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RESPONSES TO GLOBAL CLIMATE CHANGE

Adaptation and the physiology of ocean acidification

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1. Ocean acidification, caused by the uptake of atmospheric CO₂, is a threat to marine biodiversity, potentially rivalling the threat imposed by rising temperatures in some marine ecosystems. Although a growing body of literature documents negative effects of acidification on marine organisms, the majority of this work has focused on the effects of future conditions on modern populations, ignoring the potential effects of adaptation and physiological acclimatization.

2. We review current literature on the potential for adaptation to elevated pCO₂ in marine organisms. Although this body of work is currently quite small, we argue that data on the physiological effects of acidification, natural variation in pH and lessons learned from previous work on thermal adaptation can all inform predictions and priorities for future research.

3. Spatially varying selection is one of the most important forces maintaining intraspecific genetic variation. Unlike temperature, pH lacks a strong and persistent global gradient, and so selection may maintain less adaptive variation for pH than for temperature. On the other hand, we are only beginning to amass long-term data sets for pH variation in natural habitats, and thus, pH gradients may be more common than previously observed.

4. Two of the most important effects of elevated pCO₂ are reduced calcification and changes in metabolism. We discuss the ways that a detailed understanding of the physiological mechanisms underlying these effects is key to predicting the capacity for acclimatization and adaptation.

5. Important priorities for future research will be to assess local adaptation to pH conditions and to measure the capacity for adaptation to future acidified conditions in natural populations. Tools for this work include traditional quantitative genetics, transcriptomics and the adaptation of ion-sensitive field-effect transistor (ISFET) technology for use in continuous seawater pH monitoring in the field.

Key-words: acclimation, calcification, climate change, contemporary evolution, ion-sensitive field-effect transistor, local adaptation, ocean acidification

Introduction

Because physiological limits (in concert with biotic interactions) shape modern species distributions, they will also play a key role in responses to future climate change, influencing both shifts in distribution and extinction risk (Somero 2012). Organisms in a changing environment are expected to undergo some combination of geographic range shifts, physiological acclimatization and/or genetic adaptation to changing conditions; those that cannot do any of these rapidly risk extinction. Rising temperatures have been the primary focus of climate change research,

but over the last decade, ocean acidification (OA), a global reduction in seawater pH due to uptake of anthropogenic CO₂, has emerged as another important effect of climate change in marine systems (Orr *et al.* 2005). The oceans have absorbed almost one-third of anthropogenic CO₂ emissions since the beginning of the industrial revolution (Sabine *et al.* 2004) resulting in a decrease in the pH of surface waters by 0.1 pH units and a reduced availability of free carbonate ions (CO₃²⁻) used by many marine invertebrates as part of the calcification process. The rate at which CO₂ is currently being emitted to the atmosphere and absorbed by the world's oceans exceeds nearly all well-documented previous emissions events, and surface waters are projected to decrease an additional 0.3–0.4 pH

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units by the end of the century (Caldeira & Wickett 2003; Orr *et al.* 2005) resulting in changes in carbonate chemistry more abrupt than any experienced during the previous 20 million years (Caldeira & Wickett 2003; Feely *et al.* 2004).

A growing body of literature documenting the effects of predicted OA conditions on marine organisms, including effects on calcification, growth, metabolism, reproduction and tolerance of other stressors (reviewed in Hofmann *et al.* 2010; Kroeker *et al.* 2010; Doney *et al.* 2012). However, as with previous work on temperature, the majority of OA research has focused on the physiological effects of simulated future conditions on modern populations, while the capacity for acclimation and genetic adaptation has received relatively little attention (but see Collins & Bell 2004, 2006; Pistevo *et al.* 2011; Sunday *et al.* 2011; Lohbeck, Riebesell & Reusch 2012; Foo *et al.* 2012).

Although the current body of literature on the scope for adaptation to OA is small, we can draw from empirical and theoretical literature to inform both our predictions and future research priorities. We can draw from the evolutionary genetics literature to make predictions about the circumstances in which populations are likely to maintain genetic variation for OA tolerance. We can also use our knowledge of the physiology of organismal responses to OA conditions to identify which traits are likely to be under selection and possible constraints on the selection response. The focus of this review is to ask: how can an understanding of the physiology and ecology of OA stress (i) inform predictions about the capacity for natural populations to evolve increased tolerance of OA conditions and (ii) shape future research priorities?

We start by reviewing current literature on the capacity of marine organisms to evolve increased tolerance of OA stress. Next, we discuss the current state of knowledge regarding natural variation in pH in different habitats, and how this knowledge can be used to predict the relative likelihood of local adaptation to pH conditions. We then discuss two key physiological processes affected by increased $p\text{CO}_2$ and how a detailed understanding of physiology is key to evaluating the potential for adaptation. We conclude with a list of future research questions and a discussion of research tools that will play important roles in this work.

Evolution from standing variation

There is a growing appreciation that evolution can occur on relatively short ecological time-scales (Carroll *et al.* 2007) and that adaptation may ameliorate extinction risk during periods of rapid environmental change (Bell & Gonzalez 2009). However, evolution does not always occur rapidly enough to outpace population decline, as past periods of environmental change (including changes in carbonate chemistry) have led to mass extinctions and ecosystem-level state shifts (Knoll *et al.* 2007; Barnosky

et al. 2012). Because current change will be rapid, the key question is under what circumstances and how rapidly is contemporary evolution likely to occur? Evolution over short time-scales can occur from standing variation or from new mutations. Although quite labour intensive, the process of quantifying standing genetic variation for a trait is relatively straightforward, using either breeding or selection experiments. Predicting the likelihood of new beneficial mutations is much more difficult, as it involves estimating rates of events that are extremely rare; however, there are also techniques for gaining insight into this question, as we will discuss below.

