Journal of Experimental Marine Biology and Ecology xxx (xxxx) xxx–xxx

Contents lists available at ScienceDirect

Journal of Experimental Marine Biology and Ecology

journal homepage: www.elsevier.com/locate/jembe

Transgenerational effects in an ecological context: Conditioning of adult sea urchins to upwelling conditions alters maternal provisioning and progeny phenotype

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ARTICLE INFO

Keywords:
Transgenerational plasticity
Marine invertebrate
Strongylocentrotus purpuratus
Maternal effects
Global change

ABSTRACT

Transgenerational plasticity occurs when the conditions experienced by the parental generation influence the phenotype of their progeny. This may in turn affect progeny performance and physiological tolerance, providing a means by which organisms cope with rapid environmental change. We conditioned adult purple sea urchins, *Strongylocentrotus purpuratus*, to combined pCO_2 and temperature conditions reflective of in situ conditions of their natural habitat, the benthos in kelp forests of nearshore California, and then assessed the performance of their progeny raised under different pCO_2 levels. Adults were conditioned during gametogenesis to treatments that reflected static non-upwelling (~650 μatm pCO_2, ~17 °C) and upwelling (~1300 μatm pCO_2, ~13 °C) conditions. Following approximately 4 months of conditioning, the adults were spawned and embryos were raised under low pCO_2 (~450 μatm pCO_2) or high pCO_2 (~1050 μatm pCO_2) treatments to determine if differential maternal conditioning impacted the progeny response to a single abiotic stressor: pCO_2. We examined the size, protein content, and lipid content of eggs from both sets of conditioned female urchins. Offspring were sampled at four stages of early development: hatched blastula, gastrula, prism, and echinopluteus. This resulted in four sets of offspring: (1) progeny from non-upwelling-conditioned mothers raised under low pCO_2, (2) progeny from non-upwelling-conditioned mothers raised under high pCO_2, (3) progeny from upwelling-conditioned mothers raised under low pCO_2, and (4) progeny from upwelling-conditioned mothers raised under high pCO_2. We then assessed the effects of maternal conditioning along with the effects of developmental pCO_2 levels on body size of the progeny. Our results showed that differential maternal conditioning had no impact on average egg size, although non-upwelling females produced eggs that were more variable in size. Maternal conditioning did not affect protein content but did have a modest impact on egg lipid content. Developing embryos whose mothers were conditioned to simulated upwelling conditions (~1300 μatm pCO_2, ~13 °C) were greater in body size, although this effect was no longer evident at the echinopluteus larval stage. Although maternal conditioning affected offspring body size, the pCO_2 levels under which the embryos were raised did not. Overall, this laboratory study provides insight into how transgenerational effects may function in nature. The impacts of parental environmental history on progeny phenotype during early development have important implications regarding recruitment success and population-level effects.

1. Introduction

Mechanisms that alter phenotypic plasticity are recognized as potential biological responses to climate change (Buckley and Kingsolver, 2012; Hoffmann and Sgro, 2011; Munday et al., 2013; Reusch, 2014; Somero, 2012), and importantly, can drive a rapid shift in physiological performance and the capacity to resist abiotic stress, a shift that occurs on ecological rather than evolutionary timescales. Recently, mechanisms involved in transgenerational plasticity (TGP) in marine metazoans have attracted interest within the research community

DOI of original article: https://doi.org/10.1016/j.jembe.2019.03.002

Abbreviations: TGP, transgenerational plasticity; CCS, California Current System; FSW, filtered seawater; hpf, hours post-fertilization; TAG, triacylglycerol; ST, sterol; PL, phospholipid; PCA, principal component analysis

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https://doi.org/10.1016/j.jembe.2019.04.006
Received 25 September 2018; Received in revised form 16 March 2019; Accepted 17 April 2019

(Chirgwin et al., 2018; De Wit et al., 2016; Hofmann, 2017; Marshall et al., 2016; Munday, 2014; Putnam and Gates, 2015; Ross et al., 2016). Here, the research goal has been to explore how and whether the environmental history of the parents, driven by maternal provisioning or epigenetic mechanisms, has a role in bolstering the resistance of progeny to future anthropogenic changes in marine ecosystems. Alternatively, TGP may be disadvantageous if environmental stress causes a reduction in offspring quality (Putnam and Gates, 2015; Shama and Wegner, 2014), or if a significant mismatch between parental and offspring environments leads to progeny that are poorly prepared for their environment (Bondurianski et al., 2012; Putnam and Gates, 2015; Reed et al., 2010). Numerous recent studies indicate that conditioning of the parental generation does alter characteristics of the progeny, and in some cases, confers resistance and tolerance to abiotic stress in a global change context (Munday, 2014; Ross et al., 2016; Shama et al., 2014). Investigating TGP in a global change context has expanded our understanding of the role of phenotypic plasticity as a first response to anthropogenic global change, and importantly, the potential of application in conservation efforts (Chakravarti et al., 2016). The goal of this study was to examine TGP, in the form of maternal effects, in early stage purple sea urchins, *Strongylocentrotus purpuratus* (Stimpson, 1857), and to contribute to insights on the 'plasticity first' hypothesis (Levis and Pfennig, 2016; Schwander and Leimar, 2011) in marine organisms.

Examples of TGP in marine metazoans have been described in the literature, with most studies conducted in the laboratory (Donelson et al., 2017; Munday, 2014; Ross et al., 2016). Examples of environmental conditioning of adults in situ have also been reported. Murray and colleagues demonstrated TGP in a wild population of Atlantic silverside, *Menidia menidia*, whose natural habitat was characterized by an annual pH decline throughout their spawning season (Murray et al., 2014). Offspring produced later in the spawning season exhibited greater tolerance to high CO_2, suggesting an effect of in situ parental exposure to seasonal acidification. Similarly, in the European squid, *Loligo vulgaris*, the phenology of egg laying (i.e., summer versus winter) influenced the response of early developmental stages to ocean acidification and warming (Rosa et al., 2014). Overall, TGP may fulfill the role of a fast-acting response mechanism to rapid environmental change, providing time for and potentially assisting adaptive evolution processes. Here, the basic concept is that the variant forms of early life stages have differential performance in a dynamic environment and, if that phenotype is heritable, it could create the grist for future adaptation, and possible evolutionary rescue (Levis and Pfennig, 2016; Marshall et al., 2016). In an eco-evolutionary context, evolution of traits is a process that occurs in response to environmental change, and thus, shifts in progeny traits via TGP is a likely mechanism of rapid response to climate change (Hendry, 2016).

An inherent challenge in understanding the significance of TGP in a global change context is that, in many cases, we often know relatively little about what early stages experience in situ. Increasingly, investigators are realizing that natural variability in the marine environment is selecting for tolerant genotypes, and that local adaptation likely plays a key role on a biogeographic scale (Hofmann et al., 2014; Kelly et al., 2017; Pereira et al., 2017; Sanford and Kelly, 2011; Vargas et al., 2017). Such observations have been made for tropical corals (Barshis et al., 2013), sea urchins (Evans et al., 2017; Kelly et al., 2013), abalone (De Wit and Palumbi, 2013) and other marine snails (Calosi et al., 2017; Gleason and Burton, 2013), and intertidal copepods (Kelly et al., 2017). Recent research has indicated that local adaptation will likely influence organismal responses to future ocean change (Calosi et al., 2017; Pereira et al., 2017).

Importantly, field observations of pH and temperature conditions are increasingly available, an advance that supports studies aimed at coupling natural conditions with experimental approaches. For example, in the California Current System (CCS), episodic upwelling dominates the region (Feely et al., 2008), and the progression of ocean acidification is expected to result in dramatic changes to ocean chemistry in this nearshore region (Gruber et al., 2012). Recently, Chan and colleagues reported that waters corrosive to aragonite, a more soluble form of calcium carbonate, currently cover large coastal areas of the CCS, and that upwelled waters already display the effects of increased anthropogenic CO_2 contributions following the Industrial Revolution (Chan et al., 2017). Oceanographic observations from the kelp forest show that pH and temperature conditions are highly variable, likely driven by biological processes such as photosynthesis and respiration, interacting with episodic upwelling (Frieder et al., 2012; Hoshijima, 2018; Hoshijima and Hofmann, 2019; Kapsenberg and Hofmann, 2016; Koweek et al., 2017). Overall, the present-day physicochemical environment of the kelp forest creates a highly variable mosaic of pH and temperature conditions that likely influences the history of adults, with gradients existing in relation to the proximity to kelp (e.g., inside versus outside kelp bed differences) (Hoshijima and Hofmann, 2019). Here, we used a simplified set of conditions as a representation of two distinct conditions that can be observed in kelp forest environments in situ. We did not incorporate the full extent of variability that is observed, but instead focused on the question: does differential conditioning of the parents result in progeny with different properties? Thus, this study represents the first steps to examine whether adult conditioning of a benthic marine invertebrate in the natural physicochemical environment of a kelp forest might induce variable performance in the progeny.

