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Temporal fluctuations in seawater $p$CO$_2$ may be as important as mean differences when determining physiological sensitivity in natural systems

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Most studies assessing the impacts of ocean acidification (OA) on benthic marine invertebrates have used stable mean pH/$p$CO$_2$ levels to highlight variation in the physiological sensitivities in a range of taxa. However, many marine environments experience natural fluctuations in carbonate chemistry, and to date little attempt has been made to understand the effect of naturally fluctuating seawater $p$CO$_2$ ($p$CO$_2^{sw}$) on the physiological capacity of organisms to maintain acid–base homeostasis. Here, for the first time, we exposed two species of sea urchin with different acid–base tolerances, Paracentrotus lividus and Arbacia lixula, to naturally fluctuating $p$CO$_2^{sw}$ conditions at shallow water CO$_2$ seep systems (Vulcano, Italy) and assessed their acid–base responses. Both sea urchin species experienced fluctuations in extracellular coelomic fluid pH, $p$CO$_2$, and $[HCO_3^-]$ in line with fluctuations in $p$CO$_2^{sw}$. The less tolerant species, $P$. lividus, had the greatest capacity for $[HCO_3^-]$ buffering in response to acute $p$CO$_2^{sw}$ fluctuations, but it also experienced greater extracellular hypercapnia and acidification and was thus unable to fully compensate for acid–base disturbances. Conversely, the more tolerant $A$. lixula relied on non-bicarbonate protein buffering and greater respiratory control. In the light of these findings, we discuss the possible energetic consequences of increased reliance on bicarbonate buffering activity in $P$. lividus compared with $A$. lixula and how these differing physiological responses to acute fluctuations in $p$CO$_2^{sw}$ may be as important as chronic responses to mean changes in $p$CO$_2^{sw}$ when considering how CO$_2$ emissions will affect survival and success of marine organisms within naturally assembled systems.

Keywords: acid–base balance, natural variability, ocean acidification, sea urchin, volcanic vents.
Introduction

Most studies investigating the effects of elevated pCO₂ associated with ocean acidification (OA) on marine invertebrates have focused on stable mean differences in seawater pCO₂ (pCO₂sw). Such studies have indicated a range of variability in tolerances to elevated pCO₂ between species and phyla (e.g. Harvey et al., 2013; Kroeker et al., 2013), which drive shifts in species distributions along natural pCO₂sw gradients (e.g. Calosi et al., 2013a, b). To understand species responses to elevated pCO₂, we need to know how organisms tolerate natural variations in carbonate balances (Hofmann et al., 2011). While large areas of Open Ocean vary little in seawater pCO₂ and pH, wide fluctuations occur in coastal marine habitats (e.g. Price et al., 2012; Reum et al., 2014). Upwelling systems, such as the eastern Pacific, have relatively high pCO₂ levels and aragonite under-saturation (Manzello, 2010; Harris et al., 2013). Although nearshore carbonate chemistry has wide seasonal (Hauri et al., 2012; Harris et al., 2013) and daily fluctuations (Hofmann et al., 2011), the underlying trend is for increasing CO₂ enrichment and aragonite under-saturation due mainly to burning fossil fuels (IPCC, 2013). In some instances, coastal systems, such as those in fjords and estuaries, can exceed mean atmospheric CO₂ due to high respiration rates in organic-rich conditions (Borges and Abril, 2011; Reum et al., 2014). In addition to carbon flux from coastal respiration, photosynthesis and calcification can also drive short-term fluctuations in pH, pCO₂, pH, and total alkalinity (Ap) (e.g. Truchot and Duhamel-Jouve, 1980; Mucci et al., 2011). As our understanding of nearshore carbonate chemistry increases, we must take this into account when selecting the appropriate pCO₂ levels in OA experiments (Andersson and Mackenzie, 2012; McElhaney and Busch, 2013; Reum et al., 2014).

