UNIVERSITY OF CALIFORNIA

Santa Barbara

The Role of Pre- and Post-Spawning Temperature Stress on Fertilization Dynamics within Santa Barbara Channel Sea Urchin Species

A dissertation submitted in partial satisfaction of the

requirements for the degree Doctor of Philosophy

in Ecology, Evolution, and Marine Biology

by

Terence Sebastian Leach

Committee in charge:

Professor Gretchen Hofmann, Chair

Professor Kathy Foltz

Professor Debora Iglesias-Rodriguez

June 2022

The dissertation of Terence Sebastian Leach is approved.

_____

Kathy Foltz

_____

Debora Iglesias-Rodriguez

_____

Gretchen Hofmann, Committee Chair

June 2022

The Role of Pre- and Post-Spawning Temperature Stress on Fertilization Dynamics within Santa Barbara Channel Sea Urchin Species

Terence Sebastian Leach

ACKNOWLEDGEMENTS

The work outlined in this thesis would not have been possible without an abundance of help and support. I want to first thank my graduate advisor, Dr. Gretchen Hofmann, for all the guidance she has provided me throughout my dissertation. From the moment I stepped foot on campus, I felt like I had a family who was going to look out for me. This is a testament to the amazing lab culture that you have set in the Hofmann lab. Your encouragement to develop a healthy work-life balance made me feel comfortable to make my Ph.D. the most fulfilling time of my life, both personally and academically. I would also like to thank the other members of my Ph.D. committee, Drs. Kathy Foltz and Debora Iglesias-Rodriguez for their valuable feedback and encouragement during my thesis.

Speaking of the Hofmann family, I cannot express how grateful I am to all the members of the Hofmann lab (both current and past). To Dr. Kevin Johnson, I would like to thank for setting an amazing example for what a scientist should be, someone who is not just passionate about what they do but also how this information will help others. Dr. Juliet Wong, you were my first true friend at UCSB and I am still so honored to have been taken under your wing during our shared time in the Hofmann lab. I look up to you so much and will always come to you for advice (whether you like it or not). Dr. Marie Strader, it was so much fun getting to work with you in the lab and get to watch you succeed through academia. The world needs more Jannine Chamorros in science, you are one of the most caring individuals I know on top of being a fantastic researcher. To Sam Bogan, you have been such an amazing friend and colleague, someone who I always felt I could just start

chatting up about science and life whenever. I expect that to continue, so keep your phone close!

To Xochitl Clare and Amelia Ritger, thank you for being great presences in the lab and for fueling my sugar addiction through candy and treats. Adriane McDonald and Erin de Leon Sanchez, I am saddened that the pandemic took away a lot of our time to work together but I am so excited to see all the amazing work on your horizons. To Logan Kozal (the better half of "Togan"), I cannot imagine going through graduate school without you. I am taking so much away from you as my graduate career comes to an end. I am SO proud of you and amazed by all your accomplishments. I am going to miss you so much and I request that we always live on the same coast (at least). Finally, I would like to thank Umi Hoshijima, the most talented and genuine person I have ever had the privilege to meet. I will never forget you and the impact you had on me and the other members of the Hofmann lab.

Lastly, I would like to thank my family for their support and love. To my parents, Jill and Fred, thank you for being such great role models for me, I could never have made it this far without you. Also, after completing this thesis work, I finally feel confident in thanking both of you for the maternal (and paternal) investments you have made. Additional thanks to my siblings, Fredrick and Alex, for rooting for my success and allowing me to still be your annoying little brother. My biggest thanks goes to my partner, Jordan, who has been right by my side through all the challenges and accomplishments I have experienced on the road to my Ph.D. Thank you for your flexibility and unwavering support. I cannot believe all the things we have accomplished together in these past 6 years. You are everything I could ever want in a partner and friend, while being the best mother to Miles. I love you so much!

VITA OF TERENCE SEBASTIAN LEACH
June 2022

EDUCATION

2016-present **University of California Santa Barbara, Santa Barbara, CA**
Ph.D. Candidate in Ecology, Evolution, and Marine Biology
Advisor: Dr. Gretchen Hofmann
Expected Graduation Date: Spring 2022

2012-2016 **University of Washington, Seattle, WA**
Bachelor of Science in Ecology, Evolution & Conservation Biology
Minor in Marine Biology

PREVIOUS RESEARCH EXPERIENCE

2016-present **Graduate Student Researcher**
Ecology, Evolution, and Marine Biology, UC Santa Barbara
Advisor: Dr. Gretchen Hofmann

FELLOWSHIPS & AWARDS

2018-2021 Ford Foundation Fellowship – Predoctoral Competition

2019 Ellen Schamberg Burley Graduate Scholarship, UC Santa Barbara

2016-2018 UC Santa Barbara Regents Fellowship

2018 EEMB Departmental Grant Award, UC Santa Barbara

2017 Friday Harbor Laboratories Travel Award, University of Washington

2017 EEMB Departmental Grant Award, UC Santa Barbara

2014-2015 University of Washington Mary Gates Research Scholarship

2012-2013 Washington Research Foundation – Distinguished Undergraduate Scholar

PUBLICATIONS

Leach TS, BuyanUrt B and Hofmann GE (2021) Exploring impacts of marine heatwaves: Paternal heat exposure diminishes fertilization success in the purple sea urchin (*Strongylocentrotus purpuratus*). *Marine Biology.* Springer Nature. doi: 10.1007/s00227-021-03915

