

REVIEW

Biochemical adaptation to ocean acidification

Jonathon H. Stillman^{1,2,*} and Adam W. Paganini¹**ABSTRACT**

The change in oceanic carbonate chemistry due to increased atmospheric P_{CO_2} has caused pH to decline in marine surface waters, a phenomenon known as ocean acidification (OA). The effects of OA on organisms have been shown to be widespread among diverse taxa from a wide range of habitats. The majority of studies of organismal response to OA are in short-term exposures to future levels of P_{CO_2} . From such studies, much information has been gathered on plastic responses organisms may make in the future that are beneficial or harmful to fitness. Relatively few studies have examined whether organisms can adapt to negative-fitness consequences of plastic responses to OA. We outline major approaches that have been used to study the adaptive potential for organisms to OA, which include comparative studies and experimental evolution. Organisms that inhabit a range of pH environments (e.g. pH gradients at volcanic CO_2 seeps or in upwelling zones) have great potential for studies that identify adaptive shifts that have occurred through evolution. Comparative studies have advanced our understanding of adaptation to OA by linking whole-organism responses with cellular mechanisms. Such optimization of function provides a link between genetic variation and adaptive evolution in tuning optimal function of rate-limiting cellular processes in different pH conditions. For example, in experimental evolution studies of organisms with short generation times (e.g. phytoplankton), hundreds of generations of growth under future conditions has resulted in fixed differences in gene expression related to acid–base regulation. However, biochemical mechanisms for adaptive responses to OA have yet to be fully characterized, and are likely to be more complex than simply changes in gene expression or protein modification. Finally, we present a hypothesis regarding an unexplored area for biochemical adaptation to ocean acidification. In this hypothesis, proteins and membranes exposed to the external environment, such as epithelial tissues, may be susceptible to changes in external pH. Such biochemical systems could be adapted to a reduced pH environment by adjustment of weak bonds in an analogous fashion to biochemical adaptation to temperature. Whether such biochemical adaptation to OA exists remains to be discovered.

KEY WORDS: Comparative physiology, Experimental evolution, Conservation of function, Protein, Membrane, Plasticity, Acclimation, Acclimatization

Introduction

The marine carbonate system is driven by exchange of atmospheric CO_2 with ocean surface waters, by physiological processes (i.e. photosynthesis and respiration) and by geochemical processes. On long timescales (100s to 1000s of years), marine carbonate systems

are likely to be in equilibrium with atmospheric CO_2 . But on short timescales, other carbonate system drivers are likely to be more important, especially in coastal waters (Fig. 1). Natural variability of pH varies throughout coastal and pelagic regions of Earth's oceans. Open ocean pH tends to be stable, but surface water pH and carbonate chemistry varies across the Earth's oceans (Takahashi et al. 2014). In shallow habitats on temperate rocky shores, pH routinely fluctuates by ≥ 0.5 units between day and night, and by ≥ 1 pH unit seasonally (Wootton et al., 2008). In estuaries, pH is also highly dynamic, changing with tides, respiration–photosynthesis cycles and runoff (Duarte et al., 2013, Fig. 1). On coral reefs, pH varies by up to 0.5 pH units during day–night cycles (Birkeland et al., 2008). Finally, pH changes rapidly by up to 0.5 pH units during upwelling of CO_2 -rich water on the California coast (Feely et al., 2008; Hofmann et al., 2011). The Intergovernmental Panel on Climate Change predicts that mean global surface ocean pH will decline ~ 0.35 units by the end of the century under the most carbon-intensive scenario, a phenomenon known as 'ocean acidification' (OA) (Gattuso et al., 2014, Pörtner et al., 2014, Hennige et al., 2014).

Certainly, there have been major acidification events through Earth's history (Fig. 1), and those events are associated with major changes in marine communities (Table 1; Hönisch et al., 2012; Pelejero et al., 2010). Widespread extinction of calcifying marine organisms occurred during the Palaeocene–Eocene thermal maximum (PETM, 56 Ma), that had rapid rates of ocean warming (OW) and OA (Hönisch et al., 2012). On geological time scales, PETM OA and OW changed slower than the present rates of change (Fig. 1). As a result of the present extremely rapid rates of change in temperature and pH, and the coupling of changes in pH with changes in other aspects of ocean habitat conditions (e.g. dissolved oxygen, salinity, alkalinity), none of those events are a perfect parallel to what is happening at present (Hönisch et al., 2012). Thus, it is reasonable to conclude that OA will exert strong selective pressures on marine organisms and communities, but predicting the consequences of those selective pressures is limited from the geological record.

Acidification can cause clearly unfavorable conditions to the cellular milieu. Such disruptions result in proton gradients that disrupt cellular homeostasis. Thus it is expected that adaptive potential could involve shifting acid–base balance or redox potential of cells, impacting metabolic pathway flux (Rokitta et al., 2012). Internal acidosis that results from H^+ build-up can be buffered by HCO_3^- acquired through the dissolution of existing CaCO_3 skeletons or shells (Green et al., 2004; Manno et al., 2007; Orr et al., 2005; Wheatly and Henry, 1992). However, making such adjustments to internal HCO_3^- can have profound effects on osmotic and ionic regulation (Larsen et al., 2014) as well as integrated organismal function such as behavior (Nilsson et al., 2012).

