

Reviewer 1

I would like to congratulate the authors for this complete study that incorporates both field and laboratory experiments. The study, in general, is well-written and does not show important methodological failures. However, I have some specific comments and doubts that I would like authors
5 could respond to.

The information provided in the introduction is sufficient to understand the necessity to perform this research. However, I recommend the authors to try to re-order the paragraphs, because there are some paragraphs that are totally disconnected from the others making it difficult to follow the storyline. For example, the paragraph starting at L98, in my opinion, would fit better at the beginning
10 when the authors explain the study system.

Author response

**The authors agree with the reviewer's suggestion. The Introduction section has been restructured with the first paragraph focusing on the environmental conditions present in the Baltic Sea, the second paragraph introducing the ecological importance of calcifying mussels and what is currently
15 known about how the environment impacts calcification rates and the following paragraphs introducing the known mechanisms of how salinity and carbonate chemistry impacts calcification. These revisions have been made in L42-126.**

Specifically, at L51 and following it would nice that authors explain more about the ecosystem function of the study species. Authors only make a small notification about that, surely there are studies about
20 the ecological importance of this species and the formed-beds along the Baltic Sea.

Author response

**We have provided more details in the Introduction of the ecological dominance and role calcifying mussels play in benthic Baltic Sea ecosystems. Consideration has been given to the degree of ecosystem services provided by biogenic mussel reefs compared to other biogenic habitats and several ecological functions provided by mussels have also been mentioned (L52-61). Finally, the
25 potential for mussel aquaculture as a means of regional remediation of eutrophication has also been mentioned (L61-63), highlighting the dominant ecological role played by marine calcifying mussels in the Baltic Sea.**

Also, I would like to know if the authors have information if these ecosystem functions change along
30 the gradient (salinity), and if the abundance of this species is sensitive to the gradient informed. This information is interesting to highlight the effects of environmental changes on the different Baltic sea mussel populations.

Author response

**The authors have reworded the introduction to reflect the documented change in growth rates biomass and abundance of Baltic *Mytilus* down to salinities of 5 (L66-69). The functional contribution of Baltic *Mytilus* to ecosystems services has been commented on in relation to the salinity gradient and emphasis has been put on the fact that ecosystem function drops drastically below salinities of 5 when calcifying mussels are no longer present. This revised section of the introduction further highlights the ecological importance of Baltic *Mytilus* and how potential
40 changes in growth rates, biomass and abundances can have large ecological consequences.**

L131: what is based on the diet supply used? Is it based on field measurements, previous feeding rates reported. Please, add a reference.

Author response

45 **The rationale behind the choice of this feeding regime has been clarified in the methods section (L193-194) based on previous studies investigating feeding rates in Baltic *Mytilus*, and the accompanying study has been referenced (Riisgård et al., 2013).**

L142: Authors pointed out that they use 1600 animals by experimental replicate. The authors did monitor the oxygen availability in the aquarium. I am worried that this animal density could affect the oxygen supply to the experimental aquarium, or change the pH conditions as a product of mussel
50 respiration. The experimental replicates were bubbled while both experiments lasted?

Author response

55 **The methods section has been rewritten to include steps taken by authors to ensure animal densities in both experiments did not impact seawater chemistry through respiration, calcification or food addition. Due to minor differences in initial mean sizes between both cohorts of mussels in either experiment, we aimed to ensure biomass density per aquaria were comparable between both experiments (Table S2; L354-356). The methods section now states that experimental aquaria were aerated in the bicarbonate experiment (L206) but not in the calcium experiment (L239). The frequency of water chemistry monitoring and water changes has been clarified with mention of the maximum acceptable deviation in pH, $[Ca^{2+}]$ and $[HCO_3^-]$ between water changes (L216-218 and
60 L240-241). Water chemistry monitoring revealed mean pH deviated by between 0.04 and 0.12 units between water changes, in line with the pH variation observed at field monitoring sites (Table 1). Changes in experimental pH values before and after water changes (presented in table S2) have been described in the results section (L351-353). Oxygen saturation levels in experimental aquaria were not measured, however due to minor (< 0.1) deviations in pH values between water changes
65 in experimental aquaria we are confident oxygen levels in the bicarbonate ion experiment did not drop significantly due to the high number of animals (See our response to reviewer 2's comment, L245-268 of this document).**

L150 and following: Why the duration of both experiments was not the same? How authors can avoid the time accumulated effects of living in stressful environments. Even if the authors calculated a rate
70 (by day), it is not comparable. I think that this an important issue to discuss as to compare both experiments as the results can be under or overestimate. The authors measured the calcification rates at the end of each experiment, right? This was no clear to me.

