Vol. 10: 11–21, 2010
doi: 10.3354/ab00266

AQUATIC BIOLOGY
Aquat Biol

Published online June 22

Impact of medium-term exposure to CO_2 enriched seawater on the physiological functions of the velvet swimming crab *Necora puber*

Daniel Small[1,*], Piero Calosi[1], Daniel White[2], John I. Spicer[1], Steve Widdicombe[2]

[1]Marine Biology and Ecology Research Centre, University of Plymouth, Drake Circus, Plymouth PL4 8AA, UK
[2]Plymouth Marine Laboratory, Prospect Place, West Hoe, Plymouth PL1 3DH, UK

ABSTRACT: Ocean acidification (OA) is predicted to play a major role in shaping species biogeography and marine biodiversity over the next century. We tested the effect of medium-term exposure to OA (pH 8.00, 7.30 and 6.70 for 30 d) on acid–base balance in the decapod crab *Necora puber*—a species that is known to possess good extracellular buffering ability during short-term exposure to hypercapnic conditions. To determine if crabs undergo physiological trade-offs in order to buffer their haemolymph, we characterised a number of fundamental physiological functions, i.e. metabolic rate, tolerance to heat, carapace and chelae [Ca^{2+}] and [Mg^{2+}], haemolymph [Ca^{2+}] and [Mg^{2+}], and immune response in terms of lipid peroxidation. *Necora puber* was able to buffer changes to extracellular pH over 30 d exposure to hypercapnic water, with no evidence of net shell dissolution, thus demonstrating that HCO_3^- is actively taken up from the surrounding water. In addition, tolerance to heat, carapace mineralization, and aspects of immune response were not affected by hypercapnic conditions. In contrast, whole-animal O_2 uptake significantly decreased with hypercapnia, while significant increases in haemolymph [Ca^{2+}] and [Mg^{2+}] and chelae [Mg^{2+}] were observed with hypercapnia. Our results confirm that most physiological functions in *N. puber* are resistant to low pH/hypercapnia over a longer period than previously investigated, although such resistance comes at the expenses of metabolic rates, haemolymph chemistry and chelae mineralization.

KEY WORDS: Ocean acidification · Physiology · Metabolic depression · *Necora puber* · Thermal tolerance · Carbon capture and storage

Resale or republication not permitted without written consent of the publisher

INTRODUCTION

Atmospheric carbon dioxide (CO_2) levels are predicted to reach 1000 ppm by 2100 and exceed 1900 ppm by 2300 (Caldeira & Wickett 2003, Raven et al. 2005). The oceans have absorbed almost half of the anthropogenically produced CO_2 emitted to date (Sabine et al. 2004), causing a negative effect on ocean chemistry via a reduction in pH and disruption of the carbonate system (Fabry et al. 2008)—a process termed ocean acidification (OA). Surface water pH has already dropped by 0.1 unit (Orr et al. 2005) and current predictions forecast reductions of 0.5 to 0.7 units by the years 2100 and 2300, respectively (Caldeira & Wickett 2003). Wootton et al. (2008) have warned that declines are already more than an order of magnitude greater than previously expected. An associated net decline in ocean [CO_3^{2-}] and related changes in aragonite and calcite saturation states (Feely et al. 2004, Orr et al. 2005, Andersson et al. 2008) would create waters that are potentially corrosive to calcium carbonate and could compromise organisms' calcification rates and the integrity of calcium carbonate structures (see Findlay et al. 2009). Marine organisms could already be experiencing sudden and severe acidification events, such as the periodic upwelling of undersaturated aragonite waters observed in coastal ecosystems off the northwest USA (Feely et al. 2004). These events could become more common and more severe as the oceans absorb more CO_2. In addition, the future use of geo-

*Email: daniel.small@students.plymouth.ac.uk

© Inter-Research 2010 · www.int-res.com

logical storage to reduce CO_2 emissions (Holloway 2005) brings with it the risk of possible leaks from pipelines and subseabed storage sites, and considerable subsequent localised impact on benthic communities even in shallow water ecosystems (Blackford et al. 2009, Kano et al. 2010), possibly including the intertidal zone.

The coastal ecosystem is a focal point for ocean productivity and human activity (Wootton et al. 2008), thus there is a pressing need for studies that quantify the impacts of high CO_2 on coastal marine species (Raven et al. 2005, Widdicombe & Spicer 2008, Wootton et al. 2008). However, the vast majority of previous work has almost exclusively focused on potentially nontolerant species (see Widdicombe & Spicer 2008). In order to understand and predict the impacts of OA on organism survival and ultimately on global marine biodiversity, it is essential to investigate what makes some species tolerant (Widdicombe & Spicer 2008). The velvet swimming crab *Necora puber* has been identified as a potentially tolerant species to OA, as it is able to compensate for extracellular acidosis, with bicarbonate HCO_3^- anion exchange playing an important role in restoring acid–base balance during short-term exposure to low pH/hypercapnia (Spicer et al. 2007). While this is consistent with what is known for other decapod crustaceans (Cameron & Iwama 1987, Pörtner et al. 2004), no other information on the effect of hypercapnia on this species is known to date. For example, while internal regulation may be achieved by *N. puber*, this is an energetically expensive process (Cameron & Mangum 1983) with associated costs. OA is known to affect several functions of marine invertebrates including metabolic rates (Reipschläger & Pörtner 1996, Langenbuch & Pörtner 2002), haemolymph [Mg^{2+}] (Sartoris & Pörtner 1997), heat tolerance (Metzger et al. 2007, Walther et al. 2009) and immune responses (Bibby et al. 2008). In order to build a comprehensive picture on the likely responses of a species to prolonged exposure to hypercapnia, it is necessary to monitor a wide range of physiological parameters (see Wood et al. 2008 for examples).

Necora puber is ecologically important in rocky shore intertidal habitats as it feeds on keystone intertidal species such as limpets (Silva et al. 2008), thereby influencing the structure and diversity of intertidal communities (Griffin et al. 2008). It is also commercially important in Europe (Freire & González-Gurriarán 1995, Wilhelm & Mialhe 1996, Henderson & Leslie 2006, Robson et al. 2007). Finally, the vertical distribution range of *N. puber* along the shore reaches into the intertidal area, and certain populations may at times be exposed to extreme temperatures (Hopkin et al. 2006) and already occurring fluctuations in external pH (Agnew & Taylor 1986).

In the current study, we characterise the acid–base compensation ability of *Necora puber* after a longer exposure period (30 d) than has previously been achieved and identify the potential mechanisms of HCO_3^- buffering used by this species. Most importantly, we examine whether any sustained elevated acid–base haemolymph buffering ability shown by *N. puber* comes at the expense of other fundamental physiological functions and the overall fitness of this organism. To achieve this, a series of key physiological functions were measured in adult *N. puber* that were exposed to hypercapnia for 30 d.

