Journal of the Marine Biological Association of the United Kingdom, 2010, 90(7), 1379–1384.
doi:10.1017/S0025315410000457

Short-term exposure to hypercapnia does not compromise feeding, acid–base balance or respiration of *Patella vulgata* but surprisingly is accompanied by radula damage

HANNAH K. MARCHANT, PIERO CALOSI AND JOHN I. SPICER

Marine Biology and Ecology Research Centre, University of Plymouth, Drake Circus, Plymouth, Devon, PL4 8AA, UK

The effect of short-term (5 days) exposure to CO_2-acidified seawater (year 2100 predicted values, ocean pH = 7.6) on key aspects of the function of the intertidal common limpet Patella vulgata *(Gastropoda: Patellidae) was investigated. Changes in extracellular acid–base balance were almost completely compensated by an increase in bicarbonate ions. A concomitant increase in haemolymph Ca^{2+} and visible shell dissolution implicated passive shell dissolution as the bicarbonate source. Analysis of the radula using SEM revealed that individuals from the hypercapnic treatment showed an increase in the number of damaged teeth and the extent to which such teeth were damaged compared with controls. As radula teeth are composed mainly of chitin, acid dissolution seems unlikely, and so the proximate cause of damage is unknown. There was no hypercapnia-related change in metabolism (O_2 uptake) or feeding rate, also discounting the possibility that teeth damage was a result of a CO_2-related increase in grazing. We conclude that although the limpet appears to have the physiological capacity to maintain its extracellular acid–base balance, metabolism and feeding rate over a 5 days exposure to acidified seawater, radular damage somehow incurred during this time could still compromise feeding in the longer term, in turn decreasing the top-down ecosystem control that* P. vulgata *exerts over rocky shore environments.*

Keywords: acid–base balance, hypercapnia, mollusc, ocean acidification, oxygen uptake, radula

Submitted 7 June 2009; accepted 1 November 2009; first published online 2 June 2010

INTRODUCTION

Atmospheric carbon dioxide (CO_2) has risen 31% in relation to pre-industrial levels (Houghton *et al.*, 2001) with 30% of anthropogenically-produced CO_2 absorbed by the surface layer of the oceans (Raven *et al.*, 2005). This has resulted in a reduction of 0.1 pH units in surface waters (Haugan & Drange, 1996), with a further 0.3–0.5 pH drop expected by 2100 (Caldeira & Wickett, 2003), although pH decline appears to proceed at a much faster rate than previously predicted with important ecological consequences for near shore ecosystems (Wootton *et al.*, 2008). Marine invertebrates that construct exoskeletons, or other structural components, from calcite and aragonite are likely to be particularly sensitive to pH changes (Pörtner *et al.*, 2004; Orr *et al.*, 2005), and it is recommended that any further research into the effects of CO_2-acidified seawater on marine invertebrates be carried out on such keystone and model species. Consequently the effect of mildly low pH/hypercapnic seawater on a key intertidal species, the limpet *Patella vulgata* Linnaeus 1758, was investigated. Intertidal organisms are, in general, thought to be able to compensate internally for exposure to environmental hypercapnia (Pane & Barry, 2007; Widdicombe & Spicer, 2008) as they have evolved acid–base regulatory mechanisms to deal with pH fluctuations, frequently experiencing pH lower than 7.5, and extremes as low as 6.5 in tidepools during low tide (Morris & Taylor, 1983). *Patella vulgata* is thus expected to display good acid–base compensation and little perturbation of key functions like feeding and metabolism when exposed to pH = 7.5. Consequently, we measured (for the first time) pH, dissolved CO_2 (and thus calculated bicarbonate), divalent ion concentration of the haemolymph fluid, together with metabolic rates and feeding in response to exposure to the concentration of dissolved CO_2 predicted by the end of the century (Caldeira & Wickett, 2003). Feeding was included as some preliminary experiments indicated that rates of feeding in *P. vulgata* might decrease in acidified seawater. Furthermore, a preliminary investigation was carried out using scanning electron microscopy (SEM) to determine whether low pH/hypercapnia could significantly affect this function, via alteration to the morphology of the feeding organ (the radula), which is predominantly a chitinous structure (Mann *et al.*, 1986 and references within). Although on this basis the radula perhaps would not be regarded as directly sensitive to acidified seawater, the

