Effects of Seawater Acidification on Gene Expression: Resolving Broader-Scale Trends in Sea Urchins

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Abstract. Sea urchins are ecologically and economically important calcifying organisms threatened by acidification of the global ocean caused by anthropogenic CO2 emissions. Propelled by the sequencing of the purple sea urchin (Strongylocentrotus purpuratus) genome, profiling changes in gene expression during exposure to high pCO2 seawater has emerged as a powerful and increasingly common method to infer the response of urchins to ocean change. However, analyses of gene expression are sensitive to experimental methodology, and comparisons between studies of genes regulated by ocean acidification are most often made in the context of major caveats. Here we perform meta-analyses as a means of minimizing experimental discrepancies and resolving broader-scale trends regarding the effects of ocean acidification on gene expression in urchins. Analyses across eight studies and four urchin species largely support prevailing hypotheses about the impact of ocean acidification on marine calcifiers. The predominant expression pattern involved the down-regulation of genes within energy-producing pathways, a clear indication of metabolic depression. Genes with functions in ion transport were significantly over-represented and are most plausibly contributing to intracellular pH regulation. Expression profiles provided extensive evidence for an impact on biomineralization, epitomized by the down-regulation of seven spicule matrix proteins. In contrast, expression profiles provided limited evidence for CO2-mediated developmental delay or induction of a cellular stress response. Congruence between studies of gene expression and the ocean acidification literature in general validates the accuracy of gene expression in predicting the consequences of ocean change and justifies its continued use in future studies.

Introduction

Since the onset of the industrial revolution, human activities have been rapidly adding carbon dioxide (CO2) to Earth’s atmosphere (Caldeira and Wickett, 2003), and atmospheric CO2 levels are now higher than at any point in the past 800,000 years (Lüthi et al., 2008). In addition to causing the global warming responsible for rises in atmospheric and ocean temperature, anthropogenic CO2 is also responsible for shifts in the chemical composition of the global ocean. Approximately one-quarter to one-half of atmospheric CO2 is dissolved into our world’s oceans (Sabine et al., 2004; Boyd, 2011), where it reacts with seawater to increase the concentration of hydrogen ion (H+) and decrease the concentration of carbonate ion (CO32−) (Caldeira and Wickett, 2005). This process, termed ocean acidification, has already reduced global ocean pH by 0.1 units since pre-industrial times (IPCC, 2007), and is predicted to decrease seawater pH by 0.45 units over the next century and by 0.77 units over the next 300 years. This anticipated rate of change is faster than any experienced in the last 300 million years (Caldeira and Wickett, 2003, 2005; Honisch et al., 2012).

In the context of such rapid and widespread change, understanding the consequences of ocean acidification has emerged as a major priority in marine science research (Raven et al., 2005; National Research Council, 2010). Spanning sub-disciplines from oceanography to ecology, investigators are using a variety of scientific tools to forecast the effects of continued ocean acidification on a range of marine organisms. Genomic approaches have become an informative way to investigate responses to ocean acidification and have become more prevalent in recent years. The advent of RNA sequencing now allows genomic-based inquiry in marine organisms with little or no previous sequence information (e.g., De Wit and Palumbi, 2013) and
genome sequencing of ecologically and economically important marine organisms impacted by declines in ocean pH, such as the purple sea urchin (Strongylocentrotus purpuratus) (Stimpson, 1857) (Pearse, 2006; Sodergren et al., 2006; Anderson et al., 2011), Pacific oyster (Crassostrea gigas) (Zhang et al., 2012), and coccolithophore Emiliania huxleyi (Benner et al., 2013) have further encouraged the use of genomics in ocean acidification research. A common application of nucleic acid sequence information involves tracking changes in gene expression in organisms exposed to near-future pCO2 scenarios. The philosophy behind this approach is simple: marine organisms exposed to high pCO2 seawater will induce compensatory changes in physiology that are facilitated in part by changes in gene expression (Gracey, 2003; Gracey and Cossins, 2003), and knowledge of the numbers, magnitude of change, and function of genes regulated by seawater pCO2 informs questions surrounding the ability of these organisms to cope with ocean change (Evans and Hofmann, 2012). For example, functional analyses of genes regulated by seawater pH assists in identifying biological processes most strongly influenced by ocean acidification and provides mechanistic understanding of differential sensitivity to seawater pCO2 among marine species or populations (Evans et al., 2013; Granados-Cifuente et al., 2013). Analyses of gene expression may also highlight potential evolutionary responses to shifts in seawater chemistry, as the genes that contribute to short-term plastic responses to ocean acidification also represent probable targets of natural selection driving the evolution of tolerance toward more acidic oceans (Whitehead and Crawford, 2006; Crawford and Oleksiak, 2007; Whitehead, 2012).

Efforts to characterize ocean-acidification-induced changes in gene expression have been implemented using both small- and large-scale approaches across a variety of marine taxa. From candidate gene approaches that target a small number of genes with well-described functions, to high-throughput approaches that track expression across the entire transcriptome or proteome, expression-based ocean acidification studies have been performed in corals (e.g., Moya et al., 2012; Vidal-Dupiol et al., 2013), crabs (e.g., Fehsenfeld et al., 2011), coccolithophores (e.g., Jones et al., 2013), oysters (e.g., Tomanek et al., 2011; Dineshram et al., 2012; Liu et al., 2012), mussels (e.g., Hüning et al., 2013), barnacles (e.g., Wong et al., 2011), and other important marine species (e.g., Hauton et al., 2009; Crawford et al., 2011). Sea urchins have emerged as an ideal system for monitoring changes in gene expression caused by ocean acidification. Urchin embryos, larvae, and adults all construct calcium carbonate skeletons and are therefore predicted to be susceptible to the decreases in carbonate ion concentration that accompany ocean acidification (Dupont et al., 2010; Hofmann et al., 2010; Kroeker et al., 2010; Byrne et al., 2011, 2013). The importance of the purple sea urchin as a laboratory model used around the world expedited the sequencing of its genome and stimulated the use of urchins in genetic experiments that study the effects of ocean acidification. This combination of ecological relevance and experimental power has led to the publication of eight manuscripts that analyzed gene expression during exposure to acidified seawater in four species of urchins (Strongylocentrotus purpuratus, Lytechinus pictus, Hemicentrotus pulcherrimus, and Paracentrotus lividus). While these studies are undoubtedly informative, gene expression data are sensitive to differences in experimental methodology, which prevents the genetic response to ocean acidification from being directly compared even between studies using the same species. For example, pronounced differences in treatment regimes between studies almost certainly influence the number and type of genes deemed responsive to seawater pCO2. Martin et al. (2011) detected increases in the expression of three key genes involved in calcification in Mediterranean urchin (P. lividus) larvae cultured in low pH seawater, a result that was not replicated in studies of purple sea urchins (S. purpuratus) that used lower pCO2 treatment levels (Todgham and Hofmann, 2009; Evans et al., 2013). Discrepancies such as these ultimately hinder the ability of researchers to resolve common or divergent responses to ocean acidification between species or populations. Other experimental parameters, such as method of detecting differential expression (e.g., quantitative polymerase chain reaction (qPCR) vs. microarray vs. RNA sequencing), statistical approaches (supervised approaches including Student’s t-tests and analyses of variance vs. unsupervised approaches such as principal components analysis), and life stage (early stage vs. adult) also influence gene expression, with these differences again limiting broader-scale conclusions about the effects of ocean acidification on gene expression.

