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The ocean acidification seascape and its relationship to the performance of calcifying marine invertebrates: Laboratory experiments on the development of urchin larvae framed by environmentally-relevant pCO_2/pH

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ABSTRACT

Variation in ocean pH is a dynamic process occurring naturally in the upwelling zone of the California Current Large Marine Ecosystem. The nearshore carbonate chemistry is under-characterized and the physiology of local organisms may be under constant challenge from cyclical changes in pH and carbonate ion concentration of unexpectedly high magnitude. We looked to environmental pH conditions of coastal upwelling and used those values to examine effects of low pH on 4-arm larvae of purple sea urchin *Strongylocentrotus purpuratus*. We deployed a pH sensor at a nearshore shallow benthic site for 3 weeks during summer 2010 to assess the changes in pH in the Santa Barbara Channel, a region considered to have relatively less intense upwelling along the US Pacific Coast. Large fluctuations in pH of up to 0.67 pH units were observed over short time scales of several days. Daily pH fluctuations on a tidal pattern followed temperature fluctuations over short time scales, but not over scales greater than a day. The lowest pH values recorded (~7.70) are lower than some of those pH values predicted to occur in surface oceans at the end of the century. In the context of this dynamic pH exposure, larvae were raised at elevated pCO_2 levels of 1000 ppm and 1450 ppm CO_2 (pH 7.7 and 7.5 respectively) and measured for total larval length (from the spicule tip of the postoral arm to the spicule tip of the aboral point) along the spicules, to assess effects of low pH upwelling water on morphology. Larvae in all treatments maintained normal development and developmental schedule to day 6, and did not exhibit significant differences in larval asymmetry between treatments. At day 3 and day 6, larvae in the 1450 ppm CO_2 treatment were significantly smaller ($p<0.001$) than the control larvae by only 7–13%. The observation of smaller larvae raised under high pCO_2 has an as yet undetermined physiological mechanism, but has implications for locomotion and feeding. These effects of small magnitude in these urchin larvae are indicative of a potential resilience to near-future levels of ocean acidification. Using environmental monitoring of pH to inform experimental parameters provides a means to improve our understanding of acclimatization of organisms in a dynamic ecosystem.

1. Introduction

Research on the effects of ocean acidification (OA) on organismal function has now been conducted on a variety of marine species (see Kurihara, 2008; Przeslawski et al., 2008; Doney et al., 2009; Dupont and Thorndyke, 2009; Ries et al., 2009; Hofmann et al., 2010; Crim et al., 2011-this issue). As the empirical evidence from these CO_2 manipulation experiments continues to grow, it is increasingly clear that many species are sensitive to OA conditions whereas other species are quite tolerant. Notably, the majority of these studies employ IPCC (2007) emission scenarios to determine the pCO_2 levels (or pH levels) used in the experimental design (e.g., from our laboratory: Todgham and Hofmann, 2009; O'Donnell et al., 2010). One of the ultimate goals of our physiologically-inclined research in the field of OA is to perform our laboratory experiments in the context of the actual environmental conditions experienced by the study organisms in nature; that is, to consider the ocean acidification seascape in an experimental context.

The benefits of such an approach are considerable — experimental results can be interpreted in a manner that better supports forming predictions about individual species' responses (e.g., Melzner et al., 2009) and, thus ecosystem vulnerability to OA (see Widdicombe and Spicer, 2008). Additionally, knowing environmental conditions opens the door to asking questions about physiological performance across a species' range (i.e., macrophysiology in a climate change context —

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see Chown and Gaston, 2008; Gaston et al., 2009 for a review). This contribution to the special edition on 'Global environmental change in marine ecosystems' highlights the emerging research activities in our laboratory. Here, we report data on the development and morphology of the larvae of the purple sea urchin (*Strongylocentrotus purpuratus*) from laboratory experiments that are framed within the estimated present-day environmental exposure for pelagic stages of this species (see Hauri et al., 2009). In addition, we present field observations of pH collected using a SeaFET pH sensor (Martz et al., 2010). This latter data set is preliminary but illustrates a technical approach that may be useful to marine ecologists and ecophysiologists interested in linking environmental exposures to physiological performance in the lab.

Until recently there has been a paucity of nearshore environmental pH data that are available to the research community within experimental marine biology and ecology (e.g., Yates and Halley, 2006; Yates et al., 2007; Hall-Spencer et al., 2008; Wootton et al., 2008; Chierici and Fransson, 2009; Gagliano et al., 2010). In general, high quality pCO_2 data are limited to snapshots taken during large field campaigns (e.g. World Ocean Circulation Experiment "WOCE", http://woce.nodc.noaa.gov/wdiu/; U.S. Joint Ocean Global Flux Study "JGOFS", http://www1.whoi.edu/; Climate Variability and Predictability "CLIVAR", http://www.clivar.org/), a few time-series locations (e.g. Bermuda Atlantic Time Series "BATS", http://www.bios.edu/research/bats.html; Hawaiian Ocean Times Series "HOTS", http://hahana.soest.hawaii.edu/hot/hot_jgofs.html), and occasional process studies that would best pair with a study system or study organisms in the open ocean. Furthermore, for an individual research group interested in linking environmental variables of OA to experimental biology, collecting these types of data can be a logistical and technical challenge, involving time-consuming and expensive carbonate chemistry sampling and analysis, and a relative lack of affordable and deployable sensors for pH equivalent to those available for measuring temperature (e.g. *i*buttons and StowAway® TidbiT™ loggers). The recent development of autonomous pH sensors (Seidel et al., 2008; Martz et al., 2010) has enabled the collection of pH data at study sites in a manner that can support studies on local to larger whole ecosystem scales. As of February 2011, 45 autonomous Durafet sensors have been deployed by 13 research groups at multiple sites along the North American West Coast, Pacific Islands, Gulf of Mexico, and Mediterranean Sea (Martz et al., unpublished data: http://martzlab.ucsd.edu/data.html). In this study, we deployed a sensor on the near-shore benthos at a site in the Santa Barbara Channel. This site is in the southern biogeographic range of the study organism, the purple urchin *S. purpuratus*, and larvae in the water column would encounter chemistry typical of this location. In addition to being a developmental model organism, *S. purpuratus* is also a model in the study of calcium biomineralization (Wilt, 2005; Mann et al., 2008a, 2008b), thus affording us a model study organism about which there is already a substantial body of research into the formation of calcified structures. The objective of this aspect of the study was to begin to characterize the heterogeneity of coastal waters of the California Current Large Marine Ecosystem (CCLME), an upwelling-dominated system on the west coast of the U.S., and relate these values to the physiological performance and tolerances of our study organism, the purple sea urchin.

In terms of what is known regarding the pCO_2 of waters of the CCLME, the observations suggest that there is heterogeneity in the physical nature of coastal waters. Further, these observations indicate that organisms in the CCLME experience low pH and high pCO_2 values that are not expected for the open ocean for another ~100 years or longer. Globally, the Northeastern Pacific region has both the shallowest aragonite and calcite saturation depth horizons ($\Omega = 1$); on average, the West coast of North America from Alaska down to the tip of Central America is undersaturated with respect to calcite below 500 m depth (Feely et al., 2004). Specifically, Feely et al. (2008) report that in summer 2007, the aragonite saturation horizon had shoaled (>20 m) during peak upwelling on the coast of the Oregon–California state line. These investigators estimate that the aragonite saturation horizon in the eastern North Pacific has shoaled by 50–100 m since the pre-industrial era. High resolution time series by Hales et al. (2005) demonstrate high short-term dynamic variability in pCO_2 (reported as X_{CO2}) with shifting wind stress, and the large differential between offshore and nearshore conditions, with sharp pCO_2 gradients occurring over <10 km. More recently, pCO_2 levels ranging from 200 to 1400 μatm have been recorded along the Oregon coast near Cape Perpetua and Newport, OR during the period of April–October 2009 (Francis Chan, unpublished observations).