Predicting how marine organisms are likely to respond to changing ocean physical and chemical conditions over long time scales (decades to centuries) has been primarily informed by studies of plastic responses of extant organisms to OA over relatively short

¹Romberg Tiburon Center, Department of Biology, San Francisco State University, Tiburon, CA 94920, USA. ²Department of Integrative Biology, University of California Berkeley, Berkeley, CA 94709, USA.

*Author for correspondence (stillmaj@sfsu.edu)

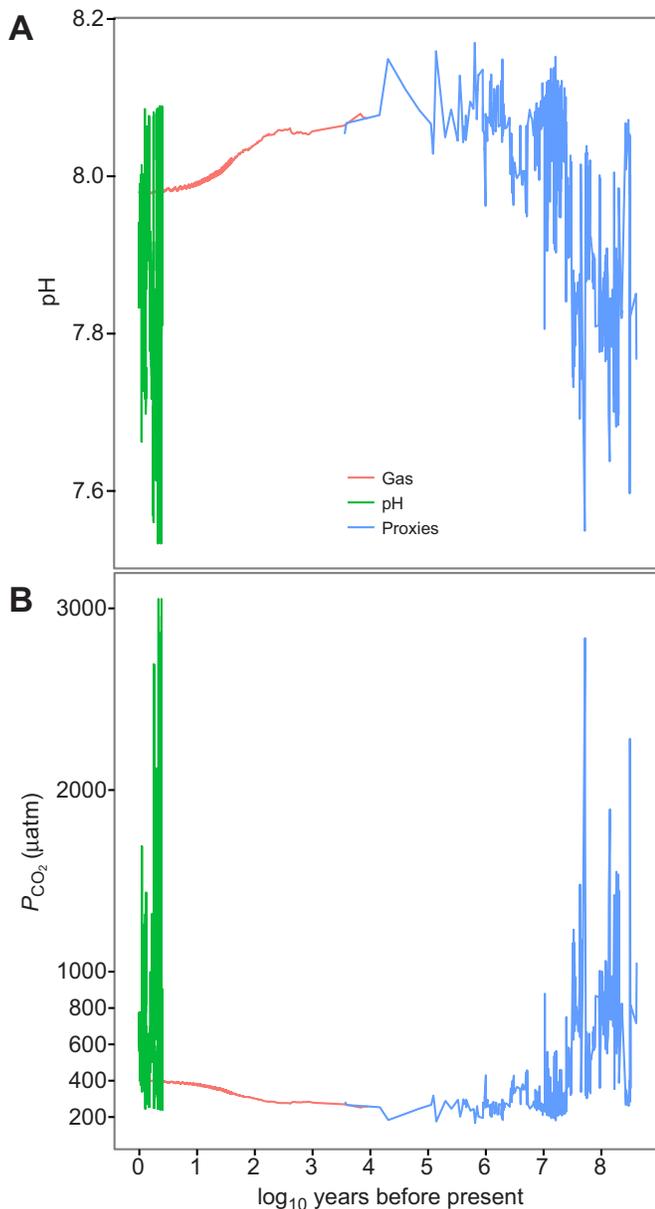


Fig. 1. Variation in ocean surface pH and P_{CO_2} over Earth's history.

(A) pH and (B) P_{CO_2} data used to make this plot were taken from the literature or environmental monitoring stations from three different types of data sources (supplementary material Table S1). 'Gas' indicates data from atmospheric monitoring stations or from gas bubbles trapped in Antarctic ice, and represents actual atmospheric P_{CO_2} . 'Proxies' indicates calculation of atmospheric P_{CO_2} from fossil or geological specimens (e.g. carbonate sediments, foraminifera tests). 'pH' indicates directly measured pH of water at a shallow coastal site in the San Francisco Estuary using a YSI pH sensor (Model 6600V2) at the Romberg Tiburon Center SF BEAMS site (37°53'29"N, 122°26'47"W; <http://sfbeams.sfsu.edu>). For comparison purposes, P_{CO_2} has been interpolated from pH data using the mean relationships between P_{CO_2} and pH and time at the Hawaii Ocean Time Series (Doney et al., 2009) using a second order polynomial curve fit $P_{\text{CO}_2} = 7721.1 \times \text{pH}^2 - 125131 \times \text{pH} + 507221$, and pH has been calculated from P_{CO_2} data using the logarithmic curve fit $\text{pH} = -0.219 \ln \times P_{\text{CO}_2} + 0.2904$. [Note, use of the linear fits presented in Doney et al., 2009 produces overestimates of acidification in extrapolation, hence the curvilinear fits were used]. Although P_{CO_2} from pH data, and vice versa, are likely inaccurate since coastal pH is dependent on other sources of protons than just CO_2 , a large fraction of the observed signal is due to biological processes, and reflects the dramatic variation in pH experienced by coastal organisms within their lifetime, which is presently equivalent to the range in the carbonate system that has been seen over the past 100 Ma.

time frames (see Kroeker et al., 2013 for summary). However, there is a growing number of recent studies that assess adaptive potential over evolutionary time scales (for review, see Sunday et al., 2014). Experimental evolution responses in microorganisms with short generation times are able to demonstrate adaptive responses (e.g. Lohbeck et al., 2013; Reusch and Boyd, 2013). Comparative studies in organisms (populations, species) living across ecological gradients in pH use a 'space for time' argument in inferring adaptive responses (Calosi et al., 2013; Pespenti et al., 2013). In this article, we review the physiological, biochemical and molecular differences observed in those responses to seek a deeper understanding of whether there is a generalizable 'biochemical adaptation' to ocean acidification.

