Effects of hypercapnia on acid–base balance and osmo-/iono-regulation in prawns (Decapoda: Palaemonidae)

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ABSTRACT: Anthropogenic carbon dioxide-induced ‘ocean acidification’ is predicted to have major implications for marine organisms. As the oceans becomes increasingly hypercapnic (elevated CO₂) and seawater pH decreases, the ability of organisms to maintain extracellular pH homeostasis (acid–base balance) may be compromised. Acid–base regulation occurs by ionic transport, where hydrogen and bicarbonate ions (HCO₃[−]) are exchanged for sodium and chloride, respectively (H⁺/Na⁺; HCO₃[−]/Cl[−]), as exemplified by decapod crustaceans. Palaemonid prawns, in particular, are efficient hypo-ionic/osmotic regulators in seawater. We demonstrate that hypercapnic exposure (0.3 kPa) results in short-term (5 to 14 d) extracellular acidosis in 2 efficient ionic/osmo-regulators (thus, acid–base regulators), i.e. *Palaemon elegans* and *P. serratus*. Complete hypercapnic compensation was observed in both species after 30 d exposure with no effect on osmotic capacity, but at the expense of extracellular acid–base alteration (alkalosis). Furthermore, the predominantly subtidal species *P. serratus* was observed to be as tolerant as the intertidal species *P. elegans*, although 2 differing mechanisms of ionic regulation may be at work, with *P. elegans* and *P. serratus* displaying lower and elevated haemolymph ion concentrations (i.e. sodium, chloride and calcium), respectively.

KEY WORDS: Hypercapnia · ‘Ocean acidification’ · Acid–base balance · Osmotic and ionic regulation · Palaemon

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INTRODUCTION

Since the beginning of the industrial revolution (ca. 1750), atmospheric levels of carbon dioxide (CO₂) have been steadily increasing. Present day levels of atmospheric CO₂ (380 ppm) are greater than pre-industrial levels (280 ppm) (Feely et al. 2004). The increase in anthropogenic CO₂, attributed to the burning of fossil fuels (IPCC 2001), has been rising at a far greater rate than previously recorded in the Earth’s history (Pearson & Palmer 2000). Under current models, continuously increasing production of CO₂ emissions (under the ‘business as-usual scenario’) are predicted to raise the current atmospheric concentrations to 540 ppm by the turn of the century, and ca. 2000 ppm by the year 2300 (Caldeira & Wickett 2003). Oceans are regarded

as natural ‘sinks’ for carbon and account for 30 to 50 % of the atmospheric carbon that has been emitted over the last 250 yr (Siegenthaler & Sarmiento 1993, Feely et al. 2004). As atmospheric carbon diffuses passively into the ocean surface waters, it alters seawater chemistry (Zeebe & Wolf-Gladrow 2001). Carbonic acid (H₂CO₃) is formed, which rapidly dissociates into bicarbonate ions (HCO₃[−]) yielding hydrogen ions (H⁺). This results in an increase in acidity (or decrease in pH). The natural buffering of this reaction occurs by carbonate ions (CO₃^{2−}) reacting with excess H⁺ ions to form HCO₃[−], which is known as the carbonate buffering system, represented by the equation (Raven et al. 2005):

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The ocean's natural capacity to buffer changes in seawater chemistry caused by CO₂ has been compromised and surface ocean pH has decreased by 0.1 units (since pre-industrial times) as a result of a 30 % increase in H⁺ ions (Caldeira & Wickett 2003, Blackford & Gilbert 2007). The reduction in ocean pH is termed 'ocean acidification'. Responsible for this rapid rise in ocean acidity is increasing hypercapnia (elevated levels of dissolved CO₂) (which drives the equilibria of Eq. 1 towards the right-hand side). This may have profound effects upon the biota, the critical limits and long-term effects of which are currently unknown (Pörtner et al. 1998, Seibel & Walsh 2003, Widdicombe & Spicer 2008).

It is likely that changes in seawater chemistry will affect the internal physiological functioning of marine organisms, such as the acid–base balance (Raven et al. 2005). Organisms that live in aquatic environments face the problem of maintaining a constant internal environment (i.e. acid–base balance in extracellular fluid) that enables cells to function efficiently and is independent of the external environment (Rankin & Davenport 1981, Seibel & Walsh 2003). Mechanisms of acid–base regulation require ion exchange with the external environment, where H⁺ and HCO₃[−] ions are used as counter-ions for sodium (Na⁺) and (Cl[−]) (HCO₃[−]/Cl[−] and H⁺/Na⁺) (Cameron & Mangum 1983, Mantel & Farmer 1983, Wheatly & Henry 1992). Ionic and osmotic regulation is defined as 'the maintenance in a body fluid of concentrations of ions (ionic) and total particle concentration (osmotic) differing to that the external medium' (Robertson 1949), and it is generally regarded that efficient ionic/osmotic regulators are efficient acid–base regulators. Examples of ionic/osmotic regulators include the decapod crustaceans (Robertson 1949). Some decapods, such as the Palaemonid prawns, have previously been shown to be efficient hyper- and hypo-ionic/osmoregulators in both low and high saline waters, respectively (Panikkar 1941, Parry 1954, Campbell & Jones 1989, Freire et al. 2003, González-Ortegón et al. 2006), and are regarded as displaying an osmoregulatory ability which is the most advanced form of genetic adaptation to osmotic change (Kinne 1971). Palaemonid prawns occur throughout the world in a wide variety of aquatic habitats; the majority inhabit freshwater, while a few are marine species, and a minority are brackish-water species (Holthuis 1950).