Given the variability in pH exposure that larvae could experience during the pelagic stage in the waters of the CCLME, we explored the effects of elevated pCO_2 on the development of larvae of the purple urchin. We predicted that purple urchin larvae would mostly develop normally with possible minor perturbations, as they may be more adapted to conditions of variable and elevated pCO_2. Evidence from other studies indicate that larvae of sea urchins show a range of negative responses (from decreased fertilization success, to smaller sizes and altered gene expression) under elevated pCO_2 levels that generally vary according to the intensity of the treatments (reviewed by Dupont et al., 2010). Though we were certain the effects on larvae were sublethal, we nonetheless expected some effect on overall performance/growth given the metabolic costs of functioning at lower pH.

Larval size and in particular, larval length and arm length, is a highly plastic character in echinoid larvae. While it is only moderately reliable as a proxy for feeding physiological state (Fenaux et al., 1994; Reitzel et al., 2004), the shape and size of the arms is directly controlled by spicule length. Multiple studies on echinoplutei have shown a consistent pattern of decreased larval and arm length resulting from acidification treatments, with a wide range of responses observed among the different treatments used (Kurihara and Shirayama, 2004; Brennand et al., 2010; Dupont et al., 2010; O'Donnell et al., 2010). The mechanism responsible for reduced size has not been determined, but could result from a combination of factors including developmental delay (e.g., Dupont and Thorndyke, 2008), reduced growth from depressed metabolism, and decreased ability to deposit calcium carbonate due to greater energetic cost (Cohen and Holcomb, 2009).

In this study, we present morphometric analysis of larval endoskeletons cultured under variable pCO_2 treatments that bracket a range of environmental exposures that would be characteristic of waters in the North Pacific coastal upwelling system (see Feely et al., 2008). In addition, we provide a small data set of environmental pH collected from the Santa Barbara Channel using a SeaFET sensor (Martz et al., 2010). These are the first data in our possession that will assist us in performing manipulative laboratory CO_2 exposure culturing experiments that are framed using realistic environmental data.

2. Material and methods

2.1. Larval culturing

Gravid adult purple urchins (*S. purpuratus*) were collected by SCUBA divers off Goleta Pier, Goleta (34° 24.854 N 119° 49.711 W) at a depth of 5.5 m in November 2009, and maintained in flowing seawater aquaria (ambient temperature) until spawning at the University of California, Santa Barbara. Adult urchins were fed *ad libitum* with *Macrocystis pyrifera* until use in experiments. *S. purpuratus* has a biogeographic distribution that ranges from Alaska to the northern half of the Baja Peninsula, Mexico.

Gametes were obtained by coelomic injection of 0.5 M KCl. The eggs were resuspended in 0.35 μm filtered, UV-sterilized seawater (FSW) at ambient temperature and pCO_2 for fertilization. The sperm

was collected dry, and then diluted in FSW at ambient temperature and pCO_2 immediately prior to fertilizing a batch of eggs. The sperm from one male was used to fertilize the eggs of all 4 females used in Experiment 1 (Families 1–4), and each family was cultured separately, with a single bucket per treatment per family. One male and one female were used for Experiment 2 (Family 5); the embryos were equally and randomly distributed among three replicate buckets per treatment to control for possible bucket effects. Fertilization success for all batches of eggs was >90%. The embryos were counted and then stocked prior to first cleavage into buckets filled with treated seawater at 300,000 per vessel (~20 embryos ml^{-1}).

The larvae were sampled at 3 days and 6 days in both experiments. The larvae were removed from vessels by siphoning through a hole in the bucket lid to minimize the disturbance of vessel lids and to minimize gas exchange between the bucket headspace and the atmosphere. The larvae were immediately concentrated by reverse siphoning, counted volumetrically and then distributed to sample tubes for fixation.

2.2. CO_2 mixing system

The CO_2 mixing system as described in Fangue et al. (2010) was used to manipulate pCO_2 levels in experimental treatment containers. An additional modification to the reported larval culture vessel design was added to maintain high levels of pCO_2 in seawater: the gas mixture used to equilibrate the seawater in the reaction vessels was also simultaneously pumped into the headspace of the larval culture vessels, keeping the headspace pCO_2 at the same concentration as the treated water.

2.3. Seawater chemistry

Total Alkalinity (TA), pH, temperature and salinity were measured on the first day of the experiment prior to fertilization (listed in Table 1 as "First cleavage" as these would have been the chemistry conditions for the embryos from that point onward), and subsequently on the sampling days. The experimental parameters for Experiments 1 and 2 are shown in Table 1. Temperature was measured in each bucket during water sampling with a calibrated wire-thermocouple (Thermolyne PM 20700/Series 1218). Temperature in the experimental vessels was 15.6 ± 1.1 °C in both experiments. Salinity of incoming seawater (33.1–33.2) was measured with a benchtop digital salinity meter (YSI 3100 Conductivity). The methods for CO_2 analysis as modified from the Standard Operating Procedures (SOPs) for pH (SOP 6b) and Total Alkalinity (SOP 3b) (Dickson et al., 2007) are reported in Fangue et al. (2010). All other carbonate parameters (including pCO_2) were calculated in CO2SYS for MS Excel (Pierrot et al., 2006) using the constants of Mehrbach et al. (1973) as refit by Dickson and Millero (1987).

During Experiment 2, ambient seawater pCO_2 was higher than during Experiment 1 (~500 ppm CO_2 vs. ~700–900 ppm CO_2). These conditions made it more difficult to control pCO_2 levels in the "control" treatment and a stable value in the range of 370 ppm was not obtained, thus resulting in a higher pCO_2 level of ~430 ppm in the "control" treatment culture vessels during Experiment 2.

2.4. Morphometric analysis

The larvae were fixed in 1% $NaBO_3$-buffered formalin in seawater and stored at 4 °C until analysis. The larvae were stored up to 1 month after collection. The larvae oriented with their dorsal side down against the slide were digitally photographed (n = 30) under bright field DIC illumination at 10× on a compound microscope (Olympus BX50) with attached digital camera (Infinity Lite). Digital images were calibrated using a stage micrometer for the 10× objective in ImageJ (v. 4.6). Total larval length was measured on both sides of each imaged individual from the spicule tip of the postoral arm to the spicule tip of the aboral point. Spicule length was taken as the length of the whole larva due to minor tissue shrinkage (observed protrusion of the tips of the postoral arm rods in larger larvae).

Table 1
Water chemistry for larval experiments. Carbonate parameters in the larval culture vessels are shown for two experiments. pH and Total Alkalinity (TA) were the measured parameters and the remaining parameters were calculated by CO2SYS. pH was measured in each larval culture vessel (n = 4 in Experiment 1 and n = 3 in Experiment 2) and TA was measured in the mixing reservoir for each treatment. Mean values and SD across larval culture vessels are shown at each sampling time point, and the last column shows the mean of means for each experiment. Control values for Experiment 2 were higher due to low pH (7.83–7.73)/high pCO_2 (700–900 ppm) of incoming ambient seawater.