One way to overcome methodological differences between studies and reveal common patterns across datasets is to perform a meta-analysis. Meta-analyses have proven effective in finding predominant trends within ocean acidification data, despite individual studies reporting conflicting results (Ries et al., 2009; Hendriks et al., 2010; Kroeker et al., 2010, 2013). Although a sufficient mass of gene expression data now exists to enable comparative analyses of the genetic response to ocean acidification in sea urchins, we are unaware of any efforts to do so. In this study, we perform meta-analyses of genes deemed significantly differentially expressed in sea urchins exposed to simulated ocean acidification as a means of identifying major outcomes across these studies. We use the compiled gene expression data to address five prevailing hypotheses regarding the effects of ocean acidification on sea urchins and marine organisms in general: exposure to high pCO2 seawater (i) depresses metabolism, (ii) impairs calcification, (iii) disrupts pH homeostasis, (iv) delays development; and
finally, (v) future $p$CO$_2$ regimes will exceed organism tolerance limits. Our results largely support these five hypotheses and reassert the vulnerability of sea urchins in future oceans (Wittmann and Pörtner 2013). We also aim to identify a core response to seawater acidification in urchins that could be used to increase our understanding of species-specific responses to ocean acidification and develop more accurate biomarkers of exposure to low pH seawater. We resolved a set of 26 genes common to multiple studies that further emphasizes the impact of ocean acidification on metabolism, calcification, and pH regulation.

Materials and Methods

Data selection

We searched the biological literature for studies that tracked changes in gene expression in sea urchins exposed to acidified seawater. Literature searches were conducted using the ISI Web of Science database and different combinations of relevant keywords that included ocean acidification, seawater acidification, hypercapnia, sea urchin, urchin, echinoderm, gene expression, gene, genomics, transcriptomics, and proteomics. Literature searches were performed until 21 September 2013. We retained studies regardless of the method used to measure gene expression, the $p$CO$_2$/pH exposure level used, or the life stage or species investigated. Database searches using these criteria returned eight manuscripts that were used in subsequent analyses (see Table 1).

Compilation of differentially expressed gene lists

Each of the eight manuscripts were parsed for genes that the authors considered significantly differentially expressed in response to seawater acidification. Differentially expressed genes from Evans et al. (2013) and Padilla-Gaminó et al. (2013) were extracted from lists contained in the online supplementary information accompanying those manuscripts. Differentially expressed genes from the remaining six manuscripts were extracted from figures, tables, or other sources present in each manuscript. Using this gene expression data, we complied four gene sets that were used in downstream analyses: (i) the complete list of all differentially expressed genes, (ii) genes down-regulated by high $p$CO$_2$ seawater, (iii) genes up-regulated by high $p$CO$_2$ seawater, and (iv) genes differentially expressed in two or more studies.

To generate the list of genes differentially expressed in at least two studies, we first assigned all differentially expressed genes to GLEAN gene models (e.g., SPU_X-XXXXX) that were used to annotate the *S. purpuratus* genome (as described in Oliver et al., 2010; e-scores <0.001). This conversion step allowed us to use a common set of identifiers throughout our analyses and was necessary because the software used to determine intersection of gene sets requires an exact character match in the chosen identifier (Oliveros, 2007). Gene names could not be used to determine intersection because annotations of the same gene

### Table 1

<table>
<thead>
<tr>
<th>Publication</th>
<th>Species</th>
<th>Life stage</th>
<th>$p$CO$_2$ (μatm)</th>
<th>Method of detection$^1$</th>
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<tr>
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<td><em>Strongylocentrotus purpuratus</em></td>
<td>gastrula, echinoplutei</td>
<td>435, 813, 1255</td>
<td>microarray (28,036 genes)</td>
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<td><em>Strongylocentrotus purpuratus</em></td>
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<td>380, 1000, 1350</td>
<td>qPCR (7 genes)</td>
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<td>Kurihara et al. (2012)</td>
<td><em>Hemicentrotus pulcherrimus</em></td>
<td>gastrula, prism, pluteus</td>
<td>380, 1000, 2000</td>
<td>qPCR (3 genes)</td>
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<tr>
<td>Martin et al. (2011)</td>
<td><em>Paracentrotus lividus</em></td>
<td>1-, 2- and 3-day embryos</td>
<td>Experiment 1: 397, 743, 1188, 1962, 3555, 6632, Experiment 2: 412, 704, 1161, 1996, 3624, 6590</td>
<td>qPCR (6 genes)</td>
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<td>O’Donnell et al. (2010)</td>
<td><em>Lytechinus pictus</em></td>
<td>echinoplutei</td>
<td>380, 540, 970</td>
<td>microarray (1057 genes)</td>
</tr>
<tr>
<td>Padilla-Gaminó et al. (2013)</td>
<td><em>Strongylocentrotus purpuratus</em></td>
<td>gastrula, echinoplutei</td>
<td>400, 1100</td>
<td>microarray (28,036 genes)</td>
</tr>
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<td>Stumpp et al. (2011a)</td>
<td><em>Strongylocentrotus purpuratus</em></td>
<td>2, 4, 7 days past fertilization</td>
<td>399, 1318</td>
<td>qPCR (27 genes)</td>
</tr>
<tr>
<td>Todgham and Hofmann (2009)</td>
<td><em>Strongylocentrotus purpuratus</em></td>
<td>early prism</td>
<td>380, 540, 1020</td>
<td>microarray (1057 genes)</td>
</tr>
</tbody>
</table>

