Biogeosciences Discuss.,
https://doi.org/10.5194/bg-2020-382-AC1, 2021
© Author(s) 2021. This work is distributed under
the Creative Commons Attribution 4.0 License.

Biogeosciences
Discussions
Open Access
EGU

Interactive comment on "Decoupling salinity and carbonate chemistry: Low calcium ion concentration rather than salinity limits calcification in Baltic Sea mussels" *by* Trystan Sanders et al.

Trystan Sanders et al.

trystanbrett@gmail.com

Received and published: 9 February 2021

Reviewer comment

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 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 when the authors explain the study system.

Author response

The authors agree with the reviewer's suggestion. The Introduction section will be restructured to improve the flow of the storyline specifically by bringing forward L98-L112.

Reviewer comment

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 the ecological importance of this species and the formed-beds along the Baltic Sea.

Author response

This is an important point raised by the reviewer. Benthic reef-forming mytilid mussels in the Baltic Sea are prominent ecosystem engineers forming extensive mussel reefs that enhance biodiversity and organic carbon flow through benthic ecosystems in the Baltic (Koivisto & Westerbom 2010; Attard et al. 2020). Baltic mussel reefs are particularly dominant at salinities below 10 practical salinity units being one of the primary habitat-forming organisms in the central and eastern Basins (Westerbom et al., 2002). Baltic Mytilus reefs are particularly important in their contribution to benthic ecosystem services in the Baltic Sea having a stronger impact on ecosystem function than both macroalgae beds and seagrass meadows (Heckwolf et al., 2020). This information regarding the functionality of these species in Baltic Sea ecosystems will be added to the introduction after L51.

Reviewer comment

Also, I would like to know if the authors have information if these ecosystem functions change along 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

Biodiversity in the central and Eastern Baltic Basins (salinity 5 – 8) is ca. 10 % that of the North Sea (salinity >32). Despite this Baltic filter feeding mussels dominate benthic ecosystems at salinities of 5 – 8, albeit at extremely reduced growth rates and body sizes. Several studies have observed reduced growth rates of Baltic Mytilus at low salinities (Sanders et al., 2018) and decreasing biomass and abundance of mussels with decreasing salinity in the Baltic Sea, both in the field and in model simulations (Westerbom et al, 2002; Westerbom et al. 2019; Liénart et al., 2020). It is therefore expected that desalination will result reduced maximum size, abundance and biomass of Baltic Mytilus which will have negative impacts on the associated ecosystem services provided by Baltic mytilid mussels. Comparisons of carbon flow between the Bothnian Sea (filter feeding mussels present) and the Bothnian Bay (absence of filter feedings mussels) reveals significantly reduced ecosystem function upon the loss of filter feedings bivalves at salinities below 5 (Elmgren & Hill, 1997). This highlights the potential ecological impacts of loss of calcifying, filter feedings bivalves in the Central Baltic Sea with predicted desalination. This information on the reduced size and contribution to ecosystem function shall also be added to the introduction at L54 when the impacts of salinity on mussel growth are introduced. The drastic reduction in ecosystem services upon the complete loss of calcifying bivalves in the Baltic Sea, will also be mentioned in this section.

Reviewer comment

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

This feeding regime was used to ensure saturated feeding conditions of 10 000 phytoplankton cells m-1 (Riisgård et al., 2013) based on Baltic Mytilus clearance rates reaching maximums at or above 6-7000 cells ml-1. This justification will be clarified in L131 and the relevant reference will be added to the manuscript.

Reviewer comment

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 respiration. The experimental replicates were bubbled while both experiments lasted?

Author response

In the HCO3- experiment, ambient air was aerated through all aquaria to ensure fully aerated seawater (L148). Oxygen concentration was not measured during experiments; however pH was monitored (a proxy for carbon dioxide partial pressure) 3-4 x weekly in the HCO3- experiment to ensure pH did not deviate by more than 0.1 units from target values due to mussel respiration or calcification between water changes. The frequency of water changes was increased to 3 x weekly during the HCO3- experiment as AT started to increase through the addition of alkalinity in food culture.

