EFFECTS OF OCEAN ACIDIFICATION ON JUVENILE CLAMS *RUDITAPES DECUSSATUS*

P. Range [1]*, L. Chícharo [1], M. A. Chícharo [1], R. Ben-hamadou [1], D. Piló [1], D. Matias [2], S. Joaquim [2] and A. P. Oliveira [2]
[1] Centro de Ciências do Mar, Universidade do Algarve, 8005-139 Faro, Portugal - prange@ualg.pt
[2] Instituto Nacional de Recursos Biológicos (INRB-IPIMAR)

Abstract

We report the preliminary findings of a 3 month-long experiment aimed at investigating the effects of elevated pCO_2 and reduced pH on the juvenile stages of the clam *Ruditapes decussatus*. The concentration of CO2 in seawater was manipulated by diffusing pure CO_2, to attain two reduced pH treatments (-0.4 and -0.7 pH units), which were compared to seawater with natural pH levels. We found no differences among treatments in the size or weight of the clams. The natural pH treatment showed significantly larger mortality than the acidified treatments. The peak of mortality coincided with the occurrence of sporadic spawning events, which were not observed in the most extreme acidification treatment. This suggests that the increased survival under acidified conditions may be associated with a delay in the reproductive cycle of the clams.

Keywords: Carbon, Bivalves, Coastal Waters, Physiology, Ph

Introduction. Anthropogenic emissions of carbon dioxide (CO_2) are changing the carbonate chemistry of seawater, causing decreases in pH and in the saturation state of carbonate minerals, which are major components for biological calcification. Whether and how this process of "ocean acidification" will affect marine organisms is currently a topic of great concern. The detrimental effects of acidic waters on bivalves have long been identified (1) and a critical pH value (<7.0) has even been proposed in relation to marine bivalves (2). More recent studies indicate that future CO_2 concentrations, predicted by the IPCC scenarios, can significant reduce calcification (3) and growth (4,5) of bivalves. Other aspects of bivalve physiology previously shown to be affected by reduced pH include acid-base regulation (4), respiration (4) and reproductive output (6). Here we report the preliminary findings of a 3 month-long experiment aimed at investigating the effects of elevated pCO_2 and reduced pH on the juvenile stages of the clam *Ruditapes decussatus*.

Methods. The experiment started on 09/06/2009 and ended on 23/10/2009. The rearing system was open, supplied with a continuous flow of filtered natural seawater to minimize the interference from the metabolic waste-products of the organisms. Seawater chemistry was manipulated by diffusing pure CO_2, to attain two reduced pH treatments (-0.4 and -0.7 pH units), which were compared to seawater with natural pH levels. Each level of pH had 3 replicate tanks with clams, each stocked with 100 juvenile clams. Temperature and salinity were not controlled, but were continuously monitored, along with pH. Combined with measurements of total alkalinity and dissolved oxygen, taken at regular intervals, these variables allowed the estimation of the amount of carbonates in the seawater. The bivalves were fed in continuous flow with an adequate mixture of microalgae. The response of the juvenile clams was measured in terms of mortality, growth, biochemical composition, calcification and microstructure of the shell. The physiological response was also evaluated, in terms of feeding behaviour and metabolic rates.

Results and Discussion. Values of pH recorded during the experiment were maintained within the planned intervals (Mean ± SD): pH -0.7 (7.50 ± 0.12); pH -0.4 (7.81 ± 0.06); pH CT (8.14 ± 0.09). During the first 75 days the clams grew from 10.2 ± 2.29 mm to 12.4 ± 2.51 mm (shell-length, mean ± SD). We found no differences among treatments in terms of the average size or weight of the clams. After 75 days there was a significant difference in survival between the natural pH and the two acidified treatments: pH -0.4 = pH -0.7 > pH CT (Figure 1). In contrast, the RNA/DNA condition index revealed an opposite pattern: pH CT > pH -0.4 > pH -0.7.

Considering the reported effects of acidic seawater on bivalves, the decreased mortality of *R. decussatus*juveniles under reduced pH conditions was an unexpected result. Some earlier studies (1,7) did not specifically addressed increasing CO_2 concentrations and other methods of acidification were used, so their results should be interpreted with caution. The peak mortality in our experiment occurred in the same tanks where a spawning event was observed, just a few days before. No spawning was observed in the pH -0.7 treatment, which suggests the increased survival in that treatment may be associated with a delay in the reproductive development of the clams. The pattern of reduced growth under experimental conditions of reduced pH, previously reported for other species of bivalves (4,5), was also not apparent in our study. It is important to note, however, that the pH reductions imposed in our experiment, although within the range of values projected by IPCC, were not as severe as in those previous studies. Further clarification and interpretation of the preliminary findings reported here is expected when the full dataset is available.

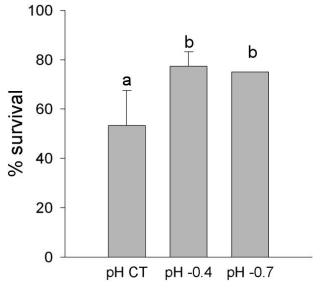

Fig. 1. Mean (+ SD; n=3) survival after 95 days for each of the 3 pH levels considered; letters identify groups of means that are significantly different (SNK tests, $p<0.05$)

Acknowledgements

We would like to thank the following people for their help at various stages of this study: Ana Amaral, Alexandra Leitão, Margarete Ramos, Maurício Teixeira and Eloise de Sá. This is a contribution of the ACIDBIV project, which is part of the CIRCLE Med projects, funded by the Regional Ministry of Innovation and Industry of the Galician Government, the Italian Ministry for Environment, Land and Sea, and the Foundation for Science and Technology of Portugal, in the framework of Circle ERA Net project (which is funded by the European Commission 6th Framework Programme).

References

1 - Loosanoff, V.L. & Tommers, F.D., 1947. Effect of Low Ph Upon Rate of Water Pumping of Oysters, Ostrea Virginica. *Anatomical Record* 99, 668-669.

2 - Bamber, R.N., 1990. The Effects of Acidic Seawater on 3 Species of Lamellibranch Mollusk. *Journal of Experimental Marine Biology and Ecology* 143, 181-191.

3 - Gazeau, F. et al., 2007. Impact of elevated CO2 on shellfish calcification. *Geophysical Research Letters* 34 (7), L07603.

4 - Michaelidis, B., Ouzounis, C., Paleras, A. & Portner, H.O., 2005. Effects of long-term moderate hypercapnia on acid-base balance and growth rate in marine mussels Mytilus galloprovincialis. *Marine ecology progress series.* 293, 109.

5 - Berge, J.A., Bjerkeng, B., Pettersen, O., Schaanning, M.T. & Oxnevad, S., 2006. Effects of increased sea water concentrations of CO2 on growth of the bivalve Mytilus edulis L. *Chemosphere* 62, 681-687.

6 - Kurihara, H., 2008. Effects of CO2-driven ocean acidification on the early developmental stages of invertebrates. *MARINE ECOLOGY-PROGRESS SERIES* 373, 275-284.

7 - Calabrese, A. & Davis, H.C., 1970. Tolerances and requirements of embryos and larvae of bivalve molluscs. *Helgoland Marine Research* 20, 553-564.