We thank the reviewers for the useful and constructive comments, which helped to clarify a number of points in our manuscript and focus the discussion on the main findings. Our responses including the modifications made to the manuscript are detailed below

Response to comments by Referee # 1

REVIEWER COMMENT 1 by Referee #1

General issue:

Based on the abstract, I expected to read results on changes in the community, not a single species. My general feeling form the manuscript was that the authors used importance of the larger project, KOSMOS, was one of the main selling points of this article. The results are interesting enough in themselves, particularly the difference in response from the laboratory study to field/mesocosm study. The references to KOSMOS and other publications resulting from that project detracted from the results in this study. In particular, this study only reports the response of a single organism and not a community response. For example, in the abstract, "The response of organisms to future ocean acidification has primarily been studied in single-species experiments, whereas the knowledge of community-wide responses is still limited. To study responses of the Baltic Sea pelagic community to a range of future CO_2-scenarios, six â´Lij 55 m3 pelagic mesocosms were deployed in the northern Baltic Sea in June 2012. In this specific study we focused on the tolerance, development and subsequent settlement process of the larvae of the benthic key-species Macoma balthica when exposed to different levels of future CO_2." The authors state that the majority of studies report single-species experiments, that the mesocosms were used to study the community response, but that this study focuses on a single species. This can easily be addressed.

Author response:

It is clear that the original abstract lead to some misunderstanding and the sentence that seems to have caused most of the confusion here ("The response of organisms to future ocean acidification has primarily been studied in single-species experiments, whereas the knowledge of community-wide responses is still limited") was moved to the discussion, highlighting needs for future studies.

The point that needs to be clarified is that most experiments have been single-species experiments in the lab, with only that one particular species included in the experimental setup (often even with filtered seawater), while in this study the response of a single species was studied while the species was still in its natural community. To highlight this point, we changed the following two sentences: "To study responses of the Baltic Sea pelagic community to a range of future CO_2-scenarios, six ~ 55 m3 pelagic mesocosms were deployed in the northern Baltic Sea in June 2012. In this specific study we focused on the tolerance, development and subsequent settlement process of the larvae of the benthic key-species Macoma balthica when exposed to different levels of future CO_2."

NEW VERSION: "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".

Also, "Tolerance and development" were changed to "survival and growth" to accommodate comments from the second reviewer.

REVIEWER COMMENT 2 by Referee #1

The decline in abundance in the control mesocosms is not accounted for. Do the authors have a suggestion as to why this occurred?

Further, were samples taken from within the bay, outside of the mesocosms to control for the mesocosms themselves? These data become particularly relevant when the control mesocosms behave unexpectedly.

Author response:
The decline in the control mesocosms is considered to be within normal mortality patterns, and is discussed e.g. on page 20421 lines 11-13. What remained unexplained is the pattern in the control mesocosm M1 during days -3 to -1, where unaccounted for variation was found. We hypothesize this to be a sampling issue or an artifact caused by a mesocosm maintenance method (bubbling to destroy the halocline on day -3). This discussion was added to the manuscript.

Samples taken from the bay have unfortunately not been analysed. However, we do not think these data would provide a reliable control setting as in the bay the larvae are part of a dynamic open system (predation, transport, production of new larvae occurring), whereas in the mesocosms the community is fixed at the start of the experiment.

REVIEWER COMMENT 3 by Referee #1
I found the use of M1-8 confusing, as it was not stated (outside of table 1), which mesocosm had which fCO_2 value. I suggest referring to the mesocosms not by Mx but by CO_2 level.

Author response:
The way of referring to the mesocosms was changed to as suggested by the reviewer.

REVIEWER COMMENT 4 by Referee #1
P20422 L 4-5: Is it possible from the samples collected to determine if shell thickness was reduced, resulting in an animal that is too light to settle? The delayed development/lengthened time to settlement is an interesting result and should be

investigated in more detail, ideally in this publication. This would then rule in or out a lighter shell as the cause of the animal not being able to settle.

Author response:

The shells (average size < 300 µm) are unfortunately too small and fragile to handle (remove, weigh) with the methods we have access to. We fully agree that this is a topic that should be investigated in more detail.

REVIEWER COMMENT 5 by Referee #1

It is really interesting that the M. balthica responded differently to elevated CO_2 compared to the previous laboratory experiments. The authors should include a discussion as to why this may have occurred.

