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Kelp-associated variability in seawater chemistry during a marine heatwave event connects to transgenerational effects in the purple urchin *Strongylocentrotus purpuratus*

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ABSTRACT: Giant kelp *Macrocystis pyrifera* provides the foundation for immense biodiversity on the coast of California, USA. Kelp forests can change seawater retention time, altering water chemistry, including pH and dissolved oxygen (DO), as well as the magnitude and predictability of variability in the same properties. Environmental heterogeneity across space and time could drive organismal performance and processes such as transgenerational plasticity (TGP), where parental experience modifies the offspring phenotype, potentially conferring tolerance to future environmental stress. We monitored environmental variability by deploying temperature, pH, and DO sensors inside and outside a temperate kelp forest in the Santa Barbara Channel (SBC) throughout the gametogenesis period of a key herbivore, the purple urchin *Strongylocentrotus purpuratus*. Over the 6 mo period, pH and temperature were slightly elevated inside the kelp forest, accompanied by more predictable, low-frequency variability relative to outside. Adult *S. purpuratus* were conditioned inside and outside the kelp spanning gametogenesis. The urchins were spawned and their larvae were raised under high (1053 µatm) and low pCO_2 (435 µatm) at 15°C in the laboratory to assess their physiological response to the maternal and developmental environments. Larvae raised under high pCO_2 were more susceptible to acute thermal stress; however, within each larval treatment, progeny from outside-conditioned mothers had a 0.4°C higher lethal temperature (LT_{50}). Our results indicate that heterogeneity in abiotic factors associated with kelp can have transgenerational effects in the field, and interactions between factors, including temperature and pH, will impact purple urchins as local variability associated with marine heatwaves and upwelling evolves with climate change.

KEY WORDS: Transgenerational plasticity · *Strongylocentrotus purpuratus* · *Macrocystis pyrifera* · pH · Marine heatwave · Coastal variability · Sea urchin · Kelp forest

1. INTRODUCTION

Environmental heterogeneity in the context of rapid global climate change and ocean acidification (OA) could have important consequences for species persistence and adaptation (Schindler et al. 2015, Morelli et al. 2016, Webster et al. 2017). As average pCO_2 concentrations and marine sea surface temperatures (SSTs) rise, stochastic variability in pH and temperature is also influenced by climate change. Marine heatwaves (MHWs) are increasing in frequency and duration (Frölicher et al. 2018, Barkhordarian et al. 2022, Jacox et al. 2022) and pH regimes can vary greatly over short distances and fluctuate rapidly. Some of the most variable pH environments are found in temperate waters, eastern boundary cur-

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rent upwelling systems, and nearshore productive habitats such as macrophyte habitats (Hofmann et al. 2011). In the California Current System (CCS), episodic upwelling brings cold water, low in pH and dissolved oxygen (DO) but rich in nutrients, up from depth. The frequency, duration, and extent of upwelling is predicted to increase with climate change, extending the footprint of undersaturated waters with respect to aragonite (Diffenbaugh et al. 2004, Feely et al. 2008). In the Santa Barbara Channel (SBC), in the southern CCS, pH and oxygen levels vary on diurnal and seasonal scales, as well as on 'event' scales (Frieder et al. 2012, Kapsenberg & Hofmann 2016). The lowest pH events can be paired with warm temperatures driven by overnight respiration in the summer, or cold temperatures during strong upwelling (Kapsenberg & Hofmann 2016). The interaction between physical drivers, such as upwelling, and biological drivers, such as primary production, makes it critical to consider site-specific differences in dynamic ecosystems like the CCS.

Kelp forests can impact flow and modify local water chemistry through photosynthesis (Gaylord et al. 2007, Krause-Jensen et al. 2016), elevating mean pH and DO levels while also increasing diurnal variability due to photosynthesis and respiration (Delille et al. 2009, Frieder et al. 2012, Kapsenberg & Hofmann 2016, Hirsh et al. 2020). This process has piqued interest in whether macrophytes provide potential refuges from OA (Morelli et al. 2016, Nielsen et al. 2018, Woodson et al. 2019, Ricart et al. 2021), and further, how differences in the magnitude and variability of pH and DO influence organismal performance (Mcleod et al. 2011, Wahl et al. 2018). Since the change in mean pH around macrophytes comes with a corresponding increase in variability, it has mixed consequences for processes such as calcification. Impacts can vary between taxa and ontogenetic timing with consequences for community assemblage (Cornwall et al. 2018, Kapsenberg et al. 2018, Wahl et al. 2018). The spatial heterogeneity and temporal variability of macrophyte habitats with respect to pH, DO, and temperature creates a mosaic where conditions can range in hospitability across short distances (Krause-Jensen et al. 2015, Starko et al. 2022).

Given this existing abundance of small-scale physicochemical variability, plasticity may play a key role for species persistence under global change. Phenotypic plasticity is critical to survival in variable environments and can be sustained in a population by selection (Via 1993). Mechanistically, transgenerational plasticity (TGP), where parental environment modifies the phenotype of the offspring without affecting the genotype, could be particularly important in conferring tolerance to environmental stressors (Donelson et al. 2018). TGP can be derived from maternal effects such as nutrient, hormone, protein, and lipid provisioning to eggs, or from paternal effects mediated through sperm (Munday 2014). Males and females may pass epigenetic markers such as DNA methylation, histones, or small RNAs to their offspring, driving phenotypic plasticity through heritable changes in gene expression (Gavery & Roberts 2010, Munday 2014, Hofmann 2017). It is necessary to describe the abiotic conditions that promote TGP and align investigations with ecologically relevant stressors and the duration of their exposure (Burgess & Marshall 2014, Bautista & Crespel 2021). Characterizing both the magnitude and predictability of variability will be critical for considering its implications for plasticity, the conditions which drive its evolution, and the resulting consequences for population vulnerability (Burgess & Marshall 2014, Fox et al. 2019, Bitter et al. 2021).

Environmental variability may align with particular life history stages. In the SBC, the spring period of strongest upwelling, and thus variation in pH, coincides with when larvae of the purple sea urchin *Strongylocentrotus purpuratus* are in the water column (Strathmann 2017). Early life stages are thought to be highly vulnerable with respect to environmental stressors for most marine organisms (Kurihara 2008, Byrne 2011). As a dominant herbivore, *S. purpuratus* has a major influence on kelp forest ecosystems in the CCS. In addition to the variability in pH and DO associated with upwelling and kelp forests, purple urchins and the kelp that supports them are experiencing increased thermal stress from MHWs, which may impact biotic interactions and recruitment patterns (Okamoto et al. 2020, Starko et al. 2022). A recent study found that female adult urchins conditioned inside a kelp forest during gametogenesis produced eggs with increased protein content compared to mothers conditioned outside the kelp, indicating elevated maternal provisioning (Hoshijima & Hofmann 2019). Previous laboratory-based studies have shown that conditioning adult purple urchins to different pH and temperature regimes during gametogenesis has transgenerational effects on size, lipid content, gene expression, and DNA methylation of their larvae raised under different pCO_2 conditions (Wong et al. 2018, 2019, Strader et al. 2019). However, aside from Hoshijima & Hofmann (2019), little work has been done to look at this phenomenon in the field.

While most TGP studies are conducted in the laboratory, temporal variation in environmental con-

ditions around reproduction can have transgenerational consequences *in situ* in Atlantic silverside *Menidia menidia* and European squid *Loligo vulgaris* (Murray et al. 2014, Rosa et al. 2014), as can spatial temperature variation in California mussels *Mytilus californianus* (Waite & Sorte 2022). In the field, high-frequency fluctuations, regular cycles, and event-scale shifts associated with upwelling or MHWs interact to define the acclimation environment. This temporal mosaic is further complicated spatially across habitat types and depths. It is difficult for lab studies to mimic these many axes of variation and their correlations accurately; therefore, it is critical to supplement lab studies of TGP with field studies in dynamic areas such as kelp forests. Studies have explored ecological changes in recruitment dynamics and community assemblage across a kelp gradient (Duggins et al. 1990, Schroeter et al. 1996, Carrasco et al. 2017), but few have examined the physiological consequences of the abiotic gradient, as we do here mechanistically via TGP.

In this study, we investigated TGP driven by field acclimation using the natural heterogeneity within and outside a kelp forest. To accomplish this, sensors were deployed to monitor DO, temperature, and pH inside and outside a giant kelp *Macrocystis pyrifera* forest in the SBC. Adult *S. purpuratus* were simultaneously acclimatized on the kelp forest benthos in close association with the sensor arrays. Caged adult sea urchins were conditioned in the field for 6 mo during their gametogenesis period, then spawned in the laboratory in order to assess the physiological response of their larvae to pCO_2 stress (Fig. 1). This field to lab experiment allowed us to examine

