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Review

Ocean acidification research in the 'post-genomic' era: Roadmaps from the purple sea urchin *Strongylocentrotus purpuratus*

Tyler G. Evans [a,*], Jacqueline L. Padilla-Gamiño [b], Morgan W. Kelly [c], Melissa H. Pespeni [d], Francis Chan [e], Bruce A. Menge [e], Brian Gaylord [f], Tessa M. Hill [g], Ann D. Russell [h], Stephen R. Palumbi [i], Eric Sanford [f], Gretchen E. Hofmann [j]

[a] Department of Biological Sciences, California State University East Bay, Hayward, CA 94542, USA
[b] Department of Biology, California State University Dominguez Hills, Carson, CA 90747, USA
[c] Department of Biological Sciences, Louisiana State University, Baton Rouge, LA 70803, USA
[d] Department of Biology, University of Vermont, Burlington, VT 05405, USA
[e] Department of Integrative Biology, Oregon State University, Corvallis, OR 97331-2914, USA
[f] Department of Evolution and Ecology and Bodega Marine Laboratory, University of California Davis, Bodega Bay, CA 94923, USA
[g] Department of Geology and Bodega Marine Laboratory, University of California Davis, Bodega Bay, CA 94923, USA
[h] Department of Geology, University of California Davis, Davis, CA 95616, USA
[i] Department of Biology, Stanford University, Hopkins Marine Station, Pacific Grove, CA 93950, USA
[j] Department of Ecology, Evolution and Marine Biology, University of California Santa Barbara, Santa Barbara, CA 93106-9620, USA

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ABSTRACT

Advances in nucleic acid sequencing technology are removing obstacles that historically prevented use of genomics within ocean change biology. As one of the first marine calcifiers to have its genome sequenced, purple sea urchins (*Strongylocentrotus purpuratus*) have been the subject of early research exploring genomic responses to ocean acidification, work that points to future experiments and illustrates the value of expanding genomic resources to other marine organisms in this new 'post-genomic' era. This review presents case studies of *S. purpuratus* demonstrating the ability of genomic experiments to address major knowledge gaps within ocean acidification. Ocean acidification research has focused largely on species vulnerability, and studies exploring mechanistic bases of tolerance toward low pH seawater are comparatively few. Transcriptomic responses to high pCO_2 seawater in a population of urchins already encountering low pH conditions have cast light on traits required for success in future oceans. Secondly, there is relatively little information on whether marine organisms possess the capacity to adapt to oceans progressively decreasing in pH. Genomics offers powerful methods to investigate evolutionary responses to ocean acidification and recent work in *S. purpuratus* has identified genes under selection in acidified seawater. Finally, relatively few ocean acidification experiments investigate how shifts in seawater pH combine with other environmental factors to influence organism performance. In *S. purpuratus*, transcriptomics has provided insight into physiological responses of urchins exposed simultaneously to warmer and more acidic seawater. Collectively, these data support that similar breakthroughs will occur as genomic resources are developed for other marine species.

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* Corresponding author. Tel.: +1 510 885 3475; fax: +1 510 885 4747.
E-mail address: tyler.evans@csueastbay.edu (T.G. Evans).

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1. Introduction

Ocean acidification has emerged as a global-scale consequence of human activity. Approximately one-quarter to one-half of the carbon dioxide (CO_2) released into the atmosphere from the combustion of fossil fuels, deforestation, and other anthropogenic sources is absorbed by our world's oceans (Sabine et al., 2004; Boyd, 2011). While dissolution of CO_2 into the marine environment removes this greenhouse gas from the atmosphere and curbs the rate of temperature increase (Doney et al., 2009; Gruber, 2011), it forms carbonic acid in seawater, which lowers ambient pH and decreases the abundance of carbonate ions (CO_3^{2-}) (Caldeira and Wickett, 2003). Average surface ocean pH has already decreased by more than 0.1 units below the pre-industrial average of 8.17. By the year 2100, pH is expected to change by −0.13, −0.22, −0.28, or −0.42 pH units under emission scenarios that put atmospheric CO_2 levels at 421, 538, 670, or 936 ppm, respectively (Fig. 1). At such high sustained CO_2 concentrations, these changes in ocean chemistry will take thousands of years to be buffered by the natural dissolution of calcium carbonate from sediments and tens to hundreds of thousands of years to be eliminated completely by the weathering of rocks on land (Hoegh-Guldberg et al., 2014). Paleontological records indicate that the current average rate of pH change over the last century in the global ocean (>0.1 pH units per century) is unprecedented, and at least 10 times faster than any event within the last 65 to 300 million years (Ridgwell and Schmidt, 2010; Hönisch et al., 2012). Slower events in geological history provide robust evidence that ocean acidification will have a profound effect on marine organisms and ecosystems (Hoegh-Guldberg et al., 2014).

