Vol. 15: 73–86, 2012
doi: 10.3354/ab00408

AQUATIC BIOLOGY
Aquat Biol

Published online April 3

Impact of exposure to elevated pCO_2 on the physiology and behaviour of an important ecosystem engineer, the burrowing shrimp *Upogebia deltaura*

Penelope J. C. Donohue[1,*], Piero Calosi[1], Adam H. Bates[1], Bonnie Laverock[2], Samuel Rastrick[1], Felix C. Mark[3], Anneli Strobel[3], Steve Widdicombe[2]

[1]Marine Biology and Ecology Research Centre, School of Marine Science and Engineering, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK

[2]Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth PL1 3DH, UK

[3]Alfred Wegener Institute for Polar Marine Research, Am Handelshafen 12, 27570 Bremerhaven, Germany

ABSTRACT: There is an increasing need to predict the effects of global climate change on ecologically important marine organisms and a demand for proactive solutions to reduce CO_2 emissions. CO_2 sequestration is one such method. While this offers a practical solution, recognition should be given to the potential for considerable localised effects on marine organisms in the event of leakage. This laboratory study quantifies the impact of exposure to elevated pCO_2 conditions on the physiological and behavioural responses of a relatively tolerant marine organism. Burrowing shrimps *Upogebia deltaura* were exposed to CO_2-enriched seawater for 35 d to treatments of 1396 µatm (pH 7.64), 2707 µatm (pH 7.35) and 14 110 µatm (pH 6.71). CO_2 levels represented scenarios which included coastal ocean acidification and extremely elevated CO_2 associated with geological CO_2 sequestration leaks. Results were compared with those from shrimps maintained in a control treatment (pH 7.99). *U. deltaura* appeared to be tolerant to elevated pCO_2 predicted to occur in the year 2100 (1396 µatm, pH 7.64). However, at 2707 µatm (pH 7.35) shrimps experienced extracellular acidosis, but no difference in haemolymph bicarbonate concentration, suggesting they have little or no buffering capacity, although there was no evidence of other physiological costs in terms of metabolism, osmotic regulation, shell mineralogy, growth and overall activity. At pH 6.71, before 100% mortality occurred, significant differences in activity were observed compared with shrimps in other pH treatments. Results suggest deleterious consequences for benthic ecosystems in the event of a CO_2 sequestration leakage.

KEY WORDS: Ocean acidification · Ecosystem engineer · Oygen consumption · Homeostasis · Haemolymph regulation · CO_2 sequestration · Carbon capture and storage · CCS · *Upogebia deltaura* · Upwelling

INTRODUCTION

One of the greatest challenges currently facing marine ecosystems is that of anthropogenically accelerated global climate change (GCC). This phenomenon includes ocean acidification (OA), which results from an increased absorption of rising atmospheric CO_2 into the oceans (Meehl et al. 2007) and leads to an accelerated lowering of global ocean pH. This period of accelerated change is in contrast to the stability of the past 25 million years, where ocean pH has remained between 8.3 and 8.0 (Caldeira & Wickett 2003). Since

*Email: p.donohue.1@research.gla.ac.uk

the beginning of the industrial revolution OA has led to a reduction in ocean pH by 0.1 unit, and a further reduction of ~0.3 to 0.4 pH units is predicted by the end of this century (Orr et al. 2005); it is this accelerated rate of change that is the cause of most concern for marine systems. Furthermore, atmospheric pCO_2 levels are predicted to increase from the present 380 µatm to 1000 µatm around the year 2100 and exceed 1900 µatm by the year 2300 (Caldeira & Wickett 2003, Raven et al. 2005). In addition to this general decrease in surface ocean pH, particular areas of the coastal ocean can experience large increases in CO_2 levels that can last for several months, including those in upwelling zones (Feely et al. 2008, 2010), restricted fjords or bays (Thomsen et al. 2010) and areas with high riverine influence (Salisbury et al. 2008).

Recognition of the huge social, economic and political implications of anthropogenically driven climate change and OA has led to an increasing demand for proactive solutions to reduce CO_2 emissions (Widdicombe & Needham 2007). Governments are presently exploring the possibility of employing geological CO_2 sequestration to help reduce the ongoing increase in atmospheric pCO_2, in an attempt to slow down the negative effects of GCC (Blackford et al. 2009). This method of storage involves the injection of CO_2 into underground reservoir rocks, and its use has been demonstrated with success (Holloway 2005). While it is acknowledged that geological sequestration is a practical solution to CO_2 mitigation (Gibbins et al. 2006), recognition should be given to the potential for considerable localised impact on benthic marine organisms (Blackford et al. 2009) in the event of periodic leakages from these sub-seabed stores (Hawkins 2004). These acute and intense decreases in pH caused by localised CO_2 injections, and chronic exposure to lower levels of CO_2 due to anthropogenically accelerated global OA, may lead to unfavourable conditions for some benthic organisms that may affect their growth, metabolic rates, calcification, behaviour and activity levels (see Widdicombe & Spicer 2008, Wood et al. 2008, Munday et al. 2010, Small et al. 2010, de la Haye et al. 2011). In turn, this may result in high mortality rates, particularly during acute exposures, and ultimately cause a shift in the local benthic community composition and biodiversity (see Barry et al. 2004, Hall-Spencer et al. 2008, Martin et al. 2008, Widdicombe et al. 2009a, Cigliano et al. 2010, Hale et al. 2011, Kroeker et al. 2011). Examination of both physiological and behavioural responses to a range of OA scenarios is critical to further our understanding of how global environmental change may impact marine systems.

In many ecosystems there are organisms that, as a result of their specific mode of life, activity or interaction with other species, have a disproportionately large impact on the environment in which they live and the organisms that live near them; these organisms are referred to as 'ecosystem engineers' (Jones et al. 1994). Any changes in their abundance or activity can have a large impact on many of the processes that underpin essential ecosystem processes. Examples include the cycling of key nutrients within coastal and shelf sea ecosystems (Field et al. 1998, Dale & Prego 2002, Widdicombe & Needham 2007, Wootton et al. 2008) or the maintenance of biodiversity (Widdicombe et al. 2000, Laverock et al. 2010). The burrowing shrimp *Upogebia deltaura* is an abundant component of the benthic community across the majority of European coastal sediments (Haywood & Ryland 2005) and construction and maintenance of burrows by means of bioturbation and irrigation behaviour significantly influences the ecosystem. Bioturbation by shrimps significantly influences bacterial community structure and composition, both within the burrows and in surrounding surface sediment, which may then determine the microbial transformations of important nutrients (Laverock et al. 2010). In addition, the presence of burrows increases the surface area of sediment across which nutrients can pass and the number of sites for nutrient transformations such as denitrification (Mayer et al. 1995, Astall et al. 1997, DeWitt et al. 2004, Webb & Eyre 2004). However, to date, relatively few studies have focused on the response of important infaunal bioturbating organisms to changes in seawater acidity (e.g. in *Nereis diversicolor*, Widdicombe & Needham 2007, and in *Amphiura filiformis*, Wood et al. 2008, 2009, 2010).

The burrow environment is regularly exposed to elevated pCO_2 and decreased pH levels; Astall et al. (1997) recorded pH values of 8.0 to 7.6 in the laboratory in Y-shaped burrows of *Upogebia stellata*. Burrowing macrofauna may therefore demonstrate a higher tolerance to elevated pCO_2 levels than that of organisms living on the sediment surface (Widdicombe & Spicer 2008, Wood et al. 2010, Widdicombe et al. 2011). In the present study we investigated the impact of exposure to elevated pCO_2 on the physiology and behaviour of *U. deltaura* to determine the response of this species to conditions of acute and intense elevations in seawater pCO_2 and/or decreased burrow pH. Experimental treatment levels were chosen to represent a range of scenarios from coastal ocean acidification to intense environmental hypercapnia associated with leaks for sub-seabed CO_2 stores.