$^1$ Number of genes assayed in parenthesis.
$^2$ Collected from Fogarty Creek, Oregon.
$^3$ Collected from Santa Barbara, California.
$^4$ Collected from Newport Beach, California.
often use slightly different nomenclature (e.g., sodium/potassium transporting ATPase alpha subunit vs. Na\(^+\)/K\(^+\)-ATPase subunit alpha). GLEAN gene model identifiers were listed in Todgham and Hofmann (2009), Hammond and Hoffman (2012), Evans et al. (2013), and Padilla-Gamino et al. (2013). GLEAN identifiers were also listed in the transcriptomic study of *Lytechinus pictus*, as a microarray containing *S. purpuratus* sequences was used (O’Donnell et al., 2010). For the remaining three studies, GenBank sequence identifiers listed in Kurihara et al. (2012), Martin et al. (2011), and Stumpp et al. (2011a) were used in a Basic Local Alignment Search Tool (BLAST) search to annotate genes differentially expressed in *Hemichordus pulcherrimus*, *Paracentrotus lividus*, or *S. purpuratus* with GLEAN identifiers, respectively. We used the online program Venny (Oliveros, 2007) to identify overlapping GLEAN identifiers across the eight studies. Only genes with identical GLEAN gene model identifiers were considered the same gene.

**Gene functional annotation and gene set enrichment analysis**

Gene functional annotation and gene set enrichment analyses (GSEA) were performed on each of the four lists to resolve the biological significance of genes regulated by seawater pCO\(_2\) in sea urchins. Gene functional annotation serves to organize lists of genes into functionally related groups called ontologies, which assists in defining the range of molecular, cellular and physiological processes regulated by seawater acidification. To do so, each differentially expressed gene was assigned a UniProt identifier using GLEAN models to retrieve corresponding RNA sequences from the *S. purpuratus* transcriptome, ver. 3.0 (Cameron et al., 2009). These sequences were then used in a BLAST search of the Uniprot database (UniProt Consortium, 2014) to retrieve corresponding UniProt identifiers for each gene (http://www.uniprot.org/blast/). Finally UniProt identifiers were used to assign each gene to one or more ontologies using the PANTHER Database, ver. 8.0 (Thomas et al., 2003, 2006). Three different ontology databases within PANTHER were used: (i) Gene Ontology: Biological Process, (ii) Gene Ontology: Molecular Function, and (iii) PANTHER Protein Class. Integrating information across these three databases provided a method to validate results between databases and more accurately identify processes strongly affected by seawater pCO\(_2\).

UniProt identifiers and the PANTHER Database were also used to perform GSEA (Thomas et al., 2003; Thomas et al., 2006; Mi and Thomas, 2009). GSEA is a statistical tool that uses gene functional information to identify categories of genes significantly over- or under-represented within a user-defined list. Significance using the PANTHER database is determined by the binomial statistic; that is, the probability that the number of genes observed from a given ontology occurred by chance relative to the total number of genes from this ontology present in the entire *S. purpuratus* transcriptome. The same three PANTHER ontology databases were again used to summarize results. Following PANTHER recommendations, we considered ontologies with Bonferroni false-discovery rate corrected *P*-values < 0.05 to be significant (Thomas et al., 2006). A separate GSEA was performed on gene sets that excluded five genes identified as differentially expressed using qPCR (but not also differentially expressed in one of the four microarray studies), as these genes were not selected randomly and may bias results. Results of the GSEA excluding the qPCR genes are available in the supplementary material (http://www.biobull.org/content/supplemental/) for comparison. However, interpretation of results focuses on analyses performed using all genes.

An alternative approach was taken to investigate the impact of seawater pCO\(_2\) on the expression of biomineralization genes. Genes involved in urchin biomineralization are very well-characterized within the scientific literature (Oliveri et al., 2002; Livingston et al., 2006), but annotate poorly in functional databases like PANTHER due to the absence of orthologous proteins in organisms used to populate the databases. As a result, functional analyses using these databases are not appropriate for determining whether a taxa-specific process such as biomineralization is strongly affected by seawater acidification. To circumvent this issue, we used an urchin-specific list of 345 biomineralization genes used previously by Pespeni et al. (2013a) and searched for overlap between this list and the list of differentially expressed, down-regulated, up-regulated, and overlapping genes defined in this study. A Fisher’s exact test was then used to determine whether the proportion of genes involved in biomineralization from each list was higher than expected given the total number of biomineralization genes encoded in the *S. purpuratus* genome.

**Results**

**All genes regulated by seawater acidification in urchins**

Our database search yielded eight manuscripts reporting expression changes in response to elevated seawater pCO\(_2\) among four different species of sea urchin (Table 1). A total of 602 genes were considered significantly differentially expressed across these eight studies (Fig. S1) (http://www.biobull.org/content/supplemental/). The vast majority of differentially expressed genes originated from the four studies that used high-throughput microarray-based approaches (*n* = 594), while the remaining eight were obtained from studies using candidate gene approaches and qPCR. During exposure to acidified seawater, 483 genes were down-regulated (80%) while only 134 (22%) were up-regulated. Interestingly, 15 differentially expressed genes exhibited...
conflicting directions of change between different studies (Fig. S1). Forty-three of the 602 genes differentially expressed during ocean acidification in urchins were also found within the urchin-specific list of biomineralization genes, including 10 spicule matrix proteins and three isoforms of carbonic anhydrase (Table 2).

We next used gene functional annotation to determine the complete range of biological processes regulated by seawater acidification in sea urchins. We were able to functionally annotate 591 of the 602 differentially expressed genes to ontologies associated with 17 major biological processes (with most genes annotating to more than one functional category) (Fig. 1). While genes with a variety of functions were differentially expressed, genes involved in metabolism were by far the most numerous, with 251 genes (42% of total) annotating to the “metabolic process” ontology within the PANTHER Biological Process database. Given the very large number of genes contributing to this ontology, we also examined the number of genes annotating to finer-scale ontologies within the “metabolic process” parent ontology (Fig. 2A). Genes involved protein (n = 104; e.g., dipeptidase 1, 26S protease regulatory subunit 6A, matrix metalloproteinase-17); nucleic acid (n = 91; e.g., ribonuclease T2, DNA helicase INO80); carbohydrate (n = 44; e.g.,
lactate dehydrogenase, pyruvate dehydrogenase, citrate synthase, succinate dehydrogenase, isocitrate dehydrogenase); lipid \((n = 30): \text{e.g., elongation of very long chain fatty acids protein 6, long-chain-fatty-acid–CoA ligase 1}\); and amino acid \((n = 25): \text{e.g., L-asparaginase, cysteine dioxygenase type 1, cysteine/glutamate transporter}\) metabolism were all differentially expressed in large numbers. The next most frequent ontology was “cellular process” containing 149 genes (25% of total) (Fig. 2B). The cellular process ontology includes genes involved in cell communication \((n = 102): \text{e.g., MAP kinase-activated protein kinase 5}\); cell adhesion \((n = 39): \text{e.g., integrin alpha-4, protocaherin-12}\); cell motion \((n = 29): \text{e.g., alpha and beta tubulin}\); and the cell cycle \((n = 47): \text{e.g., cyclin-dependent kinase 1}\). The ontology containing the third largest number of genes was “transport” containing 112 genes (19% of total) involved in the transport of ions \((n = 54): \text{e.g., sodium/potassium-transporting ATPase subunit alpha}\); amino acids \((n = 11): \text{e.g., proton-coupled amino acid transporter 1}\); lipids \((n = 6): \text{e.g., apolipoprotein D}\); nucleotides \((n = 6): \text{e.g., ADP/ATP translocase 3}\); vitamins \((n = 9): \text{e.g., sodium-dependent vitamin C transporter}\); and other molecules (Fig 2C).