Evolutionary response possibilities

Organismal responses to changing ocean temperature and pH are likely to involve plasticity where one genotype can produce a range of phenotypes or adaptation by local or widespread selection of existing genetic diversity (Sunday et al., 2014). An evolutionary 'time machine' may exist in freshwater ecosystems, whereby diapause embryos of *Daphnia magna* trapped in sediments produced during pre-industrial atmospheric P_{CO_2} can be resurrected for study (Orsini et al., 2013). No such dormant propagules of marine animals are available to assess evolutionary changes in marine ecosystems, although algae cysts in sediments may present that opportunity (Ellegaard et al., 2013; Härnström et al., 2011). However, the 'crystal ball' of the comparative physiology approach can inform us as to how organisms are likely to respond given present phenotypes (Somero, 2010, 2011).

Three main approaches have been used to assess adaptive potential to ocean acidification (Sunday et al., 2014): (1) Short-term acclimation studies (within one generation) to assess the capacity for physiological plasticity; (2) population or species level comparative studies to assess extant genetic and physiological diversity in organisms distributed across natural gradients in pH; and (3) experimental evolution, whereby selection of extant genetic diversity or novel mutations can lead to shifts in performance over multiple generations. Here, we describe advances in each type of approach related to understanding whether biochemical systems are adapted to environmental gradients in pH.

Plasticity

Plastic responses to ocean acidification involve those made within the lifetime of an individual (Somero, 2011), or potentially that persist for multiple generations through epigenetic or maternal effects (Burggren, 2014). Studying organismal responses to anthropogenic changes in the ocean pH yields insight into physiological systems most sensitive to habitat pH and with the potential for adaptation to ocean acidification. Organisms exposed to OA exhibit a wide array of responses that vary across response variables, life history stages, geographic locations and taxa. Meta-analyses have revealed across taxa that survival and calcification are most negatively affected (Kroeker et al., 2013). Taxon-specific analysis reveals the most heavily calcifying groups (calcified algae, corals, mollusks and the larval stages of echinoderms) are most negatively affected by decreases in pH (Kroeker et al., 2013). It is likely that there are environmental pH threshold limits to plastic responses, beyond which acclimation does not occur and fitness is impaired (Dorey et al., 2013).

Reductions in pH disrupt fish olfactory senses, which reduces the ability of individuals to detect predators, prey and parental cues (Dixson et al., 2010; Munday et al., 2009; Nilsson et al., 2012).

