, 2003), whereas many coastal areas already experience large pH fluctuations reaching to considerably lower pH levels than predicted for the near future (Blackford and Gilbert, 2007; Johnson et al., 2013). The multiple environmental stressors impacting coastal areas and the local processes that impact watersheds make the precise modelling of future pH levels exceedingly challenging for these areas (Borges and Gypens, 2010; Duarte et al., 2013).

The majority of studies investigating the biological effects of future $CO_2$ levels have focused on its impacts on calcifying species and on pelagic primary producers. Pelagic calcifiers such as bivalve early life-stages are generally considered susceptible to increasing $CO_2$ levels (Kurihara, 2008; Dupont and Thorndyke, 2009), with a range of observed (mostly negative) impacts on development, survival and growth of larval stages as consequences of the $CO_2$ increase (Gazeau et al., 2013). Also the settling and survival of post-larvae are impacted by the changes in the water chemistry (Green et al., 2004, 2009; Clements and Hunt, 2014). The response of organisms to future $CO_2$ levels has traditionally been studied in experiments focusing on single species, and the community-wide responses are still not well known. In mesocosms, the natural community can be maintained to a high degree, and organismal performance can be measured in near-natural surroundings (Riebesell et al., 2010). Mesocosm studies have the additional advantages of allowing experimental manipulation of environmental factors such as $CO_2$, possibility for replication, and repeated sampling of the closed study systems over long experimental duration.

In the Baltic Sea a drop in pH of 0.5 units is estimated for the surface waters within this century (Hjalmarsson et al., 2008; Omstedt et al., 2012). Similar to coastal and estuarine areas (Duarte et al.,

2013), however, the natural pH variability in the Baltic Sea is large and regularly exceeds the estimates made for the near-future (Omstedt et al., 2009; Melzner et al., 2012; Jansson et al., 2013). For example, during the summer season pH changes of nearly one unit per day driven by changes in primary production and respiration are common in the shallow coastal areas of the northern Baltic Proper (pers. obs.). Yet, ocean acidification is likely to increase the pH fluctuations, making the occasionally experienced extreme pH levels even more pronounced, further expanding the pH range which the Baltic species are exposed to (Thomas and Schneider, 1999; Omstedt et al., 2010; Melzner et al., 2012). A key species in the Baltic Sea soft-bottom communities, the bivalve *Macoma balthica* (L.), is experiencing variable conditions throughout its life-cycle. During the larval phase, it is exposed to large pelagic diel pH-fluctuations (Jansson et al., 2013; Almén et al., 2014) followed by the harsh reducing conditions of the sedimentary system when settling into the benthic environment (Woodin et al., 1998). The tolerance of *M. balthica* to low pH conditions has so far been studied in aquarium experiments of different types and durations (van Colen et al., 2012; Jansson et al., 2013), which have shown negative effects on the early-stage bivalves. In such experiments, however, the potential impact of future environmental changes on e.g. the settlement process is challenging to study.

The aim of the whole large-scale pelagic mesocosm experiment was to study the responses of the Baltic Sea pelagic community to different future $fCO_2$-scenarios. In this specific study we wanted to explicitly shed light on 1) growth and survival of *M. balthica* larvae and 2) the subsequent settling of the post-larvae, when exposed to different levels of future $CO_2$ in their natural surroundings. Based on the results of our previous experiments (Jansson et al., 2013; van Colen et al., in prep.), we predicted the growth of the larvae to decrease along the increasing $fCO_2$ gradient and the survival and settling to be negatively impacted by the $fCO_2$ increase.

## 2 Material and methods

### 2.1 The study species

The infaunal bivalve *M. balthica* is abundant throughout the Baltic Sea, often dominating biomass in soft sediments from organic mud to sandy bottoms from the very shallow down to 190 m depth