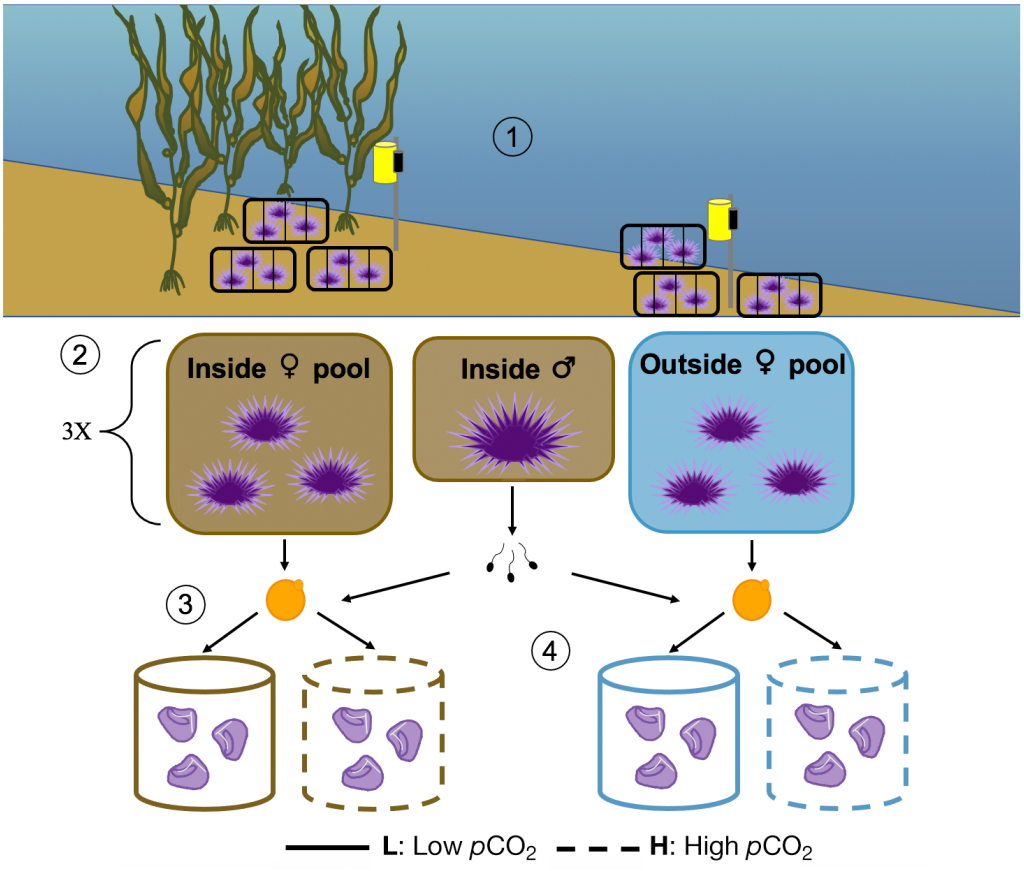


Fig. 1. Experimental design. (1) Adult purple urchins *Strongylocentrotus purpuratus* were held in cages inside (I) or outside (O) the kelp forest for 6 mo prior to spawning. pH, temperature, and dissolved oxygen sensors (yellow and black cylinders) were deployed at both sites. (2) Eggs from n = 3 mothers were pooled to create n = 3 pools from each site. (3) Each pool of eggs (n = 6) was fertilized with sperm from 1 inside-conditioned male. (4) Fertilized eggs from each pool were then divided into low pCO_2 (L: ~435 µatm), or high pCO_2 (H: ~1050 µatm) treatments to develop until the prism larval stage yielding 4 final combinations of maternal and larval treatment: larvae from inside mothers (I) raised under low pCO_2 (L), IL; larvae from inside mothers (I) raised under high pCO_2 (H), IH; larvae from outside mothers (O) raised under low pCO_2 (L), OL; and larvae from outside mothers (O) raised under high pCO_2 (H), OH

whether and how maternal environmental experience inside or outside the kelp forest influences the phenotype and performance of their offspring when exposed to low pH/high pCO_2. Adult acclimation could result in a range of larval responses to pCO_2 stress, as both positive and negative transgenerational effects have been observed in the literature, and the environmental factors inside and outside the kelp forest are complex (Fig. 2).

2. MATERIALS AND METHODS

2.1. Site instrumentation and adult conditioning

In early June 2018, a SeaFET™ pH and miniDOT™ DO sensor were deployed on a benthic mooring within the kelp forest (henceforth, 'inside') at Arroyo Quemado reef, a Santa Barbara Coastal Long Term Ecological Research (SBC LTER) site ((34° 28.040' N, 120° 07.084' W) Rivest et al. 2016). The SeaFET™ recorded pH and temperature every 30 min, and the miniDOT™ recorded DO and temperature every 10 min. A complementary pair of sensors was deployed at the base of the LTER mooring 'outside' the kelp forest (34° 27.897' N, 120° 07.179' W) with the same sampling intervals (Fig. 1). The 2 sites were 305 m apart. The sensors were replaced halfway through the experiment. There was a depth difference between the 2 moorings: inside ~9 m and outside ~14 m. The benthos inside was rocky reef while outside was sandy substrate. To calibrate the pH sensors, water samples were taken on scuba using Niskin Go-FLO sampling bottles (General Oceanics) and immediately poisoned on the boat using $HgCl_2$ at a final concentration of 0.02%. The pH of these water samples was measured in the lab using a UV spectrophotometer (Shimadzu UV-1800) and *m*-cresol purple dye, and total alkalinity was measured by titration (Mettler-Toledo T50) following the procedures outlined by Dickson et al. (2007). Point calibrations were aligned with the time and temperature when a sample was taken, and pH was adjusted to the *in situ* levels using 'CO2Calc' (Robbins et al. 2010). Calibration coefficients for the raw voltage data from the SeaFET™ were calculated using the 'seaCarb' (Gattuso et al. 2021) in R (v 4.0.3) (R Core Team 2020).

Four plastic cages reinforced with plastic garden fencing (dimensions: 50 cm × 36 cm × 20 cm; volume: 36 l; mesh size: 0.8 cm) were deployed at both sites close to the sensor arrays. Outside cages were secured ~0.5 m above the substrate using sand anchors, whereas inside cages were bolted to a rock the same height above the benthos. Purple urchins were collected from the Arroyo Quemado kelp forest (Califor-

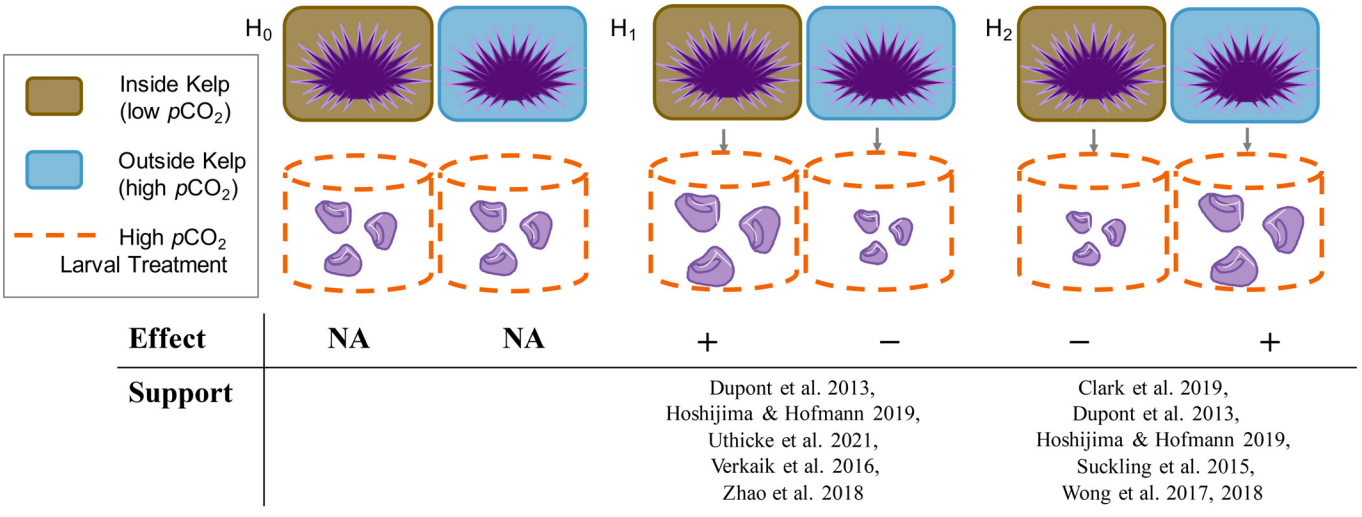


Fig. 2. Possible directions for transgenerational effects of conditioning inside and outside kelp forest on purple urchin larval development under high pCO_2. Based on current support from the literature, transgenerational impacts may align with different hypotheses: H_0: Conditioning inside and outside a kelp forest has no transgenerational impact. H_1: Negative effect from high pCO_2: urchins that were acclimated outside the kelp forest suffer from higher pCO_2 and lower oxygen, and they pass down damage or are unable to provision offspring proficiently, while urchins that were acclimated inside the kelp forest may have a more hospitable environment of low pCO_2 and higher dissolved oxygen, allowing them to provision their eggs more than those acclimated outside the kelp forest, creating more resilient larvae. H_2: Positive effect of priming urchins that were acclimated inside the kelp forest that are naïve to higher pCO_2 and lower oxygen and do not confer changes to offspring upregulating pathways to help them cope while urchins acclimated outside the kelp forest that experience more acidic, low-oxygen conditions may be primed by poor conditions and confer resilience to these same stressors to their offspring through maternal provisions or epigenetic modifications

nia Department of Fish and Wildlife Scientific Collecting permit #SC-9228) and randomly assigned to the 8 cages, ~12 in each (Fig. 1). The urchins were held in these cages from early June through early December during their gametogenesis period and fed an excess of kelp every 2 wk to control for the effects of food availability at each site. Every 2 wk, divers opened the cages, visually checked the health and number of urchins, removed any dead urchins and remaining kelp, and filled the cage with fresh giant kelp collected from Arroyo Quemado Reef (the inside site). Several urchins (n = 1–2) at each site died in the first ~2 wk, presumably from the stress of collection. After this, there was no mortality at either site.