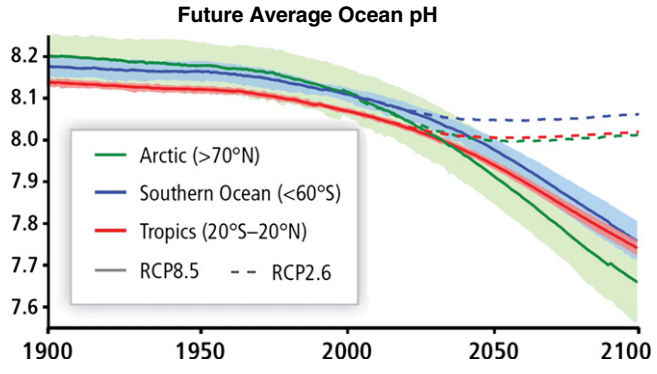


Fig. 1. Intergovernmental Panel on Climate Change predictions for future ocean acidification. Predictions are derived from 11 Coupled Model Intercomparison Project Phase 5 (CMIP5) Earth System models under Representative Concentration Pathway (RCP) 8.5. Time series of surface pH shown as the mean (solid line) and range (shaded area) of models, given as area-weighted averages over the Arctic Ocean (green), the tropical oceans (red), and the Southern Ocean (blue) for both RCP 8.5 (solid line) and RCP 2.6 (dashed line). RCP 8.5 assumes greenhouse gas emissions continue to rise throughout the 21st century. RCP 2.6 assumes that global annual greenhouse gas emissions peak between 2010–2020, with emissions declining substantially thereafter. Figure adapted from: The Ocean. In: Climate Change 2014: Impacts, Adaptation, and Vulnerability. Part B: Regional Aspects. Contribution of Working Group II to the Fifth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press. Fig. 30.7, page 1673.

Shifts in the chemical composition of seawater caused by the dissolution of CO_2 have important consequences for marine life (Fabry et al., 2008; Pörtner, 2008). Marine calcifiers depend on adequate concentrations of carbonate to precipitate shells, spines and skeletons (Hofmann et al., 2010) and thus face an uncertain future should inputs of CO_2 continue to decrease average surface ocean pH by the additional 0.13–0.42 units predicted for the end of this century (e.g. Caldeira and Wickett, 2003; Orr et al., 2005). Moreover, reductions in seawater pH can influence acid–base regulation internal to organisms (Pörtner, 2008), as well as organism behavior in some taxa (Nilsson et al., 2012). The realization that a broad-range of ecologically and economically important marine species will be affected by ocean acidification has placed a strong impetus on characterizing responses to increasing seawater pCO_2 in contemporary marine populations (Raven et al., 2005; Hofmann and Todgham, 2010; National Research Council, 2010). A key question currently facing the scientific community is whether and how quickly organisms can compensate for the effects of ocean acidification. As stated in the most recent report from the Intergovernmental Panel on Climate Change (IPCC) "the limits to acclimatization or adaptation capacity with regard to ocean acidification are presently unknown" (Hoegh-Guldberg et al., 2014).

Future studies of ocean acidification are certain to capitalize on the data-generating power and newfound accessibility and affordability of genomic approaches (Wang et al., 2009). In what some researchers have termed the 'post-genomic' era (Cossins et al., 2006; Dow, 2007; Strange, 2007), genome-scale data can be obtained within the budget and expertise of a single lab, a monumental shift from the costly and time-consuming sequencing projects performed by consortiums of scientists in the past (e.g. Sea Urchin Genome Sequencing Consortium et al., 2006). Recent genome sequencing of key marine organisms impacted by declining seawater pH, including the coral *Acropora digitifera* Dana (1846) (Shinzato et al., 2011), the Pacific oyster *Crassostrea gigas* Lamarck (1818) (Zhang et al., 2012), and the coccolithophore *Emiliania huxleyi* Lohman (1902) (Benner et al., 2013), have encouraged the use of 'omics' technology in ocean acidification research and approaches that exploit nucleic acid sequence information are being increasingly recognized as relevant to questions surrounding ocean change (Evans and Hofmann, 2012). For example, the advent of RNA sequencing has facilitated transcriptome-wide analyses of the response to ocean acidification in species with little or no previous sequence information (De Wit et al., 2012). RNA sequencing also allows detection of both differences in transcript abundance and variation in transcript coding sequences to be derived from a single sequencing experiment, meaning that both short-term plastic and long term evolutionary responses to seawater acidification can be inferred from the same data set (Wang et al., 2009).

The purpose of this review is to highlight the potential for genomic-based inquiry to address unanswered questions surrounding the biological consequences of ocean acidification. While ocean acidification research has grown almost exponentially over the last decade, there still

exists unevenness within the scientific literature that limits our ability to predict the response of marine organisms to increasing seawater pCO_2 (Wernberg et al., 2012). A recent meta-analysis of ocean acidification experiments demonstrates that research performed to date focuses largely on short-term phenotypic responses and single-stressor experiments (Wernberg et al., 2012). Ocean acidification research has also tended to emphasize the identification of vulnerable species, rather than those species that may be capable of tolerating future ocean change (Melzner et al., 2009). As a result, traits underlying tolerance of low pH seawater, the potential to adapt to a high pCO_2 ocean, and the effects of simultaneous exposure to multiple axes of ocean change remain understudied. Experiments that address these knowledge gaps are likely to drive ocean acidification research in the near future. This review presents the purple sea urchin *Strongylocentrotus purpuratus* Stimpson (1857) as a case study that illustrates how the development of genomic resources can assist in filling these knowledge gaps and increase our understanding of physiological and evolutionary responses to ocean acidification. The purple sea urchin has been a major experimental system for over a century (Jasny and Purnell, 2006), and the use of *S. purpuratus* in laboratories around the world expedited the sequencing of its genome (Sea Urchin Genome Sequencing Consortium et al., 2006). The ocean acidification research community was quick to take advantage of resources stemming from the urchin genome project, as sea urchins construct calcium carbonate skeletons and are considered vulnerable to the decreases in carbonate ion concentration that accompany ocean acidification (Byrne et al., 2011, 2013; Dupont et al., 2010; Hofmann et al., 2010; Kroeker et al., 2010). As technology promotes the development of similar genomic resources for other species affected by ocean acidification, researchers may look toward studies in *S. purpuratus* to estimate the scientific value of these endeavors or as blueprints for experimental design. Considerable insight gained from genomic studies of ocean acidification in urchins argues for the expansion of genomic resources to a wider-range of marine organisms.