Although large differences in pH tolerance have been observed both within and among taxonomic groups (Kroeker *et al.* 2010) so far, only a handful of studies have quantified standing variation in single species for resistance to future acidified seawater conditions. Sunday *et al.* (2011) used a breeding experiment to measure phenotypic and genetic variation for larval growth rates under OA conditions in a sea urchin (*Strongylocentrotus franciscanus*) and in a mussel (*Mytilus trossulus*) and then used these data, along with demographic information, to predict future rates of evolution under OA conditions. Interestingly, there were greater rates of predicted evolution in the sea urchin even though this species had much lower rates of population turnover. These results highlight the importance of genetic data, given that the *a priori* prediction would have been lower rates of evolution for the longer-lived species. Variation among genotypes in responses to OA conditions has also been observed in bryozoans (Pistevo *et al.* 2011), oysters (Parker *et al.* 2012) and coccolithophores (Langer *et al.* 2009).

Another approach to measuring the scope for adaptation is experimental evolution, where populations of organisms are exposed to simulated future conditions over many generations and then compared to control populations for signs of adaptation. Where possible (typically with short-lived organisms that can be maintained in the laboratory), these types of experiments can provide additional insights, because responses are based on both standing variation and new mutations that occur over the time frame of the experiment. Interestingly, after 1000 generations of exposure to elevated CO_2 , the green alga *Chlamydomonas* failed to evolve any specific adaptations to high CO_2 conditions, suggesting constraint on the response of photosynthesis to increased CO_2 (Collins & Bell 2004).

By contrast, 500 generations under high CO_2 conditions led to substantial adaptive evolution in the calcification response of the coccolithophore alga *Emiliania huxleyi* (Lohbeck, Riebesell & Reusch 2012). Although calcification rates were not completely restored under high CO_2 conditions, they were still up to 50% higher in adapted as compared to nonadapted strains. Because coccolithophores are dominant calcifiers in the world's oceans, these results are relevant for forecasting the effects of OA on global biogeochemical cycles (Ridgwell *et al.* 2009).

Although there are data on standing variation in OA responses for a handful of species, we also know that patterns of environmental variability are key to maintaining intraspecific variation in the form of local adaptation, where local genotypes have higher fitness in their home environment relative to foreign genotypes (Kawecki & Ebert 2004; Sanford & Kelly 2010). We can therefore make predictions about the likelihood of standing variation for OA resistance in different habitats based on what we know about regional and historical variation in pH, the ‘OA seascape’ (Yu *et al.* 2011).

THE OCEAN ACIDIFICATION SEASCAPE

Local adaptation is most likely when the scale of environmental variation is coarse-grained, relative to the scale of gene flow (Kawecki & Ebert 2004) (Fig. 1). A key question then is the spatial and temporal scale of pH variation and associated carbonate saturation variability in the world’s oceans. Early discussions of OA tended to focus on future changes in mean pH. However, in the last few years, deployment of high-resolution pH sensors into a variety of habitats and oceanographic provinces has revealed daily fluctuations up to 0.3 pH units in some areas and remarkably invariant conditions in others (Hofmann *et al.* 2011; Fig. 2). Organisms from low-pH and fluctuating habitats may already be experiencing pH values forecast for the end of this century, albeit perhaps irregularly, or during certain seasons. Seawater chemistry also varies over large and small spatial scales. Low-pH ‘hot spots’ can be caused by upwelling of deeper acidified water onto continental shelves (Feely *et al.* 2008) and on smaller scales by high levels of localized recurring heterotrophic respiration (Marshall *et al.* 2008; Wootton, Pfister & Forester 2008). Local adaptation to pH conditions is especially likely if physical and biological forces promote consistent differences in pH among sites. By contrast, temporally varying conditions or spatial variation on a finer scale than that of

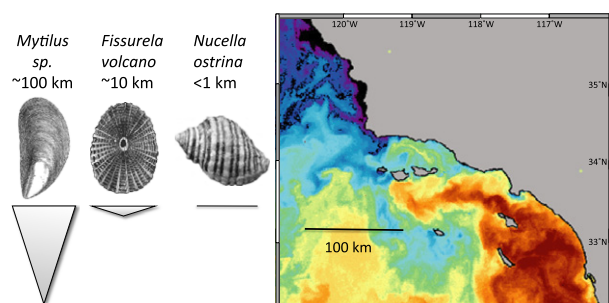


Fig. 1. Scales of dispersal for three calcifying marine invertebrates vs. possible scale of variation in pH. Map shows sea surface temperatures for the Pacific Coast of North America in May, 2000. Purple shows areas of upwelling, which brings acidified waters onto the continental shelf (Feely *et al.* 2008). The likelihood of local adaptation to acidified conditions is higher for organisms with dispersal distances that are small to intermediate relative to the spatial scale of pH variation. (SST image courtesy of NOAA).