	First cleavage	4-arm pluteus (day 3)	4-arm pluteus (day 6)	Mean
Experiment 1 — 12/16/2009–12/22/2009				
pH				
Control	8.06 ± 0.03	8.07 ± 0.00	8.07 ± 0.00	8.07
Medium	7.63 ± 0.03	7.67 ± 0.00	7.68 ± 0.00	7.67
High	7.52 ± 0.02	7.53 ± 0.00	7.54 ± 0.01	7.53
TA				
Control	2214.3	2213.1	2223.3	2215.6
Medium	2222.9	2209.8	2224.3	2218.0
High	2216.3	2208.6	2214.6	2214.0
pCO_2				
Control	379.6 ± 26.7	370.1 ± 2.3	369.6 ± 3.0	371.7
Medium	1145.2 ± 87.2	1033.7 ± 12.7	1034.5 ± 9.5	1057.0
High	1518.6 ± 87.7	1456.3 ± 16.2	1454.4 ± 35.9	1469.2
Ω_{ara}				
Control	2.38 ± 0.12	2.43 ± 0.02	2.46 ± 0.02	2.42
Medium	1.00 ± 0.07	1.09 ± 0.01	1.10 ± 0.01	1.07
High	0.77 ± 0.04	0.80 ± 0.01	0.81 ± 0.01	0.80
Ω_{cal}				
Control	3.72 ± 0.19	3.79 ± 0.02	3.84 ± 0.03	3.78
Medium	1.56 ± 0.11	1.69 ± 0.02	1.72 ± 0.01	1.67
High	1.21 ± 0.06	1.26 ± 0.01	1.27 ± 0.02	1.25
Experiment 2 — 3/26/2010–4/1/2010				
pH				
Control	8.00 ± 0.00	8.03 ± 0.01	8.02 ± 0.00	8.02
High	7.52 ± 0.00	7.56 ± 0.01	7.55 ± 0.01	7.54
TA				
Control	2243.2	2240.7	2246.0	2243.3
High	2243.6	2240.7	2236.7	2240.3
pCO_2				
Control	454.7 ± 1.8	412.6 ± 7.0	429.1 ± 3.9	432.2
High	1543.1 ± 7.3	1366.6 ± 27.7	1412.2 ± 20.9	1440.6
Ω_{ara}				
Control	2.22 ± 0.01	2.22 ± 0.03	2.20 ± 0.02	2.21
High	0.82 ± 0.01	0.84 ± 0.02	0.83 ± 0.01	0.83
Ω_{cal}				
Control	3.45 ± 0.01	3.47 ± 0.04	3.44 ± 0.03	3.45
High	1.27 ± 0.01	1.31 ± 0.03	1.29 ± 0.02	1.29

2.5. SeaFET deployment

We deployed a SeaFET pH sensor from 22 July to 17 August 2010 at Mohawk Reef, Santa Barbara (34° 23.66 N, 119° 43.80 W), on sandy substrata approximately 50 m outside (seaward) of the kelp forest at a depth of 8 m. The SeaFET is an autonomous data logger based on a modified Honeywell Durafet™ pH sensor (Martz et al., 2010). The sensor was mounted approximately 0.5 m above the substratum onto a previously-installed base station used by the Santa Barbara Coastal Long Term Ecological Research Project (SBC LTER; http://sbc.lternet.edu/). Mohawk Reef has been an SBC LTER study site since 2001 over which time the project has accrued a large dataset including both oceanographic (e.g. temperature, salinity, turbidity, current velocity) and biological (e.g. kelp production, density of kelp forest biota, phytoplankton abundance) metrics. As such, this wealth of data

warranted Mohawk Reef as an excellent location to begin measuring environmental variation in pH using the SeaFET sensor.

During this deployment, the SeaFET recorded measurements of pH and temperature every 10 min, with each individual measurement averaged over a 30 s period. The SeaFET holds a calibration over long periods once the sensor is deployed and the electrodes are conditioned to seawater (Martz et al., 2010). We therefore calibrated the sensor using a discrete sample collected *in situ* during the deployment. The water sample was collected adjacent to the sensor on 4 August 2010 by SCUBA divers using a 5 L Niskin sampling bottle. From this sample, a 500 mL water sample was returned to the laboratory and analyzed for salinity, Total Alkalinity, and pH (see Section 2.3, Seawater chemistry). This single point calibration approach is justified when the sensor obeys the Nernst equation and the temperature component of the standard potential has been previously characterized; both of which have been repeatedly demonstrated for SeaFET sensors (Martz et al., 2010).

2.6. Statistical analyses

Morphometric comparisons within a biological family were analyzed by t-test (no bucket replicates, within each separate family, Experiment 1) and nested ANOVA (replicates within Family 5 between treatments, Experiment 2 day 3), except where treatment replicates were unbalanced, in which case a non-parametric test (Kruskal–Wallis, Experiment 2 day 6) had to be applied to test between treatments. Statistics were calculated using Excel 2007 and R v.2.11.0.

3. Results

3.1. Nearshore shallow water in-situ pH

In order to ascertain in-situ variability of pH at a nearshore coastal location in Southern California, a SeaFET sensor was deployed for 3.5 weeks at 8 m depth off Santa Barbara, CA. Data recorded by the SeaFET indicated that Mohawk Reef experienced a fluctuating thermal environment (Fig. 1a) with temperature changes of up to 4 °C over a 24 h period. This may be due in part to a tidally driven fluctuation of the thermocline at this site (M. Brzezinski, pers. comm.). Based on the measured values for Total Alkalinity (2257.3 μmol kg^{-1}), pH (7.8852 ± 0.0022 SD, Temp = 25°C, n = 3), and salinity (33.1) we determined *in situ* pH at 14.1 °C to be 8.0477 for the calibration sample (calculated on the total proton scale using CO2SYS). High frequency measurement of pH and temperature at this shallow water benthic location revealed highly dynamic changes over diurnal and weekly scales (Fig. 1a). Changes of 0.67 pH unit magnitude were observed over this deployment, and changes of up to 0.32 pH units were observed over a 24 h period. The time-averaged pH was 7.933 ± 0.119 (± SD) over the recording period. Over this limited-duration deployment, pH and temperature were typically positively correlated in sub-daily patterns but not at lower frequencies. There was clearly a strong tidal control on both daily and weekly pH trends. Because the SeaFET uses a passively flushed copper screen over the pH sensor, and due to the thermal mass of the instrument, rapid changes in pH and temperature occurring around the measurement interval (10–30 min) may cause pH and temperature to lag one another on sub-hourly timescales.

In situ pCO_2, aragonite and calcite saturation state (Ω_{ara}, Ω_{cal}) were calculated by combining pH and temperature measured by the sensor (Fig. 1a) with the measured salinity of 33.1 and Total Alkalinity of 2257 μmol kg^{-1} from a discrete bottle sample (Fig. 1b–c). An error of ± 50 μmol kg^{-1} was applied to the Total Alkalinity value to bracket the errors in our calculations due to changes in salinity and Total Alkalinity and the error bars for the corresponding estimates are plotted in Fig. 1b–c. The corresponding range in estimated pCO_2 over