2. Knowledge gap 1: what traits promote survival in a low pH ocean?

2.1. Coastal upwelling: future pH in a present day ecosystem

The rapid change in seawater chemistry predicted for the global ocean over the next century is at least an order of magnitude faster than has occurred for millions of years (Doney and Schimel, 2007), and the current concentration of atmospheric CO_2 is higher than experienced on Earth for at least the past 800,000 years (Lüthi et al., 2008). For most marine communities these shifts in ocean chemistry represent major deviations from conditions experienced over the course of their recent evolutionary history (Hönisch et al., 2012; Pelejero et al., 2010). However, some contemporary marine ecosystems already experience significant variation in pH and carbonate saturation states as a result of naturally occurring oceanographic processes (e.g. Andersson and Mackenzie, 2012; Hofmann et al., 2011; Thomsen et al., 2010). Exploiting naturally more acidic marine environments as analogs for future oceans has proven extremely informative in the context of ocean acidification research. Sites where natural volcanic CO_2 vents radically lower seawater pH to as low as 6.6 ± 0.5 have been utilized as "natural laboratories" to investigate the structures and functions of future marine ecosystems under different pH scenarios (Hall-Spencer et al., 2008; Kroeker et al., 2011; Kroeker et al., 2013a,b). While these unique environments highlight the ecological consequences of increasing ocean CO_2 concentrations, this research (and ocean acidification research in general) has tended to emphasize species vulnerability rather than seeking to identify mechanisms that underlie the ability of the relatively few remaining organisms to acclimatize to these more acidic environments. As a consequence, knowledge regarding the physiological underpinnings of resistance toward seawater acidification is limited (Melzner et al., 2009).

Natural variation in pH/pCO_2 occurring as a consequence of coastal upwelling in the Northeast Pacific Ocean provides an opportunity to discover how resident species cope with what can be dramatic changes in seawater chemistry (Hofmann et al., 2011; Yu et al., 2011), and identify potential mechanisms underlying low pH tolerance (Evans et al., 2013). Coastal upwelling is a dominant oceanographic process in the Northeast Pacific caused by seasonally strong winds that push surface waters offshore, allowing subsurface waters to rise or upwell. These deeper waters have accumulated the products of organic respiration and are therefore rich in CO_2 and low in pH and carbonate ions (Fassbender et al., 2011; Feely et al., 2008; Hauri et al., 2009; Gruber et al., 2012), creating changes in seawater chemistry that replicate those occurring with increased anthropogenic CO_2 emissions several decades into the future (Hauri et al., 2009). Upwelling-driven declines in seawater pH can be sizeable, temporarily exposing resident species to pH conditions not expected to occur in other marine habitats for at least one hundred years (Hoegh-Guldberg et al., 2014). Upwelling is particularly strong along the coast of Oregon. High-frequency measurements of pH dynamics (for technical information see Martz et al., 2010) within an intertidal site in coastal Oregon demonstrate that upwelling events influence pH dynamics in the intertidal zone, exposing this biodiverse habitat to pH transients as low as 7.47 and daily-averaged pH values as low as 7.80 (Fig. 2; Evans et al., 2013). Previous research has repeatedly shown that pH declines of this magnitude can be problematic for a variety of marine organisms (Dupont et al., 2010; Kroeker et al., 2010; Ries et al., 2009). However, purple sea urchins have persisted in the Northeast Pacific through this period of strong upwelling that began some 12–15 million years ago (Jacobs et al., 2004; Vermeij, 1989). Persistent seasonal exposure to low pH seawater over these geological timescales may have selected for increased tolerance in marine populations inhabiting sites of natural pH variation (Pansch et al., 2014), and investigation of *S. purpuratus* from upwelling zones will assist in identifying traits associated with low pH tolerance. Organisms living within variable pH zones may also be living close to low pH tolerance limits and therefore be threatened by the further decreases in ocean pH caused by anthropogenic ocean acidification. This trend has been demonstrated previously with respect to temperature for organisms inhabiting intertidal zones that experience highly variable temperature regimes (Stillman, 2003; Tomanek, 2008). Ocean acidification is expected to progress rapidly in the Northeast Pacific, with models predicting widespread and persistent undersaturation of carbonate in the nearshore 10 km by 2050 (Gruber et al., 2012).

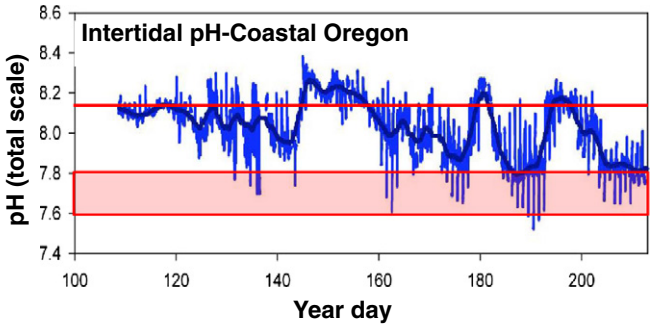


Fig. 2. Temporal variation in seawater pH in an intertidal habitat in Coastal Oregon. Data were collected from April 19, 2011 (year day 108) to July 31 2011 (year day 211). Daily minimum and maximum pH (total scale; blue lines) and three-day running mean pH (dark blue line) are shown. pH measurements were recorded every 10 min during sensor immersion. Solid red line denotes pre-industrial average global ocean pH. Shaded red box denotes range of average global ocean pH predicted for the year 2100 by the Fifth Assessment Report of the Intergovernmental Panel on Climate Change (Hoegh-Guldberg et al., 2014).

