

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)

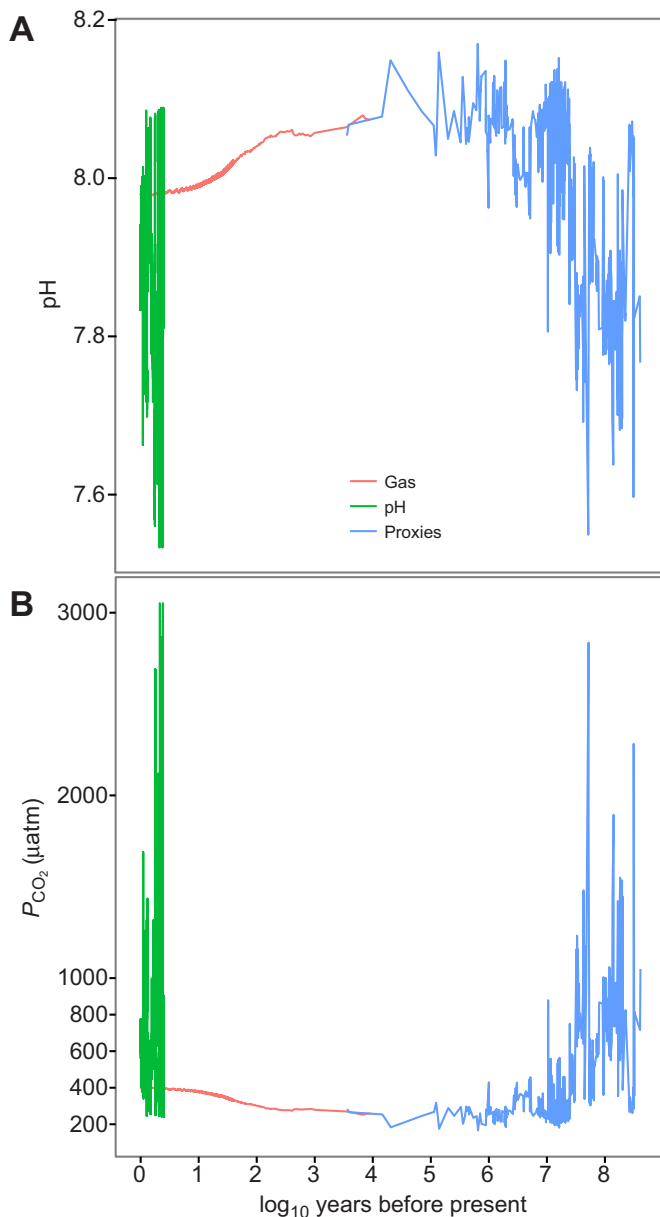


Fig. 1. Variation in ocean surface pH and P_{CO_2} over Earth's history.

(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 (Doney 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).

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.

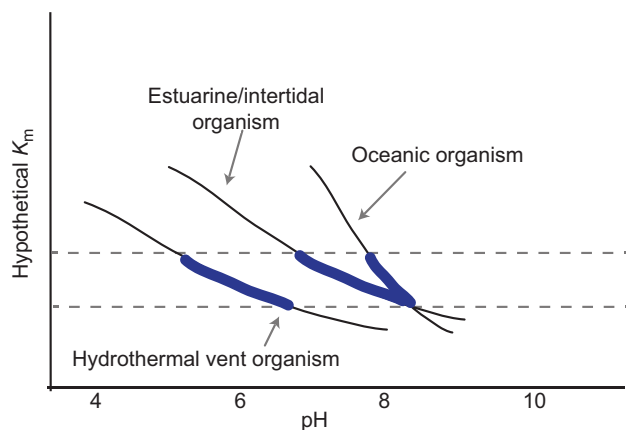


Fig. 2. Hypothetical data of enzyme substrate binding affinity (K_m) from organisms adapted to different pH environments. Low pH=hydrothermal vent; variable pH=estuarine/intertidal; high pH=oceanic. We hypothesize that biochemical adaptation to environmental pH would follow similar 'rules' as biochemical 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.

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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.