Here, we conditioned adult *S. purpuratus* to two divergent temperatures and pCO_2 levels during gametogenesis that represent end-member conditions in the kelp forest during upwelling and non-upwelling (i.e., relaxed) conditions (Kapsenberg and Hofmann, 2016; Koweek et al., 2017). To examine if maternal conditioning to different pCO_2 levels and temperatures elicited a transgenerational effect, the offspring of differentially conditioned mothers were tested using two pCO_2 levels at one temperature representative of conditions at the time of year the embryos and larvae would be in the water column, in this case, 15 °C. Thus, offspring of the females conditioned under the upwelling and non-upwelling treatments were raised at the same temperature, but under either a low or high pCO_2 level. Sea urchins are vulnerable to high pCO_2 (i.e., low pH) conditions, particularly during their early development (Byrne, 2011; Byrne et al., 2013b; Byrne and Przeslawski, 2013; Clark et al., 2009). Exposure to high pCO_2 levels can affect body size (Byrne et al., 2013a; Kurihara and Shirayama, 2004; O'Donnell et al., 2009; Sheppard Brennand et al., 2010), internal acid-base balance (Miles et al., 2007), metabolic processes (Padilla-Gamiño et al., 2013; Stumpp et al., 2011b), and gene expression (Evans et al., 2013; Pespeni et al., 2013; Stumpp et al., 2011a; Wong et al., 2018). Therefore, investigating how maternal conditioning influences the response of the progeny to high pCO_2 conditions represents a valuable and ecologically relevant opportunity to explore TGP in *S. purpuratus*.

In a previous study using a similar experimental design and laboratory conditions, we found maternal conditioning affected the transcriptome of the progeny, and gene expression patterns suggested that the offspring of upwelling-conditioned mothers may be primed to respond to high pCO_2 levels (Wong et al., 2018). In this study, we report the size, protein content, and lipid content of the eggs from differently conditioned females to evaluate maternal provisioning and egg quality. We also report a response variable in the form of body size that was assessed across four stages of early development to evaluate changes in performance of the progeny relative to the conditioning of the adults. In a companion study, DNA methylation was examined as an epigenetic mechanism that potentially mediates transgenerational and intragenerational plasticity in *S. purpuratus* (Strader et al., 2019). Although transgenerational effects can be maternal, paternal, or a combination of both, only maternal contributions to TGP were examined. Specifically, we examined an average maternal effect across a pool of genotypes, using an experimental approach that mimics female mass spawning events that occur in nature. Our results indicated that there is a transgenerational effect that links environmental conditioning of the

mothers to changes in phenotype of the early life stages. In nature, such an outcome could drive differences in progeny dispersal and fitness.

2. Material and methods

2.1. Animal collection and conditioning

Approximately 90 adult purple sea urchins, *S. purpuratus*, were hand-collected on SCUBA on October 5, 2016 from a site in the Santa Barbara Channel near Goleta Beach, Goleta, CA (34° 24.840′ N, 119° 49.742′ W). Urchins were transported to the Hofmann Lab at UC Santa Barbara, where they were held in flow-through seawater tanks for approximately 2.5 weeks prior to adult conditioning. To begin the adult conditioning trial, urchins were randomly sorted into 20-gal glass treatment tanks (8–13 urchins per tank). There were three replicate tanks for each of two conditioning treatments, for a total of 6 treatment tanks. Adults were held under two combined pCO_2 and temperature experimental conditions: (1) a non-upwelling treatment (N: ~650 µatm pCO_2, ~17 °C) and an upwelling treatment (U: ~1300 µatm pCO_2, ~13 °C). These two static conditions represent ecologically relevant values observed using ocean sensors deployed in the Santa Barbara Channel (Kapsenberg and Hofmann, 2016; Rivest et al., 2016). Adult urchins were maintained at these two conditioning treatments for approximately four months, and were fed an excess of kelp blades (*Macrocystis pyrifera*) three days per week.

Conditioning treatments were maintained using a flow-through CO_2 mixing system modified from Fangue et al. (2010) to reach the target pCO_2 levels, and two Delta Star® heat pumps with Nema 4× digital temperature controllers (AquaLogic, San Diego, CA, USA) to control the temperature. Treated seawater flowed to each adult conditioning tank at a rate of 20 L/h. Aquaria were fitted with a small fountain pump (Total Pond, 70gph) to aid in water mixing. Every one to two days, temperature (Omega HH81A), salinity (YSI-3100) and spectrophotometric pH (Shimadzu UV-1800 and m-cresol dye) were analyzed according to best-practice procedures outlined by Dickson et al. (2007b). Total alkalinity was measured every three to five days (Mettler-Toledo T50) according to best-practice procedures outlined by Dickson et al. (2007a).

Measured spectrophotometric pH, total alkalinity, salinity, and temperature were used to calculate in-situ carbonate chemistry parameters using CO_2calc (Robbins et al., 2010) with equilibrium constants from Mehrbach and colleagues (Mehrbach et al., 1973) refit by Dickson and Millero (1987). As total alkalinity was taken less periodically than the other parameters, calculations were conducted using the last recorded alkalinity sample.

2.2. Spawning and egg assessment

At the conclusion of adult conditioning, spawning was induced via injection of 0.53 M KCl into the coelom. Sperm was collected dry and stored on ice until activation. Eggs from each female were collected in filtered seawater (FSW) (i.e., UV-sterilized seawater filtered to 0.35 µm). A subset of eggs ($n > 100$) from each individual female was visually inspected for maturity (i.e., ova that lack large, visible germinal vesicles) and tested for male-female compatibility to ensure the use of only high quality, viable eggs. Nine females from each maternal treatment were selected to contribute eggs for egg analyses and subsequent embryo culturing (Methods Section 2.3). Prior to conducting fertilizations, eggs from each individual female were sampled, resulting in eggs from 18 separate females for analysis (i.e., $n = 9$ females per maternal treatment). Size, total protein, and lipid content were measured for the eggs.

2.2.1. Egg morphometric analyses

Samples for morphometric analyses were preserved by adding 4% formalin in 0.01 M phosphate buffered saline (PBS) to an equal volume of seawater with eggs, fixing the samples in a final concentration of 2% formalin in seawater. Formalin preservation has been shown to cause eggs to shrink slightly in some echinoderm species (Lessios, 1987). However, all eggs were preserved using the same formalin and methods, so any formalin effects should have affected all eggs equally. Samples were stored at 4 °C until imaged, for no longer than two months. Eggs were digitally photographed under bright field DIC illumination on a compound microscope (Olympus BX50) with an attached digital camera (Infinity Lite) using Infinity Capture software (version 6.2.0). Digital images were calibrated using a stage micrometer for the 20× objective using ImageJ (National Institutes of Health, USA). Eggs ($n \geq 20$) from each of 18 separate females (i.e., eggs from $n = 9$ females from each maternal treatment) were photographed for morphometric analyses. All digital images were analyzed using ImageJ. Eggs were measured by their average diameter and total 2-dimensional (2D) area (see Supplementary material, Fig. S1). The average diameter of the eggs was determined by taking the average of three independent diameter measurements for each egg.

2.2.2. Protein quantification

To preserve samples for protein quantification, approximately 5000 eggs were placed in a 1.5 mL Eppendorf tube. The samples were pelleted by centrifugation, excess seawater was carefully removed using a pipette, and the tube was immediately frozen in liquid nitrogen and stored at −80 °C. Three tubes of eggs from each of the 18 separate females (i.e., eggs from n = 9 females from each maternal treatment) were used to quantify protein content. Protein samples were extracted using methods 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 (Sigma-Aldrich) using a Sonic Dismembrator 550 (Fisher Scientific). Samples were shaken on ice for 15 min and centrifuged for 20 min at 14,000 rpm. The retained supernatant was diluted 1:2 to 1:4 in deionized water and total soluble protein was quantified at 562 nm on a microplate reader (Bio-Rad) using a BCA protein assay kit following manufacturer's instructions (Catalog number 23225, Pierce Biotechnology, Rockford, IL, USA). Protein density (ng/nL) and energy content from protein (mJ) were estimated (see Supplementary material).

