



# Survival and settling of larval *Macoma balthica* in a large-scale mesocosm experiment at different $f\text{CO}_2$ levels

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**Abstract.** Anthropogenic carbon dioxide ( $\text{CO}_2$ ) emissions are causing severe changes in the global inorganic carbon balance of the oceans. Associated ocean acidification is expected to pose 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  $\text{CO}_2$  scenarios using six  $\sim 55 \text{ m}^3$  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  $\text{CO}_2$ . The size and time to settlement of *M. balthica* increased along the  $\text{CO}_2$  gradient, suggesting a developmental delay. With ongoing climate change, both the frequency and extent of regularly occurring high  $\text{CO}_2$  conditions are likely to increase, and a permanent pH decrease will likely occur. The strong impact of increasing  $\text{CO}_2$  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  $\text{CO}_2$  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 pose 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  $\text{CO}_2$  levels have focused on their impacts on calcifying species and on pelagic primary producers. Pelagic calcifiers such as bivalve early-life stages are generally considered susceptible to increasing  $\text{CO}_2$  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  $\text{CO}_2$  increase (Gazeau et al., 2013). Also, the settling and survival of post-larvae are impacted by the changes in the water chem-

istry (Green et al., 2004, 2009; Clements and Hunt, 2014). The response of organisms to future CO<sub>2</sub> 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<sub>2</sub>, 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 (A. Jansson, personal observation, 2014). However, 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 lifecycle. 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 *f*CO<sub>2</sub> 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<sub>2</sub> in their natural surroundings. Based on the results of our previous experiments (Jansson et al., 2013; van Colen et al., 2016), we predicted the growth of the larvae to decrease along the increasing *f*CO<sub>2</sub> gradient and the survival and settling to be negatively impacted by the *f*CO<sub>2</sub> 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). The 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<sup>-3</sup> are measured in the Baltic Sea, with a settling population of around 30 000 m<sup>-2</sup> each year, at peak settling even up to 300 000 m<sup>-2</sup> (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<sup>3</sup> 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 (*f*CO<sub>2</sub>). 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 5 days before closing the mesocosms on 17 June (day -5, 5 days before CO<sub>2</sub> manipulation). To seal the bottom of each mesocosm, a 2 m 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 3.5 min right after closure destroyed the halocline present inside the bags. The mesocosms were manipulated with filtered (50 µm), CO<sub>2</sub>-saturated seawater as described by Riebesell et al. (2013a) on 4 consecutive days (days 0–3) to establish a range of four *f*CO<sub>2</sub> target treatments (600–1650 µatm) and two ambient blind manipulated

**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 $f\text{CO}_2$ ( $\mu\text{atm}$ )	Ambient/control	Ambient/control	600	950	1300	1650	Ambient
$f\text{CO}_2$ ( $\mu\text{atm}$ )	319	321	469	857	1072	1347	282
pHT	7.94	7.94	7.80	7.59	7.51	7.43	7.99
$\Omega$ aragonite	1.07	1.06	0.77	0.47	0.39	0.33	1.19
$\Omega$ calcite	1.92	1.91	1.39	0.84	0.71	0.59	2.14

mesocosms (Table 1). On day 15  $f\text{CO}_2$  was readjusted inside the treated mesocosms to counteract outgassing of  $\text{CO}_2$ . 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 (conductivity, temperature and depth) 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 analysing samples with a Cary 100 (Varian) spectrophotometer (Dickson et al., 2007). The details of the procedure ( $f\text{CO}_2$  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  $\mu\text{m}$  mesh size by pulling the net vertically from 17 m depth to the sea surface. Net hauls were taken from the mesocosms on 11 sampling days: prior to the first  $\text{CO}_2$  addition (days –3, –2, –1), on the day of the first  $\text{CO}_2$  addition (day 0), and

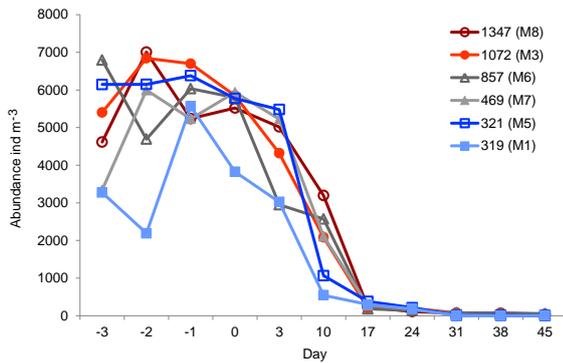
after the first  $\text{CO}_2$  addition in a 7-day rhythm (days 3, 10, 17, 24, 31, 38, 45). Mesozooplankton samples were preserved in 70 % ethanol. The larvae of *M. balthica* were counted in the whole sample under a stereo microscope (WILD M3B). For size range determination, on average 70 individuals 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 metre, 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., 2016). 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 three 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