Examples of palaemonids around coastal shallow UK waters include *Palaemon elegans* (Rathke), which is normally found in intertidal rock pools, and *P. serratus* (Pennant), which inhabits the subtidal or rock pools on the lower shore (Smaldon 1979). Both palaemonids can co-occur; however, *P. serratus* is restricted to the lower shore/subtidal areas due to abiotic factors (Berglund 1982). The physiology of *P. elegans* has been shown to

more tolerant of the conditions associated with rock pools, such as daily and seasonal fluctuations in oxygen, pH, temperature (Truchot & Duhamel-Jouve 1980, Morris & Taylor 1983, Taylor & Spicer 1991) compared with *P. serratus* (Taylor & Spicer 1991).

The aim of the present study was to investigate the short-term effects of hypercapnia (due to CO₂-acidified seawater exposure) on the relationship between osmotic and ionic regulation and acid–base balance in 2 palaemonids (Decapoda: Palaemonidae) that exhibit efficient hypo-osmo/ionoregulatory ability. One is primarily high shore in its distribution (*Palaemon elegans*) and the other is low shore/subtidal (*P. serratus*).

MATERIALS AND METHODS

Animal collection and maintenance. *Palaemon elegans* was collected from tidal pools using hand-held nets and *P. serratus* from LWS (low water spring) using baited creels at Mount Batten, Plymouth, UK (50° 21' 26 N, 4° 07' 36 W) during November 2008. Only adults were used in the experiments described below. The body length (BL, rostrum to telson) of each was measured: *P. elegans* mean BL 41.48 ± 3.0 mm and *P. serratus* mean BL 61.2 ± 10.7 mm. All prawns were maintained in a number of static holding aquaria (volume = 100 l) each containing filtered (10 µm carbon-filtered), continually aerated artificial seawater (ASW) (Instant Ocean®, J&K Aquatics; salinity of 32, 15 ± 1°C, pH 7.94 ± 0.01) under a 12 h light:12 h dark photoperiod.

Experimental design. Prawns were exposed to either control seawater (normocapnic: 0.1 kPa CO₂) or hypercapnic seawater (0.3 kPa CO₂) for 5, 14 or 30 d (n = 13 for each exposure period). The CO₂-enriched seawater for the experimental period was prepared as follows. 'Scrubbed air' (produced by bubbling through 2 mol l^{−1} NaOH) was mixed with CO₂ to produce a nominal CO₂ concentration. The resultant gas mixture was dried and, subsequently, measured using a CO₂ analyser (Licor, LI-7000) and supplied to the seawater in the experimental tanks. The control (normocapnic) air was supplied to the seawater in the experimental tanks without any CO₂ added. The gas volume added to each tank (irrespective of treatment) was 0.2 l min^{−1}. Details of seawater physico-chemical parameters for the 30 d exposure period are presented in Table 1. Prawns were held individually in aquaria (volume = 2 l) and fed artificial feed pellets (0.07 ± 0.001 g) equivalent to 3 % body mass for crustaceans (McGaw & Reiber 2000, Dissanayake et al. 2008) (CrustaGran, Dennerle) every 2 d. Water was changed within 18 h after feeding.

Haemolymph sampling. Acid–base studies using extracellular fluid (blood or haemolymph) have previ-

Table 1. *Palaemon elegans* and *P. serratus*. Physico-chemical parameters (mean $\pm$ 1 SE) over the 30 d exposure period (n = 13 for each parameter). Water temperature ($^{\circ}$ C) and salinity were 15 ± 0.13 and 32 ± 0.1 , respectively