Understanding how organisms cope with their current environment can provide valuable insights into how species will respond to future OA. Specifically, the fluctuations in pCO₂ experienced by coastal species in many habitats are far greater in magnitude than the predicted long-term increase in mean CO₂ due to OA (Hofmann et al., 2010; Joint et al., 2011). Gaining a better understanding of the effect of fluctuating carbonate chemistry on marine organisms may provide basic insights into variations in OA tolerance found in physiological observations (Dupont and Portner, 2013).

The ability to maintain extracellular homeostasis is important in determining tolerance to elevated pCO₂sw (Whiteley, 2011). Most organisms are able to regulate their extracellular pH through acid–base buffering mechanisms, but this can be energetically costly (Portner et al., 2004). Differences in extracellular pH regulatory capabilities and the ability to regulate metabolic rates between species indicate that certain taxa, e.g. teleost fish, brachyuran crustaceans, and cephalopod molluscs, will be more tolerant of elevated pCO₂ than others, including bivalve molluscs and echinoderms (Melzner et al., 2009). Interspecies variability of acid–base regulation capabilities in closely related and/or co-habiting species may underpin comparative sensitivities to OA (e.g. Pane and Barry, 2007; Bressan et al., 2014), which are important if we are to predict community and ecosystem responses to OA. It has been recognized that species living in distinctly different pCO₂ environments have differences in acid–base regulatory capabilities, for example, shallow water species are more able to compensate for acid–base disturbances than deep-water species (Pane and Barry, 2007). While such studies demonstrate variations in acid–base regulatory capabilities within phyla, they do not reveal the community or ecosystem effects of differing species sensitivities. It is these differences in sensitivities to elevated pCO₂ which drive decreases in species richness and increased dominance of tolerant species at naturally acidified vent sites (Kroeker et al., 2011).

Understanding how closely related species living in the same habitat deal with natural pCO₂ conditions would form a basis to our understanding of how communities will be affected by future climate change. Differences in physiological regulatory capabilities have been suggested to drive differences in distribution patterns between closely related species across natural gradients in pCO₂sw, such as those found at volcanic CO₂ vent sites (e.g. Suggett et al., 2012; Calosi et al., 2013a, b). However, to date, all such studies on comparative growth and physiology have focused on chronic mean differences in pCO₂sw (e.g. Suggett et al., 2012; Calosi et al., 2013a, b), despite pCO₂sw at vent sites exhibiting considerable variation within the space of hours to days (Boatta et al., 2013). To date, we have little understanding of how short-term natural fluctuations in pCO₂sw affect animal acid–base regulation and so distribution at vent sites. An example of this can be seen in the distributions of the sea urchin species used in the present study, Arbacia lixula and Paracentrotus lividus, at the CO₂ vent sites off Isola Vulcano (Sicily, Italy). Despite a greater capacity for bicarbonate buffering in response to stable elevated pCO₂sw, P. lividus decreases in population density at higher pCO₂sw closer to the vent sites compared with A. lixula (Calosi et al., 2013a). This indicates that the ability to buffer stable increases in mean pCO₂sw, via increased [HCO₃⁻] is not the primary physiological response driving distribution at the vent sites. The present study investigates the hypothesis that for species such as P. lividus, an increase in acute natural fluctuations in pCO₂sw may pose as great or a greater challenge to maintaining acid–base regulation as chronic mean elevations in pCO₂sw due to the constant need to up- or down-regulate possibly costly bicarbonate buffering responses. Changes in the distribution of related species across present natural pCO₂sw gradients at volcanic seep sites, or, in response to future climate change may have as much to do with physiological responses to increased acute fluctuations in pCO₂sw as with chronic mean elevations in pCO₂sw.

To test this hypothesis, P. lividus and A. lixula collected from control areas were exposed to acute natural fluctuations in pCO₂sw generated by the volcanic CO₂ vent. The coelomic fluid acid–base balance (pH, pCO₂sw, and [HCO₃⁻]sw) of P. lividus and A. lixula was measured every 6 h over the course of 4 d (90 h, total of 16 time points) in an attempt to understand the effect of fluctuating pCO₂sw conditions on the distribution of A. lixula compared with P. lividus. Individual responses in acid–base parameters to changes in pCO₂sw were determined along with differences in bicarbonate and non-bicarbonate buffering capacity between the two species. Bicarbonate and non-bicarbonate buffering capacity were determined by davenport models where bicarbonate buffering is indicated by increases in [HCO₃⁻], above the non-bicarbonate buffer line (NBL) and changes in non-bicarbonate buffering are indicated by changes in the slope of the NBL. Changes in non-bicarbonate buffering were also linked to changes coelomic fluid protein concentration.