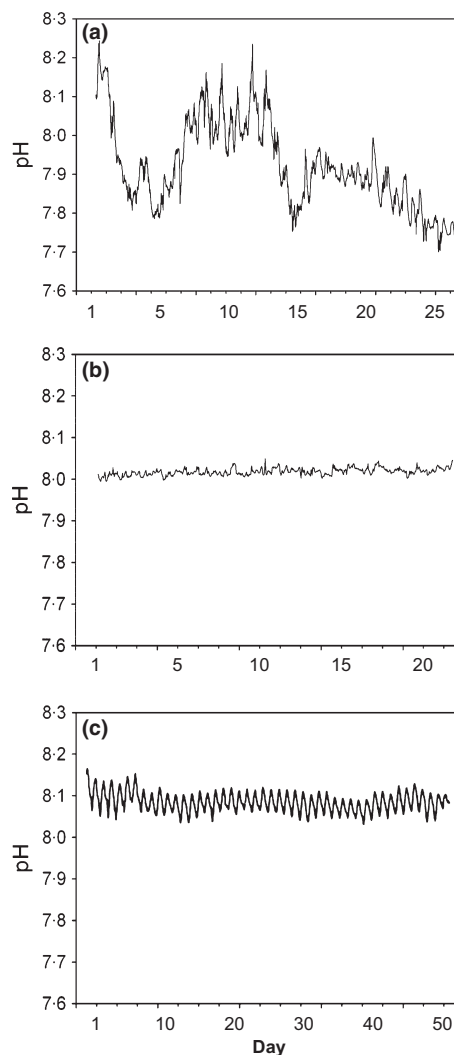


Fig. 2. Continuous pH measurements for three different habitats using a modified version of the Honeywell DuraFET[®], an ion-sensitive field-effect transistor (ISFET), with an integrated data logger and power supply (modified from Hofmann *et al.* 2011). (a) Santa Barbara Channel, USA, July 25–August 20, 2010. (b) McMurdo Sound, Antarctica, October 29–November 17, 2010. (c) Moorea, French Polynesia, January 31–March 21, 2011.

organismal dispersal are expected to promote phenotypic plasticity (Levins 1968).

We can also start to predict the likelihood of local adaptation to pH conditions based on what we know about the geography of thermal adaptation (Conover *et al.* 2006; Sanford & Kelly 2010). For example, local adaptation to temperature is more common among organisms with restricted dispersal (Sanford & Kelly 2010). This will probably be true for local adaptation to pH, although theory also predicts that local adaptation should be greatest under conditions of low to intermediate (rather than very low) levels of gene flow (Alleaume-Benharira, Pen & Ronce 2006).

There are some broad similarities between patterns of temperature and pH variation. In both cases, global means are expected to change rapidly, but in both cases, fine-scale

temporal and spatial variation may greatly exceed the expected magnitude of changes in global means. For temperature, the extremes of daily and yearly cycles, rather than the means, are often the part of the temperature signal most closely tied to species' stress responses (Helmuth 2009). An important priority for OA research will be to determine the relative importance of extremes and their duration vs. mean pH values in their effects on organismal physiology.

There are also some significant differences between patterns of temperature and pH variation. One of the most important is the strong latitudinal gradient in temperature and the lack of a comparable global gradient in pH. Mean sea surface temperatures vary $\sim 30^\circ\text{C}$ from the equator to the poles (Locarnini *et al.* 2006), whereas the projected change in mean surface temperature in most of the world's oceans is $2\text{--}3^\circ\text{C}$ over the next century (IPCC 2007). By contrast, the magnitude of the global gradient in pH is about the same as the expected change in pH due to OA (Orr *et al.* 2005; Fig. 3). Because of the linear temperature gradient, species distributions are expected to shift poleward with increasing temperatures (with some important exceptions; see Helmuth *et al.* 2002). Local adaptation to broad latitudinal temperature gradients is also common, leading to the prediction that gene flow from equatorward, more tolerant populations will facilitate adaptation to climate change in poleward populations (Pease, Lande & Bull 1989). Because the global gradient in temperature is steep relative to the projected warming, an allele that confers local adaptation has a much shorter distance to travel to keep pace with rising temperatures, as compared to declining pH. Because pH lacks a steep linear gradient, the effects of pH changes are much more difficult to predict. Rather than shifts in geographic range, it is likely that changes in pH will lead to localized extinctions at pH 'hot-spots', such as areas of increased upwelling around headlands. Increased 'phenotype environment mismatch' around such sites may also reduce population connectivity (Marshall *et al.* 2010). Notably, habitats with large fluctuations in pH, such as the California Current Ecosystem, may be among the first to have pH cross thresholds of tolerance for the biota in coastal marine ecosystems (Gruber *et al.* 2012). However, we know relatively little about the scale and persistence of pH gradients in most ecosystems, making it difficult to evaluate how close populations are to these thresholds, or the likelihood that local adaptation is maintaining the variation that would allow adaptation to future conditions. Detailed information on natural temporal and spatial variation in pH, and tests of local adaptation to pH conditions, will be critical priorities for future OA research.

The physiology of ocean acidification

Assessing the capacity for an evolutionary response to OA requires that we first understand what traits are likely to be under selection. Will acidification affect fitness primarily