Treatment Species	Exposure period (d)	Seawater pH	Seawater pCO ₂ (kPa)	Seawater total alkalinity (μ mol kg ⁻¹)	Calcite saturation	Aragonite saturation	Haemo-lymph pCO ₂ (kPa)	Haemolymph [HCO ₃ ⁻] (mmol l ⁻¹)
Control (0.1 kPa)								
<i>P. elegans</i>	5	7.98 $\pm$ 0.01	0.08 $\pm$ 0.02	2853 $\pm$ 3.57	3.12 $\pm$ 0.05	1.99 $\pm$ 0.03	0.29 $\pm$ 0.01	2.99 $\pm$ 0.13
	14	7.96 $\pm$ 0.01	0.09 $\pm$ 0.01	3085 $\pm$ 2.51	3.28 $\pm$ 0.04	2.09 $\pm$ 0.02	0.22 $\pm$ 0.01	3.01 $\pm$ 0.13
	30	7.95 $\pm$ 0.01	0.12 $\pm$ 0.02	3685 $\pm$ 2.99	3.79 $\pm$ 0.05	2.42 $\pm$ 0.03	0.44 $\pm$ 0.01	6.80 $\pm$ 0.40
<i>P. serratus</i>	5	7.95 $\pm$ 0.01	0.11 $\pm$ 0.02	2829 $\pm$ 26.87	2.85 $\pm$ 0.31	1.82 $\pm$ 0.20	0.16 $\pm$ 0.01	2.14 $\pm$ 0.20
	14	7.89 $\pm$ 0.01	0.11 $\pm$ 0.02	3283 $\pm$ 1.76	3.36 $\pm$ 0.03	2.15 $\pm$ 0.02	0.22 $\pm$ 0.01	2.92 $\pm$ 0.01
	30	7.89 $\pm$ 0.02	0.14 $\pm$ 0.05	3657 $\pm$ 7.98	3.38 $\pm$ 0.12	2.16 $\pm$ 0.07	0.17 $\pm$ 0.01	3.23 $\pm$ 0.10
Hypercapnia (0.1 kPa)								
<i>P. elegans</i>	5	7.40 $\pm$ 0.02	0.33 $\pm$ 0.13	2715 $\pm$ 7.24	0.86 $\pm$ 0.04	0.05 $\pm$ 0.03	0.25 $\pm$ 0.01	2.85 $\pm$ 0.30
	14	7.51 $\pm$ 0.01	0.31 $\pm$ 0.06	3357 $\pm$ 4.00	1.36 $\pm$ 0.03	0.87 $\pm$ 0.05	0.28 $\pm$ 0.01	3.04 $\pm$ 0.05
	30	7.49 $\pm$ 0.01	0.38 $\pm$ 0.06	3823 $\pm$ 4.53	1.47 $\pm$ 0.03	0.93 $\pm$ 0.02	0.66 $\pm$ 0.01	12.21 $\pm$ 0.09
<i>P. serratus</i>	5	7.36 $\pm$ 0.01	0.36 $\pm$ 0.10	2699 $\pm$ 5.25	0.78 $\pm$ 0.02	0.50 $\pm$ 0.01	0.55 $\pm$ 0.01	6.99 $\pm$ 0.49
	14	7.51 $\pm$ 0.01	0.32 $\pm$ 0.10	3363 $\pm$ 6.38	1.34 $\pm$ 0.05	0.86 $\pm$ 0.03	0.22 $\pm$ 0.01	3.58 $\pm$ 0.01
	30	7.51 $\pm$ 0.03	0.37 $\pm$ 0.10	3855 $\pm$ 14.76	1.59 $\pm$ 0.16	1.02 $\pm$ 0.10	0.37 $\pm$ 0.01	7.66 $\pm$ 0.24

ously been employed on catheterised individuals (e.g. fish, cuttlefish and large decapods) (Rahn & Baumgardner 1972, Pane & Barry 2007, Gutowska et al. 2010) to avoid problems associated with hyperventilation or hypoxia. In the present study, individual prawns could not be catheterised due to the small body size. Extracellular fluid was therefore collected as described by Taylor & Spicer (1991). Individuals were collected from each tank containing exposure water (either 0.1 or 0.3 kPa) (no flight reactions were noted). Haemolymph was extracted using an ice-chilled Hamilton microsyringe (volume = 50 μ l) on unanaesthetised individuals after thoroughly drying the cephalothorax using absorbent paper; the microsyringe needle was inserted directly into the pericardial cavity between the thorax and first abdominal segment (sampling time within 10 s) (Campbell & Jones 1989). The extracted haemolymph was then treated as follows.

Biological endpoints. All measurements were taken within 10 s of haemolymph sampling from each individual. Haemolymph osmolality was estimated for samples (volume = 8 μ l) using a vapour pressure osmometer (Wescor 5500) (Campbell & Jones 1989, Spicer et al. 2007). Results were expressed as osmotic capacity (or hypo-OC), defined as the difference between the osmolalities of haemolymph and the medium (ASW) (Charmantier & Anger 1999, Lignot et al. 2000) as palaemonids hypo-osmoregulate in seawater (Panikkar 1941, Parry 1954). Total carbon dioxide content of haemolymph (CCO₂ mmol l⁻¹) was measured using a CO₂ analyser (Ciba-Corning 965) (Miles et al. 2007, Spicer et al. 2007). Haemolymph partial pressure of CO₂ (pCO₂) was then calculated from measured values for CCO₂ and pH using a modified form of the Henderson-Hasselbalch equation, as employed by Miles et al.