Strader ME, Kozal LC, **Leach TS**, Wong JM, Chamorro JD, Housh MJ, Hofmann GE (2020) Examining the role of DNA methylation in transcriptomic plasticity of early stage sea urchins: Developmental and maternal effects in a kelp forest herbivore. *Frontiers in Marine Science* https://doi.org/10.3389/fmars.2020.00205

Strader ME, Wong JM, Kozal LC, **Leach TS**, Hofmann GE (2019) Parental environments alter DNA methylation in offspring of the purple sea urchin, *Strongylocentrotus purpuratus*. *Journal of Experimental Marine Biology and Ecology.* 517:54-64. https://doi.org/10.1016/j.jembe.2019.03.002

Wong JM, Kozal LC, **Leach TS**, Hoshijima U, and Hofmann GE (2019) Transgenerational effects in an ecological context: Conditioning of adult sea urchins to upwelling

conditions alters maternal provisioning and progeny phenotype. *Journal of Experimental Marine Biology and Ecology*. https://doi.org/10.1016/j.jembe.2019.04.006

Carlson MCG, McCary ND, **Leach TS** and Rocap G (2016) Pseudo-nitzschia challenged with co-occurring viral communities display diverse infection phenotypes. *Frontiers in Microbiology* 7:527. https://doi.org/10.3389/fmicb.2016.00527

PRESENTATIONS

Leach TS, BuyanUrt B and Hofmann GE. *Oral*. "If you can't stand the heat, stay out of the gonad: Paternal heat stress diminishes fertilization success in the purple sea urchin". Western Society of Naturalists. November 5-8 2020

Hofmann GE and Leach TS. *Oral*. "Professional development series promotes inclusion and community: Report from the site REU at UC Santa Barbara". Aquatic Sciences Meeting. San Juan, PR. February 23 – March 2 2019

Leach TS, Strader ME, Hofmann GE. *Oral*. "Investigating the role of maternal conditioning on offspring performance and DNA methylation patterns in the purple sea urchin". Aquatic Sciences Meeting. San Juan, PR. February 23 – March 2 2019

Leach TS, Kozal LC, Wong JM, Hofmann GE. *Poster*. "Maternal and paternal investments alter offspring phenotype in the purple sea urchin, *Strongylocentrotus purpuratus*". Long Term Ecological Research (LTER) All Scientists Meeting. Asilomar, CA. September 30 – October 4 2018

Leach TS, Wong JM, Kozal LC, Hofmann GE. *Poster*. "Mahalo, Mama: The role of DNA methylation in the transgenerational plasticity of purple urchins". Larval Biology Symposium. Honolulu, HA. August 10-13 2017

Leach TS, Wong JM, Hofmann GE. *Poster*. "Exploring transgenerational effects in purple sea urchins: Using DNA Methylation to link adult condition to progeny performance". Western Society of Naturalists. Monterrey, CA. November 10-13 2016.

Leach T, Guannel M, Carlson M, Rocap G. *Oral*. "Uncovering the evolutionary history of *Pseudo-nitzschia*: Connecting physiology and molecular phylogeny" 2014 Ocean Sciences Meeting. Honolulu, HA. February 23-28 2014.

Leach T, Guannel M, Carlson M, Rocap G. *Oral*. "Uncovering the evolutionary history of *Pseudo-nitzschia"*. Oceans and Human Health Joint NOAA/UW Seminar. Seattle, WA. March 2013.

TEACHING

Fall 2021	**Lecturer**, Introductory Biology 3, University of California Santa Barbara, USA
Fall 2021	**Head Teaching Assistant**, Introductory Biology Laboratory 2LL, University of California Santa Barbara, USA
Spring 2021	**Teaching Assistant**, Introductory Biology Laboratory 2LL, University of California Santa Barbara, USA

Fall 2020	**Reader**, Introductory Biology Laboratory 2LL, University of California Santa Barbara, USA
Spring 2020	**Teaching Assistant**, Introductory Biology Laboratory 2LL, University of California Santa Barbara, USA
Spring 2018	**Teaching Assistant**, Introductory Biology Laboratory 2LL, University of California Santa Barbara, USA

MENTORSHIP

Summer 2019-Summer 2020	**Graduate Mentor,** Gorman Scholars Program University of California Santa Barbara Mentee: Buyanzaya BuyanUrt (UCSB undergraduate) Project Title: *Examining the Effects of Varying* pH *Conditions on the Early Development of the Painted Sea Urchin,* Lytechinus pictus
Summer 2018, 2019	**Super Mentor,** Ocean Global Change REU University of California Santa Barbara
Summer 2018	**Graduate Mentor,** Ocean Global Change REU University of California Santa Barbara Mentee: Carlos Estrada (Chico State University undergraduate) Project Title: *Effects of Rearing Temperature on Physiology and Morphology of Painted Sea Urchin,* Lytechinus pictus
Summer 2017	**Super Mentor**, UC LEADS Program University of California Santa Barbara

PROFESSIONAL DEVELOPMENT & TRAINING

Research

Fall 2020-2021	**Virtual Lab Meeting Program**, RCN for Evolution in Changing Seas
Summer 2018	**Environmental Genomics**, Mount Desert Island Biological Laboratory, ME, July 13 - 21
Summer 2017	**Comparative Invertebrate Embryology**, Friday Harbor Labs, University of Washington, WA, June 12 – July 14

Teaching

Summer 2021	**Lead TA Institute,** University of California, Santa Barbara, CA, August 18 – 27
Spring 2020	**University Teaching,** University of California Santa Barbara, CA, March 29 – June 11

PUBLIC & PROFESSIONAL SERVICE

Outreach

2019-2021	**Elementary School Volunteer** La Cañada Elementary School, Lompoc, CA