**Figure 1.** Larval abundance in the water column of the individual mesocosms over time.

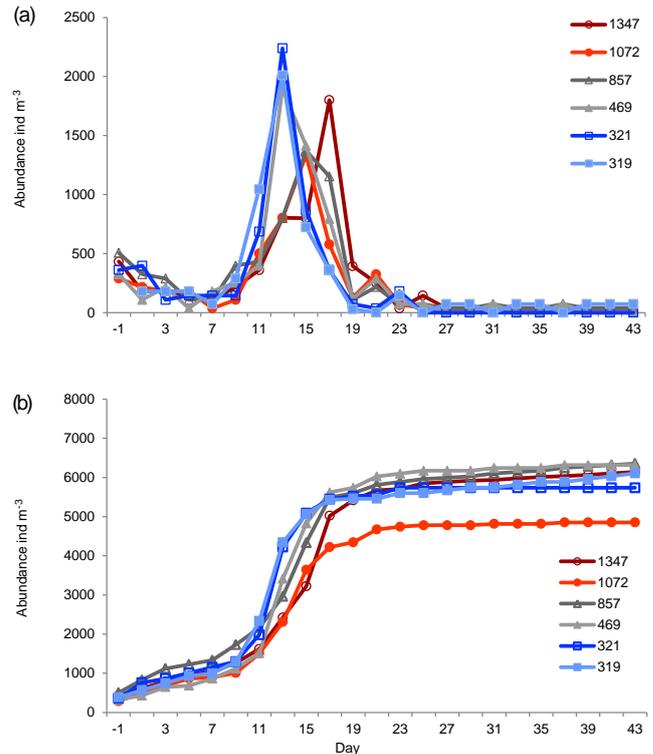
change between each sampling day and comparing the timing of decreasing abundances. This was done by calculating Spearman correlation ranks for each time point. To analyse the differences in post-larval settling between the mesocosms, we performed a chi-square test to compare the cumulative abundances 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  $f\text{CO}_2$  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 R software (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  $f\text{CO}_2$  values (presented in Table 1). Data are presented as means  $\pm$  SE.

### 3 Results

#### 3.1 Abiotic conditions in the mesocosms

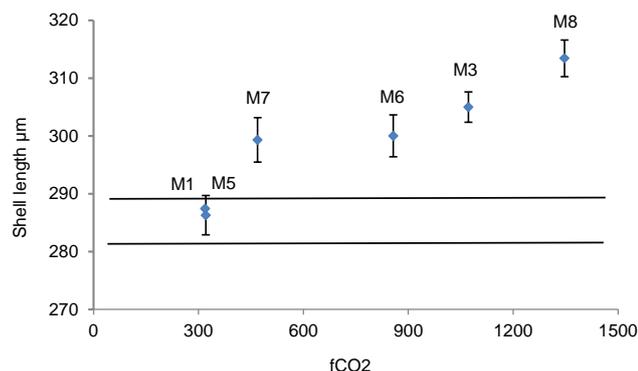
Water temperature varied from 8 to 16 °C during the experiment, following the natural conditions in the bay. Salinity was on average 5.7 and total alkalinity on average 1550 mmol kg<sup>-1</sup> at the closing of the mesocosms. Both parameters remained fairly constant during the experiment in all mesocosms (Paul et al., 2015). Initial pHT after closing of the mesocosms and before the CO<sub>2</sub> 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.



**Figure 2.** (a) The abundance of settled individuals per cubic metre water mass enclosed in the different mesocosms over the course of the experiment. (b) The cumulative abundance of settled *M. balthica* per cubic metre of individual mesocosm volume.

#### 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<sup>-3</sup>), except in the 319  $\mu\text{atm}$  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<sub>2</sub> manipulation, by day 10, the larval abundance had decreased strongest in the two ambient mesocosms, with a > 80 % decrease in abundance in comparison to the 35–50 % decrease in the two highest  $f\text{CO}_2$  mesocosms (> 1000  $\mu\text{atm}$ , Spearman  $r = -0.83$ ,  $p < 0.05$ ). Consequently, on day 10 the highest abundance was measured in the highest  $f\text{CO}_2$  mesocosm (3194 ind. m<sup>-3</sup>) and the lowest abundances in both ambient mesocosms (319–321  $\mu\text{atm}$ ) (545 resp. 1064 ind. m<sup>-3</sup>). A strong decrease in abundance (> 85 %) occurred a week later (day 10 to 17) in all the high, > 400  $f\text{CO}_2$ , mesocosms, with up to a 93 % decrease found in the 1347  $\mu\text{atm}$  mesocosm (Spearman  $r = 0.94$ ,  $p < 0.05$ ). From day 17 onwards, the abundances were low in all of the mesocosms (Fig. 1).