Material and methods

Animal collection

On 18 May 2013, A. lixula and P. lividus (n = 18 per species) were collected from a 2–3 m depth in Ponente Bay, Vulcano well away from the effects of seabed CO₂ vents (coordinates = 38°25.185’N, 14°57.074’E). Sea urchins were immediately transported in fully
Table 1. Seawater chemistry throughout the exposure period, measured in the experimental tanks at each time point (means ± s.e.).

<table>
<thead>
<tr>
<th>Seawater parameter</th>
<th>Control treatment</th>
<th>Fluctuating treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH (NBS)</td>
<td>8.04 ± 0.01*</td>
<td>7.72 ± 0.07*</td>
</tr>
<tr>
<td>pH max</td>
<td>8.12</td>
<td>8.04</td>
</tr>
<tr>
<td>pH min</td>
<td>7.95</td>
<td>7.14</td>
</tr>
<tr>
<td>A$_r$ (mEq kg$^{-1}$)</td>
<td>2.53 ± 0.01</td>
<td>2.54 ± 0.01</td>
</tr>
<tr>
<td>Temperature (°C)</td>
<td>19.6 ± 0.2</td>
<td>19.5 ± 0.2</td>
</tr>
<tr>
<td>Salinity</td>
<td>382.2 ± 0.1</td>
<td>382.2 ± 0.1</td>
</tr>
<tr>
<td>pCO$_2$ (μatm)</td>
<td>608.8 ± 19.1*</td>
<td>1742.3 ± 352.7*</td>
</tr>
<tr>
<td>pCO$_2$ max</td>
<td>779.3</td>
<td>5497.4</td>
</tr>
<tr>
<td>pCO$_2$ min</td>
<td>492.5</td>
<td>599</td>
</tr>
<tr>
<td>Ω$_{CaCO_3}$</td>
<td>3.97 ± 0.09*</td>
<td>2.34 ± 0.28*</td>
</tr>
<tr>
<td>Ω$_{Ar}$</td>
<td>2.59 ± 0.06*</td>
<td>1.52 ± 0.18*</td>
</tr>
<tr>
<td>HCO$_3^-$ (μmol kg$^{-1}$)</td>
<td>2102 ± 9*</td>
<td>2295 ± 28*</td>
</tr>
<tr>
<td>CO$_3^{2-}$ (μmol kg$^{-1}$)</td>
<td>170.1 ± 4.1*</td>
<td>100.2 ± 12.0*</td>
</tr>
</tbody>
</table>

*Significant differences between treatments (p < 0.05).

...from 4.2 to 3.0, as mEq kg$^{-1}$ from the slope of the curve HCl volume vs. pH. Total alkalinity measurements were corrected using standards provided by A.G. Dickson (batch 99 and 102). Parameters of the carbonate system (pCO$_2$, CO$_3^{2-}$, HCO$_3^-$) and saturation state of calcite and aragonite were calculated from pH, A$_r$, temperature and salinity using the free-access CO$_2$SYS (Lewis and Wallace, 1998) with constants provided by Mehrbach et al. (1973) refitted by Dickson and Millero (1987) and K$_S$O$_4$ constants from Dickson (1990). Water chemistry throughout the exposure period is shown in Table 1.