While functional annotation is an appropriate method to identify the full range of biological processes affected by seawater acidification, these analyses do not consider the relative proportions of genes changing within specific ontologies. For example, the \textit{S. purpuratus} genome encodes for many more genes annotating to metabolic ontologies than to other ontologies; consequently, it may be expected that a large number of metabolic genes will respond to seawater acidification simply due to their abundance in the genome. To resolve this issue and identify processes most strongly impacted by seawater acidification in urchins, we performed GSEA, which identifies ontologies where the number of contributing genes are in significantly higher proportion relative to their total number in the \textit{S. purpuratus} genome. Using GSEA we identified two biological processes most strongly influenced by \(p\text{CO}_2\) in sea urchins: ion 242 T. G. EVANS AND P. WATSON-WYNN

Figure 1. Functional annotation of genes regulated by seawater \(p\text{CO}_2\) in sea urchins. The number of differentially expressed genes annotating to major ontologies using the Gene Ontology: Biological Process database. Blue, all differentially expressed genes; red, down-regulated genes; green, up-regulated genes; purple, genes differentially expressed in two or more studies.
transport and metabolism (Table 3). Ontologies associated with ion transport were the most numerous and had the lowest \( P \)-values in the GSEA. Significantly over-represented ontologies relating to ion transport included “ion transport,” “cation transport,” and “transport” from the Biological Processes database; “transporter activity,” “transmembrane transporter activity,” and “cation transmembrane transporter activity” from the Molecular Function database; and “transporter” and “cation transporter” from the Protein Class database. A large number of ontologies clearly linked to metabolic changes were also significantly over-represented (Table 3). These data indicate that the metabolic genes present in the functional annotation were not simply the result of a larger number of metabolic genes in the *S. purpuratus* genome. Significantly over-represented metabolic ontologies from the Biological Process database included “generation of precursor metabolites and energy,” “carbohydrate metabolic process,” “oxidative phosphorylation,” “respiratory electron transport chain,” and “tricarboxylic acid cycle.” A significantly higher proportion of genes involved in biomineralization were differentially expressed in urchins exposed to high \( pCO_2 \) seawater than would be expected based upon the number of biomineralization genes encoded in the *S. purpuratus* genome (Fisher’s exact test \( P < 0.001 \); Fig. S2; http://www.biolbull.org/content/supplemental/).

**Genes down-regulated by seawater acidification in urchins**

We performed similar analyses on the set of genes down-regulated by \( pCO_2 \) to identify biological processes repressed by seawater acidification. Since input gene lists for down-regulated genes and all differentially expressed genes were largely the same (483 of 602 genes, or 80% similarity), functional annotation of down-regulated genes mirrored that
of the complete list, with genes annotating most frequently to “metabolic process” (n = 194), “cellular process” (n = 114), and “transport” (n = 100) ontologies (Fig. 1). The same trend was apparent in the GSEA performed on down-regulated genes, with the majority of significantly over-represented ontologies functioning in ion transport and metabolism (Figs. S3, S4; http://www.biolbull.org/content/supplemental/). Thirty-eight down-regulated genes are involved in urchin biomineralization, including seven spicule matrix proteins and two carbonic anhydrases (Fig. S2). This is again a significantly higher proportion than would be expected based upon the number of biomineralization genes encoded in the *S. purpuratus* genome (Fisher’s exact test *P* < 0.001).

**Genes up-regulated by seawater acidification in urchins**

We next performed analyses on the 134 up-regulated genes as a means to resolve processes activated in response to seawater acidification. Far fewer ontologies were significantly over-represented among genes up-regulated as compared to genes down-regulated by seawater acidification, a function of the much lower number of input genes (134 up-regulated genes compared to 483 down-regulated genes). However, four ontologies were significantly over-represented among the set of up-regulated genes: “cellular calcium ion homeostasis” and “homeostatic process” from the Biological Process database, “cation transmembrane transporter activity” from the Molecular Function database, and “cation transporter” from the Protein Class database (Fig. S3). Despite the induction of many genes involved in metabolism, ontologies with apparent metabolic functions were not significantly over-represented among up-regulated genes. Nine of the 134 genes up-regulated during ocean acidification in urchins were involved in biomineralization (Fig. S2), which again was a significantly higher proportion than expected by chance (Fisher’s exact test *P* <0.001).

**Genes regulated by seawater acidification in more than one study**

A total of 26 genes were common to at least two studies, five genes were differentially expressed in at least three studies, and one gene, Na\(^+\), K\(^-\)-ATPase alpha subunit, was

<table>
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<tr>
<th>Biological Process</th>
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**Table 3**

Significantly over-represented metabolism and ion transport ontologies among the set of all differentially expressed genes
differentially expressed in four studies. No genes were found to intersect in more than four studies (Fig. S1). Strikingly, 25 of the 26 overlapping genes were down-regulated (94%), while only spicule matrix protein SM30 was induced by seawater acidification in multiple studies. Genes annotating to metabolic and ion transport ontologies were most frequent among the 26 overlapping genes: 10 genes annotated to “transport,” including the Na$^{+}$, K$^{+}$-ATPase alpha subunit and H(+)-transporting ATPase beta subunit; and seven genes annotated to “metabolic process,” including succinyl-CoA synthetase alpha subunit and cytochrome c oxidase subunit IV precursor (Fig. 1). GSEA revealed that ontologies associated with ion transport and metabolism were significantly over-represented, and several ontologies also over-represented in the complete list of differentially expressed genes were present, including “oxidative phosphorylation,” “generation of precursor metabolites and energy,” “respiratory electron transport chain,” “cation transport,” and “cation transmembrane transporter activity” (Table 4). “Cellular calcium ion homeostasis” was the only ontology significantly over-represented in both the set of overlapping genes and the set of up-regulated genes. We did not perform separate GSEA on subsets of up- or down-regulated genes from within the 26 overlapping genes given the low number of up-regulated genes in this set ($n = 1$) and the high degree of similarity between the set of down-regulated genes and all overlapping genes (25 of 26 genes). Seven of the 26 genes (39%) common to at least two studies intersected with the list of urchin biomineralization genes, including H(+)-transporting ATPase beta subunit and msp130 protein (i.e., spicule matrix protein 130) (Fig. S2). Once again, this represents a significantly higher proportion than would be expected by chance (Fisher’s exact test $P < 0.001$).