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Supplementary material

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

References

- Applebaum, S. L., Pan, T.-C. F., Hedgecock, D. and Manahan, D. T. (2014). Separating the nature and the allocation of energy in response to global change. *Integr. Comp. Biol.* **54**, 284-295.
- Barcelos e Ramos, J., Biswas, H., Schulz, K. G., LaRoche, J. and Riebesell, U. (2007). Effect of rising atmospheric carbon dioxide on the marine nitrogen fixer *Trichodesmium*. *Global Biogeochem. Cycles* **21**, 177-186.
- Barker, S., Elderfield, H. (2002). Foraminiferal calcification response to glacial-interglacial changes in atmospheric CO₂. *Science* **297**, 833-836.
- Barton, A., Hales, B., Waldbusser, G. G., Langdon, C. and Feely, R. A. (2012). The Pacific oyster, *Crassostrea gigas*, shows negative correlation to naturally elevated carbon dioxide levels: Implications for near-term ocean acidification effects. *Limnol. Oceanogr.* **57**, 698-710.
- Bates, N. R. (2007). Interannual variability of the oceanic CO₂ sink in the subtropical gyre of the North Atlantic Ocean over the last 2 decades. *J. Geophys. Res. Oceans* **112**, C09013.
- Beaufort, L., Probert, I., de Garidel-Thoron, T., Bendif, E. M., Ru  z-Pino, D., Metzl, N., Goyet, C., Buchet, N., Coupel, P., Grelaud, M., Rost, B., Rickaby, R. E.M. and de Vargas, C. (2011). Sensitivity of coccolithophores to carbonate chemistry and ocean acidification. *Nature* **476**, 80-83.
- Bednar  ek, N., Feely, R. A., Reum, J. C. P., Peterson, B., Menkel, J., Alin, S. R. and Hales, B. (2014). *Limacina helicina* shell dissolution as an indicator of declining habitat suitability owing to ocean acidification in the California Current Ecosystem. *Proc. R. Soc. B* **281**, 20140123.
- Beerling, D. J., Lomax, B. H., Royer, D. L., Upchurch, G. R. and Kump, L. R. (2002). An atmospheric pCO₂ reconstruction across the Cretaceous-Tertiary boundary from leaf megafossils. *Proc. Natl. Acad. Sci. USA* **99**, 7836-7840.
- Benner, I., Diner, R. E., Lefebvre, S. C., Li, D., Komada, T., Carpenter, E. J. and Stillman, J. H. (2013). *Emiliania huxleyi* increases calcification but not expression of calcification-related genes in long-term exposure to elevated temperature and PCO₂. *Philos. Trans. R. Soc. Lond. B Biol. Sci.* **368**, 20130049.
- Bice, K. L., Birgel, D., Meyers, P. A., Dahl, K. A., Hinrichs, K.-U. and Norris, R. D. (2006). A multiple proxy study of Cretaceous upper ocean temperatures and atmospheric CO₂ concentration. *Paleoceanography* **21**.
- Birkeland, C., Craig, P., Fenner, D., Smith, L., Kiene, W. E. and Riegl, B. (2008). Geologic setting and ecological functioning of coral reefs in American Samoa. In *Coral Reefs of the USA* (ed. B. Riegl and R. E. Dodge) pp. 741-765. Amsterdam, The Netherlands: Springer.
- Breitwieser, G. E., Altamirano, A. A. and Russell, J. M. (1987). Effects of pH changes on sodium pump fluxes in squid giant axon. *Am. Physiol. Soc.* **253**, C547-C554.
- Brinkhuis, H., Bujak, J. P., Smit, J., Versteegh, G. J. M. and Visscher, H. (1998). Dinoflagellate-based sea surface temperature reconstructions across the Cretaceous-Tertiary boundary. *Paleogeogr. Paleoclimatol. Paleoecol.* **141**, 67-83.
- Burggren, W. W. (2014). Epigenetics as a source of variation in comparative animal physiology - or - Lamarck is lookin' pretty good these days. *J. Exp. Biol.* **217**, 682-689.
- Calosi, P., Rastrick, S. P. S., Graziano, M., Thomas, S. C., Baggini, C., Carter, H. A., Hall-Spencer, J. M., Milazzo, M. and Spicer, J. I. (2013). Distribution of sea urchins living near shallow water CO₂ vents is dependent upon species acid-base and ion-regulatory abilities. *Mar. Pollut. Bull.* **73**, 470-484.
- Carter, H. A., Ceballos-Osuna, L., Miller, N. A. and Stillman, J. H. (2013). Impact of ocean acidification on metabolism and energetics during early life stages of the intertidal porcelain crab *Petrolisthes cinctipes*. *J. Exp. Biol.* **216**, 1412-1422.
- Ceballos-Osuna, L., Carter, H. A., Miller, N. A. and Stillman, J. H. (2013). Effects of ocean acidification on early life-history stages of the intertidal porcelain crab *Petrolisthes cinctipes*. *J. Exp. Biol.* **216**, 1405-1411.
- Clapham, M. E. and Payne, J. L. (2011). Acidification, anoxia, and extinction: A multiple logistic regression analysis of extinction selectivity during the Middle and Late Permian. *Geology* **39**, 1059-1062.
- Cohen, A. S., Coe, A. L. and Kemp, D. B. (2007). The late Paleocene - early Eocene and Toarcian (early Jurassic) carbon isotope excursions: a comparison of their time scales, associated environmental changes, causes and consequences. *J. Geol. Soc.* **164**, 1093-1108.
- Collins, S. and Bell, G. (2004). Phenotypic consequences of 1,000 generations of selection at elevated CO₂ in a green alga. *Nature* **431**, 566-569.
