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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.

C. Hauton et al.

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This comprehensive review has raised a number of issues regarding the manuscript which we are seeking to address within an appropriate revision. The referee initially identifies that, with the limited number of genes studied in this work, the manuscript should only be considered as preliminary data. We agree entirely with this statement and did not intend for any other impression to be given. We felt it was clear from the discussion that this manuscript presents the first data from what is a larger, and on-going, study; however, we are re-looking at the manuscript discussion to see if this can be made clearer.

The referee makes a valid point about the timescale of the response of the hsp70 gene regulation; pointing out the discrepancy between our short-term heat shock experiment, which was only intended as a positive control to confirm the function of the

transcribed gene product, and our longer-term exposure to more acidic sea water. We acknowledge this point but do not feel that it is necessarily a fault with the approach we have taken. We have revised the text to clarify this issue. In this preliminary study we sought to investigate the response of two candidate genes to long-term perturbation of the environment pH. We have been able to show that the hsp70 gene is not transcriptionally upregulated after 14-days or 28-days, which would indicate that the product of this gene has no role in the long term physiological tolerance to acid base disturbance. We can make no comment about the short term shock response to changes in pH but agree that the HSP70 protein may have a role in that short term response. The revised manuscript will clarify this.

The referee suggests that our initial intention was to use the expression of the gapdh gene as an endogenous reference to correct our hsp70 data. We can categorically state that this was never the plan. As the referee correctly states, there are significant challenges associated with the identification of an appropriate endogenous reference or references; ensuring consistent expression in all organisms across the experiment. As a consequence, and for this first study, we elected to pursue a published strategy of absolute quantification. We agree with the comment that to confirm our observations it would be logical to study the expression of genes coding for other key metabolic enzymes; this is just one of many next steps; we are currently working on.

The reviewer questions our quantification strategy, arguing that it does not account for RNA quality or RT efficiency. We acknowledge that, with the approach we have taken, we are not able to put much weight on the absolute numbers of transcript copies, focussing instead on the changes in transcript abundance expressed as an equivalent copy number. This does not invalidate our conclusions. We would argue that the approach we have taken has been published previously and, whilst any method used has its limitations, as long as those limitations are understood valid conclusions can be made. As we now summarize in the revised discussion; now that we have tested an amenable model system to support this type of research another development is to

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test a range of endogenous reference genes to support further study. RNA integrity (by electrophoresis) was assessed in preliminary extractions that were carried out in advance of this study; however it is true that the samples used were not tested individually for integrity.

We disagree with the statement that the data would be better analyzed using a two way analysis of variance. Having carefully consulted a number of texts (e.g. Sokal and Rolff, 1995; Underwood, 1997) we are confident that we have designed a nested experiment with repeat batches of random samples nested within the main factor of pH. The batches represent an experimental convenience rather than a second fully manipulated factor (as would be the case for a two-way analysis of variance). We did not include any data on the fold increase in expression of the gapdh gene and this was an oversight. The mean fold increase in expression of the gapdh gene was recorded as 2.65; this has been added to the revised manuscript.

We have taken on board the referees other comments regarding the clarity of the discussion and also the additional minor points and will include them within the revised manuscript.

Sokal, RR & Rohlf FJ (1995) Biometry. 3rd Edition. W.H. Freeman and Company

Underwood AJ (1997) Experiments in Ecology. Cambridge University Press.

Interactive comment on Biogeosciences Discuss., 6, 919, 2009.