High pCO₂ affects body size, but not gene expression in larvae of the California mussel (Mytilus californianus)

Morgan W. Kelly¹*, Jacqueline L. Padilla-Gaminío², and Gretchen E. Hofmann³

¹Department of Biological Science, Louisiana State University, Baton Rouge, LA 70803, USA
²Department of Biology, California State University, Dominguez Hills, Carson, CA, USA
³Ecology, Evolution, and Marine Biology, University of California, Santa Barbara, Santa Barbara, CA, USA

*Corresponding author: tel: + 1 225 578 0224; fax: + 1 225 578 2597; e-mail: morgankelly@lsu.edu


Received 25 June 2015; revised 20 September 2015; accepted 23 September 2015.

Many studies have reported reductions in body size and calcification rates for marine larvae exposed to ocean acidification conditions. However, the physiological mechanisms driving these effects, and mechanisms underlying body size variation in general, are poorly understood. Here, we combine transcriptome sequencing with bulked segregant analysis to assess the physiological response to acidification in larvae of the California mussel, Mytilus californianus, and to explore physiological basis of variation in larval size. We reared three families of M. californianus larvae under ambient (~35 μatm, pH_total 8.1) and high (~1300 μatm, pH_total 7.6) pCO₂ conditions, then passed larvae through a mesh filter, separating each family × pCO₂ treatment into fractions of larvae with large vs. small body sizes. We sequenced larval mRNA for each family × treatment × body size combination, and assembled a de novo transcriptome. We then mapped reads from each library to this assembly to identify effects of high pCO₂ on gene expression, and to identify transcriptomic differences between small vs. large larvae of the same age class. Although larvae reared under elevated pCO₂ were smaller, we observed no consistent effect of elevated pCO₂ on gene expression. Nevertheless, 1225 transcripts, primarily related to metabolism, were differentially expressed between large vs. small larvae, regardless of CO₂ treatment. We conclude that the observed reduction in larval body size under high CO₂ may be driven by a direct effect of the environment on phenotype, unmediated by changes in gene expression. Because M. californianus has evolved in the context of seasonal upwelling, exposure to 1300 μatm pCO₂ may not produce the large stress-mediated effects on gene expression that might be expected for an organism exposed to conditions far outside those of its typical environment.

Keywords: bivalve, gene expression, global change biology, ocean acidification, physiology, RNA-seq, transcriptomics.

Introduction

Ocean acidification (OA) occurs through the absorption of atmospheric carbon dioxide, and reduces both ocean pH and the availability of free carbonate ions (CO₃²⁻) used by many marine invertebrates for calcification (Hofmann et al., 2010). Laboratory experiments across a variety of taxa have demonstrated negative effects of OA conditions, ranging from reductions in size and fecundity to increased mortality (Kroeker et al., 2010). However, relatively little is known about the underlying physiological mechanisms for these effects.

One of the most widespread consequences of exposure to high pCO₂ is a reduction in the size of developing larvae. This has been observed in taxa ranging from brittle stars (Dupont et al., 2008) to sea urchins (Kelly et al., 2013) and mussels (Gaylord et al., 2011), and may carry over into later life stages, long after exposure to high pCO₂ has ended (Hettinger et al., 2012). CO₂-driven reductions in larval size may be due to lower overall growth rates stemming from metabolic depression (Lannig et al., 2010; Seibel et al., 2012). Alternately, reductions in size may stem more specifically from reduced calcification (and, hence, reduced skeletal growth), driven by lower availability of carbonate ions and increased energetic cost of calcification (Watson et al., 2012; Waldbusser et al., 2013). One way to address competing hypotheses regarding the physiological effects of OA conditions is with gene expression data, which provide a global view of physiological responses (Evans and Hofmann, 2012; Whitehead, 2012), identifying which stressors...
(salinity, pH, temperature, etc.) have the biggest effects on physiology (Chapman et al., 2011), and which physiological processes are most affected. For example in sea urchins, exposure to high pCO2 led to down-regulation of spicule matrix 30 alpha protein, a major component of the larval skeleton, but not to metabolic depression (Padilla-Gaminho et al., 2013). This suggests that the observed reductions in growth rates were a specific consequence of reduced calcification rather than generalized metabolic depression. However, despite their utility in providing a comprehensive view of physiological responses, transcriptomic data in the context of OA conditions are available for a relatively small number of taxa (Moya et al., 2012; Evans et al., 2013; Harms et al., 2014; Vidal-Dupiol et al., 2014).