2.2.3. Lipid content

Eggs for lipid extractions were preserved using the same methods described for protein quantification (5000 eggs per tube). Three tubes of eggs from each of the 18 separate females (i.e., eggs from n = 9 females from each maternal treatment) were analyzed for lipid content. Lipids were extracted from frozen egg samples following the methods described by Sewell (2005), with some modifications. Each sample was sonicated on ice for three 25-s bursts. Samples were transferred to 1 mL glass V-vials (Wheaton) and combined with 250 µL of methanol, 75 µL of chloroform, and 50 µL of ketone internal standard (1 µg/µL in 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; 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 loading the samples, the extract was dried down using nitrogen gas and re-suspended in 50 µL of chloroform. The lipid classes were separated and quantified using an Iatroscan MK-6/6 s thin layer chromatography/flame ionization system and silica gel S-4 Chromarods (Parrish, 1987, 1999). For the chromatographic separation, 10 µL of sample was added to each of two duplicate rods. The rods were developed under a double development system modified from Parrish (1999). Following sample application, the rods were first

developed in hexane:diethyl ether:acetic acid (98.95:1.0:0.05 by volume) for 25 min, dried for 5 min at 60 °C in a drying oven, and partially scanned through the ketone peak. The rods were then developed in hexane:diethyl ether:acetic acid (79:20:1 by volume) for 40 min, dried in the same manner, and fully scanned. Chromatographs were collected using the software Peak Simple (version 4.54; SRI Inc.). Raw values were calibrated using a multilevel calibration curve as outlined by (Matson et al., 2012). Free fatty acid (palmitic acid) was excluded from our calibration solutions as it is not detected in unfed larvae and eggs of *S. purpuratus* (Meyer et al., 2007a).

Although ketone (KET; 3-hexadecanone) was included as an internal standard as a means of calculating percent yield, it was ultimately not used to adjust the final lipid concentrations because we found that the concentration of ketone in our lipid extractions was not a reliable indicator of total yield (see Supplementary material for additional detail). Aliphatic hydrocarbon (Nonadecane) was consistently at or below the level of detection for this method (Matson et al., 2012), and was not used in our quantification of lipids. Therefore, total lipid content was represented by one energy storage lipid class: triacylglycerols (TAG; tripalmitin), and two structural lipid classes: sterol (ST; cholesterol) and phospholipid (PL; L-α-phosphatidylcholine). Lipid density (ng/nL) and energy content from lipids (mJ) were also estimated (see Supplementary material).

2.2.4. Principal component analysis

A principal component analysis (PCA) biplot was used to visualize the data from all three analyses in tandem to provide an overall assessment of the eggs. The PCA biplot was created in R (version 3.3.3) using a combination of data from eggs of each female urchin. The data used to generate the biplot included: 1) morphometric data (i.e., average egg diameter), 2) protein data (ng of protein per egg), and 3) lipid data (ng of total lipid per egg). The average of each data set was combined by individual female identity (n = 9 females from each maternal treatment). Groupings (i.e., eggs from either non-upwelling (N) or upwelling (U) females) were tested using a PERMANOVA technique.

2.3. Embryo and larval culturing

An equal number of eggs from each female were collected and gently pooled together by treatment. This was meant to simulate the mixture of genotypes that occurs during mass spawning events in nature, while also ensuring that each female contributed equally to the pools of eggs. This resulted in two pools of eggs, in which one pool was composed of eggs from 9 females acclimated to non-upwelling conditions and one pool was composed of eggs from 9 females acclimated to upwelling conditions. Because this study is focused on only the maternal contributions to TGP, and to somewhat limit genotypic diversity that would otherwise confound analyses, only a single male conditioned to non-upwelling conditions was crossed with both groups of females, resulting in only half- and full-sibling progeny (Fig. 1). Sperm was collected dry and activated with FSW. Dilute, activated sperm was added to each pool of eggs until at least 95% fertilization success was reached.

Embryos from each cross were divided and transferred into either low pCO_2 (L: ~450 µatm) or high pCO_2 (H: ~1050 µatm) conditions. All embryos were raised at the same temperature, ~15 °C, to isolate the observed response to one factor (i.e., different pCO_2 levels) as well as to ensure synchronous development and consistent sampling across all stages. This experimental design resulted in four sets of treatments: (1) progeny of non-upwelling mothers raised in low pCO_2 conditions (NL), (2) progeny of non-upwelling mothers raised in high pCO_2 conditions (NH), (3) progeny of upwelling mothers raised in low pCO_2 conditions (UL), and (4) progeny of upwelling mothers raised in high pCO_2 conditions (UH) (Fig. 1). Approximately 150,000 embryos were added to each culture vessel immediately after verification that fertilization success was ≥95%. The embryos for each treatment (i.e., NL, NH, UL and UH) were cultured in triplicate (i.e., 12 total culture vessels). Each culture vessel was composed of two, nested 5-gal buckets with a flow-through rate of 6 L/h. Each vessel was fitted with a small paddle attached to a 12-V motor to aid in gentle mixing of the cultures. Temperature, salinity, and pH measurements were taken daily for the cultures, following the same procedure implemented during adult conditioning.

Once culturing commenced, embryos and larvae were sampled from each culture vessel at four discrete stages of early development: hatched blastula, gastrula, prism, and early echinopluteus. To maintain consistent pCO_2 and temperature conditions, seawater was filtered for embryos or larvae and immediately replenished to the culture vessels during sampling. Additionally, due to the flow-through nature of our culturing system, treated seawater was continuously added to the culture vessels at 6 L/h during all sampling processes. As a result, it was not feasible to accurately determine the concentration of embryos and larvae within each culture vessel at each developmental stage, and therefore, mortality rates could not be accurately assessed throughout development.

Generally, the developmental schedule adhered to what might be expected for *S. purpuratus* (Strathmann, 1987). At 15 °C, blastulae were sampled at ~18 h post-fertilization (hpf). The blastula stage was designated by the development of cilia and the occurrence of hatching and swimming. At ~30 hpf, the gastrula stage was characterized by the formation and extension of the archenteron to 1/2 of the total body length. At ~45 hpf, the archenteron had grown towards one side of the body and become tripartite. At this time, the prism stage was designated by the first development of skeletal rods and the formation of a pyramid-like body shape. Finally, at ~70 hpf, the early echinopluteus stage was sampled, by which time the larvae had developed defined internal structures, including the mouth, esophagus, stomach, anus, anterolateral and postoral skeletal body rods, as well as the early formation of feeding arms. While echinopluteus larvae are capable of feeding, larvae were sampled immediately upon reaching this stage. As such, it was not necessary to feed the larvae and potential starvation did not impact the findings of this study.

2.4. Morphometric analyses of embryos and larvae

Samples for morphometric analyses were preserved by the same methods used to preserve eggs. Digital images were calibrated using a stage micrometer for the 10× objective using ImageJ (National Institutes of Health, USA). Embryos and larvae from each treatment replicate were photographed for morphometric analyses ($n \geq 30$ per culture vessel) at each developmental stage.

All digital images were analyzed using ImageJ to determine various size metrics for each developmental stage (see Supplementary material, Fig. S1). The hatched blastula stage was oriented such that a full lateral view was visible, evident by uneven thickness of walls on the animal versus vegetal poles of the embryo. The maximum length of each hatched blastula, measured from the animal to vegetal end, was determined as well as the 2D area. When photographing the gastrula stage, larvae were oriented such that the full side profile of the archenteron was visible and aligned with the center of the vegetal plate. The gastrula stage was measured by length (i.e., the maximum distance from the anterior to posterior end, when measured across the center of the archenteron) and by 2D area. Prism stage larvae were photographed in a lateral view, and length was determined by measuring the tip of the body rod to the tip of the postoral rod. When photographing the echinopluteus stage, larvae were oriented such that the dorsal side was down against the microscope slide, with the postoral arms facing upwards. The length of the left postoral arm of each echinoplutei was measured, from the spicule tip of the postoral arm to the spicule tip of the aboral point (following Yu et al., 2011).