(2007). Haemolymph (extracellular) pH and seawater pH was measured using a micro pH electrode equilibrated at 15 $^{\circ}$ C (Mettler Toledo Inlab423) inserted directly into the sample *in vitro* within seconds of collection. Using the pH and CCO₂ data, values for bicarbonate concentration [HCO₃⁻] and the saturation states for calcium and aragonite were calculated for each time point (5, 14 and 30 d) using the CO2SYS program (Pierrot et al. 2006) and are presented in Table 1.

Haemolymph ion concentrations. Haemolymph concentrations of ions (sodium Na⁺, calcium Ca²⁺, potassium K⁺ and magnesium Mg²⁺) were measured, after appropriate dilution with double de-ionised water, using an inductively-coupled plasma optical emission spectrophotometer (ICP-OES, Varian 725 ES). An independent check standard, prepared from different stock solutions to those used for the calibration standards, was used to confirm instrumental performance throughout the analytical procedure. The analysis was conducted under ISO 9001:2000 certification. Chloride concentrations were determined spectrophotometrically at 480 nm using a microtitre plate format with ferric nitrate and mercuric thiocyanate, and using sodium chloride as a standard solution (Merchant 2009).

Statistical analysis. Analysis of variance (ANOVA) tests were performed to test for differences (as revealed by Student-Newman-Keuls, SNK, tests) in (1) exposure level (normocapnia control or hypercapnia) or (2) time (5, 14, or 30 d) using GMAV for Windows® (Underwood 2005). Prior to analysis, data was tested for normality using Cochran's test and transformed where necessary (square-root or log-transformed where the data range was either within the same order of magnitude or within different orders of magnitude, respectively) (see 'Results').

RESULTS

Haemolymph pH and osmotic capacity (hypo-OC)

Changes were observed in the osmotic capability of *Palaemon elegans* only after 14 d (5 d: $F_{1,24} = 0.08$, $p = 0.78$; 14 d: $F_{1,24} = 15.85$, $p < 0.001$; 30 d: $F_{1,24} = 0.38$, $p = 0.38$) (Fig. 1) and 5 d in *P. serratus* (5 d: $F_{1,24} = 150.18$, $p < 0.001$; 14 d: $F_{1,24} = 2.67$, $p = 0.12$; 30 d: $F_{1,24} = 1.03$, $p = 0.32$), with hypo-osmotic capacity reaching similar levels to that of normocapnic individuals after 30 d in both species. With respect to haemolymph pH, a significant decrease was observed in hypercapnia-exposed individuals of both species compared to respective con-

Fig. 1. *Palaemon elegans* and *P. serratus*. Relationship between osmotic capacity and haemolymph pH (mean $\pm$ SE) in (A) *P. elegans* and (B) *P. serratus* showing time course (in d) of haemolymph acid–base alterations over a 30 d hypercapnic exposure (0.3 kPa). Open symbols and filled symbols indicate control (normocapnic) and hypercapnic treatments, respectively. (A) 0 d (control value), ($\square, \circ, \diamond$) 5, 14 and 30 d, respectively, and time course of values from hypercapnia-exposed individuals are labelled ($n = 13$)

trols (after 5 d). Following 14 d hypercapnia exposure, haemolymph pH values returned to pre-exposure levels in *P. serratus*; however, in *P. elegans*, hypercapnia resulted in further sustained haemolymph acidosis. After 30 d hypercapnia exposure, however, there was a significant haemolymph alkalosis, compared with control individuals, in both species (control vs. hypercapnia exposure: *P. elegans*: $F_{2,72} = 31.77$, $p < 0.001$; *P. serratus*: $F_{2,72} = 5.2$, $p < 0.01$).

Hypercapnia-exposed *Palaemon serratus* individuals experienced greater variation in internal pH (Δ pH) (on average) compared with hypercapnic *P. elegans* individuals (Δ pH = 0.23 compared to Δ pH = 0.2 in *P. serratus* and *P. elegans*, respectively). There was no difference in haemolymph pH between controls from the start to the end of the exposure (0 and 30 d) (*P. elegans*: $F_{1,24} = 0.07$, $p = 0.80$; *P. serratus*: $F_{1,24} = 0.07$, $p = 0.44$). Thirty d hypercapnic exposure resulted in a new 'steady state' in osmotic capacity, similar to that of normocapnic (control) individuals; however, at the expense of significant acid–base balance shift to haemolymph alkalosis.

Haemolymph ion concentrations

Sodium. With regard to sodium ion concentration in *Palaemon elegans*, the only significant differences were observed after 14 d, with hypercapnic individuals displaying lower sodium concentrations compared to normocapnic individuals ($F_{2,72} = 11.37$, $p < 0.001$) (Fig. 2A). Conversely, in *P. serratus*, hypercapnia-exposed individuals had greater haemolymph sodium ion concentrations after 5 and 14 d exposure (hypercapnia vs. normocapnia: square root-transformed: $F_{1,72} = 15.99$, $p < 0.001$; time: square root-transformed: $F_{2,72} = 10.84$, $p < 0.001$) (Fig. 2B).