Table 1. A summary of ocean acidification events through Earth's history

Event/interval	Evidence and mechanisms		Evidence for seawater chemistry changes				OA score
	Estimated duration	Geological evidence for carbon cycle disruption	Inferred biotic evidence for OA	P_{CO_2}	pH	Surface ocean saturation state	
Future (1850 to present)	160 years	Depletion of fossil fuel reserves, land use changes	?	↑ From 280 ^c to 390 ppm ^d	↓	↓	↑ ~0.7°C in surface ocean ^g
Last deglaciation (17.8–11.6 ky BP)	6.2 ky	Lysocline changes ^h	Lighter planktonic foraminifer shells ⁱ , decreasing coccolith mass ^j	↑ Ice cores ^k	↓ δ ¹¹ B ^l	↓ δ ¹¹ B and assumed alkalinity	↑ Microfossils and geochemical proxies ⁿ
Oligocene/Pliocene (34–2.6 Ma)	10 ⁵ –10 ⁶ years, steady state	Lysocline changes: Oligocene ^o , Miocene ^p	NA	↑ δ ¹¹ B (Pliocene), alkenone δ ¹³ C ^q	↓ Pliocene δ ¹¹ B ^r ; NA >3.5 Ma	NA	↑ ^s
Paleocene and Eocene hyperthermals (58–51 Ma)	Event onset 5–19 ky ^t	Deep-sea CaCO ₃ dissolution ^u , volcanism in the North Atlantic and Caribbean (2x10 ⁶ km ³ basalt ^v)	Benthic foraminifer extinction ^w , turnover in planktonic foraminifers ^x , deformities of coccoliths ^y , demise of coralgal reefs ^z	↑ Geochemical modeling ^{aa}	↓ Geochemical modeling ^{bb}	↓ Geochemical modeling ^{cc}	↑ Foraminifer δ ¹⁸ O, Mg/Ca, TEX86 ^{dd}
Cretaceous/Paleogene (65 Ma)	Instantaneous (impact), <1 My (volcanism) ^{ee}	Asteroid impact ^{ee} and Deccan flood basalts (2x10 ⁶ km ³ basalt), shallow ocean CaCO ₃ dissolution ^{ff}	Extinction of planktonic foraminifers and coccolithophores ^{gg} but not benthic foraminifers ^{ww} or non-calcifying phytoplankton ^{mm}	↑ Stomatal index ^{gg}	NA	↔ Rise in CCD may be due to extinction of calcifiers, not dissolution ^{hh}	↑ Dinocyst assemblages suggest initial cooling followed by extended warming ⁱⁱ
Cretaceous (100–65 Ma)	35 My, steady state	Extensive chalk deposits contradict low CaCO ₃ saturation	NA	↑ Paleosol δ ¹³ C, stomatal index ^{jj}	NA	↔ Geochemical modeling ^{kk}	↑ ^{ll}
Jurassic and Cretaceous OAEs (183 Ma, 120 Ma, 93 Ma)	>100 ky, with pulses potentially as short as 650 to <20,000 years ^{mm}	Volcanism associated with Karoo Traps (2.5x10 ⁶ km ³ basalt) and emplacement of Ontong-Java Plateau (9.1x10 ⁵ km ³ basalt), δ ¹³ C excursion ⁿⁿ	Deformities, variations in size and abundance of planktonic calcifiers ^{oo} , some extinctions (mollusks, echinoderms, brachiopods ^{pp})	↑ Paleosol δ ¹³ C, stomatal index, liverwort δ ¹³ C, alkenone δ ¹³ C ^{jj}	NA	NA	↑ OAE2: Mg/Ca, δ ¹⁸ O and TEX86 ^{qq} , OAE1b: δ ¹⁸ O ^{rr}
Triassic/Jurassic (~200 Mya)	600 ky, pulses as short as 20 ky ^{ss}	Central Atlantic Magmatic Province (>2x10 ⁶ km ³ basalt), reduced carbonate preservation ^{tt}	Collapse of reefs and mass extinction of scleractinian corals ^{uu}	↑ Stomatal index and pedogenic carbonates ^{jj}	NA	NA	↑ Inferred from elevated P_{CO_2} and δ ¹⁸ O ^{vv}
Permian/Triassic (252.3 Mya)	600 ky, pulses shorter than 20–40 ky ^{ww}	Siberian trap basalts (4x10 ⁶ km ³ basalt), shelf carbonate dissolution ^{xx}	Selective extinction of organism probably sensitive to hypercapnia ^{yy}	↑ Geochemical modeling ^{zz}	NA	↔ Disputed ^{aaa}	↑ Inferred from geologic proxies and modeling ^{bbb}

^aDe'ath et al., 2009; ^bde Moel et al., 2009; ^cIndermühle et al., 1999; ^dTans and Keeling, 2010; ^eBates, 2007; ^fKleypas et al., 2006; ^gFarrell and Praeli, 1989; ^hBarker and Elderfield, 2002; ⁱBeaufort et al., 2011; ^jMonnin et al., 2001; ^kFoster, 2008; ^lHönisch and Hemming, 2005; ^mKiessling and Claeys, 2001; ⁿMARGO project members, 2009; ^oPälike et al., 2006; ^pHolbourn et al., 2007; ^qPagani et al., 2010, 2005; ^rSeki et al., 2010; ^sSeki et al., 2010; ^tSeki et al., 2010; ^uDowsett et al., 2005; ^vFlower and Kennett, 1993, 1994, 1995; ^wLawrence et al., 2009; ^xCui et al., 2011; ^yZachos et al., 2010; ^zZeebe et al., 2009; ^{aa}Stap et al., 2009; ^{ab}Zachos et al., 2004; ^{ac}Svensen et al., 2004; ^{ad}Thomas, 2007; ^{ae}Kelly et al., 1998; ^{af}Raffi and De Bernardi, 2008; ^{ag}Scheibner and Speijer, 2008; ^{ah}Zeebe et al., 2009; ^{ai}Ridgwell and Schmidt, 2010; ^{aj}Uchikawa and Zeebe, 2010; ^{ak}Ridgwell and Schmidt, 2010; ^{al}John et al., 2008; ^{am}Zachos et al., 2003; ^{an}Sluijs et al., 2010; ^{ao}Schulte et al., 2010; ^{ap}Ekdale and Bromley, 1984; ^{aq}Beerling et al., 2002; ^{ar}D'Hondt, 2005; ^{as}Brinkhuis et al., 1998; ^{at}supplementary material Table S1; ^{au}Ridgwell, 2005; ^{av}Bice et al., 2006; ^{aw}Cohen et al., 2007; ^{ax}Jenkyns, 2010; ^{ay}Erba et al., 2010; ^{az}Mattioi et al., 2009; ^{ba}Cohen et al., 2007; ^{bb}Bice et al., 2006; ^{bc}Cohen et al., 2007; ^{bd}Forster et al., 2007; ^{be}Forster et al., 2007; ^{bf}Erbacher et al., 2001; ^{bg}Ruhl et al., 2010; ^{bh}Schaller et al., 2011; ^{bi}Crne et al., 2011; ^{bj}Kiessling et al., 2009; ^{bk}McElwain et al., 1999; ^{bl}Korte et al., 2009; ^{bm}Kamo et al., 2003; ^{bn}Shen et al., 2011; ^{bo}Sobolev et al., 2011; ^{bp}Payne et al., 2007; ^{bq}Knoll et al., 2007; ^{br}Clapham and Payne, 2011; ^{bs}Svensen et al., 2009; ^{bt}Payne et al., 2007; ^{bu}Wignall et al., 2009; ^{bv}Kidder and Worsley, 2004.