**Figure 3.** Larval sizes in different  $f\text{CO}_2$  levels on day 10. Data are presented as means  $\pm$  SE;  $n = \text{ca. } 70$  individuals. The horizontal lines indicate the range of average larval sizes on day 0.

### 3.3 The abundance of settled individuals

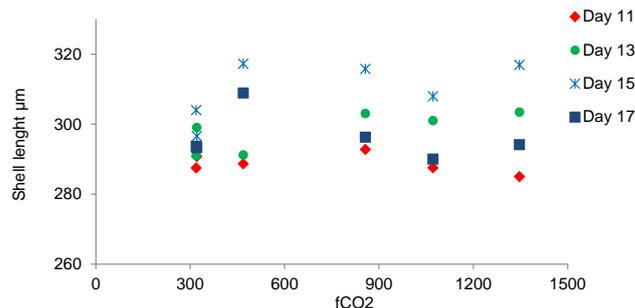
The abundances of settled individuals differed significantly between mesocosms and sampling days of the main settling period (days 9–17, chi-square  $\chi^2 = 1168.588$ ,  $df = 25$ ,  $p < 0.001$ ). The graphical post hoc tests showed three distinct settling peaks of *M. balthica*. In the ambient and near-ambient ( $< 500 \mu\text{atm}$ )  $f\text{CO}_2$  mesocosms, a large increase in the abundance of settled individuals was found between days 9 and 13, with 71, 74 and 54 % of all the individuals having settled by day 13. In comparison, only 39 and 47 % of the individuals had settled during that time period in the two highest (1072–1347  $\mu\text{atm}$ )  $f\text{CO}_2$  mesocosms (Fig. 2a and b). In the 857 and 1072  $\mu\text{atm}$   $f\text{CO}_2$  mesocosms, a smaller settling event occurred on days 11–15, and in the highest  $f\text{CO}_2$  mesocosm the settling peaked on day 17, whereafter the settling soon ceased in all mesocosms. On average  $6130 \pm 240$  individuals settled in the mesocosms during the course of the experiment, with the exception of the 1072  $\mu\text{atm}$   $f\text{CO}_2$  mesocosm, where only ca. 4850 individuals settled (Fig. 2b).

### 3.4 Larval sizes in the water column

On day 0, larval size in the water column was on average  $287 \pm 23 \mu\text{m}$  with no difference found between the mesocosms. After 10 days of exposure to different  $f\text{CO}_2$  levels, the average size of the larvae in the water column (0–17 m) varied from 286 to 313  $\mu\text{m}$ , increasing significantly along the increasing  $f\text{CO}_2$  gradient ( $R^2 = 0.78$ ,  $F = 14.47$ ,  $p = 0.019$ , Fig. 3) with ca. 10 % larger larvae still in the water column in the two highest  $f\text{CO}_2$  mesocosms (1072 and 1347  $\mu\text{atm}$ ).

### 3.5 The sizes of settled individuals

On average  $> 80 \%$  of the individuals settled in the mesocosms during days 11 to 17. No significant differences were found in the sizes of the settled individuals in the



**Figure 4.** Sizes of the settled individuals exposed to different  $f\text{CO}_2$  levels on days 11, 13, 15 and 17. Data are presented as means,  $n = \text{ca. } 35$  at each data point. For clarity, SE (standard error) is not shown.

different  $f\text{CO}_2$  levels at any of these investigated time points (Fig. 4). On days 11 and 13 the average size within the mesocosms varied between 285 and 303  $\mu\text{m}$ , and on days 15 and 17 the average size varied between 293 and 317  $\mu\text{m}$ .

## 4 Discussion

In this study we investigated the effects of different future  $\text{CO}_2$  scenarios on the larval survival, growth and settling of a Baltic Sea benthic key species *M. balthica* in a large-scale mesocosm setting. We found that *M. balthica* settled later along the increasing  $f\text{CO}_2$  gradient of the mesocosms. Moreover, an indication that *M. balthica* larvae settled at a larger size in the high  $f\text{CO}_2$  treatments was also observed, possibly indicating that at increasing  $f\text{CO}_2$  a sufficient mass for settling is not reached until a larger shell length has been attained.