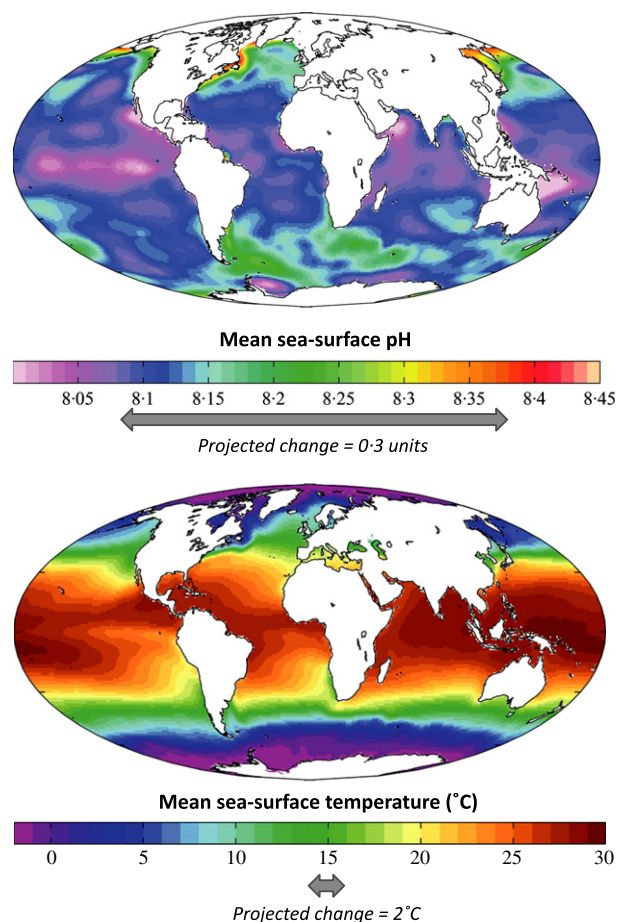


Fig. 3. Global gradient in mean sea surface temperature (a) and pH (b) (modified from Locarnini *et al.* 2006) compared to the projected change in global means by 2100 under business as usual scenarios (IPCC 2007). Global gradient in mean sea surface temperature is large relative to projected change (30° vs. $2\text{--}3^\circ\text{C}$), whereas the global gradient in pH is of roughly the same magnitude as the projected change (0.3 pH units, Orr *et al.* 2005; Locarnini *et al.* 2006).

through its effects on calcification rates? Or are there other traits of equal or greater importance? We will discuss two well-studied effects – reductions in calcification and changes in metabolism – and the ways that a detailed understanding of physiological pathways is key to understanding the capacity for adaptation.

CALCIFICATION

Of all of the physiological processes likely to be affected by OA, calcification has received the most attention due to its close relationship with dissolved inorganic carbon (DIC). Dissolved CO_2 reacts with seawater to increase the abundance of hydrogen ions (H^+) and bicarbonate ions (HCO_3^-) with a concurrent decrease in the concentration of carbonate ions (CO_3^{2-}). With increasing dissolved CO_2 , seawater chemistry eventually crosses the threshold for the saturation states of aragonite and calcite (two mineral phases of calcium carbonate) that support biogenic

calcification. These forms begin to dissolve in low-pH environments, leading to a net loss of calcareous structures exposed to seawater or severe reductions in calcification and growth rates (see Anthony *et al.* 2008; Fabry *et al.* 2008; Cohen & Holcomb 2009; Hofmann *et al.* 2010). Numerous studies have shown a link between increasing $p\text{CO}_2$ and decreased calcification. However, for the purposes of further discussion, we will focus on two ecologically important calcifying groups: coccolithophores and scleractinian corals.

COCCOLITHOPHORES

Coccolithophores are unicellular algae covered in calcitic plates found throughout most of the world's oceans. This group plays an important role in global geochemical cycles, and so estimating changes in calcification rates is important for forecasting changes in future global carbon cycles (Ridgwell *et al.* 2009). There has been substantial debate, however, not just about the magnitude but also the direction of the predicted effects of OA on coccolithophores; most laboratory experiments have found decreased rates of calcification, but with substantial variation in results both among and within species (Iglesias-Rodriguez *et al.* 2008; Langer *et al.* 2009). One explanation for these conflicting results is the existence of intraspecific variation in calcification responses, so that studies with different strains produce different outcomes.

Another complication for forecasting the feedbacks between coccolithophores and the future atmosphere is the complex relationship between calcification and CO_2 uptake. Coccolithophores use bicarbonate (HCO_3^-) as the substrate for calcification, leading to net production of CO_2 . Thus, if increased dissolved CO_2 leads to decreased calcification, there should be a negative feedback, where increased atmospheric CO_2 leads to decreased CO_2 production by coccolithophores. On the other hand, CaCO_3 seems to ballast faecal pellets, increasing rates of sinking, and so decreased calcification could lead to decreased rates of deep-water carbon storage (Ridgwell *et al.* 2009).

Calcification rates in coccolithophores may respond to increased CO_2 through adaptation, acclimatization or turnover among species or strains with different calcification rates. As discussed earlier, *E. huxleyi* showed substantial adaptive evolution in calcification rates after 500 generations under high $p\text{CO}_2$ conditions (Lohbeck, Riebesell & Reusch 2012). At least one study has also demonstrated acclimation in coccolithophores in the form of changes in the calcification response under high $p\text{CO}_2$ over a 7-day experiment (Casareto *et al.* 2009). Nevertheless, current data suggest that strain turnover will have the most dramatic effect on calcification rates in this group. Beaufort *et al.* (2011) show that calcification rates in coccolithophores over the last 40 000 years are tightly linked to atmospheric CO_2 , but that changes in calcification are driven primarily by species turnover, with more heavily calcified strains dominating under lower $p\text{CO}_2$ conditions.

Nevertheless, the authors also report a highly calcified strain of *E. huxleyi* from upwelling habitats off the coast of Chile, and another recent study reports a shift towards a highly calcified strain of *E. huxleyi* during the winter, when pH and CaCO_3 saturation states are the lowest (Smith *et al.* 2012). These results highlight our need to understand the fitness consequences of calcification: why are strains with lower calcification rates generally favoured by increased $p\text{CO}_2$, and what is driving the apparent exceptions to this pattern in *E. huxleyi*? Finally, strain turnover, acclimatization and adaptation in response to high $p\text{CO}_2$ in coccolithophores all strongly caution against using the results of short-term laboratory experiments to make long-term forecasts of the effects of OA on global biogeochemical cycles.

