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Discussions

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Interactive comment on "Controlled experimental aquarium system for multi-stressor investigation: carbonate chemistry, oxygen saturation, and temperature" *by* E. E. Bockmon et al.

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General Response to Referee Comments

We would like to thank the reviewers for their comments, and believe that we will be able to improve our paper significantly by attending to many of the concerns expressed.

Our manuscript described a (less than perfect) prototype system for ocean acidification experiments that enabled the oxygen and dissolved carbon dioxide concentrations in the experimental tanks to be varied independently from one another. As chemists (Bockmon & Dickson), we have focused much of the manuscript on the chemical characterization of the system while it is operating; however, the system has proved valu-

able for a variety of biological experiments (performed by co-authors: Frieder, Navarro & White-Kershek) and we have used measurements made during such experiments to better understand the factors affecting the performance of such a system while it is in use. The paper aims to describe what we have done during the period this system has been operating together with what we have learned from our trials, as well as indicating ways in which we would modify our approach in the future based on our experiences.

Many of the comments made by reviewers point out places where we have either not made ourselves as clear as we have hoped, or ask for further information. We would typically plan to address both aspects in any invited revision. Some of the reviewer comments address more general aspects of the experimental design of laboratory ocean acidification experiments and, where appropriate, we will work to ensure we address these more clearly in our discussion. Nevertheless we believe that a careful discussion of pseudo-replication in such experiments (a concern of at least one of the reviewers) is outside the scope of this manuscript, and shall modify our text to remove the offending term altogether.

Included with the following responses, comments from the referees are given in italics.

Responses to Individual Reviews

Referee 1

General comments

1: I am not sure whether the statement that the stability of the system presented here better for shorter than longer experiments is true. If so, possible reasons should be discussed. Nevertheless, the larger variability in the four weeks experiment seems to be to a large extent by poorer temperature control. Also, the deliberate change in oxygen (O2) and carbon dioxide (CO2) concentrations in the air used for equilibration adds to this. In this respect, I would be interested to know why the change from 1500 to 1600 ppmv is just seen as a small pH drop on the same day while the change in

oxygen is persistent from then on until the end of the experiment.

In any revision, we shall work to make clear the stability of the system over the observed time-scales, and will explicitly discuss our current understanding of the factors controlling stability in the system that is described.

2: Another interesting observation is the unexplained pH drop in one of the 'ambient' replicates during the four weeks experiment. Also, at the beginning both of these replicates do show a pH off-set for a couple of days. Apparently something went wrong and it would be helpful to speculate about potential candidates, e.g. change in water turnover time, imperfect/different equilibration at the membrane interface...

(see #1)

3: It seems that target CO2 and O2 levels in air (these values should be reported for all treatments in both experiments) differ from the actual levels reached in seawater. For instance, in the four weeks experiment seawater pCO2 at low pH was calculated to be about 1350 μatm, while the air had a mixing ratio of 1600 ppmv. Potential explanations, such as CO2 loss by out-gassing, or imprecise mixing by the mass-flow controllers should be discussed. Also, why do the 'ambient' treatments have higher pCO2 than one might have expected (again please report the mixing ratios of the air used for equilibration).

In a system such as that described here where kinetics plays a role in controlling the water concentrations, it is virtually certain that the concentrations achieved are not identical to those in the gases that are being introduced. We shall ensure that any revision makes this clear, and explicitly addresses the concerns noted here.

4: Finally, I would like to see some recommendations/discussions on 1) a suitable biomass to water volume ratio (e.g. in μmol organic C per L), avoiding significant biological impact on carbonate chemistry, and 2) on suitable combinations of seawater turn-over, replenishment and air-flow rates.

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We would hesitate to recommend a particular biomass to water volume ratio. The real concern is the rate at which the biomass is influencing the water composition (e.g., using oxygen and producing CO_2, etc.), and what rates can be handled effectively by the control system (including water replenishment). We will work in any revision to make this clearer.