Corresponding author:
P. Calosi
Email: piero.calosi@plymouth.ac.uk

general appearance of radular teeth, the number of broken teeth, and the extent to which they were broken, in individual limpets exposed to either normocapnic or hypercapnic conditions were compared. *Patella vulgata* is described as a keystone species due to the role of patellid limpets generally as the dominant grazers on north-west Atlantic rocky shores and because they function in 'top-down' control of ecosystem dynamics (Hawkins & Hartnoll, 1983; Jonsson *et al.*, 2006), so if acidification compromises the function of this intertidal (and thus considered tolerant) species, the consequences for intertidal ecology are likely to be of broad relevance for rocky shore ecosystems and their biotic communities.

MATERIALS AND METHODS

Limpet collection and maintenance

Adult individuals of the common limpet *Patella vulgata* (>4.0 cm length, >3.5 cm width, >2.5 cm height) were hand collected from just above mean low water springs, on the rocky shore at Mount Batten, Plymouth, UK (50°21.34′N 04°07.45′W) during January and June 2008. Individuals were collected attached to substrate bearing their home scar and taken to the laboratory at the University of Plymouth, within 1 hour of collection, inside insulated containers filled with damp macroalgae. Upon arrival limpets were distributed amongst a number of large aquaria (volume = 20 l) supplied with recirculating (and filtered) natural seawater (S = 33, T = 15°C). Rocks and boulders from the collection site covered with microalgae were placed in the aquaria to provide food for the limpets, and arranged such that the limpets had the opportunity for voluntary immersion. Shell epibiota was removed manually, except for a red encrusting film which was not possible to clean off without damage occurring. Such cleaning was necessary to both reduce epibiont respiration and allow visual detection of any hypercapnic-related shell dissolution, as this phenomenon has been documented previously in *Patella caerulea* exposed to pH 7.4 around volcanic CO_2 vents (Hall-Spencer *et al.*, 2008). Only one mortality was recorded during the holding period.

Experimental set-up and seawater acidification

The largest individuals of *P. vulgata* were randomly assigned either to a normocapnic (nominal pH = 8.2, N = 10) or hypercapnic (nominal pH = 7.6, N = 10) treatment. Adults of *P. vulgata* were kept individually, on their original substrate, in containers with screw lids (volume = 0.5 l) filled with artificial seawater (Instant Ocean, S = 35 throughout), and aerated using glass Pasteur pipettes fitted into each lid with compressed air for the normocapnic or compressed air enriched with CO_2 for the hypercapnic treatment. Seawater acidification was achieved using the method employed by Ellis *et al.* (2009). The pH was measured using a pH meter (Inlab 413SG electrode and Sevengo meter, Mettler Toledo, Switzerland) employing the British National Scale (BNS). Total CO_2 (TCO_2) was measured using a CO_2 analyser (965D, Corning Limited, UK). Additional carbonate system parameters (pCO_2, alkalinity, calcite and aragonite saturation, HCO_3^-, and CO_3^{2-}) were calculated using CO_2SYS (Pierrot *et al.*, 2006), with dissociation constants from Mehrbach *et al.* (1973) refit by Dickson & Millero (1987) and KSO_4 using Dickson (1990). To avoid accumulation of waste products and alteration of water pH and O_2, water was replaced every eight hours.

Measurement of metabolic and feeding rates

Rates of O_2 uptake, used as proxy for metabolic rate, were obtained for individuals (pH 8.2, N = 6; pH 7.6, N = 10). Oxygen uptake was determined by using a well-established closed respirometer technique (see Spicer & Eriksson, 2003). Upon completion of these measurements individuals were removed from their shells, and weighed (FP-203 Fisherbrand, Fisher Scientific UK Ltd, UK). Digital photographs of exposure chambers (control and experimental) were taken after an 8 hour period before a water change was performed (between 0800 and 1600 daily for the entire duration of the experiment) and total area (μm^2) of accumulated faeces for each individual for each day was calculated using AnalySIS Digital, which was used as estimate of individuals' feeding rates (pH 8.2, N = 9, pH 7.6, N = 9).