MATERIALS AND METHODS

Animal collection. Adult individuals of *Necora puber* (intermoult male, carapace width 45.88 ± 7.32 mm) were collected from the lower intertidal zone at Mount Batten Beach (Plymouth, UK; 50° 21′ 34″ N, 04° 07′ 45″ W) on 20 Aug 2008 during spring tide. Crabs were transported to the Plymouth Marine Laboratory in plastic buckets containing damp macroalgae within 30 min of collection. Upon arrival, they were transferred to holding aquaria (1000 l) containing filtered, constantly aerated seawater (T = 16°C, salinity = 34.6). Crabs were held for a maximum of 3 d before use in any experiment. They were not fed during this time.

Experimental set-up. Twelve plastic aquaria (4 l each) were haphazardly allocated to 1 of 3 different pH treatment levels (i.e. 4 aquaria level^{-1}). The treatment levels were nominally pH 8.0 (control), 7.3, and 6.7. Experimental pH levels were chosen to mimic values predicted for the year 2100 (Caldeira & Wickett 2003, but see Wootton et al. 2008) and levels potentially resulting from CO_2 storage or pipeline leaks (Blackford et al. 2008). Considering the greater drop in pH already experienced by marine organisms in the intertidal area (Agnew & Taylor 1986), the potential impact of upwellings on [CO_2] (Feely et al. 2004), and the fact that pH appears to be decreasing at a greater rate (see Wootton et al. 2008) than is predicted by mathematical models (e.g. Caldeira & Wickett 2003, Raven et al. 2005), we have chosen a slightly lower pH (7.3) than that predicted for 2100 by Caldeira & Wickett (2003).

Each aquarium was continuously supplied with seawater from the appropriate header tank at a constant rate (30 ml min^{-1}) using peristaltic pumps. 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 2 small mesh bags (40 × 50 mm, made from 0.5 mm diameter mesh net) containing activated carbon to prevent ammonia accumulation, and a small aquarium pump (Cascade 300 Inter-

nal Filter, Penn-Plax) to ensure adequate water circulation. Nominal temperature for the exposure period was 17°C.

At the start of the exposure period, 48 individuals were haphazardly assigned to 1 of the 3 pH treatment levels (16 ind. treatment^{-1}), ensuring an equal distribution of body sizes across the different treatments. Each crab was marked on the carapace using nail polish (Claire's Accessories) for identification. The 16 individuals allocated to each treatment were randomly assigned to 1 of the 4 designated aquaria, with 4 individuals being placed into each aquarium. Four individual compartments were constructed within each aquarium using a plastic garden mesh (square mesh shape, 20 mm width, Endsleigh Garden Center), which minimised conflict and interactions between individuals. The exposure period was 30 d. Preliminary experiments indicated that feeding the crabs during the first 2 wk of exposure resulted in elevated ammonia excretion and high mortality, so crabs were not fed until 15 d into the experiment. After this time, crabs were fed once with fresh mussels (2 g) that were placed in the crab's cage and left for 30 min, after which all uneaten material was removed. Qualitative observations indicated no differences in feeding behaviours or consumption between treatments.

Experimental monitoring. Throughout the exposure period, water samples were taken daily from the header tanks and from each of the exposure aquaria, and pH, total CO_2 (TCO_2), temperature, and salinity were measured. pH was measured with a pH meter (Inlab 413SG pH electrode and Sevengo pH meter, Mettler Toledo) using the USA National Bureau of Standards (NBS), TCO_2 using a CO_2 analyser (965D, Corning Diagnostics), and temperature and salinity using a handheld multimeter (TA 197 LF, WTW). Additional carbonate system parameters (pCO_2, alkalinity, calcite and aragonite saturation (Ω_{calc} and Ω_{arag}, respectively), [HCO_3^-], and [CO_3^{2-}]) were calculated using CO2SYS (Pierrot et al. 2006) with dissociation constants from Mehrbach et al. (1973) refitted by Dickson & Millero (1987) and KSO_4 values from Dickson (1990). Over the course of the 30 d exposure, there were 12 mortalities, all of which could be attributed to accidental interactions between individuals, leaving 36 individuals spread over the 3 treatments: 11 at nominal pH 8.0, 13 at pH 7.3 and 12 at pH 6.7.

All individuals were maintained in their designated treatment water during the test for the determination of metabolic rates and thermal limits. This prevented the possibility that the outcome of our tests would be affected by the recovery of stressed individuals that were returned to ambient seawater. All oxygen probes were recalibrated before each set of trials using crabs from different water treatments. At the end of each test, each individual was returned to its experimental aquarium and left for at least 1 h before being tested for another variable or being sacrificed to obtain tissue samples.

Determination of metabolic rates. In order to determine *Necora puber* metabolic rate responses to hypercapnia at the end of the 30 d exposure period, resting rates of O_2 uptake (used as a proxy for metabolic rate) were measured using the method described by Spicer & Eriksson (2003) for larvae of the Norway lobster *Nephrops norvegicus*. Two different sized respirometers were used: 750 ml for small individuals (<30 g body mass) and 1500 ml for larger individuals (>30 g body mass). Each incubation chamber was equipped with a magnet that was capped with a perforated Petri dish, and placed over an underwater magnetic stirrer (Rank Brothers) to ensure even mixing of O_2. Individuals were allowed to rest for 30 min before the respirometers were closed and left for 20 min, and pO_2 was measured before and after using an O_2 electrode (1302 Oxygen Electrode, Strathkelvin Instruments) that was connected to a calibrated meter (Oxygen Meter 781, Strathkelvin Instruments). Percent O_2 saturation was never allowed to fall under 70% to avoid hypoxia and oxygen uptake was expressed as µl O_2 (mg wet mass)$^{-1}$ h^{-1}.

Determination of upper thermal tolerance. Tolerance to heat in adult individuals of *Necora puber* exposed to hypercapnic conditions for 30 d was determined by measuring upper thermal limits using predefined endpoints as proxies: loss of righting response (LRR) and onset of spasms (OS), in approximately half of the individuals per treatment (5, 6 and 6 ind. at nominal pH 8.0, 7.3 and 6.7, respectively), which were haphazardly selected. LRR was defined as the temperature at which crabs were unable to right themselves after 1 min of being inverted onto their dorsal surface (Lutterschmidt & Hutchinson 1997), while OS was defined as the temperature at which spasms first occurred (Lutterschmidt & Hutchinson 1997). A computer-controlled water bath (LTC 6-30, Grant Instruments) that was operated using custom-made software (Grant Coolwise Cooler Control Software, Grant Instruments) was attached to an external cylindrical shaped glass dish, which was filled with seawater. The dish was wrapped in a fine aquarium tubing (5 mm ∅), which formed a tight coil around the bottom section and sidewall of the dish. Distilled water heated by the bath was passed through the tube coil and then recirculated to the water bath. The entire apparatus was enclosed in insulating foam (2 mm thick) to ensure a good level of thermal isolation and thus an efficient warming of the seawater inside the dish. The temperature of the seawater that was inside the dish was raised at a constant rate of 0.75 ± 0.10°C min^{-1} and constantly monitored

using a thermometer (HH806AU, Omega Engineering).