- Crne, A. E., Weissert, H., Gorican, S. and Bernasconi, S. M. (2011). A biocalcification crisis at the Triassic-Jurassic boundary recorded in the Budva Basin (Dinarides, Montenegro). *Geol. Soc. Am. Bull.* **123**, 40-50.
- Cui, Y., Kump, L. R., Ridgwell, A. J., Charles, A. J., Junium, C. K., Diefendorf, A. F., Freeman, K. H., Urban, N. M. and Harding, I. C. (2011). Slow release of fossil carbon during the Palaeocene-Eocene Thermal Maximum. *Nature Geosci.* **4**, 481-485.
- D'Hondt, S. (2005). Consequences of the Cretaceous/Paleogene mass extinction for marine ecosystems. *Annu. Rev. Ecol. Syst.* **36**, 295-317.
- de Moel, H., Ganssen, G. M., Peeters, F. J. C., Jung, S. J. A., Kroon, D., Brummer, G. J. A. and Zeebe, R. E. (2009). Planktic foraminiferal shell thinning in the Arabian Sea due to anthropogenic ocean acidification? *Biogeosciences* **6**, 1917-1925.
- D  ath, G., Lough, J. M. and Fabricius, K. E. (2009). Declining Coral Calcification on the Great Barrier Reef. *Science* **323**, 116-119.
- Dixon, M. (1953). The effect of pH on the affinities of enzymes for substrates and inhibitors. *Biochem. J.* **55**, 161-170.
- Dixon, D. L., Munday, P. L. and Jones, G. P. (2010). Ocean acidification disrupts the innate ability of fish to detect predator olfactory cues. *Ecol. Lett.* **13**, 68-75.
- Doney, S. C., Fabry, V. J., Feely, R. A. and Kleypas, J. A. (2009). Ocean acidification: the other CO₂ problem. *Ann. Rev. Mar. Sci.* **1**, 169-192.
- Dorey, N., Melzner, F., Martin, S., Oberh  nsl, F., Teyssi  , J.-L., Bustamante, P., Gattuso, J.-P. and Lacoue-Labarthe, T. (2013). Ocean acidification and temperature rise: effects on calcification during early development of the cuttlefish *Sepia officinalis*. *Mar. Biol.* **160**, 2007-2022.
- Dowsett, H. J., Chandler, M. A., Cronin, T. M. and Dwyer, G. S. (2005). Middle Pliocene sea surface temperature variability. *Paleoceanography* **20**.
- Duarte, C. M., Hendriks, I. E., Moore, T. S., Olsen, Y. S., Steckbauer, A., Ramajo, L., Carstensen, J., Trotter, J. A. and McCulloch, M. (2013). Is ocean acidification an open-ocean syndrome? Understanding anthropogenic impacts on seawater pH. *Estuaries Coasts* **36**, 221-236.
- Dupont, S. and Portner, H.-O. (2013). Marine science: Get ready for ocean acidification. *Nature* **498**, 429.
- Ekdale, A. A. and Bromley, R. G. (1984). Sedimentology and Ichnology of the Cretaceous-Tertiary Boundary in Denmark - Implications for the Causes of the Terminal Cretaceous Extinction. *J. Sediment. Petrol.* **54**, 681-703.
- Ellegaard, M., Ribeiro, S., Lundholm, N., Andersen, T. J., Berge, T., Ekelund, F., H  rnstr  m, K. and Godhe, A. (2013). Using the sediment archive of living dinoflagellate cysts and other protist resting stages to study temporal population dynamics. In *Biological and Geological Perspectives of Dinoflagellates* (ed. J. M. Lewis, F. Marret and L. Bradley), pp. 149-153. London, UK: TMS Special Publications, Geological Society.
- Erba, E., Bottini, C., Weissert, H. J. and Keller, C. E. (2010). Calcareous Nannoplankton Response to Surface-Water Acidification Around Oceanic Anoxic Event 1a. *Science* **329**, 428-432.
- Erbacher, J., Huber, B., Norris, R. and Markey, M. (2001). Intensified thermohaline stratification as a possible cause for an oceanic anoxic event in the Cretaceous period. *Nature* **409**, 325-327.
- Evans, T. G., Chan, F., Menge, B. A. and Hofmann, G. E. (2013). Transcriptomic responses to ocean acidification in larval sea urchins from a naturally variable pH environment. *Mol. Ecol.* **22**, 1609-1625.
- Falkowski, P. (2012). Ocean science: the power of plankton. *Nature* **483**, S17-S20.
- Farrell, J. W. and Prell, W. (1989). Climatic change and CaCO₃ preservation: An 800,000 year bathymetric reconstruction from the central equatorial Pacific Ocean. *Paleoceanography* **4**, 447-466.
- Feely, R. A., Sabine, C. L., Hernandez-Ayon, J. M., Ianson, D. and Hales, B. (2008). Evidence for upwelling of corrosive "acidified" water onto the continental shelf. *Science* **320**, 1490-1492.
- Feely, R. A., Sabine, C. L., Lee, K., Berelson, W. M., Kleypas, J. A., Fabry, V. J. and Millero, F. J. (2004). Impact of anthropogenic CO₂ on the CaCO₃ system in the oceans. *Science* **305**, 362-366.
- Fendler, K., Grell, E. and Bamberg, E. (1987). Kinetics of pump currents generated by the Na⁺, K⁺-ATPase. *FEBS Lett.* **224**, 83-88.
- Flower, B. P. and Kennett, J. P. (1993). Middle Miocene ocean-climate transition - high-resolution oxygen and carbon isotopic records from Deep-Sea Drilling Project Site 588A, Southwest Pacific. *Paleoceanography* **8**, 811-843.
- Flower, B. P. and Kennett, J. P. (1994). The Middle Miocene climatic transition - East Antarctic ice-sheet development, deep-ocean circulation and global carbon cycling. *Paleogeogr. Paleoclimatol. Paleoecol.* **108**, 537-555.