(Segerstråle, 1960; Elmgren et al., 1986; Bonsdorff, 2006). The spawning of *M. balthica* occurs when water temperature has reached approximately 7°C (Caddy, 1967). The planktonic life stage (ca. 6 weeks) ends when the individual has reached a sufficient size and developmental stage (including increased mobility of the foot) to metamorphose and settle to the seafloor (Caddy, 1969). A majority of the very newly settled bivalves encountered in the Baltic Sea have a size of 250–300 µm (Ankar, 1980; Elmgren et al., 1986; Olafsson, 1989). Peak settling in the northern parts of the Baltic Sea typically occurs in July. During the pelagic larval phase, abundances of up to 12 000 larvae $m^{-3}$ are measured in the Baltic Sea, with a settling population of around 30 000 $m^{-2}$ each year, at peak settling even up to 300 000 $m^{-2}$ (Ankar, 1980; Elmgren et al., 1986; Bonsdorff et al., 1995). *M. balthica* is an important prey organism, and has a central role in sediment reworking and bioturbation, contributing to the overall health and functioning of the benthic ecosystem (Michaud et al., 2006). In the species-poor northern Baltic Sea, this species is essential to the functioning of the benthic ecosystem through these key processes (Villnäs et al., 2012; Norkko et al., 2013).

**2.2 Experimental set-up**

Six pelagic mesocosms (KOSMOS, Riebesell et al. 2013a) of ~55 $m^3$ were deployed in the western Gulf of Finland (59° 51.5' N, 23° 15.5' E) on 12 June 2012 to study responses of the Baltic Sea plankton community to increased fugacity of carbon dioxide ($fCO_2$). The mesocosm bags were lowered down to a depth of 17 m to enclose the natural plankton community, excluding organisms larger than 3 mm by a mesh installed at the top and bottom of the cylindrical bags. With the bags fully submerged below the sea surface, water and organisms inside the bags could exchange with the surrounding water mass for five days before closing the mesocosms on 17 June (day -5, 5 days before $CO_2$ manipulation). To seal the bottom of each mesocosm, a two meter long sediment trap funnel collecting settling particles and organisms was installed by divers to replace the 3 mm mesh. The top end of the bags was simultaneously pulled above the sea surface to fully isolate the enclosed water bodies. Bubbling the systems with compressed air for three and a half minutes right after closure destroyed the halocline present inside the bags. The mesocosms were manipulated with filtered (50 µm), $CO_2$-saturated seawater as described by Riebesell et al. (2013a) on four consecutive days (day 0–3) to establish a range

of four $fCO_2$ target treatments (600–1650 µatm) and two ambient blind manipulated mesocosms (Table 1). On day 15 $fCO_2$ was readjusted inside the treated mesocosms to counteract outgassing of $CO_2$. For a more detailed description of the experimental set-up, manipulations and maintenance of the mesocosms please see Paul et al. (2015).

### 2.3 Sampling the mesocosms

#### 2.3.1 Water parameters

CTD profiles were measured daily with a handheld self-logging CTD60M probe (Sea and Sun Technology) from 0.3 down to 18 m (mesocosms) and to 30 m (surrounding bay) with sensors for salinity, temperature, dissolved oxygen, PAR (photosynthetic active radiation) and pH. Details on the sensors and their accuracy are described in Schulz and Riebesell (2013). Depth-integrated water samples (IWS, HYDRO-BIOS Kiel) were collected regularly (daily to every other day, see Paul et al., 2015) from all mesocosms and the surrounding water body to measure e.g. total pH (pHT), total alkalinity (TA) and dissolved inorganic carbon (DIC) for determining the inorganic carbon components, and chlorophyll *a* to follow the development of the phytoplankton bloom. pHT was determined by analyzing samples with a Cary 100 (Varian) spectrophotometer (Dickson et al., 2007). The details of the procedure ($fCO_2$ was calculated from measured DIC and pHT) are described in Paul et al. (2015). CTD pH measurements were corrected to pH on the total scale by linear correlations of mean water column potentiometric pH measurements to spectrophotometric pHT measurements. Exact details of all sampling procedures, equipment used and sample analyses are described in Riebesell et al. (2013a), Schulz et al. (2013) and Paul et al. (2015).

#### 2.3.2 Water column: Mesozooplankton sampling and quantification of *M. balthica* larvae

Mesozooplankton samples from the six mesocosms were taken with an Apstein net of 17 cm diameter and 100 µm mesh size by pulling the net vertically from 17 m depth to the sea surface. Net hauls were taken from the mesocosms on eleven sampling days: prior to the first $CO_2$ addition (days -3, -2, -1), on the day of the first $CO_2$ addition (day 0), and after the first $CO_2$ addition in a seven day rhythm (days 3, 10, 17, 24, 31, 38, 45). Mesozooplankton samples were preserved in 70% ethanol. The larvae of *M.*

*balthica* were counted in the whole sample under a stereo microscope (WILD M3B). For size range determination, on average 70 individuals were measured from each mesocosm on days 0 and 10. The individuals were photographed using a dissecting microscope connected to a Nikon DS-Fi2 camera system, and sizes were determined by measuring shell lengths using the Nikon DS camera interface. Zooplankton abundance was calculated as individuals per cubic meter, assuming 100% filtering efficiency of the net. For more details on mesozooplankton sampling and processing see Lischka et al. (2015).