Individuals were allowed to settle for 30 min before the start of experiments. During the experiments, water temperature and individuals were monitored continuously using protocols in Cuculescu et al. (1998) until the temperature of occurrence of LRR and OS was reached.

Measurement of haemolymph acid–base status. Key haemolymph acid–base parameters (pH and TCO_2) were measured in the remaining 19 individuals (6, 7 and 6 ind. at nominal pH 8.0, 7.3 and 6.7, respectively). Haemolymph (~800 µl) was extracted by inserting a hypodermic syringe (1 ml) that was attached to a 26 G needle directly into the infrabranchial sinus via the arthrodial membrane between the 2nd and 3rd abdominal segments or via the arthrodial membrane at the base of the 4th pereiopod. Haemolymph was carefully extracted in order to obtain clean, clear samples, and placed into a microcentrifuge tube (Eppendorf, 1.6 ml). The sample was immediately analysed for TCO_2 by pipetting a 100 µl subsample into a CO_2 analyser (965D, Corning Diagnostics). Haemolymph pH was also immediately measured by immersing a micro-pH probe (Micro-Inlab pH combination electrode, Mettler Toledo) that was linked to a pH meter (Seven Easy pH meter, Mettler Toledo) into the remaining haemolymph. The shape of the base of the microcentrifuge tube used to hold the sample fitted the shape of the pH probe, allowing anaerobic measurements. Haemolymph pCO_2 and HCO_3^- were calculated using Eqs. (1) and (2) respectively (both from Truchot 1976):

$$pCO_2 = TCO_2/\alpha(10^{pH-pK'1} + 1) \quad (1)$$

$$[HCO_3^-] = TCO_2 - \alpha\, pCO_2 \quad (2)$$

where α is the solubility coefficient of CO_2 in crab haemolymph (0.376 mmol l^{-1} kPa^{-1}, Truchot 1976) and $pK'1$ is the first dissociation constant of carbonic acid in *C. maenas* haemolymph (6.027, Truchot 1976). Once these procedures were accomplished, the remaining haemolymph was placed on ice to prevent clotting prior to being frozen in liquid nitrogen and stored for further analyses.

Measurement of oxidative stress and divalent ions. Levels of immune response were evaluated by measuring concentrations of lipid peroxide (LPO) in all haemolymph samples. These were determined directly in untreated haemolymph samples (100 µl) using a diagnostic kit (LPO-CC Kit; K-ASSAY, Kanimaya Biomedical Company). The haemolymph samples were then analysed for $[Ca^{2+}]$ and $[Mg^{2+}]$ as follows: subsamples (10 µl) were diluted in acid washed volumetric flasks (2 ml) by a factor of 200 using ultrapure water and analysed using an atomic absorption spectrometer (Spectr AA 50, Varian).

Determination of shell mineralization. In order to determine whether hypercapnia caused an alteration to the shell mineralogy of *Necora puber*, all 36 individuals were dissected and the right chelae dactyl and carapace retained and frozen for analysis of $[Ca^{2+}]$ and $[Mg^{2+}]$. The samples were scrubbed clean of all soft organic material before being freeze dried at −50°C for 24 h. They were then stored in a desiccation chamber before ~70 mg was taken, weighed, digested in 2 ml nitric acid (70% concentration, trace analysis grade), diluted to 50 ml with ultrapure water, and analysed to determine $[Ca^{2+}]$ and $[Mg^{2+}]$ using an atomic absorption spectrometer (Spectr AA 600, Varian).

Statistical analysis. All data were first analysed with a Kolmogorov-Smirnov test for normality, followed by a Levene's test of equality of error variances to test for homogeneity. If assumptions were met, a 1-way nested analysis of covariance (ANCOVA), in combination with a Tukey-Kramer post hoc test, was employed to analyse the data. If the assumptions of normality or homogeneity were not met, the data were $\log_{10}$ transformed before being reanalysed. pH was nested within tank, and body mass used as a covariant. Where no significant effect of tank or body mass ($p < 0.05$) was found, these factors were excluded from analysis.

RESULTS

Experimental conditions

Means and SDs of seawater physical parameters measured from the header tanks and experimental aquaria throughout the 30 d, along with any significant differences between treatment levels, are summarised in Table 1.

The pH was significantly lowered between the control pH 7.85 (nominal pH 8.0) and both hypercapnic conditions: pH 7.26 (nominal pH 7.30) and pH 6.69 (nominal pH 6.70). The slight differences in CO_2 between the aquaria and header tanks are likely due to the respiration of crabs, and bacteria in the tubes supplying water. Temperature and salinity did not differ significantly, while $[HCO_3^-]$ in the aquaria increased significantly under hypercapnia and $[CO_3^{2-}]$ significantly decreased. Alkalinity did not increase significantly with pH, while Ω_{calc} and Ω_{arag} decreased. Field temperature ranges and thermal profiles are presented in Table A1. The average (±SD) temperature at the top of the vertical zonation of *Necora puber* in the intertidal area was 13.80 ± 2.07°C, with a maximum of 19.20°C, and the average (±SD) temperature at spring low water was 14.44 ± 0.79°C, with a maximum of 18.20°C. While the experimental conditions were at

Table 1. Water physical parameters (mean ± SD) measured throughout the 30 d exposure period for experimental aquaria and header tanks. Different superscript letters represent significant differences among treatments (based on the Tukey-Kramer test), separately for experimental aquaria and header tanks