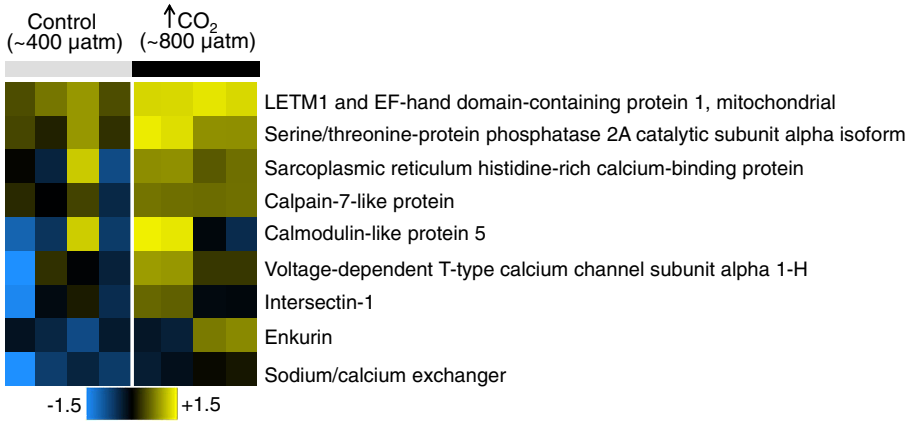
2.2. Potential mechanisms for maintaining homeostasis in a low pH ocean

Transcriptomics represents a powerful tool to uncover potential mechanisms underlying low pH tolerance. The capacity to cope with ocean acidification will be determined at least in part by phenotypic plasticity, the degree to which an organism can reversibly alter its phenotype, and these plastic responses are implemented through changes in gene expression (Evans and Hofmann, 2012; Whitehead, 2012). Sequence resources developed for *S. purpuratus* facilitate tracking gene expression across the entire transcriptome, providing a comprehensive and unbiased perspective of the response to ocean acidification in this species. Evans et al. (2013) characterized the transcriptomic response to increased seawater pCO_2 (~800 μatm, pH = 7.8) across 28,036 putative genes in *S. purpuratus* embryos spawned from adults collected within an intertidal site in Oregon that experiences upwelling-driven fluctuations in seawater pH. Assuming that urchins able to recruit and survive within this area of strong upwelling possess an enhanced ability to cope with high pCO_2 seawater, identifying genes regulated by pCO_2 in this population of urchins will provide insight into mechanisms underlying tolerance of lower pH seawater. While a more robust experimental design would directly compare the response to seawater acidification between urchin populations inhabiting regions of stably high pH with those living within low and variable pH regimes, Evans et al. (2013) nonetheless highlight candidate genes that may contribute to low pH tolerance. Gene expression patterns suggest that modifying the transport of calcium (Fig. 3) and sodium are major components of the acclimatory response to ocean acidification in urchins inhabiting upwelling zones, and in turn, the ability to regulate the transport of these ions may be an important trait for maintaining homeostasis in more acidic oceans.

Larval sea urchins construct delicate but functionally important skeletons composed of calcium carbonate. Modifying the transport of calcium plausibly represents an adaptation to promote calcification in more acidic seawater. Genes up-regulated following exposure to high pCO_2 seawater in gastrulae from coastal Oregon include multiple calcium transporters and binding proteins (Fig. 3A) and were statistically enriched for calcium-related functions (Fig. 3B; Evans et al., 2013). Calcium is the primary cation used in the biomineralized structures of sea urchins and the large amounts needed to sustain skeletogenesis must be transported intracellularly from seawater prior to being combined with carbonate and deposited into the skeletal matrix (Wilt, 2002). Skeletogenesis is impaired by calcium channel blockers, presumably by inhibiting calcium uptake from seawater (Hwang and Lennarz, 1993; Mitsunaga et al., 1986). Thus changes in the abundances of calcium transporters (including a sodium/calcium exchanger, a voltage-dependent T-type calcium channel and a calcium/hydrogen antiporter (LETM1 and EF-hand domain-containing protein 1)) and binding proteins (including calmodulin and sarcoplasmic reticulum histidine-rich calcium-binding protein) (Fig. 3A) may increase the abundance of intracellular calcium and promote skeletogenesis in a carbonate-limited environment.

S. purpuratus embryos from Oregon also modify the expression of multiple genes involved in sodium transport following exposure to high pCO_2 seawater, including the sodium/potassium transporting ATPase (Evans et al., 2013). Adjusting sodium transport likely enhances pH tolerance by sustaining the transmembrane gradients that provide the chemical energy necessary to remove excess protons. pH compensation in congeneric green sea urchins (*Strongylocentrotus droebachiensis* Müller 1776) is dependent on sodium, supporting a buffering mechanism that uses energy derived from transmembrane sodium gradients to export hydrogen ions (Stumpp et al., 2012). Acid–base regulation in teleost fish, crustaceans, and cephalopods also rely on energy derived from sodium gradients to export protons (Melzner et al., 2009), and the powerful ionoregulatory systems of these taxa are thought to underlie their broad tolerance of changes in seawater pCO_2. Enhanced pH tolerance in urchins and other marine species that experience

A



B

Gene Ontology	Database
Calcium channel protein	Protein Class
Calcium binding protein	Protein Class
Intracellular calcium sensing protein	Protein Class
Calmodulin	Protein Class
Voltage-gated calcium channel activity	Molecular Function
Calcium ion binding	Molecular Function
Calmodulin binding	Molecular Function