### 2.3.3 Sediment traps: collection of material, subsampling and quantification of settling *M. balthica*

The sediment traps were emptied every second day using a gentle vacuum to pump the samples through a silicon tube into sampling flasks at the sea surface (for more details see Boxhammer et al., 2015). Subsamples of 20 mL were taken with a pipette of the homogeneously mixed samples (on average 2.5 L) and preserved in 4% buffered formalin for quantification and size determination of settling bivalves. Abundance and size range determinations of settled bivalves were made on 3 replicates of 1 mL subsamples. *M. balthica* collected in the sediment traps included settled individuals as well as individuals that had died in the water column or in the sediment trap after settling. However, the gaping shells of individuals that were dead at the time of sampling were identified in the preserved samples and such individuals were not counted. Individuals that were assessed to be living at the time of sampling were counted and photographed using a dissecting microscope connected to a Nikon DS-Fi2 camera system. During the main settling period (days 11, 13, 15 and 17) on average 35 individuals were measured from each mesocosm. Sizes were determined by measuring shell lengths using the Nikon DS camera interface.

## 2.4 Numerical analysis

The abundance of bivalve larvae in the water column of each mesocosm over time was compared by calculating a rate of change between each sampling day and comparing the timing of decreasing abundances. This was done by calculating Spearman correlation ranks for each time point. To analyse the differences in post-larval settling between the mesocosms, we performed a chi-square test to

compare the cumulative abundances of settled individuals on days 9, 11, 13, 15, 17 and 19. Graphical post-hoc tests were performed to identify differences between mesocosms.

The sizes of both the larvae in the water column and the post-larvae in the settling traps in the different $fCO_2$ levels were compared by a linear regression model. To standardize the comparisons, they were conducted on average sizes of a batch of individuals measured in each mesocosm. The residuals of the regressions adhered to the assumption of normality. All analyses were performed in the software R (version 3.0.2; R Development Core Team, 2012). The differences were considered significant at $p < 0.05$ for all tests. The data for the carbonate system parameters are shown as averages until day 17 (the settling period of *M. balthica).* The graphs are based on actual $fCO_2$ values (presented in table 1). Data are presented as means ± SE.

## 3 Results

### 3.1 Abiotic conditions in the mesocosms

Water temperature varied from 8°C to 16°C during the experiment, following the natural conditions in the bay. Salinity was on average 5.7 and total alkalinity on average 1550 mmol $kg^{-1}$ at the closing of the mesocosms. Both parameters remained fairly constant during the experiment in all mesocosms (Paul et al. 2015, this issue). Initial pHT after closing of the mesocosms and before the $CO_2$-manipulations was ca. 8.2 in the mesocosms and the bay. Average pHT levels and other parameters of each mesocosm over the course of the experiment are shown in table 1.

### 3.2 Larval abundance

After the closing of the mesocosms (day -3 to -2), some unexplained variation was found in the abundance of bivalve larvae (Fig. 1). On day 0, however, the abundances in the water column were relatively similar within the mesocosms (5522-5936 ind. $m^{-3}$), except in the 319 µatm ambient mesocosm. This is likely due to a sampling issue or an artifact caused by a mesocosm maintenance method (bubbling to destroy the halocline on day -3). During the first week after the $CO_2$-manipulation, by day 10, the larval abundance had decreased strongest in the two ambient mesocosms, with > 80%