Variable	Nominal pH 8	7.3	6.7
Aquaria			
pH	7.85 ± 0.11^{A}	7.26 ± 0.11^{B}	6.69 ± 0.11^{C}
TCO_2 (mmol l^{-1})	1.71 ± 0.20^{A}	1.93 ± 0.19^{B}	2.30 ± 0.29^{C}
Salinity	34.6 ± 0.10	34.6 ± 0.10	34.6 ± 0.10
Temperature (°C)	17.30 ± 1.40	17.30 ± 1.40	17.30 ± 1.30
pCO_2 (µatm)	734.00 ± 244.46^{A}	3205.68 ± 794.70^{B}	12341.23 ± 3898.90^{C}
Alkalinity (µEq kg^{-1})	1795.78 ± 206.20	1851.50 ± 180.32	1878.31 ± 225.66
Calcite saturation (Ω_{calc})	1.73 ± 0.46^{A}	0.49 ± 0.14^{B}	0.14 ± 0.03^{C}
Aragonite saturation (Ω_{arag})	1.12 ± 0.30^{A}	0.32 ± 0.09^{B}	0.09 ± 0.02^{C}
HCO_3^- (mmol l^{-1})	1.61 ± 0.19^{A}	1.80 ± 0.17^{B}	1.86 ± 0.22^{B}
CO_3^{2-} (mmol l^{-1})	0.072 ± 0.019^{A}	0.020 ± 0.006^{B}	0.005 ± 0.001^{C}
Header tanks			
pH	8.05 ± 0.05^{A}	7.32 ± 0.05^{B}	6.42 ± 0.10^{C}
TCO_2 (mmol l^{-1})	1.66 ± 0.27^{A}	1.95 ± 0.24^{B}	2.42 ± 0.37^{C}
Salinity	34.6 ± 0.10	34.6 ± 0.00	34.6 ± 0.1
Temperature (°C)	19.50 ± 0.30^{A}	20.30 ± 0.20^{B}	20.10 ± 1.20^{C}
pCO_2 (µatm)	437.67 ± 85.06^{A}	2828.92 ± 404.58^{B}	21467.15 ± 4703.81^{C}
Alkalinity (µEq kg^{-1})	1830.17 ± 286.59^{AB}	1899.85 ± 230.47^{A}	1733.56 ± 284.16^{AB}
Calcite saturation (Ω_{calc})	2.76 ± 0.57^{A}	0.63 ± 0.11^{B}	0.08 ± 0.03^{C}
Aragonite saturation (Ω_{arag})	1.79 ± 0.37^{A}	0.41 ± 0.07^{B}	0.05 ± 0.02^{C}
HCO_3^- (mmol l^{-1})	1.53 ± 0.24^{A}	1.83 ± 0.22^{AB}	1.73 ± 0.28^{B}
CO_3^{2-} (mmol l^{-1})	0.115 ± 0.023^{A}	0.026 ± 0.005^{B}	0.003 ± 0.001^{C}

the high end of this range (mean ±SD: 17.30 ± 1.30 or ±1.40°C, see Table 1), they were within the range that *N. puber* experiences naturally. Furthermore, at the end of autumn 2008, the UK experienced lower than normal temperatures for this time and our environmental measurements were, thus, lower than average seasonal values (see Table A2 for 2007–2008 sea surface temperature and atmospheric temperature profiles). There was no significant difference in body mass (wet weight) among individuals after 30 d exposure to hypercapnia (F_2 = 2.050, p = 0.145).

Oxygen uptake

The oxygen uptake of *Necora puber* decreased significantly in individuals that were exposed to hypercapnic conditions (F_2 = 9.764, p < 0.001) (Fig. 1, Table 2) for 30 d. While a marginal decrease in oxygen consumption was observed between control and medium hypercapnic conditions (p = 0.090) (Fig. 1), the respiration rates of crabs that were kept under the lowest pH conditions were significantly lower than those of crabs under control and medium hypercapnic conditions (p < 0.05) (Fig. 1). Finally, there was a significant effect of body mass on oxygen uptake (F_2 = 12.145, p = 0.001).

Upper thermal tolerance

The upper thermal tolerance (UTT, Table 2) of *Necora puber* was not significantly affected by either level of hypercapnia investigated, for both LRR (F_2 = 1.542, p = 0.248) and OS (F_2 = 2.058, p = 0.165). We detected a reduction in UTT–LRR variation around the mean with decreased pH.

Extracellular acid–base balance

While no significant difference in haemolymph pH was detected (F_2 = 1.797, p = 0.198), an increasing variability was observed with decreasing pH (Fig. 2A, Table 2). Haemolymph TCO_2 increased significantly with decreasing pH (F_2 = 4.650, p = 0.026) (Fig. 2B, Table 2). A significant increase in haemolymph TCO_2 between the control and both hypercapnic treatments was detected (F_2 = 4.650, p = 0.026) (Fig. 2B, Table 2), although no significant difference was observed between the 2 hypercapnic treatments (p > 0.05) (Fig. 2B, Table 2). In addition, a signif-

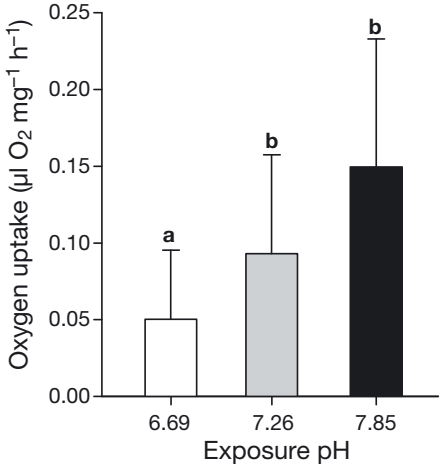

Fig. 1. *Necora puber*. Rates of oxygen uptake of adult individuals after 30 d exposure to control and low pH/hypercapnic conditions. Significant differences in mean rates of oxygen uptake (p ≤ 0.05) are indicated by different letters based on the Tukey-Kramer test

Table 2. *Necora puber*. Haemolymph acid–base parameters and key physiological functions (mean ± SD) measured in adult individuals after 30 d exposure to control and low pH/hypercapnia. UTT: Upper thermal tolerance—measured as loss of righting response (LLR) and onset of spasms (OS); LPO: haemolymph lipid peroxidation

Parameter	pH 7.85	pH 7.26	pH 6.69
pH	7.73 ± 0.11	7.66 ± 0.18	7.53 ± 0.22
TCO_2 (mmol l^{-1})	3.32 ± 0.77	6.60 ± 2.59	11.93 ± 8.34
pCO_2 (kPa)	0.19 ± 0.04	0.41 ± 0.08	0.88 ± 0.36
$[HCO_3^-]$ (mmol l^{-1})	2.63 ± 0.75	5.07 ± 2.52	8.66 ± 7.58
Oxygen uptake (µl O_2 mg^{-1} h^{-1})	0.150 ± 0.083	0.098 ± 0.060	0.057 ± 0.041
UTT – LRR (°C)	32.18 ± 0.55	32.55 ± 0.45	32.58 ± 0.14
UTT – OS (°C)	33.02 ± 0.16	33.08 ± 0.18	33.38 ± 0.49
LPO (nmol ml^{-1})	8.83 ± 2.73	7.20 ± 3.89	6.47 ± 3.08
Haemolymph $[Ca^{2+}]$ (mmol l^{-1})	3.16 ± 2.56	12.72 ± 5.17	11.37 ± 2.51
Haemolymph $[Mg^{2+}]$ (mmol l^{-1})	11.72 ± 2.74	14.50 ± 1.76	14.52 ± 2.12
Chelae $[Ca^{2+}]$ (mmol g^{-1} dry mass)	3.46 ± 0.25	3.49 ± 0.29	3.20 ± 0.64
Chelae $[Mg^{2+}]$ (mmol g^{-1} dry mass)	0.234 ± 0.025	0.264 ± 0.020	0.273 ± 0.021
Carapace $[Ca^{2+}]$ (mmol g^{-1} dry mass)	3.25 ± 0.10	3.15 ± 0.35	3.10 ± 0.15
Carapace $[Mg^{2+}]$ (mmol g^{-1} dry mass)	0.271 ± 0.019	0.266 ± 0.035	0.274 ± 0.014