2.2. Time series analysis

Temperature observations from the miniDOT^{TM} oxygen sensors were used in all analysis. Power spectral density (PSD) estimates were conducted following the methods of Hoshijima & Hofmann (2019). Time series analysis for DO and temperature was conducted on observations from 10 June to 8 October 2018 to avoid artifacts due to sensor conditioning at the beginning of the deployment and bio-fouling at the end. Due to a data gap between the first and second deployment, PSD estimates for pH were calculated separately for each half from 8 June to 21 September and 28 September to 30 October, and a mean spectrum was computed by weighting each by their duration. A 24 h moving-average filter was applied to remove the diurnal signal, and beta (β) statistics were calculated as the negative slope of the $\log_{10} - \log_{10}$ spectral density to measure 'environmental color', a characterization of predictability based on the structure of residual variation around a mean trend (Marshall & Burgess 2015, Hoshijima & Hofmann 2019). Using NOAA 1/4° Daily Optimum Interpolation Sea Surface Temperature (OISST) data (Huang et al. 2021) for the region between 34.25 and 34.5° N and between 120.5 and 119.5° W, MHW events were calculated using the R package 'heatwaveR' (v 0.4.5) (Schlegel & Smit 2018) as events where SST exceeded the 90th percentile of climatological observations, based on a 38 yr dataset (1982–2019), for at least 5 d.

2.3. Adult spawning and larval culturing

Adult urchins were recovered on 4 December after 6 mo of field conditioning. Urchins from each cage were kept in separate bags and submerged in flowing ambient seawater until spawning 1 wk later. Spawning, gamete collection, and quality screening was performed following Wong et al. (2019). Spawning was induced by injection of 0.53 M KCl into the coelom. Sperm was collected and stored dry on ice until activation. Eggs were collected from each female by inverting the female over a beaker filled with UV sterilized filtered seawater (FSW). Egg concentrations were calculated by counting 3 aliquots (5 µl each) of eggs per female under a microscope. The coefficient of variation (CV) for the 3 aliquots was less than 10% for all females, and the average count was used to determine pooling volumes to ensure females contributed an equal number of eggs. Eggs were visually checked for quality, indicated by uniformity and sphericity, during counting, and females with significant numbers of eggs containing visible germinal vesicles were deemed immature and excluded. Sperm from potential males was activated in FSW, checked for motility, and then used to fertilize a subset of eggs from each female to ensure male–female compatibility. Three females with the highest-quality eggs were selected from each of 6 cages (1 cage was excluded from each site due to the bag failing post collection, leading to uncertainty about their source site). Test diameters were measured for females that contributed eggs. Eggs from each of the 3 females were pooled in equal numbers to create 3 egg pools per site, each representing a cage. To reduce genetic diversity and control functional variation between individuals sired with sperm with different environmental history, eggs were fertilized with the sperm from 1 inside-conditioned male (Fig. 1). All larvae were thus a mix of full and half siblings. Dilute activated sperm was added slowly to each pool of eggs until 95% fertilization was reached. Half of the embryos from each pool were then added to a high (H) pCO_2 (1053.18 ± SD 14.24 µatm) and low (L) pCO_2 (~435 ± 6.23 µatm) 12 l culture vessel for a final concentration of 10 embryos ml^{-1}. This yielded 3 culture vessels for each of 4 treatment groups: embryos from mothers conditioned inside the kelp forest raised under high pCO_2 (IH), embryos from mothers conditioned inside the kelp forest raised under low pCO_2 (IL), embryos from mothers conditioned outside the kelp forest raised under high pCO_2 (OH), embryos from mothers conditioned outside the kelp forest raised under low pCO_2 (OL).

2.4. CO_2 mixing system and seawater chemistry

Larval cultures were held constant at a mean ± SD of 15.01 ± 0.52°C and a salinity of 33.2 ppt, reflective

of the collection site during the last weeks of adult conditioning. Temperature was maintained using a Delta Star heat pump with a Nema 4× digital temperature controller (AquaLogic). A flow-through CO_2 system was modified from the design of Fangue et al. (2010) to create the desired carbonate chemistry parameters. To generate filtered seawater (FSW), incoming seawater was UV- and 0.35 µm filter-sterilized. Desired pCO_2 treatments were created by mixing CO_2 gas with CO_2 scrubbed dry air in the appropriate ratios using mass flow controllers (Sierra Instruments) and injecting the gas mixture into 2 reservoir tanks using Venturi injectors. The 2 reservoir tanks then fed 6 culture vessels per treatment at a rate of 6 l h^{-1} using irrigation drippers. Each culture vessel consisted of 2 nested 12 l buckets: the inner bucket had holes covered with 30 µm mesh allowing water to flow to the outer bucket and overflow without losing any larvae. Temperature, pH, and salinity were measured daily for each bucket to confirm uniform conditions across each treatment. Temperature was measured using a thermocouple (Omega HH81A), and salinity was measured using a conductivity meter (YSI-3100). pH was measured following SOP 6b using a spectrophotometer and *m*-cresol purple dye (Dickson et al. 2007). Total alkalinity (2236.89 ± SD 0.17 µmol kg^{-1}) was measured from incoming water samples poisoned with $HgCl_2$ following the SOP 3b procedure (Dickson et al. 2007). 'CO2calc' (Robbins et al. 2010) was used to calculate carbonate chemistry parameters using the equilibrium constants from Mehrbach et al. (1973) refit by Dickson & Millero (1987).

2.5. Egg and larval sampling

Prior to conducting fertilizations, eggs were sampled from all 18 females that would mother offspring (n = 9 females per treatment). From each female, 1000 eggs estimated by volume were placed in 6 replicate tubes for protein and lipid quantification. Eggs were centrifuged to remove excess seawater and flash frozen with liquid nitrogen. For morphometric analysis, 1000 eggs estimated by volume were preserved per female by addition of formalin (in 0.01 M phosphate-buffered saline with borax) to the same volume of eggs in FSW for a final concentration of 2% formalin and stored at +4°C. The offspring from each bucket were also sampled as prism larvae, an early echinopluteus stage of pre-feeding larvae, at ~45 h post fertilization. The prism stage was defined by the archenteron merging to one side of the body and becoming tripartite, the first development of skeletal rods, and a pyramid like shape. Prism larvae (~1000 per bucket per stage) were preserved in a final concentration of 2% formalin in FSW (larval concentrations were determined in the same manner as for eggs, described in Section 2.3 using 20 µl aliquots of larvae after larvae were concentrated for sampling from each bucket using a 30 µm mesh to reduce the volume of FSW). Some larvae were also sampled at the prism stage and were immediately used for live assessment in an acute thermal stress trial and whole-animal respirometry.

2.6. Thermal tolerance

To assess the effect of maternal conditioning and larval pCO_2 treatment on response to acute thermal stress, larvae from each treatment were subjected to a range of acute heat shocks for 1 h and then scored as alive or dead following a procedure adapted from Hammond & Hofmann (2010). FSW (3.5 ml) with ambient pCO_2 in scintillation vials was brought to temperature across a gradient from 24.8 to 33.4°C. At the prism stage, ~3333 larvae from each of the 3 larval cultures per treatment were combined into an aggregate pool. Larvae from each pool were gently mixed before 1000 larvae (0.5 ml) were pipetted into 10 treatment vials per treatment across the temperature gradient and were then incubated for 1 h. Control larvae were held at 15°C. After 1 h, all vials were transferred to a 15°C cold room, and scoring was performed immediately. A volume of 1 ml was loaded onto a rafter slide, and the larvae were viewed through a compound microscope with the 4× objective. The first 100 larvae seen were scored as either alive (denoted by swimming and/or cilia movement) or dead. All scoring was blind to treatment and temperature. The survivorship curve for each treatment group was used to calculate the lethal temperature at which 50% of the individuals died, LT_{50}, using logistic regression with the 'MASS' package (Venables & Ripley 2013) in R (v 4.0.3). A generalized linear model fitted using a logit link function was used to analyze differences in thermal tolerance, including vial temperature, maternal treatment, and larval treatment as fixed effects. A Wald chi-squared test was used to assess the significance of each factor with the 'car' package (v 3.0-10) (Fox & Weisberg 2019), and post hoc tests to compare individual treatments were conducted in the 'lsmeans' package (Lenth 2013) using the Tukey method to adjust for multiple comparisons. An α level of 0.05 was used for all statistical tests.

2.7. Respirometry

To assess the effect of maternal conditioning and larval pCO_2 treatment on larval metabolism, oxygen consumption rates of prism-stage larvae were measured using the method outlined by Marsh & Manahan (1999) with minor modifications. A gradient of larvae (n = 100–600) from each culture bucket (n = 3 per treatment, n = 12 total) were loaded into 5 ground-glass µBOD vials per culture bucket containing a known volume of high or low pCO_2 FSW with no headspace. One blank vial per culture bucket was also loaded with high or low pCO_2 FSW corresponding the larval treatment. End-point oxygen concentrations for each vial were measured following a 5–7 h incubation. Water (~315 µl) from each µBOD vial was loaded into an optode cell using a gas-tight syringe. Oxygen concentration was measured using a fiber optic oxygen probe inside the cell (Micro TX3; PreSens). The probe reading was calibrated using a 2-point (0 and 100%) calibration with 0.01 g ml^{-1} Na_2SO_3 solution and aerated 0.2 µm re-filtered FSW. Baseline respiration over the 5–7 h incubation, determined from blank vials, was subtracted from each µBOD vial measurement. Oxygen consumption rates per individual (in pmol O_2 h^{-1} $larva^{-1}$) were then calculated from a standard curve of oxygen consumption rate generated from the cumulative oxygen consumption over time at each larval concentration (n = 100–600). Oxygen consumption rates per individual were divided by larval volume, which was calculated as:

$$\left(\frac{\text{Mean body length}^3}{3}\right) \quad (1)$$

Average oxygen consumption rate per unit volume (pmol O_2 h^{-1} mm^{-3}) for each bucket was used for analysis. Distribution normality was tested using a Shapiro-Wilk test, and homogeneity of variance between treatment groups was confirmed using Bartlett's test. Differences in size-corrected respiration rates across treatments were tested using an ANOVA including the maternal and larval treatment as well as their interaction as fixed effects in base R (v 4.0.3).

