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Interactive comment on "Technical Note: Artificial coral reef mesocosms for ocean acidification investigations" *by* J. Leblud et al.

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Interactive comment on "Technical Note: Artificial coral reef mesocosms for ocean acidification investigations" by J. Leblud et al.

Anonymous Referee 1 Received and published: 6 December 2014

First I want to point out that the technician of our team (who has a good experience in coral husbandry and mesocosm design) and myself performed this review together. This technical note describes methods used to maintain artificial coral reef communities in aquarium during several years. The particularity of this method is that it was performed in a closed system (i.e., with reduced water changes) and that part of the control of the chemistry was mediated trough

biological control. The manuscript is generally well written and presents an interesting scientific approach to reef tank husbandry. Nevertheless, the methods presented here are maybe not highly novel and the authors' view of established equilibrium is only a relative term. The authors obviously know how to take care of a reef tank and are showing it with good data that backs up the idea that it is possible to replicate coral reefs away from the ocean. However, the authors tend to make a leap by saying that the tank is its own established ecosystem whose nutrients are perfectly cycled and doesn't need any form of natural seawater input (food addition, water changes, filtering, etc. are similar to seawater inputs). I have included below a list of specific comments.

Thanks for this detailed review and for your pertinent remarks. Two comments about (1) the fact that it is not highly novel, and (2) the relativity of the equilibrium.

(1) We do not claim novelty in the separate techniques used to manage the ecosystem, which are clearly well-known in aquariology. We do not claim as novelty to keep hermatypic corals, or an artificial coral reef community in a closed system. But we claim novelty, or more exactly originality, in the demonstration that it is possible to match reasonably well, in a closed system, fluctuation of the major physico-chemical parameters that occur in a natural coral reef. It includes temperature, pH, pCO2, pO2, inorganic nitrogen and orthophosphates. It includes daily variations of these parameters. We stress also that we obtained such a result in a setup that we document carefully enough to allow replicating it by colleagues as a starting point for OA experiments in so-called minicosms. To us, this is worth a technical note in biogeosciences. The manuscript is edited to make that clearer, especially in the discussion.

(2) Regarding the equilibrium, equilibrium of nutrients, their recycling is a key aspect. As one can see in Table 1 water change has no impact on the orthophosphates concentration since it is about the same in the fresh seawater (0.5μM) and in the mesocosms. Yet, we succeed in stabilizing them at sub-micromolar concentrations over several years. We edited the manuscript and added a table (Table 1) that quantify

inputs of N and P to make this more clear. The internal recycling of N and P in the minicosms is indeed a key aspect. So, such clarifications were required.

Please see the text updates in the attached pdf file.

Specific comments:

Introduction - The introduction is rather long and the tone is overall negative for the other studies (i.e. everything was bad before), maybe the authors should think about revising it.

Both the introduction and the negative tone were reduced.

-p15466: l-15-18: I don't think that high flow rate was the reason for the use of HCl. Bubbling pure CO2 is very efficient to decrease pH and can be used in open flow system.

The authors indeed didn't mentioned this information. It was removed from the text.

Design: - p15468: l-20-25: I don't really understand what is the goal of the "main tank". It will be very helpful to explain the role plaid by this tank and why it is important to have a separate "main tank" and a "sump".

The text was changed to provide clearly this information at p5:l-132-148. In short, the community can be adjusted in the main tank, even when experiments are run in the other units. The sump is a technical simplification that eases the management of the flow between the different units and provide space for technical devices.

- p15470: l-05-10: Could you indicate what was the maximal PAR value in the tanks?

Maximum PAR was 450+/-30 μmol.m-2.s-1. It was added to the text at p6:l-170.

- p15470: l-05-10:These bulbs are known to change spectrum after 4-6 months of use. Did you switch them out?

Yes indeed, T5 bulbs were replaced every 6 months. It was added to the text at p6:l-171. It should be noted that each bulb operates 8h per day thanks to the progressive dawn and dusk produced by lighting on and off these bulbs in 3 groups (see. Fig. 3). Lifespan is thus larger than lighting operated 12h per day for each bulb.

- p15470: l-20: Which aquarium were equipped with the speed Tunze, the main tanks or the experimental tanks?

All aquaria are equipped with Tunze pumps. It was clarified in the text at p6:l-179.

-p15471: Why did you choose to couple a refugium for each experimental tank rather than using a larger one on the sump/main tank? It seems that the use of one main refugium would have limited the differences between experimental tanks.

The refugia were connected to each experimental tank because the flow rate between main tank and the experimental tanks was too low to allow a correct O2 dial cycle. Buffering of pO2 required slightly higher flow rate than the one we can reasonably obtain for keeping the pH at its desired level in the highest pH condition. Also, it helps in the fine-tuning of the system to have both flow rates independent from each other. Hence, the selected design with one separate refugium for each experimental aquarium was chosen; it allows to decouple flow rates for pH/pCO2 (main tank to experimental aquaria) and for pO2 (experimental aquaria to refugia).

-p15472: l-13-16: This is a long statement between parenthesis, reformulate.