MATERIALS AND METHODS

Collection and maintenance of burrowing shrimp

Intermoult adult individuals of *Upogebia deltaura* (weight = 5.9 ± 0.78 g [mean ± SE]) were collected in July 2009 from an area of subtidal muddy sand (water depth = 12 m) in Jenny Cliff Bay, Plymouth, UK (50° 20′ 93″ N, 04° 07′ 61″ W). A box corer, 0.1 m^2 in size, was used to collect the sediment, which was immediately sorted by hand to retrieve the shrimps. Upon collection, the shrimps were immediately transferred to individual mesh-covered sample containers and placed in plastic buckets containing free-flowing seawater for transportation to the CO_2 mesocosm facility at the Plymouth Marine Laboratory, Plymouth. Upon arrival at the laboratory, shrimps were gently blotted to remove excess water, weighed with a digital scale (Fisher Scientific, model SG-202, European Instruments) and placed in individual transparent plastic tubes (20 cm in length, 26 or 30 mm diameter). The plastic tubing was designed to mimic the shrimp burrow and was covered at each end with a mesh net (mesh size, 0.5 mm diameter). Each tube was then placed into individual 3 l aquaria with seawater. In the laboratory, Astall et al. (1997) determined the burrows of *U. deltaura* were between 17 and 23 mm in diameter, which was constant throughout the burrow, and contained occasional turning chambers. In the present study, 2 sizes of tubes, made from clear plastic tubing (Endsleigh Garden Centre, Plymouth), that allowed unrestricted movement within the tube, were used: 200 mm × 26 mm diameter and 260 mm × 30 mm diameter for small (<6 g) and large (>6 g) shrimps, respectively. All aquaria were supplied with a continuous flow of natural seawater (temperature [T] ≈ 15°C, S = 35), consistent with environmental data recorded at the time of collection from the seabed (depth = 11.4 m, T = 14.8°C, S = 35.1). Seawater was collected from the Eddystone area, 20 km offshore from Plymouth. Aquaria were then covered with a lid and blackout fabric; this ensured shrimp were maintained in the dark in the absence of sediment-based burrows. Shrimps were held for no more than 1wk before starting the experiment and were not fed during this time.

Experimental design

The experimental treatments were nominally maintained at pH 8.0 (control), 7.6, 7.3 and 6.7. These levels were chosen to mimic predicted values that potentially could be encountered by benthic organisms under ocean acidification scenarios (pH 7.6) and during upwellings and CO_2 storage leaks (pH 7.3 to 6.7) (Feely et al. 2008, 2010, Blackford et al. 2009, Thomsen et al. 2010). Twenty-four 3 l plastic aquaria were allocated randomly to 1 of the 4 different pH treatment levels. Each aquarium was supplied individually and continuously with gravity-fed natural seawater at a constant rate (10 ml min^{-1}) from the appropriate header tank. This ensured complete water turnover approximately every 5 h and prevented the build-up of any waste products such as additional dissolved CO_2 from respiration (Widdicombe et al. 2009b, Hale et al. 2011). Seawater acidification was achieved by bubbling pure CO_2 gas through the water in the header tanks exactly as described by Widdicombe & Needham (2007). Each aquarium was supplied with a small mesh bag (mesh size, 0.5 mm diameter) containing 3 g of activated carbon to prevent ammonia accumulation (Small et al. 2010). Nominal temperature for the exposure period was 15°C and salinity was 35. At the start of the exposure period, 24 shrimps were randomly assigned to 1 of 4 pH levels (6 individuals per nominal treatments of pH 6.7, 7.3, 7.6 and 8.0 [control]), to ensure, as much as possible, an even distribution of weights across the 4 treatments: in fact, no significant difference in wet weights (g) of individuals was found across all treatments ($F_{2,15}$ = 0.255, p = 0.778). Each of the 24 shrimps were placed in individual aquaria and used as stated previously. The exposure period was 35 d. Shrimp were fed by adding 0.2 ml Instant Algae (~400 million cells) (Shellfish Diet 1800, Reed Mariculture) to the natural seawater in the header tanks (450 l) every 5 d for the duration of the experiment.

Experimental monitoring

Throughout the exposure period water samples were removed daily from each of the 24 exposure aquaria to measure pH, salinity, temperature and dissolved inorganic carbon (DIC). pH was measured according to US National Bureau of Standards with a pH meter (Sevengo, Mettler Toledo) and a pH electrode (Seven Easy, InLab micro electrode), DIC was measured with a carbon dioxide analyser (965D, Corning Diagnostics), and temperature and salinity were measured using a hand-held conductivity meter (Multi350, WTW). Additional carbonate system parameters (pCO_2, alkalinity, calcite and aragonite saturation, [HCO_3^-] and [CO_3^{2-}]) were calculated from pH and total DIC values by using the software program

CO2SYS (Pierrot et al. 2006) with dissociation constants from Mehrbach et al. (1973) refit by Dickson & Millero (1987) and [KSO_4] using Dickson (1990). Over the course of the 35 d exposure there were 6 mortalities, all of which occurred in the pH 6.7 treatment. Two replicates were also lost in the pH 7.3 treatment due to an unexpected alteration of the experimental conditions in 2 aquaria, leaving 16 individuals (6 individuals in each of the nominal treatments pH 7.6 and 8.0 and 4 individuals in nominal treatment pH 7.3).

Determination of shrimp activity

Three individual shrimps from each of the 4 treatments were filmed at random times during each day of the 35 d exposure so that video footage could be analysed to ascertain any difference in shrimp behaviour between treatments. During filming individual aquaria containing each shrimp (within a plastic tube and seawater at the respective pH) were removed from the experimental set-up and transferred directly to the filming set-up. The filming set-up consisted of a hooded compartment covered with black-out material, which allowed for filming under dark conditions, and a video camera mounted on a tripod. Filming was done using infrared vision. Shrimps were filmed individually, and once moved into position within the filming set-up, were given 10 min to recover from any imposed handling stress and then filmed for a further 10 min. During filming the shrimp were kept at their experimental pH treatment conditions to prevent any potential recovery influencing test outcomes. Preliminary experiments indicated that experimental pH was maintained over the 20 min total filming period without continuous supply from the header tanks. Eight behavioural end-points were identified from preliminary analysis of the video footage: (1) no movement: no visible movement; (2) pleopod beating: the abdomen and the telson were motionless, pleopods were moving in a fanning motion; (3) walking: movement up and down or around the circumference of the tube using pereopods; this included turning (180° somersault to change direction); (4) flexing: movement of the abdomen and telson, curling in towards the cephalothorax in a flexing motion; (5) cleaning: scratching or brushing the cephalothorax, abdomen, telson and other appendages using the pereopods (usually the fifth pair); (6) cleaning with pleopod beating: cleaning simultaneously accompanied with pleopod beating; (7) cleaning with flexing: cleaning simultaneously accompanied with flexing; (8) feeding: movement of the mandible and/or maxilla, and/or use of the pereopods to 'feed'. Video recordings were then watched in real time and the time the shrimp spent carrying out each behaviour was recorded with a hand-held pocket observer (Workabout Pro-C, Psion Teklogix). The percentage of time each shrimp spent engaged in each activity category was then determined.

Determination of metabolic rate

To determine the response of *Upogebia deltaura* metabolic rate to exposure to elevated pCO_2 conditions at the end of the 35 d exposure period, O_2 uptake (used as a proxy of routine metabolic rate [RMR]) was measured using closed bottle respirometers. Individual shrimp were placed in a blacked out 300 ml respirometer. The respirometer vessels were left open for the first 15 min to allow the shrimps recovery time from handling. The respirometers were then closed for a further 15 min. The [O_2] was measured immediately before closing and immediately after the 15 min incubation time, by using an O_2 electrode (1302, Strathkelvin Instruments) connected to a calibrated oxygen meter (781, Strathkelvin Instruments) and expressed as µl O_2 mg (wet mass)$^{-1}$ h^{-1}. The size of the respirometers was such that O_2 saturation did not go below 79% during the incubation period, thus ensuring individuals did not experience hypoxic conditions. All individuals remained in the designated treatment water throughout the experimental period to prevent the effect of any potential recovery influencing the test outcomes. All individuals were returned to the experimental aquaria and left for at least 24 h before other physiological tests were carried out.

