Biogeosciences Discuss., 6, S195–S196, 2009
www.biogeosciences-discuss.net/6/S195/2009/

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.

Received and published: 25 February 2009

Sam Dupont and Mike Thorndyke have made relevant observations regarding the pH range used in this study. The pH variability of the estuary was taken from published values from an earlier study and was not measured at the time of collection; this was an oversight which in future studies will be corrected. As we discuss in the manuscript, and raised by these reviewers the use of a control pH 8.1 that was above that measured in the estuary probably explains the reduced survival in the control treatment after 28 days. The discussion needs to be adjusted to explain this more clearly. The water used for this experiment was initially taken from the same estuary but maintained in a large volume recirculating aquarium before use in the experiment.

The revised manuscript will include additional data on the carbonate chemistry and this will be used to describe the parameters of the microcosm system more effectively.

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The dietary regime of the collected amphipods was not determined. The use of ad libitum conditions was an operational decision for this study. It is correct that in a food limited environment the negative effects of exposure to high pCO2 might be more pronounced. This next step, including the production of a detailed energy budget of adaptation to low pH in this species, is the focus of ongoing experimental and modelling research in the laboratory.

Some discussion of the effects of changes in gene expression are offered in the manuscript although we were concerned not to over-interpret the data or conclusions. The explanation of the current conclusions will be reconsidered in the revised manuscript. As part of ongoing research the effects (or otherwise) of subtle changes in the transcription of genes are being investigated at multiple levels of organization within the amphipods, adopting a systems approach. The outcome of this continued study will allow us to make more robust conclusions as to the relevance of changes in gene expression.

The use of NTCs in this study was simply to confirm that there was no genomic contamination of each PCR sample that would have produced erroneous data. NTC data were not used for quantification. An established technique for absolute quantification was used that does not rely on the determination of appropriate constitutively expressed endogenous reference genes (gene transcript levels are corrected against total RNA in the sample). Concerns over the proof of consistent expression of endogenous references meant that a relative expression approach was not pursued. However, and now that this species has been shown to be amenable to study and of potential value as a model system, efforts are underway to screen a panel of endogenous reference genes to permit relative quantification studies using multiple reference genes in the future.

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