Chloride. Chloride regulation in *Palaemon elegans* matched the pattern of sodium regulation with significantly lower chloride concentrations in hypercapnic individuals compared with control individuals after 14 d ($F_{2,72} = 5.60$, $p < 0.01$) (Fig. 3A). In *P. serratus*, conversely, a different pattern was observed, with significantly higher haemolymph concentrations observed after 14 d (log-transformed: $F_{2,72} = 9.13$, $p < 0.001$) (Fig. 3B).

Calcium. No difference in calcium concentrations were observed after 5 d exposure in *Palaemon elegans* (Fig. 4A). After 14 d, hypercapnic *P. elegans* individuals displayed significantly lower calcium concentrations compared with controls, matching the pattern seen with sodium and chloride; however, there were no differences in calcium concentrations between control and hypercapnia-exposed individuals after 30 d ($F_{2,72} = 8.81$, $p < 0.001$). Conversely, with *P. serratus*, hyperregulation of internal calcium was observed in hypercapnic individuals after both 5 and 14 d exposure. After

Fig. 2. *Palaemon elegans* and *P. serratus*. Haemolymph sodium concentrations (mean $\pm$ SE) ($mmol\ l^{-1}$) in (A) *P. elegans* and (B) *P. serratus*. ○ and ● indicate control (normocapnic) and hypercapnic treatments, respectively. * $p < 0.05$, ** $p < 0.001$ ($n = 13$)

30 d, there were no significant differences between normocapnic and hypercapnic individuals (square root-transformed: $F_{2,72} = 3.45$, $p < 0.05$) (Fig. 4B).

Potassium. The only significant differences in internal potassium concentrations in *Palaemon elegans* were after 5 d (log-transformed: $F_{1,72} = 4.51$, $p < 0.05$) (Fig. 5A). With regard to *P. serratus*, however, there were no significant differences between hypercapnic and normocapnic individuals (Fig. 5B).

Magnesium. There were differences in haemolymph magnesium concentrations, with hypercapnic individuals of both species displaying significantly lower magnesium concentrations compared with control individuals after 5 and 14 d (only in *Palaemon elegans*) (*P. elegans*: log-transformed: $F_{1,72} = 2.50$, $p < 0.001$; *P. serratus*: log-transformed: $F_{2,72} = 6.08$, $p < 0.001$) (Fig. 6A,B).

Haemolymph ion ratios

Palaemon serratus exhibits a rapid compensation for hypercapnic acidosis as shown by a 2- and 4.7-fold change increase in bicarbonate:calcium and bicarbon-

Fig. 3. *Palaemon elegans* and *P. serratus*. Haemolymph chloride concentrations (mean $\pm$ SE) ($mmol\ l^{-1}$) in (A) *P. elegans* and (B) *P. serratus*. ○ and ● indicate control (normocapnic) and hypercapnic treatments, respectively. * $p < 0.05$, ** $p < 0.001$ ($n = 13$)

ate:chloride exchanges at 5 d compared to *P. elegans* (Table 2). However, after 14 d, an increase in bicarbonate buffering occurs in *P. elegans* as indicated by a 4- and 5-fold increase in bicarbonate exchange compared to calcium and chloride ions, respectively (Table 2). Differing mechanisms of ion regulation are expected to occur between the 2 species as indicated by the lower ion concentrations in *P. elegans* and the increasing ion concentrations in *P. serratus* over time (Figs. 2–6) and compared to the respective controls and *in situ* values (Parry 1954, Ramirez de Isla Hernandez & Taylor 1985) (Table 3).

DISCUSSION

Haemolymph acidosis was observed in both species after 5 d, coupled with an increase in hypo-osmotic capacity after 5 d in *Palaemon serratus* and after 14 d in *P. elegans*. After 14 d, extracellular pH in *P. elegans* was still depressed but was not significantly different from the control in *P. serratus*. Hypercapnia-related changes to extracellular pH may be linked to altered

Fig. 4. *Palaemon elegans* and *P. serratus*. Haemolymph calcium concentrations (mean $\pm$ SE) ($mmol\ l^{-1}$) in (A) *P. elegans* and (B) *P. serratus*. ○ and ● indicate control (normocapnic) and hypercapnic treatments, respectively. * $p < 0.05$, ** $p < 0.001$ ($n = 13$)

ionic regulation, as indicated by decreased (*P. elegans*) and elevated (*P. serratus*) ion concentrations in haemolymph, thereby acting as a compensatory mechanism of ion exchange to buffer changes in internal pH. Although the exact mechanism is as yet unclear, ion regulatory changes may result from an up-regulation of Na^+/K^+ -ATPase, the enzyme responsible for ion exchange, as shown previously for palaemonids and other decapods (Freire et al. 2007, Mendonça et al. 2007, Ituarte et al. 2008, Masui et al. 2009). Palaemonids actively pump out sodium and chloride ions (in seawater) in order to regulate the ionic composition of haemolymph in seawater (*P. serratus* $459\ mmol\ l^{-1}$ at $15^\circ C$; *P. elegans* 320 and $398\ mmol\ l^{-1}$ at 10 and $20^\circ C$, respectively) (Parry 1954, Ramirez de Isla Hernandez & Taylor 1985), thereby maintaining the internal medium hypo-osmotic to that of the external medium (seawater) (Table 3).