2016-2019	**Family Ultimate Science Exploration (FUSE) Volunteer** University of California-Santa Barbara
Spring 2019	**Kindergarten Volunteer** Washington Elementary School, Santa Barbara, CA
February 2018	**California Forum for Diversity in Graduate Education – Bio Panelist** California State University – Channel Islands
2016	**Day Cruise Outreach for K-12 Teachers with NOAA on R/V Shearwater** NOAA Channel Islands National Marine Sanctuary

University Service

Spring 2018, Fall 2019	**EEMB Professional Development Course Panelist** University of California-Santa Barbara Imparted advice about my experiences within the department, topics included: forming a committee, talking to your advisors, work-life balance, etc.
Fall 2018	**New Graduate Student Orientation: Grad Success Series Panelist** University of California-Santa Barbara Answered questions about what to expect during the graduate school experience and what resources are available to promote success

PROFESSIONAL AFFILIATIONS

Western Society of Naturalists, Long Term Ecological Research (LTER) Network

ABSTRACT

The Role of Pre- and Post-Spawning Temperature Stress on Fertilization Dynamics within Santa Barbara Channel Sea Urchin Species

by

Terence Sebastian Leach

Marine heatwaves (MHWs) are becoming an increasingly pervasive threat to marine ecosystems. Projected increases in the frequency and intensity of future MHW events highlight the need to assess the adaptive capacity of marine organisms to such extreme events. While the massive mortality caused by MHWs is frequently observed, the sublethal consequences for reproduction and development serve as understudied areas of research. Phenotypic plasticity, the ability of an organism to change its physiology and/or morphology without alteration to the genome, presents an avenue in which the effects of MHWs can be transduced across life history stages. In this dissertation, I explored the impact of elevated temperatures associated with MHW events on the fertilization process and early development of two species of sea urchin found in the Santa Barbara Channel (SBC).

First, I used the purple urchin species, *Strongylocentrotus purpuratus*, to measure the effects of male thermal history on fertilization and early development. In the lab, male urchins were acclimated for one month to either non-MHW or MHW-like temperature conditions, as determined by temperature data collected in the SBC during past MHW events. Fertilization trials and offspring rearing were then conducted at both temperatures to

assess sperm and developmental performance. While sperm appeared robust to elevated temperatures during fertilization, sperm produced by MHW-acclimated males had overall diminished fertilization capacity as compared to those acclimated to non-MHW temperatures. For early developmental stages, the effects of paternal temperature were masked by the influence of developmental thermal conditions, where elevated temperatures resulted in larger offspring. Alternatively, the thermal physiology of offspring was significantly influenced by the interaction of paternal and developmental temperatures. Here, offspring had a higher thermal tolerance when their developmental temperature matched that experienced by their sire, possibly hinting at an adaptive paternal effect.

The influence of fertilization temperature on later developmental success was also explored in the summer-spawning sea urchin species, *Lytechinus pictus*. For this experiment, fertilization trials were performed under one of three temperatures representing: ambient, current MHW, and future MHW temperature. The resulting embryos from each fertilization treatment were then split and raised under the three temperatures. Here, elevated temperatures during fertilization had largely negative effects on fertilization success and the performance of later developmental stages. The number of successfully hatched and gastrulated embryos, representing key stages of development in echinoderms, was significantly lower in embryos produced from fertilizations at both MHW conditions than those from fertilizations under ambient conditions. Overall, the results of this dissertation highlight the importance of the reproductive response to extreme events like MHWs. The carry-over effects within and across generations seen here, show that the stress response of individual life history stages should not be studied in isolation, and that the negative effects of MHW events may persist long after these events have passed.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

I. Introduction

Statement of the problem

Human activities, such as the production and addition of carbon dioxide (CO_2) to the atmosphere, are driving unprecedented climatic changes in both terrestrial and aquatic ecosystems (IPCC 2022). On top of more gradual changes in the environment (e.g., ocean warming and acidification, sea level rise, decreased precipitation) is the increasing frequency and intensity of extreme events (Harris et al. 2018). These extreme events, including aerial heatwaves, cyclones, and wildfires, have already led to irreversible losses of biodiversity and loss of critical habitat (Goss et al. 2020; Wu et al. 2022). Together, the intensity and frequency of these more short-term, anomalous events will be exacerbated by the gradual shift in baseline environmental conditions. As such, it has never been more critical to identify vulnerable ecosystems and populations in hopes of creating more efficient climate change assessments and mitigation strategies.

In the ocean, anthropogenic CO_2 has driven atmospheric warming which has been linked directly to warming of the surface oceans along with the increased frequency of marine heatwave (MHW) events. Under current projections, global sea surface temperatures are expected to rise by 1.5-2.0 ^{o}C within the coming decades under even the most conservative greenhouse gas emission scenarios (IPCC 2022). Coupled with this predicted warming is the projected increased frequency and intensity of MHW events, defined as a discrete period of anomalously high temperatures (compared to an area's historical baseline) lasting longer than 5 days (Hobday et al. 2016; Oliver et al. 2021). Such events are predicted to become a decadal certainty by the end of the century, with these events characterized by increased

frequency (every two days), duration (126-152 days), and intensity (maximum temperatures ~3.5 ^{o}C higher than the 99th percentile of temperatures from an area's baseline) under the high emission scenario (IPCC 2022). Current warming and extreme heat events have already been shown to affect marine organisms by: altering biogeographic distributions (Byers and Pringle 2006; Sanford et al. 2019), altering phenological and reproductive cycles (Montes-Hugo et al. 2009; Pineda et al. 2018; Shanks et al. 2020), and impacting the health of ecosystem engineers (Gaitan-Espitia et al. 2014; Rogers-Bennett and Catton 2019), especially coral reefs (Hughes et al. 2003, Hoegh-Guldberg et al. 2007). Additionally, the short, intense thermal stress characterizing MHW events is more likely to push species past their physiological limits, leading to large-scale mortality events (Wernberg et al. 2016; Harvell et al. 2019; Seuront et al. 2019).