CORALS

Corals have received significant attention by OA researchers due to their ecological importance in tropical systems and due to a number of early studies that demonstrated dramatic effects of altered carbonate chemistry on their growth and rates of calcification (see Hoegh-Guldberg *et al.* 2007). Recent experiments have also demonstrated a substantial role for increased CO_2 in triggering coral bleaching (expulsion of algal endosymbionts and subsequent loss of productivity and/or death of the host) (Anthony *et al.* 2008). It had been assumed that bleaching was caused mainly by thermal stress, but in an experimental manipulation of both temperature and CO_2 , Anthony *et al.* (2008) demonstrated a substantially larger effect of CO_2 than temperature on bleaching in one coral species and a strong interaction between temperature and CO_2 in another. High levels of CO_2 also led to decreased photosynthesis in both species, possibly due to effects on photoprotective mechanisms.

Coral hosts associate with multiple strains and genotypes of *Symbiodinium*, some of which confer local adaptation to temperature and greater resistance to temperature-induced bleaching (see Howells *et al.* 2012). The flexibility of the host-symbiont relationship raises the possibility of coral adaptation or acclimatization to environmental change via shifts in their dominant symbionts. The possibility of symbiont-mediated changes in OA resistance in corals has not yet been tested directly, but recent work has shown dramatic differences among symbiont types in the responses of free-living cells to elevated CO_2 (Brading *et al.* 2011). This suggests that symbiont type is likely to affect coral responses to elevated $p\text{CO}_2$ and also that elevated $p\text{CO}_2$ may shift the relative abundances of different symbiont types in the environment, thereby changing their rate of uptake by corals. Direct tests of the effects of symbiont type on performance under elevated CO_2 are an important area for future research. Finally, there is evidence that genetic differences in coral hosts contribute to local adaptation and intraspecific variation in stress responses, suggesting that corals themselves may have at least some capacity to evolve increased

tolerance (Meyer *et al.* 2009; Császár *et al.* 2010; Pandolfi *et al.* 2011).

As with coccolithophores, longer-term experiments on corals demonstrate the need for caution when interpreting the results of shorter experiments. During a 1-week exposure to elevated $p\text{CO}_2$, the cold-water coral *Lophelia pertusa* exhibited a >25% decline in growth with a drop in pH as little as 0.1 unit. By contrast, after longer-term (6 month) exposure, corals in elevated $p\text{CO}_2$ showed an increase in growth rate over controls with no measurable change in metabolism, suggesting complete acclimation of this species to elevated $p\text{CO}_2$ over time (Form & Riebesell 2012).

UNDERSTANDING THE MECHANISM

Both corals and coccolithophores illustrate the ways that a detailed understanding of physiology informs our understanding of potential for adaptation. In coccolithophores, calcified scales are produced entirely within intracellular compartments. This means that the calcification environment is not directly influenced by external pH, and also suggests that coccolithophores already utilize substantial machinery for intracellular pH regulation to maintain the conditions necessary for calcification (Taylor *et al.* 2011). Recent work also shows that corals exert substantial control over pH at the site of calcification, with internal pH falling at only half the rate of external seawater under acidified conditions (McCulloch *et al.* 2012). Because in both corals and coccolithophores, calcification is under biological, rather than strictly chemical control, there also exists the strong possibility of biological variation in this process and hence the capacity for adaptation.

METABOLISM

The documented effects of OA conditions on metabolism include both increases (e.g. Wood, Spicer & Widdicombe 2008; Lannig *et al.* 2010) and decreases (e.g. Rosa & Seibel 2008; Albright & Langdon 2011) in metabolic rates in response to high $p\text{CO}_2$. Nevertheless, these short-term physiological responses will not necessarily predict the effects of *adaptation* to OA conditions on metabolism. Comparisons across closely related species often show that taxa adapted to stressful environments have lower basal metabolic rates, leading to the general prediction that stress adaptation will lead to lowered metabolism (Hoffmann & Parsons 1991). However, laboratory selection for increased stress resistance within individual species has yielded mixed responses with respect to metabolism, suggesting that the effects of stress adaptation on metabolism will probably depend on the specific stressor (Djawdan, Rose & Bradley 1997).

Changes in metabolism are probably an important mediator of tolerance in some species. For example, boosting the energetic status of corals through increased heterotrophic feeding appears to counteract the negative effects of OA conditions on calcification (Cohen & Holcomb 2009). In other cases, changes in metabolism appear to be a pas-

sive response to elevated CO_2 . For example, decreased pH lowers the oxygen affinity of blood in the jumbo squid and the resulting decrease in metabolism is a direct consequence of oxygen stress, suggesting that it is not under organismal control (Rosa & Seibel 2008). An important area for future research will be to identify changes in gene expression associated with hypercapnic effects on metabolism. This will help us to understand whether these changes are a direct consequence of elevated CO_2 or part of a more complex response and thus potentially under greater organismal control.

Interestingly, baseline metabolic rate may itself play an important role in organisms' vulnerability to OA. In a recent review, Melzner and colleagues point out that many organisms with higher metabolic rates (fish, cephalopods, decapod crustaceans) appear to be less affected by elevated $p\text{CO}_2$ (Melzner *et al.* 2009). One possible explanation is that these organisms produce large amounts of CO_2 as a product of their own cellular respiration and are therefore already well adapted to regulating extracellular pH.

The effects of elevated CO_2 on metabolism highlight the potential importance of multiple stressors in organismal responses to climate change (Boyd 2011), particularly because metabolism is directly affected by environmental conditions such as temperature. Increased metabolic rate is expected to narrow thermal tolerance windows by decreasing aerobic scope (Portner & Farrell 2008), and an increasing number of studies have documented synergistic effects of elevated CO_2 and temperature (reviewed in Byrne 2011). In general, metabolic effects are likely to be one of the most important mediators of trade-offs between OA tolerance and other traits.