Haemolymph acid–base parameters, divalent ion content and protein content

Haemolymph (volume = 100–500 µl) was collected anaerobically using 1 ml hypodermic syringes, equipped with a 21 g needle that was inserted, via the foot, anaerobically into the overlying pallial sinus. Immediately after sampling, TCO_2 of anaerobically-drawn haemolymph (50 µl) was determined in a CO_2 analyser (965D, Corning Limited, UK) using a 50 µl subsample. Simultaneously, extracellular pH was estimated (anaerobically) at 16°C by direct insertion of a microelectrode pH probe (Inlab 413SG electrode and Sevengo meter, Mettler Toledo, Switzerland) using a microcentrifuge tube (volume = 0.6 ml, Eppendorf). Bicarbonate ion concentrations ($[HCO_3^-]$) from individual limpets' haemolymph were calculated using the Henderson–Hasselbach equation in the form (Spicer *et al.*, 1988):

$$(1)[HCO_3^-] = TCO_2 - \alpha(pCO_2)$$

where α is the solubility coefficient of CO_2 in seawater (0.0499 mmol l^{-1} $Torr^{-1}$ at salinity 35 and temperature 15°C) and $pCO_2 = TCO_2/\alpha(10^{pH-pK_i} + 1)$. pK_i is the negative log of the dissociation constant of carbonic acid in seawater (6.04, salinity 35 and temperature 15°C) (Truchot, 1976). The remaining haemolymph was transferred to a microcentrifuge tube (volume = 1.5 ml, Eppendorf) and kept at 4°C until calcium ($[Ca^{2+}]$) and magnesium ($[Mg^{2+}]$) concentrations in the haemolymph were determined using AAS (GBC Double Beam and Varian Spectra 50, respectively). Protein concentration of haemolymph sub-samples (1.5 µl) was determined employing the A_{280} assay using a Nanodrop spectrophotometer (measurement in triplicate). For all above measures at pH 8.2, N = 10, at pH 7.6, N = 9.

Scanning electron microscopy of radula

Complete radulas were carefully removed manually from all limpets immediately after haemolymph sampling was carried out. Radulas were stored for no more than 7 days wrapped in aluminium foil. Two haphazardly selected

https://doi.org/10.1017/S0025315410000457 Published online by Cambridge University Press

subsections (~4 mm length) of the radula were extracted from the anterior region, mounted and gold spattered prior to SEM analysis (JEOL JSM 5600 LV). Images of worn teeth were overlaid onto comparable images of non-worn teeth from the same subsection and the amount of tooth lost (nm^2) was calculated using AnalySIS Digital Imaging Solutions v.5. Average percentage of worn lateral teeth per each individual limpet was calculated and data were arcsine transformed before statistical analysis (pH 8.2, N = 9, pH 7.6, N = 9).

Statistical analyses

Statistical analyses were conducted using Minitab v.15, SPSS v.16.0 and StatView as appropriate. Data were normally distributed in all cases ($P > 0.05$). Student's *t*-test (not assuming equal variances) was employed to test for possible differences among physiological and integrative parameters measured at control and experimental low pH/hypercapnia. Repeated measure analysis of variance was employed to analyse limpets' feeding rates. Values are presented as means ± SE.

RESULTS

Water pH was stable in normocapnic (8.24) and hypercapnic (7.53) treatments throughout the experimental period. Mean temperature was 13.8°C for both control and low pH and salinity remained constant at 35 (see Table 1 for experimental conditions).

Patella vulgata showed an ability to fully compensate its haemolymph pH when exposed to low pH/hypercapnic seawater for 5 days, as no significant effect of low pH/hypercapnia on this physiological parameter was found when compared to control conditions ($t_{16} = -1.217$, $P > 0.05$; see Figure 1A and Table 1). However, TCO_2 and bicarbonate ion concentration ($[HCO_3^-]$) were significantly greater in hypercapnic treatment compared to the control ($TCO_2 - t_{12} = -5.276$, $P = 0.0002$; see Figure 1B and Table 1; $([HCO_3^-]) - t_{17} = -3.530$, $P = 0.003$; see Figure 1C and Table 1), suggesting that haemolymph acidification was compensated, via increased dissolution of bicarbonate.