In the Ca2+ experiment, stock artificial seawater was equilibrated with atmospheric pCO2 and pO2 before water changes. Experimental aquaria were not actively aerated during the experiment as biomass per ml in experimental aquaria was lower (Table S2) and preliminary monitoring of pH revealed no detectable impact of mussel respiration on seawater pH. pH and ATÂň were still monitored 2-3 x per week (immediately before and after water changes) to ensure respiration or calcification did not cause deviations from target values. Tables 1 and 2 show that pCO2 throughout experiments did not

differ hugely from field pCO2 values (Table 3).

The rational behind the frequency of carbonate chemistry monitoring with relation to respiration and biomass density in experimental aquaria will be clarified in L148 of the methods section.

Reviewer comment

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 (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

Experimental durations were not identical due to practical limitations during experimentation. Since organisms were slightly larger (older) in the Ca2+ experiment compared to the HCO3- experiment (different times of spawning and sampling cohort of juveniles), a shorter experimental period was still sufficient to allow a significant increase in shell mass over the experimental period and significant differences between treatments. A longer experimental duration in the Ca2+ experiment may indeed result in different results both within the experiment and between experiments, however methodological limitations prevented this from being realised. 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 when both experiments are comparing the same species, sample population and life stage.

Shell mass was estimated at the beginning of the experiment (from the shell length-shell mass relationship in Fig. S1) and then directly measured at the end of both experiments (2 time points) Since the experiments were (relatively) short compared to

the field monitoring (12+ months), a linear increase in shell growth was employed for calculating calcification in both experiments, as opposed to a power curve (eg. the relationship modelled for field calcification rates). This makes the comparison of both experiments possible as calcification rates (increase in CaCO3 mass over time) are derived from the same function for both experiments. Section 2.4 will be expanded to clarify that 2 time points were used to measure calcification and that a linear relationship was used to derive calcification rates for both experiments.

Of course, we cannot disregard the potential effects of differential accumulation of stress effects between both experiments, however we took relevant steps to maximise comparability between both experiments:

1: We used mussels originating from the same population and life stage in both experiments. 2: We utilised the same experimental design in both experiments (5 x 3 treatments with 4 replicates). 3: We measured calcification using identical methods between both experiments. 4: To ensure food was not a limiting factor we maintained saturated food conditions in both experiments with comparable availability of phytoplankton cells per unit biomass during experiments. 5. We conducted regular water changes at sufficient frequencies to ensure seawater chemistry was not deviating significantly from target values due to metabolism and calcification thus ensuring respiratory build-up of CO2 or depletion of dissolved inorganic carbon/calcium did not impact calcification rates. 6. Most importantly, we ensured seawater alkalinity was at natural levels across all experiments and salinity treatments. Most other studies that expose calcifying organisms to desalination experimentally dilute seawater using distilled water which also dilutes seawater alkalinity. At salinities below 10, this results in extremely low alkalinities (< 1000 μmol kg-1) which may negatively impact calcification synergistically in low salinity treatments.

These main points highlighting comparability between both experiments will be made clearer in L166-177 in the methods section and discussed at the end of section 4.1 in the discussion.

Reviewer comment

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 denso-dependency potential effects on the results observed.

Author response

The reviewer is right to point out that the number of mussels ml-1 was quite different 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. Mean biomass per L was 13.2 mg l-1 at the beginning of the Ca2+ experiment, and 51.5 mg-1 at the beginning of the HCO3- experiment, thus within the same order of magnitude. By the end of the HCO3- experiment, biomass per litre of seawater ranged from 32.6 – 537.1 mg l-1 between the most extreme treatments. This highlights that the range of values within a single experiment exceeds the differences between both experiments showing that relatively, both experimental approaches achieved comparable biomass per litre. We argue that monitoring of clearance rates during the experiment and ensuring saturated feeding conditions, combined with regular water chemistry monitoring and water changes, largely minimised the denso-dependent effects across treatments and experiments. In the methods section (L166-177) of the manuscript, the measures taken by the authors to minimise denso-dependant impacts between experiments will be discussed specifically focusing on the 6 points mentioned in the previous comment.

Reviewer comment

L190 and following. Suddenly, the authors show that a field experiment was also per-

formed. 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.

Author response

The reviewer makes a good point here about a strength of the study which could be better communicated. The rational underlying the field experiments will be included in the introduction, specifically by bringing forward L98-112 to the start of the introduction. The methods section will be reordered, starting with the field monitoring methodologies of both abiotic conditions and field calcification rates.