Trade-offs

The magnitude and direction of the evolutionary response to OA will ultimately depend on trade-offs, which occur when a relationship between two traits prevents them from being simultaneously optimized. Trade-offs occur because genetic variation and natural selection are usually multivariate, and evolution depends on the degree to which genetic variation is aligned with the 'multivariate direction of selection' (Blows & Hoffmann 2005). For example, a population might possess genetic variation for tolerance of OA and genetic variation for tolerance of thermal stress, but if there is a negative correlation between these two traits, it may not be possible to evolve substantially increased tolerance of OA and thermal stress simultaneously. Multiple sources of selection may also act in opposing ways on the same trait. For example, OA conditions may favour increased calcification during an organism's larval stage, but increased energy spent on calcification might lead to lower fecundity as an adult. Thus, to understand how organisms might adapt to OA stress, we need to understand the multiple sources of selection acting on traits related to OA tolerance and genetic correlations among OA tolerance and other traits.

One approach to measuring genetic correlations is to measure two or more traits (e.g. OA tolerance and thermal tolerance) in many individuals of known pedigree. Alternatively, we can measure genetic correlations indirectly using selection experiments. Selecting for increased OA tolerance in a population of organisms will cause changes in other traits if they are genetically correlated with OA tolerance. For example, selection for improved performance (higher growth rates) under high $p\text{CO}_2$ conditions in *E. huxleyi* led to correlated responses in calcification rates, cell size and organic carbon content of cells (Lohbeck, Riebesell & Reusch 2012). Thus far, there is little data on genetic correlations with OA tolerance, but it is a safe assumption that evolutionary responses to OA conditions will lead to unexpected changes in other traits.

There are comparatively more data on multivariate sources of selection. In particular, studies examining the combined effects of OA and other stressors highlight the potential interactions among multiple sources of selection in the natural environment. In a study on oysters, Lannig *et al.* (2010) find evidence for an interaction between the effects of temperature and elevated $p\text{CO}_2$ in *Crassostrea gigas*, with no effect of elevated $p\text{CO}_2$ on metabolic rate at 15°, but sharp increase in metabolic rate for CO_2 -exposed oysters relative to normocapnia at 25°. Synergistic effects of elevated temperature and $p\text{CO}_2$ on calcification rates have been observed for both coralline algae (Martin & Gattuso 2009) and corals (Anthony *et al.* 2008). OA stress has also been shown to affect upper thermal tolerance limits, by narrowing aerobic scope in fish (Munday, Crawley & Nilsson 2009).

The synergistic effects of CO_2 and temperature suggest that there may be a physiological trade-off between tolerances of these two stressors, with increased energy dedicated to maintenance of intracellular pH leading to a narrowing of aerobic scope. But it is important to note that synergistic effects do not necessarily imply a physiological trade-off. For example, there is a strong synergistic effect of elevated CO_2 and UV radiation on photosynthesis and calcification in coralline algae, but this is probably due to the loss of the calcified layer with decreased pH, increasing sensitivity to UV radiation (Gao & Zheng 2010). This means that a genetic change leading to a decreased sensitivity to elevated CO_2 would likely also produce a decreased sensitivity to UV radiation. Thus, our interpretation of interaction effects, and whether they imply a trade-off for tolerance of different stressors, depends critically on understanding the physiological mechanisms of tolerance.

Maternal effects

Maternal effects are nongenetic effects of the maternal parent on offspring phenotype. Although they are under-studied in marine systems, there is an increasing recognition of their importance (Marshall 2008), and several studies have demonstrated maternal effects on larval responses to OA conditions (Sunday *et al.* 2011; Parker *et al.* 2012; M. W. Kelly J.L. Padilla-Gamiño & G.E. Hof-

mann, unpublished data). Maternal effects are a form of trans-generational plasticity and should not be confused with adaptation, but they are also important to consider in studies of adaptation for several reasons. First, maternal effects will tend to inflate estimates of genetic variance. This is true within populations, but is especially problematic for comparisons between populations; offspring of field-caught parents spawned in the laboratory may appear to show local adaptation, when in fact they are expressing phenotypic effects of parental environments rather than true genetic differences among populations. Maternal effects may transmit negative effects of maternal environment to the offspring, but they may also be a source of resilience, transmitting acclimatization to environmental change across generations. For example, offspring of adult oysters that had been acclimatized to high $p\text{CO}_2$ conditions had better performance under high $p\text{CO}_2$ than the offspring of nonacclimatized adults (Parker *et al.* 2012). As with short-term evolution, maternal effects imply that the results of short-term studies on single life stages should be interpreted with caution.

Questions for future research

As more studies begin to examine the scope for adaptation to acidified conditions, we see several important questions to be addressed:

1. What are the physiological mechanisms underlying tolerance of acidification stress?
2. How much variation exists among and within populations for tolerance traits?
3. How does acidification interact with other stressors? Will trade-offs in tolerance of multiple stressors constrain adaptive responses?
4. What is the important part of the pH signal? Will organisms primarily be affected by changes in mean pH values, or by changes in the extremes and their duration?
5. What are the spatial and temporal scales of natural variation in pH, and how do these compare to scales of gene flow and organismal life spans?

Tools for future research

TRANSCRIPTOMICS

Although a large body of research has now documented morphological and physiological effects of OA conditions in a diversity of taxa, we know comparatively little about the physiological mechanisms underlying these responses. Gene expression data are one way to obtain a global view of physiological responses (Evans & Hofmann 2012; Whitehead 2012). Transcriptomic data can be used to identify which stressors (salinity, pH, temperature, etc.) have the biggest effects on physiology (Chapman *et al.* 2011) and can be used to identify genes underlying variation among individuals (Mackinder *et al.* 2011).

