

Interactive comment on “Measuring gross and net calcification of a reef coral under ocean acidification conditions: methodological considerations” by S. Cohen and M. Fine

Anonymous Referee #1

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On a first view the paper promises an interesting, complex experimental design and question addressed. Authors aimed at investigating the "role of coral tissue in determining the vulnerability of the reef-building coral *Stylophora pistillata*" by comparing net calcification of intact corals with dissolution of bare skeleton and net calcification and gross calcification of intact corals under long term exposure to reduced pH.

Unfortunately, the manuscript is very confusing and experiments and analyses were not carried out in a consistent enough way to actually address the questions of tissue function and response or acclimation to OA:

The incubation for TA without prior exposure time to reduced pH was carried out in

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an open system while the other experiments were carried out in a closed system, and the "reduced" initial pH-level was 7.19 in the open system (with an $\omega < 1$), while in the closed system it was 7.49 ($\omega > 1$). The closed system approach resulted in a drastic change of the carbonate chemistry (as shown in Table S3). Specifically the pCO₂-level should have been maintained constant during incubation (f.ex. by bubbling). These drastic changes led to rather uncontrolled conditions with respect to the carbonate chemistry and render any interpretation with respect to OA greatly ambiguous. Here definitely an open system with pCO₂ bubbling would have been required.

Independent of this major flaw using a closed system approach, the manuscript is confusing as it is hard to follow with respect of experimental design (net, gross calcification, light/dark incubations etc, dissolution of bare skeleton and which reduced pH level 7.19 or 7.49, and open and closed system).

Other comments:

p. 8243, line 18: "true" calcification should be deleted - net and gross calcification can clearly be defined and what in the end would be the "true" calcification is a matter of debate as one may also state, that only the calcium accreted after a certain time is the "true" calcification and this would be "net".

p. 8244, line 18 ... "only the oral ectoderm is in direct contact with seawater" I doubt this is true, seawater can access via the mouth and coelenteric to the endodermal tissue. Then there is some believe that the calciblastic layer might be replenished (from time to time) with seawater... this does still not contradict, that the tissue layer form a barrier where chemical gradients can be obtained between calciblast and ambient seawater.

p. 8247 chapter 2.2.4 – this is unclear. authors should be more specific – what time of day and was there light and dark incubations carried out – may be Table 3 can be complemented with respect to the repeated measurements being carried out with a same specimens

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p. 8248, - line 11ff – NBS scale is “out-dated” and total scale should have been measured - line 25 instead of “numerous times” authors should give number or time interval for measuring

Results 3.1:

as pointed out in my major concern, the comparison of net calcification between non-exposed and exposed to reduced pH prior to incubation is not valid because two systems (open-closed and two different pH levels were used).

Why the carbonate chemistry for dark incubations is not given? This is important, and if data are available, this could “save” the MS and help for interpretation of results

Results 3.2

line 17 what is meant by 1:1 regression slope – a linear regression ?

Discussion 4.1.

this discusses the obvious and is not related to author’s findings as they state themselves in the last sentence

Discussion 4.3.1

- The first paragraph could be more specific/quantitative and related to the results the second paragraph discusses the major problem of using a closed system approach, the last sentence is superfluous – it is not that more intercomparison studies are needed, but that they are carried out with the appropriate approach and analyses.

Authors discuss at length some mechanistic explanations why results are variable, however, they do not discuss, that interestingly, the TA method appears to provide consistently higher calcification results than the ^{45}Ca (although not significant) and what this means with respect to determination of “net” and “gross” calcification – f.ex. the TA could have been influenced by inorganic nutrient uptake during photosynthesis (I guess nutrients were not determined from incubations). Adversely, if dissolution takes

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place, newly incorporated ^{45}Ca would also dissolve again, meaning, that the ^{45}Ca method does not measure gross calcification either, but - at most - has less dissolution effects (as also older skeletal parts prior to labelling dissolve)

The conclusion with respect to “acclimation” is not valid with the experimental results and flaws pointed out above.

At least at this stage, I cannot recommend publication. The paper would need some major “straightening out” and writing more “to the point”, but then still the problem of the closed system approach with the immense change in carbonate chemistry during incubations persists versus the open system of “non-acclimatized” corals and a lower reduced pH-level (omega <1) than in the closed “acclimatized” treatments (omega >1).

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