2.8. Morphometric analysis

Eggs and larvae preserved in 2% formalin were photographed on a compound microscope (Olympus BX50) with an attached digital camera (Motic 10MP) using the Motic Images Plus software. Images were calibrated using a stage micrometer with the 10× objective and analyzed using ImageJ (National Institutes of Health, USA). Average diameter was calculated for 35 eggs from each of the 18 females (n = 9 per treatment) using 3 roughly orthogonal diameters. Prism larvae were photographed from a lateral view where both the tip of the body rod and branching point of the postoral rod were in focus. Spicule length was measured as the length from the tip of the body rod to the branching point of the postoral rod, and body length was measured as the top of the arch to the top of the pyramid parallel to the ventral plane (Fig. A1 in the Appendix). Spicule and body length for each individual were used to calculate the spicule:body length ratio. Approximately normal distributions were confirmed through histograms and qqplots, and homogeneity of variance was confirmed between treatment groups using Bartlett's tests. A linear mixed effects model (LMM) was used to compare size between treatments with maternal and larval treatment and their interaction as fixed effects and pool as a random effect. A Wald chi-squared test was used to assess the significance of each factor in the 'car' package (v 3.0-10) (Fox & Weisberg 2019). For egg morphometrics, the inverse (1/average diameter) was used to adjust for skew in the distribution before performing an ANOVA. Maternal treatment was treated as a fixed effect while maternal identity was treated as a random effect. Statistical tests were run in R (v 4.0.3) using the 'lme4' (Bates et al. 2015) and 'lmerTest' (Kuznetsova et al. 2015) packages. Post hoc tests were conducted in the 'lsmeans' package (Lenth 2013) using the Tukey method to adjust for multiple comparisons.

2.9. Protein quantification

Total protein was extracted from frozen egg samples (n = 3 tubes per female) using the method described by Wong et al. (2019), modified from Byrne et al. (2008) and Prowse et al. (2008). Samples were sonicated on ice for 20 s in 100 µl homogenization buffer (20 mM Tris-HCl [pH 7.6]; 130 mM NaCl, 5 mM EDTA) containing 1% Triton X and 1% protease inhibitor cocktail using a Sonic Dismembrator 550 (Fisher Scientific). Samples were shaken on ice for 15 min and centrifuged for 20 min at 18000 × *g*. After extraction, the retained supernatant of total soluble protein was quantified at 562 nm on a microplate reader (Bio Rad) using a BCA protein assay kit following the manufacturer's instructions (Catalog number 23225, Pierce Biotechnology). For protein and lipid measurements, distribution normality was tested using Shapiro-Wilk tests, and homogeneity of variance between treatment groups was confirmed by

Bartlett's tests using R base packages. Differences in biochemical content of inside and outside eggs were assessed using Welch's 2-sample *t*-tests.

2.10. Lipid content analysis

Total lipid was extracted from frozen egg samples (n = 3 tubes per female) following the methods described by Wong et al. (2019) based on Sewell (2005), the only difference being that no internal standard was needed. Each sample was sonicated on ice for 25 s bursts (n = 3). Samples were then transferred to glass V-vials and combined with 250 µl of methanol and 125 µl of chloroform (Parrish 1987). After vigorous shaking, V-vials were centrifuged for 5 min at 4°C. Using a pulled Pasteur pipette, both the aqueous and chloroform layers were transferred to a clean V-vial, and chloroform and water were added to a final volume ratio of 4:3:2 (water:chloroform:methanol). After centrifuging under the same conditions, only the bottom chloroform layer was transferred and stored under nitrogen at −20°C. Immediately prior to quantification, total lipid extracts were dried in glass vials using nitrogen gas. Total lipid was quantified using a spectrophotometric method (Marsh & Weinstein 1966). Briefly, 500 µl of sulfuric acid were added to each of the dried samples; the vials were then covered with aluminum foil caps and heated in a furnace at 200°C for 15 min. The vials were allowed to cool for 15 min, and then 2.5 ml of water were added to each, followed by an additional 15 min cooling period. The absorption of each re-constituted lipid sample was read on a UV spectrophotometer (Shimadzu UV 1800) at 375 nm using disposable cuvettes. A standard curve of known mass of lipid ranging from 25 to 300 µg was prepared in the same way and measured alongside each batch of samples. The lipid profile of the standards was composed of the major lipid classes found in *Strongylocentrotus purpuratus* eggs in the ratios reported by Wong et al. (2019) (51% triacylglycerol, 38% phospholipid, 11% cholesterol). Principal component analysis was conducted on all egg metrics (size, protein, and lipid content) and maternal test diameter in R (v 4.0.3) using 'ggfortify' (Tang et al. 2016) and base packages.

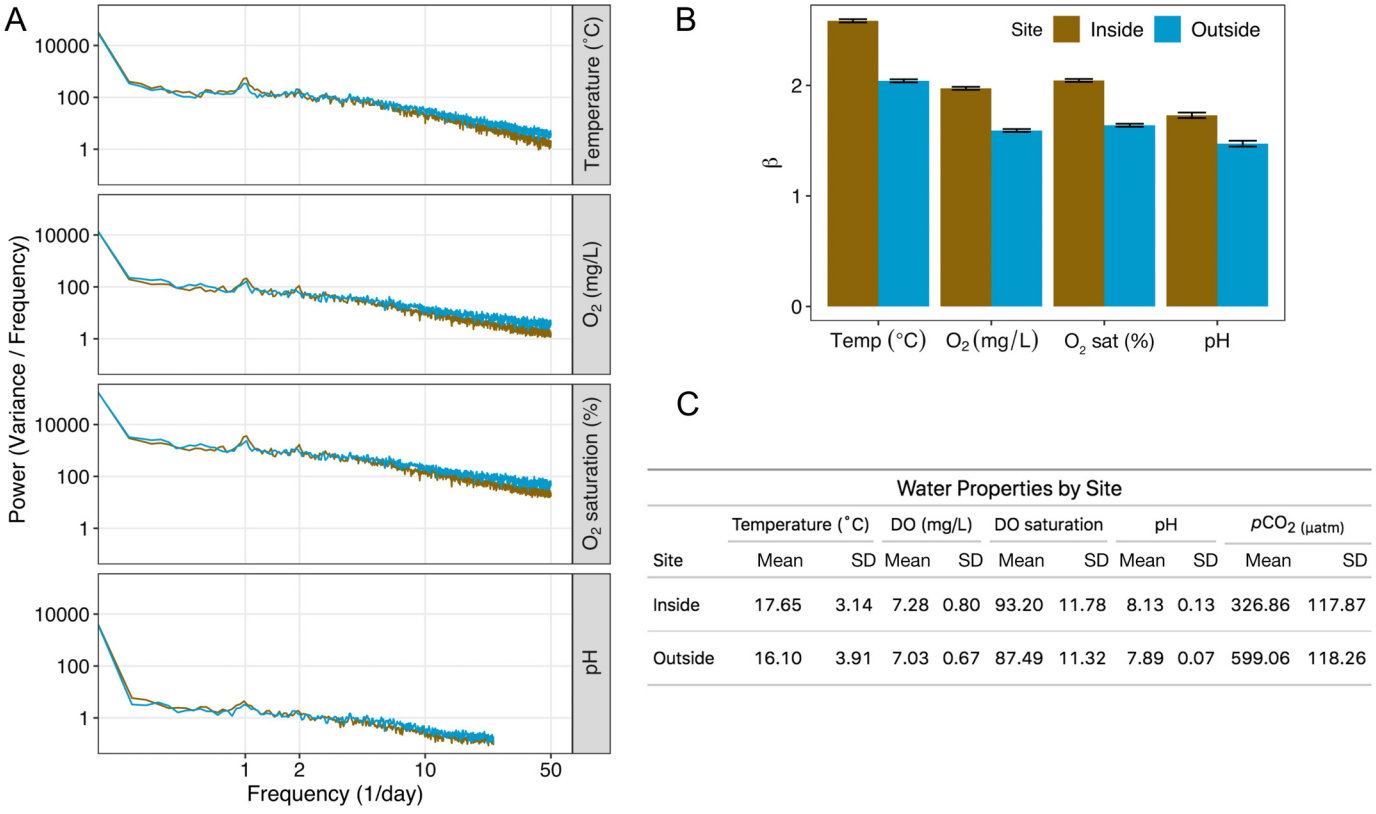
3. RESULTS

3.1. *In situ* water properties

The average *in situ* water temperature varied between the inside and outside sites for the time period analyzed, with the shallower inside site warmer by approximately 1.5°C (17.65 ± SD 3.14°C) than the deeper outside site (16.10 ± 3.91°C) (Fig. 3). The average (±SD) DO concentration and saturation were also higher inside the kelp forest (7.28 ± 0.80 mg l^{-1}, 93.20 ± 11.78%) than outside (7.03 ± 0.67 mg l^{-1}, 87.49 ± 11.32%). With regard to pH, on average, seawater inside the kelp forest tended to be more alkaline than was measured outside the kelp; pH was 8.13 ± 0.13 inside the kelp forest vs. 7.89 ± 0.07 outside (Fig. 3). Lastly, there was a greater degree of high-frequency variation in temperature, DO (concentration and saturation), and pH outside the kelp forest compared with inside. β-values, a metric of environmental color, were higher inside the kelp forest relative to outside for all parameters, indicating a tendency to lower-frequency and more predictable variation over time (Fig. 3). Multiple MHW events occurred during the conditioning period of the experiment (Fig. 4).