Fig. 3. Induction of genes and significantly over-represented ontologies involved in calcium homeostasis in Oregon urchins exposed to acidified seawater. (A) Hierarchical clustered heatmap of the nine calcium transport and binding proteins up-regulated by *S. purpuratus* gastrulae in high pCO_2 (~800 μatm) seawater. The heatmap displays the normalized $\log_2$-ratio for each gene (rows) across each replicate (n = 4) at the two pCO_2 exposure levels (columns) with yellow hues representing up-regulated genes and blue hues down-regulated genes. (B) Significantly over-represented gene ontologies involved in calcium homeostasis among the set of genes up-regulated by Oregon *S. purpuratus* gastrulae in high pCO_2 (~800 μatm) seawater.

natural pH variation is likely not without cost (Collard et al., 2014). The transmembrane movement of ions, including sodium and calcium, can account for up to 77% of metabolism in larval urchins (Leong and Manahan, 1997) and if additional ion transport is indeed required to maintain homeostasis in future oceans, the so-called 'costs of living' for these critical early life stages are certain to increase.

Conclusions regarding the mechanistic basis of low pH tolerance in *S. purpuratus* are currently limited by the absence of comparative experiments. That is, transcriptomic responses to high pCO_2 seawater in Oregon urchins must be compared to responses from populations inhabiting regions of stable and high pH. In the absence of these comparative data, it remains ambiguous as to which transcriptomic responses represent evolutionary innovations that enhance low pH tolerance, and which are simply part of a conserved physiological response to seawater acidification shared by all populations. However, results from a long-term, common-garden study of distant populations of adult purple sea urchins hint at divergent transcriptional responses to pH between populations inhabiting areas of high and low intensity upwelling (Pespeni et al., 2013a,b). Following three years of acclimation to ambient conditions in Central California (Pacific Grove, California, USA), expression of genes functioning within biomineralization and metabolic pathways were consistently higher in urchins collected from Southern California (La Jolla, California, USA), which are protected from upwelling-driven declines in pH, than in urchins from Oregon (Boiler Bay, Oregon, USA) that experience more frequent and intense upwelling (Pespeni et al., 2013a). A meta-analysis of ocean acidification-induced changes in gene expression indicates that modifying the expression of genes involved in metabolism and biomineralization is the primary transcriptional response to seawater acidification in urchins (Evans and Watson-Wynn, 2014). Given that expressions of genes from these same functional categories were altered between northern and southern populations held in a common-garden, these data suggest northern and southern populations of *S. purpuratus* are responding differently to the novel pH conditions encountered in central California and are adapted to their local pH regimes. Oregon urchins seemed relatively insensitive to pH fluctuations compared with congeners in southern California that experience little seawater pH variation in the wild.

3. Knowledge gap 2: will marine organisms evolve in response to ocean acidification?

The ability of marine organisms to persist in more acidic oceans will depend not only on phenotypic plasticity, but also evolutionary adaptation (Kelly and Hofmann, 2013; Pespeni et al., 2013b,c; Sunday et al., 2014). Whereas many ocean acidification experiments have assessed phenotypic responses using short-term experiments (Kroeker et al., 2013c), few studies have considered the potential for evolution (Kelly et al., 2013; Stockwell et al., 2003; Sunday et al., 2014). Evaluating the capacity of organisms to adapt to low pH seawater is being increasingly recognized as necessary to understanding the consequences of ocean acidification (Sunday et al., 2014). Contemporary genomic approaches, such as RNA sequencing, are poised to provide substantial insight into evolutionary responses to ocean acidification and may also represent the most feasible method to study adaptation to ocean change considering alternative approaches, such as experimental evolution (Collins et al., 2014), are difficult to implement in organisms like sea urchins that have long generation times (Ebert and Southon, 2003). Genomic resources for *S. purpuratus* stimulated their use in studies of evolutionary responses to ocean acidification (Pespeni et al., 2013a,c) and these evolutionary genomic studies serve as blueprints for future experiments performed in species for which genomic information can now be more easily obtained.

3.1. Adapting to ocean acidification

Rapid evolution depends more on existing genetic variation than new mutations (Hermisson and Pennings, 2005; Kirkpatrick and Barton, 1997; Lande and Shannon, 1996), and as such, marine species capable of adapting to ocean acidification most quickly are those with large populations that exist across an environment with variable pH (Pespeni et al., 2013c). The biogeographic range of *S. purpuratus* stretches northward along the west coast of North America from Baja California to Alaska (Tegner, 2001), a vast expanse of coastline in which environmental factors influencing pH vary considerably. Autonomous pH sensors placed within various urchin habitats along the United States west coast demonstrate that there are distinct latitudinal differences in upwelling intensity, with sites north of Point Conception, California, USA experiencing stronger and more frequent upwelling, and consequently lower annual pH, than sites further south (Hauri et al., 2013; Kelly et al., 2013). Quantitatively, 20% of the pH values recorded by sensors in Oregon fell below pH 7.8, compared with less than 2% of the pH values recorded for sites in Central and Southern California, respectively (Fig. 4A,B; Hofmann et al., 2014). As a consequence of these very different pH conditions, natural selection should act upon northerly *S. purpuratus* populations to promote and sustain alleles with greater fitness in more acidic seawater.