CCD, calcite compensation depth; NA, not available; OAE, oceanic anoxic events.

These changes are mediated through the direct effects of pH regulation on GABA neurotransmitter pathways. Shifts in acid–base regulation alter the gradients of anions (Cl^- and HCO_3^-) in neuronal membranes, resulting in a reversed current flow through GABA receptors (Hamilton et al., 2014; Nilsson et al., 2012). The impacts of reduced pH on fish olfaction can persist across generations (Welch et al., 2014), suggesting limited phenotypic plasticity in physiological systems involving olfaction. Olfactory neuron architecture is probably similar across fish. Thus, future studies that compare the acid–base regulatory physiology and GABA receptor responses in fish showing different behavioral responses to pH could illuminate an important example of biochemical adaptation to ocean acidification. Such studies could compare across individuals with different behaviors in response to changes in environmental pH (Welch et al., 2014) or among fish adapted to different pH environments.

An important consideration is that pH is not changing in isolation. Increased climate warming due to elevated atmospheric P_{CO_2} is concomitantly changing sea surface temperature and those factors must be considered in combination for ecologically and evolutionarily realistic conclusions to be drawn (Harvey et al., 2013). The effect of OA is generally interactive with the effect of a temperature (Harvey et al., 2013). Physiological energetics may be the basis for the interactive effects, because shifts in energy partitioning between growth and maintenance have been observed for each environmental driver independently. Interactive effects of acidification and temperature variation have been observed in the intertidal porcelain crab, *Petrolisthes cinctipes* (Paganini et al., 2014). Acclimation to increasing pH variability caused an elevation in thermal tolerance, but no concomitant increase in respiration rate (Paganini et al., 2014). In contrast, pH variability had strong interactive effects with the effect of temperature on metabolic rate and thermal tolerance, and thus, sensitivity to pH is context dependent (Paganini et al., 2014). For coastal organisms living in dynamic pH habitats (Duarte et al., 2013), incorporating responses to environmental variability in pH and temperature is an important aspect of understanding plastic and adaptive responses (Dupont and Portner, 2013).

Comparative studies

Populations living across natural ecological gradients in pH demonstrate that decreased calcification under low pH is a real-world phenomenon, not just one that is observed in laboratory experimentation. For example, *in situ* shell dissolution of the coastal snail *Limacina helicina* is accelerated at relatively more-acidified sites in the California Current Large Marine Ecosystem (CCLME) (Bednaršek et al., 2014). Sea urchin species distributed across pH gradients at naturally occurring CO_2 vents in the Mediterranean Sea have shown that local adaptation to high P_{CO_2} environments leads to an adaptive response, resulting in a high buffering capacity of intracellular fluid (Calosi et al., 2013). In this case, this physiological differentiation is what leads to the species distribution patterns. Interspecific comparisons of species adapted to different pH habitats offer insight into how organisms differ in their tolerance for such changes. Organismal tolerance for pH stress is shown when comparing congeners from habitats with different selective pressures, for example, porcelain crabs distributed across the intertidal–subtidal vertical gradient (Stillman and Somero, 2000). Comparisons of porcelain crabs in the genus *Petrolisthes* show how less-thermally-tolerant species exhibit higher mortality with concurrent decreases in exoskeleton $[\text{Ca}^{2+}]$ when exposed to pH stress than congeners from more stable (e.g. subtidal) temperature

habitats (Page and Stillman, 2014). Variation in coccolithophore calcification has been seen across species and strains distributed across global-scale physico-chemical gradients, whereby coccolith mass is inversely correlated with P_{CO_2} (Beaufort et al., 2011), highlighting the importance of comparing similar taxa distributed across large gradients in ocean chemistry conditions in order to understand adaptive potential to ocean acidification.

Intraspecific comparisons between individuals from different habitats allow inferences to be drawn about the plasticity of the physiological responses organisms may have to OA. Local adaptation and differential selection of specific genotypes under acidified conditions has been shown to govern allele frequency of top candidate genes for OA responses (Pespeni et al., 2013). Purple urchin larvae (*Strongylocentrotus purpuratus*) locally adapted to less-acidic sites show this increase in allele frequency when exposed to pH stress, indicating that the adaptive capacity may be a result of standing genetic variation across the spatial-temporal habitats (Pespeni et al., 2013). Intraspecific comparisons with differing thermal habitats show how thermal plasticity can shape responses to pH stress. Populations of intertidal *Concholepas concholepas* snails from warmer habitats increase their aerobic capacity, resulting in higher levels of molecular chaperones when exposed to pH stress (Lardies et al., 2014). These responses are indicative of how thermal plasticity across populations can govern the tolerance limits of acidification stress, possibly by inducing similar pathways.