Hypercapnia exposure due to increased CO_2 concentration, as shown here, resulted in species-specific responses in 2 efficient osmotic/ionic regulators. There

Fig. 5. *Palaemon elegans* and *P. serratus*. Haemolymph potassium concentrations (mean $\pm$ SE) ($mmol\ l^{-1}$) in (A) *P. elegans* and (B) *P. serratus*. ○ and ● indicate control (normocapnic) and hypercapnic treatments, respectively. * $p < 0.05$, ** $p < 0.001$ ($n = 13$)

was a general pattern of increased haemolymph ionic concentrations (particularly Na^+ , Cl^- and Ca^{2+}) in *Palaemon serratus*, and decreased ionic concentrations in *P. elegans* compared to respective controls. Specifically regarding Ca^{2+} , an increase in haemolymph $[Ca^{2+}]$ could arise from exoskeleton dissolution. In decapod crustaceans, dissolution of the exoskeleton yields bicarbonate (and thus calcium), which may be used for buffering of acid–base alterations. This has been observed in the blue crab under hypercapnic conditions, although the carapace played a minor role in hypercapnic buffering, whereas branchial ionic exchanges (at the gills) accounted for most of the compensatory exchanges (Cameron 1985). Spicer et al. (2007) demonstrated a 20% decrease in osmolality in hypercapnia-exposed individuals of the decapod crustacean species *Necora puber*, with a associated rise in Ca^{2+} and Mg^{2+} ions in haemolymph (2 to 6 d). Although these authors could not ascertain the exact mechanism of elevated Ca^{2+} and Mg^{2+} ions in their study, they claim bicarbonate buffering could be 'sourced' from

Fig. 6. *Palaemon elegans* and *P. serratus*. Haemolymph magnesium concentrations (mean $\pm$ SE) (mmol l⁻¹) in (A) *P. elegans* and (B) *P. serratus*. $\circ$ and $\bullet$ indicate control (normo-capnic) and hypercapnic treatments, respectively. * $p < 0.05$, ** $p < 0.001$ ($n = 13$)

the seawater rather than the exoskeleton. In our study, we also could not identify the exact mechanism or origin of elevated divalent ions. The comparative ratio between $[\text{HCO}_3^-]$ and ΔCa^{2+} (as calculated by Henry et al. 1981) for both palaemonid species could elucidate the magnitude of the ion transport mechanism (Table 2). The compensation in hypercapnic acidosis with concomitant change in hypo-osmotic capacity may be linked to exchanges of bicarbonate, calcium and chloride. *P. serratus* exhibited a faster compensation for hypercapnic acidosis as shown by a 2- and 4.7-fold increase in bicarbonate:calcium and bicarbonate:chloride exchanges at 5 d compared to *P. elegans*, and also by the lower ion concentrations in *P. elegans*

and the increased ion concentrations in *P. serratus* over time compared to respective controls (Figs. 2–6, Table 3). Carbonic anhydrase plays a role in supplying counter-ions (H^+ and HCO_3^-) for ionic exchange through the hydration of respired CO_2 and plays an important role in CO_2 excretion and acid–base balance (Henry & Cameron 1983, McMahon et al. 1984). It could be hypothesised that under hypercapnia exposure any upregulation in carbonic anhydrase (and also Na^+/K^+ -ATPase, as mentioned previously) could serve to compensate for hypercapnic changes in acid–base balance as a result of active ion transport (Henry & Wheatly 1992, Seibel & Walsh 2003), although 90 % of bicarbonate sourced is ‘sourced’ from seawater (Cameron 1985).