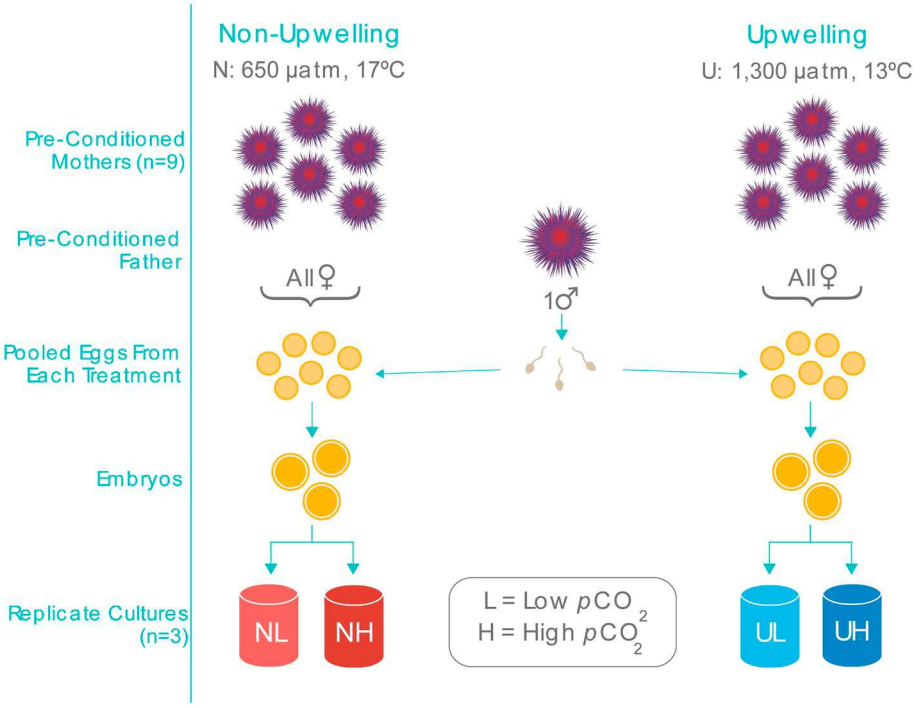

Fig. 1. Cross design of adult urchins conditioned under treatments that mimic non-upwelling (N: ~650 µatm pCO_2, ~17 °C) or upwelling (U: ~1300 µatm pCO_2, ~13 °C) conditions observed in the kelp forest ecosystem (Kapsenberg and Hofmann, 2016; Rivest et al., 2016). Embryos were raised at the same temperature (~15 °C) under low pCO_2 (L: ~450 µatm) or high pCO_2 (H: ~1050 µatm) conditions. This resulted in four embryo treatments: 1) progeny of females that experienced non-upwelling conditions raised under low pCO_2 levels (NL), (2) progeny of females that experienced non-upwelling conditions raised under high pCO_2 levels (NH), (3) progeny of females that experienced upwelling conditions raised under low pCO_2 levels (UL), and (4) progeny of females that experienced upwelling conditions raised under high pCO_2 levels (UH).

2.5. Statistical analyses

Statistical analyses were performed using JMP Pro (version 11.2.0) and R (v. 3.4.1). The morphometric, protein, and lipid data were tested to verify approximately normal distributions. A Levene test was used to test for unequal variance between treatments. A one-way analysis of variance (one-way ANOVA) was used to compare the means between egg samples with experimental treatment (i.e., non-upwelling or upwelling) set as a fixed factor and female identity set as a random effect. A Best Linear Unbiased Predictor (BLUP) (Robinson, 1991) was used to evaluate the random factor effect. A two-way analysis of variance (two-way ANOVA) was used to examine body size in embryos and larvae by setting maternal treatment (i.e., non-upwelling or upwelling) and offspring treatment (i.e., low or high pCO_2) as fixed factors and testing for an interaction between maternal and offspring treatment. For all developmental stages (i.e., blastula, gastrula, prism, echinopluteus), culture vessel replicate identity was set as a random effect in the model.

3. Results

3.1. Outcome of the adult conditioning phase and raising cultures of offspring

Adult urchins were successfully conditioned to the two experimental treatments (i.e., either simulated non-upwelling or upwelling treatment conditions) for a 4-month acclimation period. During this period, under controlled pCO_2 and temperature conditions (Table 1), there was no mortality and adults remained healthy and fecund. Although the seawater chemistry was somewhat variable due to respiration processes by the adults, a clear separation between the non-upwelling and upwelling treatment remained throughout the conditioning period (Fig. S2). In addition, the embryo culturing experiment was successful with pCO_2 conditions remaining stable throughout the 3-day culturing period (Table 1, Fig. S2). All culture buckets displayed synchronous development with each batch of embryos reaching key stages on the appropriate development schedule for *S. purpuratus* raised at 15 °C (Strathmann, 1987).

3.2. Assessment of the eggs

3.2.1. Egg morphometric analyses

There was no significant effect of parental history on the average egg size. Here, average egg diameter and 2D area were highly correlated ($r^2 = 0.924$). Only the analyses for average egg diameter are presented here (see Supplementary material, Fig. S3 for 2D area results). The average diameters of the eggs produced by the 9 non-upwelling females differed by which female produced them ($F_8 = 58.5399$, $p < .0001$). The 9 upwelling females also produced eggs that differed in size between females ($F_8 = 8.1425$, $p < .0001$). After accounting for size differences between the eggs of different females by treating female identity as a random effect, there was no apparent effect of maternal treatment (i.e., conditioning to non-upwelling versus upwelling) on average egg diameter ($F_{1,16} = 0.1658$, $p = .6892$) (Fig. 2A). On average, eggs from non-upwelling females (N) were 0.09103 ± 0.002535 mm in diameter while eggs from upwelling females (U) were 0.09138 ± 0.002196 mm in diameter (Table 2). In

Table 1
Average (± standard deviation) carbonate chemistry parameters and temperatures for adult conditioning (N or U) and offspring culturing (L or H). The pCO_2 and Ω_{Arag} were calculated from measured pH, salinity (33.1 ± 0.1), and total alkalinity values (2211.25 ± 7.10 µmol kg^{-1}).

	pH_{total}	pCO_2 (µatm)	Ω_{Arag}	Temperature (°C)
Non-upwelling (N)	7.87 ± 0.11	651 ± 242	1.74 ± 0.34	17.0 ± 0.1
Upwelling (U)	7.57 ± 0.08	1330 ± 300	0.79 ± 0.14	12.9 ± 0.2
Low pCO_2 (L)	7.99 ± 0.01	446 ± 12.4	2.03 ± 0.05	15.0 ± 0.11
High pCO_2 (H)	7.66 ± 0.01	1050 ± 37.7	1.02 ± 0.03	14.9 ± 0.05

