

## ***Interactive comment on “Ocean acidification increases the sensitivity and variability of physiological responses of an intertidal limpet to thermal stress” by Jie Wang et al.***

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Received and published: 10 May 2017

The Wang et al ms is an interesting study of the impact of warming and acidification on physiological responses. The main significant effect was seen with the molecular biology – the hsp response. Some analyses of the other parameters measured (eg. heart rate) were equivocal. I suggest reduce the emphasis on the latter and concentrate on the hsp data. Reduce the text on non significant results. I have questions on methods that need to be addressed before a full picture of the outcomes of the work can be assessed.

Introduction

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L. 42-45 – Not quite correct there are many studies that show that moderate increase in temperature – within projections – reduces/ameliorates the negative effect of acidification.

At the end of the introduction more context is needed about the region, species and approaches used. Some of this is in the first section of the methods and can be moved here. Also provide some predictions/hypotheses at the end of the introduction. How would you expect the limpets to respond with respect to hsp, heart rate, ABT etc.

## Methods

Is 7 days a sufficient “acclimation” time – why was this selected. It seems that the limpets were placed directly in treatment – is this a shock? I do not think that with a 7 day experiment much can be said about post-acclimation, (eg. discussion) some justification is needed for this – perhaps there are other studies that have determined this for other limpets.

The sample  $n=100$  per acclimation treatment that is a big sample size, so how many in total  $\sim 400$ ? How many containers were the limpets in? To use as independent data each limpet would have to be housed in several containers. What was the density of the limpets in each container? These animals have distinct density dependent behaviour – shown in many studies and this may influence outcome. It is not clear to me what was done with the 100's of limpets when only  $\sim 10$  were used for the experimental measures – perhaps I am missing something?

Show a photo of the artificial rock.

How were the  $n=10$ ,  $n=9-11$  limpets selected for hsp and heart rate respectively. Were the latter in separate containers during this measurement?

Use of CV is not mentioned in the stats section – also state why used.

## Results

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Just provide stats for significant results, so give the ANOVA results for the heart rate and post hoc for the heart rate but not the ABTs. For the latter just give mean and SE, say non significant and cite stats table. Same for the next paragraph.

Fig 2 – why are there no error bars on the post data – best to state why in the legend. Interesting that the hsp data was significant with just n=10 per treatment. Usually n=20 is the minimum.

### Discussion

Paragraph 1 can be reduced – some of this is introduction type text Only speak to the significant results and make this clear. State that higher thermal sensitivity to . . . was indicated by increased heart rate.

It will be good to state what the CVs actually indicate. Overall perhaps for some measures the sample size was too low.

The hsp text could be expanded with regard to the species and methods comparisons. For instance a lot of the work by Tomanek and colleagues involves other intertidal molluscs and on different heights on the shore etc. Are there any other studies of limpets etc.

For the hsp – the sample size may have been too low to discern between constitutive and induced expression.

What studies have used gene expression –vs- protein expression. This might influence the comparisons being made. Just because the gene is expressed we really do not know if the protein is also expressed.

General comments –

L. 21 state 7 days

For a short results section – 6 pages of references seems excessive –

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L. 35 Scheffers et al could be deleted

L 46-49 – This is a general sentence – one ref will suffice

L. 96-97 can delete much of this detail (eg falling high tide)

L. 367 – this is a discussion paper – not fully peer review - delete

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Interactive comment on Biogeosciences Discuss., doi:10.5194/bg-2017-47, 2017.

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