3.2. Thermal tolerance

Using acute thermal tolerance trials, we found an effect of both maternal conditioning environment and developmental pCO_2 treatment on the capacity of larvae to deal with an additional environmental stressor, temperature. Specifically, LT_{50}, the lethal temperature at which 50% of the larvae died from a 1 h exposure, varied by up to 1.0°C across treatment groups (Fig. 5). The effect of both maternal (Wald chi-squared test: $\chi^2 = 9.5$, df = 1, p = 0.002) and larval treatment ($\chi^2 = 17.3$, df = 1, $p < 0.0001$) were significant, along with vial temperature ($\chi^2 = 3224.7$, df = 1, $p < 0.0001$). The interaction between maternal and larval treatment was not significant ($\chi^2 = 0.5$, df = 1, p = 0.483). LT_{50} varied by ~0.6°C between larvae raised under the low (L) versus high (H) larval pCO_2 treatment, within each maternal treatment (Fig. 5, Table 1). Within each larval pCO_2 treatment, larval LT_{50} varied by 0.4°C across mothers conditioned outside (O) versus inside (I) the kelp forest (Fig. 5, Table 1). Within the same larval treatment (H or L), those from inside (I) mothers had lower thermal tolerance, while within a maternal group (O or I), those exposed to high pCO_2 (H) had lower thermal tolerance (Fig. 5). Larvae from mothers conditioned outside the kelp forest raised under low pCO_2 (OL) had the highest LT_{50} (±SD) at 29.9 ± 0.1°C. Those from inside mothers who were raised under high pCO_2 conditions (IH) had the lowest LT_{50} at 28.9 ± 0.1°C. Larvae from outside mothers raised under high pCO_2 conditions (OH), and those from inside mothers raised under low



Water Properties by Site

	Temperature (°C)		DO (mg/L)		DO saturation		pH		pCO_2 (µatm)	
Site	Mean	SD	Mean	SD	Mean	SD	Mean	SD	Mean	SD
Inside	17.65	3.14	7.28	0.80	93.20	11.78	8.13	0.13	326.86	117.87
Outside	16.10	3.91	7.03	0.67	87.49	11.32	7.89	0.07	599.06	118.26

Fig. 3. Temperature (°C), dissolved oxygen (concentration in mg l^{-1} and saturation in %), and pH inside (brown) and outside (blue) of a kelp forest environment, shown as (A) power spectra, (B) calculated β indexes (±SE), and (C) mean (±SD) values for the deployment of temperature (°C), dissolved oxygen (concentration, mg l^{-1} and saturation,%), pH, and pCO_2 (µatm) inside and outside of a kelp forest environment

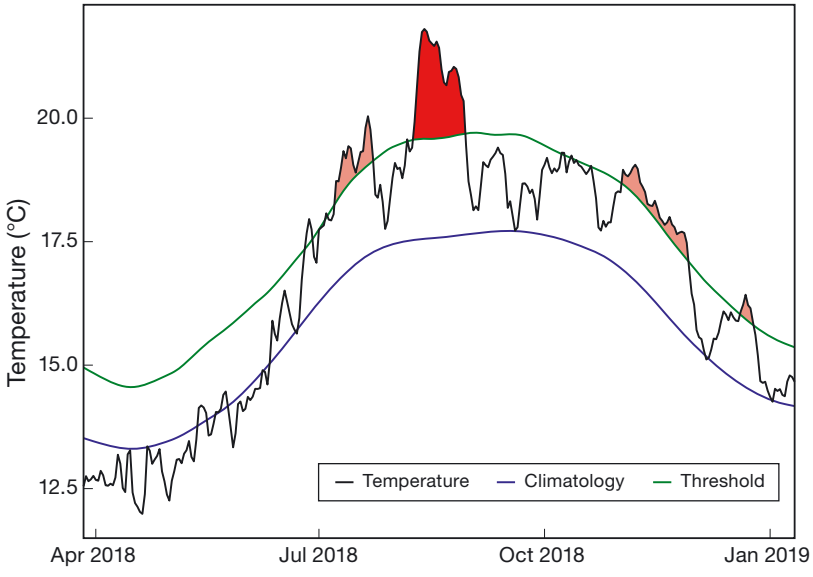


Fig. 4. Marine heatwave (MHW) events during the deployment period calculated using 'heatwaveR'. Long-term climatology (blue line), seasonal variation threshold at the 90th percentile (green line), and MHW events (red areas) were determined from sea surface temperature encompassing the region of the Santa Barbara Channel containing Arroyo Quemado Reef. Darker red shade denotes most severe event in the time frame

pCO_2 (IL) had intermediate LT_{50}, at 29.3 ± 0.1 and 29.5 ± 0.1°C, respectively. The same trends held for calculated LT_{10} and LT_{25} values, although overall variation was more extreme, with a difference of 1.4°C between the LT_{25} values and a difference of 2°C between the LT_{10} values of IH and OL larvae (Table 1). In pairwise contrasts between treatments, using a Tukey correction, all treatments were significantly different except for OH and IL, which displayed intermediate LT_{50} values, (IL–IH $p < 0.001$, IL–OL $p < 0.001$, IL–OH $p = 0.693$, IH–OL $p < 0.0001$, IH–OH $p = 0.012$, OL–OH $p < 0.0001$).

3.3. Metabolic rate

Using whole-animal respirometry, we saw that larval pCO_2 treatment seemed

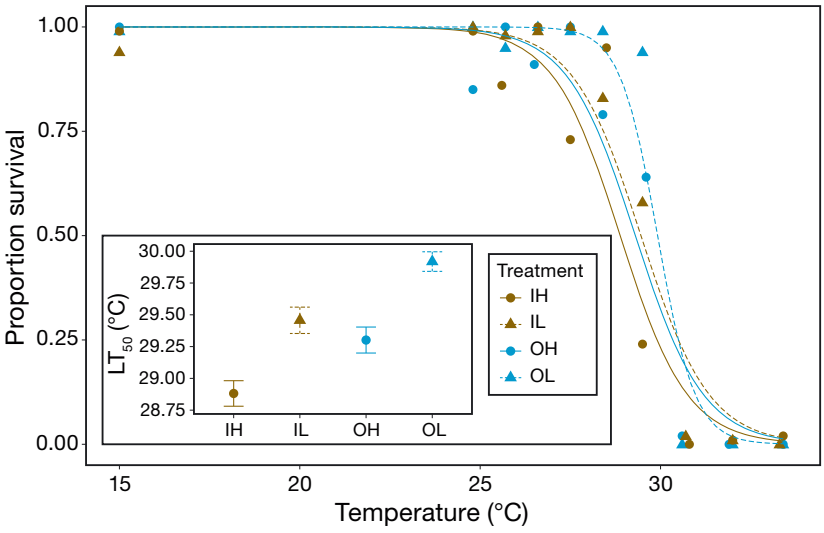


Fig. 5. Effect of maternal (I: inside or O: outside kelp forest) and larval (H: high pCO_2 or L: low pCO_2) treatment on *Strongylocentrotus purpuratus* prism larval thermal tolerance, measured as proportion survival after a 1 h acute heat shock and recovery. Acute heat shock temperature is shown on the *x*-axis. Maternal group is shown by color and larval treatment is shown by pattern; IL: brown, solid line; OL: blue, solid line; IH: brown, dashed line; OH: blue, dashed line. Inset displays the calculated lethal temperature (LT_{50}) for each treatment ± SD

Table 1. Lethal temperature (LT_{50}, LT_{25}, and LT_{10}) values for echinopluteus larvae from different maternal and larval treatments. Adult purple urchins were held in cages inside or outside of kelp forest for 6 mo prior to spawning. Urchin larvae were then reared under low pCO_2 (~435 µatm) or high pCO_2 (~1050 µatm) conditions. Values are given as mean ± SD

	Temperature treatment		
Mother – Offspring	LT_{10}	LT_{25}	LT_{50}
Inside – Low	27.4 ± 0.2	28.4 ± 0.1	29.5 ± 0.1
Inside – High	26.8 ± 0.2	27.9 ± 0.1	28.9 ± 0.1
Outside – Low	28.8 ± 0.1	29.3 ± 0.1	29.9 ± 0.1
Outside – High	27.2 ± 0.2	28.3 ± 0.1	29.3 ± 0.1

to have a greater impact on offspring from mothers conditioned inside the kelp than those from mothers conditioned outside, although the difference was not statistically significant. Larvae from inside-conditioned mothers raised under high pCO_2 conditions (IH) had the highest metabolic rate per unit size (mean ± SE: 17 094.86 ± 926.65 pmol O_2 h^{-1} mm^{-3}); the rate for IL larvae was lower (14 812.77 ± 744.60 pmol O_2 h^{-1} mm^{-3}) (Fig. 6). The metabolic rates for both groups of larvae from outside mothers were similar under both larval pCO_2 levels (15 415.00 ± 1651.14 pmol O_2 h^{-1} mm^{-3} for OH larvae and 15 250.96 ± 717.69 pmol O_2 h^{-1} mm^{-3} for OL larvae) (Fig. 6). Without size correction, the pattern was similar (IH: 12.11 ± 0.66 pmol O_2 h^{-1}; IL: 11.05 ± 0.56 pmol O_2 h^{-1}; OH: 10.56 ± 1.13 pmol O_2 h^{-1}; OL: 10.89 ± 0.51 pmol O_2 h^{-1}). There was no effect of maternal treatment (ANOVA: $F_{1,8}$ = 0.015, p = 0.906), larval treatment ($F_{1,8}$ = 2.212, p = 0.175), or their interaction ($F_{1,8}$ = 0.009, p = 0.927).

