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Discussions

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.

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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 3 years. Monitoring of sea-

water 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 emethods 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!

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 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

The reviewer makes an important point here. From the beginning of the experiment, pH and carbonate chemistry were monitored 3-4 x per week to observe the impacts of metabolism and calcification on experimental seawater conditions. Resultingly, water changes were initiated 2 x weekly at the beginning of the experiment as this was found to be sufficient to prevent significant deviations of more than 0.2 mmol kg-1 Ca2+ and 200 μmol kg-1 HCO3- in seawater chemistry due to biological activity. The depletion of HCO3- and Ca2+ due to calcification in both experiments was also partially compensated by the addition of phytoplankton food which was cultured in filtered Kiel fjord water at a salinity of 16 ([HCO3-] = 1883 μmol kg-1, [Ca2+] = 4.99 mmol kg-1). The frequency of water changes was increased to 3 x weekly towards the second half of the experiment because the requirement to add more phytoplankton food resulted in

increasing HCO3 concentrations in treatment aquaria by more than 200 μmol kg-1. Water changes in the calcium experiment were kept at a frequency of twice weekly as the combination of food addition and calcification did not cause deviations in Ca2+ concentrations by more than 0.2 mmol kg-1 across all treatments.

Tables 1 & 2 present mean values from each experimental treatment (4 x replicate aquaria) over the course of the experiment. Measurements were taken immediately before and after water changes to present the range of experimental water chemistries within treatments (This will be clarified in section 2.2 L148 and L163). In the HCO3- experiment (1600 animals), aquaria were equilibrated with ambient air at atmospheric pO2 and pCO2 equilibrium (L148) to ensure air saturation. In the Ca2+ experiment, 2-3 weekly monitoring of experimental seawater chemistry revealed minimal impacts of biological activity on pH (ie. pHNBS standard errors of less than 0.1 pH units), even though these 60 ml aquaria were not aerated during experiments. Oxygen was not measured, but changes in pH (resulting from CO2 or net acid excretion) was used as a proxy for the impacts of metabolism on seawater chemistry. Values for pCO2 in both experiments (table 1 & 2) revealed laboratory experiments were remarkably similar to field conditions across all 3 sites and salinities, and pH did not vary by more than 0.1 units between water changes across all treatments and experiments. Ammonia excretion was not quantified, however given a conservatively high ammonia excretion rate in Baltic mussels of 20 μg NH4 per gram dry weight hr-1 (Tedengren & Kautsky 1987), the more biomass dense HCO3- experiment (mean biomass 52 mg dry weight per litre) would have resulted in maximum ammonia concentration of 0.08 mg L-1 immediately prior to a water change after 3 days accumulation. This value of 0.08 mg L-1 is far below the LC50 value for juvenile Mytilus edulis of 0.39 mg NH4 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.

The rational behind the frequency of water changes and the monitoring of pH as a proxy for monitoring the impacts of respiration and calcification on seawater chemistry

and the monitoring of [Ca2+] and [HCO3-], will be clarified in methods section 2.2.

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 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 Ca2+ exp and 51.5 mg/L during the HCO3- exp, it does not seem comparable to me.

Author response

Mortality rates did exhibit patterns in relation to seawater chemistry. Mortality rates were highest at low pH/[HCO3-] and slightly higher at low salinities (6) compared to higher salinities of 11 and 16 (see attached Figure 1). Table S2 presents the standard deviation of mortality rates across all treatments in the experiment. Dead organisms were not replaced as this would introduce issues related to sizes and differential exposure times of organisms within treatments. Feeding regimes were chosen in such a way to ensure saturated feeding conditions in all treatments (>10 000 phytoplankton cells ml-1). To correct for larger biomasses in certain experimental treatments, feeding frequencies were increased to prevent energy intake from becoming limiting. Clearance rates in each aquaria were monitored (L172) every 2 weeks to ensure sufficient frequencies of feeding as biomass in aquaria increased (growth) or decreased (mortality). This information is presented (L168) but will be expanded for the sake of clarification.

Table S2 presents total biomass 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 HCO3- experiment to highlight that biomass and biomass per ml varied by a higher degree within the HCO3Âň- experiment that between both experiments. The mean values for each experiment (13.2 mg/L and 51.5 mg/L) are therefore within the same order of magnitude. As mentioned in our responses to the comments by Reviewer 1, measures were taken to ensure maximum comparability between both experiments (See author responses to reviewer 1). The header for Table S2 will be clarified to reflect exactly what these data represent.

Reviewer comment

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?