197 decrease in abundance in comparison to the 35-50 % decrease in the two highest $fCO_2$ mesocosms
198 (>1000 µatm) (Spearman r =−0.83, $p < 0.05$). Consequently, on day 10 the highest abundance was
199 measured in the highest $fCO_2$ mesocosm (3194 ind. $m^{-3}$) and the lowest abundances in both ambient
200 mesocosms (319-321 µatm) (545 resp. 1064 ind. $m^{-3}$). A strong decrease in abundance (> 85 %)
201 occurred a week later (day 10 to 17) in all the high, >400 $fCO_2$, mesocosms, with up to a 93% decrease
202 found in the 1347 µatm mesocosm (Spearman r =0.94, $p < 0.05$). From day 17 onwards, the abundances
203 were low in all of the mesocosms (Fig. 1).
204
205 **3.3 The abundance of settled individuals**
206 The abundances of settled individuals differed significantly between mesocosms and sampling days of
207 the main settling period (days 9-17, chi-square $\chi^2 = 1168.588$, $df = 25$, $p < 0.001$). The graphical post-
208 hoc tests showed three distinct settling peaks of *M. balthica*. In the ambient and near-ambient (<500
209 µatm) $fCO_2$ mesocosms a large increase in the abundance of settled individuals was found between days
210 9-13, with 71 %, 74 % and 54 % of all the individuals having settled by day 13. In comparison, only 39
211 % and 47 % of the individuals had settled during that time period in the two highest (1072-1347 µatm)
212 $fCO_2$ mesocosms (Fig. 2a and b). In the 857 and 1072 µatm $fCO_2$ mesocosms, a smaller settling event
213 occurred on days 11-15 and in the highest $fCO_2$ mesocosm the settling peaked on day 17, where after the
214 settling soon ceased in all mesocosms. On average 6130 ± 240 individuals settled in the mesocosms
215 during the course of the experiment, with the exception of 1072 µatm $fCO_2$ mesocosm where only ca.
216 4850 individuals settled (Fig. 2b).
217
218 **3.4 Larval sizes in the water column**
219 On day 0, larval size in the water column was on average 287 ± 23 µm with no difference found
220 between the mesocosms. After 10 days of exposure to different $fCO_2$ levels, the average size of the
221 larvae in the water column (0-17 m) varied from 286 µm to 313 µm, increasing significantly along the
222 increasing $fCO_2$ gradient ($R^2 = 0.78$, $F = 14.47$, $p = 0.019$, Fig 3) with ca. 10 % larger larvae still in the
223 water column in the two highest $fCO_2$ mesocosms (1072 and 1347 µatm).
224

225 **3.5 The sizes of settled individuals**

226 On average > 80% of the individuals settled in the mesocosms during days 11 to 17. No significant
227 differences were found in the sizes of the settled individuals in the different $fCO_2$ levels at any of these
228 investigated time points (Fig. 4). On days 11 and 13 the average size within the mesocosms varied
229 between 285 µm to 303 µm, and on days 15 and 17 the average size varied between 293 µm to 317 µm.
230
231 **4 Discussion**

232 In this study we investigated the effects of different future $CO_2$ scenarios on the larval survival, growth
233 and settling of a Baltic Sea benthic key species *M. balthica* in a large-scale mesocosm setting. We found
234 that *M. balthica* settled later along the increasing $fCO_2$ gradient of the mesocosms. Moreover, an
235 indication that *M. balthica* larvae settled at a larger size in the high $fCO_2$ treatments was also observed,
236 possibly indicating that at increasing $fCO_2$ a sufficient mass for settling is not reached until a larger shell
237 length has been attained.
238
239 During the week after first $CO_2$ manipulation (day 3 to day 10) settling of *M. balthica* occurred faster in
240 the ambient and middle $fCO_2$ mesocosms (319 to 469 µatm) than in the higher $fCO_2$ mesocosms.
241 Consequently, the main settling peak occurred ca. 6 days earlier in these mesocosms (<500 µatm).
242 When comparing the sizes of the larvae, we found that the ones remaining in the water column on day
243 10 had an average size of 290 µm in both ambient mesocosms, whereas in the other mesocosms ($fCO_2$
244 >400 µatm), the sizes of the remaining larvae were 300-315 µm. We hypothesise that in the ambient
245 $fCO_2$ the bivalves settled at the expected size (<300 µm), and thus only the smaller larvae remained in
246 the upper water column when the settling was reaching its peak. In the high $fCO_2$ treatments the
247 development of the *M. balthica* larvae might have been compromised and/or delayed as on day 10,
248 despite being relatively large (>300 µm), a large part of the bivalves remained in the upper water
249 column without initiating settlement.
250
251 The observed inconsistency between the growth and settling of the early-stage bivalves can be explained
252 by proximate factors that regulate settling. For successful metamorphosis and settling from the