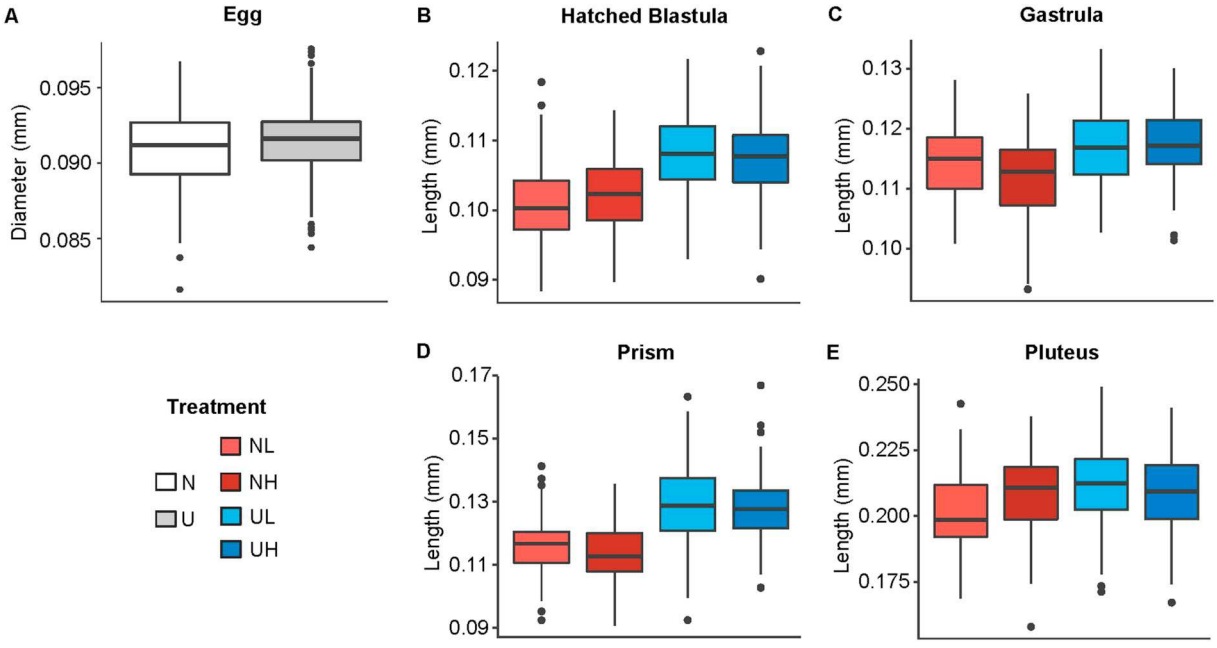

Fig. 2. Size differences across developmental stages of offspring, with error bars showing standard error. A. The average diameter of eggs from female urchins conditioned under either non-upwelling (N) or upwelling (U) treatments. The average length of developmental stages, including B. hatched blastula, C. gastrula, D. prism, and E. echinopluteus larvae stages of each of the four treatment types (i.e., NL, NH, UL, and UH).

Table 2
Average (± standard deviation) size, protein content, and lipid content of eggs produced by females conditioned to the non-upwelling treatment (N) and females conditioned to the upwelling treatment (U).

	Non-upwelling (N)	Upwelling (U)
Diameter (mm)	0.09103 ± 0.002535	0.09138 ± 0.002196
Total protein (ng/egg)	36.10 ± 10.95	42.88 ± 8.79
Triacylglycerol (ng/egg)	8.68 ± 3.66	10.36 ± 2.47
Phospholipid (ng/egg)	6.41 ± 1.96	7.89 ± 1.58
Sterol (ng/egg)	1.91 ± 0.83	2.44 ± 0.68
Total lipid (ng/egg)	17.01 ± 5.90	20.69 ± 4.08

examining variance across all eggs produced by both sets of females, the eggs produced by non-upwelling females exhibited more variance in size ($F_{1,651} = 7.9524$, $p = .0049$).

3.2.2. Protein quantification

There was no significant effect of maternal treatment on total protein content in the eggs ($F_{1,16} = 2.2991$, $p = .1490$), although on average, eggs from upwelling-conditioned females had more total protein (average of 42.88 ± 8.79 ng/egg) than eggs from non-upwelling-conditioned females (average of 36.10 ± 10.95 ng/egg) (Table 2). The variability of protein content did not differ between eggs from non-upwelling females and eggs from upwelling females ($F_{1,52} = 1.2793$, $p = .2632$).

3.2.3. Lipid content

The lipid content (ng per egg) was determined by measuring the concentration of three lipid classes: triacylglycerols (TAG), sterol (ST), and phospholipids (PL), in which the total lipid content was the combination of the three classes measured. After accounting for variability between eggs from different females of the same conditioning treatment, there were modest differences in lipid content between the eggs from urchins conditioned to different maternal treatments. Specifically, there was a significant difference in the concentration of phospholipids (PL), in which eggs from females conditioned to the upwelling treatment had on average a higher concentration of PL (7.89 ± 1.58 ng/egg) than those from females conditioned to the non-upwelling treatment (6.41 ± 1.96 ng/egg) ($F_{1,16} = 4.6935$, $p = .0454$) (Fig. 3). There was no significant treatment effect on triacylglycerol content (TAG) ($F_{1.17} = 2.3072$, $p = .1474$), sterol content (ST) ($F_{1,16} = 3.0881$, $p = .0978$), or total lipid content (TAG + PL + ST) ($F_{1,16} = 0.0762$). However, there was a clear trend overall in which eggs from females that experienced upwelling conditions had a higher lipid content of all lipid classes (Fig. 3). On average, eggs produced by upwelling-conditioned females had a total lipid content of 20.69 ± 4.08 ng/egg, while eggs produced by non-upwelling-conditioned females had a total lipid content of 17.01 ± 5.90 ng/egg (Table 2). There was equal variance of

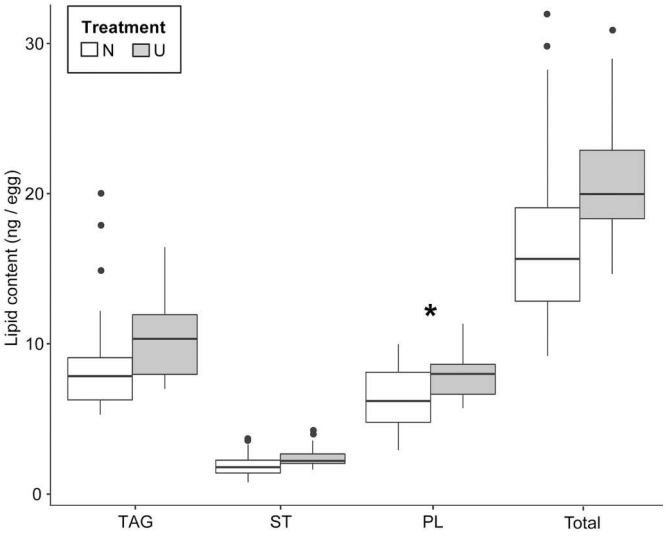

Fig. 3. Lipid content (ng/egg) of eggs from females conditioned to the non-upwelling treatment (N) and females conditioned to the upwelling treatment (U), with error bars showing standard error. Three lipid classes were measured including triacylglyerols (TAG; tripalmitin), sterol (ST; cholesterol) and phospholipids (PL; L-α-phosphatidylcholine). Total is the total of all three lipid classes. Any significant difference between treatments is marked with an asterisk (*).

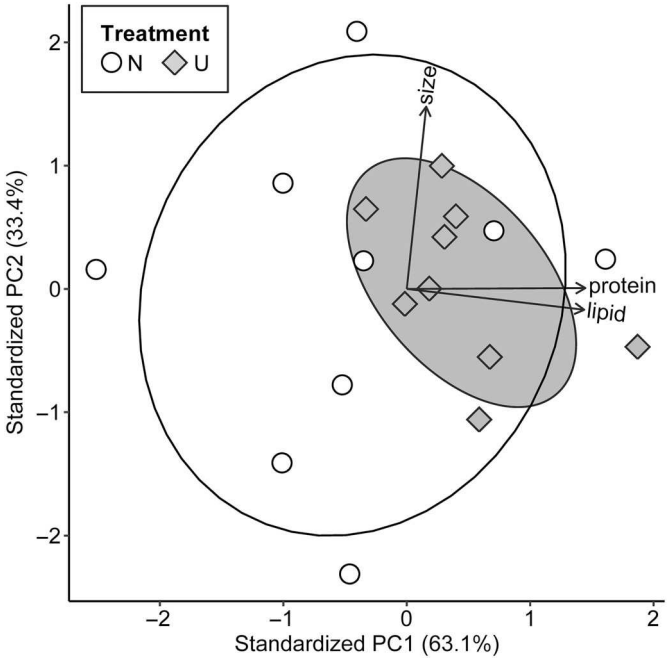

Fig. 4. A PCA biplot of the eggs from females conditioned to the non-upwelling treatment (N) and from females conditioned to the upwelling treatment (U), using a combination of the egg data, including: 1) size (average egg diameter), 2) protein (average ng of protein per egg), and 3) lipid (average ng of total lipid per egg).

total lipid content between the eggs from the two sets of females for every lipid class ($p > .05$).

3.2.4. Principal component analysis

A PCA biplot of the combined size (average diameter), protein (ng/egg of total protein), and lipid data (ng/egg of total lipid) for the eggs showed that Principal Components 1 and 2 (PC1 and PC2) cumulatively explain a majority of the variance in the data (96.5%) (Fig. 4). A PERMANOVA showed that the grouping identity of the eggs (i.e., eggs from females conditioned to non-upwelling versus upwelling) were not significantly associated with PC1 ($F_{1,16} = 4.11$, $p = .05962$) or PC2 ($F_{1,16} = 0.04294$, $p = .8385$). However, the eggs from the females conditioned to upwelling are generally clustered more closely to one another than are the eggs from females conditioned to non-upwelling (Fig. 4). As noted earlier, only morphometric analyses showed a significant difference in variance between the two groups of eggs, in which eggs from non-upwelling females were significantly more variable in size.