Specific Comments

1: P.3432, L.5 Why should ocean warming and deoxygenation complicate the anthropogenic impact on organisms. What is probably meant is that two combined stressors complicate the interpretation.

We agree, and will clarify this.

2: P.3432, L.14 What is meant with 'control results'?

Will reword to make our meaning clear.

3: P.3433, L.13 Next to 'bubbling' there are also other methods to perfectly mimic ocean acidification, e.g. combined additions of acid and sodium-(bi)carbonate.

We were quoting a particular cited recommendation; however, the reviewer is correct that other approaches can adequately mimic the addition of CO_2 gas to seawater.

4: P.3435, L.13 If the boron/salinity relation is mentioned here, influencing the calculations of carbonate from total alkalinity, inorganic nutrients such as phosphate and silicate should be mentioned as well.

In this system where nutrient levels are relatively low (coastal seawater) and there is significant water exchange ensuring they stay that way, it is unnecessary to worry about the effects of such nutrient levels on alkalinity. We will try to make this clear.

5: P.3435, L.13 and below The discussion of controlling two parameters and assuming total alkalinity constant is a bit confusing. Regardless any changes in total alkalinity, target levels for pCO2 and O2 (ignoring small changes in solubility due to likely changes

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in salinity) are unaffected. For pH, of course, it matters.

Our intent was to point out that it is essential to control two CO_2-system parameters to achieve control of the CO_2-composition of the water in the tank. We will endeavor to make this clearer.

6: P.3436, L.10 Dissolved inorganic carbon is also not affected by changes in temperature and is conservative with respect to mixing.

Here we are justifying considering total alkalinity as one of our two "controlled variables". Controlling total dissolved inorganic carbon is not a practical approach (though it is also p and T independent and mixes conservatively).

7: P.3436, L.23 What is meant with 'x 50 fiber'?

This provides an identification of the exact type of Liqui-Cel used. We will use parentheses to make this clearer.

8: P.3437, L.20 'Quickly' is relative and depends on the biomass to seawater ratio.

Agreed. We shall reword to clarify this.

9: P.3438, L.4 'In addition...', I did not understand what was meant here.

We shall reword this to make our meaning clear.

10: P.3438, L.20 and below Explain better, as now it seems that the increase in atmospheric pCO2 would just have to be added on top of the natural fluctuations.

Agreed. Will do.

11: P.3439, L.5 and below Maybe a few more details on the total alkalinity and pH measurements employed could be given.

We shall add appropriate citations to the Dickson et al. (2007) guide, as we do follow those protocols.

12: P.3439, L.12 Why would an 'ambient' treatment be typical for a California coastal upwelling event? Under such conditions I would expect high CO2 in combination with low O2. I was also puzzled that the opposite combination of high CO2 and high O2 (low CO2 and low O2) was chosen in experiment S32. Which processes would lead to that unusual situation?

We shall clarify this language. However, we should point out that in experiments designed to identify the role of both CO_2 and O_2 changes there is no a priori reason to only study the high-CO_2/low-O_2 situation.

13: P.3439, L.25 Respiration decreases the pH. During night time, when I would expect the biggest effect of respiration on carbonate chemistry, pH, however, actually goes up.

We agree, but although the pH does decrease into the evening in Figure 4, it also increases again during the night. Insofar as this is not a system with significant photosynthesis it is not clear to us that the pH fluctuations should be light-dominated, rather temperature probably plays a larger role.

14: P3439 L28 What is meant with 'the influence of outside factors on the carbonate chemistry'?

We will rewrite this to identify the factors we are referring to.

15: P.3440, L.16 Having a quick look at Nam et al. 2011, it seems that they describe a typical high CO2 low O2 environment (see also comment #12).

See response to #12 above

16: P.3441, L.22 Remove space within μatm.

Yes!