- Flower, B. P. and Kennett, J. P.** (1995). Middle Miocene deep-water paleoceanography in the Southwest Pacific - relations with east Antarctic ice-sheet development. *Paleoceanography* **10**, 1095–1112.
- Foo, S. A., Dworjanyn, S. A., Poore, A. G. B. and Byrne, M.** (2012). Adaptive capacity of the habitat modifying sea urchin *Centrostephanus rodgersii* to ocean warming and ocean acidification: performance of early embryos. *PLoS ONE* **7**, e42497.
- Forster, A., Schouten, S., Moriya, K., Wilson, P. A. and Damste, J. S. S.** (2007). Tropical warming and intermittent cooling during the Cenomanian/Turonian oceanic anoxic event 2: Sea surface temperature records from the equatorial Atlantic. *Paleoceanography* **22**, PA1219.
- Foster, G. L.** (2008). Seawater pH, P_{CO_2} and [CO₃²⁻] variations in the Caribbean Sea over the last 130kyr: A boron isotope and B/Ca study of planktic foraminifera. *Earth Planet. Sci. Lett.* **271**, 254–266.
- Gattuso, J.-P., Brewer, P., Hoegh-Guldberg, O., Kleypas, J. A., Pörtner, H.-O. and Schmidt, D.** (2014). Ocean acidification. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change*. (ed. C. B. Field et al.), pp. 129–131. Cambridge, UK: Cambridge University Press.
- Gledhill, D. K., Wanninkhof, R., Millero, F. J. and Eakin, M.** (2008). Ocean acidification of the Greater Caribbean Region 1996–2006. *J. Geophys. Res. Oceans* **113**, C10031.
- Green, M. A., Jones, M. E., Boudreau, C. L., Moore, R. L., Westman, B. A., Gerber, R., Teegarden, G. and Marinelli, R.** (2004). Dissolution mortality of juvenile bivalves in coastal marine deposits. *Limnol. Oceanogr.* **49**, 727–734.
- Hamilton, T. J., Holcombe, A. and Tresguerres, M.** (2014). CO₂-induced ocean acidification increases anxiety in Rockfish via alteration of GABA A receptor functioning CO₂-induced ocean acidification increases anxiety in Rockfish via alteration of GABA A receptor functioning. *Proc. R. Soc. B* **281**, 20132509.
- Härnström, K., Ellegaard, M., Andersen, T. J. and Godhe, A.** (2011). Hundred years of genetic structure in a sediment revived diatom population. *Proc. Natl. Acad. Sci. USA* **108**, 4252–4257.
- Harvey B. P., Gwynn-Jones, D. and Moore P. J.** (2013). Meta-analysis reveals complex marine biological responses to the interactive effects of ocean acidification and warming. *Ecol. Evol.* **3**, 1016–1030.
- Hennige, S., Roberts, M., Williamson, P., Aze, T., Barry, J., Bellerby, R., Brander, L., Byrne, M., Gattuso, J.-P. et al.** (2014). An updated synthesis of the impacts of ocean acidification on marine biodiversity. Montréal, Canada: Convention on Biological Diversity.
- Hochachka, P. W. and Somero, G. N.** (2002). *Biochemical Adaptation: Mechanism and Process in Physiological Evolution*. New York, NY: Oxford University Press.
- Hofmann, G. E., Smith, J. E., Johnson, K. S., Send, U., Levin, L. A., Micheli, F., Paytan, A., Price, N. N., Peterson, B., Takeshita, Y. et al.** (2011). High-frequency dynamics of ocean pH: a multi-ecosystem comparison. *PLoS ONE* **6**, e28983.
- Holbourn, A., Kuhnt, W., Schulz, M., Flores, J. A. and Andersen, N.** (2007). Orbitally-paced climate evolution during the middle Miocene "Monterey" carbon-isotope excursion. *Earth Planet. Sci. Lett.* **261**, 534–550.
- Holum, J. R.** (1998). *Fundamentals of General, Organic, and Biological Chemistry*, 6th edn. New York, NY: John Wiley & Sons.
- Hönisch, B. and Hemming, N. G.** (2005). Surface ocean pH response to variations in PCO₂ through two full glacial cycles. *Earth Planet. Sci. Lett.* **236**, 305–314.
- Hönisch, B., Ridgwell, A., Schmidt, D. N., Thomas, E., Gibbs, S. J., Sluijs, A., Zeebe, R., Kump, L., Martindale, R. C., Greene, S. E. et al.** (2012). The geological record of ocean acidification. *Science* **335**, 1058–1063.
- Hutchins, D. A., Mulholland, M. R. and Fu, F.** (2009). Nutrient cycles and marine microbes in a CO₂-enriched ocean. *Oceanography* **22**, 128–145.
- Indermühle, A., Stocker, T. F., Joos, F., Fischer, H., Smith, H. J., Wahlen, M., Deck, B., Mastroianni, D., Tschumi, J., Blunier, T., Meyer, R. and Stauffer, B.** (1999). Holocene carbon-cycle dynamics based on CO₂ trapped in ice at Taylor Dome, Antarctica. *Nature* **398**, 121–126.
- Jenkyns, H. C.** (2010). Geochemistry of oceanic anoxic events. *Geochem. Geophys. Geosyst.* **11**, Q03004.
- John, C. M., Bohaty, S. M., Zachos, J. C., Sluijs, A., Gibbs, S., Brinkhuis, H. and Bralower, T. J.** (2008). North American continental margin records of the Paleocene-Eocene thermal maximum: Implications for global carbon and hydrological cycling. *Paleoceanography* **23**, PA2217.
- Kamo, S. L., Czamanske, G. K., Amelin, Y., Fedorenko, V. A., Davis, D. W. and Trofimov, V. R.** (2003). Rapid eruption of Siberian flood-volcanic rocks and evidence for coincidence with the Permian-Triassic boundary and mass extinction at 251 Ma. *Earth Planet. Sci. Lett.* **214**, 75–91.
- Kelly, D. C., Bralower, T. J. and Zachos, J. C.** (1998). Evolutionary consequences of the latest Paleocene thermal maximum for tropical planktonic foraminifera. *Paleogeogr. Paleoclimatol. Paleoecol.* **141**, 139–161.
- Kelly, M. W., Padilla-Gamiño, J. L. and Hofmann, G. E.** (2013). Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin *Strongylocentrotus purpuratus*. *Glob. Chang. Biol.* **19**, 2536–2546.