The major physiological changes in 2 closely related species which inhabit different environments (rock pools and subtidal areas) could provide fundamental insights into the adaptive responses and small scale phenotypic evolution in decapod crustacean species. The differential pattern of haemolymph ions (Na^+ , Cl^- and Ca^{2+}) observed here and, particularly, decreased $[\text{Ca}^{2+}]$ in *Palaemon elegans* and elevated $[\text{Ca}^{2+}]$ in *P. serratus* could signal species-specific differences in the mechanisms used to compensate for hypercapnic conditions. The gills of Palaemonid prawns are the most vital organs in osmoregulation (sodium and chloride ions), as the renal organs play an unimportant role (Panikkar 1941, Parry 1954, Freire et al. 2003). In seawater, palaemonids compensate for passive salt influx by active salt excretion and active water uptake, thereby achieving hypo-osmotic regulation in seawater, although the exact mechanism is unknown (Freire et al. 2008). The increased hypo-osmotic capability (i.e. lowering of osmolality), as seen here, may thus arise by either a combination of active secretion of ions and/or active transport of water against the osmotic gradient, as indicated by a lower ion concentration in *P. elegans* (Panikkar 1941), which would account for the observed differential pattern of ion regulation. Augusto et al. (2009) observed a decrease in haemolymph osmolality and a concomitant increase in both sodium and chloride haemolymph ion concentrations linked to an increase in muscle tissue water content in the intertidal *P. northropi* when acutely exposed to lower salinities. The elevated ionic concentrations (Na^+ , Cl^- and Ca^{2+}) and lowered osmolality (as observed here) may occur via similar mechanisms when palaemonids are exposed to lower salinities (Augusto et al. 2009), as *P. serratus* also retains this capability. Long-term hypercapnia exposure (30 d) had no effect on osmotic capacity, as values were similar to those of control individuals, suggesting acclimatory effects, since osmolality values are lower than those obtained *in situ* (0 d) and from previous studies (calculated from Panikkar 1941, Janas & Spicer 2008).

Table 2. *Palaemon elegans* and *P. serratus*. Average changes in ion concentration (mmol l⁻¹) with hypercapnia exposure over time. Ratio values ca. 1 indicate equivalent changes in ion, >1 represent greater loss in primary exchange ion and <1 represent greater increase in secondary exchange ion

Species	Time (d)	ΔHCO_3^-	ΔCa^{2+}	ΔCl^-	$\text{HCO}_3^-:\text{Ca}^{2+}$	$\text{HCO}_3^-:\text{Cl}^-$
<i>Palaemon elegans</i>	5	0.95	1.03	1.08	0.93	0.88
	14	1.01	0.24	0.20	4.28	5.06
	30	1.80	1.07	0.67	1.68	2.69
<i>Palaemon serratus</i>	5	3.27	1.80	0.80	1.81	4.10
	14	1.23	2.37	3.57	0.52	0.34
	30	2.35	1.00	0.88	2.36	2.66

Table 3. *Palaemon elegans* and *P. serratus*. Ionic composition of seawater (mmol l⁻¹) and extracellular fluid (expressed as percentage of seawater) for *P. elegans* and *P. serratus*

Ions		Na ⁺	Cl ⁻	Ca ²⁺	K ⁺	Mg ²⁺
Seawater (mmol l ⁻¹) ^a		418	541	17	9	76
<i>Palaemon elegans</i> ^a	10°C	77	57	206	83	5
	20°C	95	83	165	86	9
<i>Palaemon elegans</i> (hypercapnia, 0.1 kPa) ^b	5 d	94	102	82	301	4
	14 d	14	9	12	178	3
	30 d	49	23	47	211	13
<i>Palaemon serratus</i> ^c	15°C	85	85	106	85	20
<i>Palaemon serratus</i> (hypercapnia, 0.1 kPa) ^b	5 d	133	78	124	1056	9
	14 d	230	146	206	589	9
	30 d	201	50	147	556	7

^aRamirez de Isla Hernandez & Taylor (1985), ^bPresent study, ^cParry (1954)

Haemolymph acid–base disturbances in aquatic decapod crustaceans have been well-documented, with regard to temperature (Whiteley et al. 1995), salinity (Whiteley et al. 2001) and hypercapnia (Cameron 1985, Cameron & Iwama 1987). Short-term hypoxia and hypercapnia exposure (pO₂ = 10 torr, 1.33 kPa; pCO₂ = 0.74 torr, 0.10 kPa for 3 to 6 h) has previously been shown to cause significant deviations in acid–base disturbance in both *Palaemon elegans* and *P. serratus* (Taylor & Spicer 1991). Significant alterations in blood parameters were found in *P. serratus*, with haemolymph alkalosis after 3 to 6 h and significant acidosis observed after a further 3 h, even after return to normoxic conditions. In *P. elegans*, however, haemolymph alkalosis was negated by a return to normal levels when transferred to normoxic conditions. A significant increase in haemolymph L-lactate concentrations was observed in both species after hypoxia exposure and was assumed to be a result of anaerobic metabolism (Taylor & Spicer 1987). The marked recovery observed in *P. elegans* is perhaps due to the species-specific ability to metabolise L-lactate accumulated under hypoxic conditions, in contrast to *P. serratus*. In the present study, a significant haemolymph acidosis was also observed in both species, which could have arisen as a result of both decreased external pH