During the week after the first  $\text{CO}_2$  manipulation (day 3 to day 10), settling of *M. balthica* occurred faster in the ambient and middle  $f\text{CO}_2$  mesocosms (319 to 469  $\mu\text{atm}$ ) than in the higher  $f\text{CO}_2$  mesocosms. Consequently, the main settling peak occurred ca. 6 days earlier in these mesocosms ( $< 500 \mu\text{atm}$ ). When comparing the sizes of the larvae, we found that the ones remaining in the water column on day 10 had an average size of 290  $\mu\text{m}$  in both ambient mesocosms, whereas in the other mesocosms ( $f\text{CO}_2 > 400 \mu\text{atm}$ ), the sizes of the remaining larvae were 300–315  $\mu\text{m}$ . We hypothesize that in the ambient  $f\text{CO}_2$  the bivalves settled at the expected size ( $< 300 \mu\text{m}$ ), and thus only the smaller larvae remained in the upper water column when the settling was reaching its peak. In the high  $f\text{CO}_2$  treatments the development of the *M. balthica* larvae might have been compromised and/or delayed as on day 10; despite being relatively large ( $> 300 \mu\text{m}$ ), a large part of the bivalves remained in the upper water column without initiating settlement.

The observed inconsistency between the growth and settling of the early-stage bivalves can be explained by proximate factors that regulate settling. For successful meta-

morphosis 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 seafloor (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  $f\text{CO}_2$  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 to 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  $\text{CO}_2$ -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 that 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, 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-wide responses that take into account ecological processes such as competition, predation and the effect of/on different trophic levels, several species interactions need to be tested simultaneously. The interactions between factors such as increasing  $\text{CO}_2$  and predation is a topic for future studies, but it is likely that individuals stressed by high  $\text{CO}_2$  would also suffer higher predation rates.

In a previous aquarium experiment conducted with newly hatched larvae (ca. 150  $\mu\text{m}$ ) from the same bay (Jansson et al., 2013), both the growth and survival of the larvae were

found to be negatively impacted by decreasing pH. In this mesocosm experiment, however, survival was not found to be affected, and it was not possible to study growth in the same level of detail as in a laboratory experiment. Other typical consequences of pH decrease found in early-stage bivalves are e.g. delayed and/or abnormal development (Kurihara et al., 2008; Talmage and Gobler, 2010; Crim et al., 2011), reduced calcification (Miller et al., 2009) and higher mortality (Talmage and Gobler, 2009; Crim et al., 2011; van Colen et al., 2012). The settling of post-larvae to the seafloor may be impacted by the changes in the water chemistry created by  $\text{CO}_2$  increase (Green et al., 2004; Cigliano et al., 2010; Clements and Hunt, 2014). The major part of ocean acidification research has been conducted by studying the response of single species, with a few studies focusing on the interactions between a small number of species, whereas studies on intact communities have so far only rarely been conducted (but see e.g. work done at  $\text{CO}_2$  vents by Hall-Spencer et al., 2008, or Kroeker et al., 2011, and previous/other mesocosm studies by Christen et al., 2013, and Riebesell et al., 2013b). For species such as *M. balthica*, a mesocosm setting provides an excellent platform to study the development and succession of pelagic early-life stages resulting in recruitment into the benthic system, which cannot be studied in a simple, small-scale aquarium experiment. The direct and indirect factors that essentially impact the early-life success of a bivalve, e.g. natural food quality and quantity, can be incorporated into a mesocosm setting in a more comprehensive way. In the case of future ocean acidification, potential changes in phytoplankton dynamics due to increased  $\text{CO}_2$  levels are likely to have consequences for the other trophic levels. The growth of nanoplankton and diatom species (< 20  $\mu\text{m}$ ), which are the main food particles of larval bivalves (Bos et al., 2006), has been shown to benefit from changing  $\text{CO}_2$  conditions (e.g. Engel et al., 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  $f\text{CO}_2$  mesocosms was, however, found later on during the experiment (day 16 onwards; Crawford et al., 2016; 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 (A. Jansson, personal observation, 2014). For areas such as this, accurate modelling of the future pH change is generally challenging. However, 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<sub>2</sub> 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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## References