Exposure to hypercapnia resulted in an increase in extracellular $[Ca^{2+}]$ in respect to the control ($t_{17} = 4.760$, $P < 0.001$, see also Table 2), although the magnitude of the effect was relatively small ($\Delta = 2.18$ mmol l^{-1}). No significant difference was detected between control and low pH for haemolymph protein content, extracellular $[Mg^{2+}]$, O_2 uptake (maximum $t_{10} = 1.478$, $P > 0.05$; see also Table 2), and for rates of feeding ($F_{1,52} = 0.137$, $P > 0.05$; see also Table 3 for further details).

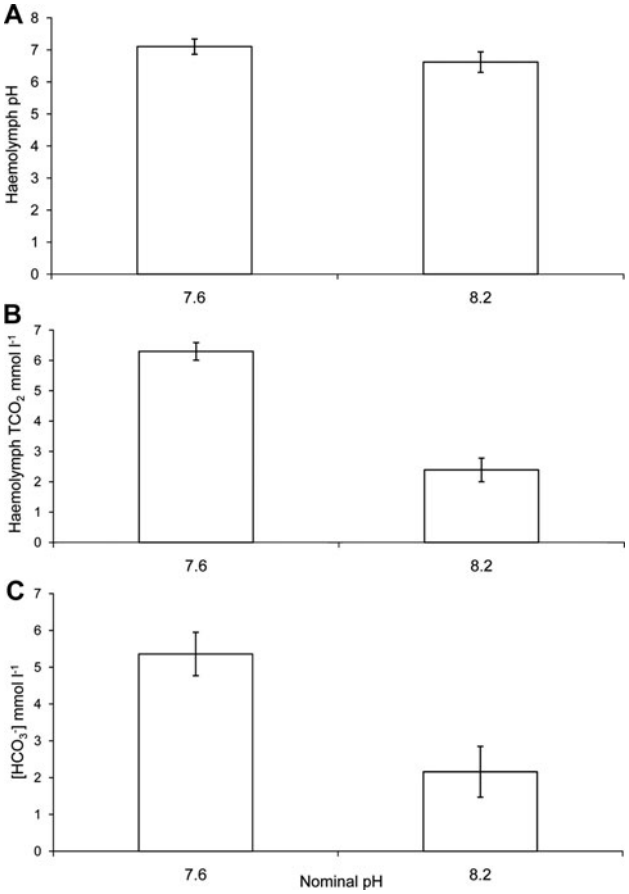

Fig. 1. Haemolymph parameters of the common limpet *Patella vulgata*: (A) pH; (B) TCO_2; and (C) $[HCO_3^-]$ during exposure to low pH/hypercapnic seawater. Histograms are mean values ± SE. Significant differences ($P < 0.05$) between normocapnic (pH 8.2) and hypercapnic treatment (pH 7.6) are highlighted with an *.

Table 2. Mean divalent ion and protein concentration in haemolymph fluid (± SE) of individuals of the common limpet *Patella vulgata* exposed for 5 days to normocapnic (pH 8.2) and hypercapnic (pH 7.6) conditions.

Nominal pH		Calcium (mmol l^{-1})	Magnesium (mmol l^{-1})	A_{280} protein assay (mg μl^{-1})
8.2	Mean	15.25	53.72	3.3
	SE	0.36	0.48	0.44
7.6	Mean	17.73	52.98	3
	SE	0.36	0.79	0.29

Marked shell dissolution was visible in the hypercapnic treatment but not in the control (Figure 2). Radula of the limpets from the low pH/hypercapnia treatment appeared to

Table 1. Measured (salinity, temperature, total CO_2 (TCO_2) and pH) and calculated physico-chemical parameters (total alkalinity (TA), pCO_2, saturation state (Ω) of calcite and aragonite, concentration of bicarbonate $[HCO_3^-]$ and carbonate $[CO_3^{2-}]$ ions) for microcosms of seawater throughout the entire duration of the experiment. Mean values ± SE are given.