In this study, we sought to use whole transcriptome sequencing to identify physiological effects of OA conditions in larvae of the California mussel, *M. californianus*, and the molecular mechanisms underlying variation in larval size. The California mussel is a foundation species in the rocky intertidal zone of the eastern Pacific, creating the physical habitat used by a diverse assemblage of species (Suchanek, 1992). Previous work has shown that exposure to high pCO2 conditions produces *M. californianus* larvae with smaller, thinner shells that are more vulnerable to breakage (Gaylord et al., 2011), suggesting that the effect of low pH on the growth of larval shells may be a key bottleneck in the response of this species to future acidification.

Because of the taxonomically widespread effects of OA on larval size, we were also interested in mechanisms underlying body size variation in general, and whether the same mechanisms (e.g. reduced metabolism) underlie body size variation under ambient and high pCO2 conditions. To identify gene expression differences responsible for phenotypic variation in size among *Mytilus* larvae under ambient and high pCO2, we combined transcriptome sequencing with bulked segregant analysis (BSA). In BSA, DNA (or in this case mRNA) is pooled among individuals sharing a particular trait value (e.g. large body size), and compared against a pool of individuals sharing an opposing trait value (e.g. small body size), with the expectation that on average, allele frequencies (or in this case gene expression) will differ only for loci or transcripts linked to the focal trait (Michelmore et al., 1991).

We chose larval body size as the focal trait for this study, because declines in body size have been observed for *M. californianus* larvae and many other taxa in experiments simulating OA conditions (Kroeker et al., 2010; Gaylord et al., 2011). Larval size in marine invertebrates is tied to fitness through feeding rate and risk of predation (Allen, 2008), and reductions in body size have been an emergent response to climate change across multiple systems and stressors (Sheridan and Bickford, 2011). We also focused on size because effective use of BSA requires the accurate phenotyping of a large number of individuals, and so body size lends itself well to this type of study.

Here, we report the results of the first study to combine high throughput sequencing with BSA in the context of physiological responses to OA. We reared *M. californianus* under ambient and high pCO2 conditions in laboratory cultures, used filters to separate larvae into pools of different body sizes, and then sequenced pools of mRNA for each treatment × body size combination to identify effects of high pCO2 on gene expression, and gene expression differences between large and small larvae of the same age class. While we observed substantial gene expression differences between large and small larvae, we were unable to detect any consistent effect of high pCO2 on gene expression.

### Methods

#### Field collection and larval culture

We collected adult mussels from Campus Point, Goleta, CA (34.40°N; 119.84°W), and brought them back to the laboratory where they were maintained in flowing seawater at 15°C before spawning. To induce spawning, we scraped the mussels clean, and following Trevelyan and Chang (1983), immersed them in filtered seawater (FSW) with 30 mmol hydrogen peroxide buffered with 17 mmol TRIS for 1.5 h with periodic agitation. After 1.5 h, we rinsed mussels in FSW, then moved them to individual cups to monitor them for the release of gametes. We moved eggs from single females to clean 1 l beakers of FSW, where they were fertilized with 1 ml of dilute sperm suspension from a single male. We split the embryos from each family (the offspring of one male and one female) into high and low pCO2 rearing conditions. We stocked larval culture buckets with 200,000 embryos each, at a density of 10 larvae ml⁻¹. Due to cost limitations imposed by next-generation sequencing efforts, we were not able to perform culture replicates (multiple buckets per family within a CO2 treatment). However, previous work with this culturing set-up has shown it to be robust to container effects (Ya et al., 2011).