planktonic phase to the benthos, the individuals need to reach a sufficient size or weight and developmental stage, including increased mobility/appearance of the foot (Caddy, 1969; Drent, 2002). Shell growth alone, the growth measure used in our experiment as in many other studies, does not automatically reflect the overall biomass production and developmental stage of the organism (Lewis and Cerrato, 1997; Wood et al., 2008). In undersaturated conditions, calcification of the shell might be compromised so that even though shell length reaches its typical size for settling, shell thickness is reduced. This could be a factor that restricts the gaining of necessary mass to settle to the sea floor (Waldbusser et al., 2010). During the entire experiment, undersaturation with respect to aragonite occurred in all mesocosms apart from the two ambient mesocosms, and the three highest $fCO_2$ treatments were also undersaturated with respect to calcite (Table 1). It is also likely that at decreased pHT levels shell growth was occurring at the cost of tissue development and biomass increase. Unfortunately we were not able to measure soft tissue weight of collected larvae due to the very small size. Larvae that stay longer in the water column, e.g. due to slower growth or delayed development, face a higher risk of predation. The population dynamics of a bivalve species is largely dependent on successful settlement and recruitment of the post-larvae, and dispersal of larval and post-larval stages (Pedersen et al., 2008; Pineda et al., 2009; Valanko et al., 2010). As larval mortality of planktonic invertebrates is also generally high (yet variable; estimates range from 3–23% daily), mainly due to predation and environmental factors (Pineda et al., 2009), a reduced survival of the early-life stages, as found in the present study, is alarming. As the key species of the soft-bottom ecosystems of the Baltic Sea, *M. balthica* is an essential contributor to the overall health and functioning of the benthic ecosystem. Future $CO_2$-mediated changes to this species' population size might thus affect the diversity and ecosystem functioning of the area.

Some other important factors that impact the settlement process, but cannot be mimicked in this mesocosm setup include, e.g., sediment type and quality, cues from adult conspecifics and water movements that can prevent or facilitate the settlement process (Woodin et al., 1986). Some limits to ecosystem realism also arise from the exclusion of factors such as currents and large predators, which impact the natural succession and dispersion patterns of the species. To understand complex, system-

281 wide responses that take into account ecological processes such as competition, predation and the effect
282 of/on different trophic levels, several species interactions need to be tested simultaneously. The
283 interactions between factors such as increasing $CO_2$ and predation is a topic for future studies, but it is
284 likely that individuals stressed by high $CO_2$ also would suffer higher predation rates.
285
286 In a previous aquarium experiment conducted with newly hatched larvae (ca. 150 µm) from the same
287 bay (Jansson et al., 2013), both the growth and survival of the larvae were found to be negatively
288 impacted by decreasing pH. In this mesocosm experiment, however, survival was not found to be
289 affected, and it was not possible to study growth in the same level of detail as in a laboratory
290 experiment. Other typical consequences of pH decrease found in early-stage bivalves are e.g. delayed
291 and/or abnormal development (Kurihara et al., 2008; Talmage and Gobler, 2010; Crim et al., 2011),
292 reduced calcification (Miller et al., 2009) and higher mortality (Talmage and Gobler, 2009; Crim et al.,
293 2011; van Colen et al., 2012). The settling of post-larvae to the seafloor may be impacted by the changes
294 in the water chemistry created by $CO_2$ increase (Green et al., 2004; Cigliano et al., 2010; Clements and
295 Hunt, 2014). The major part of ocean acidification research has been conducted by studying the
296 response of single species, with a few studies focusing on the interactions between a small number of
297 species, whereas studies on intact communities have so far only rarely been conducted (but see e.g.
298 work done at $CO_2$ vents by Hall-Spencer et al., 2008 or Kroeker et al., 2011 and previous/other
299 mesocosm studies by Christen et al., 2013; Riebesell et al., 2013b). For species such as *M. balthica*, a
300 mesocosm setting provides an excellent platform to study the development and succession of pelagic
301 early-life stages resulting in recruitment into the benthic system, which cannot be studied in a simple,
302 small-scale aquarium experiment. The direct and indirect factors that essentially impact the early life
303 success of a bivalve, e.g. natural food quality and quantity, can be incorporated in a mesocosm setting in
304 a more comprehensive way. In the case of future ocean acidification, potential changes in phytoplankton
305 dynamics due to increased $CO_2$ levels are likely to have consequences for the other trophic levels. The
306 growth of nanoplankton and diatom species (< 20 µm), which are the main food particles of larval
307 bivalves (Bos et al., 2006), has been shown to benefit from changing $CO_2$ conditions (e.g. Engel et al.,
308 2008; Feng et al., 2009; Meakin and Wyman, 2011; but see also e.g. Tortell et al., 2002), potentially