**Determination of coelomic fluid acid–base balance**

The acid–base balance of sea urchin coelomic fluid was determined following the methods in Calosi et al. (2013a). Briefly, coelomic fluid (vol. = 100 μl) was extracted anaerobically from each individual using a gas-tight syringe (Gas-tight 1710 100 μl syringe with an RN 22S gauge needle, Hamilton Co., Bonaduz, Switzerland) inserted 1 cm into the coelomic cavity via the peristomial membrane at 6 h intervals for 90 h (4 d, 16 time points). Total bound and dissolved CO$_2$ (TCO$_2$) was analysed instantly in a subsample of coelomic fluid (vol. = 30 μl) using a TCO$_2$ analyser (956D TCO$_2$ Analyser, Corning Diagnostics, Cambridge, MA, USA). For determination of coelomic fluid pH, the remaining coelomic fluid (vol. = 70 μl) was rapidly (within 5 s) injected into a 0.5 ml microcentrifuge tube (TUL-649-010L, Fisher Scientific, Loughborough, UK) and placed into a micro pH probe (Micro-Infab pH combination electrode, Metter Toledo, Leicester, UK) connected to a pH meter (Seven Easy pH meter, Metter Toledo) forming a gas-tight seal between the air and sample (after, Rastrick et al., 2014).

An additional 150 μl of coelomic fluid was extracted from nine freshly collected reference individuals from the collection site of _A. lixa_ and _P. lividus_, and immediately frozen for later determination of coelomic fluid protein concentration ([protein], NBL, and the first apparent dissociation constants of carbonic acid (pK$_A$) in urchin coelomic fluid. NBL were determined after Rastrick et al. (2014), modified from Spicer et al. (1988). Briefly, the 100 μl coelomic fluid sample from each reference individual was injected into a glass diffusion chamber and equilibrated to 0.1, 1.0, and 2.5% CO$_2$ (mixed with O$_2$-balanced N$_2$) in turn, using gas supplied by a gas mixing pump (Wösthoff pump, Wösthoff GmbH, Bochum, Germany). The sample was constantly measured for pH using a micro pH electrode connected to a pH meter. Samples were agitated with a magnetic stirrer and considered equilibrated when the pH value stopped decreasing (circa 15 min). At equilibration pH, the pH value was read and TCO$_2$ measured in a subsample (vol. = 30 μl) using a TCO$_2$ analyser. This was repeated for each pCO$_2$ level. NBLs were constructed using the Henderson–Hasselbalch equation in the following forms:

\[
pCO_2 = \frac{TCO_2}{\alpha(10^{\text{pH}-pK'_A} + 1)},
\]

\[
[HCO_3^-] = 10^{\text{pH}-pK'_C} \cdot \alpha pCO_2,
\]

where \( \alpha \) is the CO$_2$ solubility coefficient in seawater at 20°C (0.3293 mmol l$^{-1}$ kPa$^{-1}$; Spicer et al., 1988; after Harvey, 1955) and \( pK'_C \) calculated as 6.03 for _P. lividus_ and 5.91 for _A. lixa_ at 20°C using Equation (3):

\[
pK'_C = pH\left(\frac{\text{Log}_{10}(TCO_2 - \alpha pCO_2)}{\alpha pCO_2}\right).
\]
The effect of fluctuating \( p\text{CO}_{2} \) on urchin coelomic fluid acid–base balance

There was a significant three-way interaction between site, species, and \( p\text{CO}_{2} \) on \( p\text{CO}_{2} \) (Table 2). The relationship between \( p\text{CO}_{2} \) and \( p\text{CO}_{2} \) in \( P. \text{lividus} \) was significantly different from the fluctuating and the control treatments (Table 2). Similarly, no significant difference between the two treatments (Table 2). In contrast, \( A. \text{lixula} \) showed no significant difference between the two treatments (Table 2). There was also a significant three-way interaction between site, species, and \( p\text{CO}_{2} \) on \( p\text{CO}_{2} \) (Table 2). The relationship between \( p\text{CO}_{2} \) and \( p\text{CO}_{2} \) in \( P. \text{lividus} \) was significantly different from the fluctuating and the control treatments (Table 2). This is because both species show some significant increase in \( [\text{HCO}_3^-] \), as seawater \( p\text{CO}_{2} \) increases; however, this response was significantly greater in \( P. \text{lividus} \) compared with \( A. \text{lixula} \) (where a bicarbonate response was extremely limited) when exposed to natural fluctuations in \( p\text{CO}_{2} \) (Table 2). While fluctuating \( p\text{CO}_{2} \) had a significant effect on the coelomic acid–base status of both species, the effect was greater on \( P. \text{lividus} \) than \( A. \text{lixula} \), as demonstrated by the average slopes of the regressions (Table 2) performed on the \( p\text{H}_\text{a} \), \( p\text{CO}_{2} \), and \( [\text{HCO}_3^-] \) of all individuals against \( p\text{CO}_{2} \) (Table 2). The \( p\text{H}_\text{a} \) of both \( A. \text{lixula} \) and \( P. \text{lividus} \) showed a negative relationship with \( p\text{CO}_{2} \); however, the average \( B \) for the regression of \( p\text{H}_\text{a} \) against \( p\text{CO}_{2} \) in \( P. \text{lividus} \), 