### Genes not strongly affected by seawater acidification

We were also interested in determining the effect of seawater acidification on genes involved in development and the cell stress response. Exposure to elevated $p$CO$_2$ negatively affects development in marine embryos and larvae, including echinoderms (Dupont and Thorndyke, 2009; Dupont et al, 2010). We hypothesized that impaired development caused by exposure to seawater acidification in urchins would alter the expression of developmentally regulated genes, and in turn, these genes would be detected as differentially expressed when compared to expression levels in urchins raised under control conditions. Seventy-nine genes regulated by seawater acidification in urchins functionally annotated to the “developmental process” ontology (Fig. 1). However, GSEA showed that ontologies relating to development were not significantly over-represented among any of the four gene sets analyzed (Fig. S3).

As organisms approach environmental tolerance limits, a conserved set of genes functioning to repair and prevent cellular damage are induced (Kültz, 2003, 2005; Petrak et al., 2008; Wang et al., 2009a; Evans and Hofmann, 2012). Little evidence for cellular damage or the induction of a cellular stress response was apparent in the urchin expression data. Most notably, 80% of genes affected by seawater acidification were down-regulated, a pattern that does not support the induction of genes involved in the cellular stress response. The set of up-regulated genes was not significantly over-represented for ontologies with apparent functions in the cellular stress response, and there was very limited evidence that cellular function was severely compromised. Four genes indicative of macromolecular damage were induced: heat shock protein 40, DNAJb11 (molecular chaperones involved in re-folding damaged proteins; Feder and Hofmann, 1999), ubiquitin (which targets irreparable

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<th>Bonferroni Corrected $P$-Value</th>
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proteins for destruction; Dutton and Hofmann 2008; Hofmann and Somero 1995), and caspase activation and recruitment domain 15 (part of the signaling cascade that ultimately causes apoptosis; Festjens et al., 2006) (Fig. S1).

Discussion

Inherent problems with comparing changes in gene expression caused by ocean acidification

As ecologically and economically important marine calcifiers, sea urchins have emerged as ideal models for studying the effects of ocean acidification. Many researchers have exploited nucleic acid sequence resources provided by the Strongylocentrotus purpuratus genome to undertake studies that monitor shifts in gene expression triggered by exposure to acidified seawater. However, much like studies of ocean acidification in general, differences in experimental methods, target species or populations, and statistical approaches make direct comparison of results problematic. Comparisons of gene expression data between studies are a necessity to properly relate new results to that of previous work, but most of these comparisons are made in the context of major caveats that reduce their impact. For example, four studies analyzing changes in gene expression caused by seawater acidification in urchins used qPCR to evaluate shifts in mRNA abundance for a small number of genes (Table 1). Enhanced sensitivity of qPCR compared to alternative approaches like microarrays could lead to genes being reported as differentially expressed in one study, but unchanged in another (Wang et al., 2006). The trade-off associated with qPCR-based inquiry is that while sensitive, expression can only be tracked across a small number of pre-selected genes, which prevents discovery of novel genes with potentially important roles in the response to seawater acidification. Four other studies used higher-throughput transcriptomic approaches to examine genes regulated by seawater pCO2 in urchins (Table 1). These studies eliminate biases associated with gene selection and are capable of monitoring expression across the transcriptome, but are themselves associated with methodological discrepancies that make comparing gene lists challenging. For example, O’Donnell et al. (2010) used a S. purpuratus microarray platform to track acidification-induced changes in gene expression in the painted urchin, Lytechinus pictus. This approach, termed heterologous hybridization, limits the number of genes in which expression data can be acquired because L. pictus mRNAs will not bind strongly to divergent S. purpuratus sequences spotted on the array (Renn et al., 2004; Buckley, 2007). Statistical approaches to analyzing high-throughput and candidate gene expression also differ dramatically. Whereas a small number of genes can be assessed using standard statistical approaches, analyzing high-throughput expression data requires more specialized techniques. In most cases, arbitrary filtering criteria are applied to high-throughput datasets to reduce the number of genes used in downstream statistical tests (Tseng et al., 2001; Hackstadt and Hess, 2009), with the consequence of ignoring genes with low levels of expression but potentially important roles in the response to ocean acidification. For example, both microarray and RNA-sequencing datasets are often filtered by removing genes whose fluorescence intensity or sequence count, respectively, is below a certain threshold in all samples. Consequently, genes are often eliminated from analyses due to low expression in a single replicate. Importantly, low expression in one sample could reflect minor technical discrepancies in sample preparation or processing just as easily as it could a biological response or lack thereof. The emergence of RNA-sequencing technology provides enhanced sensitivity compared to other transcriptomic techniques such as microarrays (Wang, Z., et al., 2009), but seems to result in the identification of a far greater number of significantly differentially expressed genes compared to microarray experiments (Sirbu et al., 2012). This difference in sensitivity is a potentially important issue considering that the number of differentially expressed genes is often used as an indicator of how strongly an organism is affected by environmental change.

Meta-analyses have been used successfully to resolve broader-scale trends within the sometimes contradictory scientific literature of ocean acidification (Ries et al., 2009; Kroeker et al., 2010, 2013; Hendriks et al., 2010), and here we performed a meta-analysis of eight studies monitoring gene expression in sea urchins in order to resolve key questions surrounding the effects of ocean acidification. A central goal was to determine whether gene expression data collected to date support five prevailing hypotheses about the effects of ocean acidification on echinoderms. Gene expression profiles in urchins strongly support that exposure to high pCO2 seawater will depress metabolism, impact biomineralization, and disrupt pH homeostasis. Expression patterns also provide inconclusive evidence that ocean acidification slows developmental rate, and little evidence that short-term exposure to future ocean pH regimes will exceed organism tolerance limits.