Referee 2

Section 3434-01: The text correctly states that many OA experiments manipulate car-

bonate chemistry in a header tanks and then distribute the manipulated water to replicate tanks containing the study organisms. The authors then imply that their system will be more precise than this method because each tank in their system is individually manipulated using a Liqui-Cel membrane contactor. However, it does not necessarily follow that using a separate system on each tank will be more precise than treating the water once and then distributing to multiple tanks. In fact, it is easy to imagine that the use of multiple treatment units, each controlling a single tank, might be less precise because of small differences in flow rates of water or gases, or differences in biological activity within tanks. A comparative analysis within the same laboratory using the same techniques (ie. Liqui-Cel used to manipulate a header tank versus separate Liqui-Cels for each tank) would seem necessary to know whether this system is more precise, or not.

Our revised manuscript will more clearly discuss the benefits we perceive in our approach of controlling individual tanks as opposed to simply using a single controlled "header tank". We agree that we have not the data to support the assertion made here, though we feel it is, in principle, so.

Section 3438-02: (Shortened) While the proposed design has the potential to overcome one pseudoreplication problem (i.e. a single source of chemistry manipulation for each treatment), it has the potential to contribute further to the most significant pseudoreplication problem in OA studies –lack of independence of samples due to inadequate tank replication and rearing of many animals in the same tank . . . Obviously, the design could be improved by increasing the number of replicate tanks, as stated by the authors. However, given the costs of the Liqui-Cel units this may not be financially possible . . . A cost effective alternative would be to use the Liqui-Cel membrane contactors in a header-tank or pre-treatment system . . . The use of multiple tanks would greatly reduce the potential for tank-effects in one or two replicates to bias the results, it would increase statistical power if tank means were used, and it provides the option to have many tanks from which only one organisms is reared or sampled (the ideal

method to overcome sampling pseudoreplication). The authors may wish to consider discussing this alternative.

There is – we agree – a need for an informed discussion of the trade-offs involved in designing high-quality ocean acidification experiments. We feel, however, that we are not well-qualified to provide that discussion here. As noted above, we plan to remove all reference to "pseudo-replication" in any revised manuscript, discussing instead why we feel that individual tank control is desirable for effective chemical control. Also, we should like to point out that if the chemistry is not controlled adequately in such experiments, one has not saved money; one has wasted it.

Section 3438-05: The text suggests that the proposed system is superior to a header tank system because of the problem associated with inference of carbonate chemistry based on measurements made in header tanks. I would argue that all studies, whether header-tank or other designs, should measure and report carbonate chemistry at the tank level. After all, that is what the organisms experience. The implication that the current system is superior because chemistry is controlled and reported at the tank level is misplaced in assuming that studies using header tanks only report the chemistry for the header tanks and not the replicate rearing tanks. What we really need to do is insist that studies report the chemistry at the tank level, regardless of the method used to manipulate the chemistry.

We agree whole-heartedly with the comment that whatever the experimental design it is essential to measure the carbonate chemistry directly where the organisms are living (at the tank level in such experiments) and will make that point more clearly.

Referee 3

As we point out in our introduction above, the emphasis of this manuscript has been on describing our system, characterizing its behavior, and sharing what we have learned as a result about the factors that influence our system (and almost certainly others). We will work to minimize unnecessary speculation in our revision.

As we mention in our response to referee 2 we perceive benefits in controlling the various tanks individually. Essentially the composition of an individual tank at a particular time will be reflect the source water to that tank and its rate of replacement in the tank, the rate of gas exchange from that tank (which will be affected by the stirring in the tank even if other factors are equal), and the rate of biological processes in that particular tank. In the header-tank approach only the source water is controlled; thus if the other factors are significant and vary from tank to tank there will be variability in CO_2 composition from tank to tank, and perhaps with time.

Yes, we do believe that it will be harder to perform well-controlled experiments without a constant supply of seawater. We will make clearer why we feel this is so.

Specific Comments

l. 177 (3437-20): can you present data for a closed system set up, what would a suitable volume/biomass ratio be?