In terms of our egg metrics, total protein content had a high positive correlation with total lipid content ($r = 0.9151$). Additionally, protein content and lipid content both have a positive correlation with PC1 ($r = 0.7073$ and 0.7027, respectively), which represents the most variability in the data (63.1%). Protein and lipid content show nearly no relationship to the size of the eggs ($r = 0.08445$ and 0.004050, respectively). Size has a high, positive correlation with PC2 ($r = 0.9935$), which represents 33.4% of the variability in the data. In general, eggs from females conditioned to upwelling (U) appeared to have more protein and lipids per egg (Fig. 4). In contrast, egg size did not appear to differ between the upwelling and non-upwelling eggs, other than by tighter clustering of the upwelling eggs.

3.3. Morphometric analyses of embryos and larvae

Although the average size of the eggs was not significantly affected by maternal conditioning, treatment effects became evident during early development upon examining average maternal effects across a pool of embryo genotypes. Here, the results for hatched blastula length are presented, but the morphometric results for hatched blastula 2D area were very similar (see Supplementary material, Fig. S3). There was a significant maternal treatment effect on the lengths of hatched blastulae ($F_{1,8} = 53.7797$, $p < .0001$) (Fig. 2B). The progeny of upwelling-conditioned mothers (UL and UH) were, on average, 6.5% longer than the progeny of non-upwelling-conditioned mothers (NL and NH) at the hatched blastula stage. While there was an effect of maternal treatment, there was no significant effect of offspring treatment (i.e., raised under high versus low pCO_2 at ~15 °C) on blastula body size ($F_{1,8} = 0.3008$, $p = .5987$). There was also no significant interaction between maternal and offspring treatment at the blastula stage ($F_{1,8} = 0.6219$, $p = .4536$).

The effect of differential maternal conditioning on embryo size was also evident at the gastrula stage (Fig. 2C). We found a significant effect of maternal treatment (non-upwelling versus upwelling) on the length of the gastrulae ($F_{1,8} = 18.0220$, $p = .0025$). The morphometric results for gastrula 2D area are reported in the Supplementary material (Fig. S3). Like the hatched blastula stage, progeny at the gastrula stage were greater in length if their mothers experienced upwelling conditions (U: ~1300 µatm pCO_2, ~13 °C) during gametogenesis (i.e., UL and UH), and were, on average, 3.9% longer than the progeny of mothers that experienced non-upwelling conditions (N: ~650 µatm pCO_2, ~17 °C) (i.e., NL and NH). There was no effect of offspring treatment (i.e., raised under high versus low pCO_2 at ~15 °C) on gastrula length ($F_{1,8} = 0.1064$, $p = .7522$). There was not a significant interaction between maternal and offspring treatment (i.e., an interaction between maternal conditioning and the response of the offspring to their environment) ($F_{1,8} = 4.6172$, $p = .0621$).

The same morphometric patterns observed at the gastrula stage remained evident at the prism stage (Fig. 2D). There was a significant maternal treatment effect on prism skeletal rod length ($F_{1,8} = 44.6129$, $p = .0002$). The skeletal rods in offspring of upwelling-conditioned mothers were, on average, 11.6% longer than the progeny of non-upwelling-conditioned mothers. There was no effect of offspring treatment (i.e., raised under high versus low pCO_2 at 15 °C) on prism rod length ($F_{1,8} = 0.06152$, $p = .4563$). Lastly there was no significant interaction between maternal and offspring treatment ($F_{1,8} = 0.7419$, $p = .4152$).

While maternal conditioning to upwelling versus non-upwelling conditions during gametogenesis impacted body size at the hatched blastula, gastrula, and prism stages, this effect was no longer evident later in development. There was no significant effect of maternal treatment ($F_{1,8} = 1.2122$, $p = .3023$), offspring treatment ($F_{1,8} = 0.0363$, $p = .8535$), or significant interaction between maternal and offspring treatment ($F_{1,8} = 1.0591$, $p = .3329$) on the arm length of the echinopluteus stage larvae (Fig. 2E).

4. Discussion

Transgenerational effects have been demonstrated to influence the performance of marine invertebrate embryos and larvae in an environmental change context (Donelson et al., 2017; Ross et al., 2016). In this study, we investigated whether adult sea urchins that experience different conditions during gametogenesis might produce offspring with varying responses to different pCO_2 conditions during development. One working hypothesis here is that in kelp forest ecosystems, adult urchins likely perform gametogenesis across a range of seawater conditions (see Chan et al., 2017; Hoshijima, 2018; Hoshijima and Hofmann, 2019), and this could point to the role of kelp, upwelling, and altered physicochemical conditions influencing ecological and evolutionary dynamics via TGP. In a previous study, differential gene expression in gastrula-stage embryos of *S. purpuratus* was shown to vary significantly with the conditioning treatment of the adults (Wong et al., 2018). In the present laboratory experiment, we investigated whether conditioning of the adults to combined pCO_2 and temperatures that are simple representations of upwelling versus non-upwelling conditions in

a coastal kelp forest would lead to a change in phenotype of the early life history stages exposed to elevated pCO_2 levels. Here, we were able to detect a maternal effect on offspring phenotype. However, we were unable to measure a maternal effect on offspring response to stress because the offspring pCO_2 treatment alone failed to elicit an observable change in phenotype. Nonetheless, TGP may provide rapid resilience to environmental stress in the early life stages of echinoderms.

Because the timing of adult conditioning is likely an important factor in TGP (Ross et al., 2016; Uthicke et al., 2013), we conditioned adult *S. purpuratus* at the onset of seasonal gonadal development as a means of capturing the entirety of gametogenesis. The adult conditioning period reported here appears to have been of sufficient duration to elicit observable differences in offspring phenotype. Here, we report four salient findings: 1) maternal conditioning did not affect average egg size, though there was a difference in the variability of egg sizes produced, 2) maternal conditioning affected egg lipid content, 3) there was an effect of maternal conditioning on body size during the early development of the embryos (transgenerational effect), but no detectable effect of offspring pCO_2 treatment on body size (intragenerational effect).

4.1. Impact of differential maternal conditioning on eggs

In this study, we found that differential maternal conditioning to combined pCO_2 and temperatures representative of upwelling or non-upwelling conditions in the kelp forest environment affected some, but not all, of the response variables used to measure the females' eggs. Egg size has been associated with a variety of important developmental characteristics, including larval duration (Strathmann, 1985; Wray and Raff, 1991) and juvenile success (Emlet and Hoegh-Guldberg, 1997). Other studies have found that maternal exposure to either high pCO_2 levels or low temperatures can increase egg sizes. In the Antarctic sea urchin *Sterechinus neumayeri*, adults conditioned to lowered pH conditions for 17 months produced larger eggs than adults conditioned under average pH levels (Suckling et al., 2015). Regarding temperature TGP effects on oocyte size, females exposed to colder temperatures have been observed to produce larger eggs in a variety of organisms (Atkinson et al., 2001; Baldanzi et al., 2015; Blanckenhorn, 2000; Feiner et al., 2016; Shama, 2015). In contrast, we found that maternal exposure to the high pCO_2 level and low temperature associated with the upwelling treatment had no significant effect on the average size of the eggs, although this observation may be subject to egg size variability between females. In fact, urchins that experienced non-upwelling conditions produced eggs that were more variable in size than those that experienced upwelling conditions.

Intraspecific variability in egg size occurs within and between populations of sea urchins in nature (George et al., 1990; Hagström and Lönning, 1967). It is possible that the natural size variability that exists in *S. purpuratus* egg production was evident under relaxed pCO_2 and temperature conditions (i.e., non-upwelling), but the elevated pCO_2 level, lower temperature, or the combination of both factors decreased the variability of the eggs produced by the upwelling-conditioned females. Variability in egg size can also be affected by differences in genetic factors of the adult females that produced the eggs (Moran and McAlister, 2009). Although adult urchins were randomly sorted into their conditioning treatments, we cannot rule out that the non-upwelling treatment may have harbored more genetic variability. Thus, the difference in egg size variability between the maternal treatments could also be caused by genetic variability and/or variability in gene-environment interactions.