Measurement of haemolymph acid–base status and total osmolality

Key haemolymph acid–base parameters (pH and total CO_2 (Cco_2)) were measured in all individuals at the end of the 35 d exposure period. Haemolymph was extracted by inserting a 100 µl Hamilton syringe dorsally, directly into the pericardium via the arthrodial membrane between the cephalothorax and the abdomen; the individual was immobilised in a vertical position to allow gravity to assist in recovery of the sample. Haemolymph was extracted carefully to obtain clean, clear and anaerobic samples. Samples were carefully but rapidly placed in a 1.6 ml Eppendorf tube and 2 operators worked together to minimise the handling time. Operator 1 immediately

analysed haemolymph for CCO_2 by pipetting a 50 µl subsample into a carbon dioxide analyser (965D, Corning Diagnostics), and then measured osmolality by pipetting a 10 µl haemolymph subsample into a vapour pressure osmometer (5520 VAPRO, Wescor). Operator 2 measured haemolymph pH (in less than 10 s after extraction) by immersing a micro-pH probe (Micro-InLab pH combination electrode, Mettler Toledo) in the haemolymph (see Miles et al. 2007, Spicer et al. 2007, Marchant et al. 2010, Small et al. 2010). The micro-pH probe was attached to a calibrated pH meter (Seven Easy pH Meter, Mettler Toledo). Haemolymph pCO_2 and $[HCO_3^-]$ were calculated with the Henderson-Hasselbach equation in the following forms (see Spicer et al. 2007):

$$pCO_2 = CCO_2 / \alpha\ (10^{pH-pK'1} + 1) \quad (1)$$

$$[HCO_3^-] = CCO_2 - \alpha\ pCO_2 \quad (2)$$

where α is the solubility coefficient of CO_2 in crab haemolymph (taken from Spicer et al. 2007 as 0.376 mmol l^{-1} kPa^{-1}, calculated from Truchot's 1976 values for haemolymph from the European shore crab *Carcinus maenas*, kept at S = 35, T = 15°C), and $pK'1$ is the first apparent dissociation constant of carbonic acid (6.027 from Truchot 1976), also using values from *C. maenas* haemolymph at S = 35, T = 15°C, see also Spicer et al. 2007, Small et al. 2010). The remaining haemolymph was placed in ice to prevent clotting and water evaporation before being frozen and stored for further analysis.

Determination of haemolymph ions

To determine whether elevated pCO_2 caused an alteration in extracellular ion regulation in *Upogebia deltaura*, a 10 µl subsample of haemolymph from all 16 surviving individuals was diluted to 2 ml with ultra pure water. The samples were then analysed for $[Ca^{2+}]$, $[Mg^{2+}]$, $[K^+]$ and $[Na^+]$, by using an atomic absorption spectrometer (725-ES, ICP optical emission spectrometer, Varian).

Determination of shell mineralisation

To determine whether elevated pCO_2 caused an alteration to shell mineralogy of *Upogebia deltaura*, all 16 surviving individuals were dissected and the left chela, abdominal somites and telson were retained and frozen for analysis of $[Ca^{2+}]$, $[Mg^{2+}]$, $[K^+]$ and $[Na^+]$. The samples were scrubbed clean of all soft organic material before being freeze-dried at –50°C for 48 h. Samples were then weighed and digested as follows. The abdominal somites and the telson were analysed together and the whole left chela (dactylus and propodus) was analysed separately. Each sample was weighed and placed in a 50 ml glass beaker with 3 ml of nitric acid (70% concentration, trace analysis grade) to digest the sample. The beakers were then covered with a watch glass and left at room temperature for 60 min to allow easily oxidised material to be digested. Samples were then placed on a hotplate (Hotplate, S & J Juniper) and gently heated to boiling for at least 1 h so that samples were fully digested. All samples were then transferred into acid-washed 25 ml volumetric flasks and diluted to 25 ml with ultra pure water. The samples were then analysed for $[Ca^{2+}]$, $[Mg^{2+}]$, $[K^+]$ and $[Na^+]$ by using an atomic absorption spectrometer (725-ES, ICP optical emission spectrometer, Varian).

Statistical analyses

All physiological data were analysed with SPSS 17.0. Data were found to meet assumptions for normality of distribution (Kolmogorov-Smirnov test: $p \geq 0.05$) and homogeneity of variance (Levene's test: $p \geq 0.05$). Where required, data were transformed by using either $\log_{10}$ (for seawater pCO_2) or square-root (for seawater calcite and aragonite saturation, and $[CO_3^{2-}]$). A 1-way ANOVA test was used to investigate differences in whole-organism ecophysiological responses and shell mineralogy between the control and elevated pCO_2 treatments. Differences between means were considered to be significant when $p \leq 0.05$. If a significant difference between treatments was found a Tukey-Kramer post hoc test was used to allow for multiple comparisons. When assumptions of ANOVA were not met (Levene's and Kolmogorov-Smirnov tests: $p \geq 0.05$; shell calcification; abdominal somites and telson $[Ca^{2+}]$ and $[Na^+]$) the nonparametric Kruskal-Wallis test was used. All physiological parameters were preliminarily tested against pH treatment level in combination with body mass as covariate using analysis of covariance (ANCOVA). Where body mass was not found to co-vary with a physiological parameter ($p \geq 0.05$) it was removed from further analysis. To assess the effect of elevated pCO_2 on the overall activity of *Upogebia deltaura* a permutational multivariate ANOVA (PERMANOVA) approach (Anderson 2001, McArdle & Anderson 2001) was adopted with the 8 different categories (see 'Determination of shrimp activity') of shrimp activity

Table 1. Mean ± SD of seawater physico-chemical parameters measured in or calculated for the experimental aquaria throughout the 35 d exposure period. Different letters accompanying values within a column represent significant differences between treatments according to ANOVA. Variables calculated from pH and total DIC using CO2SYS (Pierrot et al. 2006) with dissociation constants from Mehrbach et al. (1973) refit by Dickson & Millero (1987) and [KSO_4] using Dickson (1990) are indicated by an asterisk (*). DIC: dissolved inorganic carbon; TA: total alkalinity; Ω_{cal}: calcite; Ω_{ara}: aragonite saturation

Treatment	Salinity	Temperature (°C)	pH	DIC (µmol kg^{-1})	TA* (µEq kg^{-1})	pCO_2* (µatm)	$[HCO_3^-]$* (µmol kg^{-1})	$[CO_3^{2-}]$* (µmol kg^{-1})	Ω_{cal}*	Ω_{ara}*
pH 8.0 (control)	35.5 ± 0.09	14.0 ± 0.2	7.99 ± 0.05^a	1870.03 ± 98.79^a	2004.67 ± 100.57	606.53 ± 95.01^a	1749.24 ± 99.80^a	99.67 ± 9.49^a	2.37 ± 0.22^a	1.52 ± 0.14^a
pH 7.7	35.5 ± 0.07	14.0 ± 0.1	7.64 ± 0.04^b	2055.43 ± 57.88^b	2087.98 ± 56.43	1395.77 ± 147.47^b	1960.33 ± 56.36^b	50.36 ± 5.12^b	1.19 ± 0.12^b	0.77 ± 0.08^b
pH 7.3	35.5 ± 0.01	14.0 ± 0.1	7.35 ± 0.03^c	2120.40 ± 21.58^b	2058.21 ± 53.76	2707.42 ± 168.70^c	1991.41 ± 49.72^b	26.24 ± 2.60^c	0.62 ± 0.06^c	0.40 ± 0.04^c
pH 6.7	35.4 ± 0.16	14.3 ± 0.1	6.71 ± 0.09^d	2411.78 ± 82.29^c	1988.06 ± 82.04	14109.50 ± 2674.66^d	1970.69 ± 79.63^b	6.91 ± 1.82^d	0.16 ± 0.04^d	0.11 ± 0.03^d

being considered as individual variables. For each shrimp, activity data collected at the different time points were pooled to give the average % time each individual shrimp spent engaged in each of the 8 activities over the course of the experiment. These averaged activity data were square-root transformed to ensure the assessment of overall activity was not unduly dominated by a few frequently observed activities. PERMANOVA+ routines (beta version, Anderson et al. 2008), which are an 'add-in' to the PRIMER 6 software, were used to carry out formal tests for the main effect of pH treatment level. Where a significant effect was observed, pair-wise PERMANOVA was used to determine where significant differences existed between the different pH levels. Where significant effects were identified, SIMPER analysis (PRIMER 6 software) was used to determine which shrimp activities may have contributed most (in terms of percent time) to the potential differences in activity amongst treatments.