impacting the capacity of the larvae to survive in a changing environment via consequences in their energy balance. In this study, no significant changes were detected in the phytoplankton abundance or the total chlorophyll *a* concentration within the mesocosms during the main occurrence of *M. balthica* larvae in the water column (until days 10 and 17). An increase in the abundance of phytoplankton and Chl *a* concentration in the highest $fCO_2$ mesocosms was, however, found later on during the experiment (day 16 onwards; Crawfurd et al., 2015; Paul et al., 2015). By the time the differences in phytoplankton abundance started emerging, most of the *M. balthica* larvae had already settled from the water column.

The Baltic Sea is a unique system to study future ocean acidification. Large pH fluctuations that already occur seasonally in the northern Baltic Sea in the shallow coastal areas, primarily due to changes in productivity (Thomas and Schneider, 1999; Schneider et al., 2003), result in high pH values of up to 8.4 during daytime and low pH values such as 7.4 during respiration at night (pers. obs.). For areas such as this, accurate modelling of the future pH change is generally challenging. Yet, future ocean acidification is predicted to permanently decrease the pH and thus shift the pH range the organisms are exposed to towards lower values (Omstedt et al., 2010). In our study we found negative effects of increasing $CO_2$ levels on the settling and early development of *M. balthica*. The impact on the success of these early-stage bivalves is alarming as these stages are crucial for sustaining viable populations. A failure in their recruitment would ultimately lead to negative effects on the population, and considering the key role *M. balthica* has in the Baltic Sea, also for the functioning and resilience of the benthic ecosystem.

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Table 1. Carbonate system parameters in the mesocosms during the experiment (average values on days 0-17, the main settling period of *M. balthica*).

| | M1 | M5 | M7 | M6 | M3 | M8 | Bay |
|---|---|---|---|---|---|---|---|
| Target $fCO_2$ (µatm) | ambient/control | ambient/control | 600 | 950 | 1300 | 1650 | ambient |
| $fCO_2$ (µatm) | 319 | 321 | 469 | 857 | 1072 | 1347 | 282 |
| pHT | 7.94 | 7.94 | 7.80 | 7.59 | 7.51 | 7.43 | 7.99 |
| Ω aragonite | 1.07 | 1.06 | 0.77 | 0.47 | 0.39 | 0.33 | 1.19 |
| Ω calcite | 1.92 | 1.91 | 1.39 | 0.84 | 0.71 | 0.59 | 2.14 |