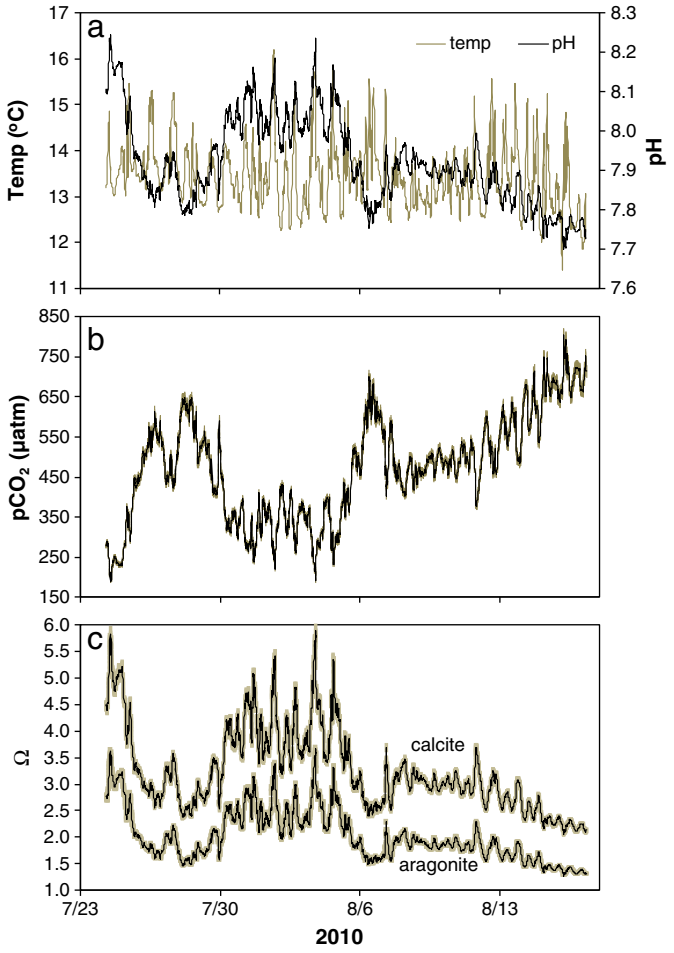

Fig. 1. Time series of pH and temperature (a) from field deployment (22 July–17 August 2010) of SeaFET sensor, estimated pCO_2 (b), and estimated aragonite and calcite saturation state (c, Ω) based upon measured in-situ pH. (a) pH is shown in black with the y-axis on the right. Temperature recorded simultaneously by the sensor is shown in gray on the left y-axis. The SeaFET is operated with two independent reference electrodes (Martz et al., 2010). During this deployment, agreement between the two reference electrodes was better than 0.01 pH unit and we report here pH data based on the internal reference electrode only. Estimated pCO_2 (b), and estimated aragonite and calcite saturation state (c, Ω). Upper line is calcite saturation. Lower line is aragonite saturation. Estimates of carbonate parameters are based upon the measured in situ pH and temperatures in a, and using the salinity of 33.1 and a Total Alkalinity of 2257 ± 50 μmol kg^{-1} as measured from the bottle sample used for SeaFET calibration. An error of ± 50 μmol kg^{-1} (gray) was applied to the Total Alkalinity value to bracket errors in our calculation due to potential variation in salinity and Total Alkalinity. A first-order estimate of the variability in $CaCO_3$ saturation state is possible based on temperature and pH data because, at this study location, these two parameters capture nearly all of the variability in Ω. Although continuous salinity data were not available during this deployment, based on archived salinity and temperature data (http://sbc.lternet.edu/data/index.html), salinity at the Mohawk Reef site is expected to fluctuate by ~0.3 ppt over the season and time scale of our sensor deployment where temperature varied by 4.5 °C. Based on the North Pacific regional Total Alkalinity–Salinity–Temperature relationship (TA = f(S,T); TA_{ST}) for the open ocean (Lee et al., 2006) the water mass changes in alkalinity over this range of salinity and temperature might be expected to be ~24 μmol kg^{-1}, reflecting a relatively small error in Ω of ~0.03 when Ω is calculated from pH assuming constant Total Alkalinity. Variation in pCO_2 is at most ± 23 μatm around the estimated pCO_2 values when using the bracketed Total Alkalinity values over the *in-situ* temperature range at the lowest pH recorded values. Because Mohawk Reef is readily flushed and not dominated by calcifying organisms, the observed variability in pH is likely due to *in situ* changes and circulating gradients in temperature and CO_2 content driven by photosynthesis and respiration of organic matter, rather than by large changes in alkalinity. Using a constant salinity introduces errors in K_{sp}, resulting in Ω errors of 0.01–0.02 over the expected range of salinity of 0.3 ppt, which is about half the error associated with actual changes in Total Alkalinity due to water mass changes, as predicted by the TA_{ST}. In the absence of a second CO_2 system measurement, a more desirable approach to estimating saturation state would be to use a TA_{ST} relationship derived from local data, yet such data are not currently available. Because the equations given by Lee et al. (2006) are representative of the open ocean and do not extend to our coastal study site, we use their relationship here as a guide to bracket TA errors, rather than directly using TA values predicted by their equation.

the recording period was 188–804 μatm (Fig. 1b). The saturation state estimates yielded by this approach ranged from 5.9 to 2.1 for calcite and 3.7 to 1.3 for aragonite (Fig. 1c). Based on the relative sensitivity of saturation state to the expected ranges of salinity and TA and observed ranges in pH and temperature, pH–temperature variability drove about 90% of the change in Ω, with the other 10% accounted for by salinity and TA.

3.2. Larval morphometrics

We cultured larvae at experimentally high levels of dissolved pCO_2 that corresponded to environmental levels occurring on the California coast during upwelling. Overall, larvae exposed to higher pCO_2 were smaller than larvae grown in atmospheric control levels of CO_2. Total larval length ($n = 30$ per culture) was measured at day 3 and day 6 in two experiments (Fig. 2a–e). Larvae raised at 1000 ppm and 1450 ppm CO_2 were significantly smaller than larvae raised at 370 ppm CO_2 (control) in Families 1–4 at days 3 and 6 (Fig. 2a–d, pairwise 2-tail t-tests of unequal variance, $p<0.001$ in all comparisons). In Family 5 at day 3, there were significant differences between treatments (nested ANOVA, $p<0.0002$), and no significant differences between replicate containers within treatments or replicate/treatment interactions (Fig. 2e). An equipment malfunction occurred on day 4 in the 430 ppm CO_2 (control) replicate A culture, resulting in abnormally depressed growth — this culture was excluded from analysis. In the day 6 larvae, the mean lengths between replicates in the control treatment were not significantly different, while replicate C in the 1450 ppm CO_2 was significantly smaller from the other replicates (one-way ANOVA, $p<0.001$). Given those constraints, differences in larval length at day 6 were still highly significant when compared between treatments by the Kruskal–Wallis test ($p \ll 0.001$).

Larvae reared in all treatments appeared normal during the course of both experiments, with significant growth occurring between days 3 and 6 in the absence of food in all treatments (pairwise 2-tail t-tests of unequal variance, $p \ll 0.001$ in all comparisons for Families 1–4; Kruskal–Wallis tests, $p \ll 0.001$ for Family 5). Absolute asymmetry between the left and right larval lengths of individuals was not significantly different between treatments (and replicates) at days 3 and 6 in 4 of 5 families (ANOVA, $p>0.1$–0.9; data not shown). Only Family 1 larvae at day 6 had significantly different asymmetry between treatments (medium pCO_2 < control, high pCO_2).

4. Discussion

This study presents two elements in the exploration of the ocean acidification seascape, and emphasizes how such variation affects the performance and physiology of resident organisms within the CCLME. The two salient findings of this study are: (1) pH variation was high in waters that urchin larvae would encounter in this portion of their biogeographic range, and (2) the magnitude of perturbations in these larvae was of small magnitude even at pCO_2 levels exceeding 'worst case' estimates for the end of this century.