Treatment		Salinity	Temperature (°C)	TCO_2 (μmol kg^{-1})	pH	TA (μEq kg^{-1})	pCO_2 (μatm)	Ω calcite	Ω aragonite	HCO_3^- (μmol/kg)	CO_3^{2-} (μmol/kg)
Control	Mean	35	13.84	2644.64	8.24	2942.69	418.61	5.48	3.51	2398.65	229.81
	SE	–	0.09	166.89	0.02	182.41	28.75	0.41	0.27	150.31	17.37
Acidified	Mean		13.83	3316.82	7.53	3287.01	2803.82	1.4	0.9	3149.35	59.01
	SE	–	0.08	158.28	0.01	158.97	119.42	0.10	0.06	150.99	4.19

https://doi.org/10.1017/S0025315410000457 Published online by Cambridge University Press

Table 3. Influence of pH and day on the feeding rates of limpets (measured as total area (μm^2) of accumulated faeces for each individual for each day). Degrees of freedom (df), sum of squares (SS), F-ratio and probability level (*P*) are given.

Source	df	SS	F-ratio	*P*
pH	1	10.016	0.137	0.718
Day	4	618.969	1.264	0.296
pH*day	4	329.317	0.672	0.614

show more signs of wear than those from the control (Figure 3).

There were more broken teeth in the low pH/hypercapnia treatment compared with the control (6% compared to 26%; $t_5 = 2.58$, $P < 0.05$; Figure 4) and the teeth from the

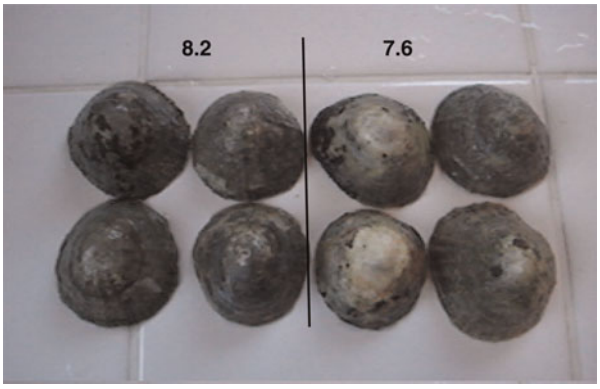

Fig. 2. Visual comparison of common limpets' shell dissolution exposed to normocapnic (left) and hypercapnic treatment (right).