Author response

75 **Experimental durations were not identical due to practical limitations during experimentation. We argue that despite the dissimilar durations of both laboratory experiments, results are still comparable due to complete acclimation of intracellular osmolality (> 2 weeks) in response to different salinities (Neufeld et al., 1996). This has been addressed in the discussion (L502-514). Resultingly, we believe that in the context of the experiments presented here, short term exposures whether 1 or 2 months, would not result in significantly different effects of carbonate chemistry
80 when both experiments are comparing the same species, sample population and life stage. Experimental durations were still long enough to detect a significant effect of seawater chemistry on calcification rates in juvenile bivalves.**

The methods for measuring and calculated calcification rates during laboratory experiments has been clarified in the methods section (L275-287) of the revised manuscript. Explanations have been

85 **given for the 2 time points when shell mass was quantified/calculated and an equation has been given (L285) for calculating calcification rates in each experiment.**

I understand that due to experimental limitations, the volume of the replicates had to be different. However, the final density (mussels ml⁻¹) is too different to compare between both laboratory experiments. This is an issue to discuss in terms of density-dependency potential effects on the results observed.

90 Author response

The reviewer is right to point out that the number of mussels ml⁻¹ was not identical between both experiments. However, since mussels at the beginning of the Ca²⁺ experiment had ~4 x the body mass of those at the beginning of the HCO₃⁻ experiment (despite both cohorts being in the juvenile life stage), we argue a better metric for comparing both experiments is the total mussel biomass per L. This metric is also more applicable for identifying the metabolic effects on seawater chemistry between both experiments. This information has also been described in the results section (L354-356).

100 **In the revised manuscript and supplementary document, Table S2 has been revised and clarified to reflect the key metrics to compare between both experiments. In the revised supplementary Table S2, the column 'No. animals per tank' now states the mean number of animals as an average of all treatments throughout each experiment, rather than presenting the initial number of animals in each experiment. This mean value has been used to calculate the mean body dry mass (BM) per litre, as opposed to using the initial number of animals to calculate this metric. Resultingly, mean BM throughout each experiment is now calculated as 13.2 mg L⁻¹ and 24.1 mg L⁻¹ for the Ca and HCO₃ experiments, respectively.**

105 **The methods section of the revised manuscript has been rewritten to emphasise the steps taken to minimise the effects of differential mussel biomass densities between both experiments and the impacts on food availability between both experiments (L245-261).**

110 **We argue that monitoring of clearance rates during the experiment and ensuring saturated feeding conditions, combined with regular water chemistry monitoring and water changes ensuring minimum deviations in water chemistry resulting from biological activity, largely minimised the density-dependent effects across treatments and experiments.**

L190 and following. Suddenly, the authors show that a field experiment was also performed. However, nor the introduction or abstract is pointed out. In my opinion, this is a stronghold of this study. Please, try to incorporate this information in the last paragraph of the introduction, as well as in the abstract.

115 Author response

The authors have re-ordered the storyline of the manuscript to first introduce the environment and field systems in the introduction, methods and results sections with descriptions and discussions of the laboratory experiments following after. The abstract now mentions the field study (L23-24 and L30-31) whilst the re-ordered introduction now introduces the field study on growth rates (L129-132) and the methods section describes the rationale behind the field experiment (L141-142).

About the field study, the authors collected the laboratory experimental mussels in Ahreenshoop, however, the authors also performed field experiments in the other two extra sites. I understand the objective of this, but this is not explained in the manuscript.

125 Author response

130 **The objective of comparing environmental conditions and calcification rates in the field have been described in the methods section (L141-142 and L161-163) of the revised manuscript. This was to follow the methodologies of previous studies and to cover the range of the steepest salinity gradient in the Southwest Baltic Sea. The reordering of the methods section also makes clearer the rational behind the choice of the 3 monitoring sites in this study.**

135 Authors, in the field experiment, estimated calcification rates from the reported SL-CaCO₃ relationship. I understand, that this is a unique relationship developed for a specific mussel population. However, after reading the introduction where authors pointed out that there are important differences along the salinity gradient. So, in my opinion, this relationship should be different among mussel populations. This could have important effects on the results. Indeed, why authors did not use the same methodology of the laboratory experiment, could improve the comparison of results.

