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Discussions

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

Anonymous Referee #2

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This paper describes a novel and effective system for the manipulation of multiple physical parameters in ocean acidification experiments. The ms does a very good job of describing the system and it well supported by data from two separate trials. The system clearly has great potential for use in ocean acidification research, whether just as an effective means to manipulate carbonate chemistry alone, or in multi-factor experiments that also manipulate oxygen saturation and temperature. This is an important methodological development that has the potential to significantly advance the field.

The chemistry component of the paper is very clear, as one might expect from this team of authors. The explanation of the apparatus and experimental design is also

very good. I do, however, have some comments about the proposed benefits of the system described in the text, and the comparison with other methods of manipulating carbonate chemistry, especially in relation to the issue of pseudoreplication. I think the paper could be improved with some more consideration of the types of pseudoreplication that are of primary concern in ocean acidification experiments and what this system can contribute to addressing this issue.

Comments

Section 3434, line 1. The text correctly states that many OA experiments manipulate carbonate 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.

Section 3438, line 2. The text states that the proposed system is superior to other designs because it "eliminates many of the pseudoreplication problems that continue to plague other ocean acidification aquarium experimental designs". 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. The proposed design, with just two replicate tanks, presumably each

containing multiple animals that will be sampled, is an example of the primary type of pseudoreplication that needs to be addressed in OA research. While the authors clearly state that there is potential to increase tank replication, with a separate Liqui-Cel for each tank, there are obvious cost constraints to this approach. My point here is simply that it seems inappropriate to claim that the current design will significantly reduce the problem of pseudoreplication in OA research when cost constraints of this design might tend to inflate the problem of inadequate tank replication.

The problem of sampling pseudoreplication is sometimes overcome by using tank means for the biological traits of interest; however this would leave just two replicate means and very low experimental power with the current design. 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 for many research groups. Furthermore, the number of units required would multiply rapidly in a multifactorial experiment that manipulated several gases (e.g. CO2 and O2) at multiple treatment levels (e.g. 3 levels each). The expense of such a system could quickly become prohibitive. A cost effective alternative would be to use the Liqui-Cel membrane contactors in a header-tank or pre-treatment system. With such a design only one unit (or two units to overcome pseudoreplication at the CO2 manipulation level) would be needed for each treatment level and the pre-treated water could then be delivered to many replicate tanks. 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.

Section 3438, line 5. 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.

These are all relatively minor issues that can easily be addressed with some simple rewording or revision. Nevertheless, they are important to avoid overstating the benefits of the system to experimental design.

Interactive comment on Biogeosciences Discuss., 10, 3431, 2013.