The impact of such extreme heat events is demonstrated by two recent heatwave events experienced on the Pacific Coast of the United States. From 2014-2016, the Pacific "blob" comprised a two-year period of anomalously warm sea surface temperatures from southern California up to Canada (Cavanaugh et al. 2019). Both lethal and sublethal effects were observed across coastal ecosystems in the following years (Rogers-Bennett et al. 2019; Sanford et al. 2019). More recently, the northern Pacific coast experienced an intense aerial and aquatic heatwave event over a relatively short period of 3 days. Occurring during the lowest tides of the year, this heat-dome caused a massive mortality event across the intertidal, the effects of which are still being elucidated (Hesketh & Harley 2022). These types of events are occurring across marine ecosystems, underscoring the pressing need for more research aimed at determining the biological consequences of exposure to such short-term, extreme climatic events.

Biological consequences of climate change

Understanding the adaptive capacity of species in response to climate change is a major research and conservation goal (Ofori et al. 2017; Thurman et al. 2020). In marine systems, for organisms not as reliant on migration and dispersal in response to unfavorable climatic conditions, adaptive capacity can be thought of as having two elements: micro-evolutionary processes (where genetic changes occur in response to selection in the environment) and phenotypic plasticity (where species change their physiology and morphology without alteration to the underlying genome) (Hoffmann and Sgro 2011; Bay et al. 2018). Since phenotypic plasticity may act more rapidly, and on ecological time scales, this class of mechanisms might allow species to cope with environmental change *in situ* (Fox et al. 2019). Additionally, these more reversible, plastic mechanisms are suggested to work in unison with evolution, serving as a buffer in which underlying genetic adaptation can have time to occur (Kronholm and Collins 2016, Fox et al. 2019).

The response of marine taxa to environmental stressors, such as elevated temperature, varies with ontogeny, highlighting the need to consider life history in projections of a species' adaptive capacity (Przeslawski et al. 2015; Thurman et al. 2020). For species with complex life history strategies, as is the case for many marine invertebrates, early life history stages appear to be the most sensitive to the environmental stressors associated with ocean warming, acidification, and hypoxia (Byrne 2011; Pandori and Sorte 2019). Elevated temperature experienced by these early stages has been documented to increase mortality, decrease growth, as well as promote stalled and abnormal development (Rahman et al. 2009; Randall and Szmant 2009; Byrne 2011; Martino et al. 2021). As many of these species are

broadcast spawners that release their gametes directly into the water column to facilitate fertilization, their gametes also have the potential to be exposed to stressor conditions.

In contrast to the sensitivity of post-fertilization life stages, the general effect of increased temperature on gametes and the resulting fertilization is more contested. Fertilization can occur with unaltered success under moderate increases in temperature for many species (Byrne et al. 2010a; Enricuso et al. 2019; Pitts et al. 2020), with several studies observing positive effects of elevated temperature on echinoderm and mollusk fertilization (Ho et al. 2013; Foo et al. 2014; Eads et al. 2016b). Alternatively, a handful of studies document decreased fertility under high temperature (Parker et al. 2010; Albright and Mason 2013), with these conflicting results even observed within the same species challenged by similar temperatures (Byrne et al. 2009; Eads et al. 2016a; Chirgwin et al. 2020). These inconsistencies highlight the variability associated with measuring fertilization success as a result of differences in methods, parental crossing designs, distances between experimental temperatures from the study organism's thermal limits, and parental environmental histories (Byrne 2011). Given the importance of fertilization for both the success of individuals and populations, it is imperative to gain a better understanding of the mechanisms through which environmental stressors, like temperature, affect the fertilization process.

Up to this point, much of the research aimed at characterizing adaptive capacity has focused on the individual responses of early life history stages to environmental stress (Byrne 2011). However, there is growing evidence to suggest that the response of specific life history stages to environmental stress are shaped by the environmental experiences faced earlier in development (intragenerational plasticity) and/or by their parents

(intergenerational plasticity) (Bonduriansky and Day 2009; Donelson et al. 2018). The consequences of either inter- or intra-generational plasticity could have large significance on how populations persist in the face of climate change, with adaptive effects serving to further buffer populations from stress but negative carry-over effects potentially exacerbating the sensitivity of already vulnerable stages (Chevin et al. 2010; Munday 2014). The mechanisms behind either process are yet to be elucidated, but there are many leading candidates, particularly for intergenerational (or parental) effects. Maternal provisioning, where a female alters the energy invested in each egg in response to her environment, has long been identified as playing a role on later offspring success (Boyd et al. 2016, Ross et al. 2016). Recent investigations into other non-genetic inheritance mechanisms such as epigenetics, though, have provided a new avenue in which both parents might contribute to the success of next generation outside of the passage of genetic information (Eirin-Lopez and Putnam 2019).