Author response

140 **Unique SL-CaCO₃ relationships were developed for each of the 3 individual mussel populations and these relationships have already been presented in the supplementary material (Fig. S3 of revised supplementary material). This has been clarified in the methods section (169-174) with emphasis on the fact that separate population specific relationships exist. Direct measurement of CaCO₃ mass was not done for the mussels sampled in the field, as population specific SL-CaCO₃ relationships were already available and the number of mussels collected, and the frequency of collections would have necessitated a significant workload. The SL-CaCO₃ relationship for the Ahrenshoop population was used to calculate the initial CaCO₃ mass in lab experiments, and direct measurements of CaCO₃ mass were done at the termination of both experiments with identical methods. This has all been rewritten in the methods section (L275-289).**

150 Authors, in the laboratory experiment, show how they burned shells in order to eliminate organic matter from the shells in order to provide CaCO₃ data and estimate calcification rates. Were there differences in the organic matter among populations? This is so important, as many previous studies have shown how marine calcifying organisms show different organic matter concentrations under different environmental conditions (lab or field). If authors could show this data would be very interesting to understand another potential factor affecting calcification rates. Indeed, shell organic matter (periostracum and inter, intra-crystalline organic matters) has a shell protection function under corrosive environments, but also as a substrate to favor crystallization and biomineralization processes.

Author response

160 **The reviewer makes an excellent and very important point here. In the laboratory experiments, initial CaCO₃ mass was calculated using a population specific SL-CaCO₃ relationship, whereas CaCO₃ mass in each treatment was measured using the muffle furnace method described in the methods section. Subsequently, there is no initial measure of shell organic (periostracum or shell matrix proteins) to calculate changes in shell organic content during the course of both experiments. The aim of this study was to investigate changes in inorganic CaCO₃ deposition with salinity and carbonate chemistry, rather than changes in total shell composition or shell organic content. Subsequently, this data is unfortunately not available from this study. Understanding how shell organic content may be modulated in Baltic mussel shells in light of predicted climate change is an important point for understanding the fate of calcifying Baltic mussels. A discussion on the importance of considering shell organic content and structure has been included (L541-550) with**

170 **reference to its implications for adaptive responses of shell formation in marine calcifiers as well as the energetic cost of shell production.**

How many times Chl-a was measured during the field experiment?

Author response

175 **The number of Chl-*a* measurements are as follows: Usedom: 25 data points; Ahrenshoop: 25 data points; Kiel: 28 data points. All Chl-*a* monitoring occurred from January 2015 – December 2017. This information has been added to the header of Table 1 in the revised manuscript.**

I do not have major comments on the results section.

180 The discussion section is clear and also identified the major limitations of the study which is appreciated. Results are broadly discussed from many points of view, however as it was pointed out above, I miss a discussion of other potential causes that could determine the results. Indeed, biomineralization processes not only incorporate CaCO₃ precipitation but they incorporate the secretion of periostracum/shell organic matter which has an important function on biomineralization. Also, the entire biomineralization process is energetically expensive because of the secretion of these shell organic compounds. It would be nice if the authors develop this idea as potential causes of the results observed in order to complete the discussion section. If authors can show shell organic matter by treatment, this could help a lot to understand the results. Indeed, this could be a future research topic to develop. In addition, some methodological limitations of the study (pointed out above) such as density-dependency are not discussed in the discussion.

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Author response

190 **We have included a discussion on the potential for changes in shell organic content to impact the observed results in terms of increasing energetic costs of calcification (L541-550). Whilst all authors agree the importance of considering the impacts of density-dependent effects on the observed results, we have amended the manuscript to clearly show that measures were taken to minimise the impacts of differential biomass concentrations in experimental aquaria between treatments and experiments. These include measures to ensure comparable food availability between both experiments (See Table S2 and L356-357), minimising the impacts of biological activity and food addition on pH and pCO₂ (Table 2 and 3; and Section 2.5).**

195

FIGURES AND TABLES.

200 I suggest changing the order of figures, first showing the environmental conditions of field study sites, and then the results of calcification rates.

Author response

205 **We have re-ordered the presentation of the figures, tables and results section. The environmental monitoring and field study is presented and discussed first, followed by the results from the laboratory experiments. This reflects changes made to all sections of the revised manuscript to reflect the change in the storyline order.**

In table 1, I noticed that there are important differences in pH conditions among experimental treatments, how could that affect the calcification rates?