Similarly, diversity of phenotypic plasticity within populations can have significant effects on how species respond to OA. Brood-specific responses have been shown to be beneficial in regards to OA (Carter et al., 2013; Ceballos-Osuna et al., 2013), which are especially vital since early life stages (e.g. embryonic, larval) can be the most vulnerable to environmental stress (Miller et al., 2013). In porcelain crab larvae and embryos, individuals from some broods show a metabolic reduction in response to lowered pH, whereas in other broods, the same life stages are largely unaffected (Carter et al., 2013; Ceballos-Osuna et al., 2013). Brood-specific variation in response to lowered pH could be due to genetic variability among parents, though maternal effects related to environmental exposure during or prior to oogenesis could also play a role; differentiating between genetic and epigenetic or maternal effects remains an important challenge in determining the sources of plasticity.

Increased phenotypic and genetic variation for larval size of coastal invertebrates in future CO_2 conditions has been shown to be key in understanding relative evolutionary potentials across a large number of species (Sunday et al., 2011). Increases in larval size can produce faster evolutionary responses to pH stress despite having lower rates of population turnover (Sunday et al., 2011). On a population level, the degree to which phenotypic plasticity is an important aspect of tolerance to ocean acidification may be related to standing genetic diversity. For example, increased tolerance for acidification is shown in urchins (Foo et al., 2012; Kelly et al., 2013) because of standing genetic diversity.

The capacity for adaptive responses through selection of genetic variation that leads to acidification-tolerant phenotypes has been demonstrated in many taxa, principally through studies where breeding designs (e.g. North Carolina breeding design) allow for partitioning of phenotypic diversity into genetic and environmental components (Lynch and Walsh, 1998). This approach has been useful for identification of sea urchin genotypes that produce embryos that are more resistant to OA and warming, potentially as a result of maternal provisioning (Foo et al., 2012), and larvae with growth that is less impacted by OA (Kelly et al., 2013; Sunday et al., 2011). Variation in responses to OA and OW at urchin early life

stages are also evidenced at the molecular level, through differential regulation of gene expression (Evans et al., 2013; Padilla-Gamiño et al., 2013; Todgham and Hofmann, 2009), which has also been shown in abalone (Zippay and Hofmann, 2010).

Mechanisms that are responsible for calcification are paramount to also understanding adaptive shifts to OA. Purple urchin larvae, *Strongylocentrotus purpuratus*, reared under high P_{CO_2} were found to exhibit broad-scale decreases in gene expression in four major cellular processes: biomineralization, cellular stress response, metabolism and apoptosis; underscoring that physiological processes beyond calcification and biomineralization are impacted greatly (Todgham and Hofmann, 2009).

Oysters are economically important organisms that have had a huge influence on the attention given to ocean acidification in the public sector because of the sensitivity to OA during their early life stages (Barton et al., 2012). When water in oyster hatcheries is acidified, largely because of variation in pH across the CCLME, early 'D' stage larvae suffer high mortality (Barton et al., 2012). Selective breeding of oysters has great potential to diminish OA impacts on growth and energetics by selection of genotypes that are most fit under future OA conditions (Applebaum et al., 2014; Parker et al., 2011). However, there are potentially trade-offs in oyster biology between being well adapted to OA and other life history characteristics. For example, bryozoan clonal isolates exhibited correlated life history traits and trade-offs of those traits with tolerance to OA and warming (Pistevos et al., 2011). Clearly, there is much that remains to be learned about correlated traits that may have fitness consequences (or advantages) in addition to tolerance to OA conditions. Demonstration of the potential for existing genetic diversity contributing to the resilience of these and other species to a changing ocean suggests that conserving locally adapted populations in low- or variable-pH environments should be emphasized.

Experimental evolution

Adaptive responses to OA in marine organisms have focused on phytoplankton, including diatoms and coccolithophores, because of their importance in the ocean's food webs and biogeochemical cycles and their short generation times (Tatters et al., 2013; Falkowski, 2012). Coccolithophores are generally thought to have reduced inorganic carbon content under OA, though laboratory studies indicate a remarkable diversity in the responses of individual genotypes to future conditions (see Benner et al., 2013 for review). Because coccolithophores can be cultured for hundreds of generations under controlled conditions, they have been used in studies of experimental evolution to assess their adaptive potential to OA (Reusch and Boyd, 2013). The coccolithophore *Emiliania huxleyi* is a particularly well studied species, with remarkable diversity in how it responds to OA across genotypes (Langer et al., 2009). Studies of specific strains held under different conditions for different lengths of time illustrate the potential for coccolithophores to make plastic and adaptive responses to OA (Benner et al., 2013; Langer et al., 2009; Lefebvre et al., 2012; Lohbeck et al., 2012, 2013; Schlüter et al., 2014). *E. huxleyi* typically exhibited a plastic response after 8 generations at high P_{CO_2} and an adaptive response after 500 generations under the same high P_{CO_2} conditions (Lohbeck et al., 2014). After 500 generations under OA, *E. huxleyi* can adaptively regulate genes responsible for cytosolic pH regulation (upregulation of proton pumps and bicarbonate transporters) and subsequently increase its growth and calcification (Lohbeck et al., 2012, 2013, 2014). Shifts in cell size have been observed following experimental evolution under elevated P_{CO_2} in the freshwater green algae

Chlamydomonas (Collins and Bell, 2004). The accumulation of mutations in genes involved with carbon-concentrating mechanisms is believed to be responsible for the adaptive shifts in *Chlamydomonas* (Collins and Bell, 2004).