- Almén, A.-K., Vehmaa, A., Brutemark, A., and Engström-Öst, J.: Coping with climate change? Copepods experience drastic variations in their physicochemical environment on diurnal basis, *J. Exp. Mar. Biol. Ecol.*, 460, 120–128, 2014.
- Ankar, S.: Growth and production of *Macoma balthica* (L.) in a northern Baltic soft bottom, *Ophelia*, 1, 31–48, 1980.
- Blackford, J. C. and Gilbert, F. J.: pH variability and CO<sub>2</sub> induced acidification in the North Sea, *J. Mar. Syst.*, 64, 229–241, 2007.
- Bonsdorff, E.: Zoobenthic diversity-gradients in the Baltic Sea: Continuous post-glacial succession in a stressed ecosystem, *J. Exp. Mar. Biol. Ecol.*, 330, 383–391, 2006.
- Bonsdorff, E., Norkko, A., and Boström, C.: Recruitment and population maintenance of the bivalve *Macoma balthica* (L.) – factors affecting settling success and early survival on shallow sandy bottoms, in: *Biology and ecology of shallow coastal waters*, edited by: Eleftheriou, A., Ansell, A. D., and Smith, C. J., Proceedings of the 28th European Marine Biological Symposium, Fredensborg, Olsen and Olsen, 253–260, 1995.
- Borges, A. V. and Gypens, N.: Carbonate chemistry in the coastal zone responds more strongly to eutrophication than to ocean acidification, *Limnol. Oceanogr.*, 55, 346–353, 2010.
- Bos, O. G., Hendriks, I. E., Strasser, M., Dolmer, P., and Kamer-mans, P.: Estimation of food limitation of bivalve larvae in coastal waters of north-western Europe, *J. Sea Res.*, 55, 191–206, 2006.
- Boxhammer, T., Bach, L. T., Czerny, J., and Riebesell, U.: Technical note: Sampling and processing of mesocosm sediment trap material for quantitative biogeochemical analysis, *Biogeosciences*, 13, 2849–2858, doi:10.5194/bg-13-2849-2016, 2016.
- Caddy, J.: Maturation of gametes and spawning in *Macoma balthica* (L.), *Can. J. Zool.*, 45, 955–965, 1967.
- Caddy, J.: Development of mantle organs, feeding, and locomotion in postlarval *Macoma balthica* (L.) (Lamellibranchiata), *Can. J. Zool.*, 47, 609–617, 1969.
- Caldeira, K. and Wickett, M.: Anthropogenic carbon and ocean pH, *Nature*, 425, 365–365, 2003.
- Christen, N., Calosi, P., McNeill, C. L., and Widdicombe, S.: Structural and functional vulnerability to elevated pCO<sub>2</sub> in marine benthic communities, *Mar. Biol.*, 160, 2113–2128, doi:10.1007/s00227-012-2097-0, 2013.
- Cigliano, M., Gambi, M. C., Rodolfo-Metalpa, R., Patti, F. P., and Hall-Spencer, J. M.: Effects of ocean acidification on invertebrate settlement at volcanic CO<sub>2</sub> vents, *Mar. Biol.*, 157, 2489–2502, 2010.
- Clements, J. C. and Hunt, H. L.: Influence of sediment acidification and water flow on sediment acceptance and dispersal of juvenile soft-shell clams (*Mya arenaria* L.), *J. Exp. Mar. Biol. Ecol.*, 453, 62–69, 2014.
- Crawford, K. J., Brussaard, C. P. D., and Riebesell, U.: Shifts in the microbial community in the Baltic Sea with increasing CO<sub>2</sub>, *Biogeosciences Discuss.*, doi:10.5194/bg-2015-606, in review, 2016.
- Crim, R. N., Sunday, J. M., and Harley, C. D. G.: Elevated seawater CO<sub>2</sub> concentrations impair larval development and reduce larval survival in endangered northern abalone (*Haliotis kamtschatkana*), *J. Exp. Mar. Biol. Ecol.*, 400, 272–277, 2011.
- Dickson, A. G., Sabine, C., and Christian, J. (Eds.): Guide to Best Practices for Ocean CO<sub>2</sub> Measurements, PICES Special Publication 3, 191 pp., <http://aquaticcommons.org/1443/> (last access: 29 April 2016), 2007.
- Drent, J.: Temperature responses in larvae of *Macoma balthica* from a northerly and southerly population of the European distribution range, *J. Exp. Mar. Biol. Ecol.*, 275, 117–129, 2002.
- Duarte, C. M., Henriks, I. E., Moore, T. S., Olsen, Y. S., Steckbaer, A., Ramajo, L., Carstensen, J., Trotter, J. A., and McCulloch, M.: Is ocean acidification an open ocean syndrome?, Understanding anthropogenic impacts of seawater pH, *Estuar. Coast.*, 36, 221–236, 2013.
- Dupont, S. and Thorndyke, M. C.: Impact of CO<sub>2</sub>-driven ocean acidification on invertebrates early life-history – What we know, what we need to know and what we can do, *Biogeosciences Discuss.*, 6, 3109–3131, doi:10.5194/bgd-6-3109-2009, 2009.
- Elmgren, R., Ankar, S., Marteleur, B., and Ejdung, G.: Adult interference with postlarvae in soft sediments: the *Pontoporeia-Macoma* example, *Ecology*, 67, 828–836, 1986.
- Engel, A., Schulz, K. G., Riebesell, U., Bellerby, R., Delille, B., and Schartau, M.: Effects of CO<sub>2</sub> on particle size distribution and phytoplankton abundance during a mesocosm bloom experiment (PeECE II), *Biogeosciences*, 5, 509–521, doi:10.5194/bg-5-509-2008, 2008.
- Feng, Y., Hare, C. E., Leblanc, K., Rose, J. M., Zhang, Y., DiTullio, G. R., Lee, P. A., Wilhelm, S. W., Rowe, J. M., Sun, J., Nemcek, N., Gueguen, C., Passow, U., Benner, I., Brown, C.,