No, we have no data for a "closed system"; as we have been blessed with a constant supply of seawater we saw no need to. Furthermore we would hesitate to recommend a volume / biomass ratio for the same reasons articulated in response 4 to referee #1.

l. 193 (3438-06): this also infers that per replicate, an individual membrane contactor is needed. Given the price of these cartridges, a multi-replicate set up would render quite costly?

Yes, Liqui-Cel cartridges are not cheap. However, they are very effective and we believe that the benefits outweigh the costs in the context of these experiments.

l. 197 (3438-10): do the 1500µatm refer to the system described here or is this a general comment?

The value of 1500 μatm refers to experiments conducted in the system described here.

ll. 201/211(3438-14/24): shorten paragraph, this is not particularly relevant here.

We disagree. This text highlights the relevance of the ability to control the two gases independently. We will assess if we can make the point more usefully elsewhere in the manuscript.

l. 230 (3439-11): please correct spelling: M. galloprovincalis

Spelling corrected.

l. 237 (3439-19): please provide more information: how many larvae per bucket, how many buckets (replicates?)? Insofar as the intent of this manuscript is to describe the experimental aquarium system we have limited our discussion of the biological details of the example experiments.

We shall consider whether more detail is relevant.

l. 243 (3439-25): if a respiration signal is seen in the pH of the water, doesn't this indicate too little oxygenation/water turnover? Should a system like the one described here not aim to exclude exactly this?

Agreed. As noted in our introduction above, the system described is imperfect. We will note this explicitly, and in our discussion indicate the way we plan to deal with the problems we have identified.

ll. 244-257 (3439-25/3440-10): since your system does not seem to include such a feedback system to control water parameters, this paragraph is rather speculative and cannot be backed up with respective data. I would suggest to shorten or delete it.

As mentioned above, such comments are indicative of our thinking as to how to deal with identified problems. Although not backed up by data here, we are confident of this approach and feel that readers of the manuscript will benefit from a discussion of the potential benefits of active feedback.

l. 270 (3440-23): As you have mentioned the issue of pseudoreplication, please provide some more information here: what does 'discrete samples' really mean? How

many discrete samples, what kind of replication does this include, how many tanks and membrane contactors were involved in the experiment?

As noted above in our introduction, and in a response to referee #2, we plan to remove any reference to the contentious discussion of "pseudo-replication", instead concentrating on the benefits of controlling the tanks individually and monitoring the composition of these tanks directly.

l. 275-303 (3440-27/3441-27): this is a discussion of why the system is not as stable as it was initially described. This is a bit unfortunate, as even room temperature seemed to influence the systems' stability. The data presented here leave the impression of preliminary trial or troubleshooting experiments and does not go down well to support the concept of this set up.

As noted, this system is imperfect. Yet, it has provided adequate stability for the studies described. The goal of this manuscript is to describe the system and characterize its performance. As we have a lot of performance data, the problems are as clear as the successes. We try to discuss these honestly and critically.

ll. 306-315 (3442-02/11): this is pretty speculative as it's not backed up by data, I would recommend to shorten this paragraph.

We agree that the discussion of an expanded system foreshadows future developments we are working on rather than being solely a recapitulation of the simple prototype described here. However, we feel that this is a relevant part of our discussion.

ll. 316-329 (3442-12/25): I would suggest to shorten this paragraph as well, as it is in part redundant (cf. introduction).

We disagree that the small amount of repetition here is problematic. However, we will consider if we can improve this section by rewriting it in part.

ll. 330-337 (3442-26/3443-05): please cite the references for the experiments you refer to in this paragraph.

At present these experiments are not available in the primary literature. We will, however cite the appropriate student theses describing the more complete experiments. Other studies were exploratory in nature and have not yet been followed up.

l. 456 (3447-Fig 1): please explain the n=8 stated here. How was the setup of 4 membrane contactors (2 replicates per treatment?) used to create an n=8? Did you use a total of 16 contactors to avoid pseudoreplication (cf. l. 190)?

The referee has misunderstood. The n=8 refers to the number of separate samples of water taken from the tank for chemical analysis, and thus helps indicate the likely significance of the mean and standard deviation reported.