- Kidder, D. L. and Worsley, T. R.** (2004). Causes and consequences of extreme Permo-Triassic warming to globally equable climate and relation to the Permo-Triassic extinction and recovery. *Paleogeogr. Paleoclimatol. Paleoecol.* **203**, 207–237.
- Kiessling, W. and Claeys, P. A.** (2001). A geographic database approach to the K/T boundary. In: *Geological and biological effects of impact events* (ed. E. Buffetaut and C. Koeberl) pp. 33–140. Berlin: Springer.
- Kiessling, W., Roniewicz, E., Villier, L., L'onide, P. and Struck, U.** (2009). An early Hettangian coral reef in southern France: Implications for the end-Triassic reef crisis. *Facies* **24**, 657–671.
- Kleypas, J. A., Feely, R. A., Fabry, V. J., Langdon, C., Sabine, C. L. and Robbins, L. L.** (2006). Impacts of Ocean Acidification on Coral Reefs and Other Marine Calcifiers: A Guide for Future Research, report of a workshop held 18–20 April 2005, St. Petersburg, FL, sponsored by NSF, NOAA, and the US Geological Survey, **88**.
- Knoll, A. H., Barnbach, R. K., Payne, J. L., Pruss, S. and Fischer, W. W.** (2007). Paleophysiology and end-Permian mass extinction. *Earth Planet. Sci. Lett.* **256**, 295–313.
- Korte, C., Hesselbo, S. P., Jenkyns, H. C., Rickaby, R. E. M. and Spotl, C.** (2009). Palaeoenvironmental significance of carbon- and oxygen-isotope stratigraphy of marine Triassic-Jurassic boundary sections in SW Britain. *J. Geol. Soc.* **166**, 431–445.
- Kroeker, K. J., Kordas, R. L., Crim, R., Hendriks, I. E., Ramajo, L., Singh, G. S., Duarte, C. M. and Gattuso, J.-P.** (2013). Impacts of ocean acidification on marine organisms: quantifying sensitivities and interaction with warming. *Glob. Chang. Biol.* **19**, 1884–1896.
- Langer, G., Nehrke, G., Probert, I., Ly, J. and Ziveri, P.** (2009). Strain-specific responses of *Emiliana huxleyi* to changing seawater carbonate chemistry. *Biogeosciences* **6**, 2637–2646.
- Lardies, M. A., Arias, M. B., Poupin, M. J., Manríquez, P. H., Torres, R., Vargas, C. A., Navarro, J. M. and Lagos, N. A.** (2014). Differential response to ocean acidification in physiological traits of *Concholepas concholepas* populations. *J. Sea Res.* **90**, 127–134.
- Larsen, E. H., Deaton, L. E., Onken, H., O'Donnell, M., Grosell, M., Dantzer, W. H. and Weihrauch, D.** (2014). Osmoregulation and excretion. *Compr. Physiol.* **4**, 405–573.
- Lawrence, K. T., Herbert, T. D., Brown, C. M., Raymo, M. E. and Haywood, A. M.** (2009). High-amplitude variations in North Atlantic sea surface temperature during the early Pliocene warm period. *Paleoceanography* **24**, PA2218.
- Lefebvre, S. C., Benner, L., Stillman, J. H., Parker, A. E., Drake, M. K., Rossignol, P. E., Okimura, K. M., Komada, T. and Carpenter, E. J.** (2012). Nitrogen source and PCO₂ synergistically affect carbon allocation, growth and morphology of the coccolithophore *Emiliana huxleyi*: potential implications of ocean acidification for the carbon cycle. *Glob. Chang. Biol.* **18**, 493–503.
- Lohbeck, K. T., Riebesell, U. and Reusch, T. B. H.** (2012). Adaptive evolution of a key phytoplankton species to ocean acidification. *Nat. Geosci.* **5**, 346–351.
- Lohbeck, K. T., Riebesell, U. and Reusch, T. B. H.** (2014). Gene expression changes in the coccolithophore *Emiliana huxleyi* after 500 generations of selection to ocean acidification. *Proc. R. Soc. B Biol. Sci.* **281**, 20140003.
- Lohbeck, K. T., Riebesell, U., Collins, S. and Reusch, T. B. H.** (2013). Functional genetic divergence in high CO₂ adapted *Emiliana huxleyi* populations. *Evolution* **67**, 1892–1900.
- Lynch, M. and Walsh, B.** (1998). *Genetics and Analysis of Quantitative Traits*. Sunderland, MA: Sinauer Associates, Inc.
- Manno, C., Sandrini, S., Tositti, L. and Accornero, A.** (2007). First stages of degradation of *Limacina helicina* shells observed above the aragonite chemical lysocline in Terra Nova Bay (Antarctica). *J. Mar. Syst.* **68**, 91–102.
- MARGO project members** (2009). Constraints on the magnitude and patterns of ocean cooling at the Last Glacial Maximum. *Nature Geosci.* **2**, 127–132.
- Mattioli, E., Pittet, B., Petitpierre, L. and Mailliot, S.** (2009). Dramatic decrease of pelagic carbonate production by nannoplankton across the Early Toarcian anoxic event (T-OAE). *Glob. Planet. Change* **65**, 134–145.
- McElwain, J. C., Beerling, D. J. and Woodward, F. I.** (1999). Fossil plants and global warming at the Triassic-Jurassic boundary. *Science* **285**, 1386–1390.
- Miller, N. A., Paganini, A. W. and Stillman, J. H.** (2013). Differential thermal tolerance and energetic trajectories during ontogeny in porcelain crabs, genus *Petrolisthes*. *J. Therm. Biol.* **38**, 79–85.
- Monnin, E., Indermühle, A., Dillenbach, A., Flückiger, J., Stauffer, B., Stocker, T. F., Raynaud, D. and Barnola, J.-M.** (2001). Atmospheric CO₂ Concentrations over the Last Glacial Termination. *Science* **291**, 112–114.
- Munday, P. L., Dixon, D. L., Donelson, J. M., Jones, G. P., Pratchett, M. S., Devitsina, G. V. and Døving, K. B.** (2009). Ocean acidification impairs olfactory discrimination and homing ability of a marine fish. *Proc. Natl. Acad. Sci. USA* **106**, 1848–1852.
- Nilsson, G. E., Dixon, D. L., Domenici, P., McCormick, M. I., Sørensen, C., Watson, S.-A. and Munday, P. L.** (2012). Near-future carbon dioxide levels alter fish behaviour by interfering with neurotransmitter function. *Nat. Clim. Change* **2**, 201–204.