- and Hutchins, D. A.: Effects of increased  $p\text{CO}_2$  and temperature on the North Atlantic spring bloom, I. The phytoplankton community and biogeochemical response, *Mar. Ecol.-Prog. Ser.*, 388, 13–25, 2009.
- Fabry, V. J., Seibel, B. A., Feely, R. A., and Orr, J. C.: Impacts of ocean acidification on marine fauna and ecosystem processes, *ICES J. Mar. Sci.*, 65, 414–432, 2008.
- Feely, R. A., Sabine, C. L., Lee, K., Berelson, W., Kleypas, J., Fabry, V. J., and Millero, F. J.: Impact of anthropogenic  $\text{CO}_2$  on the  $\text{CaCO}_3$  system in the oceans, *Science*, 305, 362–366, 2004.
- Gazeau, F., Parker, L. M., Comeau, S., Gattuso, J., O'Connor, W. A., Martin, S., Pörtner, H., and Ross, P. M.: Impacts of ocean acidification on marine shelled molluscs, *Mar. Biol.*, 160, 2207–2245, 2013.
- Green, M. A., Jones, M. E., Boudreau, C. L., Moore, R. L., and Westman, B. A.: Dissolution mortality of juvenile bivalves in coastal marine deposits, *Limnol. Oceanogr.*, 49, 727–734, 2004.
- Green, M. A., Waldbusser, G. G., Reilly, S. L., and Emerson, K.: Death by dissolution: sediment saturation state as a mortality factor for juvenile bivalves, *Limnol. Oceanogr.*, 54, 1037–1047, 2009.
- Hall-Spencer, J. M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S. M., Rowley, S. J., Tedesco, D., and Buia, M.-C.: Volcanic carbon dioxide vents show ecological effects of ocean acidification, *Nature*, 454, 95–99, 2008.
- Hjalmarsson, S., Wesslander, K., Anderson, L. G., Omstedt, A., Perttilä, M., and Mintrop, L.: Distribution, long-term development and mass balance calculation of total alkalinity in the Baltic Sea, *Cont. Shelf Res.*, 28, 593–601, 2008.
- Hönisch, B., Ridgwell, A., Schmidt, D. N., Thomas, E., Gibbs, S. J., Sluijs, A., Zeebe, R., Kump, L., Martindale, R. C., Greene, S. E., Kiessling, W., Ries, J., Zachos, J. C., Royer, D. L., Barker, S., Marchitto Jr., T. M., Moyer, R., Pelejero, C., Ziveri, P., Foster, G. L., and Williams, B.: The geological record of ocean acidification, *Science*, 335, 1058–1063, 2012.
- Jansson, A., Norkko, J., and Norkko, A.: Effects of reduced pH on *Macoma balthica* larvae from a system with naturally fluctuating pH-dynamics, *PLoS One*, 8, e68198, doi:10.1371/journal.pone.0068198, 2013.
- Johnson, Z. I., Wheeler, B. J., Blinbery, S. K., Carlson, C. M., and Ward, C. S.: Dramatic variability of the carbonate system at a temperate coastal ocean site (Beaufort, North Carolina, USA) is regulated by physical and biogeochemical processes on multiple timescales, *PLoS ONE*, 8, e85117, doi:10.1371/journal.pone.0085117, 2013.
- Kroeker, K. J., Micheli, F., Gambi, M. C., and Martz, T. R.: Divergent ecosystem responses within a benthic marine community to ocean acidification, *P. Natl. Acad. Sci. USA*, 108, 14515–14520, 2011.
- Kurihara, H.: Effects of  $\text{CO}_2$ -driven ocean acidification on the early developmental stages of invertebrates, *Mar. Ecol.-Prog. Ser.*, 373, 275–284, 2008.
- Kurihara, H., Asai, T., Kato, S., and Ishimatsu, A.: Effects of elevated  $p\text{CO}_2$  on early development in the mussel *Mytilus galloprovincialis*, *Aquat. Biol.*, 4, 225–233, 2008.
- Lewis, D. E. and Cerrato, R. M.: Growth uncoupling and the relationship between shell growth and metabolism in the soft shell clam *Mya arenaria*, *Mar. Ecol.-Prog. Ser.*, 158, 177–189, 1997.