We cultured larvae in a flow-through CO2-mixing system described by Fangue et al. (2010). pCO2 exposure levels were based on predictions for the California Current Large Marine Ecosystem, where anthropogenic inputs of CO2 are expected to drive surface pH down to 7.6 during upwelling in only a few decades (Gruber et al., 2012). Temperatures in seawater tables were held at 15°C using a Delta Star heat pump and a Nema 4x digital temperature controller (Aqualogic, San Diego, CA, USA). We measured temperature, salinity, and pH daily for each culture according to best-practice procedures outlined by Dickson et al. (2007), and described in detail in Fangue et al. (2010). Temperature was measured using a wire thermocouple (Thermolyne PM 207000/Series 1218), and salinity was measured using a conductivity meter (YSI 3100). pH was measured on a total scale, following the standard operating procedure (SOP) 6b (Dickson et al., 2007) using a spectrophotometer (Bio Spec-1601, Shimadzu) and dye m-cresol purple (Sigma-Aldrich) as the indicator. Total alkalinity (TA) was measured every 3 d following the SOP 3b (Dickson et al., 2007). Both pH and alkalinity were assessed for accuracy using certified reference materials from Dickson ( Scripps Institution of Oceanography), Batch 8 (pH 8.0923 + 0.0004) and Batch 103 (TA = 2232.94 + 0.79 mmol kg⁻¹) for pH and alkalinity, respectively. Parameters of pCO2, Ω(calc) and Ω(calc) were estimated using CO2calc (Robbins et al., 2010), with the carbonic acid dissociation constants of Mehrbach et al. (1973).

Temperature, salinity, and carbonate parameters of seawater used in experimental treatments are shown in Supplementary Table S1. While we did not explicitly measure survival, we did not observe evidence of mortality differences (e.g. visibly dead larvae or noticeable declines in density) between treatments. We measured the effects of high pCO2 on growth in six families, and used larvae from three of these families for subsequent RNA-seq analyses. While we had intended from the start of the experiment to sequence the transcriptomes of only three families, we reared six families under each pCO2 treatment in case of any failed cultures, to ensure that we would have adequate samples for subsequent transcriptomic analyses. The three families used for transcriptomic analyses were chosen haphazardly from the original six.

At 63 h post-fertilization (swimming veliger stage), we concentrated larvae using reverse filtration, then pass all the larvae for
a single culture through a 64-μm mesh sieve, separating each culture into fractions of larvae with diameters less than and greater than 64 μm, respectively. We used this combination of larval age and filter mesh size, because pilot experiments indicated that this was the combination that most reliably produced size-fractionated pools of larvae with substantial numbers of larvae in each pool. The size fraction containing smaller larvae also typically had fewer larvae, but still included a minimum of ~30 000 individuals. The size-fractioned samples were then concentrated to remove excess seawater, and immediately flash frozen.

Morphometrics
We preserved a sample of larvae from each bucket before size fractionation, and a sample from each size-fractionated sample in buffered formalin for subsequent morphometric analysis. We measured shell length (maximum axis parallel with the hinge) for 360 larvae (n = 30 per cross per treatment). Body size has been shown to decrease under high pCO2 in this and other species (Gaylord et al., 2011; Yu et al., 2011) and is an important component of larval fitness (Allen, 2008). We made morphometric measurements on photographs of D-hinge larvae (63 h after fertilization) under ×20 compound magnification (Olympus, BX50, Lumenera, Infinity Lite). We tested for an effect of pCO2 treatment on larval shell length in a paired t-test, with families as replicates (N = 6).

Library construction and RNA sequencing
We created cDNA libraries for sequencing from three families of M. californianus, each reared under high and low pCO2 treatments, with each family × pCO2 treatment separated into fractions of large (≥64 μm diameter) vs. small (<64 μm diameter) larvae (12 libraries total). We extracted total RNA from all (30 000–150 000) larvae in each sample using guanidine isothiocyanate (Chomczynski and Sacchi, 1987). Following extraction, we processed RNA to remove tRNA and degraded fragments using an RNeasy Mini Kit according to manufacturer’s instructions (Cat. no. 74104, Qiagen, Valencia, CA, USA). RNA yield and purity were assessed by measuring A260 and A260/A280 ratio, respectively, with a NanoDrop spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA), then using a Bioanalyzer (Agilent, Santa Clara, CA). We constructed cDNA libraries using the Illumina TruSeq RNA sample prep kit (Illumina, San Diego, CA, USA), following the manufacturer’s instructions. Each library was given an individual barcode adapter, then all 12 libraries were pooled and sequenced using paired end, 100 base reads in an Illumina HiSeq 2500 (Illumina) at the UC Davis Genome Center Core Facility.

