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Acute extracellular acid–base disturbance in the burrowing sea urchin *Brissopsis lyrifera* during exposure to a simulated CO_2 release

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ABSTRACT

We tested the hypothesis that as infaunal organisms are regularly exposed to elevated CO_2, burrowing sea urchins will demonstrate a lower sensitivity to massive CO_2 release than has previously been recorded for epifaunal organisms. Infaunal urchins *Brissopsis lyrifera* were exposed to CO_2 acidified sea water (nominal pH 7.8 (control), 7.3, 6.5 and 5.9; T = 10 °C, S = 34) for 12 h and aspects of their extracellular acid–base balance measured every 2 h. In common with epifaunal urchins *B. lyrifera* exhibited an uncompensated respiratory acidosis in its extracellular fluid, but was more sensitive to CO_2 acidification than epifaunal urchins. The lower extracellular pH of *B. lyrifera* may indicate a higher metabolism than epifaunal urchins and this could explain the heightened sensitivity of this species to elevated CO_2. Thus, the results of this present study do not support our original hypothesis. Instead we suggest an alternative hypothesis that as infaunal organisms are exposed naturally to high levels of CO_2, they may already be closer to the limits of their physiological performance. Thus any further CO_2 increase could compromise their function. As a result of this sensitivity, infaunal urchins may be more at risk from an accidental release of CO_2 from geological sub-seabed storage sites, or from the deliberate injection of CO_2 into deep water masses, than their epifaunal counterparts.

1. Introduction

Atmospheric CO_2 concentrations continue to increase as a result of anthropogenic input (Solomon et al., 2007). Even if emissions were dramatically reduced today, the CO_2 already present in the atmosphere would continue to be absorbed into the oceans further reducing the pH of the sea water, an effect termed 'Ocean Acidification' (Orr, 2011). A number of "geo-engineering" mitigation strategies have been proposed not as an alternative to reducing CO_2 emissions but in an attempt to alleviate ongoing effects until positive effects of reduced atmospheric CO_2 concentrations take effect (Lal, 2008). One approach is ocean storage, i.e. the direct injection of CO_2 into deep waters or geological storage sites (Drange and Haugan, 1992; Herzog et al., 1996; Lal, 2008). However, this approach has risks associated with it (Seibel and Walsh, 2003) in the form of rapid reduction in seawater pH for example through direct CO_2 injection onto the seafloor (Bernhard et al., 2009) or potential localised acidification associated with leakage from geological storage (Blackford et al., 2009).

Due to their reliance on a calcareous skeleton and a limited ability to buffer chemical changes in their extracellular fluids, echinoderms have been highlighted as one of the animal groups that could be most at risk from exposure to CO_2 acidified sea water (Dupont et al., 2010). Certainly the epifaunal echinoids *Psammechinus miliaris* and *Strongylocentrotus dröebachiensis* have shown a pronounced extracellular respiratory acidosis when exposed to seawater pH's of the magnitude that could be encountered as a result of leakage from a geological site (Miles et al., 2007; Spicer et al., 2011; although cf. Stumpp et al., in press). However, it may not be the same for infaunal sea urchins. To a certain extent even *P. miliaris* naturally experiences short-term hypercapnic (associated with hypoxia) conditions when it occurs in intertidal rock pools (Spicer, 1995). It is possible that infaunal organisms may experience, and so be pre-adapted to, more sustained, chronic and pronounced elevated CO_2 (Widdicombe et al., 2011). The pH in the uppermost centimetres of sediment can range from pH 6.5 to 8.2, and the PCO_2 from 0.4 to 16 000 μatm; pH profile with depth typically showed a sharp decrease in these top few centimetres, and then becomes invariant with depth (Widdicombe et al., 2011).

Consequently, the current study investigated changes in extracellular acid–base balance and chemistry in response to acute exposure (12 h) to CO_2 acidified sea water with pH's as low as 5.5. Reductions in seawater pH as a result of acute CO_2 storage leakage have been estimated to anywhere between 1 and 4 pH units (Seibel and Walsh, 2003) and so the CO_2 levels we have chosen for this experiment are not unreasonable (see also Herzog et al., 1996). The aim of our experiment was to determine how such changes compared with epifaunal urchins seeking support for the hypothesis that infaunal organisms should display a greater ability to respond to elevated CO_2 than epifaunal organisms (Widdicombe and Spicer, 2008; Widdicombe et al., 2011).

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Due to the difficulties of catheterising, or even locating, large numbers of urchins when buried in sediment, a preliminary experiment was carried out to investigate how the presence or absence of sediment might disturb extracellular acid–base balance. Furthermore, as urchins were collected by dredging and grab we also investigated whether or not collection method might affect the acid–base condition of urchins.

2. Materials and methods

2.1. Collection

Brissopsis lyrifera ranging from 14.4 g to 75.4 g (blotted wet mass) were collected using a naturalist dredge, or Day grab, from muddy sediment at a depth of 70 m in Oslofjord, Norway (59° 43 018 N, 10° 34 357E) during October, 2005. The large size range used was unavoidable if enough urchins to carry out the experiments described below were to be secured. We thought this justified as previous work suggested that there is no significant relationship between measured acid–base parameters and urchin size (Spicer et al., 2011). All urchins were returned to the mesocosm facility of the Norwegian Institute for Water Research (NIVA), at Solbergstrand Marine Research Station (MFS) on the eastern shore of Oslofjord. The facility is described in detail by Berge et al. (1986).

Urchins were either used immediately in a preliminary experiment to determine how collection method and being kept in sediment affected the extracellular pH and TCO_2 of these burrowing urchins or, in the case of those urchins to investigate the effects of acidified sea water on extracellular pH, TCO_2, and divalent ions, maintained in the facility for 6 days (T = 10 °C, Salinity = 34‰, unfed) until used in the experiment described below.