Author response

This is an interesting point raised by the reviewer. It is true that that calcification rates at a salinity of 6 in the HCO3- experiment are significantly lower than calcification rates at a salinity of 6 in the Ca2+ experiment even at comparable [Ca2+]. A reason for this may be that the animals in the HCO3- experiment were younger/smaller, and therefore more sensitive to adverse changes in seawater carbonate chemistry/pH. This has been suggested to be related to higher calcification rates relative to body mass in larval and juvenile mussels compared to adults (Thomsen et al., 2015). Older, larger juveniles in the Ca2+ experiment may therefore be more resilient to low salinity/pH/[Ca2+], which may also explain the lack of mortality in this experiment. The high genetic diversity observed in the sampled experimental population (Ahrenshoop) could also result in genetic differences between the cohorts which may explain differential tolerances to salinity 6 between both experiments (Stuckas et al., 2017).

Statistical analyses in the Ca2+ experiment do suggest similar impacts of low salinity (6) between both experiments however, as a significant interaction effect between salinity and [Ca2+] may result from lower calcification rates at a salinity of 6 compared to 11 and 16 (Table S6). The differences in calcification rates at a salinity of 6 between both experiments will be discussed in terms of mortality rates and potential genetic differences between cohorts in section 4.3 the revised manuscript.

Reviewer comment

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 that fitting one model to data from multiple experiments has its limitations. However, we argue, experiments were performed in such a way to maximise comparability. Calcification rates were comparable between both experiments at high salinities and [HCO3-]/[Ca2+] as well as in the field at Ahrenshoop and Usedom sites, suggesting that both experiments simulated natural environmental conditions equally well.

Fig. 3 depicts both experiments separately (black triangles and red dots), however to visualise both experiments more transparently, each salinity treatment (6, 11 and 16) will also be highlighted in Fig 3 (and Figs S4 and S5) in the revised manuscript.

Reviewer comment

5) It seems that you over-determined carbonate chemistry during the field survey by measuring pH, CT and AT. It is not clear to me if AT data showed (i.e. Fig. 7) are the ones measured or derived from pH and CT, maybe to clarify. Finally, how do computed AT and measured AT compare? This could be a nice way to identify DOC contribution

no?

Author response

AT, CT and pH were all determined from field samples, however only pH and CT were used to calculate other carbonate chemistry parameters due to the potential impacts of dissolved organic matter on AT, as the reviewer mentioned. AT data is not shown in Fig. 7, but rather the subsequent carbonate chemistry parameters calculated from field measurements of pH and CT. The reviewer makes an interesting point in comparing measured AT from field samples, and calculated AT from field CT and pH. However, the potential contribution of DOM towards AT is complex, as this organic alkalinity contribution (Aorg) is not a linear function of DOC, but rather dependent on various parameters such as pH. Previous studies found that Aorg ranged from 22–58 μmol kg-1, and developed the 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 AT and calculated AT would not help us to better understand the Aorg 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.

Figures

Figure 1: Total mortality rates from an initial abundance of 1600 animals per aquarium for each treatment (N = 4) over the course of the 70-day bicarbonate ion manipulation experiment. Linear fits are shown for each salinity (6, 11 and 16).

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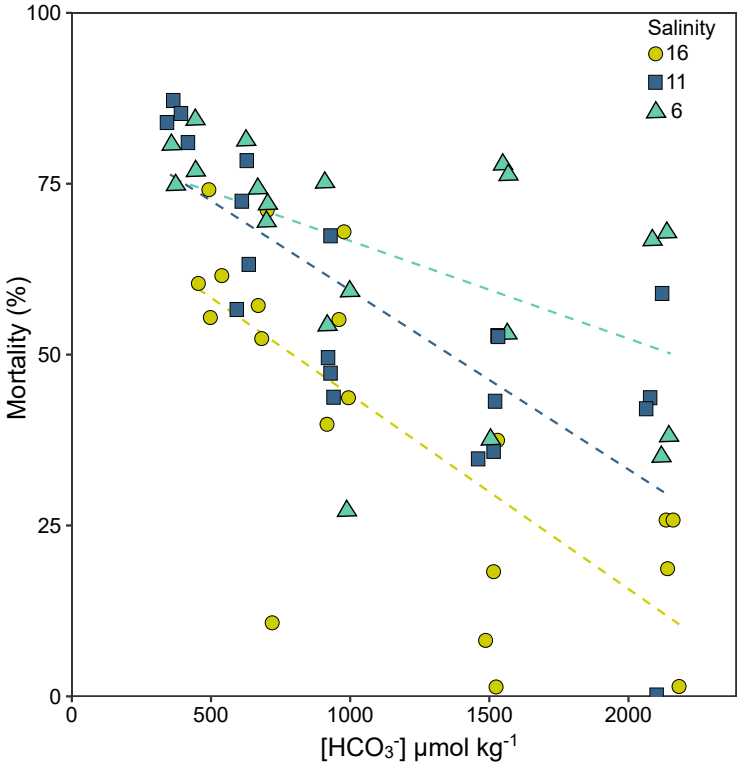
Salinity
16
11
6
Mortality (%)
100
75
50
25
0
0
500
1000
1500
2000
[HCO3-] µmol kg-1

Fig. 1.