We assessed raw sequence quality using FastQC (v 0.10.1, www.bioinformatics.bbsrc.ac.uk/projects/fastqc/), then trimmed low-quality and adapter sequences using trimgalore (v. 3.23, http://www.bioinformatics.babraham.ac.uk/projects/trim_galore/), a wrapper script around Cutadapt (v.1.2; Martin, 2011) and FastQC, under the default settings. Initial QC assessment indicated substantial mitochondrial sequence contamination in our samples, mostly from the mitochondrial 16s rRNA gene. The polyA selection used by the Illumina TruSeq kit should remove the majority of ribosomal RNA in most organisms; however, ribosomal RNA may be polyadenylated post-transcription. In particular, mitochondrial RNA may be internally adenylated at specific loci (Shepard et al., 2011), consistent with our finding of over-represented sequences from specific internal positions of the mitochondrial 16s rRNA gene. To remove mitochondrial rRNA sequence contamination from our dataset, we used bowtie 2–2.1.0 (Langmead et al., 2009) to filter out all sequences that aligned to either the maternal (JX486124.1) or paternal (JX486123.1) mitochondrial genomes. Filtering for quality, adapter contamination and mitochondrial rRNA, resulted in 80 million clean read pairs.

Transcriptome assembly
We assembled the transcriptome using Trinity v. 2013-8-25 (Grabherr et al., 2011) with default parameter settings. Reads from all 12 libraries were combined into a single assembly. To reduce transcript redundancy, we used CD-HIT (Fu et al., 2012) to collapse the assembly to contigs with <98% similarity. To remove additional mistakes and rare variants, we also filtered all contigs that did not have expression support that averaged at least one transcript per million per library. We BLASTed all contigs in the final transcriptome assembly against the NCBI non-redundant nucleotide database, with an e-value cutoff of 10−4, using stand-alone blast tools, v. 2.2.28 (Altschul et al., 1990).

Gene expression analysis
To measure gene expression, reads from each sample were mapped to the final transcriptome assembly using the default settings of RSEM v. 1.2.7 (Li and Dewey, 2011). We investigated patterns of gene expression using the R Bioconductor package, limma (Law et al., 2014; Ritchie et al., 2015). Limma employs an empirical Bayes method to estimate fold-changes in expression, and has recently been shown to be more robust to false positives than methods that rely heavily on fitting a negative binomial distribution to the data (Rocke et al., 2015). In limma, we fit a series of nested generalized linear models to the data, testing for an effect of family, larval size (nested within family), and CO2 treatment (nested within size and family) on gene expression. To identify genes differentially expressed between treatments, we set the threshold for false discovery to FDR <0.05 (Benjamini and Hochberg, 1995).

We assessed our power to detect differential gene expression, given the number of reads per library and given the observed dispersion among samples within the same treatment using the software Scotty (Busby et al., 2013). We assigned functional annotations and tested for enrichment in GO terms in differentially expressed genes using BLAST2GO (Conesa and Gómez, 2008), and assessed significance using Fisher’s exact test after correcting for multiple comparisons (FDR; Benjamini and Hochberg, 1995).

Results
Morphometrics
Rearing at elevated (1310 μatm) pCO2 resulted in 63-h-old D-hinge stage larvae that were 4% smaller than those reared under ambient (345 μatm) pCO2 conditions (paired t-test, p = 0.004, N = 6 families; Figure 1 and Table 1). We did not observe any differences between treatments in larval developmental stage, nor did we observe substantial numbers of deformed or abnormal larvae in any of our treatments.

Transcriptome
We assembled 80 million pairs of 100 bp reads. This assembly, collapsed to all contigs <98% similarity, contained 52 173 contigs, with an N50 of 1620 bp and a GC content of 33.05%. To remove additional mistakes and rare variants, we also filtered contigs that did not have an average expression support of at least one read per million per library. The final assembly contained 34 073 unique contigs. We blasted this assembly to the nr. database, and obtained
significant blast hits for 40.5% of contigs, with 81.0% of top blast hits going to other mollusc sequences.

**Gene expression**

Overall patterns of gene expression differed among families (343 differentially expressed transcripts, FDR = 0.05), between large and small larvae within families (1225 differentially expressed transcripts, FDR = 0.05), but not among PCO2 treatments, within families (0 differentially expressed transcripts, FDR = 0.05), or among PCO2 treatments within size classes (0 differentially expressed transcripts, FDR = 0.05). The lack of observed differences in gene expression persisted, regardless of the stringency of our FDR cutoff. There were still no differentially expressed genes even at an FDR cutoff of 0.5, and the top 10 genes with the greatest probability of differential expression between PCO2 treatments all had adjusted p-values > 0.95.