icant positive effect of hypercapnia on haemolymph pCO_2 was detected (F_2 = 7.873, p = 0.000) (Fig. 2C, Table 2), with values at all treatment levels increasing significantly with decreasing pH ($p < 0.05$) (Fig. 2C, Table 2). Finally, although an increase in haemolymph $[HCO_3^-]$ was observed with decreasing pH, no significant difference was found between treatments (F_2 = 2.319, p = 0.131) (Fig. 2D, Table 2).

Haemolymph lipid peroxidation, $[Ca^{2+}]$ and $[Mg^{2+}]$

After 30 d exposure to hypercapnia, haemolymph levels of LPO in adult *Necora puber* did not differ significantly among the 3 treatments (F_2 = 0.718, p = 0.505) (Table 2). Haemolymph $[Ca^{2+}]$ was significantly affected by medium-term exposure to hypercapnia (F_2 = 10.372, p = 0.002) (Fig. 3A, Table 2), while no significant difference was found between the 2 hypercapnic treatments (p = 0.805) (Fig. 3A, Table 2). There was a marginally significant effect of hypercapnia on haemolymph $[Mg^{2+}]$ (F_2 = 3.381, p = 0.063) (Fig. 3B, Table 2).

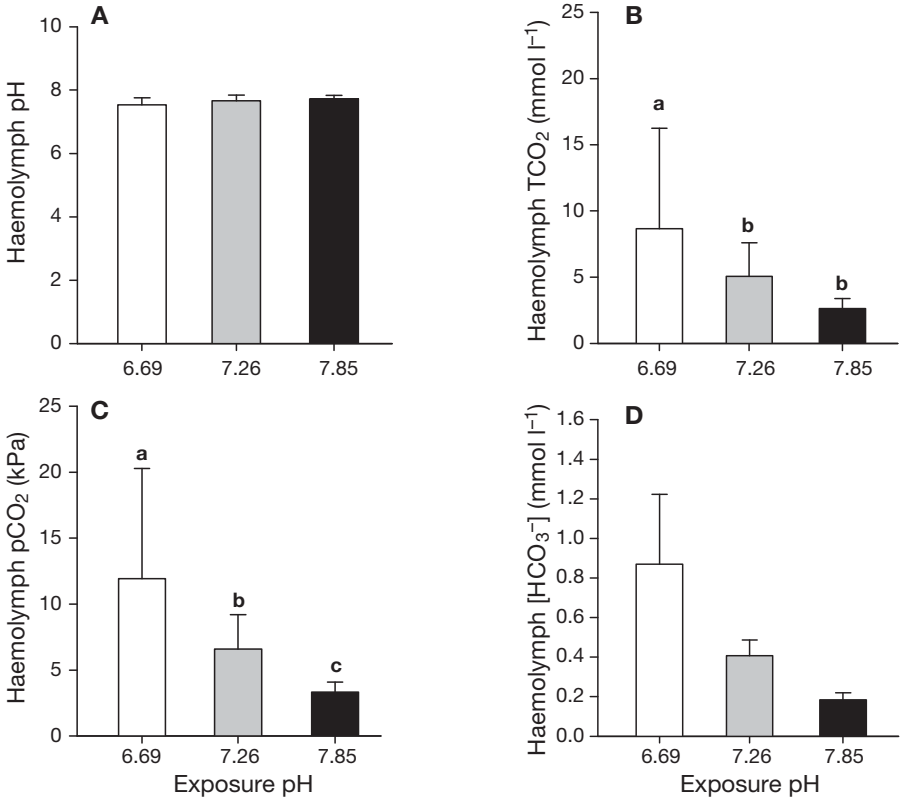

Fig. 2. *Necora puber*. Haemolymph acid–base balance parameters measured in adult individuals after 30 d exposure to control and low pH/hypercapnic conditions: (A) pH, (B) TCO_2, (C) pCO_2, (D) $[HCO_3^-]$. Significant differences in mean haemolymph acid–base balance parameters ($p \leq 0.05$) are indicated by different letters based on the Tukey-Kramer test

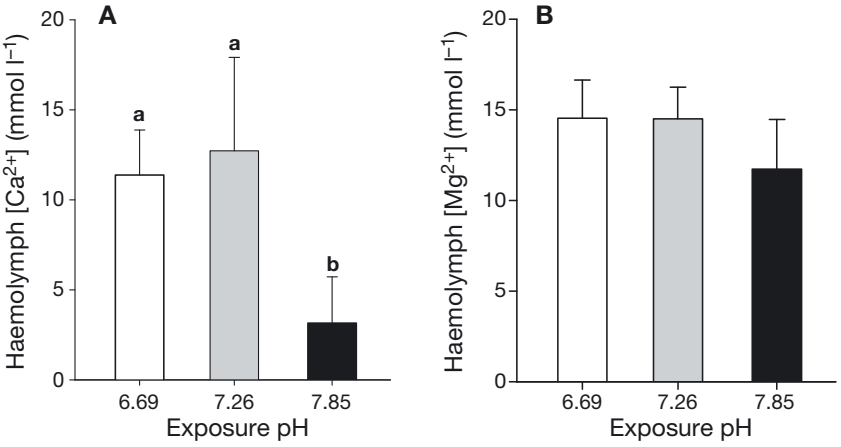

Fig. 3. *Necora puber*. Haemolymph (A) [Ca^{2+}] and (B) [Mg^{2+}] measured in adult individuals after 30 d exposure to control and low pH/hypercapnic conditions. Significant differences in mean haemolymph [Ca^{2+}] and [Mg^{2+}] ($p \leq 0.05$) are indicated by different letters based on the Tukey-Kramer test