Author response:

On page 20422 we wrote "In a previous experiment conducted with newly hatched larvae (ca. 150 µm) 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 survival was, however, not affected, while it was not possible to study growth in the same level of detail as in the laboratory experiment. Nevertheless, we still maintain that increased fCO_2 had severe negative effects on the larvae also in this experiment. This clarification was added to the manuscript.

REVIEWER COMMENT 6 by Referee #1

From your data, the "performance" of M. balthica was not actually reduced with increasing CO_2. Mortality was not increased, at least the number of settling individuals was the same, an increase in deformities was not reported or abnormal development other than the delay in settlement, the cause of which is also unknown. The final comments on p20424, are therefore not valid based on the current results. The negative comments should be toned down. The delay in settlement could very well have negative impacts, either on the individuals or on the community. If the authors believe

this to be the case, then the potential impacts should be discussed in more detail during the discussion.

Author response:

The negative conclusions were toned down and we expanded the discussion on potential consequences for communities. However, we still maintain that increased fCO_2 had a negative effect on the larvae.

REVIEWER COMMENT 7 by Referee #1

Technical comments:

1. P20412 L5: ": : :the system is already at present". Remove "at present" from the sentence.

2. P20412 L13-15. We found that the settling of M. balthica was delayed along the increasing CO_2 gradient of the mesocosms. Also, when exposed to increasing CO_2 levels larvae settled at a larger size, indicating a developmental delay. These two sentences are unclear. At first reading, they express the same result. These could be combined eg: The size and time to settlement of M. balthica increased along the CO_2, suggesting a developmental delay.

3. P20412 l 25: "before" is not needed in this sentence as it is implied by "geological past".

4. P20413l3: Similar to above "already" and "at present" suggest the same thing. Pick one.

5. 20413 l 15: "of post-larvae are"

6. 20416 l 18: Please write CTD in full, at least for the first use.

7. 20419 L4: Word reversal "total alkalinity measured on: : :"

8. 20421 L7 "an indication that M. balthica: : :" This result was observed; therefore the word indication should be removed.

9. P20422 L2: Replace "is" with "does"

10. P20423 L26: "Already at present: : :". As previously in the introduction, only one of these is necessary.

Author response:

These details were corrected according to the suggestions of the reviewer.

Response to comments by Referee #2

General comments:

Using large scale mesocosm units this study explores the role of future pH conditions on the settlement process of the benthic key species Macoma balthica. Indeed, the authors suggest that the settling of M. balthica larvae was delayed with increasing CO_2 levels. The role of ocean acidification is somewhat of a hot topic within the scientific community. In recent years a large number of publications have been published. That being said, most of the published literature is based on laboratory studies and to a lesser extent on natural or, as in this case, mesocosm experiments. I think this manuscript deserves publication. However, before publication the authors should consider the comments given below.

The authors should consider their aims of the study, the results obtained and conclusions drawn. As is evident below, it is not always clear why some aims have been included, how the aims were tested or what results support (or not) the aims.

REVIEWER COMMENT 1 by Referee #2

Abstract Page 20412, line 11: tolerance and development? The authors state that they focus on the tolerance, development and subsequent settlement process. The settlement process is clearly visible, however, tolerance and development is not discussed nor any results given or conclusions made on the topics. If still considered a focus of the study then please add results, discussion and conclusions. If not please delete from abstract.

Author response:

Tolerance and development" refers to "survival and growth (measured as average shell size of the community); this was clarified throughout the manuscript.

REVIEWER COMMENT 2 by Referee #2

Introduction Page 20413, lines 26-29: ”The disadvantages of limited ecosystem realism that arise from the exclusion of factors such as currents and large predators, which impact the natural succession and dispersion patterns of the species, nevertheless have to accounted for when interpreting the results.” This all sounds perfectly fine, but did you actually do so in this study? Did you take this into account? I couldn’t find any information on how this was done in the discussion? Please add how these factors could have influence your results

Author response:

The role of factors such as currents or predators can naturally not be quantified in this study, but a short comment on their potential effects was added to the manuscript.

REVIEWER COMMENT 3 by Referee #2

Page 20414, lines 4-5 and throughout manuscript: How are the references sorted, not chronologically and not alphabetically?

Author response:

The references are sorted chronologically; this was corrected throughout the manuscript.

REVIEWER COMMENT 4 by Referee #2

Page 20414, line 11: Omstedt et al., 2010 is not available in the reference list, please add

Author response:

This missing reference was added to the reference list.

REVIEWER COMMENT 5 by Referee #2

Page 20414, line 14: Almen or as in the reference list Almén?

Author response:
This typo was corrected.