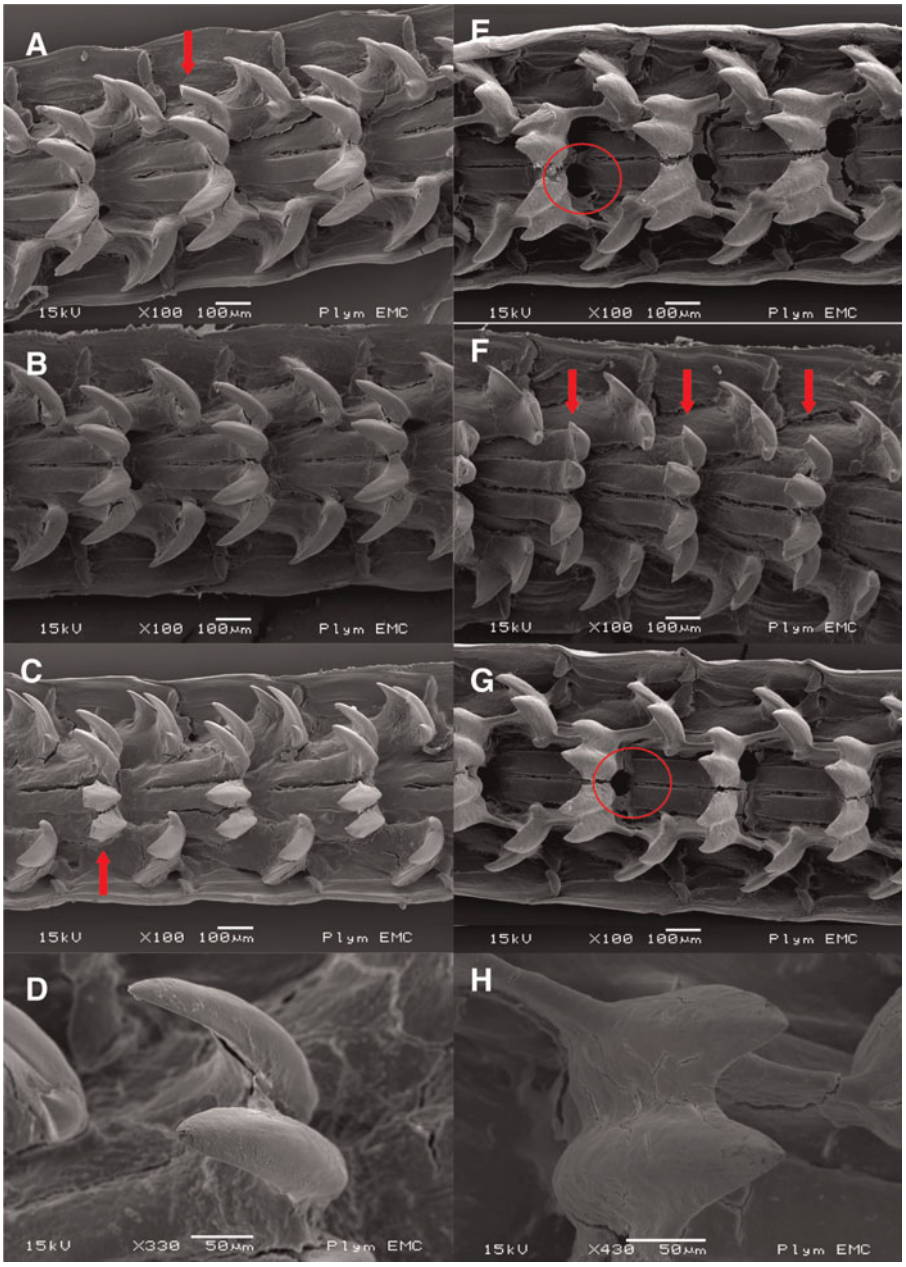

Fig. 3. Representative subsections from the anterior radula of common limpets after exposure to normocapnic (A–D) and hypercapnic treatment (E–H). Red arrows highlight rows with worn teeth; red circles highlight holes in the base of the radula.

https://doi.org/10.1017/S0025315410000457 Published online by Cambridge University Press

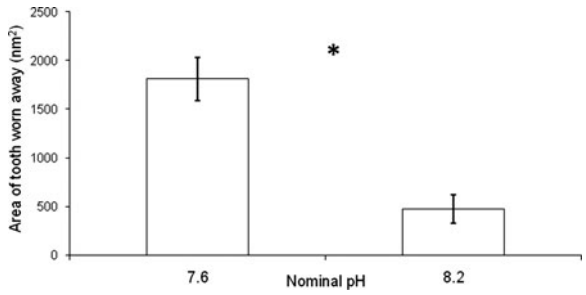

Fig. 4. Comparison of total area worn away on broken teeth of common limpets after exposure to normocapnic and hypercapnic treatment. Histograms represent mean values ± SE. Significant differences ($P < 0.05$) between control and experimental treatment are highlighted with an *.

hypercapnic treatment were significantly more worn than teeth from normocapnic treatment ($t_{14} = -5.096$, $P = 0.0002$; Figure 4).

DISCUSSION

Patella vulgata showed near complete compensation of extracellular acid–base balance, via concomitant increase in haemolymph [Ca^{2+}] and bicarbonate after 5 days exposure to low pH/hypercapnia (pH 7.53), yet no significant alteration of extracellular [Mg^{2+}] was observed. Such compensation does not seem to be so well developed in bivalve molluscs (Lindinger *et al.*, 1984; Michaelidis *et al.*, 2005), although the experimental conditions employed were not strictly comparable. The Ca^{2+}:bicarbonate stochiometry, and the shell dissolution in *P. vulgata* (which has been observed in limpets exposed to acidified water *in situ*; Hall-Spencer *et al.*, 2008) suggests that, as with other molluscs, passive shell dissolution was the main source of bicarbonate; and clearly could not be due to changes in feeding patterns. Certainly good compensation is consistent with the notion that intertidal animals should be better adapted to hypercapnia, than subtidal ones (Pane & Barry, 2007; Widdicombe & Spicer, 2008) but we can say no more at present.