Marine organisms live in a complex multi-driver environment and in the future, phytoplankton are likely to have to cope with OA concomitantly with warming, shifts in the nitrogen cycle, and potentially other environmental changes. Future P_{CO_2} levels are expected to increase the NH_4^+/NO_3^- ratio in surface waters via a doubling of N_2 fixation rates by *Trichodesmium* (Barcelos e Ramos et al., 2007; Hutchins et al., 2009). Calcification and carbon fixation of *E. huxleyi* are more sensitive to nitrogen source (NH_4^+ versus NO_3^-) than elevated P_{CO_2} , (Lefebvre et al., 2012). Those two environmental drivers interactively alter the ratios of calcification and photosynthesis products of particulate inorganic and organic carbon (Lefebvre et al., 2012). Interestingly, warming seems to ameliorate the negative effects of OA under long-term culture (Benner et al., 2013). The multivariate responses to changes in temperature, P_{CO_2} and nitrogen source remain to be examined.

Studies on non-calcifying phytoplankton have also provided insight into the possible effects of OA on community interactions and productivity. For example, community structure in a mixed dinoflagellate assembly did not shift under OA conditions in a manner that suggested adaptation or acclimation of individual community members (Tatters et al., 2013). Rather, increases in the fitness of specific strains were attributed to biotic interactions (Tatters et al., 2013).

Is there biochemical adaptation to environmental pH?

An aspect largely lacking in the above-mentioned studies is consideration of how rate-limiting biochemical processes involved in responses to environmental pH may be conserved in organisms adapted to differing pH habitats. Classic examples in biochemical adaptation to temperature, for example, have beautifully illustrated that the Michaelis–Menten substrate binding affinity (K_m) is conserved at physiological temperatures across organisms from a wide range of normal body temperatures (see Hochachka and Somero, 2002 for review). Conservation of K_m allows enzymatic reaction rates to be maximally sensitive to small changes in metabolites (Hochachka and Somero, 2002). It is not known whether biochemical adaptation of proteins, especially those on epithelial surfaces in contact with seawater, may conserve function across a wide range of environmental (including boundary layer) values of pH.

The widespread adaptive response of K_m to temperature may be due to the fact that for ectothermic poikilotherms changes in environmental temperatures alter the entire organisms' intracellular environment in which proteins must function properly. In contrast, as environmental pH changes, intracellular pH is strongly buffered (Hochachka and Somero, 2002). However, epithelial tissues in contact with seawater, such as gill epithelia or keratocytes, have proteins on the apical surface that experience environmental pH (Calosi et al., 2013). Those membrane proteins may have altered function under OA. For example, if transmembrane enzymes involved with acid–base regulation (e.g. the H^+ -ATPase transporter (VHA) or anion exchanger in chloride cells) experience variation in external environmental pH, their rates of H^+ pumping or Cl^- transport could change. Fish scale keratocyte behavior and wound healing could be pH sensitive and adapted across organisms living at a range of environmental pH. Changes in function of those processes would alter the potential for acid–base regulation by establishing an imbalance between the intracellular acid–base

environment and the extracellular pH environments in which the organisms have adapted. Do we expect that the pH-sensitive functional properties of epithelial tissue membrane proteins are conserved across organisms adapted to different pH environments?

Direct correlation of how pH affects K_m of enzymes is largely dependent on multiple factors including: (a) substrate type; (b) ionization state of the binding site; (c) an organism's ability to regulate internal pH; and (d) enzyme function (Purich, 2009). For example, the optimal pH for the K_m of pepsin (enzyme located in the acidic human gut) is 1.5, whereas K_m for trypsin located in the more alkaline small intestine is optimal at pH 7.5 (Holum, 1998). pH can affect the ionization state of the binding site (Holum, 1998), and changes in intracellular pH may also change the shape of the active site and alter catalysis (Holum, 1998). Changes in pH can also alter the properties of the substrate such that it has altered binding properties. A reasonable assertion is that enzyme, substrate or cofactor structure related to binding and kinetic properties are optimal at a certain range of pH.

There may be inadequate shifts in environmental pH across marine environments to drive changes in protein structure–function relationships because of perturbation of hydrogen bonds, ionic interactions or other weak bonds. The intracellular P_{CO_2} , HCO_3^- and pH changes that occur during normal physiological processes are greater than the changes in global surface ocean pH expected to occur in the next several hundred years (Tresguerres et al., 2010). Parietal cells, for example, function in an external pH of ~ 3 (Rabon et al., 1983), though may be buffered from the direct effects of the huge increase in $[H^+]$ by mucus (Schreiber et al., 2000). Nonetheless, it is possible that small changes in environmental pH could alter the function of those proteins. We hypothesize that enzymes in organisms adapted to different pH environments display similar conservation of K_m as for patterns of adaptation to temperature (Fig. 2). At a common measurement pH, K_m may be highest in aquatic organisms ranging from very acidic environments [e.g. hydrothermal vents, pH 5–7 (Tunnicliffe et al., 2009)], highly

variable pH environments [e.g. estuarine, coastal, shallow CO_2 vent, pH 6.1–8.6 (Duarte et al., 2013)] or stable high-pH environments [i.e. open ocean, pH 8.1–8.2 (Hofmann et al., 2011); Fig. 2]. Additionally, differences in the sensitivity of K_m to pH may differ across taxa, allowing conservation of enzyme sensitivity across physiological environmental pH (Fig. 2). The shape of each organism's line in Fig. 2 is dependent on a theoretical increase in K_m (or a decrease in binding affinity) with an increase in acidity (lower pH). The figure also assumes that the ionization state of the binding sites of these theoretical enzymes will be largely negatively affected by an increase in $[H^+]$ because of conformational changes that have been shown to take place under acidic conditions for various enzymes (Dixon, 1953).