The pervasive effects of ocean acidification

An important conclusion from this study is that the impact of ocean acidification on urchins extends beyond biomineralization and pH regulation. Functional annotation of 591 genes regulated by seawater pCO2 indicates that ocean acidification is a pervasive environmental variable that affects a wide range of biological phenomenon not typically associated with it, from reproduction to cell adhesion to the immune response (Fig. 1). Mechanistic bases for susceptibility or resilience toward future acidification could lie within these largely overlooked processes, and urchin expression data underscore the need to evaluate a wider
Range of biological processes in future studies. Trends in the scientific literature imply that the ocean acidification research community has already reached a similar conclusion and is actively exploring the consequences of seawater pCO₂ on processes outside calcification and pH regulation. For example, a link between ocean acidification and immune function has been demonstrated repeatedly (Henroth et al., 2011, 2012; Matozzo et al., 2012), including a recent study in the green sea urchin, *S. droebachiensis*, that suggests a direct link between extracellular pH and phagocyte numbers (Dupont and Thorndyke, 2012). Byssal threads used to anchor mussels to rocks within the intertidal are weakened and compromise attachment to substratum when secreted in more acidic seawater (O’Donnell et al., 2013), data that provide at least some indication as to why the expression of genes encoding adhesive proteins may be modified by ocean acidification in sea urchins. However, mechanistic understanding of these newly discovered responses is sparse, and questions surrounding the nature of pCO₂-regulated changes in, for example, cell communication, are at this point speculative. These unanswered questions illustrate that searching for impacts beyond calcification and pH regulation will continue to be an important step in understanding the consequences ocean acidification.

**Biological processes most strongly affected by ocean acidification**

The expression of a disproportionate number of genes involved in metabolism, ion transport, and biominalization were modified by seawater acidification in sea urchins, providing clear evidence that these three processes will be most strongly affected by changes in ocean pCO₂. This conclusion is congruent with well-established outcomes regarding the physiological impact of ocean acidification on marine organisms—specifically, that exposure to high pCO₂ seawater depresses metabolism (Pörtner, 2008), triggers compensatory movements of ions across cell membranes (Stumpf et al., 2012), and influences biominalization (Kroeker et al., 2010; Byrne et al., 2013). These conclusions validate gene expression as an accurate method to assess responses to shifting environmental conditions and endorse the use of expression-based approaches in future ocean acidification research.

**Metabolic depression.** Eighty percent of the genes differentially expressed in sea urchins exposed to acidified seawater were down-regulated, suggesting that most biological processes were repressed. By far the largest number of down-regulated genes annotated to the “metabolic process” ontology, including genes participating in the metabolism of carbohydrates, lipids, proteins, and nucleic acids (Figs. 1, 2A). These data strongly suggest that future ocean acidification will have an inhibitory effect on the metabolism of sea urchins. Results of the GSEA suggest that elevated seawater pCO₂ triggers a decrease in the production of ATP by down-regulating the expression of genes involved in the tricarboxylic acid cycle, electron transport chain, and oxidative phosphorylation. Significantly over-represented ontologies among the set of down-regulated genes included “respiratory electron transport chain,” “tricarboxylic acid cycle,” and “oxidative phosphorylation” (Fig. S3; http://www.biolbull.org/supplemental/). The importance of modulating these ATP-producing pathways is highlighted by the differential expression of succinyl CoA synthetase, which catalyzes the conversion of succinyl CoA to succinate as part of the tricarboxylic acid cycle (Li et al., 2013); and cytochrome c oxidase subunit IV, the last enzyme in the electron transport chain (Arnold, 2012). These two genes were among only 26 others significantly differentially expressed in two studies. Furthermore, these metabolic pathways are closely connected, as the tricarboxylic acid cycle supplies precursor metabolites to the electron transport chain, and the electron transport chain is a fundamental component of ATP production via oxidative phosphorylation (Leninger, 1999).

ATP production is tightly coupled to organism energy demands, thus a decline in ATP production is an indication that urchins either exhibit a reduced physiological demand for ATP in high pCO₂ seawater or strategically suppress metabolism as a means to extend the duration of tolerance until more favorable conditions return (Todgham and Hofmann, 2009). We hypothesize that the down-regulation of genes involved in ATP production reflects metabolic depression rather than declining ATP requirements in more acidic seawater. Adaptive responses to the environment are energetically expensive endeavors that require large amounts of ATP to fuel processes such as protein synthesis (Kültz, 2003, 2005). The ATP-dependent processes involved in acclimating to changes in seawater pCO₂ are unlikely to be mediated by down-regulating genes involved in the cell’s primary ATP production pathways (i.e., oxidative phosphorylation). Alternatively, metabolic depression occurs through the coordinated suppression of ATP-generating pathways (Guppy and Withers, 1999) and has been observed in diverse organisms exposed to acidified seawater, including corals (Nakamura et al., 2011), sipunculids (Reipschläger and Pörtner, 1996), mussels (Michaelidis et al., 2005), crabs (Carter et al., 2013), and scallops (Schalkhauser et al., 2012). Metabolic depression is considered an adaptive strategy that allows organisms to temporarily extend the duration of tolerance by inhibiting energetically expensive processes (Fabry et al., 2008). In purple sea urchins, the species of choice for five of the eight studies analyzed here, temporary metabolic depression may promote survival during episodic upwelling events that expose nearshore environments in the northeast Pacific to transient declines in seawater pH that can reach as low as
7.4 (Evans et al., 2013). However, metabolic depression provides only short-term relief from the negative effects of seawater acidification, and the use of this strategy to cope with permanently high pCO2 seawater is unlikely. Vital metabolic functions cannot be repressed over the long-term without negative consequences for growth and development, and the obvious trend toward metabolic depression in urchins raises uncertainty as to whether these organisms have the capacity to acclimatize to oceans permanently high in pCO2. The potential vulnerability of urchins to conditions expected in future oceans is supported by their reduced abundance in the low pH marine ecosystems surrounding volcanic CO2 vents (Hall-Spencer et al., 2008; Kroeker et al., 2011) and by the fact that urchins are considered to be among the poorest acid-base regulators (Calosi et al., 2013). However, caution should be exercised in extrapolating trends observed in short-term experiments, like those evaluated here, to chronically high pCO2 oceans. The difficulty in implementing long-term ocean acidification experiments has resulted in a paucity of data on the capacity of marine populations to acclimate to long-term increases in seawater acidity. The potential for misstep in using brief exposures to infer long-term survival in future oceans is illustrated in a study of the presumably vulnerable cold-water coral Lophelia pertusa, which exhibited a decline in calcification during a one-week exposure to low pH seawater, but was actually able to increase calcification rates over the course of a 6-month exposure (Form and Riebesell, 2012). The physiological response to seawater acidification also differed temporally over the course of a 2-month exposure in the mussel Mytilus edulis (Thomsen and Melzner, 2010). More long-term studies will be necessary to determine whether urchins can reverse metabolic depression and acclimatize to permanently high pCO2 conditions. Discretion is also needed when using contemporary genotypes to assess fitness in future oceans. Accumulating evidence suggests that purple sea urchins will be capable of rapidly adapting to more acidic oceans (Kelly et al., 2013; Pespeni et al., 2013b). In this case evolution, rather than phenotypic plasticity, may allow urchin populations to persist in a more acidic ocean.