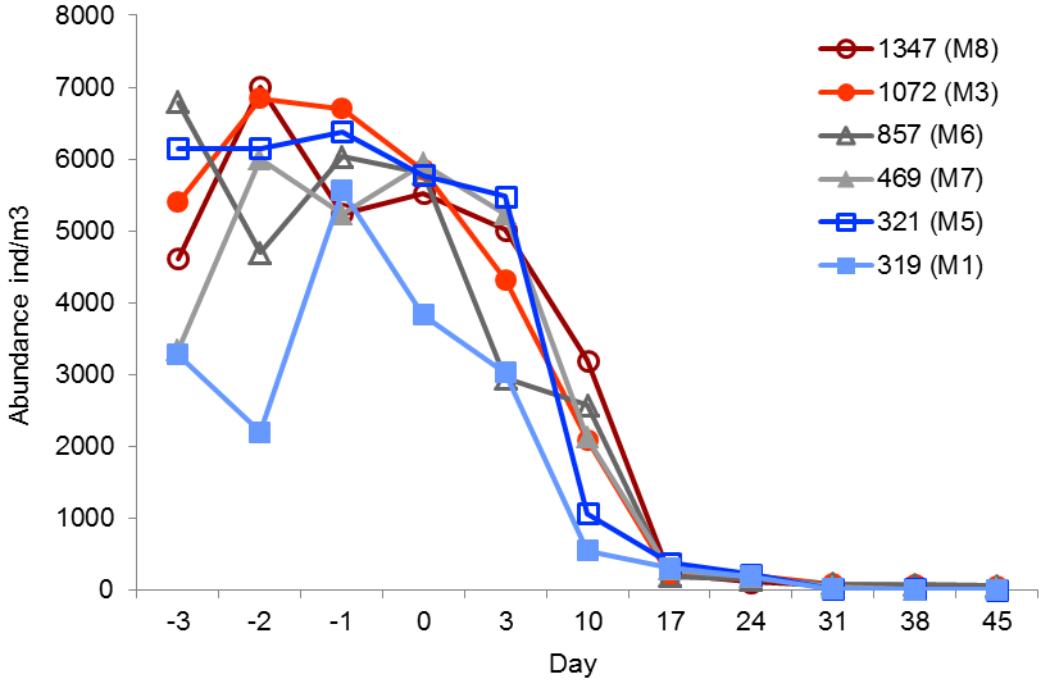
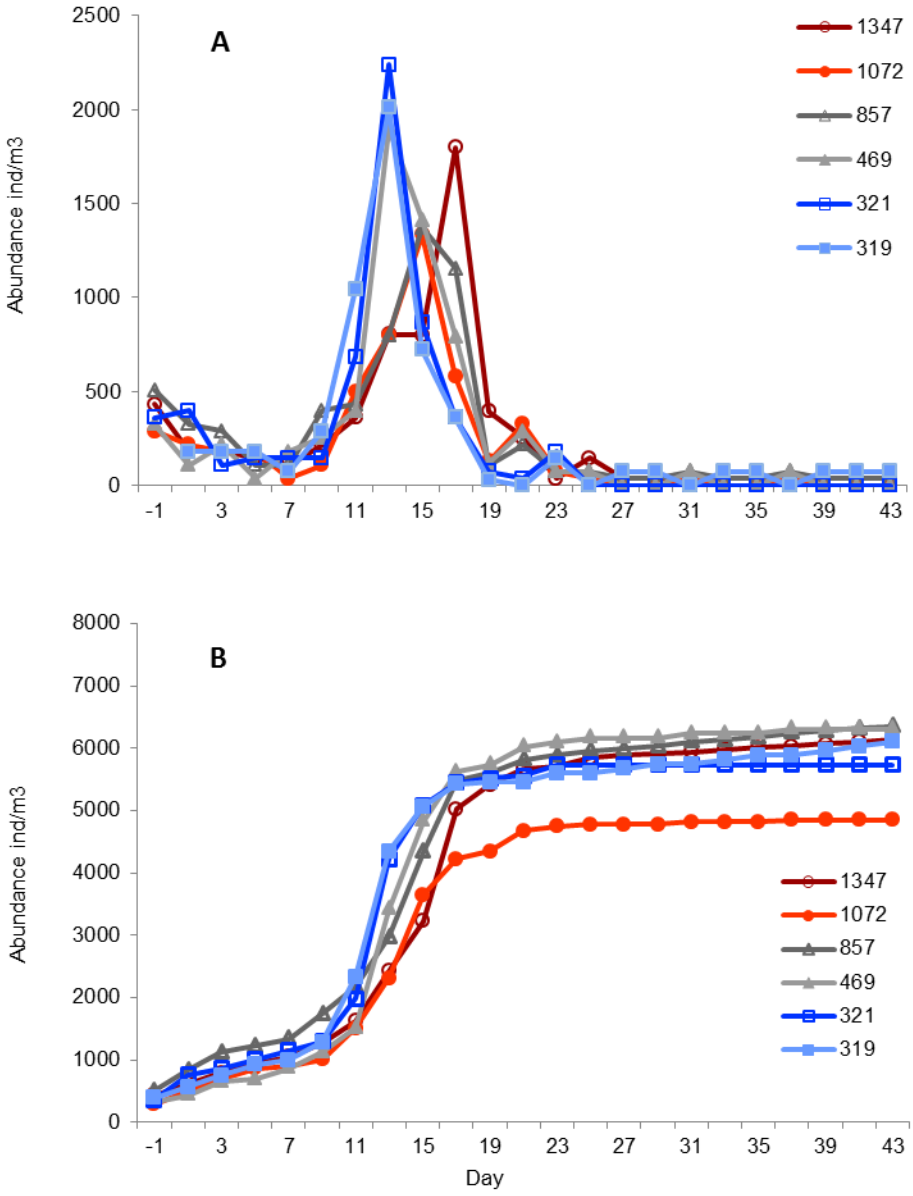
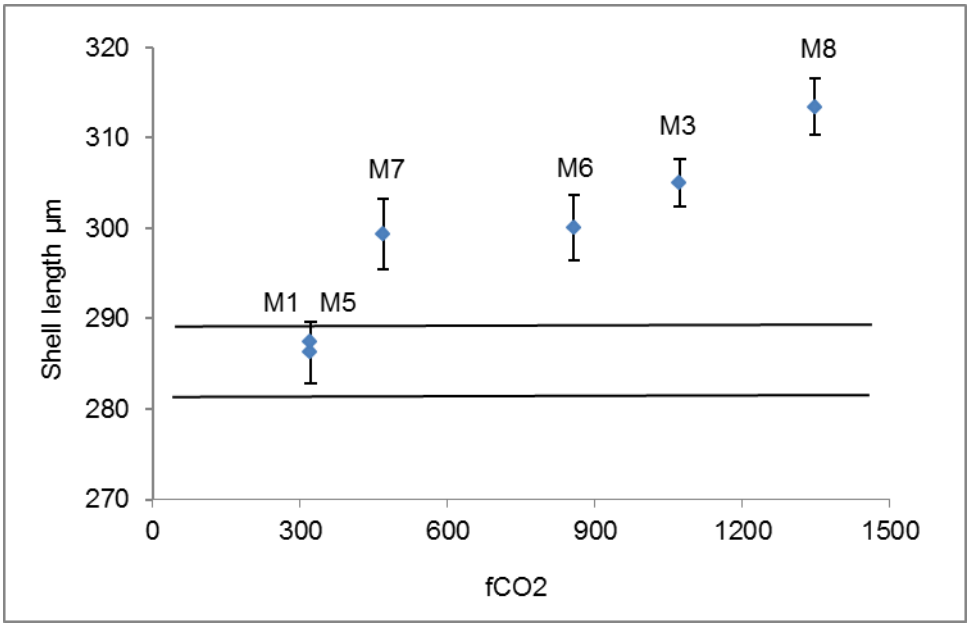