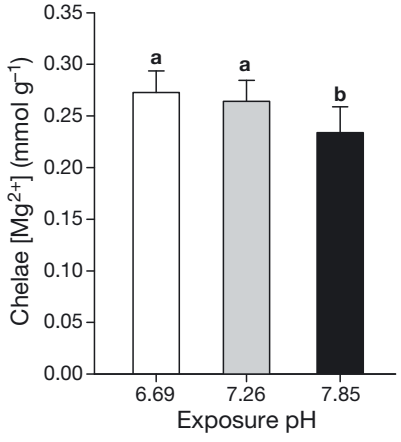

Fig. 4. *Necora puber*. Chelae [Mg^{2+}] measured in adult individuals after 30 d exposure to control and low pH/hypercapnic conditions. Significant differences in mean chelae [Mg^{2+}] ($p \leq 0.05$) are indicated by different letters based on the Tukey-Kramer test

Carapace and chelae [Ca^{2+}] and [Mg^{2+}]

[Ca^{2+}] and [Mg^{2+}] in the carapace of adult *Necora puber* exposed to 30 d hypercapnia did not vary significantly among pH treatment levels (minimum $F_2 = 1.227$, $p = 0.306$) (Table 2), nor did [Ca^{2+}] in the chelae ($F_2 = 1.601$, $p = 0.217$) (Fig. 4, Table 2). Chelae [Mg^{2+}], however, increased significantly with hypercapnia ($F_2 = 9.592$, $p = 0.001$; Fig. 4, Table 2), with no significant difference between the 2 hypercapnic treatments ($p < 0.05$).

DISCUSSION

This study confirmed that *Necora puber* is able to compensate for external acidosis of its extracellular fluids over a longer term (30 d) than previously tested, without incurring extra costs in terms of thermal tolerance, immune response, or carapace mineralogy. However, a reduction in metabolic activity at the lowest pH level was associated with changes in haemolymph chemistry (i.e. [Ca^{2+}] and [Mg^{2+}]) and chelae [Mg^{2+}]. These findings are discussed within the broader context of the effect of OA on rocky shore intertidal organisms and communities.

Adult individuals of *Necora puber* showed the ability to effectively regulate their extracellular fluid pH over 30 d exposure to hypercapnic conditions, with haemolymph pH and [HCO_3^-] being comparable across treatments. Our findings differ from those of Spicer et al. (2007) who recorded a significant increase in haemolymph [HCO_3^-] over short-term exposure to hypercapnia. Bicarbonate ions acquired from shell dissolution are thought to be used for the regulation of internal pH in marine invertebrates (see Spicer et al. 1988, Cameron & Iwama 1989, Pörtner et al. 2004, Michaelidis et al. 2005, Pane & Barry 2007) and terrestrial decapod crustaceans (Henry et al. 1981). However, while we reported an increase in haemolymph [Ca^{2+}] with hypercapnia (within the same range reported by Spicer et al. (2007) for *N. puber*), neither a significant decrease in carapace or chelae [Ca^{2+}], nor increase in haemolymph [HCO_3^-] were detected. Thus, shell dissolution appears to be less important for haemolymph buffering during exposure to low pH/hypercapnia in *N. puber* in the medium term when compared to the short term (Cameron 1985, Spicer et al. 2007). We can thus conclude that during longer-term exposures, the surrounding seawater is used as an alternative source of HCO_3^- (see Spicer et al. 2007), which actually increases with decreasing pH (see Table 1). It is likely that active extracellular compensation in *N. puber* occurs via the exchange of H^+ and Cl^- for Na^+ and HCO_3^- across the gills (Cameron 1978, 1985, Truchot 1979, Henry et al. 1981, Spicer et al. 2007). Consequently, although *N. puber* is able to maintain a pH buffering capacity during medium-term exposure, this regulation of extracellular pH will likely represent an energetically expensive process (Cameron & Mangum 1983) that may generate additional physiological costs.

In the current study, oxygen consumption decreased significantly with hypercapnia together with a marginally significant increase in haemolymph [Mg^{2+}]. This is perhaps not surprising, as previous studies have demonstrated an inverse relationship between crus-

tacean activity levels and haemolymph [Mg^{2+}] (Boardman & Collier 1946, Walters & Uglow 1981, Morritt & Spicer 1993, Spicer et al. 1994, Watt et al. 1999). Since even slight changes in haemolymph [Mg^{2+}] can result in a considerable change in intracellular [Mg^{2+}] (see Morritt & Spicer 1993, Sartoris & Pörtner 1997), a decrease in oxygen consumption has been proposed as a mechanism for conserving ATP levels and regulating intracellular pH; this would allow an organism to withstand or recover from extreme environmental conditions (Sartoris & Pörtner 1997) such as hypercapnia (Pörtner et al. 1998, Langenbuch & Pörtner 2002). Although the haemolymph [Mg^{2+}] measured here was lower than that observed for *Necora puber in situ* (Watt et al. 1999), our values are similar to those measured under laboratory conditions by Spicer et al. (2007). Additionally, we also detected an increase in chelae [Mg^{2+}],which may indicate an increase in the aragonitic fraction of the chelae, and a possible reduction in chelae strength (which was not measured here).

All other physiological functions measured in this study were unaltered by prolonged exposure to hypercapnia. Tolerance to heat was not affected, contrary to what was found for the edible crab *Cancer pagurus* (Metzger et al. 2007) and the spider crab *Hyas araneus* (Walther et al. 2009). The thermal tolerance values for *Necora puber* presented here, after accounting for methodological and acclimatisation differences (see Garland & Adolph 1991, Terblanche et al. 2007, Chown et al. 2009), are comparable to those of Hopkin et al. (2006). In addition, even when methodological differences—we used passive endpoints (Lutterschmidt & Hutchison 1997), while Metzger et al. (2007) used 'pejus' and 'critical' temperatures (Frederich & Pörtner 2000), as well as our slower ramping rate (see Terblanche et al. 2007)—are accounted for, our results appear to indicate a greater resilience to combined OA and warming in *N. puber* than in the intertidal *C. pagurus*, and the subtidal *H. araneus* (Walther et al. 2009). Finally, no significant change in the levels of haemolymph LPO (an indicator of oxidative stress and disruption of ionic regulation in crustaceans; Monserrat et al. 2003) was observed. Thus, the immune response of *N. puber* (based on this single proxy) appears not to be significantly affected by OA, contrary to what has been reported for other marine invertebrates (Burgents et al. 2005, Tanner et al. 2006, Bibby et al. 2008).