A hierarchical clustering analysis of gene expression patterns revealed clustering by family, then by size class, but no clustering by PCO2 treatment (Figure 2), nor was there any significant correlation among overall patterns of gene expression within PCO2 treatments (Supplementary Figure S1). Many of the 1225 transcripts whose expression pattern differed between large and small larvae were classified as metabolic genes, but this set of genes was not enriched for any specific functional categories (Figure 3, Fisher’s exact test, p > 0.05).

Given the observed dispersion among replicates, our estimated our statistical power to detect a twofold difference in expression between PCO2 treatments at p < 0.05 was 50%, whereas our power to detect a fourfold change in expression was 80%. Our powers to detect two- and fourfold differences in expression between size classes were 60 and 90%, respectively.

**Discussion**

We found that Mytilus larvae reared under elevated PCO2 were smaller, but we found no evidence that these differences in size were driven by an effect of high PCO2 on gene expression. The lack of any observed effect of high PCO2 on gene expression is surprising, given that we observed phenotypic effects (smaller body size) under high PCO2, and given that studies in other taxa have documented substantial effects of OA conditions on gene expression, especially for genes related to metabolism and biomineralization pathways (Kurihara et al., 2012; Moya et al., 2012; Benner et al., 2012).
Effects of high $pCO_2$ on California mussels

2013; Padilla-Gamiño et al., 2013; Lobbeck et al., 2014). However, at least one other study failed to document strong effects of high $pCO_2$ on gene expression in marine larvae: Evans et al. (2013) exposed sea urchin larvae to elevated $pCO_2$ (pH 7.6, as in this study) and detected little effect on gene expression. Stronglylocentrotus purpuratus larvae exhibited an even greater reduction in size at pH 7.6 (9.6%, Kelly et al., 2013) than the one we observe here (4%) in M. californianus. The lack of concordance between genotypic and phenotypic data highlights the importance of integrating both types of information when seeking to understand and predict the biological consequences of OA.

The lack of observed differences in gene expression between $pCO_2$ treatments could have been driven partly by the lack of power. After adapter trimming and removal of sequence contamination, we mapped an average of 6.7 million reads per sample to the reference transcriptome, therefore, despite a lack of change in gene expression, Stronglylocentrotus purpuratus larvae exhibited an even greater reduction in size at pH 7.6 (9.6%, Kelly et al., 2013) than the one we observe here (4%) in M. californianus. The lack of concordance between genotypic and phenotypic data highlights the importance of integrating both types of information when seeking to understand and predict the biological consequences of OA.

The contrast between expression responses of Mytilus and a species like Acropora to high CO$_2$ may also be driven by differences in natural CO$_2$ fluctuations experienced by each species. In the eastern Pacific habitat of M. californianus, seasonal wind-driven upwelling produces large fluctuations in surface water pH (Yu et al., 2011; Booth et al., 2012) with transient pH values as low as 7.8 in nearshore habitats (Evans et al., 2013). Although coral reef habitats also experience daily fluctuations driven by the effects of respiration and photosynthesis, these are shorter in duration than those produced by seasonal upwelling (Kline et al., 2015; Rivest and Gouhier, 2015). Because Mytilus has evolved in the context of seasonal upwelling, beginning in the Northeast Pacific some 15–12 million years ago (Vermeij, 1989), exposure to pH 7.6 may not produce the generalized stress response in M. californianus larvae that might be expected for a species exposed to conditions far outside those of its typical environment. Upwelling is less strong in southern California (where samples were collected for this study) than further to the north, but populations of M. californianus are relatively panmictic (Levinton and Suchanek, 1978), so that southern populations likely receive substantial gene flow from northern populations that experience frequent upwelling. Given that M. californianus has evolved in the context of fluctuating CO$_2$, we might expect larvae to exhibit an adaptive plastic response to low pH, such as increased energy devoted to calcification to compensate for the lower availability of carbonate. However, it also seems possible that Mytilus larvae are always calcifying at the maximum rate possible given available energy reserves, and thus do not devote any additional resources to calcification when faced with lower carbonate availability. It is also possible that Mytilus, in general, are relatively tolerant of high $pCO_2$. Recent work with congener, M. edulis and M. galloprovincialis, showed limited impacts of high $pCO_2$ on growth and mortality (even at levels >3000 $\mu$atm), and much greater effects of factors like food availability and temperature (Thomsen et al., 2013; Gazeau et al., 2014). This is despite the fact that M. galloprovincialis, unlike M. californianus, does not originate in a high upwelling region.