Reviewer comment

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.

Author response

The 3 field monitoring sites were chosen to reflect natural populations living at the 3 experimental salinities (6, 11 and 16). Whereas the experimental population from Ahrenshoop was chosen based on this population being located in the genetic transition zone between Baltic Mytilus edulis and Baltic Mytilus trossulus (Stuckas et al., 2017), allowing the ability to investigate the impacts of desalination but also antagonistic effects of increased salinity. This will be clarified in methods section 2.5-2.7 which will be brought to the beginning of the methods section where it will introduce the natural systems being studied more clearly.

Reviewer comment

Authors, in the field experiment, estimated calcification rates from the reported SLCaCO3 relationship. I understand, that this is a unique relationship developed for a specific mussel population. However, after reading the introduction where au-

thors 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

Unique SL-CaCO3 relationships were developed for all 3 mussel populations (Sanders et al., 2018) and these relationships are presented in the supplementary material (Fig. S1). The same SL-CaCO3 relationship for the mussel population from Ahrenshoop was used in both laboratory experiments for estimating initial CaCO3 mass (before experimental exposures), as well as the field monitoring study on the Ahrenshoop population. The Kiel and Usedom populations had their own, population specific SL-CaCOÂň3Âň relationships used to calculate shell mass in the field study, exactly as pointed out by the reviewer. Throughout the field monitoring study, population specific SL-CaCO3 relationships were used to calculate shell mass from shell lengths, as the number of mussels being collected was so large. These population specific relationships are mentioned in L229, but will be made clearer in this section.

Reviewer comment

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

The reviewer makes an excellent and very important point here. In the laboratory experiments, initial CaCO3Âň 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. Unpublished work, separate from the data presented in this study for the 3 different populations revealed shell organic content (derived from ashing of shells) to be $\sim$ 5-10 %, within the range expected for marine molluscs (Palmer 1983; Thomsen et al., 2013), however these data did not reveal any differences between populations and values were variable possibly due to shell biofouling. Previous work by authors and colleagues in the Baltic Sea have shown that organic content of larger adult marine mytilid shells is higher at lower salinities, however the impact of salinity is minor compared with the effects of food availability and shell length on shell organic content (Telesca et al., 2019). However, little is known about how shell organic may differ between populations and experimental treatments in juvenile mussels. 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. The potential increase in energetic costs of shell production related to higher organic content of shells will be mentioned in L415. Additionally, the role of the periostracum in defending the shell from dissolution will be mentioned in section 4.3 of the discussion.

Reviewer comment

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

Author response

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 will be added to the header of Table 3 in the manuscript.

Reviewer comment

I do not have major comments on the results section.

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 $CaCO_3$ precipitation but they incorporate the secretion of periostracum/shell organic matter which has an important function on biomineralization. Also, the entire biomineralization process is energetic expensive because of the secretion of these shell organic compounds. It would be nice 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 denso-dependency are not discussed in the discussion.

Author response

The authors agree that including a discussion on shell organic content and energetics would strengthen the study and identify potential new avenues for research, as mentioned above. The authors also agree that density-dependant effects of animals in experimental aquaria are important to consider. However as mentioned, we took multiple steps to minimise the impacts of these effects and we strongly believe findings are comparable between both experiments due to experimental animals arising from the same population and at the same life stage, similar mean biomass per litre, regular water changes to prevent deviation in carbonate chemistry resulting from metabolic or growth impacts, and saturated feeding conditions removing any effect of differential

energy intake between experiments (See response to previous reviewer comment). As mentioned above, we will discuss how we maximised comparability between experiments and how we overcame the slight differences in mean biomass density. This information will be discussed at the end of section 4.1.

Reviewer comment

FIGURES AND TABLES.

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

The authors agree this would improve the flow and presentation of the study. As such this will be implemented in the revised manuscript. This will also reflect adjustments in the ordering of the introduction, methodologies and results sections by starting with investigating field conditions and then moving on to the laboratory methods and results.

Reviewer comment

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

Author response

The authors agree with the reviewer, and we have considered the impacts of pH on calcification rates between experimental treatments through inclusion of $[H^+]$ and $[CO_3^{2-}]$ in both ESIR and âĎęaragonite. The effects of pH have also been discussed (L339-L354) with emphasis on the co-linearity of pH, $[H^+]$ and $[CO_3^{2-}]$ and the inability to individually isolate the impacts of each parameter.