Evolutionary responses to pH in *S. purpuratus* have recently been explored using genomics, revealing insight into how the pH mosaic within the Northeast Pacific has shaped the physiology and evolution of this species. Pespeni et al. (2013c) used a genomics-based selection experiment whereby embryos from seven adult populations spanning Central Oregon to Southern California were collectively cultured in low pH seawater (~900 μatm), and changes in allele frequency monitored over time across 19,493 gene loci. The authors predicted that shifts in allele frequency would occur as a consequence of increased survival among urchins from northern populations that have been consistently exposed to upwelling-driven declines in pH during evolutionary history. Consistent with this hypothesis, striking patterns of genome-wide selection were detected following exposure to low pH seawater. A critical aspect of these genomic analyses lies in the ability to subsequently identify the biological function of genes putatively under selection. In this case, large differences in allele frequency were detected among genes that regulate biomineralization, lipid metabolism and ion homeostasis. These functional changes plausibly promote skeletogenesis, modify energetics, and support pH homeostasis, respectively, in seawater low in pH and carbonate, and are congruent with well-established outcomes regarding the physiological impact of ocean acidification on marine organisms (Byrne et al., 2013; Kroeker et al., 2010; Pörtner, 2008; Stumpp et al., 2012). Genes from these same functional categories are more likely to be differentially expressed in urchins exposed to acidified seawater (Evans and Watson-Wynn, 2014), suggesting that the same gene functions driving short-term plastic responses to ocean acidification are also the targets of natural selection (Crawford and Oleksiak, 2007; Whitehead et al., 2012; Whitehead and Crawford, 2006).

Evolved tolerance toward ocean acidification is likely to involve physiological trade-offs (Sunday et al., 2014). A growing body of evidence indicates that pCO_2 resilience in marine calcifiers is associated with increased metabolic costs and thus trade-offs in energy allocation (Holcomb et al., 2010; Thomsen et al., 2013). If these trends were to hold true in *S. purpuratus*, urchins inhabiting strong upwelling zones would indeed exhibit greater tolerance of low pH, but would incur a larger energetic burden as a consequence of ATP-dependent pH regulatory processes, such as ion transport (Vidal-Dupiol et al., 2013; Wood et al., 2008). Whether such trade-offs exist in *S. purpuratus* is unknown. Exploring the transcriptomic response to seawater acidification in urchin populations that naturally differ in their exposure to low pH seawater represents a potential method to identify trade-offs associated with enhanced pH tolerance. In *S. purpuratus*, the transcriptomic response to high pCO_2 seawater was compared between embryos

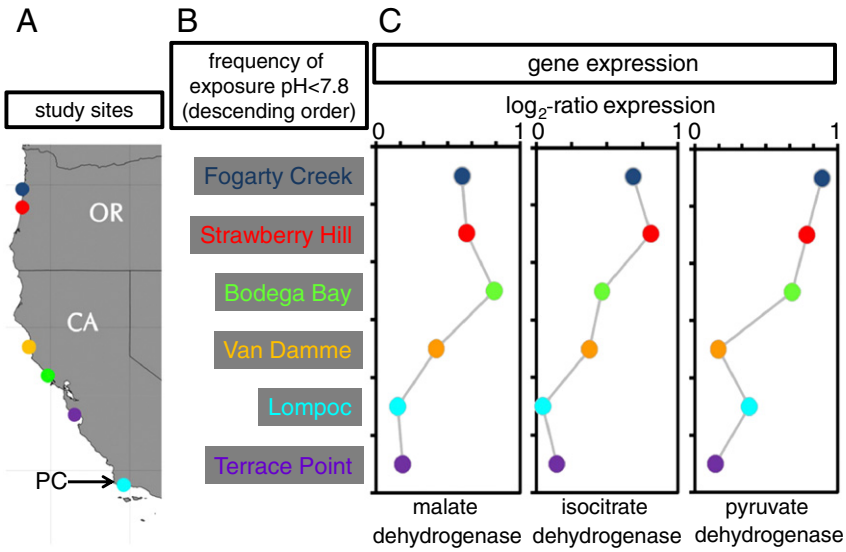


Fig. 4. Differences in the transcriptomic response to CO_2-induced seawater acidification in gastrula stage *S. purpuratus* collected from various sites along the Northeast Pacific coast. (A) Location of the six intertidal study sites where autonomous sensors recorded high frequency pH dynamics (colored dots) and location of Point Conception, CA (PC, black arrow). (B) Ranking of the six study sites based on frequency of exposure to seawater pH less than 7.8 from most frequent exposure (top) to least frequent exposure (bottom). (C) Three representative gene expression profiles (left: malate dehydrogenase, middle: isocitrate dehydrogenase and right: pyruvate dehydrogenase) illustrating population-specific responses to elevated pCO_2 among genes involved in carbohydrate metabolism. Expression refers to the normalized log_2-ratio fluorescent intensity for each gene. OR = Oregon; CA = California. Sea urchins corresponding to the Lompoc pH sensor were collected at Alegria, CA (34.4672222, longitude −120.27806), approximately 20 km south of the sensor location.

spawned from adults collected at six intertidal locations along the pH mosaic of the Northeast Pacific (Fig. 4A). Transcriptomes were parsed for genes whose change in expression was proportional to pH exposure in the wild, that is, genes were identified whose fold-change in expression following exposure to low pH seawater was strongly correlated to the magnitude of pH change across the six sampling sites (i.e. highest in the Oregon populations and lowest in the Central/Southern California populations; Fig. 4B). Functional analyses revealed that the resulting set of genes contained higher numbers of genes involved in carbohydrate metabolism (Fig. 4C; Evans T. G., Pespeni M. H. et al., *unpublished results*), suggesting that additional metabolic proteins are required to support energetically demanding homeostatic processes triggered by the larger magnitude decreases in pH occurring in more northerly habitats. This result is consistent with the high metabolic demands of ion transport necessary to maintain intracellular pH homeostasis in urchin embryos (Leong and Manahan, 1997; Stumpp et al., 2012) and metabolic trade-offs for pH resilience reported for other calcifiers (Holcomb et al., 2010; Thomsen et al., 2013). Interestingly, coastal upwelling in the Northeast Pacific also transports large amounts of nutrients to surface waters (Barth et al., 2007), which can spark increased primary productivity by phytoplankton. Thus, increased energetic demands associated with maintaining homeostasis during upwelling may be offset by exploiting this temporary increase in food availability. Recent evidence indicates that food availability has a dominant effect on the ability of marine calcifiers to function in low pH seawater (Thomsen et al., 2013). Whether or not algal productivity differs between Northern and Southern California remains unclear.