\[ \text{Table 2. Acid–base balance of control field collected } A. \text{lixula} \text{ and } P. \text{lividus.} \]

\[
\begin{array}{|c|c|c|}
\hline
\text{Species} & \text{pH}_\text{a} & \text{pCO}_2 \\
\hline
A. \text{lixula} & 7.31 \pm 0.03^* & 7.73 \pm 0.03^* \\
P. \text{lividus} & 7.08 \pm 0.02 & 7.30 \pm 0.02 \\
\hline
\end{array}
\]

\[ \text{Data are means } \pm \text{ s.e.} \]

*Significant differences between species (\( p < 0.05 \))
greater in *P. lividus* than *A. lixula* (Figure 2). Finally, when exposed to fluctuating pCO$_2$sw the standard deviation of [HCO$_3^-$], in individual *P. lividus* was significantly higher than that in *A. lividus* (t = 7.377, p < 0.001).

No mortality was observed throughout the experimental period nor were there issues with urchin viability or infection encountered due to the repeated sampling of coelomic fluid (see Calosi et al., 2013a); there was also no significant progressive acidosis throughout the experimental period showing that repeated sampling had no effect on acid–base balance.

**Discussion**

The present study demonstrates that naturally fluctuating pCO$_2$sw produced different magnitudes of bicarbonate acid–base responses in two species of sea urchin. The acid–base response of *P. lividus* in relation to fluctuating pCO$_2$sw was greater than that of *A. livula* because *P. lividus* relies on limited bicarbonate buffering capabilities for acid–base regulation, whereas *A. livula* relied on non-bicarbonate buffering capacity. The distributions of closely related species at CO$_2$ vents are driven by differences in acid–base regulatory capabilities (Calosi et al., 2013a). However, despite variations...
in carbonate chemistry being demonstrated in various ecosystems (Hofmann et al., 2011) including volcanic vent sites (Boatta et al., 2013), most studies pertaining to the comparative physiological responses between species to elevated pCO2 and in naturally acidified environments have focused on stable mean differences in pCO2ew conditions (e.g. Suggett et al., 2012; Calosi et al., 2013a, b). Understanding how species respond to natural pCO2ew conditions will help explain some of the variation in physiological responses and may provide better predictions on how species will respond to future change (e.g. Kroeker et al., 2011).

Echinoderm coelomic fluid acid–base parameters often conform according to the surrounding seawater (Farmanfarmaian, 1966; Miles et al., 2007) and they have minimal acid–base regulatory capacity when exposed to elevated pCO2 (Spicer et al., 1988; Miles et al., 2007; Appelhans et al., 2012; Calosi et al., 2013a). Despite the apparent poor acid–base and ion-regulatory mechanisms, when exposed to elevated pCO2, some species of sea urchins have been shown to exhibit bicarbonate buffering to maintain acid–base homeostasis in response to stable elevated pCO2 in laboratory (Miles et al., 2007) and field conditions (Calosi et al., 2013a). In the present study, there was no significant effect of stable pCO2ew on the coelomic fluid acid–base balance of either P. lividus or A. lixula during control incubations.