- Orr, J. C., Fabry, V. J., Aumont, O., Bopp, L., Doney, S. C., Feely, R. A., Gnanadesikan, A., Gruber, N., Ishida, A., Joos, F. et al. (2005). Anthropogenic ocean acidification over the twenty-first century and its impact on calcifying organisms. *Nature* **437**, 681-686.
- Orsini, L., Schwenk, K., De Meester, L., Colbourne, J. K., Pfrender, M. E. and Weider, L. J. (2013). The evolutionary time machine: using dormant propagules to forecast how populations can adapt to changing environments. *Trends Ecol. Evol.* **28**, 274-282.
- Padilla-Gamiño, J. L., Kelly, M. W., Evans, T. G. and Hofmann, G. E. (2013). Temperature and CO₂ additively regulate physiology, morphology and genomic responses of larval sea urchins, *Strongylocentrotus purpuratus*. *Proc. R. Soc. B Biol. Sci.* **280**, 20130155.
- Pagani, M., Liu, Z., LaRiviere, J. and Ravelo, A. C. (2010). High Earth-system climate sensitivity determined from Pliocene carbon dioxide concentrations. *Nature Geosci.* **3**, 27-30.
- Pagani, M., Zachos, J. C., Freeman, K. H., Tipler, B. and Bohaty, S. (2005). Marked Decline in Atmospheric Carbon Dioxide Concentrations During the Paleogene. *Science* **309**, 600-603.
- Paganini, A. W., Miller, N. A. and Stillman, J. H. (2014). Temperature and acidification variability reduce physiological performance in the intertidal zone porcelain crab *Petrolisthes cinctipes*. *J. Exp. Biol.* **217**, 3974-3980.
- Page, T. M. and Stillman, J. H. (2014). Effects of Lowered pH on the Exoskeleton Mineralogy of Porcelain Crabs. Masters Thesis. San Francisco State University.
- Pälike, H., Norris, R. D., Herrle, J. O., Wilson, P. A., Coxall, H. K., Lear, C. H., Shackleton, N. J., Tripathi, A. K. and Wade, B. S. (2006). The heartbeat of the oligocene climate system. *Science* **314**, 1894-1898.
- Parker, L. M., Ross, P. M. and O'Connor, W. A. (2011). Populations of the Sydney rock oyster, *Saccostrea glomerata*, vary in response to ocean acidification. *Mar. Biol.* **158**, 689-697.
- Payne, J. L., Lehmann, D. J., Follett, D., Seibel, M., Kump, L. R., Riccardi, A., Altiner, D., Sano, H. and Wei, J. (2007). Erosional truncation of uppermost Permian shallow-marine carbonates and implications for Permian-Triassic boundary events. *GSA Bull.* **119**, 771-784.
- Pelejero, C., Calvo, E. and Hoegh-Guldberg, O. (2010). Paleo-perspectives on ocean acidification. *Trends Ecol. Evol.* **25**, 332-344.
- Pespeni, M. H., Chan, F., Menge, B. A. and Palumbi, S. R. (2013). Signs of adaptation to local pH conditions across an environmental mosaic in the California Current Ecosystem. *Integr. Comp. Biol.* **53**, 857-870.
- Pistevos, J. C. A., Calosi, P., Widdicombe, S. and Bishop, J. D. D. (2011). Will variation among genetic individuals influence species responses to global climate change? *Oikos* **120**, 675-689.
- Pörtner, H.-O., Karl, D., Boyd, P., Cheung, W., Lluch-Cota S. E., Nojiri, Y., Schmidt, D. and Zaviolov, P. (2014). Ocean systems. In *Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part A: Global and Sectoral Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change* (ed. C. B. Field et al.), pp. 411-484. Cambridge, UK: Cambridge University Press.
- Purich, D. (2009). *Contemporary Enzyme Kinetics and Mechanisms*, 3rd edn. Oxford, UK: Academic Press.
- Rabon, E., Cuppoletti, J., Malinowska, D., Smolka, A., Helander, F., Mendelein, J. and Sachs, G. (1983). Proton secretion by the gastric parietal cell. *J. Exp. Biol.* **106**, 119-133.
- Raffi, I. and De Bernardi, B. (2008). Response of calcareous nannofossils to the Paleocene-Eocene Thermal Maximum: Observations on composition, preservation and calcification in sediments from ODP Site 1263 (Walvis Ridge - SW Atlantic). *Mar. Micropaleontol.* **69**, 119-138.
- Rayner, N. A., Brohan, P., Parker, D. E., Folland, C. K., Kennedy, J. J., Vanicek, M., Ansell, T. J. and Tett, S. F. B. (2006). Improved analyses of changes and uncertainties in sea surface temperature measured in situ since the mid-nineteenth century: The HadSST2 dataset. *J. Clim.* **19**, 446-469.
- Reusch, T. B. H. and Boyd, P. W. (2013). Experimental evolution meets marine phytoplankton. *Evolution* **67**, 1849-1859.
- Ridgwell, A. (2005). A Mid Mesozoic Revolution in the regulation of ocean chemistry. *Mar. Geol.* **217**, 339-357.
- Ridgwell, A. and Schmidt, D. N. (2010). Past constraints on the vulnerability of marine calcifiers to massive carbon dioxide release. *Nature Geosci.* **3**, 196-200.
- Rokitta, S. D., John, U. and Rost, B. (2012). Ocean acidification affects redox-balance and ion-homeostasis in the life-cycle stages of *Emiliania huxleyi*. *PLoS ONE* **7**, e22212.
- Ruhl, M., Deenen, M. H. L., Abels, H. A., Bonis, N. R., Krijgsman, W. and Kürschner, W. M. (2010). Astronomical constraints on the duration of the early Jurassic Hettangian stage and recovery rates following the end-Triassic mass extinction (St Audrie's Bay/East Quantoxhead, UK). *Earth Planet. Sci. Lett.* **295**, 262-276.
- Schaller, M. F., Wright, J. D. and Kent, D. V. (2011). Atmospheric PCO₂ Perturbations Associated with the Central Atlantic Magmatic Province. *Science* **331**, 1404-1409.
- Scheibner, C. and Speijer, R. P. (2008). Late Paleocene-early Eocene Tethyan carbonate platform evolution - A response to long- and short-term paleoclimatic change. *Earth Sci. Rev.* **90**, 71-102.