3.4. Morphometrics

Considering morphometrics, body length was similar across treatments, and initial skeletal development was impacted by the interaction between maternal and larval environment. At the prism stage, there was no significant effect of maternal or larval treatment or their interaction on body size (LMM: maternal-O df = 6.357, *t* = −0.939, p = 0.382, larval-L df = 412.295, *t* = 0.223, p = 0.824, maternal-O:larval-L df = 412.984, *t* = 1.570, *p* = 0.117). The average larval body

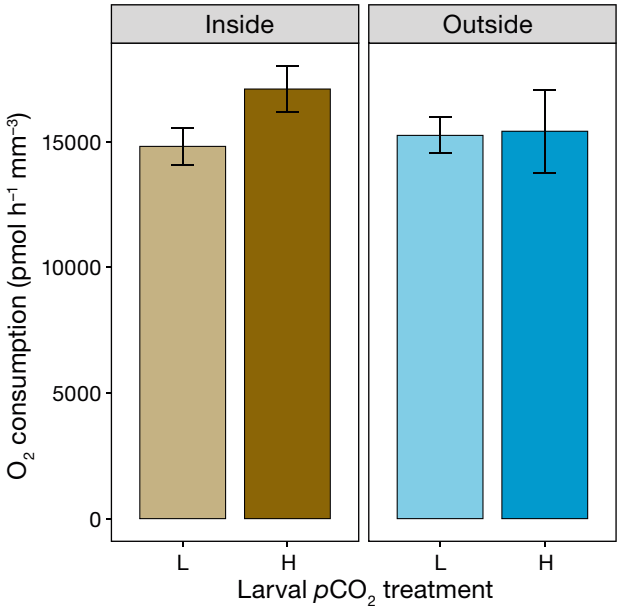


Fig. 6. Effect of maternal (I: inside or O: outside kelp forest) and larval (H: high pCO_2 or L: low pCO_2) treatment on size-corrected oxygen consumption of *Strongylocentrotus purpuratus* at the prism larval stage. The consumption rate from each culture bucket (n = 3 per treatment, n = 12 total) was calculated from a 5-point regression using µBOD vials containing a density gradient of larvae (n = 100–600 larvae per vial). The calculated consumption rates (n = 3 per treatment) were averaged to calculate the treatment average shown and size-corrected to the mean larval size in the corresponding treatment. Error bars represent standard error

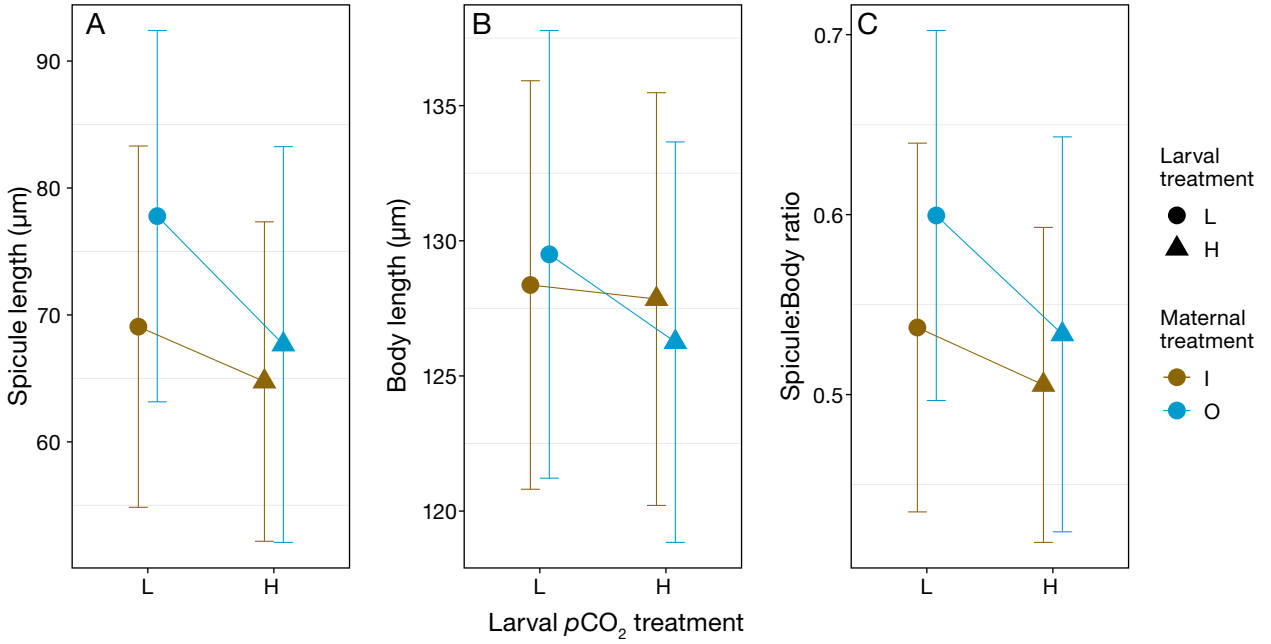


Fig. 7. Effect of maternal (I: inside or O: outside kelp forest) and larval (H: high pCO_2 or L: low pCO_2) treatment on (A) spicule length (µm), (B) body length (µm), and (C) spicule length to body length ratio. Error bars represent standard deviation

length was tightly constrained across treatments: ranging from 126.25 µm for OH larvae to 129.50 µm for OL larvae (Fig. 7B). The interaction between maternal and larval treatment was statistically significant (p = 0.028) for spicule length (maternal-O t = 0.419, df = 6.939, p = 0.688, larval-L t = 1.248, df = 412.441, p = 0.213, maternal-O:larval-L t = 2.209, df = 413.215, p = 0.028) and close to significant (p = 0.057) for spicule to body ratio (maternal-O t = 0.53, df = 4.43, p = 0.621, larval-L t = 1.31, df = 410.033, p = 0.191, maternal-O:larval-L t = 1.91, df = 410.56, p = 0.057). OL larvae had the longest spicules (77.78 ± SD 14.62 µm), followed by IL (69.08 ± 14.23 µm) and OH (67.67 ± 15.59 µm). IH larvae had the shortest spicules (64.75 ± 12.58 µm) (Fig. 7A). The ratio of spicule length to body length was highest for OL larvae (0.60 ± 0.10), followed by IL (0.54 ± 0.10) and OH (0.53 ± 0.11), while IH larvae had the lowest ratio of spicule length to body length (0.51 ± 0.09) (Fig. 7C). A Tukey test confirmed that OL larvae had significantly longer spicules (p < 0.0001) and a higher spicule to body ratio (p < 0.001) than OH larvae. All other treatments were not significantly different from one another in pairwise comparisons. For body length, OL larvae tended to be longer than OH larvae (p = 0.059), but all post hoc body length comparisons were insignificant at an alpha level of 0.05.

3.5. Eggs

With regard to traits of the eggs from females conditioned inside vs. outside the kelp, egg phenotype tended to be more variable among inside females compared to outside, but size and biochemical storage (lipid or protein) did not differ significantly between treatment groups. The eggs from inside females tended to be larger (92.49 ± 2.47 µm) than those from outside females (90.86 ± 2.25 µm). This difference in diameter was not statistically significant when taking into consideration variation between mothers of a given treatment as a random effect (ANOVA: $F_{1,16}$ = 3.6249, p = 0.075). There was no significant effect of maternal treatment on lipid content per egg (Welch's t-test, t = 0.93763, df = 14.230, p = 0.364) or size corrected per unit volume (t = 0.3694, df = 14.302, p = 0.717). There was also no significant effect of maternal treatment on protein content per egg (t = −0.36869, df = 15.248, p = 0.717) or size corrected per unit volume (t = −1.2298, df = 15.229, p = 0.237). In a PCA of egg traits and female test diameter, PC1 and PC2 accounted for 37.96 and 27.48% of the variation in the data, respectively (Fig. 8). Protein and lipid content per egg were negatively correlated in PCA space, while test diameter and egg volume were positively correlated with one another. Overall maternal treatments

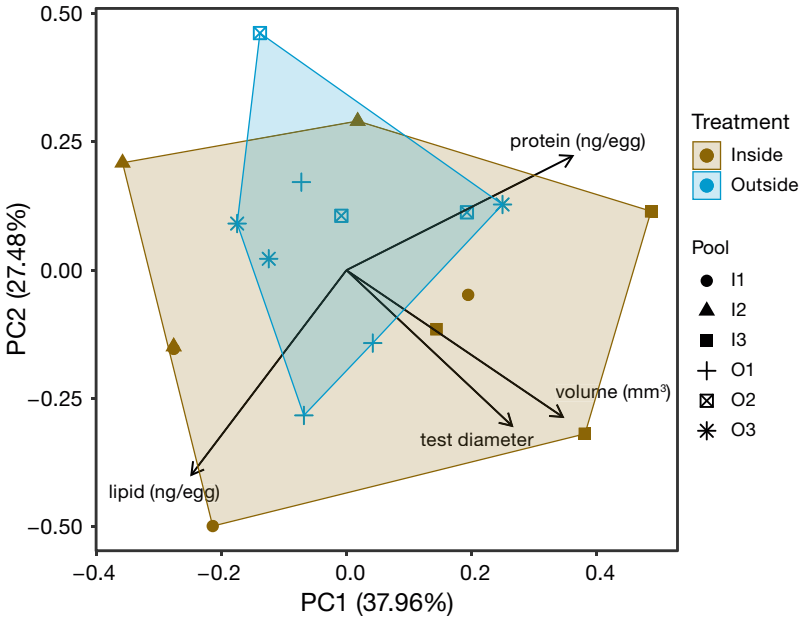


Fig. 8. Principal component analysis of *Strongylocentrotus purpuratus* egg measurements including egg volume, protein, and lipid, averaged to match individual female test diameter measurements. Different egg pools are shown by symbols

did not separate in PCA space, but the outside treatment encompassed less variation than the inside treatment.