Na^+ and K^+ pump fluxes are affected by changes in intracellular pH, not by changes in environmental pH (Breitwieser et al., 1987; Fendler et al., 1987). This is largely due to the ATP-binding site of the transporter being located inside the cell where changed environmental pH would not change the conformational structure of the enzyme. However, when changes in external pH drive shifts in acid–base regulation that are transduced to shifts in internal pH or bicarbonate concentration, the intracellular acid–base environment could also be altered (Larsen et al., 2014). Because acidic intracellular pH inhibits Na^+ and K^+ influx and reduces V_{max} for cation efflux (Breitwieser et al., 1987), there may be selective pressure for K_m of enzymes to be conserved across environmental pH. We feel that this is an area of epithelial physiology that has been largely unaddressed and is ripe for research in light of recent advances in our understanding of the function of ion pumps, ion channels and pH sensors in epithelial tissues involved with acid–base regulation (Larsen et al., 2014; Tresguerres, 2014; Tresguerres et al., 2014). Such epithelial physiology studies could use the classic biochemical adaptation comparative approach that George Somero so strongly taught: comparing pH effects on structural and functional properties of homologous proteins from organisms living across a wide gradient of pH environments.

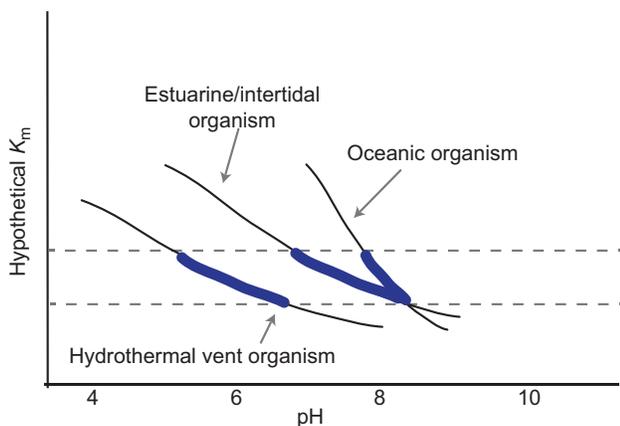


Fig. 2. Hypothetical data of enzyme substrate binding affinity (K_m) from organisms adapted to different pH environments. Low pH=hydrothermal vent; variable pH=estuarine/intertidal; high pH=oceanic. We hypothesize that biochemical adaptation to environmental pH would follow similar 'rules' as biochemical adaptation to temperature (see Hochachka and Somero, 2002), whereby biophysical properties of enzymes are conserved within a narrow range (dashed lines) across physiological environmental pH values typically experienced, as indicated by the thickened blue regions on each curve. These patterns of biochemical adaptation are expected to be greatest for processes involved with protein structure–function changes associated with binding or catalysis at external epithelial surfaces. Whether such a biochemical adaptation to environmental pH does exist remains to be determined.

Conclusions

Continued industrialization and reliance on fossil fuels is predicted to change ocean temperature and chemistry (namely, pH) in an unprecedented manner. Understanding the persistence of populations of marine organisms in future altered environments requires an understanding of extant phenotypic plasticity under realistic environmental conditions and the potential for adaptation. Adaptation potential can be inferred from existing genetic diversity related to patterns of local adaptation across present gradients in environmental pH, and the potential for adaptive shifts through evolutionary processes as assessed by comparative studies. Evidence from such studies suggests that marine organisms do have the potential to adapt to changes in ocean pH. Whether that adaptive potential translates to real-world environments as they become warmer, more acidic and change in other ways associated with how human beings are shaping our planet, remains to be seen. Conservation efforts should work to protect existing genetic diversity by targeting rare marine habitats that show variation in the physical and chemical properties reflecting conditions predicted to be widespread in the future.

Acknowledgements

We thank George Somero for providing us with an opportunity to celebrate his influence on how we think about physiological and biochemical adaptation to the environment and for teaching us how to have a whole lot of fun being scientists. We also thank The Company of Biologists for providing funding for the George Somero

retirement celebration symposium, and Lars Tomanek and Jason Podrabsky for their roles in organizing the symposium.

Competing interests

The authors declare no competing or financial interests.

Author contributions

J.H.S. and A.W.P. conceived the study, collected and analyzed the data and wrote the manuscript.

Funding

This material is based upon work supported by the National Science Foundation [grant no. 1041225] to J.H.S.

Supplementary material

Supplementary material available online at <http://jeb.biologists.org/lookup/suppl/doi:10.1242/jeb.115584/-DC1>

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