The impacts of OA at the ecosystem or community level will be a result of impacts at the organism level (Widdicombe & Spicer 2008). *Necora puber*, *Cancer pagurus,* and *Carcinus maenas* are key predators with important interactions that affect predator density and diversity, resource partitioning, and ecosystem diversity and function (Griffin et al. 2008). *Necora puber* appears to be more tolerant to medium-term exposure to hypercapnia than *C. maenas* (Truchot 1975, 1979) and to the synergistic effects of extreme upper temperatures and hypercapnia than *C. pagurus* (Metzger et al. 2007). This would have implications for predator interactions and thus for ecosystem function and diversity (Griffin et al. 2008). Also, *N. puber* showed little sign of decreased calcification or shell dissolution, whereas its preferred prey species *Littorina littorea* and *Mytilus edulis* (Griffin et al. 2008) have been shown to exhibit reduced shell thickening responses to predator presence (Bibby et al. 2007) and experience shell dissolution and decreased calcification (Lindinger et al. 1984, Gazeau et al. 2007), respectively. Finally, since *N. puber* incurs metabolic costs with hypercapnia, a limitation on the top-down control produced by predators on their prey species may occur (Widdicombe & Spicer 2008).

Acknowledgements. We thank H. Findlay for guidance in using the CO2SYS program; H. Wood for insights into investigating calcification levels; R. Haslam for technical assistance; and C. Smith, C. Moore, L. Fisher, and E. De Clive-Lowe for help in collecting crabs. This work was carried out while P.C. was on an RCUK Research Fellowship to investigate ocean acidification. S.W. acknowledges support from the NERC funded program Oceans 2025 (Theme 3 – Coastal and shelf processes) and the European Project on Ocean Acidification (EPOCA) funded by the European Community's Seventh Framework Programme (FP7/2007-2013) under grant agreement no. 211384.

LITERATURE CITED

Agnew DJ, Taylor AC (1986) Seasonal and diel variations of some physico-chemical parameters of boulder shore habitats. Ophelia 25:83–95

Andersson AJ, Mackenzie FT, Bates NR (2008) Life on the margin: implications of ocean acidification on Mg-calcite, high latitude and cold-water marine calcifiers. Mar Ecol Prog Ser 373:265–273

Bibby R, Cleall-Harding P, Rundle S, Widdicombe S, Spicer J (2007) Ocean acidification disrupts induced defences in the intertidal gastropod *Littorina littorea.* Biol Lett 3: 699–701

Bibby R, Widdicombe S, Parry H, Spicer J, Pipe R (2008) Effects of ocean acidification on the immune response of the blue mussel *Mytilus edulis.* Aquat Biol 2:67–74

Blackford JC, Jones N, Proctor R, Holt J (2008) Regional scale impacts of distinct CO_2 additions in the North Sea. Mar Pollut Bull 56:1461–1468

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

Boardman DL, Collier HOJ (1946) The effect of magnesium deficiency on neuro-muscular transmission in the shore crab, *Carcinus maenas.* J Physiol 104:377–383

Burgents JE, Burnett KG, Burnett LE (2005) Effects of hypoxia and hypercapnic hypoxia on the localization and the

elimination of *Vibrio campbellii* in *Litopenaeus vannamei*, the Pacific white shrimp. Biol Bull 208:159–168

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

Cameron JN (1978) Effects of hypercapnia on blood acid-base status, NaCl fluxes, and trans-gill potential in freshwater blue crabs *Callinectes sapidus*. J Comp Physiol 123: 137–141

Cameron JN (1985) Compensation of hypercapnic acidosis in the aquatic blue crab, *Callinectes sapidus*: the predominance of external sea water over carapace carbonate as the proton sink. J Exp Biol 114:197–206

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

Cameron JN, Iwama GK (1989) Compromises between ionic regulation and acid–base regulation in aquatic animals. Can J Zool 67:3078–3084

Cameron JN, Mangum CP (1983) Environmental adaptations of the respiratory system: ventilation, circulation, and oxygen transport. In: Vernberg FJ, Vernberg WB (eds) The biology of Crustacea, Vol 8. Academic Press, New York, p 43–63

Chown SL, Jumbam KR, Sørensen JG, Treblanche JS (2009) Phenotypic variance, plasticity and heritability estimates of critical thermal limits depend on methodological context. Funct Ecol 23:133–140

Cuculescu M, Hyde D, Bowler K (1998) Thermal tolerance of two species of marine crab, *Cancer pagurus* and *Carcinus maenas*. J Therm Biol 23:107–110

Dickson AG (1990) Standard potential of the reaction: and the standard acidity constant of the ion HSO_4^- in synthetic sea water from 273.15 to 318.15 K. J Chem Thermodyn 22: 113–127

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

Fabry VJ, Seibel BA, Feely RA, Orr JC (2008) Impacts of ocean acidification on marine fauna and ecosystem processes. ICES J Mar Sci 65:414–432

Feely RA, Sabine CL, Lee K, Berelson W, Kleypas J, Fabry VJ, Millero FJ (2004) Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science 305:362–366

Findlay HS, Wood HL, Kendall MA, Spicer JI, Twitchett RJ, Widdicombe S (2009) Calcification, a physiological process to be considered in the context of the whole organism. Biogeosciences Discuss 6:2267–2284

Frederich M, Pörtner HO (2000) Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in the spider crab *Maja squinado*. Am J Physiol 279: R1531–R1538

Freire J, González-Gurriarán E (1995) Feeding ecology of the velvet swimming crab *Necora puber* in mussel raft areas of the Ría de Arousa (Galicia, NW Spain). Mar Ecol Prog Ser 119:139–154

Garland TJ, Adolph SC (1991) Physiological differentiation of vertebrate populations. Annu Rev Ecol Syst 22:193–228

Gazeau F, Quiblier C, Jansen JM, Gattuso JP, Middelburg JJ, Heip CHR (2007) Impact of elevated CO_2 on shellfish calcification. Geophys Res Lett 34:L07603

Griffin JN, De La Haye KL, Hawkins SJ, Thompson RC, Jenkins SR (2008) Predator diversity and ecosystem functioning: density modifies the effect of resource partitioning. Ecology 89:298–305

Henderson S, Leslie B (2006) Survival of discarded velvet crabs (*Necora puber*). NAFC Marine Centre Fisheries Development Note 23

Henry RP, Kormanik GA, Smatresk NJ, Cameron JN (1981) The role of $CaCO_3$ dissolution as a source of HCO_3^- for the buffering of hypercapnic acidosis in aquatic and terrestrial decapod crustaceans. J Exp Biol 94:269–274

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

Hopkin RS, Qari S, Bowler K, Hyde D, Cuculescu M (2006) Seasonal thermal tolerance in marine Crustacea. J Exp Mar Biol Ecol 331:74–81

Kano Y, Sato T, Kita J, Hirabayashi S, Tabeta S (2010) Multiscale modeling of CO_2 dispersion leaked from seafloor off the Japanese coast. Mar Pollut Bull 60:215–224

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

Lindinger MI, Lauren DJ, McDonald DG (1984) Acid–base balance in the sea mussel, *Mytilus edulis*. III. Effects of environmental hypercapnia on intra- and extracellular acid–base balance. Mar Biol Lett 5:371–381