Biochemical composition (e.g., amount of protein and lipid) are often used to assess egg quality and maternal provisioning (Jong-Westman et al., 1995; Falkner et al., 2006; George et al., 1990; McAlister and Moran, 2012). We did not find that egg size was correlated to egg biochemical composition. Although egg energy content generally scales with size across echinoderm species, at finer intraspecific scales, this relationship can be quite weak (Moran and McAlister, 2009). Therefore, egg size does not necessarily provide a reliable indication of energetic content and quality (Jong-Westman et al., 1995; George et al., 1990; McAlister and Moran, 2012; McEdward and Morgan, 2001; Thompson, 1983). In this study, there was no observable effect of maternal conditioning on the total protein content of the eggs. However, there was a maternal treatment effect on the lipid content of the eggs, in which upwelling-conditioned females produced eggs with more lipids. This increase in lipid content could be caused by either the low temperature, the high pCO_2 level, or a combination of both factors. Given our experimental design, however, it is not possible to identify which component(s) of the upwelling maternal treatment contributed to the increase in egg lipid content. Nevertheless, in nature, sea urchins are likely exposed to both factors simultaneously due to the cold, low pH conditions characteristic of upwelled waters.

Our results indicate that females that experience upwelling conditions increase maternal provisioning of lipids to their eggs, particularly in the form of phospholipids, a structural lipid class. Phospholipids regulate membrane morphology, are involved in nuclear envelope formation during mitosis, and modulate the activity level of integral membrane proteins, including ion channels of the plasma membrane (Falkenburger et al., 2010; Larijani et al., 2014). Although not statistically significant, we also observed a trend in which upwelling-conditioned females provisioned more triacylglycerols (Fig. 3). Triacylglycerols are energy storage lipids used by echinoderms to fuel pre-feeding development (Falkner et al., 2006; Meyer et al., 2007b; Prowse et al., 2008; Sewell, 2005; Villinski et al., 2002). Therefore, an increase in provisioning of lipids to the eggs can impact performance, growth, and cellular morphology during embryonic and larval development.

Comparing our data to other studies on *S. purpuratus*, the average size of the eggs reported here (0.091 mm in diameter) are larger than what has previously been reported from other observations (0.078–0.084 mm) (Chase, 1935; Levitan, 1993; Moore, 1943). Additionally, because formalin preservation can cause echinoderm eggs to shrink slightly (Lessios, 1987), the actual sizes of the eggs we measured may be even larger than reported. Similarly, the amount of total protein measured here was greater than what has been reported in *S. purpuratus* (Matson et al., 2012; Meyer et al., 2007a; Shilling and Manahan, 1990). However, other studies used the Bradford assay (Sapan et al., 1999), which differs from the methods employed in this study. Furthermore, egg protein content can vary by the abundance and protein content of the adult urchins' diet during gametogenesis (Jong-Westman et al., 1995; George et al., 1990; Hammer et al., 2006). The total lipid content per egg reported here is greater than that reported by Matson et al. (2012), but less than what has been reported by Meyer et al. (2007b). However, for this study, the lipid concentrations were not adjusted using an internal ketone standard. Additionally, adults were not acclimated to controlled conditions prior to spawning in Matson et al. (2012) or Meyer et al. (2007a).

Importantly, much evidence suggests echinoderm egg size and quality are affected by the food quantity and quality available to the adults during gametogenesis (Bertram and Strathmann, 1998; Jong-Westman et al., 1995; George, 1996; George et al., 2001; Thompson, 1983). It is possible that the relatively large egg size and high biochemical content reported in this study were due to the feeding design adopted in this experiment. We chose to feed the adult urchins in excess throughout the conditioning period to minimize any potential effects of feeding differences between the treatments and to ensure we could obtain enough high quality gametic material to create viable embryo cultures. The effect of feeding may have dominated and diminished the visible effect of our experimental treatment on the eggs. Greater differences in egg size and biochemistry may have been observed given a more conservative feeding regime. Nevertheless, there was still an observable effect of maternal conditioning on the eggs, in which maternal provisioning of lipids was higher in females conditioned to simulated

upwelling (i.e., high pCO_2 and low temperature).

4.2. Impact of differential maternal conditioning on early developmental stages

A fundamental tenet of life-history theory is that increased offspring size is linked to increased fitness (Smith and Fretwell, 1974). Organisms with larger body sizes during early development can exhibit greater performance at later life stages (e.g., as juveniles and adults) in terms of growth, development, survivorship and reproductive output (Marshall et al., 2003; Moran and Emlet, 2001). In marine organisms with planktonic embryos and larvae, body size can be positively correlated with dispersal time and distance (Marshall and Keough, 2003) that in turn affects recruitment and species ranges. Here, we examined average maternal effects across a pool of genotypes to mimic mass spawning events that occur in nature. While we cannot rule out whether there was differential mortality between embryos from different mothers, the two progeny groups (pooled offspring of upwelling-conditioned mothers and pooled offspring of non-upwelling-conditioned mothers) exhibited distinct phenotypic differences when raised under different pCO_2 levels (low or high pCO_2) at the same temperature. We observed maternal treatment effects (i.e., transgenerational) on offspring body size, which have possible implications for progeny fitness and dispersal, but no offspring treatment effects (i.e., intragenerational) that would have allowed for an indication of differential stress response.

Although there was no significant maternal treatment effect on egg size, at the hatched blastula, gastrula and prism stages, the progeny from females that experienced upwelling conditions (U: ~1300 µatm pCO_2, ~13 °C) had larger body sizes. In general, parents that experience lower temperatures produce larger offspring (Atkinson et al., 2001; Burgess and Marshall, 2011). The production of larger offspring by mothers that experience colder environments has been attributed to increased maternal provisioning intended to offset the greater energetic costs of developing at lower temperatures (Pettersen et al., 2019). Parental exposure to low pH, however, may have a limited impact on offspring size, although this can vary somewhat depending on the duration of adult exposure (Lamare et al., 2016; Suckling et al., 2014; Suckling et al., 2015). Thus, although we cannot separate the effects of high pCO_2 from low temperature (i.e., the upwelling maternal treatment), it is possible that maternal temperature was the predominant factor eliciting transgenerational effects that facilitated larger embryo body sizes. Eggs produced by upwelling-conditioned females contained more structural lipids, which are used to construct the bodies of the embryo and larva (Prowse et al., 2008; Sewell, 2005). Therefore, an increase in maternal provisioning of lipids may have contributed to larger progeny during the blastula through prism stages.

At the echinopluteus larval stage, there was no longer a detectable maternal treatment effect on body size. In general, initial larval size is positively correlated to egg size (McEdward, 1986) and here, neither egg nor larval size was significantly different between the maternal treatments. The loss of an evident maternal effect could be explained by a difference in timing of energy usage and growth. For example, the progeny of mothers conditioned to the upwelling treatment may have spent more energy towards growth during the earlier stages of development (i.e., hatched blastula through prism stages) and slowed their growth as they reached the first larval stage. On the other hand, the progeny of mothers conditioned to the non-upwelling treatment may have grown less during their early development, ultimately catching up in size to the upwelling progeny by the time they reached the echinopluteus stage. Thus, once maternally-provided energy stores were largely exhausted and the offspring had reached their planktotrophic feeding stage, all larvae were approximately equal sizes. A more detailed analysis of how biochemical constituent quantities changed throughout development would be required to verify how progeny differ in their energy use over time. Although there is no significant maternal effect at the larval stage, the success of embryonic stages and processes, particularly gastrulation, are necessary for success in the pelagic zone, dispersal, and eventual recruitment. As such, even transgenerational effects that impact only select stages of development likely play a role in a population's success under rapid environmental change.