- Schlüter, L., Lohbeck, K. T., Gutowska, M. A., Gröger, J. P., Riebesell, U. and Reusch, T. B. H. (2014). Adaptation of a globally important coccolithophore to ocean warming and acidification. *Nat. Clim. Change.* **4**, 1024-1030.
- Schreiber, S., Nguyen, T. H., Stüben, M. and Scheid, P. (2000). Demonstration of a pH gradient in the gastric gland of the acid-secreting guinea pig mucosa. *Am. J. Physiol. Gastrointest. Liver Physiol.* **279**, G597-G604.
- Schulte, P., Alegret, L., Arenillas, I., Arz, J. A., Barton, P. J., Bown, P. R. and Bralower, T. J., Christeson, G. L., Claeys, P., Cockell, C. S. et al. (2010). The Chicxulub Asteroid Impact and Mass Extinction at the Cretaceous-Paleogene Boundary. *Science* **327**, 1214-1218.
- Seki, O., Foster, G. L., Schmidt, D. N., Mackensen, A., Kawamura, K. and Pancost, R. D. (2010). Alkenone and boron-based Pliocene PCO₂ records. *Earth Planet. Sci. Lett.* **292**, 201-211.
- Shen, S.-z., Crowley, J. L., Wang, Y., Bowring, S. A., Erwin, D. H., Sadler, P. M., Cao, C.-q., Rothman, D. H., Henderson, C. M., Ramezani, J. et al. (2011). Calibrating the End-Permian Mass Extinction. *Science* **334**, 1367-1372.
- Sluijs, A., Brinkhuis, H., Schouten, S., Bohaty, S. M., John, C. M., Zachos, J. C., Reichart, G.-J., Sinninghe Damste, J. S., Crouch, E. M. and Dickens, G. R. (2007). Environmental precursors to rapid light carbon injection at the Palaeocene/Eocene boundary. *Nature* **450**, 1218-1221.
- Sobolev, S. V., Sobolev, A. V., Kuzmin, D. V., Krivolutskaia, N. A., Petrunin, A. G., Arndt, N. T., Radko, V. A. and Vasiliev, Y. R. (2011). Linking mantle plumes, large igneous provinces and environmental catastrophes. *Nature* **477**, 312-316.
- Somero, G. N. (2010). The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine "winners" and "losers". *J. Exp. Biol.* **213**, 912-920.
- Somero, G. N. (2011). Comparative physiology: a "crystal ball" for predicting consequences of global change. *Am. J. Physiol. Regul. Integr. Comp. Physiol.* **301**, R1-R14.
- Stap, L., Sluijs, A., Thomas, E. and Lourens, L. (2009). Patterns and magnitude of deep sea carbonate dissolution during Eocene Thermal Maximum 2 and H2, Walvis Ridge, southeastern Atlantic Ocean. *Paleoceanography* **24**.
- Stillman, J. H. and Somero, G. N. (2000). A Comparative analysis of the upper thermal tolerance limits of eastern pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. *Physiol. Biochem. Zool.* **73**, 200-208.
- Suan, G., Pittet, B., Bour, I., Mattioli, E., Duarte, L. V. and Mailliot, S. (2008). Duration of the Early Toarcian carbon isotope excursion deduced from spectral analysis: Consequence for its possible causes. *Earth Planet. Sci. Lett.* **267**, 666-679.
- Sunday, J. M., Calosi, P., Dupont, S., Munday, P. L., Stillman, J. H. and Reusch, T. B. H. (2014). Evolution in an acidifying ocean. *Trends Ecol. Evol.* **29**, 117-125.
- Sunday, J. M., Crim, R. N., Harley, C. D. G. and Hart, M. W. (2011). Quantifying rates of evolutionary adaptation in response to ocean acidification. *PLoS ONE* **6**, e22881.
- Svensen, H., Planke, S., Malthes-Sorensen, A., Jamtveit, B., Myklebust, R., Eidem, T. R. and Rey, S. S. (2004). Release of methane from a volcanic basin as a mechanism for initial Eocene global warming. *Nature* **429**, 542-545.
- Svensen, H., Planke, S., Polozov, A. G., Schmidbauer, N., Corfu, F., Podladchikov, Y. Y. and Jamtveit, B. R. (2009). Siberian gas venting and the end-Permian environmental crisis. *Earth Planet. Sci. Lett.* **277**, 490-500.
- Takahashi, T., Sutherland, S. C., Chipman, D. W., Goddard, J. D., Ho, C., Newberger, T., Sweeney, C. and Murnro, D. R. (2014). Climatological disruptions of pH, pCO₂, total CO₂, alkalinity, and CaCO₃ saturation in the global surface ocean, and temporal changes at selected locations. *Mar. Chem.* **164**, 95-125.
- Tans, P. and Keeling, R. (2010). Mauna Loa CO₂ annual mean data. NOAA/ESRL Global Greenhouse Gas Reference Network.
- Tatters, A. O., Schnetzer, A., Fu, F., Lie, A. Y. A., Caron, D. A. and Hutchins, D. A. (2013). Short- versus long-term responses to changing CO₂ in a coastal dinoflagellate bloom: implications for interspecific competitive interactions and community structure. *Evolution* **67**, 1879-1891.
- Thomas, E. (2007). Cenozoic mass extinctions in the deep sea: what perturbs the largest habitat on earth? In *Geological Society of America Special Papers* (ed. S. Monechi, R. Coccioni and M. R. Rampino), pp. 1-23.
- Todgham, A. E. and Hofmann, G. E. (2009). Transcriptomic response of sea urchin larvae *Strongylocentrotus purpuratus* to CO₂-driven seawater acidification. *J. Exp. Biol.* **212**, 2579-2594.
- Tresguerres, M. (2014). sAC from aquatic organisms as a model to study the evolution of acid/base sensing. *Biochim. Biophys. Acta.* **1842**, 2629-2635.
- Tresguerres, M., Barott, K. L., Barron, M. E. and Roa, J. N. (2014). Established and potential physiological roles of bicarbonate-sensing soluble adenylyl cyclase (sAC) in aquatic animals. *J. Exp. Biol.* **217**, 663-672.
- Tresguerres, M., Buck, J. and Levin, L. R. (2010). Physiological carbon dioxide, bicarbonate, and pH sensing. *Pflügers Arch.* **460**, 953-964.