Author response

210 The authors agree that differences in pH are important to consider in the interpretation of the
results. Whilst this was discussed in the original manuscript, the revised manuscript now contains a
deeper discussion on the impacts of pH on calcification in the bicarbonate experiment (L427-443
and L521-527) and the potential impact of pH on mortality in this experiment (L525-527). This data
on mortality has been presented in Fig S10, as suggested by reviewer 2. We would also like to
emphasise that the impacts of pH are included in both SIR and ESIR calculations as $[H^+]$ (See
215 equations 2 and 3) and as such, the impacts of pH on mussel calculated have not been disregarded
(L534-536).

References

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(*Rhodomonas salina*) concentrations below and above saturation levels for reduced filtration rate,
Mar. Biol. Res., 9, 1005-1017, DOI: <http://dx.doi.org/10.1080/17451000.2012.742549>, 2013.

Reviewer 2

225 Reviewer comment

This is a very nice manuscript from Sanders and collaborators dealing with the impact of seawater chemistry on mussel growth rates. The two experiments have been well designed in order to discriminate first the impact of salinity vs. carbonate chemistry changes and second salinity vs. calcium concentration changes. These experiments have been complemented by a field survey covering over
230 3 years. Monitoring of seawater physico-chemistry and mussel growth have been performed at 3 sites along a decreasing salinity gradient towards the Central Baltic. The study is well introduced although I agree with Reviewer#1 that paragraph L98-112 should be put up front. The methods are most of the time well explained and the results properly discussed. I have no doubts that this will be a nice contribution to the Biogeosciences journal. Congratulations to the authors!

235 That being said, I have a few concerns and questions that I would like the authors to answer:

1) I have to say that I was impressed on how many individuals you could fit in 2 L containers (1600 animals, small but still. . .). Since you did not consider a flow-through system and changed the water "only" 2 to 3 times weekly, I am really wondering how would change carbonate chemistry but also ammonium and oxygen concentrations between two water changes. Table 1 and 2 are not clear to
240 me. Do these tables show the conditions in the experimental plastic aquaria and/or in the stock seawater? If measured in the aquaria, when were the samples taken? Before and/or after water changes? Were your aquaria aerated? I apologize in case I missed that in the text.

Author response

245 We have rewritten this aspect of the methods section to clearly state that water changes were
conducted at frequencies designated by regular water chemistry monitoring during experiments.
Water chemistry was measured before and after water changes in both experiments (L216 and L240)
and results revealed pH, $[HCO_3^-]$ and $[Ca^{2+}]$ did not deviate by unacceptable levels (L351-354 and
L357-359). The methods section now includes details on aeration in both experiments (L196 and
L229). Tables 2 and 3 of the revised manuscript show mean water chemistry parameters in
250 experimental aquaria during the experiment and this has also been clarified in the methods section

and in the Table headings. Although oxygen saturation was not measured in experimental aquaria, we believe continuous aeration and regular water changes prevented severe reductions in oxygen levels during experiments. We have also addressed a similar concern raised by reviewer one in our response to reviewer 1's comment (L52-63 of this document).

255 Ammonia excretion was not quantified, however given a conservatively high ammonia excretion rate in Baltic mussels of 20 $\mu\text{g NH}_4$ per gram dry weight hr^{-1} (Tedengren & Kautsky 1987), the more biomass dense HCO_3^- experiment (mean biomass 24 mg dry weight per litre) would have resulted in maximum ammonia concentration of 0.04 mg L^{-1} immediately prior to a water change after 3 days accumulation. This value of 0.04 mg L^{-1} is far below the LC_{50} value for juvenile *Mytilus edulis* of 0.39
260 $\text{mg NH}_4 \text{L}^{-1}$ after 21 days exposure (Kennedy *et al.*, 2017). Subsequently we do not believe there to be any negative impacts of ammonia build-up in either experiments. These calculations have not been included in the revised manuscript, but it has been mentioned that water change frequency was sufficient to prevent respiratory build up of CO_2 and consequent drops in pH (L241-242 and L359-360).

265 Reviewer comment

2) In Table S2, you report on a >50% mortality during the 70 days bicarbonate experiment, as well as an important range (10-75%) across treatments. Did you check whether you had some relationships between mortality rates and the imposed chemical changes? Did you replace the dead organisms? If not, what would be the effect on the amount of food available for each individual? Table S2 is not clear to me, what are these biomass data? At the start of the experiment? At the end? You mention
270 on L173 that biomass per litre was comparable between the 2 experiments while I can read that it was 13.2 mg/L during the Ca^{2+} exp and 51.5 mg/L during the HCO_3^- exp, it does not seem comparable to me.