There was no evidence of either hypo- (e.g. Pörtner *et al.*, 2004) or hyper- (e.g. Wood *et al.*, 2008) metabolism in *P. vulgata* associated with exposure to low pH/hypercapnia as was found for other marine invertebrates. Neither was there any significant change in the feeding rate. However, whilst able to maintain acid–base balance, oxygen uptake and feeding rates, there was evidence of significant hypercapnia-related damage to the radula. There was an increase in the number of damaged teeth and the extent to which the tooth was damaged. Yet, the exact mechanism that mediates this negative effect of exposure to low pH/hypercapnia in limpets is unclear. Nonetheless, we may rule out some potential mechanisms; as the radula is constructed from chitin and protein and is not calcareous, low pH-related dissolution is unlikely. Calcium has been, however, located in the radula of *P. vulgata* but not as $CaCO_3$; instead it is incorporated in the crystalline phosphatic mineral hydroxyapatite (Mann *et al.*, 1986). However, the short time scale over which this experiment was carried out (5 days) would appear to rule out the direct effect of low pH/hypercapnia in compromising the production of new teeth in some way. Certainly radula replacement is believed to be metabolically expensive (Davies *et al.*, 2005). This is significant as any physiological cost incurred as a result of radula damage during the experiment may be 'delayed', and not necessarily be detectable as alterations of feeding and metabolism during short-term exposure employed here. Further investigation is necessary to unravel possible long-term effects of exposure to low pH/hypercapnia on the physiological ecology of limpets, and to define the exact physiological mechanisms determining radular damage. It is not inconceivable that there may be some future pathological or compensatory changes in feeding and/or metabolism linked with the cost of radular repair; if so functional consequences of this damage may result in a reduction in feeding efficiency and related fitness of *P. vulgata*. Furthermore, even though maintenance of most ecophysiological functions might be unaffected by the periodic, but acute, exposure to hypercapnia in the intertidal zone, exposure to chronic levels of low pH affecting the morphology and presumably the function of the radula and the passive dissolution of the shell may still have wide-ranging consequences for the top-down ecosystem control that this organism exerts in rocky shore environments.

ACKNOWLEDGEMENTS

We thank Colette Mesher and Richard Thompson for their advice and Glen Harper, Roy Moate, Richard Ticehurst and Ann Torr for technical assistance. This work is a contribution to the Europan Project on Ocean Acidification (EPOCA) and it was undertaken while P.C. was in receipt of a RCUK fellowship to investigate ocean acidification.

REFERENCES

Caldeira K. and Wickett M.E. (2003) Anthropogenic carbon and ocean pH. *Nature* 425, 325.

Davies M.S., Proudlock D.J. and Mistry A. (2005) Metal concentrations in the radula of the common limpet, *Patella vulgata* L., from 10 sites in the UK. *Ecotoxicology* 14, 465–475.

Dickson A.G. (1990) Thermodynamics of the dissociation of boric acid in synthetic seawater from 273.15 to 318.15 K. *Deep-Sea Research* 37, 755–766.

Dickson A.G. and Millero F.J. (1987) A comparison of the equilibrium constants for the dissociation of carbonic acid in seawater media. *Deep-Sea Research* 34, 1733–1743.

Ellis R., Bersey J., Rundle S.D., Hall-Spencer J.M. and Spicer J.I. (2009) Subtle but significant effects of CO_2 acidified seawater on embryos of the intertidal snail, *Littorina obtusata*. *Aquatic Biology* 5, 41–48.

Hall-Spencer J.M., Rodolfo-Metalpa R., Martin S., Ransome E., Fine M., Turner S.M., Rowley S.J., Tedesco D. and Buia M.-C. (2008) Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. *Nature* 454, 96–99.

Haugan P.M. and Drange H. (1996) Effects of CO_2 on the ocean environment. *Energy Conversation Management* 37, 1019–1022.

Hawkins S.J. and Hartnoll R.G. (1983) Grazing of intertidal algae by marine invertebrates. *Oceanography and Marine Biology: an Annual Review* 21, 195–282.

Houghton J.T., Ding Y., Griggs D.J., Noguer M., Linden P.J. van der and Xiaosu D. (2001) Climate Change 2001: the scientific basis.

https://doi.org/10.1017/S0025315410000457 Published online by Cambridge University Press

In *IPCC Third Assessment Report: Climate Change 2001*. Cambridge: Cambridge University Press.