REVIEWER COMMENT 6 by Referee #2
Page 20414, lines 23-24: As commented on in the abstract. I do not think you present any data on tolerance and development of the larvae? How do you define development here? Size of the mussel, is that development? How did you measure tolerance? Please add additional information and data on this or consider deleting shed light on…

Author response:
Same as above: ”Tolerance and development” refers to “survival and growth” (measured as average shell size of the community); this was corrected throughout the manuscript.

REVIEWER COMMENT 7 by Referee #2
Page 20414, lines 27-28: How did you measure/calculate growth? I can’t find any information on growth measurements and calculations rather it seems as if the authors” predicted the size of the larvae to decrease along: : :”?

Author response:
Page 20414 lines 27-28. “..predicted the growth of the larvae to decrease along the increasing fCO_2 gradient”; We changed “growth” to “size”.

Material and methods
REVIEWER COMMENT 8 by Referee #2
Page 20416, line 10: Is it Riebesell et al 2013 a or b?

Author response:

2013a, this missing detail was added to the manuscript.

REVIEWER COMMENT 9 by Referee #2

Page 20416, line 20: bayc? I do not know what this is?

Author response:

Bay, this spelling mistake was corrected.

REVIEWER COMMENT 10 by Referee #2

Page 20417, line 1: Dickson et al 2007 is not found in the reference list, please add.

Author response:

This missing reference was added to the reference list.

REVIEWER COMMENT 11 by Referee #2

Page 20418, line 27: R core team 2012 is not found in the reference list, please add

Author response:

This missing reference was added to the reference list.

Results

REVIEWER COMMENT 12 by Referee #2

Page 20420, line 14: why did so few individuals settle in M3? I was not able to find anything on this in the discussion, please add

Author response:

We included a short discussion on this topic to the manuscript.

Discussion

REVIEWER COMMENT 13 by Referee #2

Page 20421, lines 7-10: “Moreover, an indication that M. balthica post-larvae settled at a larger size in the high fCO_2 treatments was also observed”. is that really true? In the results section 3.5 the sizes of settling individuals the authors state that ”no significant differences were found in the sizes of the settling individuals.” I’m confused, do they or do they not settle at a larger size in the high fCO_2?

Author response:

Our statement on Page 20421, line 7-10 refers to the size when the larvae START to settle, i.e. their size in the water column, while section 3.5. deals with the larvae that have already settled to the bottom of the mesocosm. To avoid confusion “settling individuals” was changed to “settled individuals”, throughout the manuscript.

REVIEWER COMMENT 14 by Referee #2

Page 20422, lines 1-4: ”Shell growth alone… IS NOT automatically reflecting the overall biomass production and developmental stage of the organism”. Wasn’t development one of the main aims of this paper? If so then why didn’t the authors use an appropriate measure of growth?

Author response:

To clarify, we did not measure any other modes of development in this study, so shell growth is used as a proxy. We agree that this does not automatically translate into biomass production, but provides an acceptable substitute.

REVIEWER COMMENT 15 by Referee #2

Page 20422, line 17: Pedersen et al 2008 and Pineda et al 2009 are not found in the reference list, please add

Author response:

These missing references were added to the reference list

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REVIEWER COMMENT 16 by Referee #2

Page 20423, lines 8-13: This is one of the main arguments for using mesocoms i.e. incorporating factors beyond what is possible in a laboratory experiment.

Did you actually test this? I can't find any results on this. If you didn't, why not? A quick glance at the manuscripts, currently presented for the special issue, seemingly all necessary data for e.g. food quantity and quality is available. So, as the authors state here this provides an excellent platform to study this, then why didn't they? If possible please add this to the manuscript.

Author response:

As written on page 20423 line 20-25: "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. Due to this difference in timing, the potential influence of phytoplankton abundance on *Macoma* was not analysed further. A short comment on this topic was added to the manuscript.

References

REVIEWER COMMENT 17 by Referee #2

For all references please double-check abbreviation e.g. J Marine Syst should probably be J Mar Syst? Sometimes doi, sometimes not? Compare Riebesell et al 2013b o Schulz et al 2013 one has a webpage and the other a doi. Please be consistent throughout the reference section.

Author response:

We have corrected the reference list according to the journal's standards.

Tables

REVIEWER COMMENT 18 by Referee #2

Table 1: Why aren't the averages for the whole time period presented for aragonite and calcite? On what basis are the later days excluded? Please add to the Materials and methods section.

