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Biogeosciences Discussions

Interactive comment on "The subtle effects of sea water acidification on the amphipod *Gammarus locusta*" *by* C. Hauton et al.

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In this paper, Chris Hauton and colleagues have collected a really interesting set of data to test the robustness of a species experiencing naturally high variations of pH when facing future ocean acidification (OA). This is a very important hypothesis for future predictions of what species/ecosystems are the most at risk in future oceans and what species/ecosystems are the most able to adapt. *Gammarus locusta* experiencing high variation of pH in its environment, it is reasonable to think that individuals (through acclimation) and/or population (through intraspecific diversity and microevolution) have the ability to adapt and survive future changes.

However, we have some questions and suggestions concerning the experimental design and the interpretation of the results.

- A key point for the interpretation is the pH use as a control. To ease the interpretation, it would be very helpful to have the data about pH in the natural environment for the experimental organisms and its variation (through seasons, with depth, etc. in all the environment experiences by the species at all the stages of its development). (i) The variation is a key parameter. For example, we have shown for echinoderm larvae that the species experiencing larger natural variations are more likely to be able to cope with predicted future OA (Dupont et al., submitted). (ii) In the introduction, it is mentioned that the pH varies between 6.76 and 7.95 in the studied population (this is linked to the point above concerning the natural pH range experienced by these animals at all stages of their life cycle). If this is so then why was 8.1 used as a control? - the treatment at "7.8" being closer to the maximum natural pH. This could explain why the survival is optimal at pH 7.83 and the absence of difference in HSP70 expression. (iii) If the control is closer to 7.8 than 8.1, the lower pH (7.5) should then be interpreted as a decrease of 0.2-0.3 rather than 0.5. This may change the way of interpreting these data. In fact this makes the data even more interesting if it turns out that because these animals are naturally exposed to a range of pH stresses then they are better "adapted" to predicted levels of change? (iv) It would also be useful to know the origin of the seawater used in the cultures and the experiments, is it artificial or collected from the same site where the animals are found.

- Other important data that would help interpret these results is the alkalinity in the sea-water from the sampling site and the water used for the experiments. In calcification, pH may not be the more important parameter as long as the water remains oversaturated with respect to the calcium carbonate form relevant to a given species. For example, a strong effect is only observed in mussel larvae when slight undersaturation of aragonite is associated with low pH. When low pH is associated with oversaturation, the only impact of OA is a small delay in development (Gazeau et al, in preparation). Similar results were observed in sea urchins when manipulating carbonate chemistry rather than CO2 concentration (Suarez et al., 2008).

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- In the same spirit ("being realistic"), do the authors have any data on what food is consumed in natural conditions and at what concentration? Using food "at libitum" may hide some effect by putting the individual in food conditions that compensate any potential negative impact.

The authors also assessed the sensitivity of molecular techniques to realistic CO2-induced pH changes and nicely show that subtle effects can be seen and that "*physiology and metabolism of coastal and marine species which may be overlooked in studies of whole organism response.*" If extremely interesting and relevant, it may be nice to discuss how these subtle effects are (or not) related to individual fitness and relevant to future predictions of impact of OA on marine species. This could help researcher interested in using such techniques.

Finally, it is usual to include a "housekeeping gene" (or genes) in real time PCR experiments. That is one that does not (or should not) change and this can be used as a base line control. Were any "housekeeping genes" used? NTCs are OK but not ideal.

Sam Dupont and Michael C Thorndyke

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