

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.

Anonymous Referee #2

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General comments. The manuscript is interesting because it evaluates the physiological effects caused by rising temperatures in limpets acclimated under conditions that combined ocean acidification and temperature rising. The methods, most of all, were well explained, facilitating the understanding of the experiments. However, the limpets were acclimated for a short period of time (7 days) and submitted to different heat shock treatments for a maximum period of 7 h, only once during the whole experiment. No evidence of actual acclimation of these animals was presented (methods for assessing acclimation are discussed by Peck et al. in *J. Exp. Biology* (2014) 217, 16-22, doi: 10.1242 / jeb.089946). Therefore, contrary to the authors' conclusion the results of these experiments allow predictions of future scenario in a very limited way. The

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authors argue about a large variability of the physiological response in the population based on the coefficient of variation of the analyzed parameters. However, this coefficient is derived from a standard deviation that will be reliable if obtained from large population samples, which was not the case (around 10 individuals per treatment). Therefore, this could weaken the argument about the physiological plasticity.

Specific comments Title - The authors obtained evidence that only hsp70 expression was affected in acclimated limpets under HTHC conditions. CO₂ level did not affect Q₁₀, and the highest temperature decreased Q₁₀. Therefore, the ocean acidification affected only hsp70. Then, the title does not specifically reflect the content.

The paragraph between lines 86 and 93 should be in the introduction. The determination of seawater characteristics (lines 112 - 122) should be in a separate item.

The authors should make it clear if the limpets were kept in a chamber with constant CO₂ concentration in the air during thermal shock.

On the line 267, the phrase "If only one environmental factor changed (i.e., temperature or CO₂) ..." is not sufficiently clear to me.

The discussion about why the expression of hsc70 was not affected by the treatments is insufficient. Why was this protein chosen to analysis? Is it sensitive to temperature rise in other species? Do other factors affect its expression? The discussion needs to be expanded. The conclusion and abstract must be rewritten because an incomplete acclimatization may have occurred and the experiment did not reproduce with reasonable fidelity a future scenario in which the limpets would be exposed to thermal shock.

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