RESULTS

Experimental conditions

The actual exposure pH of all treatments remained close to their nominal values: pCO_2 of 607 µatm equivalent to pH 7.99 (nominal pH 8.0), pCO_2 of 1396 µatm equivalent to pH 7.64 (nominal pH 7.6), pCO_2 of 2707 µatm equivalent to pH 7.35 (nominal pH 7.3) and pCO_2 of 14 109 µatm equivalent to pH 6.71 (nominal pH 6.7) (see Table 1). Actual exposure pH will be referred to throughout the Results and Discussion. The pH and the pCO_2 of the experimental treatments all differed significantly among each other (pH: $F_{3,20}$ = 287.42, p < 0.001; pCO_2: $F_{3,20}$ = 552.118, p < 0.001, Table 1), whilst salinity and temperature did not (maximum $F_{3,20}$ = 0.569, p = 0.580, Table 1). $[HCO_3^-]$ in the control (nominal pH 8.0) aquaria was significantly lower than in the acidified treatments ($F_{3,20}$ = 12.785, p < 0.001, Table 1). $[CO_3^{2-}]$ and calcite and aragonite saturation decreased significantly with decreasing pH (minimum $F_{3,20}$ = 332.022, p < 0.001, Table 1). Alkalinity did not differ significantly among treatments and ranged between 2090 and 1990 µEq kg^{-1} ($F_{3,20}$ = 2.249, p = 0.114, Table 1).

Mortality

A 100% mortality of shrimp was recorded for seawater treatment pH 6.71; mortalities occurred on

Days 7, 8, 12, 19, 27 and 35 of the 35 d exposure period. The average number of days alive for *Upogebia deltaura* in treatment pH 6.7 was therefore 13.14 ± 7.43 d (mean ± SE). There were no other mortalities across all other treatments. Therefore, physiological experiments examined the shrimp in treatments pH 7.35, pH 7.64, and pH 7.99 (control).

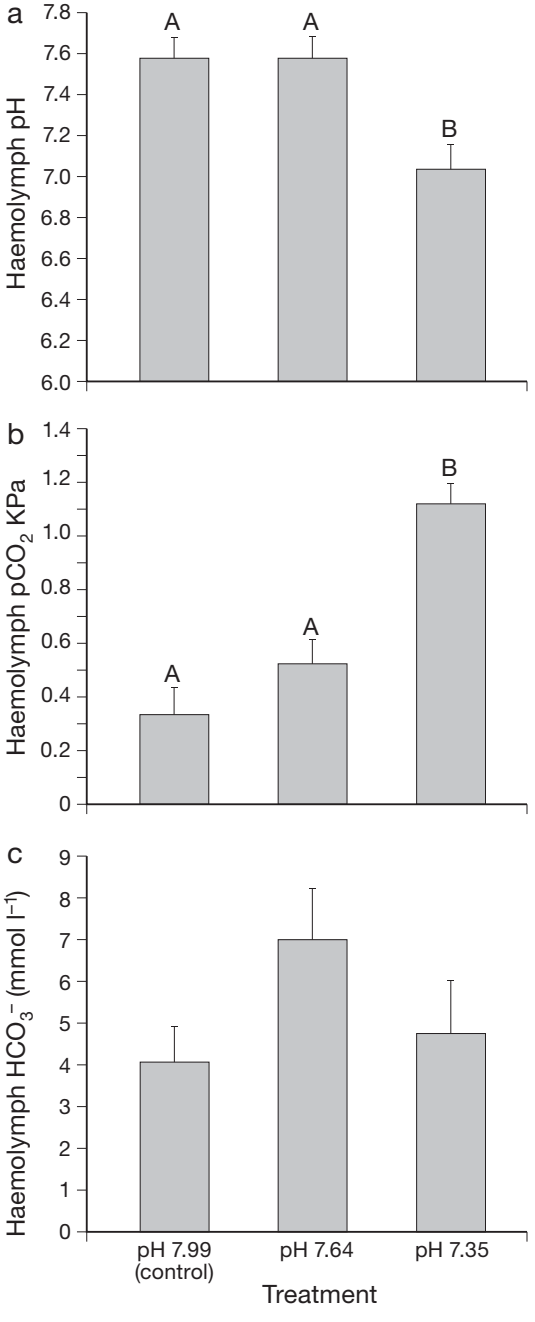

Fig. 1. *Upogebia deltaura*. Effects (mean ± SE) of seawater pCO_2 on (a) haemolymph pH, (b) haemolymph pCO_2 and (c) haemolymph $[HCO_3^-]$ after 35 d exposure. Different letters represent significant differences among treatments

Extracellular acid–base balance

Exposure to elevated environmental pCO_2 caused a significant reduction in haemolymph pH ($F_{2,12}$ = 8.812, p = 0.004, Fig. 1a) and an increase in haemolymph pCO_2 ($F_{2,12}$ = 18.041, p = < 0.001, Fig 1b). In particular, haemolymph pH of *Upogebia deltaura* exposed to treatment pH 7.34 was significantly lower and pCO_2 was significantly higher than those recorded for shrimps exposed to control conditions and pH 7.64, which did not differ between each other (Fig. 1c). There was no significant effect of acidified treatments on haemolymph HCO_3^- (bicarbonate) concentration ($F_{2,12}$ = 1.995, p = 0.179, Fig. 1c) or CCO_2 levels across all 3 treatment levels (maximum $F_{2,12}$ = 2.960, p = 0.090).

Rates of O_2 consumption and growth

Rate of O_2 consumption of adult individuals of *Upogebia deltaura* were not significantly affected by exposure to elevated pCO_2 levels ($F_{2,12}$ = 0.75, p = 0.491, Table 2). Similarly, exposure to elevated levels of pCO_2 had no significant effect on the final wet weight of shrimps (related-samples Wilcoxon signed rank test: maximum W_4 = –1.76576, p = 0.068).

Osmolality and haemolymph ions

There was no significant effect of elevated pCO_2 levels on total osmolality of haemolymph in *Upogebia deltaura* across all treatments after 35 d exposure ($F_{2,13}$ = 0.094, p = 0.911, Table 2). Similarly, a 35 d exposure to elevated pCO_2 conditions did not cause an alteration of $[Ca^{2+}]$, $[Mg^{2+}]$, $[K^+]$ and $[Na^+]$ in the haemolymph of *U. deltaura*, as no significant difference was observed between the control (pH 7.99) and low pH treatments (maximum $F_{2,13}$ = 0.987, p = 0.399, Table 2).

Shell mineralisation

Abdominal somites and telson

After a 35 d exposure to elevated pCO_2, levels of $[Ca^{2+}]$, $[Mg^{2+}]$, $[K^+]$ and $[Na^+]$ in the abdominal plates and telson of *Upogebia deltaura* did not differ significantly from the control treatment (Kruskal-Wallis test: maximum p = 0.948, Table 2). The calcification ratio ($[Ca^{2+}]$:$[Mg^{2+}]$) in the abdominal plates and tel-

Table 2. *Upogebia deltaura*. Mean ± SD of key physiological parameters measured in adult individuals after 35 d exposure to control pH and low pH/increased pCO_2. Cco_2: total CO_2

Treatment	O_2 uptake ($\mu l\ O_2\ mg^{-1}\ h^{-1}$)	Osmolality ($mmol\ l^{-1}$)	$[Na^+]$ ($mmol\ l^{-1}$)	$[K^+]$ ($mmol\ l^{-1}$)	$[Ca^{2+}]$ ($mmol\ l^{-1}$)	$[Mg^{2+}]$ ($mmol\ l^{-1}$)	C_{CO_2} ($\mu mol\ l^{-1}$)
Metabolism							
pH 7.99 (control)	0.08 ± 0.04						
pH 7.64	0.13 ± 0.11						
pH 7.35	0.14 ± 0.1						
Haemolymph							
pH 7.99 (control)		958.67 ± 55.32	342.38 ± 14.21	8.98 ± 2.53	11.15 ± 0.89	34.96 ± 1.89	4200.00 ± 1935.20
pH 7.64		953.00 ± 31.53	349.21 ± 105.49	8.27 ± 2.01	10.13 ± 1.39	33.37 ± 11.48	7209.33 ± 2944.51
pH 7.35		934.75 ± 66.81	376.02 ± 108.35	8.87 ± 2.68	10.02 ± 2.21	35.56 ± 12.02	5178.00 ± 2524.72
Abdominal somites and telson mineralisation							
pH 7.99 (control)			1.21 ± 0.39	0.22 ± 0.03	2.64 ± 0.44	0.48 ± 0.09	
pH 7.64			1.05 ± 0.16	0.22 ± 0.03	2.36 ± 0.55	0.42 ± 0.08	
pH 7.35			1.06 ± 0.09	0.20 ± 0.05	2.14 ± 1.16	0.40 ± 0.13	
Chelae mineralisation							
pH 7.99 (control)			0.66 ± 0.28	0.08 ± 0.05	5.12 ± 2.31	0.62 ± 0.27	
pH 7.64			0.76 ± 0.16	0.14 ± 0.04	7.17 ± 3.77	0.86 ± 0.42	
pH 7.35			0.84 ± 0.36	0.15 ± 0.09	5.17 ± 1.12	0.66 ± 0.09	

son of each shrimp was also calculated and no significant difference between treatments was detected (Kruskal-Wallis Test: p = 0.832, Table 2).