As measured at our Mohawk Reef site, pH is highly variable in the shallow nearshore environment over short time scales of days to less than 2 weeks, as revealed by continuous *in situ* pH measurement. Large diurnal fluctuations in pH, alkalinity and pCO_2 are often observed in habitats where water exchange is low (e.g. tide pools: Wootton et al., 2008), and where high productivity and calcification drives fluctuations (e.g. coral reefs: Yates and Halley, 2006; Yates et al., 2007). The observed fluctuations in pH followed the variation in temperature on a daily tidal cycle as the site at depth is influenced by the thermocline and internal waves (M. Brzezinski, L. Washburn, pers. comm.), and to some extent by both pelagic and benthic rates of photosynthesis and respiration. Similarly, large spatial and temporal fluctuations in pH within kelp forest habitats have been observed both in the Aleutian Islands, AK and San Diego, CA (M. Edwards, pers. comm.). This brief glimpse of benthic pH variability warrants a more thorough, longer-term characterization of the study region both spatially and temporally (e.g. tidal and seasonal scales).

For the sake of comparison, nearshore pH variability is much higher than that observed at the surface 261 km away at the offshore LTER CCE-1 mooring (Martz, unpublished data). Over the period of the deployment at Mohawk Reef, the pH at CCE-1 was no lower than 8.03, with a mean value of 8.08 ± 0.02 SD, an order of magnitude lower deviation than at the Mohawk Reef site. This large difference in offshore vs. nearshore variability reinforces the need for additional nearshore monitoring, as offshore basin-wide studies of oceanic carbonate chemistry fail to encompass the short-term dynamic variation occurring at the land–sea interface. Studies on coastal organisms using regional estimates for experimental parameterization are potentially underestimating the pH variation that the organisms naturally experience.

Based on the observed short-term fluctuations in pH and recently reported regional offshore oceanographic observations (Feely et al., 2008; Hauri et al., 2009; Alin et al., 2010) purple urchins may be experiencing a highly dynamic chemical environment on a regular basis. Across their biogeographic range, *S. purpuratus* would be exposed to a wide range of upwelling intensities, and consequently a range of local pH minima (and consequently Ω minima) during the course of a year. The SeaFET data we have presented here represent only a brief snapshot of the dynamic conditions occurring during a period of relatively relaxed conditions compared to spring upwelling, which is the period of intense upwelling in the Santa Barbara coastal region (L. Washburn, pers. comm.). While the adult urchin collection location and sensor location were 5.6 km apart, the adult urchins of this species are found at the Mohawk Reef site (P. Matson, pers. obs.), and the current and temperature regimes at both locations are very similar, though there is an occasional short-term lag in current conditions between the two sites (data for the region at Southern California Coastal Ocean Observing System (http://www.sccoos.org), and the Institute for Computational Earth System Science (http://www.icess.ucsb.edu/avhrr/avhrr.html)). Studies have previously focused on the effects of upwelling on physical transport of larvae (Pineda, 1991; Wing et al., 1995; Miller and Emlet, 1997; Botsford, 2001; Connolly et al., 2001; Shanks and Shearman, 2009), and primary productivity-fisheries yield associations (Cushing, 1971) but future research should also take into consideration the potential selective effects of low pH conditions on vulnerable species and community composition, as was presented in Wootton et al. (2008), and on vulnerable life history stages (Green et al., 2004). The seasonal nature of upwelling activity would therefore result in temporary exposure of some populations of larvae and newly settled recruits to corrosive conditions depending on local timing of reproduction. Alternately, according to the physical models, urchin larvae are recruiting during periods of upwelling relaxation (Miller and Emlet, 1997; Botsford, 2001); these events could also restore higher pH to the nearshore water column while returning larvae from offshore to settlement sites.

In order to explore the response of larvae of *S. purpuratus* to a range of pCO_2 levels they may experience in the field (as indicated by preliminary SeaFET data), we raised the larvae at pCO_2 levels ranging from 370 to 1450 ppm. Studying populations from the Santa Barbara Channel, the urchin larvae from this region of dynamic upwelling demonstrated minor morphologic changes in response to these experimentally high pCO_2/low pH conditions. Though the pH conditions recorded in the field are more moderate than those used in our experiment, the upper pCO_2 values used (~1450 ppm) were based upon reported oceanographic data from the Pacific Coast within the range of *S. purpuratus* (F. Chan, pers. comm.). These elevated values would easily cover near-future pCO_2 values occurring during upwelling before the time that atmospheric CO_2 reaches 1000 ppm under the most dire IPCC (2007) scenario. Further monitoring in the Santa Barbara Channel during periods of peak upwelling (March–May) will reveal if environmental pCO_2 exceeds our current local high