The sentence was reformulated at page p8:l-246-249.

-p15472: l-21-24: What was the average quantity of zooplankton added?

The average quantity provided was equal to 0.6$\pm$0.1 g.day-1 dry weight in each main tank. This information was added at p9:l-252.

-p15473: l-10-13: How did you control the bacterial community?

The objective of this experiment was not to control the bacterial community. The substrate, coral colonies and the other living organisms were acclimatized together during 4 months before being randomly distributed in each experimental tank at the beginning of the experiment (4 months were probably not required here, but correspond to the time we needed to get ready). Then each experimental tank and its simplified ecosystem had the opportunity to change on its own track. In order to follow these possible changes, bacterial community analyses were performed every 3 months using metagenomic techniques. It was performed both in the coral mucus and in the substrate. These results are not reported here because they are the subject of a separate, recently submitted, manuscript.

-p15472: l-20–p15473: l-13: This section as a lot of wordage for describing what people do all around the world to maintain aquariums. The water changes should also be mentioned here, as they are probably important to control the nutrient levels.

The text was synthesized at p9:l 252-263 and water change was added to the list. However, we stress here that water change is not controlling the nutrients levels: nutrients concentrations in freshly prepared seawater are indeed equal to 4.02 $\pm$ 0.20 μmol.L-1 N and 0.46 $\pm$ 0.10 μmol.L-1 P. These concentrations are slightly higher to the ones in the mesocosms (always less than 2 μmol.L-1 N, and most of the time less than 0.4 μmol.L-1 P). Water change is thus not controlling nutrient levels. Amount of N and P added by the food is roughly 2000x higher than N and P brought by water changes (a table was added that indicates average daily inputs of N and P by these means, see Table 1 in the new version of the manuscript). Also, such inputs are very limited (only fish in the main tank are fed) and in the other hand, the system contains a lot of photoautotrophs (zooxanthellae, turf algae, seaweeds) that transform inorganic N and P and an established community of decomposers and heterotrophic bacteria, fungi and archaea, that decompose organic matter, which lead us to consider that nutrients are efficiently recycled in the minicosm. This recycling is achieved in a steady way, given

the stability of N and P concentration measured in the water.

-Carbonate system: I have a problem with this section. If the goal is to let the biology impact the diel cycle of pCO2 you should not manipulate the water in the experimental tank with a pH stat (that is designed to maintain the pH constant), but rather manipulate the pCO2 of the seawater before it enters the experimental tanks.

The section was reworked at p7:l-195-197: “These daily variations are mainly driven by biological activities, such as oxygen. The aim here is to simulate these natural fluctuations while maintaining a pH shift between the two experimental aquaria for the purpose of OA experiments. Therefore, CO2 bubbling into the inflow water of the high pCO2 aquarium was used.”

- Why did you use calcium hydroxide to increase pH rather than CO2-free air? Calcium hydroxide is known to spike TA locally, which can impact the biology.

When working with fast growing coral species in closed systems, we also have to adjust the alkalinity because calcification decreases it rapidly. Ca(OH)2 additions appear to us as a good way to address both with limited technical devices. Moreover, Ca(OH)2 was added drop by drop in each experimental tank near the Tunze pumps, allowing for a fast and efficient mixing. This prevented any possible local spike of alkalinity. That said, using CO2-free air would work too.

-p15476: l-01: Please indicate the quality of your measurements (xx $\pm$SD umol from the CRM).

The information was added to the text at p10:l-300-303 : “Calibration was performed using certified reference seawater provided by A. G. Dickson (Scripps Institute of Oceanography, batches 94 120; difference between measurements and CRM before correction is lower than 24 μmol.kg-1). Repeatability of alkalinity measurements was $\pm$ 6 μmol.kg-1“

-p15476: l-24: This sentence is not clear, please reformulate.

The sentence was reformulated at p11:l-324-327: “Oxygen was monitored every two months by using Clark electrodes connected to the IKS system. At the end of the experiment, a more detailed analysis of oxygen fluxes was performed performed by recording the data continuously over a five-day period using oxygen probes in each experimental aquarium, in the sumps and in the main tanks.”

-p15479:l-8: "although it is hard...", hard in your case or in general? It is actually quit easy with a pH-stat...

In our experimental design, the pH daily variations were mainly driven by biological activities. As explained at p7l-202-205 : “Our systems do not act as pH-stats, but are only used to maintain a difference in the average pH (thus constraining the lowest value in the control and the highest level in the acidified aquaria respectively). Much of the daily fluctuation is due to biological activity in the experimental aquaria and in the refugia.”The amplitude of daily variations decreased with time in acidified conditions which is in fact a result of the pH treatment, as explained p13:l356-357. In the context of the new version of the manuscript, the sentence you mention was not pertinent and was removed.

-p15479:l-13: Why didn’t you use the pH-stat to recreate lower pCO2 at night since you were artificially manipulating the pCO2 in any case?