Chela

After a 35 d exposure to elevated pCO_2, levels of $[Ca^{2+}]$, $[Mg^{2+}]$, $[K^+]$ and $[Na^+]$ in the left chela of *Upogebia deltaura* did not differ significantly from the control treatment (pH 7.99) (maximum $F_{2,13}$ = 2.116, 337 p = 0.160, Table 2). The calcification ratio ($[Ca^{2+}]$: $[Mg^{2+}]$) in the left chela of each shrimp was also calculated and no significant difference between treatments was detected ($F_{2,13}$ = 0.671, p = 0.528, Table 2).

Activity analysis

Shrimp behaviour was significantly affected by pH (pseudo-F = 3.9483, p(perm) = 0.002). Other factors included in the analysis were sex, wet weight of the shrimps, the time of day when filming was conducted and the number of days of exposure at the time of filming, none of which were found to have a significant effect on shrimp behaviour. Pair-wise analysis revealed that the significant effect of pH on shrimp behaviour resulted from significant differences between pH 6.71 and the other pH treatment levels: pH 7.99 (t = 2.596, p(perm) = 0.006); pH 7.64 (t = 1.828, p(perm) = 0.04); pH 7.35 (t = 2.554, p(perm) = 0.004). SIMPER analysis showed that the differences in overall activity resulted predominantly from an increase in pleopod beating and a decrease in walking, flexing and cleaning behaviour in pH 6.71 compared with the other pH treatments (Fig. 2). At pH 6.71, shrimp spent an average of 35.02% of the time pleopod beating, compared with 3.13% at pH 7.99 (control), 9.47% at pH 7.64 and 5.35% at pH 7.34. In addition, at pH 6.71, shrimp spent an average of 5.34% of the time walking, compared with 19.56% at pH 7.99 (control), 11.06% at pH 7.64 and 15.63% at pH 7.35. Also, at pH 6.71, shrimp spent an average of 2.33% of the time flexing at, compared with 7.72% at pH 7.99 (control), 3.45% at pH 7.64 and 9.53% at pH 7.35. Finally, at pH 6.71 shrimp spent an average of 4.80% of the time cleaning, compared with 13.92% at pH 7.99 (control), 12.72% at pH 7.64 and 21.87% at pH 7.35.

DISCUSSION

There was no significant effect of exposure to an environmental pCO_2 of 1396 µatm (equivalent to pH 7.64) on the physiology and behaviour of adult individuals of *Upogebia deltaura* during 35 d of exposure. Physiological measurements included haemolymph acid–base parameters, oxygen consumption (used as a proxy for metabolism), osmotic regulation and shell mineralogy. However, at seawater pCO_2 of 2707 µatm (equivalent to pH 7.35) individual shrimps experienced uncompensated extracellular acidosis,

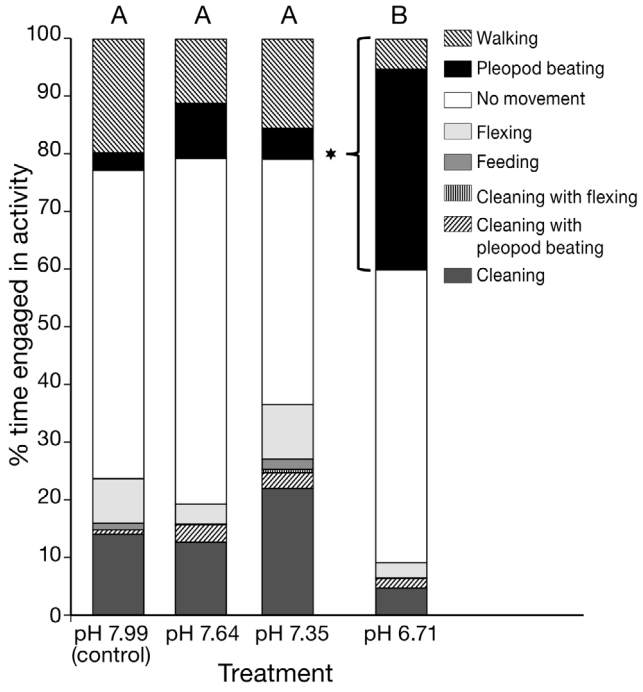

Fig. 2. *Upogebia deltaura*. Effects of seawater pCO_2 on the overall activity of shrimps during the 35 d exposure. Histograms represent the mean percentage time shrimp spent engaged in each of the 8 defined categories of activity (see 'Material and methods: Determination of shrimp activity'). Different letters represent significant differences between treatments and the asterisk (*) indicates the shrimp activities that contributed most to the observed differences between treatments

although there was no evidence of other physiological costs in terms of metabolism, osmotic regulation, growth and shell mineralogy. Furthermore, in seawater with pCO_2 of 14 109 µatm (equivalent to pH 6.71) no individual shrimp survived longer than 13 d on average. During exposure, before mortality occurred, shrimps in this treatment showed significant differences in overall activity compared with the shrimps in the other pH treatments. Our findings indicated that elevated pCO_2 conditions due to upwellings and leakages from carbon capture and storage (CCS) will probably exert severe negative effects on the behavioural performances, ecophysiological functions and survival of individuals of *U. deltaura*; however, this species appears to be able to cope with seawater pCO_2 levels similar to those predicted in coastal ocean acidification scenarios, notwithstanding possible implications for their scope for growth and reproduction (Pistevos et al. 2011, Stumpp et al. 2011) and (in the long term) survival.

Changes in environmental pH resulting from increases in ambient pCO_2 levels in seawater can lead to extracellular acidosis in marine decapod crustaceans (e.g. Pane & Barry 2007), which is characterised by a decrease in haemolymph pH due to an increase in haemolymph pCO_2 (Truchot 1979). In the present study there was no evidence of acidosis reported in adult individuals of *Upogebia deltaura* in treatment pH 7.64, and values reported for haemolymph pH, pCO_2 and $[HCO_3^-]$ were comparable with those in the control treatment. This suggests that pCO_2 levels similar to levels predicted to occur in the ocean acidification scenario for 2100 are within the physiological tolerances of this species. However, haemolymph of shrimps in treatment pH 7.35 showed a significant respiratory acidosis after a 35 d exposure (see Fig. 3.), which suggests they have a lower tolerance to elevated pCO_2 levels that would be analogous to CO_2 sequestration leakage events or levels found in upwelling zones (see Feely et al. 2010). The Davenport diagram (Fig. 3) shows a clear respiratory component to the reported acidosis in *U. deltaura* at a pH 7.35. Although the nonbicarbonate buffer line is not known for this species, the $[HCO_3^-]$ level is much lower than would be expected at this pH based on the nonbicarbonate buffer line of related crustaceans such as palaemonid shrimps (*Palaemon elegans* = −1.5 ± 0.5 mmol 0.1 pH^{-1}, Taylor & Spicer 1991), which suggests there is an additional metabolic component to the observed acidosis.