The differences in acid–base responses to fluctuating pCO2ew between the two species in the present study can be related to the differing mechanisms of acid–base regulation employed. Paracentrotus lividus relied on uncompensated bicarbonate acid–base buffering as while they exhibited a significant relationship between [HCO3⁻] and pCO2ew there were also significant fluctuations in pH and pCO2. In contrast to P. lividus, A. lixula exhibited comparatively lower fluctuations in [HCO3⁻], and experienced no significant fluctuations in pCO2 or pH in relation to fluctuating pCO2ew. The bicarbonate buffering capacity of A. lixula was to the extent of being almost non-existent. The acid–base response of A. lixula to decreasing pCO2, closely follows the non-bicarbonate buffering line (Figure 3) and therefore is due to non-bicarbonate buffering rather than HCO3⁻ regulation. This mechanism in A. lixula therefore appears to result in greater pH stability under fluctuating pCO2ew conditions. The acid–base responses observed in the present study make an interesting comparison with those of stable mean elevations in pCO2ew in the same species. When exposed to chronic exposure to high pCO2ew A. lixula also demonstrated a lower bicarbonate response than P. lividus; however, in both species, acid–base disturbances are fully compensated (Calosi et al., 2013a). Conversely, the lack of full pH compensation by P. lividus in the present study indicates that this species is unable to respond efficiently to short-term fluctuations in pCO2ew. It is therefore apparent that under fluctuating pCO2ew conditions, the ability of P. lividus to compensate pH breaks down, while the non-bicarbonate buffering capacity of A. lixula allows the maintenance of pH homeostasis. The maintenance of pH homeostasis in A. lixula

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Control</th>
<th>Fluctuating</th>
<th>Control</th>
<th>Fluctuating</th>
</tr>
</thead>
<tbody>
<tr>
<td>pH</td>
<td>7.12 ± 0.05</td>
<td>6.94 ± 0.05*</td>
<td>7.53 ± 0.04</td>
<td>7.45 ± 0.05*</td>
</tr>
<tr>
<td>CO2 (kPa)</td>
<td>0.48 ± 0.06</td>
<td>0.71 ± 0.11*</td>
<td>0.36 ± 0.03</td>
<td>0.58 ± 0.10*</td>
</tr>
<tr>
<td>[HCO3⁻] (mmol l⁻¹)</td>
<td>2.40 ± 0.10</td>
<td>2.21 ± 0.09*</td>
<td>3.57 ± 0.17</td>
<td>4.37 ± 0.23*</td>
</tr>
<tr>
<td>Seawater pH</td>
<td>7.12</td>
<td>7.12</td>
<td>7.12</td>
<td>7.12</td>
</tr>
</tbody>
</table>

Data are presented as means ± s.e., along with the minimum and maximum values observed.

*Significant effect of seawater pCO2 on acid–base parameters (p < 0.05).

![Figure 2](image-url)
may also, in part, be alleviated by comparatively low routine pH levels in this species. Throughout control incubations and in field collected individuals from the control site, A. lixula consistently had a lower pH\textsubscript{e} (7.31 ± 0.03) than P. lividus (7.73 ± 0.03) with no difference in pCO\textsubscript{2sw}. Comparatively low pH\textsubscript{e} under natural and control conditions have been reported previously for A. lixula (Calosi et al., 2013a) and have been reported in other marine organisms (e.g. Gutowska et al., 2010; Donohue et al., 2012). Although the reasons for this, and possible physiological implications, are outside the scope of the present study, what is important is the ability of A. lixula to maintain pH\textsubscript{e} homeostasis and avoid acidosis of the coelomic compartment (i.e. a decrease in pH\textsubscript{e} away from its routine value) in response to fluctuations in than pCO\textsubscript{2sw} with almost no change in [HCO\textsubscript{3}⁻].

This greater capacity for non-bicarbonate buffering in A. lixula is reflected by the steeper NBL of A. lixula compared with P. lividus, and the acid–base responses in A. lixula being closely clustered around the NBL compared with the high variation in bicarbonate response shown by P. lividus (Figure 3). Paracentrotus lividus has a significantly lower [protein], than that of A. lixula, which while low compared with other species of marine invertebrates, is representative of the low protein concentrations seen in sea urchins (e.g. Spicer et al., 1988; Miles et al., 2007). Comparative differences in [protein], between sea urchin species may be the main reason for differences in acid–base regulatory capacity, with species exhibiting higher [protein], having greater capacity for pH\textsubscript{e} regulation (Spicer et al., 1988), as demonstrated in the present study.