At the beginning of the experiment, dial variations were comparable between experimental tanks and also comparable to field conditions. As explained in the previous comment, once contrasted pH were applied, the simplified ecosystem of each experimental tank had the possibility to change and thus had a different effects on the pH amplitude of its environment.

-p15479:l-120-25: Any ideas on why the pH increased? More turf algae?

It is just a slight drift that can be significantly detected by statistical analysis, due to

very small differences in the properties of the pH probes (the pH probes were changed once during the experiment, and are thus different ones at the beginning and at the end).

-p15480: l-06: The variations in TA in the tanks were relatively important (SD> 100 umol), you should include explicitly this information in the results.

The total alkalinity was indeed fluctuating during the experiment: it is mainly due to the large calcification rates combined with the small volume of the system, leading to more difficult stabilization of this parameter. The observed variations were nevertheless always the same between control and treatment aquaria. This information was added to the results at p13:l-391-393. This is also treated in the discussion.

-p15480: l-13-16: This is not a result.

We agree, this part was removed from the results and placed in the perspectives at p.17:l-528.

-p15481: l-13-22: Could the difference between OA and ambient be due to higher photosynthesis and respiration in the OA refugia?

Yes indeed. As explained in the discussion p16:l-477-480, a change in the photosynthesis and respiration in the OA refugia may decrease the pO2 fluctuations.

-p15482: l-10-15: I think that this statement is overstated: your mesocosms are equipped with pH-stats, skimmers, CaCO3 reactors, Ca(OH)2 reactors, and you also make water changes, add zooplankton, and remove manually some organisms. In that case it is hard to claim that organisms are controlling the chemistry in your aquariums.

When working with closed systems, we have to simulate the inputs and outputs of nutrient from the adjacent ecosystems. The approach is now explained and detailed in Fig. 4. We also have to supply for calcium and carbonates, intensively used by calcifiers. Regarding the pH, as explained at p7:l-200-203, “Our systems do not act as pH-stats,

but are only used to maintain a difference in the average pH (thus constraining the lowest value in the control and the highest level in the acidified aquaria respectively). Much of the daily fluctuation is due to biological activity in the experimental aquaria and in the refugia."

-p15483: l-1-5: I would really like to see some discussion on the importance of water changes to control nutrients, etc. It seems that this part has been omitted in the manuscript.

Water change was added to the discussion at p14:l-440-441. In our test case the water change was probably important to supply micronutrients to the system, but not to remove any excess of N or P. The macronutrient concentrations of freshly prepared artificial are indeed slightly higher than in the minicosm. These concentrations were added in Table 1 to make this clearer.

-p15483: l-12: Predation keeps the biomass in the system. In your case you remove it from the system.

We don't understand the comment here. The sentence l-12 is "This simulates predation and recruitment that lack for obvious reasons in such artificial mesocosms." So, yes, there is no predation in the system, and it is simulated by manual elimination of some organisms.

- Generally the authors claim that the system is "equilibrated". I am not sure this is the correct term, as the system requires water changes, addition of zooplankton, and different types of reactor and filtering. It is maybe "artificially stable" but not "equilibrated".

Natural ecosystems are open systems, connected to other adjacent systems. Inputs and output between these different ecosystems are part of the way this work in the field. This is a dynamic equilibrium in an essentially open system. Here, we deal with a closed system that, by definition, do not allow importation and exportation from and

to other systems. So, these exchanges on the reef are replaced by the technique (see Fig. 4). The establishment of the equilibrium comes from the fact that macronutrients are mainly controlled by living organisms and not by water change, as explained at p9:l-256-261, in a presence of steady inputs and outputs that are, indeed, artificially simulated. We see no other solutions for closed systems. Still we claim for some sort of natural equilibration through the community living in the minicosms.

-p15483: l-21-30: This part is highly subjective...

This part was reworked in order to be less subjective and placed at the beginning of the discussion at p14:l-430-436: "Here minicosms are defined as intermediary systems between laboratory-based microcosms and in situ mesocosms. Particular attention must be paid to the simulation of realistic physico-chemical parameters of the environment to differentiate them from microcosms. Even more realism could be achieved mainly through the community of living organisms itself. Artificial filtration techniques cannot be avoided completely, but here they were limited as much as possible. Whether a system meets this goal or not could, and should, be assessed by comparison to a reference site in the field, including day and night fluctuations."

-p15485: l-09 and l-11: I don't think that the use of "..." is recommended.

Thanks, it was removed from the text.

-p15486: l-14-19: Was this difference in [Ca] taken into account in the calculation of the saturation states?

Yes it was. Seacarb package (Gattuso and Lavigne, 2014) allows these computations with given Ca2+ concentrations.

- p15486: l-20: See also Jury et al. 2013 (5, 1303-1325; doi:10.3390/w5031303 Water).

Thanks, Jury et al. 2013 was added at p15:l463.

-p15487:l15 - p15488:l-10: This section is very confused and should be revised.

We agree. All the discussion was intensively reworked to be clearer.

Please also note the supplement to this comment:
http://www.biogeosciences-discuss.net/11/C8860/2015/bgd-11-C8860-2015-supplement.pdf

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