(exogenous) and, to some extent, accumulation of H⁺ ions via L-lactate production as a result of anaerobic metabolism (endogenous). Unfortunately, we did not measure lactate production in the present study. A similar pattern of haemolymph acidosis with short-term hypercapnia exposure (5 to 14 d) has been previously reported in 5 decapod crustaceans: *Callinectes sapidus* (2% CO₂ ca. 15 000 ppm) (Cameron 1985, Cameron & Iwama 1987), *Cancer magister* and *Chionectes tanneri* (1% CO₂ ca. 12 800 ppm) (Pane & Barry 2007), *Carcinus maenas* (0.3 kPa pCO₂) (Truchot 1975) and *Necora puber* (6 kPa pCO₂) (Spicer et al. 2007). Compensation for respiratory acidosis was observed in these species and could be explained by the degree of osmo/ionic regulatory ability of these organisms (with the exception of the deep sea crab *Chionectes tanneri*, which is a weak acid–base regulator; Pane & Barry 2007). Those organisms that are weak osmo/ionic regulators or even osmoconformers, such as sea urchins, could possibly be more susceptible to external pH changes, as shown for the echinoid *Psammechinus miliaris*. Miles et al. (2007) observed sustained haemolymph acidosis and incomplete compensa-

tion over an 8 d period in urchins exposed to decreased seawater pH levels (7.44 to 6.14). This pattern of uncompensated acidosis has also been observed in sea urchins exposed to emersion from water and environmental hypoxia (Spicer 1995, Burnett et al. 2002), which indicates that only efficient osmotic/ionic regulators have the capacity to compensate acid–base disturbances. The control treatment in the present study was ca. 0.1 kPa (i.e. 1000 μatm CO₂), and excess (i.e. above ambient 0.04 kPa) probably resulted from bacterial proton production of nitrifying bacteria in biofiltration systems (which is commonly found in recirculating experimental systems). As such, these protons titrate total alkalinity down and cause high pCO₂ despite equilibration with ambient air. Partial pressures of CO₂ of 0.1 kPa (as used here) are similar to 'normal' conditions (0.03 to 0.19 kPa) that exist in rock pools and subtidal areas (due dense algal growth) where these 2 Palaemonid species are found (Morris & Taylor 1983, Taylor & Spicer 1991).

Present results indicate that both *Palaemon elegans* and *P. serratus* are tolerant of hypercapnic exposure and, in addition, compensation for acidosis results in long-term haemolymph alkalosis and may disrupt ionic regulation, although haemolymph osmolality was

similar to control values. While *P. elegans* may be subjected to the rapid changes in $p\text{CO}_2$ associated with intertidal rock pools (e.g. changes in abiotic factors, such as pH and CO_2), *P. serratus* is also tolerant of hypercapnia exposure, although 2 differing mechanisms of ionic regulation may be at work. In summary, hypercapnia exposure due to elevated environmental CO_2 levels by 'ocean acidification' causes a short-term hypercapnic acidosis in 2 efficient osmotic/ionic regulatory species, but compensatory effects result in significant 'off-shoot' in acid–base balance (i.e. alkalosis). Furthermore, species-specific mechanistic differences in ion regulatory ability were revealed, with the predominantly subtidal species *P. serratus* displaying elevated ionic concentrations, in particular, elevated haemolymph sodium, chloride and calcium. Present results suggests that in the short-term (in terms of days), invertebrates that possess ionic regulatory ability experience acid–base imbalances, but have the capacity to compensate for such effects. However, questions remain about the long-term effects: Can these compensatory effects be sustained (i.e. active ion transport) and at what cost to the individual? An inability to control acid–base imbalances in the extracellular fluid may lead directly to further physiological costs, e.g. metabolic suppression, reduced scope for activity and, ultimately, death (Seibel & Walsh 2003). Kurihara et al. (2008) reported significant physiological effects in the Palaemonid *P. pacificus* when exposed to long-term hypercapnic conditions (1900 ppmv CO_2 for 49 d). Growth was significantly depressed in hypercapnic individuals, leading to significant mortality, compared to control individuals. The long-term effects of 'ocean acidification' are as yet unclear; however, acid–base disturbances are apparent and may have consequences at the biochemical level. Alterations in Na^+/K^+ -ATPase enzymatic activity due to disturbances in extracellular fluid (and thus, intracellular) may lead to repercussions at the physiological level. The inability to maintain acid–base regulation and/or ionic/osmotic regulation (which is the mechanism of euryhalinity) may affect the survival of organisms in all aquatic environments (including freshwater, brackish and seawater) and may have profound effects on the salinity tolerance of euryhaline decapod crustaceans. In conclusion, it is demonstrated that as a result of hypercapnia and, subsequently, decreased environmental pH, ionic regulation in 2 osmo/ionic regulator species is affected at a cost to the individual of significant acid–base alterations in internal pH homeostasis.

Acknowledgements. This study was funded by a British Ecological Society (BES) SEP grant awarded to A.D. We thank the 3 anonymous referees whose comments have much improved the manuscript.

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