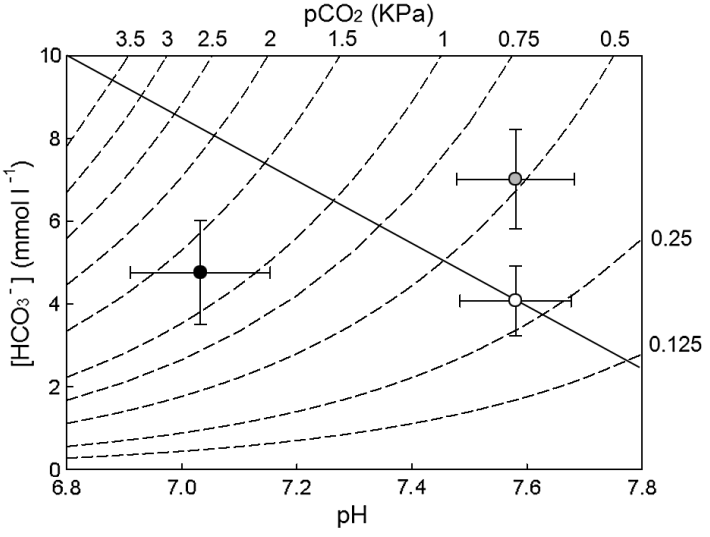

Fig. 3. *Upogebia deltaura*. Davenport diagram illustrating the relationship between haemolymph pH, $[HCO_3^-]$ and pCO_2 in shrimps after 35 d exposure to seawater at a pH of 7.99 (white), 7.64 (grey) and 7.35 (black). Solid black line shows the nonbicarbonate buffer line of a closely related crustacean, *Palaemon elegans*, of −1.5 ± 0.5 mmol 0.1 pH^{-1} (Taylor & Spicer 1991). Points represent means ± SE

Our results differ from previous studies (e.g. Spicer et al. 2007) that suggest marine decapod crustaceans are able to compensate (completely or in part), in the short term, for extracellular acidosis by increasing bicarbonate ion concentrations [HCO_3^-] in the haemolymph via shell dissolution (Truchot 1979, Cameron & Iwama 1987, Pane & Barry 2007, Spicer et al. 2007) and in the longer term by taking up bicarbonate from the surrounding seawater (Small et al. 2010) or by protein buffering (mainly haemocyanin, reviewed by Whiteley 2011). Our study showed no evidence of bicarbonate-buffering capacity in *Upogebia deltaura* when exposed to pH 7.35 for the reported extracellular acidosis, and no increase in haemolymph bicarbonate [HCO_3^-] via shell dissolution (no significant difference in shell mineralogy between the control and treatments pH 7.64 and 7.35) or uptake from surrounding seawater. Despite these considerations our results may lend further support to the conclusions of Cameron (1985) and Small et al. (2010) that buffering of extracellular pH by using [HCO_3^-] via shell dissolution may be a short-term response to elevated pCO_2 but may be unsustainable over prolonged periods of exposure, perhaps owing to the high energetic costs of ion regulation. Estimates of actual energetic costs of active ion transport associated with release or uptake of [HCO_3^-] in to the haemolymph range from 2.8 to 40% of metabolic expenditure, representing a significant energetic cost to the organism (Pannevis & Houlihan 1992, Leong & Manahan 1997).

Melatunan et al. (2011) suggested that a decrease in metabolic rate may be a mechanism for conserving energy levels and regulating intracellular pH, thereby enabling organisms to deal with fluctuations in environmental conditions (Sartoris & Pörtner 1997, Langenbuch & Pörtner 2002). This is supported by previous studies on an intertidal crustacean that reported a significant reduction in metabolic rates in response to elevated pCO_2 conditions (e.g. Small et al. 2010) and an inverse relationship between crustacean activity levels and haemolymph [Mg^{2+}] (Walters & Uglow 1981, Morritt & Spicer 1993, Wittmann et al. 2010, 2011). However, the present study reports no significant effect of elevated seawater pCO_2 (treatments pH 7.64 and 7.35) on metabolism, haemolymph cation concentrations ([Mg^{2+}], [Ca^{2+}], [K^+] and [Na^+]) and overall activity of *Upogebia deltaura*, although metabolic data should be discussed with some caution owing to the relatively small sample size employed here. In addition, there was no significant effect of elevated pCO_2 (treatments pH 7.64 and 7.35) on haemolymph osmolality in *U. deltaura*, suggesting that elevated pCO_2 conditions analogous to the predicted coastal OA scenario for 2100 may not affect ionic regulation in this species. This further supports the suggestion that pH 7.64 is within the physiological window of tolerance of *U. deltaura*. Furthermore, although there is no reported bicarbonate-buffering capacity for extracellular acidosis in shrimps exposed to treatment pH 7.35, there is no evidence of other physiological costs in terms of metabolism and growth, haemolymph cation concentration, total osmolality, or overall activity, for shrimps exposed to this treatment. In addition, it is worth noting that inherent low levels of metabolic rates in thalassinidean shrimps (Anderson et al. 1991, Stanzel & Finelli 2004), in comparison with other decapod crustaceans, may partly explain this group's natural tolerance to elevated pCO_2. This aspect of thalassinidean shrimp physiology may represent a specific adaptation of infaunal invertebrates (see Widdicombe & Spicer 2008, Melzner et al. 2009), which may already experience elevated levels of pCO_2 in nature (Astall et al. 1997, based on mesocosm observations). This has been suggested, but to a different extent, for intertidal organisms (McDonald et al. 2009, Melzner et al. 2009, Todgham & Hofmann 2009, Small et al. 2010). However, the present study also recorded a large physiological cost associated with extreme elevated pCO_2 conditions, in which there was 100% mortality and a significant change in activity (before mortality) for shrimps in treatment pH 6.71, compared with all other treatments. The shrimps spent a greater percentage of their time beating their pleopods, and less time walking and flexing. Pleopod beating behaviour in thalassinidean shrimp has been associated with the process of water renewal in the burrow (Astall et al. 1997). Upogebiids are primarily suspension feeders and the stimulus for burrow irrigation could be either nutritional or respiratory (Astall et al. 1997). Since the present study reported no significant change in weight or feeding activity, it is concluded that the stimulation for increased pleopod beating may be an attempt to reduce pCO_2 levels within the burrow and increase the diffusion gradient across the gill epithelia. As discussed above, significant respiratory, and probably metabolic, acidosis is observed at an external pCO_2 of 2707 µatm (Fig. 3) and this would be expected to greatly increase at 141 09 µatm (pH 6.71). Respiratory acidosis is known to stimulate ventilation in an attempt to reduce haemolymph pCO_2 and increase haemolymph pH. Metabolic acidosis could indicate an increased reliance on anaerobic metabolism, which could also stimulate hyperventilation (Smatresk & Cameron 1981) in an attempt to increase O_2 uptake to meet the

increased energetic demands of ion regulation. However, such mechanisms appear to be ineffective as 100% mortality was observed at an environmental pH of 6.71. Increased mortality is probably due to an increase in ATP demand coupled with a reduction of aerobic scope and the associated metabolic acidosis, and a loss of haemocyanin function at low pH (see Pörtner 2008, Melatunan et al. 2011). This suggests that elevated pCO_2 at levels analogous to CO_2 sequestration leakage events may exert a negative effect on benthic marine ecosystems.

Irrigation behaviour (pleopod beating), burrow construction and maintenance behaviours have been suggested as being important for the distribution of bacteria within the shrimp burrow and may directly influence the structure and composition of bacterial communities both within the burrow and in surrounding sediments. This in turn will determine the microbial transformations of important nutrients at the sediment–water interface (Laverock et al. 2010). Therefore, the reduction in walking and flexing behaviours, reported here for shrimps in treatment pH 6.71, may represent a reduction in burrow maintenance and building behaviour, which could lead to an alteration in the microbial transformation of nutrients. The recorded mortality of shrimps at pH 6.71 highlights the potentially significant negative consequences of upwellings and leakage from CCS. Although the present study reported no evidence of extra costs to shrimps exposed to elevated pCO_2 in treatments pH 7.64 and 7.35, it may be important to consider that, over an extended period of time, continuous exposure to extreme environmental conditions, such as elevated seawater pCO_2, may have implications for other physiological costs, such as reproduction, development and growth (e.g. Wood et al. 2008, Melatunan et al. 2011, Pistevos et al. 2011). In addition, previous studies have observed that shrimps will eventually leave their burrows when exposed to extreme environmental conditions (see Astall et al. 1997), which may be an attempt to relocate to an alternative site with more favourable conditions. However, this may increase the predation risk to shrimps and could result in a significant reduction in essential ecosystem processes, for example the cycling of key nutrients within coastal and shelf sea habitats (see Field et al. 1998, Dale & Prego 2002, Wootton et al. 2008, Laverock et al. 2010), which could result in further negative ecological consequences.

This study examined for the first time the effects of a range of elevated environmental pCO_2 conditions on both the behaviour and physiology of a relatively tolerant marine organism. *Upogebia deltaura* appears to be able to withstand exposure to pCO_2 conditions that are analogous to those predicted to occur with coastal OA scenarios, and is possibly one of the most tolerant crustaceans characterised so far. However, this species appears to function within a relatively small window of tolerance, suggesting it is probable that elevated pCO_2 conditions due to upwellings and CCS leakages could have significant negative implications for shrimp physiology and subsequently their behaviour. Widdicombe & Spicer (2008) have suggested that negative effects at the organism level would have significant implications at the community and ecosystem levels, and the high importance of *U. deltaura*, in terms of ecosystem function (e.g. Laverock et al. 2010), could therefore exacerbate the already negative effect. In addition, the examination of species that seem to demonstrate more tolerance to elevated pCO_2 conditions is an important step in increasing our understanding of how organisms may respond to elevated pCO_2 events and global climate change (Melzner et al. 20019), which will enable us to make better predictions about the ecological implications of elevated pCO_2 conditions in the oceans, and better decisions about proactive solutions to reduce CO_2 emissions.