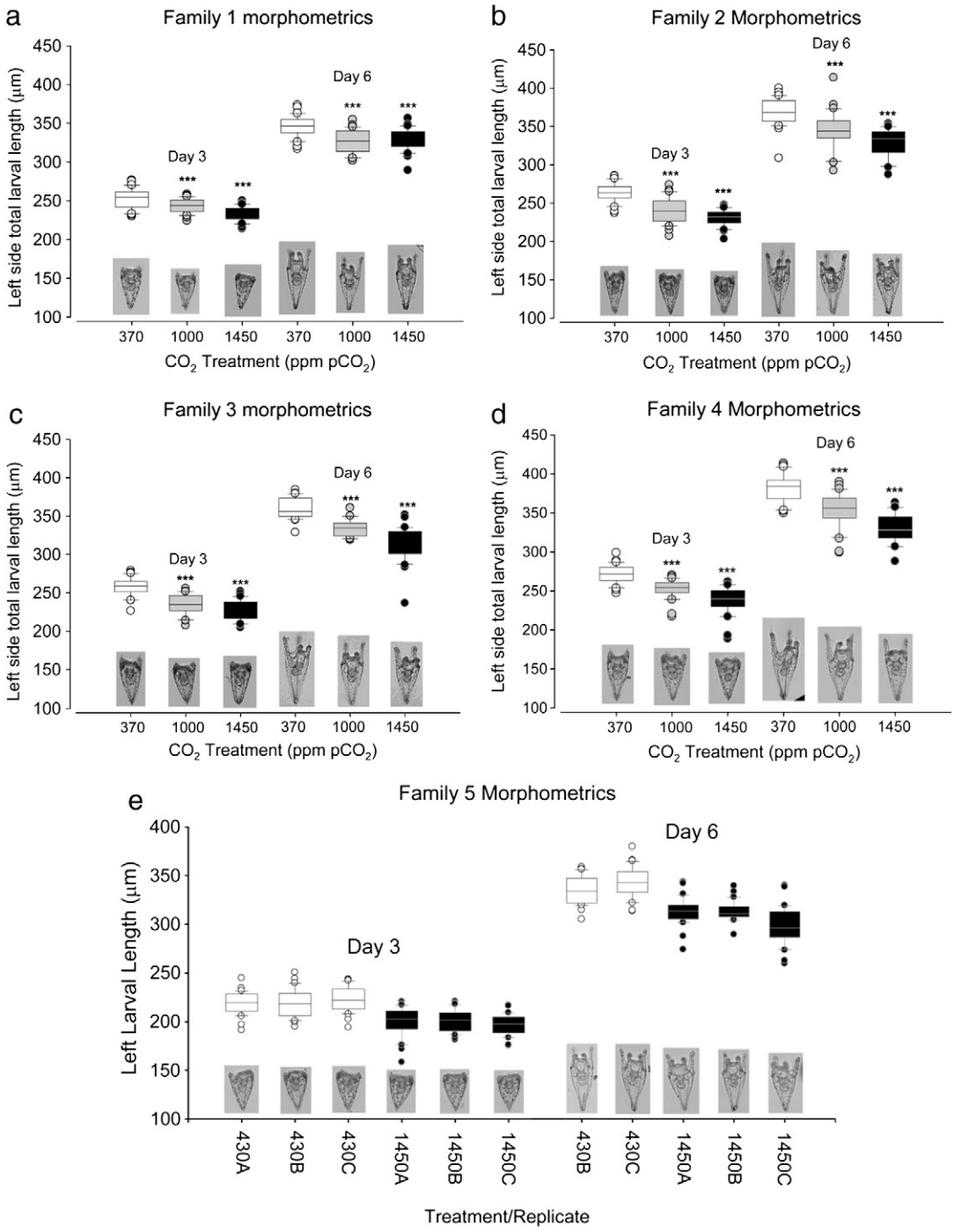

Fig. 2. a–e. Morphometrics of early 4 arm pluteus larvae under elevated CO_2. Left-side total larval length (μm) is shown for 5 families at day 3 and day 6 in 2 or 3 CO_2 treatment levels, with images of representative median-sized larvae from each sample (n = 30). (a–d) Families 1–4 were from Experiment 1. (e) Family 5 is from Experiment 2. Box plots show median values within the box, 25th and 75th percentiles at the box edges, and 10th and 90th percentiles in the error bars. The circles represent the 6 high and low outliers in each sample. ***$p < 0.001$ (t-tests in Families 1–4, and ANOVA and Kruskal–Wallis tests in Family 5). There were no significant differences between averaged right and left side larval length measurements within any sample, therefore only data from the left side is shown. An equipment malfunction occurred on day 4 in the 430 ppm (control) replicate A culture resulting in abnormally depressed growth — this culture was excluded from analysis.

estimate of 800 μatm, and for how long these events persist. The dissolved CO_2 in upwelled water contains anthropogenic CO_2 absorbed from the atmosphere 50 years ago (Feely et al., 2008); as atmospheric CO_2 increases we can anticipate that upwelled waters will have an even higher pCO_2 in the near future. Thus, scenarios of pCO_2 levels in surface waters as high as 2000 μatm may not be as distant in the future as the IPCC models predict.

Even at the highest pCO_2 treatments, larval development was normal in terms of timing and morphological appearance. Developmental rate differences were not quantified, nor was survivorship — however, the observed developmental progression and survival of cultures was within the norm typically observed for this species at this temperature range. A lack of developmental deformities at early stages for pCO_2 ~1000 ppm has been previously reported for this

species (Todgham and Hofmann, 2009), and another local species, *Lytechinus pictus*, with a similar overlapping portion of its range in southern California (O'Donnell et al., 2010). There are even reports that survival is increased in this species and its congener *S. droebachiensis* under some low pH conditions (Dupont and Thorndyke, 2008), but other species of echinoderm can have notably poor survival (e.g. *Ophiothrix* in Dupont et al., 2008).

While species variation has been demonstrated in response to different levels of increased CO_2, the sublethal effects on larvae were of small magnitude (up to 13% smaller on average, Fig. 2). Previous research on gene expression and morphometrics from this laboratory have also revealed low-intensity but broad spectrum effects of acidification at ~1000 ppm pCO_2 (Todgham and Hofmann, 2009; Hammond, 2010; O'Donnell et al., 2010; Zippay and Hofmann, 2010). Further measures of physiological indices on samples from these experimental cohorts could also reveal other signals indicative of developmental delay and/or depressed growth.

There was no significant treatment effect of CO_2 on asymmetry of early development, which also suggests that the developmental stability of this species (i.e. similar to results in *Tripneustes*, Brennand et al., 2010) is less sensitive than other species (e.g. *Ophiothrix* in Dupont et al., 2008). It should be noted that this short term study would not expose the potential for the deleterious effects on asymmetry further along in development; the asymmetric development of rudiment formation (>3 weeks at 15 °C) is under the control of H^+/K^+-ATPase (Hibino et al., 2006) which is down-regulated at prism stage under intermediate and high pCO_2 (Todgham and Hofmann, 2009). It has been noted that non-significant results do not necessarily mean a lack of effect (Havenhand et al., 2010), and in these trials, the power to detect no treatment effect in asymmetry was low (i.e. post hoc analysis suggests that $n = 791$ would be required to have sufficient power). Given the amount of individual variation observed, total length asymmetry is not a robust index of treatment effect, though the comparisons of total length differences are robust.

There is currently no definitive mechanism for the observed size differences in calcified echinoderm larvae under OA conditions. The prevailing hypotheses are slower growth (which could have multiple mechanisms), delayed development (Dupont and Thorndyke, 2008), or some interaction of both. More research, particularly with the use of precisely timed, developmental gene expression studies, will be necessary. Hammond (2010) observed a significant effect of CO_2 treatment on archenteron elongation during gastrulation (control > high CO_2); however, at 33 h post-fertilization, a higher percentage of gastrulae in the high CO_2 treatment had already deposited their initial calcite spicule crystal. This complex result does not definitively support any hypothesis.

Ultimately, the consequences of smaller larvae have potential repercussions on juvenile size and fitness (Meidel and Scheibling, 1999; Miller and Emlet, 1999; Moran, 1999; Phillips, 2002, 2004). Species will respond differently (see Ries et al., 2009) to chronic corrosive conditions along the eastern Pacific Coast, and changes in community composition are expected to occur (Wootton et al., 2008). With climate change, dynamic pH decreases may intensify simultaneously with the frequency of intense warming events. In order to gain an understanding of acclimatization of organisms in response to climate change-related effects such as OA, further study across natural environmental gradients, chemical and physical, will have to be undertaken. For many marine organisms, it is not clear to what extent physiological performance varies across a species range, although studies have demonstrated differences in performance at these scales (e.g., Stillman and Somero, 2000; Sokolova and Pörtner, 2003; Osovitz and Hofmann, 2005, 2007; Dutton and Hofmann, 2009; Stillman and Tagmount, 2009). Further, local adaptation will likely play an important role in determining the ultimate effect of a changing environment on marine populations (e.g., Findlay et al., 2009; Kuo and Sanford, 2009; Sanford and Kelly, 2011), an area that is ripe for further investigation in the study of rapid environmental change in the oceans.

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References

Alin, S.R., Feely, R.A., Dickson, A.G., Hernandez-Ayon, J.M., Juranek, L.W., Ohman, M.D., Goericke, R., 2010. Predictive relationships for pH and carbonate saturation in the Southern California Current System using oxygen and temperature data. Abstract OS23A-1570 presented at 2010 Fall Meeting, AGU, San Francisco, CA. 13–17 December.

Botsford, L.W., 2001. Physical influences on recruitment to California Current invertebrate populations on multiple scales. ICES J. Mar. Sci. 58, 1081–1091.

Brennand, H.S., Soars, N., Dworjanyn, S.A., Davis, A.R., Byrne, M., 2010. Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin *Tripneustes gratilla*. Plos One 5 (6), e11372. doi:10.1371/journal.pone.0011372.

Chierici, M., Fransson, A., 2009. Calcium carbonate saturation in the surface water of the Arctic Ocean: undersaturation in freshwater influenced shelves. Biogeosciences 6, 2421–2431.

Chown, S.L., Gaston, K.J., 2008. Macrophysiology for a changing world. Proc. R. Soc. B 275, 1469–1478.

Cohen, A.L., Holcomb, M., 2009. Why corals care about ocean acidification: uncovering the mechanism. Oceanography 22, 118–127.

Connolly, S.R., Menge, B.A., Roughgarden, J., 2001. A latitudinal gradient in recruitment of intertidal invertebrates in the northeast Pacific Ocean. Ecology 82, 1799–1813.