There is growing evidence that sires provide more than just genetic material to their offspring, giving an expanded view of transgenerational plasticity (TGP) that highlights the importance of both paternal and maternal environments on offspring performance (Crean et al. 2013, Crean and Bonduriansky 2014, Jensen et al. 2014, Marshall 2015, Guillaume et al. 2016). Paternal effects, defined here as the influence of fathers on their offspring's features through mechanisms other than the transmission of genetic alleles (Crean and Bonduriansky 2014), has largely been written off as a product of the morphology and biochemistry of sperm cells, themselves. Compared to the relatively large, cytoplasm-rich eggs they fertilize, sperm cells are lacking in the factors previously thought to underlie non-genetic inheritance: proteins and lipids (Immler 2018). The idea of epigenetic inheritance, though, has spurred a

new appreciation for paternal contributions. These epigenetic factors are expected to be present in both gametes, and there is even evidence to suggest an offspring's epigenome is more heavily influenced by that of their father than their mother (Jiang et al. 2013, Olson and Roberts 2015).

Up to this point, much of the work considering paternal effects in the context of environmental stressors starts and ends with the sperm cell, itself. Biotic and abiotic factors, such as pCO_2 (Havenhand et al. 2008, Kapsenberg et al. 2017), temperature (Chirgwin et al. 2020; Lymbery et al. 2021), salinity (Ritchie and Marshall 2013), sperm competition (Crean et al. 2013), and nutrition (Duxbury et al. 2018), have all been found to affect sperm phenotype, or the morphology and physiology that characterizes a sperm's ability to locate and fertilize an egg, and subsequent fertilization success (Marshall 2015). It is noteworthy that this group of studies surveys the impact of both the environment that the males experience during spermatogenesis as well as the environment experienced by the actual sperm cells when released on sperm phenotype. All things considered, there appears to be heavy selection under certain conditions for specific sperm phenotypes not only across males but also between sperm cells within the same ejaculate (Fitzpatrick et al. 2010). As this selection is context dependent, where variable sperm phenotypes are selected for under different conditions, gamete plasticity within males of certain species may be an adaptive strategy (Crean and Marshall 2008).

This gamete plasticity becomes more interesting when considering the potential for a paternal equivalent to anticipatory maternal effects, where dams in predictable environments can use cues to alter the resources given to their eggs in order to increase offspring fitness within that environment (Marshall and Uller 2007). As such, studies linking sperm and

offspring phenotypes in an environmental context are increasing. One of the more interesting examples comes from a study conducted in an estuarine tubeworm (Ritchie and Marshall 2013). First, this group found that within the ejaculate of a single male, only a subset of sperm is tolerant to low salinity and can subsequently fertilize eggs under these conditions. Further, sperm exposed to hyposaline conditions produced offspring that were more resistant to decreased salinity than offspring produced by sperm from the same ejaculate that was instead exposed to ambient salinity conditions. Similarly, paternal exposure to decreased salinities in a marine tubeworm had positive effects on both sperm and offspring performance when under the same hyposaline conditions (Jensen et al. 2014). The timing of paternal exposure to stress must be considered, though. One study found that acclimation of a marine tubeworm to elevated temperatures for two weeks resulted in decreased fertilization success and survival of the resulting offspring, but these negative effects were diminished after an additional 2-week exposure (Guillaume et al. 2016). Given the short timescales in which paternal effects can be observed, extreme climatic events like MHWs could disrupt the reproductive potential of populations through sire-related impacts on fertilization and development.

Study system

In order to explore the impact of temperatures observed in MHW events on fertilization and development, I chose to study two ecologically relevant sea urchin species within the Santa Barbara Channel, the purple sea urchin, *Strongylocentrotus purpuratus*, and the painted sea urchin, *Lytechinus pictus* (Pearse 2006). These sea urchin species were of particular relevance to my thesis research given their: (1) roles as developmental models, (2) ecological relevance within kelp forest communities, and (3) reproductive seasonality

aligning with the timing of MHW events experienced in the SBC. Both species have been extensively used in the laboratory as developmental models, meaning their reproduction and early development is well-characterized. In the laboratory, research on *S. purpuratus* and *L. pictus* has driven the fields of fertilization and developmental biology due to their fecundity, radial cleavage, synchronous development, and the relative ease required to spawn adults and culture their offspring (Watts et al. 2020; Nesbit et al. 2020). In addition, the sequenced *S. purpuratus* genome serves as an important link between deuterostome and protostome evolution (echinoderms are invertebrates on the deuterostome branch) and also lays the groundwork for transcriptomic and epigenetic assays to be conducted in the fields of developmental biology and eco-physiology (Consortium 2006, Evans et al. 2015). For *L. pictus*, the availability of genomic resources from the congeneric, *L. variegatus,* in addition to its rapid generation times (4-8 months) at room temperature makes this species a candidate for future work involving transgenic lines (Nesbit et al. 2019).

While *S. purpuratus* populations have a broad geographic range spanning the entire western coast of the United States, *L. pictus* have a much narrower range from Monterey Bay, California down to the Sea of Cortez in Mexico. In the areas where the two species overlap, they can be found cohabitating kelp forest communities across southern California. Ecologically, these urchin species are vital regulators of the kelp forest communities in the area (Ebert 2010; Yorke et al. 2019). As grazers of kelp recruits, urchin density, particularly that of *S. purpuratus,* influences the shift of their habitat from a rich, kelp forest to a kelp-depleted "barren" and vice versa (Dayton et al. 1992). This role in shaping kelp forest communities has made monitoring changes in *S. purpuratus* populations a key element of studies assessing the health of kelp forests following MHW events (Reed et al. 2016;

Rogers-Bennett and Catton 2019; Okamoto et al. 2020). Additionally, the seasonality of reproduction in these urchin species also leaves them vulnerable to the effects of MHW events. Across the globe, MHW events are more common during months within summer and early fall, when temperatures are already typically their highest within the SBC (Figure 1; Sen Gupta et al. 2020). This means that the early developmental stages of the summer-spawning *L. pictus* are often directly exposed to these anomalous temperatures (Strathmann 1987). On the other hand, gametogenesis begins in early fall for *S. purpuratus*, meaning that adult urchins of this species may also be carrying the effects of such events during a crucial part of their reproductive cycle. Responses to MHW events by each of these urchin species will highlight the vulnerability of each species' population in the SBC while also elucidating what elements of the urchin life cycle might be of particular relevance in future studies.