- Lischka, S., Bach, L. T., Schulz, K.-G., and Riebesell, U.: Micro- and mesozooplankton community response to increasing  $\text{CO}_2$  levels in the Baltic Sea: insights from a large-scale mesocosm experiment, *Biogeosciences Discuss.*, 12, 20025–20070, doi:10.5194/bgd-12-20025-2015, 2015.
- Meakin, N. G. and Wyman, M.: Rapid shifts in picoeukaryote community structure in response to ocean acidification, *ISME J.*, 5, 1397–1405, doi:10.1038/ismej.2011.18, 2011.
- Melzner, F., Thomsen, J., Koeve, W., Oschlies, A., Gutowska, M. A., Bange, H. W., Hansen, H. P., and Körtzinger, A.: Future ocean acidification will be amplified by hypoxia in coastal habitats, *Mar. Biol.*, 160, 1875–1888, doi:10.1007/s00227-012-1954-1, 2012.
- Michaud, E., Desrosiers, G., Mermillod-Blondin, F., Sundby, B., and Stora, G.: The functional group approach to bioturbation: II. The effects of the *Macoma balthica* community on fluxes of nutrients and dissolved organic carbon across the sediment–water interface, *J. Exp. Mar. Biol. Ecol.*, 337, 178–189, 2006.
- Miller, A. W., Reynolds, A. C., Sobrino, C., and Riedel, G. F.: Shellfish face uncertain future in high  $\text{CO}_2$  world: Influence of acidification on oyster larvae calcification and growth in estuaries, *PLoS ONE*, 4, e5661, doi:10.1371/journal.pone.0005661, 2009.
- Norkko, A., Villnäs, A., Norkko, J., Valanko, S., and Pilditch, C.: Size matters: implications of the loss of large individuals for ecosystem function, *Sci. Rep.*, 3, e2646, doi:10.1038/srep02646, 2013.
- Olafsson, E. B.: Contrasting influences of suspension-feeding and deposit-feeding populations of *Macoma balthica* on infaunal recruitment, *Mar. Ecol.-Prog. Ser.*, 55, 171–179, 1989.
- Omstedt, A., Gustafsson, E., and Wesslander, K.: Modelling the uptake and release of carbon dioxide in the Baltic Sea surface water, *Cont. Shelf Res.*, 29, 870–885, 2009.
- Omstedt, A., Edman, M., Anderson, L. G., and Laudon, H.: Factors influencing the acid–base (pH) balance in the Baltic Sea: a sensitivity analysis, *Tellus B*, 62, 280–295, 2010.
- Omstedt, A., Edman, M., Claremar, B., Frodin, P., Gustafsson, E., Humborg, C., Hägg, H., Mörth, M., Rutgersson, A., and Schurgers, G.: Future changes in the Baltic Sea acid–base (pH) and oxygen balances, *Tellus B*, 64, 1–23, 2012.
- Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F., Key, R. M., Lindsay, K., Maier-Reimer, E., Matear, R., Monfray, P., Mouchet, A., Najjar, R. G., Plattner, G.-K., Rodgers, K. B., Sabine, C. L., Sarmiento, J. L., Schlitzer, R., Slater, R. D., Totterdell, I. J., Weirig, M.-F., Yamanaka, Y., and Yool, A.: Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms, *Nature*, 437, 681–686, doi:10.1038/nature04095, 2005.
- Paul, A. J., Bach, L. T., Schulz, K.-G., Boxhammer, T., Czerny, J., Achterberg, E. P., Helleman, D., Trense, Y., Nausch, M., Sswat, M., and Riebesell, U.: Effect of elevated  $\text{CO}_2$  on organic matter pools and fluxes in a summer Baltic Sea plankton community, *Biogeosciences*, 12, 6181–6203, doi:10.5194/bg-12-6181-2015, 2015.
- Pedersen, T. M., Hansen, J. L. S., Josefson, A. B., and Hansen, B. W.: Mortality through ontogeny of soft-bottom marine invertebrates with planktonic larvae, *J. Mar. Syst.*, 73, 185–207, 2008.