Two other studies taking proteomic approaches have demonstrated broad-scale down-regulation of proteins in Pacific oysters (Crassostrea gigas) subjected to OA conditions (Dineshram et al., 2012; Timmins-Schiffmann et al., 2014). Differences between these results and those in the present study might relate to more extreme pH conditions used in those studies (pH 7.6 in the present study, vs. pH 7.5 and 7.3, respectively) or a lower tolerance of oysters, relative to mussels, to OA conditions. However, a third, intriguing possibility is that proteomics might have greater sensitivity to changes in organismal physiology than transcriptomics, highlighting the need, wherever possible, to integrate the two approaches.

Some have argued that reductions in the size of marine larvae under high $pCO_2$ are driven by metabolic depression and changes in the rate of development (Pörtner, 2008; Stumpp et al., 2011). We found strong evidence of a general relationship between metabolism and larval size: there were substantial gene expression differences between large and small larvae, and these differentially expressed transcripts were predominantly related to metabolic function. However, we found no evidence of a specific effect of high $pCO_2$ on expression of metabolic genes (or any other genes) in M. californianus larvae. Our results are consistent with recent findings of Waldbusser et al. (2015), who observed reductions in shell growth rate in Mytilus larvae driven by reductions in aragonite saturation state, but not changes in $pCO_2$ or pH, and observed effects of OA conditions on metabolism only at very low pH values (~7.4). Given these findings, it is probably unsurprising that we did not find any gene expression changes related to metabolic depression or any other global physiological changes in M. californianus larvae exposed to pH 7.6.

An important consideration when interpreting our results, or those of similar studies, is that they are essentially acute toxicity

Figure 3. Top ten biological processes represented among ontologies for transcripts that differed in expression between large and small larvae from the same family of the California mussel M. californianus.
challenges. Compared with the time-scale of our experiment and others like it, OA will occur slowly, over the next 10–20 decades. As a result, observed responses (or in our case, the lack of a response) should be interpreted with caution. Responses to OA that may occur on multi-generational time-scales include parental effects, acclimatization, and genetic adaptation, all of which will be missed by short-term studies (Kelly and Hofmann, 2013).

Transcriptomic data are useful in studies of organismal responses to climate change stressors in that they provide a global view of the physiological response (or in this case, lack of a response) to that stressor (Stillman and Armstrong, 2015). As we continue to try to understand biological responses to OA, it will be important to document which organisms are responding to OA conditions, and along which physiological axes. In this context, negative results (especially when published with their associated estimates of statistical power) will be as important as positive ones. Mytilus californianus larvae exposed to OA conditions exhibit a reduction in growth rate, but no associated changes in gene expression. The lack of transcriptomic response does not necessarily mean that M. californianus larvae will not be affected by OA conditions, merely that we cannot detect any evidence that they are compensating for these effects through changes in gene expression. More studies that integrate transcriptomic data with other phenotypic measurements, and that span multiple life stages, will be needed before we can fully appreciate the sensitivity, or potential resilience of this species to future ocean change.

Data accessibility
Illumina RNA-Seq data have been submitted to the NCBI Sequence Read Archive (SRA; accession numbers SRX1309994). Differential expression data/results, R-scripts and larval size measurements are available upon request.

Supplementary material
Supplementary material is available at the ICESJMS online version of the manuscript.

Acknowledgements
We thank B. Gaylord, M. Debiasse, and two anonymous reviewers for helpful comments on earlier versions of this manuscript. S. Bachuber and C. Sugano assisted with morphological measurements of larval mussels. This project was supported by US National Science Foundation (NSF) awards OCE-1040960 and IOS-1021536 to GEH, and by a University of California multi-campus research programme, Ocean acidification: A Training and Research Consortium (http://oceanacidification.msi.ucsb.edu/) to GEH.

References
Effects of high pCO2 on California mussels

Page 7 of 8


Handling editor: Howard Browman