Differences in [HCO\textsubscript{3}⁻], response to fluctuating conditions may be related to routine rates of activity and metabolism, as seen in crustaceans where species with routinely higher rates of activity and metabolism have higher non-bicarbonate buffering capacities due to higher levels of extracellular protein (haemocyanin; Watt et al., 1999; Whiteley, 2011). Whether the same conditions apply to urchins is currently unknown, but if true, may explain differences in pCO\textsubscript{2} sensitivity between the two species. The energetic consequences of relying on bicarbonate buffering in P. lividus may be greater than that of non-bicarbonate buffering in A. lixula. For example, Na\textsuperscript{+}/K\textsuperscript{+}-ATPase is one of a number of transport mechanisms utilized in invertebrate acid–base regulation (Reipschläger and Pörtner, 1996), the activity of which is energetically expensive accounting for up to 40% of the total energy demand in the sea urchin Strongylocentrotus purpuratus (Leong and Manahan, 1997). While there are differences between acid–base regulatory mechanisms in the two species of urchins in the present study, it must also be noted that differences in energy acquisition and utilization between the two species under elevated pCO\textsubscript{2} may also be an important aspect of determining species distributions (Binyon, 1972; Stickle and Diehl, 1987) including across natural CO\textsubscript{2} gradients. Arbacia lixula is regarded as omnivorous, while P. lividus is an herbivorous grazer (Agnetta et al., 2013) indicating higher protein acquisition in A. lixula compared with P. lividus. Non-bicarbonate buffering capacity, while perhaps initially expensive, may in the long term be less energetically expensive than constant HCO\textsubscript{3}⁻ regulation. Therefore, species such as A. lixula, which demonstrate a higher capacity of non-bicarbonate buffering and therefore a reduced reliance on HCO\textsubscript{3}⁻ regulation, may be more tolerant to not only chronic stable high pCO\textsubscript{2sw} conditions but also acute fluctuations in pCO\textsubscript{2sw} associated with natural coastal systems. This may be important in determining the resilience of a species as the ability to mobilize HCO\textsubscript{3}⁻ in an attempt to compensate pH\textsubscript{e} in response to OA may have energetic consequences that in the long term may give an advantage to species that favour non-bicarbonate protein buffering and so exhibited smaller and presumably less costly fluctuations in acid–base statues. Given the ability of P. lividus to compensate stable increases in pCO\textsubscript{2sw} (Calosi et al., 2013a), it would be expected to maintain population density closer to the vent sites. However, this present study shows that it may be the inability of P. lividus to compensate for short-term fluctuations in pCO\textsubscript{2sw} that ultimately controls its distribution at the vents. Importantly, the present study shows that natural acute fluctuations in pCO\textsubscript{2sw} associated with natural coastal systems and predicted to increases in the future are just as important in modulating this response as a chronic overall increase in pCO\textsubscript{2sw} associated with OA.

In conclusion, this study shows that the differential sensitivity of closely related species to future climate change (i.e. the “winners” and “losers”) may have as much to do with physiological compensatory responses to natural acute fluctuations in pCO\textsubscript{2sw} as with chronic changes in mean pCO\textsubscript{2sw}. Furthermore, many coastal marine habitats experience periodic and acute fluctuations in seawater carbonate chemistry, the level of variation within which is

![Figure 3](attachment:image.png)

**Figure 3.** Davenport diagrams representing coelomic fluid acid–base balance of (a) P. lividus and (b) A. lixula. Each point represents mean ± s.e. of each species at each time point. Solid line indicates non-bicarbonate buffering line. Squares indicate stable control pH conditions; triangles indicate fluctuating pCO\textsubscript{2sw} conditions.
predicted to increase. Understanding species physiological responses to variations in pCO2 during these habitats is essential in understanding the species and community responses to OA as closely related species, such as $P. lividus$ and $A. lixula$, have trophic and ecological interactions which are important in characterizing the ecology of the systems in which they reside (Bulleri et al., 1999; Privitera et al., 2008).

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