- Pineda, J., Reynolds, N. B., and Starczak, V. R.: Complexity and simplification in understanding recruitment in benthic populations, *Popul. Ecol.*, 51, 17–32, 2009.
- R Development Core Team: R: A Language and Environment for Statistical Computing, R Foundation for Statistical Computing, Vienna, Austria, <http://www.R-project.org/>, ISBN 3-900051-07-0, 2012.
- Riebesell, U., Fabry, V. J., Hansson, L., and Gattuso J.-P. (Eds.): Guide to best practices for ocean acidification research and data reporting, 260 pp., Luxembourg: Publications Office of the European Union, 2010.
- Riebesell, U., Czerny, J., von Bröckel, K., Boxhammer, T., Büdenbender, J., Deckelnick, M., Fischer, M., Hoffmann, D., Krug, S. A., Lentz, U., Ludwig, A., Mücke, R., and Schulz, K. G.: Technical Note: A mobile sea-going mesocosm system – new opportunities for ocean change research, *Biogeosciences*, 10, 1835–1847, doi:10.5194/bg-10-1835-2013, 2013a.
- Riebesell, U., Gattuso, J.-P., Thingstad, T. F., and Middelburg, J. J.: Preface: “Arctic ocean acidification: pelagic ecosystem and biogeochemical responses during a mesocosm study”, *Biogeosciences*, 10, 5619–5626, doi:10.5194/bg-10-5627-2013, 2013b.
- Schneider, B., Nausch, G., Nagel, K., and Wasmund, N.: The surface water CO<sub>2</sub> budget for the Baltic Proper: a new way to determine nitrogen fixation, *J. Mar. Syst.*, 42, 53–64, 2003.
- Schulz, K. G. and Riebesell, U.: Diurnal changes in seawater carbonate chemistry speciation at increasing atmospheric carbon dioxide, *Mar. Biol.*, 160, 1889–1899, doi:10.1007/s00227-012-1965-y, 2013.
- Schulz, K. G., Bellerby, R. G. J., Brussaard, C. P. D., Büdenbender, J., Czerny, J., Engel, A., Fischer, M., Koch-Klavnsen, S., Krug, S. A., Lischka, S., Ludwig, A., Meyerhöfer, M., Nondal, G., Silyakova, A., Stühr, A., and Riebesell, U.: Temporal biomass dynamics of an Arctic plankton bloom in response to increasing levels of atmospheric carbon dioxide, *Biogeosciences*, 10, 161–180, doi:10.5194/bg-10-161-2013, 2013.
- Segerstråle, S.: Investigations on Baltic populations of the bivalve *Macoma balthica* (L.), I. Introduction, Studies on recruitment and its relation to depth in Finnish coastal waters during the period 1922–1959, Age and growth, *Soc. Sci. Fenn. Comment. Biol.*, 23, 1–72, 1960.
- Talmage, S. C. and Gobler, C. J.: The effects of elevated carbon dioxide concentrations on the metamorphosis, size, and survival of larval hard clams (*Mercenaria mercenaria*), bay scallops (*Argopecten irradians*), and Eastern oysters (*Crassostrea virginica*), *Limnol. Oceanogr.*, 54, 2072–2080, 2009.
- Talmage, S. C. and Gobler, C. J.: Effects of past, present, and future ocean carbon dioxide concentrations on the growth and survival of larval shellfish, *P. Natl. Acad. Sci. USA*, 107, 17246–17251, 2010.
- Thomas, H. and Schneider, B.: The seasonal cycle of carbon dioxide in Baltic Sea surface waters, *J. Mar. Syst.*, 22, 53–67, 1999.
- Tortell, P. D., DiTullio, G. R., Sigman, D. M., and Morel, F. M. M.: CO<sub>2</sub> effects on taxonomic composition and nutrient utilization in an Equatorial Pacific phytoplankton assemblage, *Mar. Ecol.-Prog. Ser.*, 236, 37–43, 2002.
- Valanko, S., Norkko, A., and Norkko, J.: Strategies of post-larval dispersal in nontidal soft-sediment communities, *J. Exp. Mar. Biol. Ecol.*, 384, 51–60, 2010.
- Van Colen, C., Debusschere, E., Braeckman, U., Van Gansbeke, D., and Vincx, M.: The early life history of the clam *Macoma balthica* in a high CO<sub>2</sub> world, *PLoS ONE*, 7, e44655, doi:10.1371/journal.pone.0044655, 2012.
- Van Colen, C., Jansson, A., Saunier, A., Lacoue-Labathe, T., and Vincx, M.: Population-specific embryonic response to ocean warming and acidification along the distribution range of a marine bivalve, in preparation, 2016.
- Villnäs, A., Norkko, J., Lukkari, K., Hewitt, J., and Norkko, A.: Consequences of increasing hypoxic disturbance on benthic communities and ecosystem functioning, *PLoS One*, 7, e44920, doi:10.1371/journal.pone.0044655, 2012.
- Waldbusser, G., Bergschneider, H., and Green, M. A.: Size-dependent pH effect on calcification in post-larval hard clam *Mercenaria* spp, *Mar. Ecol.-Prog. Ser.*, 417, 171–182, 2010.
- Wood, H. L., Spicer, J. I., and Widdicombe, S.: Ocean acidification may increase calcification, but at a cost, *P. Roy. Soc. Lon. B Bio.*, 275, 1767–1773, 2008.
- Woodin, S. A.: Settlement of infauna: larval choice?, *B. Mar. Sci.*, 39, 401–407, 1986.
- Woodin, S. A., Marinelli, R. L., and Lindsay, S. M.: Process-specific cues for recruitment in sedimentary environments: Geochemical signals?, *J. Mar. Res.*, 56, 535–558, 1998.