Jonsson P.R., Granhag L., Moschella P.S., Aberg P., Hawkins S.J. and Thompson R.C. (2006) Interactions between wave action and grazing control the distribution of intertidal macroalgae. *Ecology* 87, 1169–1178.

Lindinger M.I., Lauren D.J. and McDonald D.G. (1984) Acid–base balance in the sea mussel, *Mytilus edulis*. 3. Effects of environmental hypercapnia on intracellular and extracellular acid–base balance. *Marine Biology Letters* 5, 371–381.

Mann S., Perry C.C., Webb J., Luke B. and Williams R.J.P. (1986) Structure, morphology, composition and organization of the biogenic materials in limpet teeth. *Proceedings of the Royal Society B* 227, 179–190.

Mehrbach C., Culberson C.H., Hawley J.E. and Pytkowicz R.M. (1973) Measurement of the apparent dissociation constants of carbonic acid in seawater at atmospheric pressure. *Limnology and Oceanography* 18, 897–907.

Michaelidis B., Ouzounis C., Paleras A. and Pörtner H.O. (2005) Effects of long-term moderate hypercapnia on acid–base balance and growth rate in marine mussels *Mytilus galloprovincialis*. *Marine Ecology Progress Series* 293, 109–118.

Morris S. and Taylor A.C. (1983) Diurnal and seasonal variation in physico-chemical conditions within intertidal rock pools. *Estuarine, Coastal and Shelf Science* 17, 339–355.

Orr J.C., Fabry V.J., Aumont O., Bopp L., Doney S.C., Feely R.A., Gnanadesikan A., Gruber N., Ishida A., Joos F., Key R.M., Lindsay K., Maier-Reimer E., Matear R., Monfray P., Mouchet A., Najjar R.G., Plattner G.K., Rodgers K.B., Sabine C.L., Sarmiento J.L., Schlitzer R., Slater R.D., Totterdell I.J., Weirig M.F., Yamanaka Y. and Yool A. (2005) Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* 437, 681–686.

Pane E.F. and Barry J.P. (2007) Extracellular acid–base regulation during short-term hypercapnia is effective in a shallow-water crab, but ineffective in a deep-sea crab. *Marine Ecology Progress Series* 334, 1–9.

Pierrot D., Lewis E. and Wallace D.W.R. (2006) *MS Excel 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, Tennessee, USA.

Pörtner H.-O., Langenbuch M. and Reipschlager A. (2004) Biological impact of elevated ocean CO_2 concentrations: lessons from animal physiology and earth history. *Journal of Oceanography* 60, 705–718.

Raven J., Caldeira K., Elderfield H., Hoegh-Guldberg O., Liss P.S., Riebesell U., Sheperd J. and Watson A.J. (2005) Ocean acidification due to increasing atmospheric carbon dioxide. *Royal Society Policy Document* 12/05, 1–60.

Spicer J.I. and Eriksson S.P. (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.)? *Journal of Experimental Marine Biology and Ecology* 295, 291–243.

Spicer J.I., Taylor A.C. and Hill A.D. (1988) Acid–base status in the sea urchins *Psammechinus miliaris* and *Echinus esculentus* (Echinodermata: Echinoidea) during emersion. *Marine Biology* 99, 527–534.

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

Widdicombe S. and Spicer J.I. (2008) Predicting the impact of ocean acidification on benthic biodiversity: what can animal physiology tell us? *Journal of Experimental Marine Biology and Ecology* 366, 187–197.

Wood H.L., Spicer J.I. and Widdicombe S. (2008) Ocean acidification may increase calcification rates but at a cost. *Proceedings of the Royal Society B* 275, 1767–1773.

and

Wootton J.T., Pfister C.A. and Forester J.D. (2008) Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. *Proceedings of the National Academy of Sciences*. Published online before print 24 November 2008, doi:2010.1073/pnas.0810079105.

Correspondence should be addressed to:
P. Calosi
Marine Biology and Ecology Research Centre
University of Plymouth
Drake Circus, Plymouth
Devon, PL4 8AA, UK
e-mail: piero.calosi@plymouth.ac.uk

https://doi.org/10.1017/S0025315410000457 Published online by Cambridge University Press