Author response

275 Mortality rates did exhibit patterns in relation to seawater chemistry. Mortality rates were highest at low pH/ $[\text{HCO}_3^-]$ and slightly higher at low salinities (6) compared to higher salinities of 11 and 16. This data has been included in the supplementary material (Fig S10 of revised supplementary material) and the potential causes of this discussed in the Discussion section (L525-528). Dead organisms were not replaced but quantified and changes taken into account during experimentation
280 (L214-216).

The header of Table S2 has been adjusted to clearly state it presents the standard deviation of mortality rates across all treatments in the experiment. Feeding regimes were chosen in such a way to ensure saturated feeding conditions in all treatments ($>10\,000$ phytoplankton cells ml^{-1}) and final calculations of food availability between experiments revealed cell number per unit biomass was
285 comparable between experiments (Table S2 and L354-355 of Results section). Table S2 has also been recalculated and now contains mean data over the entire experimental period, whereas it previously presented the number of animals at the beginning of the experiment. This updated value now shows mean dry body mass (BM) per L^{-1} in both experiments to be 13.2 mg L^{-1} and 24.1 mg L^{-1} for the Ca and HCO_3^- experiments, respectively. These more accurate values show that biomass densities were even more similar between experiments than presented in the originally submitted
290 manuscript.

Table S2 presents data per replicate tank as a mean value over the entire experimental period. Both experiments are presented as a comparison, as well as the range of values within the HCO_3^- experiment to highlight that biomass and biomass per ml varied by a higher degree *within* the HCO_3^-

295 **experiment than *between* both experiments. The mean values for each experiment (13.2 mg/L and 24.1 mg/L) are therefore within the same order of magnitude and the revised manuscript now clearly states in L218-220, L241-242 and Table S2, that food availability and density-dependent impacts of biological activity on water chemistry were not dissimilar between both experiments**

Reviewer comment

300 3) I believe there is one aspect (maybe related to the point above) that should be discussed. During the first experiment (bicarbonate), mussels at salinity 6 did not grow much (maybe 5 microg/d; Fig. 2a). What is the reason why they grew much better during the second experiment (Fig. 2b) even when Ca²⁺ concentrations are below ambient levels (2.5 mmol/kg), reaching rates of 20 microg/d)? Is it due to the differences in terms of experimental design?

305 Author response

The reviewer raises an interesting point here related to calcification rate differences between both experiments at a salinity of 6. The discussion has been adjusted to include reasoning behind these differences (L454-457 and L487-493). Possible explanations include maximum [Ca²⁺] in the calcium experiment being ca. 0.2 mmol kg⁻¹ below threshold values and subsequently masking the impacts of low salinity on calcification in that experiment. Potential genetic differences between cohorts and minor differences in mean animal sizes between experiments have also been discussed in the revised manuscript (L494-496).

Reviewer comment

315 4) As such, I do not believe that trying to fit any model to all data points (pooled from the two experiments) makes much sense (Fig. S4 and S5, but also Fig. 3). At least for a better view on the data, you should identify the dots depending on the experiment and salinity levels.

Author response

The authors agree and have altered Figures 6, S4 and S5 to label data points based on experiment and salinity levels for increased clarity.

320 Reviewer comment

5) It seems that you over-determined carbonate chemistry during the field survey by measuring pH, C_T and A_T. It is not clear to me if A_T data showed (i.e. Fig. 7) are the ones measured or derived from pH and C_T, maybe to clarify. Finally, how do computed A_T and measured A_T compare? This could be a nice way to identify DOC contribution no?

325 Author response

A_T, C_T and pH were all determined from field samples, however only pH and C_T were used to calculate other carbonate chemistry parameters due to the potential impacts of dissolved organic matter on measured A_T, as the reviewer mentioned. Measured A_T data is not shown in Fig. 3 of the revised manuscript, but rather the subsequent carbonate chemistry parameters calculated from field measurements of pH and C_T. The reviewer makes an interesting point in comparing measured A_T and calculated A_T from C_T and pH. However, the potential contribution of DOM towards A_T is complex, as this organic alkalinity contribution (A_{org}) is not a linear function of DOC, but rather dependent on various parameters such as pH. Previous studies found that A_{org} ranged from 22–58 μmol kg⁻¹, and developed a first mechanistic understanding of how this contribution relates to the amount and nature of DOM, as well as seawater carbonate chemistry (Kuliński et al., 2014). Simply

comparing our measured A_T and calculated A_T would not help us to better understand the A_{org} contribution to alkalinity, while a detailed analysis of this contribution is well beyond the scope of this paper. Thus, we prefer not to include the suggested comparison.

References

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