4. DISCUSSION

4.1. Summary

Our goal was to assess the transgenerational effects of field acclimation to natural variability in water properties associated with a kelp forest on the purple urchin. As a foundation to the field experiment, we did find differences in abiotic conditions inside vs. outside the kelp forest, as has been noted by other investigators (Delille et al. 2009, Frieder et al. 2012, Hoshijima & Hofmann 2019, Hirsh et al. 2020). At our SBC study site, pH and temperature were generally higher, and the variability of pH, temperature, and oxygen was more predictable inside the kelp forest, while high-frequency variation was greater outside (Fig. 3). At the biological level, these physicochemical differences aligned with differences in the physiological parameters assessed in the larvae of differentially acclimatized mothers. Larvae from mothers conditioned inside the kelp displayed lower thermal tolerance, indicating potential increased vulnerability to future MHWs (Fig. 5). Conversely, larvae from outside-conditioned mothers raised under low pCO_2 had the highest thermal tolerance. The LT_{10} values observed in this experiment still have a buffer above MHW temperatures commonly experienced in the region. However, the thermal tolerance patterns observed indicate treatment level differences in the cellular and biochemical function of the urchin larvae, which may make urchins from inside sites, or those experiencing elevated pCO_2, more vulnerable to sub-lethal thermal stress at current MHW temperatures as well as the more extreme MHWs forecasted for the future in the North Pacific (Barkhordarian et al. 2022). Overall, the fitness implications of the observed pattern of transgenerational effects on larval traits may vary based on the compounding stressors of the larval environment at critical points in development.

4.2. Conditions inside and outside the kelp forest

The difference in average pH and temperature between inside and outside was likely due to the depth difference, time of year, and thermal regime during a MHW in addition to the biological activity of the kelp. We observed colder water offshore at the outside site, likely driven by the difference in water depth (~5 m) in addition to the flow attenuation created by the kelp (Gaylord et al. 2007). This difference may have been exacerbated by a series of MHWs that occurred in 2018 during the first half of our deployments which extended the vertical stratification of summer in addition to elevating temperatures throughout the water column (Fig. 4). This depth difference may also have contributed to the pH difference between inside and outside sites, with the inside showing elevated pH, particularly for the first half of our deployment. Depth can be a large determinant of pH difference at coastal sites, sometimes exhibiting more influence than horizontal spatial variation (Frieder et al. 2012, Koweek et al. 2017).

The elevated pH inside the kelp forest was largely concentrated in the first half of our deployments when temperatures were highest and the kelp canopy was very dense. As high temperatures persisted, the kelp began to die and the pH regimes across sites converged for the second half of the condition-

ing period, a pattern consistent with decreased photosynthetic activity from the kelp. In a prior study in the SBC with a similar experimental setup, Hoshijima & Hofmann (2019) found that pH and oxygen were slightly higher outside the kelp forest relative to inside, while temperature was slightly lower outside. However, the reef at which their sensors were deployed was far shallower, and the regime comparisons took place in spring when upwelling is stronger in the SBC. We also saw cooler temperatures outside the kelp forest; yet, the temperatures were higher at both of our sites than in their study, despite the deeper depth, indicative of the time of year and the thermal anomaly during our experiment. Recent studies conducted in the Monterey Bay region have seen that small differences in pH and DO associated with kelp were outweighed by site-specific impacts based on wave exposure and currents and that the buffering effects of kelp were isolated to a narrow depth band at the canopy and diminished at depth, indicating that any potential pH refuge was unlikely to benefit benthic animals (Hirsh et al. 2020, Traiger et al. 2022). Our study looked only at the benthic water properties, as our primary goal was to co-locate the physicochemical monitoring with our biological conditioning of the adult urchins, so we are unable to compare to the surface conditions. However, the discrepancy between our findings and those of other recent studies suggests that the differences observed may be spatiotemporally specific.

The most consistent pattern in seawater chemistry was that the inside site displayed more predictable variation, weighted towards lower frequencies, while the outside site showed less predictable, high-frequency variation (Fig. 3). These findings are consistent with those seen at a different shallower site, Mohawk Reef, in the SBC in spring, indicating that this pattern is likely persistent throughout the year (Hoshijima & Hofmann 2019). The extreme physicochemical variability of coastal sites across space and time necessitates improved monitoring of pH, DO, and temperature across a range of habitats and at high temporal resolution, as it is difficult to characterize and mimic all axes of variation accurately in the lab (Waldbusser & Salisbury 2014, Baumann et al. 2015). Field-based studies that leverage natural variations and gradients will clarify how different scales of variation impact the biology of critical species, and studying transgenerational effects *in situ* will illuminate how variability impacts recruitment and fitness across generations (Murray et al. 2014, Griffiths et al. 2021).

4.3. Transgenerational effects

We raised the larvae from females acclimated to the physicochemical regimes inside and outside an SBC kelp forest under high and low pCO_2 treatments in order to assess how their maternal conditioning impacted their capacity to deal with environmental stress. We saw that the differences in maternal conditioning in combination with the larval environment impacted the thermal tolerance of offspring as well as their skeletal growth. These findings indicate that the natural heterogeneity in coastal environments can have effects across generations and that the interaction between multiple stressors may impact the local success and survival of larvae of key species.

Similar to this study, Hoshijima & Hofmann (2019) did not see a significant difference in egg size, but found less variation in egg provisioning of proteins among outside-conditioned mothers. Previous lab experiments showed that eggs from females held under cooler, more acidic conditions (similar to the outside conditions in our field experiment) tended to be larger when compared to eggs from ambient-conditioned mothers, though not significantly (Wong et al. 2018, 2019). We saw the opposite pattern comparing eggs from mothers outside the kelp forest to those from mothers inside. Colder temperatures have been associated with larger egg volumes in several species (Pettersen et al. 2019). However, it is worth noting that although we detected a temperature difference between sites, the entire region was impacted by MHWs during this experiment, causing elevated temperatures both inside and outside the kelp forest.

After development under high or low pCO_2 conditions, we found a significant effect of larval treatment on spicule development mediated by maternal treatment, with outside larvae developing under high pCO_2 displaying reduced skeletal formation. The pattern of reduced spicule growth under high pCO_2 has been seen in prior studies (Yu et al. 2011, Padilla-Gamiño et al. 2013, Strader et al. 2020). This contrasts with the lab experiment of Wong et al. (2019), who found that the maternal conditioning environment impacted skeletal growth at the prism stage while developmental pCO_2 treatment did not. In our investigation, prism larvae from outside mothers raised at low pCO_2 (OL) had significantly greater skeletal development and a significantly higher spicule to body ratio than their counterparts under high pCO_2. In contrast, at the earlier hatched blastula stage, Hoshijima & Hofmann (2019) saw that larvae which came from outside mothers but were raised under high pCO_2 conditions (OH) had larger body size than

all other treatment groups. Interestingly, in both cases, the largest embryos and larvae came from outside-conditioned mothers. The differences in the impact of the developmental treatment on body size between the 2 studies could be due the crossing design employed, or the developmental stage assessed. Larvae are generally more vulnerable to pH and temperature stress than embryos, and the onset of calcification begins between the stages investigated in these 2 studies (Przeslawski et al. 2015). In our study, this elevated performance of OL larvae was matched with elevated thermal tolerance relative to the other treatment groups.

Within each maternal treatment group, those larvae which developed under high pCO_2 were less tolerant of an additional stressor in the form of acute thermal exposure than those which developed under low pCO_2. This pattern varies from those seen by Karelitz et al. (2017), who found that larval thermal tolerance of 5 echinoderm species was not diminished by low pH exposure. In red urchins *Mesocentrotus franciscanus*, developmental pCO_2 treatment also did not impact thermal tolerance of prism larvae (Wong & Hofmann 2020). However, similar to our findings, elevated pCO_2 can impact thermal stress response in *M. fransciscanus* at the pluteus stage (O'Donnell et al. 2009). Within each larval treatment (high and low pCO_2), larvae which came from outside mothers had higher thermal tolerance than those from inside mothers. Adult exposure to warmer temperatures can increase developmental thermal tolerance in some species of urchins (Pecorino et al. 2013); however, in our study, the larvae whose mothers were at the deeper, cooler site displayed higher larval thermal tolerance. There was no significant effect of maternal treatment on larval metabolic rate; however, larval high pCO_2 exposure seemed to induce elevated metabolic rate only in larvae from in side-conditioned mothers. Effects of acidic conditions on larval metabo lic rates vary with larval stage, feeding or non-feeding, and are mediated by other stressors (Stumpp et al. 2011, Padilla-Gamiño et al. 2013).