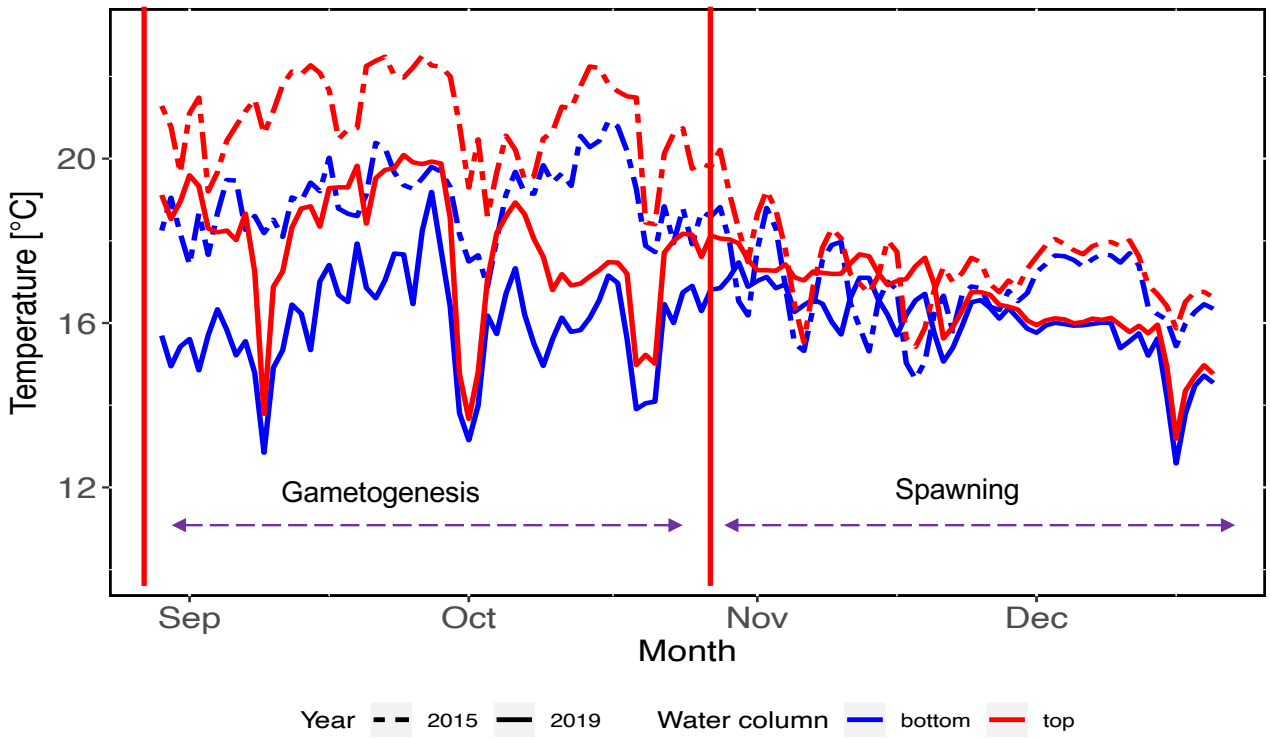


Figure 1. Correlation between the timing of *S. purpuratus* reproduction and MHW events observed in the SBC during 2015 (dashed lines) and 2019 (solid lines). Red lines represent temperatures measured at the top (pelagically) of the water column and blue lines represent temperatures measured at the bottom (benthically) of the water column.

Objective

The conceptual focus of my thesis was to investigate the role of paternal effects in an organism's adaptive capacity. This question was explored in the context of MHW events using two sea urchin species, the purple sea urchin, *S. purpuratus,* and the painted sea urchin, *L. pictus*. While paternal effects could manifest across the life history of these sea urchins, thesis research presented here focused on relevant processes within the early development of the offspring, including fertilization, hatching and gastrulation, and progression to an early larval stage. This research was guided by three (non-mutually exclusive) hypotheses (Figure 2):

H1: Gamete plasticity demonstrated under thermal stress alters fertilization success.

H2: Latent temperature effects on fertilization manifest during later development.

H3: Paternal thermal history influences offspring performance.