Crim, R.N., Sunday, J.M., Harley, C.D.G., 2011. Elevated seawater CO_2 concentrations impair larval development and reduce larval survival in endangered northern abalone (*Haliotis kamtschatkana*). J. Exp. Mar. Biol. Ecol. 400, 272–277 (this issue).

Cushing, D.H., 1971. Upwelling and production on fish. Adv. Mar. Biol. 9, 255–334.

Dickson, A.G., Millero, F.J., 1987. A comparison of the equilibrium-constants for the dissociation of carbonic acid in seawater media. Deep-Sea Res. 34, 1733–1743.

Dickson, A.G., Sabine, C.L., Christian, J.R., 2007. Guide to Best Practices for Ocean CO_2 Measurements. PICES Special Publications. 191 pp.

Doney, S.C., Fabry, V.J., Feely, R.A., Kleypas, J.A., 2009. Ocean acidification: the other CO_2 problem. Annu. Rev. Mar. Sci. 1, 169–192.

Dupont, S., Thorndyke, M.C., 2008. Ocean acidification and its impacts on the early life-history stages or marine animals. in CIESM (Ed.), Impacts of Acidification on Biological, Chemical and Physical Systems in the Mediterranean and Black Seas. No 36 in CIESM Workshop Monographs (F. Briand, Ed.), 124 pp. Monaco.

Dupont, S., Thorndyke, M., 2009. Impact of CO_2-driven ocean acidification on invertebrates early life-history — what we know, what we need to know, and what we can do. Biogeosciences Discuss. 6, 3109–3131.

Dupont, S., Havenhand, J., Thorndyke, W., Peck, L., Thorndyke, M., 2008. Near-future level of CO_2-driven ocean acidification radically affects larval survival and development in the brittlestar *Ophiothrix fragilis*. Mar. Ecol. Prog. Ser. 373, 285–294.

Dupont, S., Ortega-Martinez, O., Thorndyke, M., 2010. Impact of near-future ocean acidification on echinoderms. Ecotoxicology 19, 449–462.

Dutton, J.M., Hofmann, G.E., 2009. Biogeographic variation in *Mytilus galloprovincialis* heat shock gene expression across the eastern Pacific range. J. Exp. Mar. Biol. Ecol. 376, 37–42.

Fangue, N.A., O'Donnell, M.J., Sewell, M.A., Matson, P.G., MacPherson, A.C., Hofmann, G.E., 2010. A laboratory-based experimental system for the study of ocean acidification effects on marine invertebrate larvae. Limnol. Oceanogr.-Meth. 8, 441–452.

Feely, R.A., Sabine, C.L., Lee, K., Berelson, W., Kleypas, J., Fabry, V.J., Millero, F.J., 2004. Impact of anthropogenic CO_2 on the $CaCO_3$ system in the oceans. Science 305, 362–366.

Feely, R.A., Sabine, C.L., Hernandez-Ayon, J.M., Ianson, D., Hales, B., 2008. Evidence for upwelling of corrosive "acidified" water onto the continental shelf. Science 320, 1490–1492.