Studies that consider the response of the entire transcriptome will also help to avoid the ascertainment bias inherent in the early stages of an emerging field, where particular pathways and processes receive the most attention because they are assumed from first principles to be the most important. For example, although calcification has been a main focus of OA research, the transcriptomic response of larval sea urchins (*Strongylocentrotus purpuratus*) to OA conditions shows broad-scale downregulation of genes involved in biomineralization, cellular stress response, metabolism and apoptosis (Todgham & Hofmann 2009). This suggests that many other physiological processes are affected by OA in addition to calcification and warrant investigation.

Transcriptomic data also allow for the identification of candidate genes for further study. At the organismal level, quantitative PCR (q-PCR) can then be used to gain an in-depth understanding of environmental thresholds triggering upregulation of specific stress-related genes (Evans & Hofmann 2012). At the population level, identification of candidate genes allows quantification of intraspecific variation in physiological responses, which might reflect the genetic capacity for evolution of increased stress resistance.

QUANTITATIVE GENETICS

Even for model organisms with fully sequenced and annotated genomes, we are years away from linking genetic variation to phenotypic variation for the majority of traits. As a result, the tools of traditional quantitative genetics remain critically important to predicting the evolvability of complex traits. Quantitative genetics is the study of continuous traits and the statistical analysis of the relative contributions of genetic and environmental effects to phenotypic variation. The degree to which a trait is expected to respond to selection depends on the total amount of phenotypic variation and its narrow-sense heritability (h^2) or the proportion of variation that is controlled by additive genetic effects. Heritability can be measured via breeding or selection experiments. Because many marine invertebrates are long-lived and difficult to maintain in the laboratory through multiple generations, breeding experiments, where field-caught parents are spawned in the laboratory, are often one of the most effective methods for measuring the heritability of ecologically relevant traits (Sunday *et al.* 2011).

In our laboratory, we are using a breeding experiment to estimate genetic variation in the response to high $p\text{CO}_2$ in purple urchins (*S. purpuratus*) and to ask whether modern populations that experience different carbonate chemistry regimes differ in their responses to simulated future conditions (Fig. 4). These experiments will allow us to estimate the proportion of variation in the response to high $p\text{CO}_2$, which is due to genetic, maternal and environmental effects. If there is variation among sites, it would suggest that local adaptation might be helping to maintain variation in this trait.

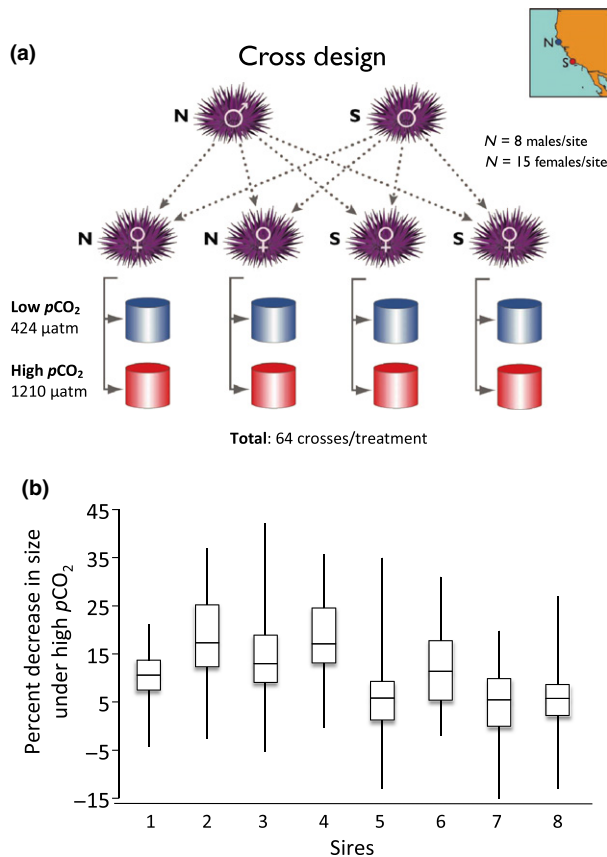


Fig. 4. Breeding experiments can be used to estimate genetic variation present for a trait, both among and within populations. (a) Cross design. We collected adult purple urchins (*Strongylocentrotus purpuratus*), from two sites in California, USA (inset), known to differ in their carbonate chemistry regimes. In the laboratory, we performed crosses according to a modified version of the North Carolina II design (Lynch & Walsh 1998), crossing each male with two females from his own site, and two females from the opposite site ($N = 8$ males/site, 15 females/site), and then split embryos from each cross into high (1210 μatm) and low (424 μatm) $p\text{CO}_2$ rearing conditions. After 5 days, we measured total larval length for all cross $\times p\text{CO}_2$ treatment combinations. (b) Box plots showing the range of CO_2 sensitivity of larvae from each of eight sires from the southern site. CO_2 sensitivity was measured as the percent decrease in larval size for high $p\text{CO}_2$, relative to the low $p\text{CO}_2$ treatment. The mean decrease in size for this site was 10.2%, but significant variation attributed to sire identity across both sites [Sire (Dam): $F_{32,1488} = 19.6$, $P < 0.0001$] provides strong evidence for genetic variation in CO_2 sensitivity (M. W. Kelly J.L. Padilla-Gamiño & G. H. Hofmann, unpublished data). Further sampling will be needed to understand whether results for these two sites are representative of their respective regional populations.