4. Knowledge gap 3: How will multiple ocean change stressors combine to influence marine organisms?

4.1. A multi-stressor ocean

In order to predict how global change will affect marine ecosystems, it is critical to understand how multiple ocean change variables interact to influence organism function (Pörtner, 2008; Wernberg et al., 2012). Anthropogenic inputs of CO_2 are not only causing the world's oceans to become more acidic, but in many cases warmer as well, and how simultaneous exposure to higher temperature and lower pH seawater will influence the performance of marine organisms is a key question within the scope of ocean change (Doney et al., 2011; Hoegh-Guldberg and Bruno, 2010; Kroeker et al., 2013c). Environmental variables can combine to affect physiology in very different ways (e.g. additive, synergistic or antagonistic; Crain et al., 2008), and the effect of multiple ocean change variables on organism function is presently under-studied in marine climate change research (Wernberg et al., 2012). Future generations of purple sea urchins will encounter seawater with substantially different temperature and pCO_2 regimes from those encountered presently. pH and temperature will merge in a complex pattern within the future Northeast Pacific Ocean. Sea surface temperatures have been increasing within the California Current over the last century (Field et al., 2006; Johnstone and Mantua, 2014), with continued moderate warming predicted for the next few decades (King et al., 2011). However, increasingly warmer sea surface temperatures may be interspersed with more frequent upwelling events that transport cold and more acidic seawater to the surface (Garcia-Reyes and Largier, 2010), causing ocean acidification to progress rapidly in this region, with rates of pH change similar to those projected for the Southern Ocean and Arctic (Gruber et al., 2012; Hauri et al., 2013; Wootton and Pfister, 2012). Genomic approaches can assist in addressing how different temperature and pH scenarios will influence the biology of the Northeast Pacific (Benner et al., 2013), and abundant sequence resources provide a means to characterize transcriptomic responses to concurrent temperature and pH change in *S. purpuratus*. Gene expression patterns demonstrate that exposure to simultaneous warming and acidification additively regulates the physiology of larval *S. purpuratus*, while highlighting the benefits of experimental designs that pair transcriptomics with other metrics of organism performance (Padilla-Gamiño et al., 2013).

4.2. A transcriptomic signature of reduced physiological performance

To explore the response of sea urchins to changes in co-occurring environmental factors, *S. purpuratus* larvae were cultured under factorial combinations of temperature (13 °C and 18 °C) and pCO_2 (400 μatm or 1100 μatm) that followed one projected ocean change scenario for

the Northeast Pacific over the next century (Hauri et al., 2009). Physiological performance was then assessed in pluteus stage larvae by monitoring metabolic rate (via oxygen consumption) and tracking gene expression with transcriptomics (28,036 genes; Padilla-Gamiño et al., 2013). Exposure to a simultaneously warmer (18 °C) and more acidic (1100 μatm) environment significantly decreased oxygen consumption in *S. purpuratus* larvae, a response not observed in urchins exposed to either low pH or higher temperature seawater alone (Padilla-Gamiño et al., 2013). Decreased oxygen consumption is predicted to promote short-term tolerance by temporarily suppressing energetically expensive physiological processes (Fabry et al., 2008), and the depression in oxygen consumption observed in *S. purpuratus* is another piece of evidence that life in future oceans will incur metabolic costs (Enzor et al., 2013; Ivanina et al., 2013). Transcriptomic analyses were performed on the same animals used in the oxygen consumption assays, and by coupling these metrics of physiological performance, a pattern of gene expression occurring coincident with metabolic depression was resolved. Metabolic depression in *S. purpuratus* occurred in parallel with down-regulation of multiple histone encoding genes: of the forty-nine genes differentially expressed in larvae raised in high temperature/high pCO_2 seawater relative to controls, twenty-seven (55%) encode for histones (Fig. 5). Previous studies indicate that modification of histone expression is not a component of the transcriptomic response to strictly seawater acidification in *S. purpuratus* (Evans et al., 2013; Padilla-Gamiño et al., 2013; Todgham and Hofmann, 2009). Histones are ancient genes that function as structural components of nucleosomes (the basic unit of DNA packaging in eukaryotes) and act as major regulators of chromatin structure, transcription and gene expression (Kouzarides, 2007; Marzluff and Duronio, 2002). Histone gene expression is altered by changes in metabolic status (Katada et al., 2012) and may therefore be acting as metabolic sensors in *S. purpuratus*, producing changes in chromatin structure that adaptively modify gene expression to better suit novel environmental conditions (Kouzarides, 2007; Su et al., 2004). Given that metabolic depression has been observed in a wide-range of marine organisms exposed to future ocean conditions (Carter et al., 2013; Michaelidis et al., 2005; Nakamura et al., 2011; Reipschläger and Pörtner, 1996; Schalkhausser et al., 2012), histone down-regulation may underlie a response to ocean change conserved across taxa. Coupling transcriptomics with other indices of physiological status increases the validity of gene expression data and should be considered an important principle in designing future transcriptomic and ocean change experiments.

