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Interactive comment on “Effect of carbonate chemistry manipulations on calcification, respiration, and excretion of a Mediterranean pteropod” by S. Comeau et al.

Anonymous Referee #2

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General comments

Apologize for posting this comment so late. I went on fieldwork (and still am in the field) just after the manuscript was finally accepted for publication in BGD.

This manuscript aims at an improved understanding on how changes in the carbonate chemistry associated with ocean acidification affect respiration, excretion and calcification rates of the Mediterranean pteropod species *Creseis acicula*.

The goal the authors have is certainly meaningful and I acknowledge their attempts to improve our understanding on how pteropods might be affected by ocean acidification and on the underlying physiological mechanisms. However, I see this work for the

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second time now and my major concern is still according to the experimental design and the statistics used to analyze the data. I am sorry to say, but the present manuscript still casts severe doubts on the conclusions the authors have drawn from their results.

In this work, two different experiments were set up and at the end the data were collectively analyzed using linear and non-linear regression. CO₂ bubbling was applied to decrease the pH in both experiments, but in the second one also alkalinity was decreased by acidification with HCl. This must have resulted in a range of different bicarbonate and carbonate ion concentrations (that are unfortunately not presented any longer in the present manuscript) which makes it impossible to disentangle the independent use of one or the other ion species.

Concerning the statistical analyses of the data set, the fundamental problem is collinearity between the independent variables (i.e. pH, HCO₃⁻, CO₃²⁻, Ω). As carbonate ion speciation and the resulting Ω depend on pH and AT they are per se highly collinear which precludes the possibility to sort out which one of these parameters truly is responsible for the observed physiological responses. Moreover, all triplicate measurements must be regarded as pseudoreplicates as they were all taken from the same experimental sub-unit, which weakens the significance of the data set even more. Fig. 2 a, c, and e, shows an off-set in the rate measurements of respiration, excretion and calcification between the two different experimental set-ups (with high and low AT) which is probably only due to the different alkalinity, i.e. the different amount of substrate carbonate ion species available to the pteropods. If the regressions were done on the two alkalinity treatments separately (i.e. regression on the low and high alkalinity treatment, respectively), the authors would find better regressions for all sub-data sets that would show that calcification rather depends on the pH but not on the carbonate ion concentrations, i.e. Ω a (or maybe a combined effect of substrate, Ω , and pH). To distinguish the effects of individual carbonate system parameters on calcification and other physiological parameters, one should conduct two different experiments, one with constant alkalinity and one with constant pH. Moreover, considering the fact

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that pteropods have actually been described to use bicarbonate for shell formation (Lalli and Gilmer 1989), the evidence given here to support the authors main conclusion that respiration, excretion and calcification rates of *C. acicula* depends on Ω_a (i.e. carbonate ion concentration) and not pH seems not convincing to me.

I am aware that pteropods are not easy to work with and low abundances often preclude an extensive experimental design including a variety of response variables. Confining the experiment to only one response variable (e.g. calcification) would have maybe allowed for a more adequate experimental design with true replicates to sort out which carbonate species is preferentially used for calcification and how calcification is affected by ocean acidification.

Overall, this manuscript suffers from an inadequate experimental design and inappropriate data/statistical analysis that led the authors to conclusions that are not supported by the data. As there is probably no chance to significantly improve the available data record, I cannot recommend this manuscript for publication in Biogeosciences.

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