Offspring pCO_2 treatment had no effect on offspring body size for any developmental stage. This was an unexpected result because generally, elevated pCO_2 levels have been shown to negatively affect body size and growth during sea urchin early development (Byrne et al., 2013a; Byrne et al., 2013b; Clark et al., 2009; Kurihara and Shirayama, 2004; Sheppard Brennand et al., 2010), particularly in developmental stages that undergo calcification processes (e.g., prism and echinopluteus), although the effects of ocean acidification can vary across stages of early development (Byrne, 2011; Ericson et al., 2010; Kurihara, 2008). However, there have been exceptions when high pCO_2 does not always cause reduced growth in during the early development of sea urchins, such as in *Pseudechinus huttoni*, in which low pH conditions have no effect on echinopluteus body size (Clark et al., 2009). In some cases, temperature and pCO_2 effects act antagonistically; temperature can offset a reduction in larval body size due to elevated pCO_2 (Byrne and Przeslawski, 2013; Przeslawski et al., 2015). Here, all the offspring were raised at the same temperature. It is possible that during this study, developmental temperature rather than developmental pCO_2 level was the dominant factor dictating intragenerational body size.

We found no significant interaction between maternal and offspring treatments for any developmental stage. In other words, adult conditioning did not impact the effect of offspring pCO_2 treatment (i.e., raised under low or high pCO_2 levels at ~15 °C) on progeny body size. Thus, because the offspring pCO_2 treatment did not elicit an observable effect on progeny body size, we were not able to measure how maternal conditioning may have affected the progeny's response to stress. While the response to pCO_2 was not detectable by examining body size, a past study suggested that maternal conditioning can affect offspring response to pCO_2 stress at the transcriptomic level (Wong et al., 2018). Future studies may include more extreme pCO_2 conditions or other abiotic stressors that can induce an observable effect on body size. In this study, only the maternal treatment affected offspring phenotype. Although we were unable to test if this maternal effect altered the offspring response to pCO_2 stress, the production of larger offspring by upwelling-conditioned mothers suggests that the fitness and dispersal potential of progeny can be affected by the environmental conditions experienced by the parental generation, thereby providing evidence of transgenerational plasticity.

4.3. Mechanisms of transgenerational plasticity

In this study, we explored mechanisms that could control the variation in phenotype of sea urchins during early development. Such mechanisms could include maternal effects, a situation where the phenotype of an individual is influenced by the phenotype or environmental experience of the mother. Provided environmental conditions are reliably predictable, the progeny's phenotype may be adjusted accordingly via maternal effects so that they are better suited to their particular environment (Bondurianski et al., 2012; Mousseau and Fox, 1998). Differential maternal provisioning by females in different CO_2 and temperature treatments has been observed in sea urchins (Foo et al., 2012; Suckling et al., 2015; Sunday et al., 2011) and copepods (Vehmaa et al., 2012). Eggs of the copepod *Acartia* sp. increased in quality over longer exposure times of the adults, which reduced negative effects of changing pH on hatching success, an observation that was attributed to better maternal provisioning (Vehmaa et al., 2012). Increased maternal provisioning, via an additional supply of nutrients and potential energy reserves, can result in larger body sizes and may functionally act to improve an organism's ability to respond to environmental stressors. Here, lipid content was higher in eggs of females conditioned to upwelling conditions (Fig. 3), which potentially

contributed to the physiological differences that were observed during the early development of the progeny (Fig. 2).

Alternatively, transgenerational plasticity may be facilitated by epigenetic mechanisms that operate to alter offspring phenotype, a process that has been proposed for invertebrates (Roberts and Gavery, 2012) and marine fish (Metzger and Schulte, 2016), although studies on marine metazoans within a global change context are rare (Hofmann, 2017). In a companion paper, we found that the maternal environment experienced by adult *S. purpuratus*, but not the developmental environment experienced by their offspring, affected the DNA methylation patterns of the progeny (Strader et al., 2019). Previous research in our group demonstrated that differential maternal conditioning also influenced the expression of genes related to epigenetic processes, such as methylation, methyltransferase activity, and histone modification (Wong et al., 2018). In many cases, epigenetic mechanisms, such as DNA modifications via methylation, operate to alter gene expression, changing the transcriptome in a manner that creates plasticity in response to new abiotic conditions (Roberts and Gavery, 2012). This has been observed in corals, in which lower levels of DNA methylation facilitated flexible gene expression across environments (Dixon et al., 2014). In addition, Putnam and colleagues suggest that environmentally-induced changes in DNA methylation may generate phenotypic plasticity in corals exposed to low pH conditions (Putnam et al., 2016). Epigenetic modifications can provide a rapid response to environmental change (Eirin-Lopez and Putnam, 2019; Hofmann, 2017), and have the potential to subsist across multiple generations, eventually contributing to long term adaptation in marine systems (Suarez-Ulloa et al., 2015).

Overall, there has been a resurgence of studying TGP and maternal effects in a global change context in marine systems (Donelson et al., 2017; Ross et al., 2016). In predicting how marine systems will be impacted by continuing global change, there are important questions regarding the interactions between TGP and evolution (Chirgwin et al., 2018; Donelson et al., 2017). TGP can alter variation in phenotype at a rate faster than, and without the need of, changes in genotype, influencing the fitness landscape and resulting evolutionary processes (Bondurianski et al., 2012; Bondurianski and Day, 2009). There exists the possibility that TGP confers resistance to progeny that acts on an ecological rather than an evolutionary timescale. This resistance is later the source of adaptation (i.e., 'plasticity first') (Levis and Pfennig, 2016; Schwander and Leimar, 2011), buying time for these organisms to respond to rapid changes in their environment. Alternatively, TGP may decelerate evolutionary processes by weakening selection on genetic variation or by providing maladaptive phenotypes when mismatches between parental and offspring environments occur (Bondurianski et al., 2012; Reed et al., 2010).

The interplay between TGP and adaptation is important for predicting future changes in marine populations, communities and ecosystems. Furthermore, there is interest in management approaches in which TGP may have a role in conservation efforts (Chakravarti et al., 2016; Evans et al., 2014; van Oppen et al., 2015). TGP has also been examined in the context of fisheries species and aquaculture practices, in which conditioning of adult broodstocks may lead to more resilient offspring (Burt et al., 2011; Gavery and Roberts, 2017; Utting and Millican, 1997). Regardless of the conceptual framework, there is still much to do to fully understand TGP, how it connects to creating phenotypic plasticity in environmentally-induced variants during development, and whether these new phenotypes bolster the adaptive capacity of marine populations in a changing ocean. Future research should continue to examine the precise mechanisms of TGP (Bondurianski and Day, 2009; Eirin-Lopez and Putnam, 2019; Jablonka and Raz, 2009), paternal contributions to TGP (Crean and Bondurianski, 2014; Crean et al., 2013; Guillaume et al., 2016), and TGP in variable and unpredictable environments in nature (Burgess and Marshall, 2014; Donelson et al., 2017).

Contributors

G.E.H. conceived of and designed the experiment. All authors conducted the culturing experiments with L.C.K., T.S.L., J.M.W., and U.H. performing the carbonate chemistry measurements and calculations. L.C.K. gathered the morphometric and protein data. L.C.K. and J.M.W. conducted the lipid extractions and analyses. Statistical analyses were performed by J.M.W., L.C.K., and U.H. The manuscript was written by J.M.W. with contributions from G.E.H.; figures were created by J.M.W. and L.C.K in consultation with all authors. Declarations of interest: none.

Funding

This research was supported by funds from the UC Climate Champion award from the University of California to G.E.H. This work was also supported by resources from the Santa Barbara Coastal Long Term Ecological Research program (NSF award OCE-1232779; Director: Dan Reed). Analytical and writing phases of the project were supported by NSF award IOS-1656262 to G.E.H. In terms of support to personnel, J.M.W. was supported by a UC Santa Barbara Regent's Fellowship and a NSF Graduate Research Fellowship under Grant No. 1650114; L.C.K. was supported by a UC Santa Barbara Chancellor's Fellowship and a NSF Graduate Research Fellowship under Grant No. 1650114; T.S.L. was supported by a UC Santa Barbara Regent's Fellowship and a Ford Foundation Predoctoral Fellowship; U.H. was supported by a NSF Graduate Research Fellowship under Grant No. 1650114. Specimens were collected in the Santa Barbara Channel under a California Scientific Collecting Permit to G.E.H. (SC-1223).

Data accessibility

All morphometric, protein, and lipid data are deposited in the Dryad Digital Repository at https://doi.org/10.5061/dryad.4nv0nb8.

Acknowledgements

The authors wish to thank Christoph Pierre, Director of Marine Operations at UC Santa Barbara, for assistance with boating and kelp collections. We also wish to thank Dr. Kevin Johnson and Maddie Housh for assistance during the experiment.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.jembe.2019.04.006.

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