Author response:

The data for aragonite and calcite were shown as averages until day 17, as the majority (>95%) of the larvae had settled until that day. Using the average of the whole experimental duration would obscure the saturation states present during the settling period of the larvae. This is the case as CO_2 was permanently outgassing from the mesocosms, slowly increasing aragonite and calcite saturation states over time. To harmonize the data presentations, from now on we also report fCO_2 values only for the settling period of *M. balthica* (day 0-17).

Figures

REVIEWER COMMENT 19 by Referee #2

Figure 3 and 4, are the graph based on actual or targeted fCO_2 values? Please add explanation to the figure legend/caption. Why the use of SE in fig 3 and SD in fig 4? Why not use the same in both figs??

Author response:

The graphs are based on actual fCO_2 values, the explanation was added to the figure legend. In the legend of Figure 4, SD was not shown for clarity. We changed "SD" to "SE" in the legend, however, to standardize the method that was used.

Survival ~~Larval development~~ 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 ~~at present~~ exposed to relatively large natural seasonal and diel pH fluctuations. ~~The response of organisms to future ocean acidification has primarily been studied in single-species experiments, whereas the knowledge of community-wide responses is still limited.~~ 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.~~To study responses of the Baltic Sea pelagic community to a range of future CO_2-scenarios, six ~55 m^3 pelagic mesocosms were deployed in the northern Baltic Sea in June 2012. In this specific study we focused on the tolerance, development and subsequent settlement process of the larvae of the benthic key-species *Macoma balthica* when exposed to different levels of future CO_2. We found that the settling of *M. balthica* was delayed along the increasing CO_2 gradient of the mesocosms. Also, when exposed to increasing CO_2 levels larvae~~

~~settled at a larger size, indicating a developmental delay.~~ 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 ~~before~~ 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~~; Orr et al., 2005~~). 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 ~~at present~~ 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 ~~is~~ 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. ~~However, 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.~~ 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. ~~The disadvantages of limited ecosystem realism that arise from the exclusion of factors such as currents and large predators, which impact the natural succession and dispersion patterns of the species, nevertheless have to be accounted for when interpreting the results.~~

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~~; Melzner et al., 2012~~). 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; ~~Melzner et al., 2012;~~ 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; Almé~~e~~n 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 (~~Jansson et al., 2013;~~ 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 this 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) the tolerance and development 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 (Bonsdorff, 2006; Segerstråle, 1960; Elmgren et al., 1986; Bonsdorff, 2006Segerstråle, 1960). 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; Elmgren et al., 1986). *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~~; Villnäs et al., 2012~~).

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~~e~~) 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).~~, Riebesell et al. (2013a) and Schulz et al. (2013).~~

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 ~~towing~~ 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), ~~at~~ 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~~ing~~ 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~~ing~~ 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~~D~~.

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 ~~measured~~ 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. ~~M1 where the abundance decreased earliest and with a steep slope~~. 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 ~~M1 and M5~~, with > 80% decrease in abundance in comparison to the 35-50 % decrease in the two highest fCO_2 mesocosms (>1000 µatm) ~~M3 and M8~~ (Spearman $r = -0.83$, $p < 0.05$). Consequently, on day 10 the highest abundance was measured in the highest fCO_2 mesocosm ~~M8~~ (3194 ind. m^{-3}) and the lowest abundances in ~~the~~ both ambient mesocosms (319 -321 µatm) ~~M1 and M5~~ (545 resp. 1064 ind. m^{-3}). A strong decrease in abundance (> 85 %) occurred a week later (day 10 to 17) in all the high, >400 fCO_2, mesocosms ~~M3, M8, M6 and M7~~, with

up to a 93% decrease found in ~~M8~~ the 1347 µatm mesocosm (Spearman r =0.94, p < 0.05). From day 17 onwards, the abundances were low in all of the mesocosms (Fig. 1).

3.3 The abundance of settled~~ing~~ individuals

The abundances of settled~~ing~~ individuals differed significantly between mesocosms and sampling days of the main settling period (days 9-17, chi-square χ^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 µatm) *f*CO_2 mesocosms ~~M1, M5 and M7,~~ a large increase in the abundance of settled~~ing~~ individuals was found between days 9-13, 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 (10720~~0~~-1347~~231~~ µatm) *f*CO_2 mesocosms ~~M8 and M3~~ (Fig. 2a and b). In the 85700 and ~~-~~ 10720~~70~~ µatm *f*CO_2 mesocosms ~~M6 and M3~~, a smaller settling event occurred on days 11-15 and in the highest *f*CO_2 mesocosm ~~M8~~ the settling peaked on day 17, where after the settling ~~number of settling individuals~~ soon ceased in all mesocosms. On average 6130 ± 240 individuals settled in the mesocosms during the course of the experiment, with the exception of 1072 µatm *f*CO_2 mesocosm ~~M3~~ 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 ± 23 µm with no difference found between the mesocosms. After 10 days of exposure to different *f*CO_2 levels, the average size of the larvae in the water column (0-17 m) varied from 286 µm to 313 µm, increasing significantly along the increasing *f*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*CO_2 mesocosms (1072 and 1347 µatm) ~~M3 and M8~~.