References

Attard, K. M., Rodil, I. F., Berg, P., Mogg, A. O. M., Westerbom, M., Norkko, A.,

Gludd, R. N.: Metabolism of a subtidal rocky mussel reef in a high-temperate setting: pathways of organic C flow, Mar. Ecol. Prog. Ser., 645, 41-54, DOI: https://doi.org/10.3354/meps13372, 2020.

Elmgren, R., Hill, C.: Ecosystem function at low biodiversity – the Baltic example, In: Ormond, R., Gage, J., Grassle, J. F., (eds), Marine Biodiversity: patterns and processes, Cambridge University Press, Cambridge, 139-336, 1997.

Heckwolf, M. J., Peterson, A., Jänes, H., Horne, P., Künne, J., Liversage, K., Sajeva, M., Reusch, T. B. H., Kotta, J.: From ecosystems to socio-economic benefits: A systematic review of coastal ecosystem services in the Baltic Sea, Sci. Total Environ., 755, 142565, DOI: https://doi.org/10.1016/j.scitotenv.2020.142565, 2021.

Koivisto, M. E., Westerbom, M.: Habitat structure and complexity as determinants of biodiversity in blue mussel beds on sublittoral rocky shores, Mar. Biol., 157, 1463-1474, DOI: 10.1007/s00227-010-1421-9, 2010.

Liénart, C, Garbaras, A., Qvarfordt, S., Sysoev, A. Ö., Höglander, H., Walve, J., Schagerström, E., Eklöf, J., Karlson, A. M. L.: Long-term changes in trophic ecology of blue mussels in a rapidly changing ecosystem, Limnol. Oceanogr., 9999, 1-17, DOI: https://doi.org/10.1002/lno.11633, 2020.

Palmer, A. R.: Relative cost of producing skeletal organic matrix versus calcification: evidence from marine gastropods, Mar. Biol., 75, 287-292, DOI: https://doi.org/10.1007/BF00406014, 1983.

Riisgård, H. U., Pleissner, D., Lundgreen, K., LArse, P. S.: Growth of mussels Mytlus edulis, at algal (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.

Sanders, T., Schmittmann, L., Nascimento-Schulze, J. and Melzner, F.: High calcification costs limit mussel growth at low salinity, Front. Mar. Sci., 5, 352,

https://doi.org/10.3389/fmars.2018.00352, 2018.

Stuckas, H., Knöbel, L., Schade, H., Breusing, C., Hinrichsen, H. H., Bartel, M., Langguth, C., Melzner, F.: Combining hydrodynamic modelling with genetics: can passive larval drift shape the genetic structure of Baltic Mytilus populations? Mol. Ecol. 26, 2765-2782, https://doi.org/10.1111/mec.14075, 2017.

Telesca, L., Peck, L. S., Sanders, T., Thyrring, J., Sejr, M. K., Harper, E. M.: Biomineralization plasticity and environmental heterogeneity predict geographical resilience patterns of foundation species to future change, Glob. Chang. Biol., 25, 4179-4193, DOI: 10.1111/gcb.14758, 2019.

Thomsen, J., Casties, I., Pansch, C., Körtzinger, A. and Melzner, F.: Food availability outweighs ocean acidification effects in juvenile Mytilus edulis: laboratory and field experiments, Glob. Chang. Biol., 19, 1017-1027, https://doi.org/10.1111/gcb.12109, 2013.

Westerbom, M., Kilpi, M., Mustonen, O.: Blue mussels, Mytilus edulis, at the edge of the range: population structure, growth and biomass along a salinity gradient in the north-eastern Baltic Sea, Mar. Biol., 140, 991-999, DOI: 10.1007/s00227-001-0765-6, 2002.

Westerbom, M., Mustonen, O., Jaatinen, K., Kilpi, M., Norkko, A.: Population dynamics at the range margin: Implications of climate change on sublittoral blue mussels (Mytilus trossulus), Front. Mar. Sci., 6, 292, DOI: https://doi.org/10.3389/fmars.2019.00292, 2019.

Interactive comment on Biogeosciences Discuss., https://doi.org/10.5194/bg-2020-382, 2020.