- Tunncliffe, V., Davies, K. T. A., Butterfield, D. A., Embley, R. W., Rose, J. M. and Chadwick, W. W., Jr.** (2009). Survival of mussels in extremely acidic waters on a submarine volcano. *Nat. Geosci.* **2**, 344-348.
- Uchikawa, J. and Zeebe, R. E.** (2010). Examining possible effects of seawater pH decline on foraminiferal stable isotopes during the Paleocene-Eocene Thermal Maximum. *Paleoceanography* **25**, PA2216.
- Welch, M. J., Watson, S.-A., Welsh, J. Q., McCormick, M. I. and Munday, P. L.** (2014). Effects of elevated CO₂ on fish behaviour undiminished by transgenerational acclimation. *Nat. Clim. Change* **2**, 2-5.
- Wheatly, M. G. and Henry, R. P.** (1992). Extracellular and intracellular acid-base regulation in crustaceans. *J. Exp. Zool.* **263**, 127-142.
- Wignall, P. B., Steve Kershaw, S., Collin, P.-Y. and Crasquin-Soleau, S.** (2009). Erosional truncation of uppermost Permian shallow-marine carbonates and implications for Permian-Triassic boundary events: Comment. *GSA Bull.* **121**, 954-956.
- 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. *Proc. Natl. Acad. Sci. USA* **105**, 18848-18853.
- Zachos, J. C., McCarren, H., Murphy, B., Rohl, U. and Westerhold, T.** (2010). Tempo and scale of late Paleocene and early Eocene carbon isotope cycles: Implications for the origin of hyperthermals. *Earth Planet. Sci. Lett.* **299**, 242-249.
- Zachos, J. C., Röhl, U., Schellenberg, S. A., Sluijs, A., Hodell, D. A., Kelly, D. C., Thomas, E., Nicolo, M., Raffi, I., Lourens, L. J. et al.** (2005). Rapid Acidification of the Ocean During the Paleocene-Eocene Thermal Maximum. *Science* **308**, 1611-1615.
- Zachos, J. C., Wara, M. W., Bohaty, S., Delaney, M. L., Petrizzo, M. R., Brill, A., Bralower, T. J. and Premoli-Silva, I.** (2003). A Transient Rise in Tropical Sea Surface Temperature During the Paleocene-Eocene Thermal Maximum. *Science* **302**, 1551-1554.
- Zeebe, R. E., Zachos, J. C. and Dickens, G. R.** (2009). Carbon dioxide forcing alone insufficient to explain Palaeocene-Eocene Thermal Maximum warming. *Nat. Geosci.* **2**, 576-580.
- Zippay, M. L. and Hofmann, G. E.** (2010). Effect of pH on gene expression and thermal tolerance of early life history stages of red abalone (*Haliotis rufescens*). *J. Shellfish Res.* **29**, 429-439.