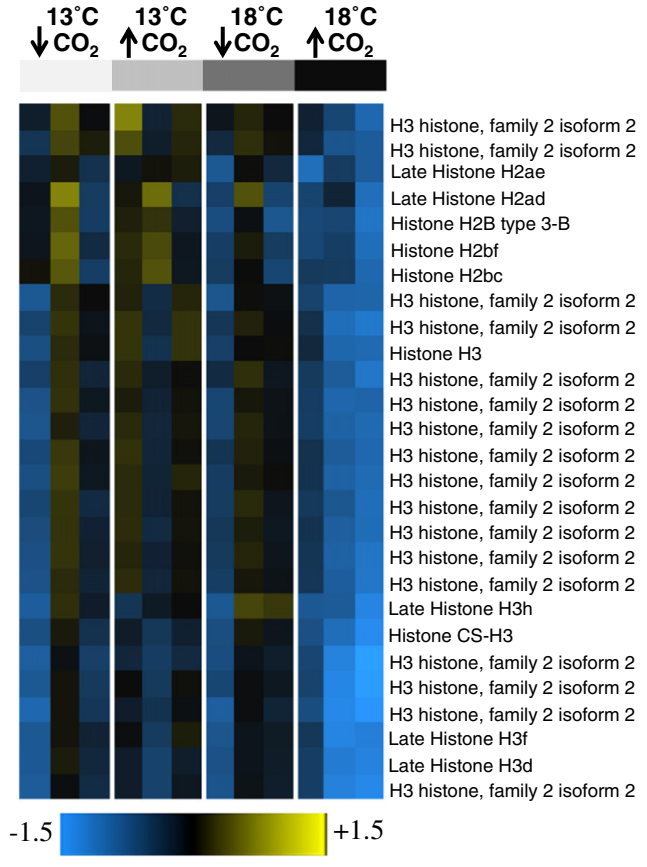


Fig. 5. Effects of simultaneously high temperature and high pCO_2 seawater on the expression of histone encoding genes in pluteus stage *S. purpuratus*. Hierarchical clustered heatmap of the twenty-seven histone encoding genes down-regulated in plutei following exposure to high temperature (18 °C), low pH seawater (1100 μatm pCO_2). The heatmap displays the normalized log_2-ratio for each gene (rows) across each replicate (n = 3) at the four temperature and pCO_2 exposures (columns) with yellow hues representing up-regulated genes and blue hues down-regulated genes. Arrows refer to control (↓ = 400 μatm) and high (↑ = 1100 μatm) pCO_2 treatments.

5. Conclusions, caveats and future directions

Advances in genome science have broadened experimental tools for ocean acidification research. The purple sea urchin has emerged as a powerful model in ocean acidification research in part because researchers have exploited resources associated with a fully sequenced genome. As genomic data are developed for other species impacted by ocean change, recent studies in *S. purpuratus* demonstrate the value of genomic approaches and can help guide future work. Whether or not calcium transporters, skeletal growth, and histone genes will prove important in physiological tolerance and genetic capacity to evolve in other species affected by ocean acidification will be revealed as similar studies in other organisms begin to fill these knowledge gaps. The possibility of organisms being adapted to local pH regimes is intriguing, and will likely remain an active area of research. Research described here hint at local adaptation in *S. purpuratus*, however whether other species whose populations occupy a similar range of pH conditions, both within the Northeast Pacific or across the global ocean, are locally adapted to pH remains unknown. The extent of local adaptation will have a profound influence on how ocean acidification will impact marine life. In species locally adapted to pH, responses to future acidification will differ among populations and predictions regarding the biological consequences of continued declines in seawater pH will become more complex. Variation among genotypes in responses to ocean acidification conditions has also been observed in bryozoans (Pistevos et al., 2011), oysters (Parker et al., 2012) and coccolithophores (Langer et al., 2009). Genomic approaches are poised to address questions surrounding local adaptation to seawater pH. The importance of epigenetic responses to abiotic change has also recently been emphasized. Transgenerational plasticity, the ability of the parental environment to alter the phenotype of their offspring, is being increasingly recognized as a potential buffer against abiotic stress and therefore relevant to predicting the biological consequences of ocean change (Munday, 2014). In green sea urchins, exposure of the parental generation to moderate levels of ocean acidification enhanced the tolerance of next generation larvae (Dupont et al., 2013). Importantly, several next generation sequencing technologies now permit the survey of genome-wide epigenetic variation at high resolution (Bell and Spector, 2011).

Like any other experimental approach, genomic experiments are associated with a set of limitations. Transcriptomic data have been consistently criticized because a large change in gene expression does not necessarily equate to a large effect on fitness, and because protein activity is most relevant to fitness, and mRNA abundance can be an unreliable indicator of protein activity (Evans, in press). RNA sequencing also requires reconstruction of full-length transcripts from short reads which poses several challenges for data analysis and can introduce bias, although computational strategies for data analysis are improving rapidly (Boley et al., 2014). For example, some transcripts will have low coverage, whereas other will be highly expressed; sequence coverage may be uneven across the transcript length; sequences that are

repeated in different genes introduce ambiguity, as well as other potential sources of error (Grabherr et al., 2011; Roberts et al., 2011). Transcript annotation is aided by the presence a reference genome, and the genomic experiments described here benefit from the completed *S. purpuratus* genome. However, de novo assembly in the absence of a reference genome is increasingly common and yields accurate and informative results (Grabherr et al., 2011). De novo transcriptome assembly has already been used to explore the biological impact of ocean acidification (De Wit and Palumbi, 2012). Ultimately, genomic approaches should emerge as an important method to understanding the traits, evolutionary capacity, and ability to respond to concurrent environmental stressors across the broad range of species that inhabit the oceans. Of course a truly comprehensive understanding of ocean acidification will only emerge from collective knowledge obtained from not only genomic-based experiments, but also those assessing performance across other levels of biological organization.

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