PHYLOGENETIC ANALYSES

Another tool for understanding how quickly or slowly a trait evolves comes from the field of phylogenetic comparative methods (Pagel 1999). By mapping character states onto a phylogenetic tree, we can ask whether a trait has very different values for closely related taxa (suggesting the trait evolves quickly) or remains unchanged across deep branches (suggesting the trait evolves slowly or is

under stabilizing selection over long periods of time). As more data on vulnerability to OA become available, it will be increasingly possible to ask whether sister taxa share similar vulnerabilities. So far, the answer appears to be mixed. A meta-analysis of OA effects on marine organisms reveals a strong effect of taxonomic group on vulnerability, suggesting that resistance to OA stress may evolve quite slowly (Kroeker *et al.* 2010). On the other hand, specific traits such as calcification rates sometimes show substantial variation in OA responses within the same taxonomic group (Iglesias-Rodriguez *et al.* 2008; Langer *et al.* 2009; Beaufort *et al.* 2011).

Phylogenetic comparative methods will also allow us to more rigorously identify traits associated with increased vulnerability to OA. Thus far, several candidates have been proposed (degree and site of calcification, metabolism, mobility) but many of these traits are highly correlated, so that it will only be possible to disentangle their effects by controlling for shared ancestry.

NATURAL EXPERIMENTS AND LOCAL ADAPTATION

Sources of high CO₂ in nature provide 'natural experiments' to ask whether organisms have the capacity to evolve tolerance of these conditions, and if so, by what physiological mechanisms this tolerance will evolve (Kroeker *et al.* 2011). Because natural features such as volcanic CO₂ vents have existed in the same place for thousands of years, they offer an advantage over short-term laboratory experiments, which may not play out over a long enough period to allow for the beneficial mutations that might arise over hundreds or thousands of generations. Importantly, such locations are being discovered and offer the chance to explore variation in the responses of different taxonomic groups and different ecosystems (Fabricius *et al.* 2011; Kroeker *et al.* 2011; Johnson *et al.* 2012). Data from these natural experiments can also complement data from controlled studies; for example, the finding that single-celled algae failed to adapt to high CO₂ after 1000 generations in the laboratory is strengthened by the fact that they also failed to do so in a natural CO₂ spring (Collins & Bell 2006). On the other hand, we should be cautious about interpreting data from these kinds of sites for two reasons. First, these anomalous sites often experience pCO₂ levels that substantially exceed those predicted in future oceans, even under the most extreme emission scenarios. Second, the spatial extent of the area in which pCO₂ is elevated is often quite small, meaning that the population of exposed organisms often receives substantial gene flow from unexposed sites, reducing the likelihood of local adaptation to the extreme conditions.

On a broader geographic scale, there are less dramatic gradients in carbonate chemistry that may also promote local adaptation to pH. For example, on the west coast of North America, there is a gradient in upwelling intensity, with sites north of Point Conception more frequently experiencing upwelling of acidified water onto the continental shelf. Locally adapted populations, if they exist, could

serve as a source of gene flow for adaptation to future conditions at other sites. Testing for the existence of local adaptation, coupled with detailed monitoring of local pH conditions, is an important area for ongoing and future research (<http://omegas.science.oregonstate.edu>).

REAL-TIME PH SENSORS

Historically, most measurements of seawater pH have been collected by research cruises, a technique that was not conducive to the collection of fine-scaled temporal or spatial data. Recent adaptation of ion-sensitive field-effect transistor (ISFET) technology for use in continuous stationary seawater monitoring (Martz, Connery & Johnson 2010) has opened the possibility for much more comprehensive data on natural variation in pH. Current data show that habitats vary in mean pH, but also even more strongly in their levels of temporal variability (Fig. 2, Hofmann *et al.* 2011). Future work will be able to couple physiology with fine-scale environmental data to ask whether populations from low-pH habitats are locally adapted, and whether species from more variable habitats exhibit greater phenotypic plasticity in their responses to pH conditions. Data regarding the heterogeneity of pH in marine ecosystems will not only facilitate laboratory selection experiments by providing environmental context, but deployment of these sensors may also begin to identify refugia to OA, a possibility that has recently been proposed for select islands in the tropical Pacific (Karnauskas & Cohen 2012).

Conclusions

Both acclimation and genetic adaptation may ameliorate extinction risk for species in a changing environment. Predicting the capacity for adaptation requires an understanding of which traits are likely to be under selection and how much genetic variation exists for these traits in natural populations. Thus far, a handful of studies have attempted to measure intraspecific genetic variation in OA responses; many more of these studies are needed before we can begin to ask whether adaptation might rescue vulnerable populations.

At previous times in the earth's history, atmospheric CO₂ has been as high as 6000 p.p.m., more than 20 times preindustrial levels, but changes in atmospheric CO₂ were usually slow enough that chemical weathering increasingly delivered alkalinity to oceans, helping to maintain elevated saturation states (Pandolfi *et al.* 2011). By contrast, the current anthropogenically driven process of acidification is expected to produce changes in carbonate chemistry more rapid than any experienced in the past 20 million years (Caldeira & Wickett 2003). This is sobering given that several of the most rapid increases in CO₂ and temperature in the earth's history were associated with mass extinctions in the oceans (Pandolfi *et al.* 2011).

Except for organisms with short generation times, the current rate of change will likely be too great for much adaptation based on new mutations to occur. However,

given enough standing genetic variation in a population, substantial adaptation to environmental change is possible even over a single generation. As a result, a priority in OA research for the next decade will be to document the extent of such variation in natural populations and consider the role of evolutionary processes in projections of the effects of OA on modern marine species.

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