Lutterschmidt WI, Hutchison VH (1997) The critical thermal maximum: data to support the onset of spasms as the definitive end point. Can J Zool 75:1552–1560

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

Metzger R, Sartoris FJ, Langenbuch M, Pörtner HO (2007) Influence of elevated CO_2 concentrations on thermal tolerance of the edible crab *Cancer pagurus*. J Therm Biol 32: 114–151

Michaelidis B, Ouzounis C, Paleras A, Pörtner HO (2005) Effects of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovincialis*. Mar Ecol Prog Ser 293:109–118

Monserrat JM, Geracitano LA, Pinho GLL, Vinagre TM, Faleiros M, Alciati JC, Bianchini A (2003) Determination of lipid peroxides in invertebrate tissues using the Fe(III) xylenol orange complex formation. Arch Environ Contam Toxicol 45:177–183

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 106:19–24

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

Pierrot D, Lewis E, Wallace DWR (2006) CO2SYS 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

Pörtner HO, Reipschläger A, Heisler N (1998) Acid–base regulation, metabolism and energetics in *Sipunculus nudus* as a function of ambient carbon dioxide level. J Exp Biol 201:43–55

Pörtner HO, Langenbuch M, Reipschläger A (2004) Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. J Oceanogr 60:705–718

Raven J, Caldeira K, Elderfield H, Hoegh-Guldberg O and

others (2005) Ocean acidification due to increasing atmospheric carbon dioxide. The Royal Society Policy document 12:05. Clyvedon Press, Cardiff

Reipschläger A, Pörtner HO (1996) Metabolism depression during environmental stress: the role of extracellular versus intracellular pH in *Sipunculus nudus.* J Exp Biol 199:1801–1807

Robson AA, Kelly MS, Latchford JW (2007) Effect of temperature on the spoilage rate of whole, unprocessed crabs: *Carcinus maenas, Necora puber* and *Cancer pagurus.* Food Microbiol 24:419–424

Sabine CL, Feely RA, Gruber N, Key RM and others (2004) The oceanic sink for anthropogenic CO_2. Science 305: 367–371

Sartoris FJ, Pörtner HO (1997) Increased concentrations of haemolymph Mg^{2+} protect intracellular pH and ATP levels during temperature stress and anoxia in the common shrimp *Crangon crangon.* J Exp Biol 200:785–792

Silva ACF, Hawkins SJ, Boaventura DM, Thompson RC (2008) Predation by small mobile aquatic predators regulates populations of the intertidal limpet *Patella vulgata* (L.). J Exp Mar Biol Ecol 367:259–265

Spicer JI, Eriksson SP (2003) Does the development of respiratory regulation always accompany the transition from pelagic larvae to benthic fossorial postlarvae in the Norway lobster *Nephrops norvegicus* (L.)? J Exp Mar Biol Ecol 295:219–243

Spicer JI, Taylor AC, Hill AD (1988) Acid–base status in the sea urchins *Psammechinus miliaris* and *Echinus esculentus* (Echinodermata: Echinoidea) during emersion. Mar Biol 99:527–534

Spicer JI, Morritt D, Taylor AC (1994) Effect of low temperature on oxygen uptake and haemolymph ions in the sand hopper *Talitrus saltator* (Crustacea: Amphipoda). J Mar Biol Assoc UK 74:313–321

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

Tanner CA, Burnett LE, Burnett KG (2006) The effects of hypoxia and pH on phenoloxidase activity in the Atlantic blue crab, *Callinectes sapidus.* Comp Biochem Physiol 114A:218–223

Terblanche JS, Deere JA, Clusella-Trullas S, Janion C, Chown SL (2007) Critical thermal limits depend on methodological context. Proc Biol Sci 274:2935–2942

Truchot JP (1975) Action de l'hypercapnie sur l'etat acide–base du sang chez le crabe *Carcinus maenas* (L.) (Crustace, Decapode). CR Acad Sci Paris 280:311–314

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

Walther K, Sartoris FJ, Bock C, Pörtner HO (2009) Impact of anthropogenic ocean acidification on thermal tolerance of the spider crab *Hyas araneus.* Biogeosciences Discuss 6: 2837–2861

Watt AJS, Whiteley NM, Taylor EW (1999) An *in situ* study of respiratory variables in three British sublittoral crabs with different routine rates of activity. J Exp Mar Biol Ecol 239:1–21

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

Wilhelm G, Mialhe E (1996) Dinoflagellate infection associated with the decline of *Necora puber* crab populations in France. Dis Aquat Org 26:213–219

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

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

Appendix

In Tables A1 and A2, *in situ* temperature data for the experimental period (16 Sept to 31 Oct 2008) from the uppermost and lowermost points of *Necora puber* vertical zonation in the intertidal area at Mount Batten Beach (Plymouth, UK; 50° 21′ 34″ N, 04° 07′ 45″ W) are shown. Temperature data in Table A1 was recorded at 1800 s intervals using 2 iButton data loggers (DS192 1L, Maxim Integrated Products) and downloaded using One Wire Viewer software (Maxim Integrated Products). The data loggers were mounted using mounting brackets that were screwed onto solid rock surfaces, avoiding direct sunlight. Data loggers A and B were positioned at the top of the range of *N. puber* at Mount Batten Beach, and at the lowest spring tide mark (0.6 m), respectively. The spring low tide area was exposed only once (at the beginning) throughout the experimental period and corresponded to the area where the experimental individuals were collected.

Table A1. Mean, maximum, minimum, mode, and median temperatures (°C) from the 2 data loggers that were placed *in situ* at Mount Batten Beach (Plymouth, Devon) between 16 Sep 2008 and 31 Oct 2008. Data loggers A and B were situated at the top of *Necora puber's* range on the shore and at spring low water respectively

	Data logger A	Data logger B
Mean	13.80 ± 2.07	14.44 ± 0.79
Max	19.19	18.18
Min	4.13	10.65
Mode	15.18	14.67
Median	14.20	14.70

Table A2. Mean, maximum, minimum, mode, and median sea surface temperatures (SST) and atmospheric temperatures (ATM) (°C) for the experimental period (16 Sept to 31 Oct 2008), and the same period in 2007. SST and ATM data were taken from www.westernchannelobservatory.org.uk/data

	2008		2007	
	SST	ATM	SST	ATM
Mean	15.88	14.24	17.28	15.2
Max	16.61	20.19	18.04	23.98
Min	15.39	6.03	16.54	4.93
Mode	16.01	14.58	17.34	16.17
Median	15.96	14.58	17.34	15.43

Editorial responsibility: Alex Rogers, London, UK

Submitted: September 16, 2009; Accepted: June 1, 2010
Proofs received from author(s): June 15, 2010