Fenaux, L., Strathmann, M.F., Strathmann, R.R., 1994. Five tests of food-limited growth of larvae in coastal waters by comparisons of rates of development and form of echinoplutei. Limnol. Oceanogr. 39, 84–98.
Findlay, H.S., Kendall, M.A., Spicer, J.I., Widdicombe, S., 2009. Relative influences of ocean acidification and temperature on intertidal barnacle post-larvae at the northern edge of their geographic distribution. Estuar. Coast. Shelf Sci. 86, 675–682.
Gagliano, M., McCormick, M.I., Moore, J.A., Depczynski, M., 2010. The basics of acidification: baseline variability of pH on Australian coral reefs. Mar. Biol. 157, 1849–1856.
Gaston, K.J., Chown, S.L., Calosi, P., Bernardo, J., Bilton, D.T., Clarke, A., Clusella-Trullas, S., Ghalambor, C.K., Konarzewski, M., Peck, L.S., Porter, W.P., Pörtner, H.O., Rezende, E.L., Schulte, P.M., Spicer, J.I., Stillman, J.H., Terblanche, J.S., van Kleunen, M., 2009. Macrophysiology: a conceptual reunification. Am. Nat. 174, 595–612.
Green, M.A., Jones, M.E., Boudreau, C.L., Moore, R.L., Westman, B.A., 2004. Dissolution mortality of juvenile bivalves in coastal marine deposits. Limnol. Oceanogr. 49, 727–734.
Hales, B., Takahashi, T., Bandstra, L., 2005. Atmospheric CO_2 uptake by a coastal upwelling system. Global Biogeochem. Cycles 19, GB1009. doi:10.1029/2004GB002295.
Hall-Spencer, J.M., Rodolfo-Metalpa, R., Martin, S., Ransome, E., Fine, M., Turner, S.M., Rowley, S., Tedesco, D., Buia, M.C., 2008. Volcanic carbon dioxide vents show ecosystem effects of ocean acidification. Nature 454, 96–99.
Hammond, L.M., 2010. Physiological Response to Environmental Variation in *Strongylocentrotus purpuratus* Early Developmental Stages, Department of Ecology, Evolution, and Marine Biology. University of California, Santa Barbara, Santa Barbara, p. 180.
Hauri, C., Gruber, N., Plattner, G.-K., Alin, S., Feely, R.A., Hales, B., Wheeler, P.A., 2009. Ocean acidification in the California Current System. Oceanography 22, 60–71.
Havenhand, J., Dupont, S., Quinn, G.P., 2010. Designing ocean acidification experiments to maximise inference. In: Riebesell, U., Fabry, V.J., Hansson, L., Gattuso, J.-P. (Eds.), Guide to Best Practices for Ocean Acidification Research and Data Reporting. Publications Office of the European Union, Luxembourg. 260 pp.
Hibino, T., Ishii, Y., Levin, M., Nishino, A., 2006. Ion flow regulates left-right asymmetry in sea urchin development. Dev. Genes Evol. 216, 265–276.
Hofmann, G.E., Barry, J.P., Edmunds, P.J., Gates, R.D., Hutchins, D.A., Klinger, T., Sewell, M.A., 2010. Ocean acidification impacts on calcifying marine organisms in marine ecosystems. Annu. Rev. Ecol. Evol. Syst. 41, 127–147.
International Panel on Climate Change (IPCC), 2007. In: Solomon, S., Qin, D., Manning, M., Chen, Z., Marquis, M., Averyt, K.B., Tignor, M., Miller, H.L. (Eds.), Climate Change 2007: The Physical Science Basis, Contribution of Working Group I to the Fourth Assessment Report of the Intergovernmental Panel on Climate Change. Cambridge University Press, Cambridge, United Kingdom and New York, NY, USA. 987 pp.
Kuo, E.S.L., Sanford, E., 2009. Geographic variation in the upper thermal limits of an intertidal snail: implications for climate envelope models. Mar. Ecol. Prog. Ser. 388, 137–146.
Kurihara, H., 2008. Effects of CO_2-driven ocean acidification on the early developmental stages of invertebrates. Mar. Ecol. Prog. Ser. 373, 275–284.
Kurihara, H., Shirayama, Y., 2004. Effects of increased atmospheric CO_2 on sea urchin early development. Mar. Ecol. Prog. Ser. 274, 161–169.
Lee, K., Tong, L.T., Millero, F.J., Sabine, C.L., Dickson, A.G., Goyet, C., Park, G.H., Wanninkhof, R., Feely, R.A., Key, R.M., 2006. Global relationships of total alkalinity with salinity and temperature in surface waters of the world's oceans. Geophys. Res. Lett. 33, L19605. doi:10.1029/2006GL027207.
Mann, K., Poustka, A.J., Mann, M., 2008a. In-depth, high-accuracy proteomics of sea urchin tooth organic matrix. Proteome Sci. 6, 33. doi:10.1186/1477-5956-6-33.
Mann, K., Poustka, A.J., Mann, M., 2008b. The sea urchin (*Strongylocentrotus purpuratus*) test and spine proteomes. Proteome Sci. 6, 22. doi:10.1186/1477-5956-6-22.
Martz, T.R., Connery, J.G., Johnson, K.S., 2010. Testing the Honeywell Durafet® for seawater pH applications. Limnol. Oceanogr.-Meth. 8, 172–184.
Mehrbach, C., Culberson, C.H., Hawley, J.E., Pytkowicz, R.M., 1973. Measurement of the apparent dissociation constants of carbonic acid in seawater at an atmospheric pressure. Limnol. Oceanogr. 18, 897–907.
Meidel, S.K., Scheibling, R.E., 1999. Effects of food type and ration on reproductive maturation and growth of the sea urchin *Strongylocentrotus droebachiensis*. Mar. Biol. 134, 155–166.
Melzner, F., Gutowska, M.A., Langenbuch, M., Dupont, S., Lucassen, M., Thorndyke, M.C., Bleich, M., Pörtner, H.O., 2009. Physiological basis for high CO_2 tolerance in marine ectothermic animals: pre-adaptation through lifestyle and ontogeny? Biogeosciences 6, 2313–2331.
Miller, B.A., Emlet, R.B., 1997. Influence of nearshore hydrodynamics on larval abundance and settlement of sea urchins *Strongylocentrotus franciscanus* and *S. purpuratus* in the Oregon upwelling zone. Mar. Ecol. Prog. Ser. 148, 83–94.
Miller, B.A., Emlet, R.B., 1999. Development of newly metamorphosed juvenile sea urchins (*Strongylocentrotus franciscanus* and *S. purpuratus*): morphology, the effects of temperature and larval food ration, and a method for determining age. J. Exp. Mar. Biol. Ecol. 235, 67–90.
Moran, A.L., 1999. Size and performance of juvenile marine invertebrates: potential contrasts between intertidal and subtidal benthic habitats. Am. Zool. 39, 304–312.
O'Donnell, M.J., Todgham, A.E., Sewell, M.A., Hammond, L.M., Ruggiero, K., Fangue, N.A., Zippay, M.L., Hofmann, G.E., 2010. Ocean acidification alters skeletogenesis and gene expression in larval sea urchins. Mar. Ecol. Prog. Ser. 157–171.
Osovitz, C.J., Hofmann, G.E., 2005. Thermal history-dependent expression of the hsp70 gene in purple sea urchins: biogeographic patterns and the effect of temperature acclimation. Exp. Mar. Biol. Ecol. 327, 134–143.
Osovitz, C.J., Hofmann, G.E., 2007. Marine macrophysiology: studying physiological variation across large spatial scales in marine systems. Comp. Biochem. Phys. A 147, 821–827.
Phillips, N.E., 2002. Effects of nutrition-mediated larval condition on juvenile performance in a marine mussel. Ecology 83, 2562–2574.
Phillips, N.E., 2004. Variable timing of larval food has consequences for early juvenile performance in a marine mussel. Ecology 85, 2341–2346.
Pierrot, D., Lewis, E., Wallace, D.W.R., Pierrot, D., Lewis, E., Wallace, D.W.R., 2006. MS Excel Program Developed for CO2 System Calculations. ORNL/CDIAC-105a. Carbon Dioxide Information Analysis Center, Oak Ridge National Laboratory, U.S. Department of Energy, Oak Ridge, Tennessee.
Pineda, J., 1991. Predictable upwelling and the shoreward transport of planktonic larvae by internal tidal bores. Science 253, 548–551.
Przeslawski, R., Ahyong, S., Byrne, M., Worheide, G., Hutchings, P., 2008. Beyond corals and fish: the effects of climate change on noncoral benthic invertebrates of tropical reefs. Glob. Change Biol. 14, 2773–2795.
Reitzel, A.M., Webb, J., Arellano, S., 2004. Growth, development and condition of *Dendraster excentricus* (Eschscholtz) larvae reared on natural and laboratory diets. J. Plankton Res. 26, 901–908.
Ries, J.B., Cohen, A.L., McCorkle, D.C., 2009. Marine calcifiers exhibit mixed responses to CO_2-induced ocean acidification. Geology 37, 1131–1134.
Sanford, E., Kelly, M.W., 2011. Local adaptation in marine invertebrates. Annu. Rev. Mar. Sci. 3, 509–535.
Seidel, M.P., DeGrandpre, M.D., Dickson, A.G., 2008. A sensor for *in situ* indicator-based measurements of seawater pH. Mar. Chem. 109, 18–28.
Shanks, A.L., Shearman, R.K., 2009. Paradigm lost? Cross-shelf distributions of intertidal invertebrate larvae are unaffected by upwelling or downwelling. Mar. Ecol. Prog. Ser. 385, 189–204.
Sokolova, I.M., Pörtner, H.O., 2003. Metabolic plasticity and critical temperatures for aerobic scope in a eurythermal marine invertebrate (*Littorina saxatilis*, Gastropoda: Littorinidae) from different latitudes. J. Exp. Biol. 206, 195–207.
Stillman, J.H., Somero, G.N., 2000. A comparative analysis of the upper thermal tolerance limits of eastern Pacific porcelain crabs, genus *Petrolisthes*: influences of latitude, vertical zonation, acclimation, and phylogeny. Physiol. Biochem. Zool. 73, 200–208.
Stillman, J.H., Tagmount, A., 2009. Seasonal and latitudinal acclimatization of cardiac transcriptome responses to thermal stress in porcelain crabs, *Petrolisthes cinctipes*. Mol. Ecol. 18, 4206–4226.
Todgham, A.E., Hofmann, G.E., 2009. Transcriptomic response of sea urchin larvae, *Strongylocentrotus purpuratus*, to CO_2-driven seawater acidification. J. Exp. Biol. 212, 2579–2594.
Widdicombe, S., Spicer, J.I., 2008. Predicting the impact of ocean acidification on benthic biodiversity: what can animal physiology tell us? J. Exp. Mar. Biol. Ecol. 366, 187–197.
Wilt, F.H., 2005. Developmental biology meets materials science: morphogenesis of biomineralized structures. Dev. Biol. 280, 15–25.
Wing, S.R., Botsford, L.W., Largier, J.L., Morgan, L.E., 1995. Spatial structure of relaxation events and crab settlement in the northern California upwelling system. Mar. Ecol. Prog. Ser. 128, 199–211.
Wootton, J.T., Pfister, C.A., Forester, J.D., 2008. Dynamic patterns and ecological impacts of declining ocean pH in a high-resolution multi-year dataset. Proc. Natl. Acad. Sci. 105, 18,848–18,853.
Yates, K.K., Halley, R.B., 2006. Diurnal variation in rates of calcification and carbonate sediment dissolution in Florida Bay. Estuaries Coasts 29, 24–39.
Yates, K.K., Dufore, C., Smiley, N., Jackson, C., Halley, R.B., 2007. Diurnal variation of oxygen and carbonate system parameters in Tampa Bay and Florida Bay. Mar. Chem. 104, 110–124.
Zippay, M.L., Hofmann, G.E., 2010. Effect of pH on gene expression and thermal tolerance of early life history stages of red abalone (*Haliotis rufescens*). J. Shellfish Res. 29, 429–439.