Acknowledgements. We thank A. Fisher and A. Atfield for their technical support with the ion analyses. P.D. acknowledges receipt of the Marine Sciences Summer Studentship awarded by the University of Plymouth. This work was undertaken whilst P.C. was in receipt of a Research Council UK Research Fellowship to investigate ocean acidification at the University of Plymouth and F.C.M. was in receipt of a British Council UK Research Exchange Programme Award. This work is a contribution to the NERC funded programme Oceans 2025 (PML Theme 3 – Coastal and shelf processes) and is a contribution to the European Project on Ocean Acidification (EPOCA), which received funding from the European Community's Seventh Framework Programme (FP7/2007–2013) under grant agreement no. 211384.

LITERATURE CITED

Anderson MJ (2001) A new method for non-parametric multivariate analysis of variance. Austral Ecol 26:32–46

Anderson SJ, Atkinson RJA, Taylor AC (1991) Behavioral and respiratory adaptations of the mud-burrowing shrimp *Calocaris macandreae* Bell (Thalassinidea: Crustacea) to the burrow environment. Ophelia 34:143–156

Anderson MJ, Gorley RN, Clarke KR (2008) PERMANOVA+ for PRIMER: guide to software and statistical methods. PRIMER-E, Plymouth

Astall CM, Taylor Ac, Atkinson RJA (1997) Behavioural and physiological implications of a burrow-dwelling lifestyle for two species of upogebiid mud-shrimp (Crustacea: Thalassinidea). Estuar Coast Shelf Sci 44:155–168

Barry JP, Buck KR, Lovera CF, Kuhnz L and others (2004) Effects of direct ocean CO_2 injection on deep-sea meiofauna. J Oceanogr 60:759–766

Blackford JC, Jones N, Proctor R, Holt J, Widdicombe S, Lowe D, Rees A (2009) An initial assessment of the potential environmental impact of CO_2 escape from marine carbon capture and storage systems. Proc Inst Mech Eng A J Power Energy 223:269–282

Caldeira K, Wickett ME (2003) Anthropogenic carbon and ocean pH. Nature 425:365

Cameron JN (1985) Acid–base homeostasis: past and present perspectives. Physiol Zool 62:845–865

Cameron JN, Iwama GK (1987) Compensation of progressive hypercapnia in channel catfish and blue crabs. J Exp Biol 133:183–197

Cigliano M, Gambi MC, Rodolfo-Metalpa R, Patti FP, Hall-Spencer J (2010) Effects of ocean acidification on invertebrate settlement at volcanic CO_2 vents. Mar Biol 157: 2489–2502

Dale AW, Prego R (2002) Physico-biogeochemical controls on benthic–pelagic coupling of nutrient fluxes and recycling in a coastal upwelling system. Mar Ecol Prog Ser 235:15–28

de la Haye KL, Spicer JI, Widdicombe S, Briffa M (2011) Reduced sea water pH disrupts resource assessment and decision making in the hermit crab *Pagurus bernhardus*. Anim Behav 82:495–501

Dickson AG, Millero FJ (1987) A comparison of the equilibrium constants for the dissociation of carbonic-acid in seawater media. Deep-Sea Res 34:1733–1743

Dickson AG (1990) Thermodynamics of the dissociation of boric-acid in potassium-chloride solutions form 273.15-K to 318.15 K. J Chem Thermodynam 22:113–127

DeWitt T, D'Andrea AF, Brown CA, Griffen B, Eldridge PM (2004) Impact of burrowing shrimp populations on nitrogen cycling and water quality in western North American temperate estuaries. In: A. Tamaki (ed) Proceedings of the symposium on ecology of large bioturbators in tidal flats and shallow sublittoral — from individual behavior to their role as ecosystem engineers. Nagasaki University, Nagasaki, p 107–118

Feely RA, Sabine CL, Hernandez-Ayon JM, Ianson D, Hales B (2008) Evidence for upwelling of corrosive 'acidified' water onto the continental shelf. Science 320:1490–1492

Feely RA, Alin SR, Newton J, Sabine CL and others (2010) The combined effects of ocean acidification, mixing, and respiration on pH and carbonate saturation in an urbanized estuary. Estuar Coast Shelf Sci 88:442–449

Field CB, Behrenfeld MJ, Randerson JT, Falkowski P (1998) Primary production of the biosphere: integrating terrestrial and oceanic components. Science 281:237–240

Gibbins J, Haszeldine S, Holloway S, Pearce J, Oakey J, Shackley S, Turley C (2006) Scope for future CO_2 emission reductions from electricity generation through the deployment of carbon capture and storage technologies. In: Schellnhuber HJ, Cramer W, Nakicenovic N, Wigley T, Yohe G (eds) Avoiding dangerous climate change. Cambridge University Press, Cambridge, p 379–383

Hale R, Calosi P, McNeill L, Mieszkowska N, Widdicombe S (2011) Predicted levels of future ocean acidification and temperature rise could alter community structure and biodiversity in marine benthic communities. Oikos 120: 661–674

Hall-Spencer JM, Rodolfo-Metalpa R, Martin S, Ransome E and others (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454: 96–99

Hawkins DG (2004) No exit: thinking about leakage from geologic carbon storage sites. Energy 29:1571–1578

Haywood PJ, Ryland JS (2005) Handbook of the marine fauna of north-west Europe. Oxford University Press, Oxford

Holloway S (2005) Underground sequestration of carbon dioxide — a viable greenhouse gas mitigation option. Energy 30:2318–2333

Jones CG, Lawton JH, Shachak M (1994) Organisms as ecosystem engineers. Oikos 69:373–386

Kroeker KJ, Micheli F, Gambi MC, Martz TR (2011) Divergent ecosystem response within a benthic marine community to ocean acidification. Proc Natl Acad Sci USA 108:14515–14520

Langenbuch M, Pörtner HO (2002) Changes in metabolic rate and N excretion in the marine invertebrate *Sipunculus nudus* under conditions of environmental hypercapnia: identifying effective acid–base variables. J Exp Biol 205:1153–1160

Laverock B, Smith C, Tait K, Osborn M, Widdicombe S, Gilbert J (2010) Bioturbating shrimp alter the structure and diversity of bacterial communities in coastal marine sediments. ISME J 4:1531–1544

Leong PKK, Manahan DT (1997) Metabolic importance of Na^+/K^+-ATPase activity during sea urchin development. J Exp Biol 200:2881–2892

Marchant HK, Calosi P, Spicer JI (2010) Short-term exposure to hypercapnia does not compromise feeding, acid-base balance or respiration of *Patella vulgata* but surprisingly is accompanied by radula damage. J Mar Biol Assoc UK 90:1379–1384

Martin S, Rodolfo-Metalpa R, Ransome E, Rowley S, Buia MC, Gattuso JP, Hall-Spencer J (2008) Effects of naturally acidified seawater on seagrass calcareous epibionts. Biol Lett 4:689–692

Mayer MS, Schaffner L, Kemp WM (1995) Nitrification potentials of benthic macrofaunal tubes and burrow walls: effects of sediment NH_4^+ and animal irrigation behaviour. Mar Ecol Prog Ser 121:157–169

McArdle BH, Anderson MJ (2001) Fitting multivariate models to community data: a comment on distance-based redundancy analysis. Ecology 82:290–297

McDonald MR, McClintock JB, Amsler CD, Rittschof D, Angus RA, Orihuela B, Lutostanski K (2009) Effects of ocean acidification over the life history of the barnacle *Amphibalanus amphitrite*. Mar Ecol Prog Ser 385: 179–187

Meehl GA, Stocker TF, Collins WD, Friedlingstein P and others (2007) Global climate projections. In: Solomon S, Qin D, Manning M, Chen Z, Marquis M, Averyt KB, Tignor M, Miller HL (eds) Climate change 2007: the physical science basis. Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge

Mehrbach C, Culberso CH, Hawley JE, Pytkowic RM (1973) Measurement of apparent dissociation-constants of carbonic-acid in seawater at atmospheric-pressure. Limnol Oceangr 18:897–907

Melatunan S, Calosi P, Rundle SD, Moody JA, Widdicombe S (2011) Exposure to elevated temperature and pCO2 reduces respiration rate and energy status in the periwinkle *Littorina littorea*. Physiol Biochem Zool 84:583–594

Melzner F, Gutowska MA, Langenbuch M, Dupont S and others (2009) Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6:2313–2331

Miles H, Widdicombe S, Spicer JI, Hall-Spencer J (2007) Effects of anthropogenic seawater acidification on acid–base balance in the sea urchin *Psammechinus miliaris.* Mar Pollut Bull 54:89–96

Morritt D, Spicer JI (1993) A brief re-examination of the function and regulation of extracellular magnesium and its relationship to activity in crustacean arthropods. Comp Biochem Physiol A Physiol 106:19–24

Munday PL, Dixson DL, McCormick MI, Meekan M, Ferrari MCO, Chivers DP (2010) Replenishment of fish populations is threatened by ocean acidification. Proc Natl Acad Sci USA 107:12930–12934

Orr JC, Fabry VJ, Aumont O, Bopp L and others (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. Nature 437:681–686

Pane EF, Barry JP (2007) Extracellular acid-base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. Mar Ecol Prog Ser 334:1–9

Pannevis MC, Houlihan DF (1992) The energetic cost of protein synthesis in isolated hepatocytes of rainbow trout (*Oncorhynchus mykiss*). J Comp Physiol B Biochem Syst Environ Physiol 162:393–400

Pierrot D, Lewis E, Wallace DWR (2006) DOS program developed for CO_2 system calculations. ORNL/CDIAC-105. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, US Department of Energy, Oak Ridge, TN

Pistevos JCA, Calosi P, Widdicombe S, Bishop JDD (2011) Will variation among genetic individuals influence species responses to global climate change? Oikos 120:675–689

Pörtner HO (2008) Ecosystem effects of ocean acidification in times of ocean warming: a physiologist's view. Mar Ecol Prog Ser 373:203–217

Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O and others (2005) Ocean acidification due to increasing atmospheric carbon dioxide. R Soc Policy Doc 12/05. Clyvedon Press, Cardiff

Salisbury J, Green M, Hunt C, Campbell J (2008) Coastal acidification by rivers: a threat to shellfish? Eos Trans Am Geophys Union 89:513–528

Sartoris FJ, Pörtner HO (1997) Increased concentrations of haemolymph Mg2+ protect intracellular pH and ATP levels during temperature stress and anoxia in the common shrimp *Crangon crangon.* J Exp Biol 200:785–792

Small D, Calosi P, White D, Spicer J, Widdicombe S (2010) Impact of medium term exposure to CO_2 enriched seawater on the physiological functions of the velvet swimming crab, *Necora puber*. Aquat Biol 10:11–21

Smatresk NJ, Cameron JN (1981) Post-exercise acid-base balance and ventilatory control in *Birgus latro*, the coconut crab. J Exp Zool 218:75–82

Spicer JI, Raffo A, Widdicombe S (2007) Influence of CO_2-related seawater acidification on extracellular acid–base balance in the velvet swimming crab *Necora puber.* Mar Biol 151:1117–1125

Stanzel C, Finelli C (2004) The effects of temperature and salinity on the ventilation behaviour of two species of ghost shrimp (Thalassinidea) from the northern Gulf of Mexico: a laboratory study. J Exp Mar Biol Ecol 312:19–41

Stumpp M, Wren J, Melzner F, Thorndyke MC, Dupont ST (2011) CO_2 induced seawater acidification impacts sea urchin larval development I: elevated metabolic rates decrease scope for growth and induce developmental delay. Comp Biochem Physiol A Mol Integr Physiol 160: 331–340

Taylor AC, Spicer JI (1991) Acid-base disturbances in the haemolymph of the prawns, *Palaemon Elegans* (Rathke) and *P. Serratus* (Pennant) (Crustacea: Decapoda) during exposure to hypoxia. Comp Biochem Physiol A Physiol 98:445–452

Thomsen J, Gutowska MA, Saphörster J, Heinemann A and others (2010) Calcifying invertebrates succeed in a naturally CO_2-rich coastal habitat but are threatened by high levels of future acidification. Biogeosciences 7:3879–3891

Todgham AE, Hofmann GE (2009) Transcriptomic response of sea urchin larvae, *Strongylocentrotus purpuratus*, to CO_2-driven seawater acidification. J Exp Biol 212:2579–2594

Truchot JP (1976) Carbon dioxide combining properties of the blood of the shore crab *Carcinus maenas* (L): carbon dioxide solubility coefficient and carbonic acid dissociation constants. J Exp Biol 64:45–57

Truchot JP (1979) Mechanisms of the compensation of blood respiratory acid-base disturbances in the shore crab, *Carcinus maenas* (L.). J Exp Zool 210:407–416

Walters NJ, Uglow RF (1981) Haemolymph magnesium and relative heart activity of some species of marine decapod crustaceans. J Exp Mar Biol Ecol 55:255–265

Webb AP, Eyre BD (2004) Effect of natural populations of burrowing thalassinidean shrimp on sediment irrigation, benthic metabolism, nutrient fluxes and denitrification. Mar Ecol Prog Ser 268:205–220

Whiteley NM (2011) Physiological and ecological responses of crustaceans to ocean acidification. Mar Ecol Prog Ser 430:257–271

Widdicombe S, Needham HR (2007) Impact of CO_2-induced seawater acidification on the burrowing activity of *Nereis virens* and sediment nutrient flux. Mar Ecol Prog Ser 341:111–122

Widdicombe S, Spicer JI (2008) Predicting the impact of ocean acidification on benthic biodiversity: What can animal physiology tell us? J Exp Mar Biol Ecol 366: 187–197

Widdicombe S, Austen MC, Kendall MA, Warwick RM, Jones MB (2000) Bioturbation as a mechanism for setting and maintaining levels of diversity in subtidal macrobenthic communities. Hydrobiologia 440:369–377

Widdicombe S, Dashfield SL, McNeill CL, Needham HR and others (2009a) Effects of CO_2 induced seawater acidification on infaunal diversity and sediment nutrient fluxes. Mar Ecol Prog Ser 379:59–75

Widdicombe S, Dupont S, Thorndyke M (2009b) Experimental design perturbation experiments: 7. Laboratory experiments and benthic mesocosm studies. In: Riebesell U, Fabry VJ, Hansson L, Gattuso JP (eds) Guide for best practices in ocean acidification research and data reporting. Eur Proj Ocean Acidification (EPOCA), Villefranche-sur-mer, p 113–122

Widdicombe S, Spicer JI, Kitidis V (2011). Effects of ocean acidification on sediment fauna. In: Gattuso JP, Hansson L (eds) Ocean acidification. Oxford University Press, Oxford, p 176–187

Wittmann AC, Held C, Pörtner HO, Sartoris FJ (2010) Ion regulatory capacity and the biogeography of Crustacea at high southern latitudes. Polar Biol 33:919–928

Wittmann AC, Storch D, Anger K, Pörtner HO, Sartoris FJ (2011) Temperature-dependant activity in early life stages of the stone crab *Paralomis granulosa* (Decapoda, Anomura, Lithodidae): a role for ionic and magnesium regulation? J Exp Mar Biol Ecol 397:27–37

Wood HL, Spicer JI, Widdicombe S (2008) Ocean acidification may increase calcification rates, but at a cost. Proc Biol Sci 275:1767–1773

Wood HL, Spicer JL, Widdicombe S (2009) The influence of hypercapnia and the infaunal brittlestar *Amphiura filiformis* on sediment nutrient flux — will ocean acidification affect nutrient exchange? Biogeosciences 6: 2015–2024

Wood HL, Spicer JL, Lowe DM (2010) Interaction of ocean acidification and temperature; the high cost of survival in the brittlestar *Ophiura ophiura*. Mar Biol 157:2001–2013

Wootton JT, Pfister CA, Forester JD (2008) Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. Proc Natl Acad Sci USA 105:18848–18853

Editorial responsibility: Hans Heinrich Janssen, Oldendorf/Luhe, Germany

Submitted: May 9, 2011; Accepted: January 9, 2012
Proofs received from author(s): March 6, 2012