The implicati ons of the larval performance metrics are likely context dependent. Purple urchin larvae reach their feeding larval stage, pluteus, after ~48 h, followed by a planktonic pelagic larval duration of 29–86 d (Strathmann 1978). The oceanographic conditions during their first lecithotrophic 48 h of initial development, or the following month(s) feeding prior to settlement will have large consequences on their survival. With the rise of MHWs and the forecasted increase in the frequency and duration of upwelling, there is an extreme range of oceanographic conditions in which larvae could develop (Feely et al. 2008, Frölicher et al. 2018). Interannual variability and slight differences in spawning time could lead to larvae recruiting under drastically different thermal, pH, and/or oxygen regimes. Larval settlement patterns for the purple urchin vary based on temperature and climate variations and are negatively correlated with SST in the SBC (Okamoto et al. 2020). In our experiment, larvae from outside-conditioned mothers showed higher acute thermal stress tolerance. As the larval stage is a major bottleneck in the lifecycle, if the larvae enter the water column during an MHW, larvae from outside mothers or deeper sites likely have increased probability of survival. OL larvae also had the greatest skeletal growth, which could be advantageous in avoiding predation or acquiring food in low-food conditions (Hart & Strathmann 1994, Hart 1995, Allen 2008, Chan et al. 2011). Therefore, the combination of stressors experienced during key times of their development might determine the benefit of any maternal transgenerational effect (Byrne & Przeslawski 2013).

This experiment isolated the effect of the physicochemical environment inside and outside a kelp forest, by feeding the urchins consistently to control for food availability. However, the availability and composition of food would vary drastically between sites in nature, with strong implications for maternal provisioning. Inside-conditioned urchins might have higher metabolic costs due to warmer temperatures but also have access to kelp, whereas outside-conditioned urchins would rely on other food sources including diatoms and encrusting algae. Urchins in barrens therefore have different, and even more diverse, microbiomes which may influence their metabolism and stress response (Marangon et al. 2021, Miller et al. 2021). The nutritional quality of kelp itself declines at warmer temperatures, which would drive urchins to eat more to meet higher metabolic costs under MHW regimes (Lowman et al. 2022). Therefore, the balance between the abiotic environment and food availability would reshape the consequences of each acclimation regime.

Overall, isolating the physiochemical differences by site, the results of this study indicate a benefit of maternal conditioning outside a kelp forest. The relative stress of a lower pH environment outside the kelp forest may have primed the offspring of those mothers with increased resilience to environmental stress, allowing them to increase skeletal development under low pCO_2 regimes and endure acute thermal stress. The relative predictability of the inside environment would be more likely to induce TGP

(Burgess & Marshall 2014). However, it is also possible that the high-frequency variability of the outside site induced epigenetic modifications, enhancing the plasticity of gene regulation to respond to rapid change which could have passed down to the offspring. Although it was not intended in the design of the study, an MHW persisted through a significant proportion of the adult field acclimation. Therefore, temperature differences across the 2 sites may likely have been the most important driver of transgenerational effects during acclimation. While temperatures were elevated at both sites, the relatively lower temperature at the deeper, less sheltered, outside site may have provided a relative thermal refuge for those mothers.

4.4. Kelp, urchins, and MHWs

One inadvertent aspect of this field experiment was that during late summer and early fall of 2018, several MHWs occurred in our study region. Specifically, from late June to December, 5 MHW events occurred on the benthos at our study site and were 7–21 d long with a total of 76 d of extreme thermal stress; 4 MHW events were detectable channel-wide using SST data (Fig. 4). This temperature regime likely interacted strongly with pH and DO and influenced our TGP results (Bautista & Crespel 2021). Giant kelp forests provide critical habitat and food as a foundation species in the CCS, and urchins shape this ecosystem as dominant herbivores. Recently, MHWs have caused shifts in kelp forest community structure in the SBC (Michaud et al. 2022). Therefore, it is essential to consider how giant kelp and key grazers such as urchins, and their ecological relationship, will be impacted by future climate change (Starko et al. 2022).

MHWs can occur in the SBC during key periods of the purple urchin life cycle such as gametogenesis (summer–fall) as well as early larval development through metamorphosis (winter) (Chamorro et al. 2023; Fig. 4). MHWs may impact the dispersal and recruitment of purple urchin larvae, influencing gene flow and population dynamics (Byrne 2011, Okamoto et al. 2020). Where settlement is not reduced, elevated temperatures may shorten pelagic larval duration, impacting dispersal (O'Connor et al. 2007, Byrne 2011). Adult exposure to MHWs may have negative effects on gonad quality or increase susceptibility to disease (Tajima et al. 1997, Uthicke et al. 2014). Sperm of male urchins exposed to MHW temperatures during spermatogenesis displays reduced fertilization success (Leach et al. 2021), underscoring the importance of considering transgenerational impacts through both parental lines, not solely the maternal line as in our study. Temperature variation across depth and small spatial scales has a significant impact on kelp resilience through MHW events, yet biotic interactions with urchin populations can regulate the capacity for cool areas to serve as kelp refugia (Starko et al. 2022). Since we found that water property differences associated with depth and macrophyte habitat can have transgenerational effects on larval performance including acute heat tolerance, this could exacerbate biotic feedbacks between kelp and urchin populations. MHWs can impact adult populations and can have transgenerational effects influencing early bottlenecks such as fertilization; even sub-lethal exposure at early life stages may have negative carryover effects at later life stages (Byrne 2011, Przeslawski et al. 2015). Overall, it is crucial to consider the interaction of MHWs, such as the one which occurred during the acclimation period of our experiment, with environmental heterogeneity in factors such as salinity, pH, and DO within and across generations, as these interactions are most often synergistic (Przeslawski et al. 2015), and conditions in the North Pacific are likely to support even more extreme MHWs in the future (Barkhordarian et al. 2022).

4.5. Concluding statements

Overall, in this study, larvae from mothers conditioned outside the kelp forest performed better on average when developing under high pCO_2 conditions, displaying increased thermal tolerance compared to their counterparts from inside-conditioned mothers. The fitness implications of the transgenerational effects observed may vary based on the compounding stressors of the larval environment at critical points in their development. It is difficult for multi-stressor lab studies to capture the full complexity of variability experienced across space and time in ecosystems such as coastal kelp forests, and therefore should be complemented by field-based monitoring and acclimation experiments.

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Appendix.

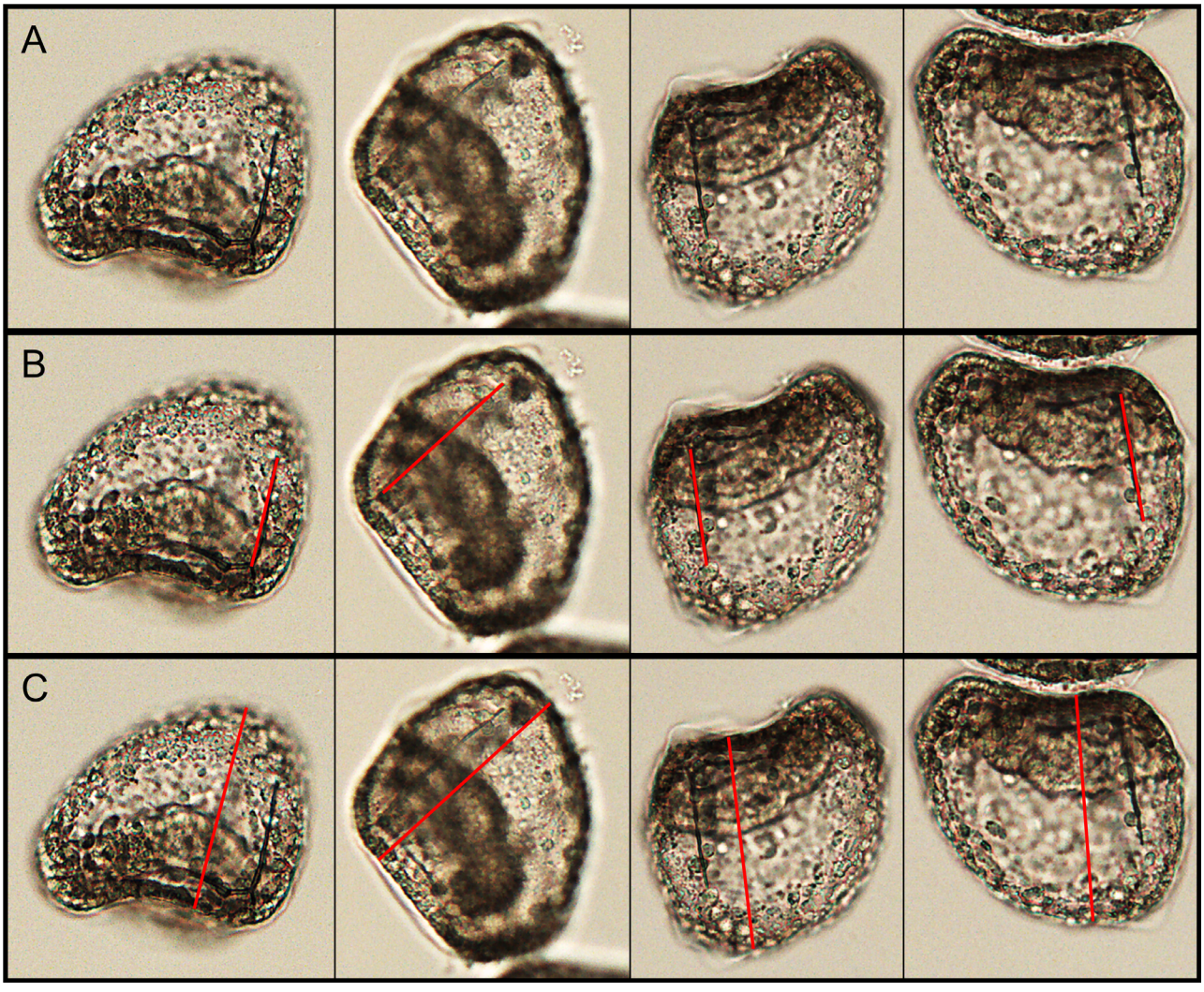

Fig. A1. (A) Prism urchin larvae, with examples of how measurements were taken for (B) spicule length and (C) body length

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