2.2. Effect of sediment and capture method on extracellular acid–base

Thirty urchins were maintained in the mesocosm holding facility for four days (T = 10 °C, Salinity = 34‰), before they were removed individually, and the extracellular (perivisceral) fluid extracted and the pH and TCO_2 of that fluid determined as described below. Twenty urchins had been trawled, with 10 placed in mesocosm aquaria without sediment and the remaining 10 in mesocosm aquaria previously filled to a depth of 10 cm with muddy sediment obtained from the same site the urchins were collected from. Ten urchins that had been collected using the Day grab were placed in mesocosm aquaria without sediment.

2.3. Exposure of urchins to CO_2 acidified sea water

Urchins (N = 125) were exposed individually in the experimental buckets to one of four CO_2 acidified sea waters (nominal pH 7.8 (control), 7.3, 6.5 and 5.9) at a temperature of 10 °C, salinity = 34‰, $O_2 \geq 70\%$ (saturated). Five individual urchins were selected haphazardly from each exposure pH and removed for sampling at each of 6 sampling times (2, 4, 6, 8, 10 and 12 h).

2.4. Seawater acidification

Seawater acidification was carried out following closely the method described in Widdicombe and Needham (2007) and Widdicombe et al. (2009), and summarised here. Carbon dioxide gas was passed through natural sea water contained within large (vol. = 450 l) reservoir tanks. The CO_2 was passed (as very fine bubbles enabling it to pass rapidly into solution) through the water and once the pH had fallen to the required level, the supply of CO_2 was stopped, via an automated feedback relay system. As the acidified water was taken from the reservoir, to supply the experimental buckets (diam. = 18.5 cm, depth = 10 cm, supply rate 70 ml.min^{-1}), it was replaced by natural sea water (pH ≈ 8.0) causing the pH in the reservoir tank to increase. This sea water was collected, via a pipe situated at 60 m in the fjord adjacent to the marine station and passed over a course filter (80 μm) before being supplied to the acidification reservoirs. The resultant increase in pH triggered the supply of CO_2 to be restarted and CO_2 continued to bubble through the water until the pH had again been reduced to the pre-set level.

From each urchin a sample of extracellular (perivisceral) fluid was collected and analysed as follows.

2.5. Extraction and analysis of perivisceral fluid

Perivisceral fluid was extracted from each individual using a 1 ml syringe with a 21 G needle inserted into the perivisceral cavity via an area slightly to one side of the Aristotle's lantern, to a depth of 5 mm at an angle of 45°. A small amount (1 ml) of extracellular fluid was withdrawn and transferred to a microcentrifuge tube (Eppendorf, vol. = 0.6 ml). Once the fluid has been extracted from the urchin its pH and CO_2 can change rapidly. Consequently sample analysis was conducted immediately after fluid collection. Firstly, the pH of the sample was determined by immersing a micro-pH probe (diam. = 3.5 mm, Mettler Toledo Inlab 423, Switzerland coupled to a pH meter: Denver 215, USA) in the fluid. Secondly, a 50 μl subsample was taken and the total CO_2 content (TCO_2) determined using an automated carbon dioxide analyser (CIBA-Corning 965, UK). Values for partial pressure of carbon dioxide (PCO_2) and the concentration of bicarbonate ($[HCO_3^-]$) in the extracellular fluid were calculated from direct measurements of TCO_2 and pH using the Henderson–Hasselbach equation in the following forms

$$PCO_2 = TCO_2/\alpha\left(10^{pH-pK'1} + 1\right) \quad (1)$$

$$[HCO_3^-] = TCO_2 - \alpha PCO_2 \quad (2)$$

Where α is the solubility coefficient of CO_2 in sea water (S = 34‰, T = 10 °C), which we assume approximates to echinoid extracellular fluid, and equals 0.04529 mol.l^{-1}.atm^{-1} (Weiss, 1974) and pK'_1 is the first apparent dissociation constant of carbonic acid based on values presented in Spicer et al. (1988).

Although concentrations of carbamate present cannot be ignored at high pH and low PCO_2 (Truchot, 1976), it was assumed that, as the effects of acidifying the environment here resulted in an acidification of extracellular body fluids, any concentrations of carbamate are negligible and so have not been calculated. Consequently our calculated values for $[HCO_3^-]$ may include very small amounts of CO_2 in other chemical forms.

After the pH of the fluid had been measured and subsamples removed for TCO_2 analysis, the remaining fluid was kept at T = −20 °C. The fluid was later thawed and analysed for calcium and magnesium concentration using atomic absorption spectrophotometry (Spectra AA 600, USA) after appropriate (i.e. to within the sensitivity range of the machine) dilution with double deionised water. Each individual urchin was sampled once thereby maintaining statistical independence for all measurements.

A non-bicarbonate buffer line was constructed for extracellular fluid by equilibrating pooled fluid from controls with gas mixtures of different, known PCO_2s, constructed using Wostoff Precision gas mixing pumps (T = 10 °C). After 15 min equilibration with each gas mixture, both the pH and TCO_2 in vitro were measured using the techniques described above. Bicarbonate was calculated using Eq. (2) above.

The TCO_2 and pH of water in some experimental buckets from each treatment was monitored haphazardly on a number of occasions throughout the experiment, employing the same measurement techniques as were used to measure perivisceral fluid from the urchins. Mean measured values for TCO_2 and pH, and values for bicarbonate concentration, total alkalinity, aragonite saturation and calcite saturation of experimental waters the urchins are immersed in (calculated using the