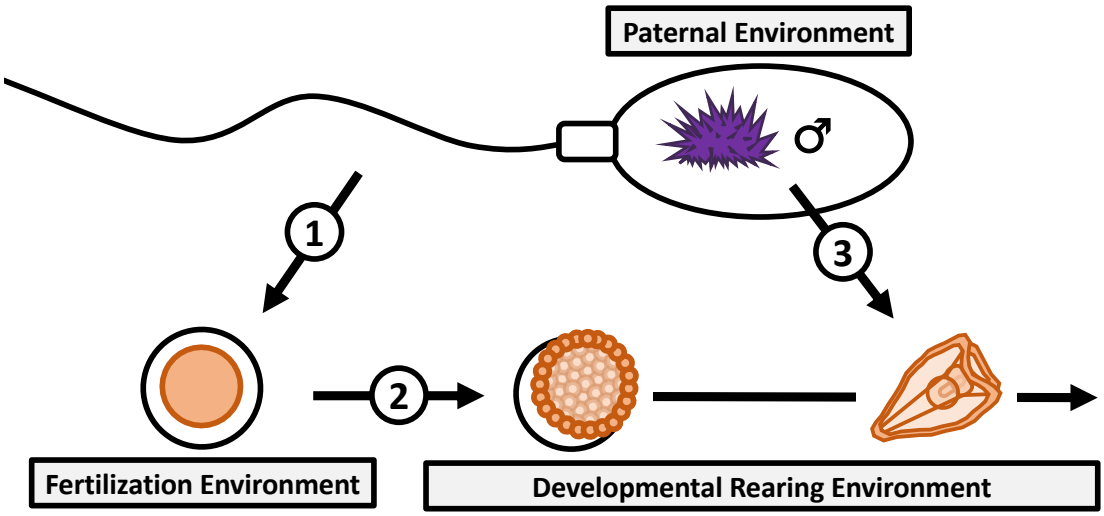


Figure 2. Conceptual diagram showing hypotheses that drove thesis research. Across two urchin species, the work described in this thesis explored the role of paternal effects in an organism's adaptive capacity. To address this question, experiments were designed using three hypotheses oriented around how the combination of three different environments (adult, fertilization, and development) can influence the degree of paternal effects observed during early development: (H1) Gamete plasticity demonstrated under thermal stress alters fertilization success, (H2) latent temperature effects on fertilization manifest during later development, and (H3) paternal thermal history influences offspring performance.

The above hypotheses were addressed using both the purple sea urchin, *Strongylocentrotus purpuratus*, and the painted sea urchin, *Lytechinus pictus* and are presented in four chapters (II-V), described below.

Chapter II: Development of methodology for quantifying fertilization success in sea urchin species

In this chapter, I develop a highly reproducible methodology for measuring fertilization success that is utilized throughout this dissertation. For the following chapters, the outlined protocol was used across both varied temperature conditions to explore the role of paternal environmental history on fertilization (Chapters III) as well as the carry-over effects of fertilization on later development (Chapter V). Given the diversity of methodologies currently used for quantifying fertilization success, I give a detailed summary of my protocol in this chapter for reproducibility in other studies seeking to explore similar questions within broadcast spawning marine taxa. In the Discussion, I compare specific areas that most often differ between methodologies and highlighted how this can influence interpretations and results.

Chapter III. Exploring impacts of marine heatwaves: Paternal heat exposure diminishes fertilization success in the purple sea urchin, Strongylocentrotus purpuratus

In this chapter, I address hypothesis #1 (H1), exploring the effects of paternal exposure to MHW-like temperatures on fertilization success in *S. purpuratus*. Here, groups of male adult urchins were acclimated for 28 days at two temperature conditions - a non-MHW treatment (14 ºC) and a MHW treatment (20 ºC), based on temperature data collected within the Santa Barbara Channel during multiple MHW events. After paternal acclimation, sperm from individual males was used in the fertilization success trials (outlined in Chapter II) with eggs pooled from females kept at the non-MHW temperature. Paternal temperature, but not fertilization temperature, was observed to have a significant influence on fertilization success, with sperm from sires acclimated to MHW-like temperatures possessing reduced

fertilization success. Results from this chapter highlight the importance of considering both male and female environmental history in projections of reproduction under climate change scenarios and suggest that MHW events may have a negative impact on fertilization in situ for *S. purpuratus* populations.

Chapter IV. Paternal Thermal History Influences Offspring Thermal Tolerance, but not Larval Size, in the California Purple Sea Urchin

In this chapter, I address hypothesis #3 (H3), investigating the impact of paternal thermal history on offspring performance within the context of marine heatwave (MHW) events. For this experiment, I used the same urchins acclimated for work outlined in Chapter III, where males were acclimated during a period in which spermatogenesis would occur under one of two temperature conditions – MHW-like (20 ^{o}C) and non-MHW (14 ^{o}C). Here, sperm from individual males were used to produce embryos that were subsequently raised under varying thermal conditions. Once offspring reached an early larval stage, the impact of paternal and offspring environments were assessed on two aspects of offspring performance: larval size and thermal tolerance. Under each rearing temperature, larval thermal tolerance was higher when rearing conditions matched that of their sire, representing one of the first examples of an adaptive paternal effect in a benthic marine invertebrate. Additionally, larvae raised under MHW-like temperatures were larger and demonstrated increased thermal tolerance than those raised under non-MHW temperatures. In the Discussion, I address the results in the context of MHW events in the Santa Barbara Channel and further connect observations in this Chapter to the results in Chapter III.

Chapter V. The Developmental Consequences of Heat Stress During Fertilization in the Summer Spawning Sea Urchin, Lytechinus pictus

In this chapter, I address hypothesis #2 (H2), exploring whether the temperature at which fertilization takes place has an impact on developmental success in the painted sea urchin, *Lytechinus pictus*. There is evidence to suggest that fertilization under stressful conditions may induce latent effects not observed until later, more vulnerable developmental stages. For summer spawning species, such as *L. pictus*, gametes and embryos are likely to experience seasonally higher temperatures during fertilization and early development as compared to other winter spawning species such as *S. purpuratus.* This work was conducted within the context of marine heatwaves (MHW), with experimental conditions representing either: (1) present-day thermal conditions in the Santa Barbara Channel (SBC), with one treatment mimicking temperatures associated with non-MHW summer conditions (17 ºC) and the other mimicking temperatures associated with observed summer MHW events (20 ºC); or (2) future high temperature extremes that could be experienced in the SBC based on projected increases in sea surface temperature over the coming decades (IPCC 2022), again comparing an ambient temperature treatment (20 ºC) to that mimicking a MHW-like temperature (24 ºC). For the experimental approach, I used pooled egg and sperm from multiple adults and then conducted fertilization success trials at each temperature (17 ºC, 20 ºC, or 24 ºC). The resulting embryos from these trials were then split and raised reciprocally under the temperatures associated with each MHW scenario (present-day: 17 ºC vs. 20 ºC; future: 20 ºC vs. 24 ºC). Embryo size, developmental success, and abnormality were assessed at two critical stages of development: hatching and gastrulation. The data presented here represent one of the first explicit examples investigating the carry-over effect of

fertilization temperature on later developmental success in an echinoderm species, and adds to a small, but growing body of literature focused on such effects in marine metazoans.