**Ion transport.** Ion transport was the second most strongly affected biological process in sea urchins exposed to low pH seawater. One quarter of the genes that could be functionally annotated were associated with ion transport (Figs. 1, 2C), and both the up-regulated and down-regulated gene sets were significantly over-represented for ontologies relating to the movement of ions across cell membranes (Table 3, Fig. S3). Differential expression of ion transporters is likely a function of pH regulation. Although urchins are considered poor acid-base regulators (e.g., Calosi et al., 2013), accumulating evidence suggests that they possess some capacity to compensate for decreases in extracellular pH. Larval green sea urchins (congeners of S. purpuratus) compensate for acid-base imbalance with a bicarbonate buffering system that uses energy derived from transmembrane sodium gradients to export hydrogen ions (Stumpf et al., 2012). A total of 15 genes involved in the transport of sodium were differentially expressed in response to high pCO2 seawater in urchins, including a K+-dependent Na+, Ca2+ exchanger, subunits of the Na+/K+-transporting ATPase, and two Na+ channels (Fig. S1; http://www.biobull.org/supplemental/). An important trend regarding the expression of transport proteins is that despite the very strong signature of metabolic depression, urchins up-regulated several ATP-dependent ion transporters. Both the alpha-3 and beta-1 subunits of the sodium/potassium-transporting ATPase and the H(+-)-transporting ATPase beta subunit increased in expression during exposure to acidified seawater. In fact, the alpha subunit of the sodium/potassium-transporting ATPase was the only gene deemed significantly differentially expressed in four studies (Fig. S1). The transmembrane movement of ions is metabolically expensive and can consume up to 77% of larval metabolism in sea urchins (Leong and Manahan, 1997). Induction of energy-demanding ion transporters within an overall framework of metabolic depression underscores the importance of ion transport in maintaining homeostasis in high pCO2 seawater. Although sodium gradients may fuel acid-base regulation, the actual transmembrane movement of bicarbonate and hydrogen ions that will ultimately help restore pH homeostasis primarily depends on two genes: the Na+/H+ and Cl-/HCO3- exchangers (Wheatly and Henry, 1992). The expressions of these transport proteins did not change in any of the eight studies analyzed here, indicating that although modifying the abundance of sodium transporters is potentially important to pH regulation, the actual movement of protons and bicarbonate required to restore acid-base homeostasis may rely solely on changes in the activity of these transporters rather than on increasing abundance.

**Biomineralization.** Biomineralization is highly sensitive to the changes in seawater chemistry expected with continued ocean acidification (Kroeker et al., 2010), and increases in seawater pCO2 have been shown to reduce calcification rates in a wide range of calcifying organisms, including urchins (Shirayama and Thornton, 2005; Ries et al., 2009; Byrne et al., 2011; Yu et al., 2011, 2013; Courtney et al., 2013). Larval urchins secrete fragile but functionally important skeletons, and consequently, the impact of ocean acidification on skeletogenesis in early-life-stage urchins has received considerable attention. Indeed, all eight of the studies analyzed here chose to investigate early life stages (Table 1). Stunted growth and impaired biomineralization appears to be a nearly universal response to future levels of seawater acidification in early-life-stage urchins: significant reductions in the length of the larval arms or supporting
skeletal rods have been documented across 13 species from tropical, temperate, and polar habitats (Byrne et al., 2013). An advantage to using gene expression in assessing biomineralization in urchins is that gene networks underlying skeletogenesis are extremely well characterized (Oliveri et al., 2002; Livingston et al., 2006). The ubiquitous effect of $pCO_2$ on biomineralization in larval urchins is consistent with patterns of gene expression resolved in this study. The complete set of differentially expressed genes (43 biomineralization genes of 602 differentially expressed), the set of up-regulated (9 biomineralization genes of 134 up-regulated genes), the set of down-regulated (38 biomineralization genes of 483 down-regulated genes), and the set of overlapping genes (7 biomineralization genes of 26 overlapping genes) all contained significantly higher proportions of genes involved in biomineralization relative to the total number of biomineralization genes encoded by the S. purpuratus genome (Fig. S2; http://www.biolbull.org/supplemental/). Thirty-eight of the 43 biomineralization genes differentially expressed in response to acidified seawater in urchins were down-regulated, a result that supports skeletogenesis being impaired.

Mechanistically, reduced biomineralization may be linked to insufficient abundance of spicule matrix proteins, critical components of the urchin endoskeleton that participate exclusively in calcification (Wilt, 2002) and function to bind and sequester Ca$^{2+}$ ions before deposition into the endoskeleton (Farach-Carson et al., 1989). Seven of the 10 differentially expressed spicule matrix proteins were down-regulated, including members of the spicule matrix protein 16, 29, 30, 50, and 130 gene families (Table 2). We hypothesize that impaired calcification, down-regulation of spicule matrix proteins, and decreased expression of other genes involved in biomineralization are occurring as part of an overall metabolic depression in urchins. This hypothesis is congruent with the predominant effect of $pCO_2$ on the expression of metabolic genes, and several studies have suggested that impaired urchin skeletogenesis in acidified seawater is primarily an energetic problem rather than a function of reduced carbonate saturation in seawater. Intracellular pH regulation is an energy-demanding process and therefore consumes resources that would normally be directed toward skeletogenesis, with negative consequences for skeletal development (Stumpp et al., 2011a; Byrne et al., 2013).