Figure1. Larval abundance in the water column of the individual mesocosms over time.




602

603 Figure 2. A. The abundance of settled individuals per cubic meter water mass enclosed in the different
604 mesocosms over the course of the experiment. B. The cumulative abundance of settled *M. balthica* per
605 cubic meter of individual mesocosm volume.

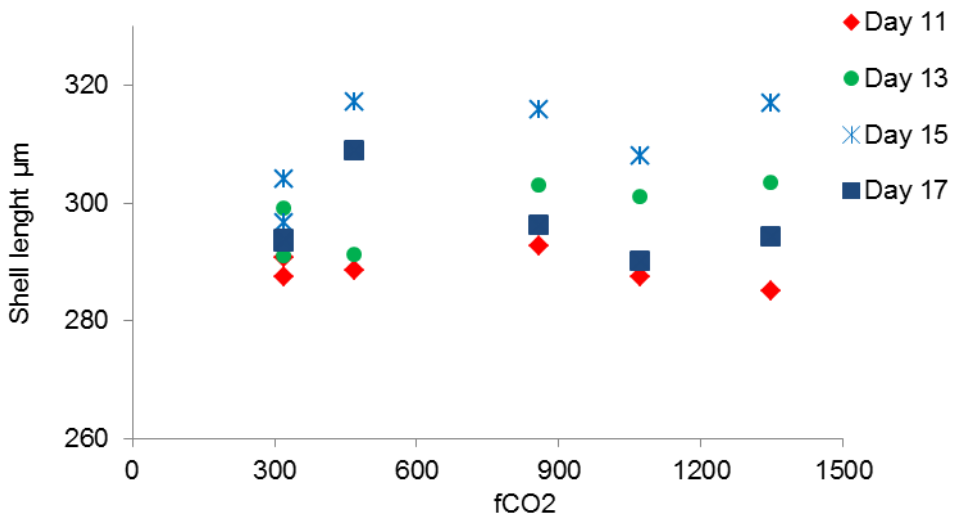



606

607 Figure 3. Larval sizes in different $fCO_2$ levels at day 10. Data is presented as means ± SE, n = ca. 70

608 individuals. The horizontal lines indicate the range of average larval sizes on day 0.

609




610

611 Figure 4. Sizes of the settled individuals exposed to different $fCO_2$ levels on days 11, 13, 15 and 17.

612 Data is presented as means, n= ca. 35 at each data point. For clarity, SE are not shown.

613