3.5 The sizes of settled~~ing~~ 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~~ing~~ individuals in the different *f*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 µm to 303 µm, and on days 15 and 17 the average size varied between 293 µm to 317 µm.

4 Discussion

In this study we investigated the effects of different future CO_2 scenarios on the larval survival, growth ~~development~~ 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 fCO_2 gradient of the mesocosms. Moreover, an indication that *M. balthica* ~~post~~ larvae settled at a larger size in the high fCO_2 treatments was also observed, possibly indicating that at increasing fCO_2 a sufficient mass for settling is not reached until a larger shell length has been attained.

During the week after first CO_2 manipulation (day 3 to day 10) settling of *M. balthica* occurred faster in the ambient and middle fCO_2 mesocosms (319 to 469 µatm) ~~M1, M5 and M7 (365-497 µatm)~~ than in the higher fCO_2 mesocosms. Consequently, the main settling peak occurred ca. 6 days earlier in these mesocosms (<~~-~~500 µatm). 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 µm in ~~the~~ both ambient mesocosms ~~M1 and M5~~, whereas in the other mesocosms ~~M7, M6, M3 and M8~~ (fCO_2 >~~-~~400 µatm), the sizes of the remaining larvae were 300-315 µm. We hypothesise that in the ambient fCO_2 the bivalves settled at the expected size (<~~-~~300 µm), and thus only the smaller larvae remained in the upper water column when the settling was reaching its peak. In the high fCO_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 µm), 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 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, ~~is~~ does not automatically reflect~~ing~~ 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 ~~performance~~ of the early-life stages, as found in the present study, is ~~thus~~ 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~~98~~). 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 CO_2 and predation is a topic for future studies, but it is likely that individuals stressed by high CO_2 also would suffer higher predation rates.

In a previous aquarium experiment conducted with newly hatched larvae (ca. 150 µm) 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 ~~for~~ early-stage bivalves are e.g. delayed and/or abnormal development (Kurihara et al., 2008; Talmage and Gobler, 2010; Crim et al., 2011; ~~Kurihara et al., 2008~~), reduced calcification (Miller et al., 2009) and higher mortality (Talmage and Gobler, 2009; Crim et al., 2011; ~~Talmage and Gobler, 2009;~~ 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 CO_2 increase (Green et al., 2004; Cigliano et al., 2010; Clements and Hunt, 2014~~; Cigliano et al., 2010~~). 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 CO_2 vents by Hall-Spencer et al., 2008 or Kroeker et al., 2011 and previous/other mesocosm studies by Christen et al., 2013; 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 in a mesocosm setting in a more comprehensive way. In the case of future ocean acidification, potential changes in phytoplankton dynamics due to increased CO_2 levels are likely to have consequences for the other trophic levels. The growth of nanoplankton and diatom species (< 20 µm), which are the main food particles of larval bivalves (Bos et al., 2006), has been shown to benefit from changing CO_2 conditions (e.g. Engel et al.,

2008~~7~~; 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. ~~Already at present, l~~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~~; Thomas and Schneider, 1999~~), 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 ~~strong~~ 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,~~43~~ the main settling period of *M. balthica*).~~, except for aragonite and calcite saturation states where the values given are averages of days 0-17).~~

	M1	M5	M7	M6	M3	M8	Bay
Target fCO_2 (µatm)	ambient/control	ambient/control	600	950	1300	1650	ambient
fCO_2 (µatm)	31965	32168	46997	85721	107207	1347231	282417
pHT	7.9489	7.9489	7.8077	7.59	7.512	7.434	7.9988
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.

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

Figure 3. Larval sizes in different fCO_2 levels at day 10. Data is presented as means ± SE, n = ca. 70 individuals. The horizontal lines indicate the range of average larval sizes on day 0.

Figure 4. Sizes of the ~~settling~~ settled individuals exposed to different fCO_2 levels on days 11, 13, 15 and 17. Data is presented as means, n= ca. 35 at each data point. For clarity, SE~~D~~ are not shown.