A reduced ability to calcify in more acidic seawater reinforces the vulnerability of urchins in future oceans (Wittmann and Pörtner, 2013). Larval skeletons are functionally important for feeding and swimming, and while the degree to which biomineralization is affected between species and habitat types varies (Byrne et al., 2013), even seemingly small impacts on skeletogenesis can reduce the fitness of urchin embryos and larvae. Food capture in larval urchins depends upon the surface area of ciliary bands that surround the larval arms. A 7% reduction in band length translates to a 16% reduction in feeding rates in the echinoid sand dollar Dendraster excentricus (Strathmann, 1971). The ability of urchin larvae to disperse could also be affected by small changes in skeletal morphology that negatively affect swimming performance (Pennington and Strathmann, 1990; Strathmann and Grunbaum, 2006). Reduced skeletogenesis may also influence ecosystem-level processes, as the inability of larval urchins to construct their skeleton may leave them at a greater risk for predation (Allen, 2008) and may even disturb energy transfer across trophic levels if decreased calcification translates into an overall decrease in larval size (Sheridan and Bickford, 2011).

The set of 125 up-regulated genes was not significantly over-represented for metabolic ontologies, further supporting the inhibitory effect of increasing seawater $pCO_2$ on urchin metabolism. Alternatively, genes up-regulated in response to acidified seawater were significantly over-represented for ontologies relating to calcium homeostasis, including several calcium transporters (e.g., similar to voltage-dependent T-type calcium channel) and binding proteins (e.g., calmodulin-like protein 5). Shifts in the expression of genes that modify the availability of calcium may represent an adaptive response to promote calcification in a carbonate-limited environment (Evans et al., 2013). Seawater is the ultimate source of calcium incorporated into urchin skeletons, and large amounts of calcium must first be imported via calcium transporters before being deposited into the skeleton (Wilt, 2002). Evidence for this potential function is strengthened by similar transcriptomic responses to high $pCO_2$ seawater in the corals Acropora millepora and Pocillopora damicornis (Moya et al., 2012; Vidal-Dupiol et al., 2013). Both studies reported increases in the abundance of calcium transporters, and both predicted that these expression changes were related to calcification, either directly via an increase in the amount available for calcification (Vidal-Dupiol et al., 2013) or indirectly through the alteration of calcium-dependent signaling pathways (Moya et al., 2012). Although the connection between calcium transport and calcification is well-supported, the up-regulation of calcium transporters and binding proteins in the context of so many other biomineralization genes being down-regulated raises several questions. For example, why would urchins choose to up-regulate genes involved in calcium homeostasis rather than spicule matrix proteins or other genes involved in biomineralization? Also why, despite changes in the expression of calcium-related genes, is skeletogenesis still impaired in the majority of urchins tested? (Byrne et al., 2013). The purpose of inducing genes involved in calcium homeostasis is also confounding considering the clear evidence for metabolic depression in urchins exposed to high $pCO_2$ seawater and that the transport of calcium, like the transport of sodium and other ions, is
ATP-dependent. Uptake of calcium from seawater is temporally variable during early urchin development (Tellis et al., 2013), and differences in the life-stage sampled between each study could plausibly lead to the differential expression of genes controlling calcium import. An alternative speculation is that fluxes in calcium are related to intracellular signaling events of which calcium is a major player (Moya et al., 2012). Given that calcium transport appears to be regulated by seawater pCO2 in both urchins and corals, experiments addressing these uncertainties may be worth pursuing.

**Processes not strongly affected by ocean acidification**

Our analyses of gene expression were informative both in terms of identifying biological processes most commonly regulated by seawater pCO2 in sea urchins, and also in defining processes not strongly affected by seawater acidification. Functional annotation showed that ocean acidification is a pervasive stressor that affects a range of biological processes; however, genes involved in two processes commonly associated with the response to ocean acidification and environmental perturbation in general did not appear to be widely affected: development and the cellular stress response.

**Development.** Numerous studies have examined the developmental consequences of ocean acidification, with developmental delay reported to occur in a wide range of species including crustaceans, molluscs, and echinoderms (Dupont and Thorndyke, 2009). While development in high pCO2 seawater has been reported to cause developmental delay in urchins (Martin et al., 2011; Stumpp et al., 2011a, b), this response is not unequivocal (Byrne et al., 2013), and other studies have failed to detect an effect on embryonic (cell cycle progression, first mitotic division, onset of DNA synthesis, and mitotic spindle formation; Place and Smith, 2012) or larval development (prism and early pluteus stages; Padilla-Gamiño et al., 2013). The genetic underpinnings of sea urchin development have been extensively studied and involve the coordinated expression of genes within well-described developmental programs (Wei et al., 2006). Should ocean acidification slow development, the expression of developmentally regulated genes should also be delayed, causing these genes to be detected as significantly differentially expressed relative to those of urchins developing at normal rates in lower pCO2 seawater. We observed limited evidence for this response in the gene expression patterns analyzed here. Ontologies relating to development were not significantly over-represented among genes differentially expressed, suggesting that development is not among the processes strongly affected by seawater acidification. Seventy-nine genes regulated by seawater acidification in urchins annotated to the “developmental process” ontology, a result that does not refute a pCO2-mediated developmental delay in urchins. However, these shifts in gene expression could also be driven by compensatory responses within specific developmental pathways rather than by an overall developmental delay. Todgham and Hofmann (2009) suggest that the differential expression of key transcription factors involved in early developmental programs reflects an effect on gene networks regulating skeletogenesis rather than an overall developmental delay, and a similar mechanism was proposed by Evans et al. (2013) as a means for promoting calcification in carbonate-limited environments. Disentangling an overall developmental delay from effects on specific developmental pathways is likely not feasible using gene expression exclusively. More directed research will be required to determine which of these two possibilities, if either, is most prevalent in urchins.

**Cellular stress response.** Environmental stress levels that approach organism tolerance limits induce a specific set of proteins that repair and prevent further macromolecular damage; these include chaperones that re-fold denatured proteins, members of protein degradation pathways, and cell cycle regulators that arrest cell division and initiate programmed cell death (Kültz, 2003, 2005; Petrak et al., 2008; Wang, P., et al., 2009). While we acknowledge that gene expression is an indirect measure of short-term organism tolerance limits, genes comprising the cellular stress response are nonetheless often used for exactly this purpose (e.g., Lockwood and Somero, 2010; Tomanek, 2012). Expression profiles in urchins suggest that short-term exposure to near-future pCO2 regimes do not approach survivable limits and that the negative impacts of ocean acidification on urchins will more likely manifest through reductions in fitness (e.g., impaired calcification, fertilization, metabolic deficits). This conclusion is largely consistent with recent meta-analyses on the effects of near-future ocean acidification on urchins (Dupont et al., 2010; Byrne et al., 2013). However, experiments conducted in urchins to date present only a snapshot of life in future oceans and provide little information as to how these animals will cope with chronic exposure to high pCO2 seawater. Some urchin species are extremely long-lived and may experience a century worth of ocean acidification in a single lifetime (Ebert and Southon, 2003).

**Acknowledgments**

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