II. Development of Methodology for Quantifying Fertilization Success in Sea Urchin Species

Overview

In this chapter, I present the methodology developed for measuring fertilization success that is utilized throughout this dissertation. For the following chapters, the outlined protocol will be used across both varied temperature and pH conditions to explore the role of paternal environmental history on fertilization (Chapters III) as well as the carry-over effects of fertilization on later development (Chapter V). Given the diversity of methodologies currently used for quantifying fertilization success, I seek to give a detailed summary of my protocol in this chapter for reproducibility in other studies seeking to explore similar questions within broadcast spawning marine taxa. In the Discussion, I compare specific areas that most often differ between methodologies and highlight how this can influence interpretations and results.

Materials and Methods

Determination of urchin sex

In order to have enough males for experimentation involving fertilization success trials, urchins were sexed by a physical induction of spawning prior to placing into acclimation tanks. Here, spawning was induced by one minute of shaking outside of water in the week following collection. This physical perturbation typically resulted in the release of a small amount of gametes, enough that the urchin could be characterized as either a male (sire) or

female (dam). Identified sires and dams were then separated into appropriately labeled tanks. All urchins were given a recovery period at ambient temperatures while being fed kelp (*Macrocystis pyrifera*) *ad libitum* for 2-3 weeks following sexing. During this recovery period, urchins were monitored closely and any individual urchin that exhibited signs of significant injury, disease, or death was promptly removed and not included in down-stream experiments. As is noted in the following chapters, this method of sex identification was not only effective, but also did not result in significant mortality.

Urchin spawning and gamete collection

For the purpose of collecting gametic material, spawning of adult sea urchins was conducted following the procedures established in Strathmann (1987). Using a 23G needle connected to a 5-mL syringe, urchins were injected with 0.53M KCl into their oral surface (specifically in the soft tissue surrounding the Aristotle's lantern). Injections were followed by physical perturbation as described above. The contraction of the gonadopore, induced by this KCl solution, results in the release of large amounts of gametic material from the aboral side of the urchin. To prevent egg-sperm contact and/or sperm contamination between males, needles and syringes were discarded after injection of each male. The amount of KCl injected into each urchin often varied by the individual's size but was typically less than 1 mL. The negatively buoyant eggs of dams were collected by balancing each urchin (aboral side down) on a 100-300 mL glass beaker filled with filtered seawater (FSW) – natural seawater that had been sterilized both through a series of mechanical filters as well as via UV radiation. Dams, atop their beakers, were moved to a cold room to keep FSW at ambient temperature conditions, ~14 °C, and given 30 minutes to release their eggs before they were

removed from their beaker. Sperm was pipetted dry, directly off the sire's aboral surface, into a 1.5mL microcentrifuge tube kept on ice.

Collection of both eggs and sperm was immediately followed by measures of gamete quantity and quality. The number of eggs spawned by each dam was estimated from 3-5 15-uL aliquots of diluted eggs taken from each dam's beaker. To obtain these aliquots, eggs were gently suspended within the collection beaker before 5 uL was pipetted out and added to 10 uL of FSW already placed on a Sedgewick rafter counting chamber. The average number of eggs counted across aliquots via microscopic observation was used to calculate the egg concentration and thus estimate the total amount of eggs collected in each dam's beaker. Measures of egg concentration were only used once the egg counts across aliquots produced a coefficient of variation (CV[1]) lower than 10%. During these quantifications of egg quantity, eggs were also observed for quality. Mature eggs in *S. purpuratus* are round without much wrinkling and do not possess a visible germinal vesicle. Sperm quality was characterized by the presence of motile sperm once activated in FSW. Sperm that were noticeably slow-moving or lacked movement at all were characterized as low quality and not used in experimentation. Quantifications of sperm count were conducted after the fertilization success trial was completed and that process is outlined in the *Protocol for measuring fertilization success* section below.

Lastly, the fertilization capacity between eggs and sperm, an indication of gamete compatibility between individuals, was observed via test fertilization trials. Using 12-well tissue culture dishes, eggs and dilute, active sperm from individual dams and sires were combined and then checked for the presence of fertilization under the microscope after 5

[1] Coefficient of variation (CV) is equal to the ratio of standard deviation to the mean.

minutes. Fertilization was characterized by the presence of a uniformly elevated fertilization membrane that was not too tight around the egg (Fig. 3). For the fertilization success trials used throughout this thesis research, a threshold of >80% successful fertilization events produced from individual egg and sperm pairings was used to represent both high quality sperm as well as acceptable compatibility between sire and dam. The eggs of all dams demonstrating high compatibility with all high-quality sires were then pooled within 1-L beakers and brought to a concentration of 500 eggs/mL. Pooled eggs were kept at the varying environmental conditions to be tested during the experiment before use in the trials. Fertilization success trials typically began ~2 hours following spawning and, depending on the number of trials run, were completed within ~6 hours post-spawning. As such, pooled eggs were mixed consistently within the beaker to prevent hypoxia from overcrowding on the bottom.

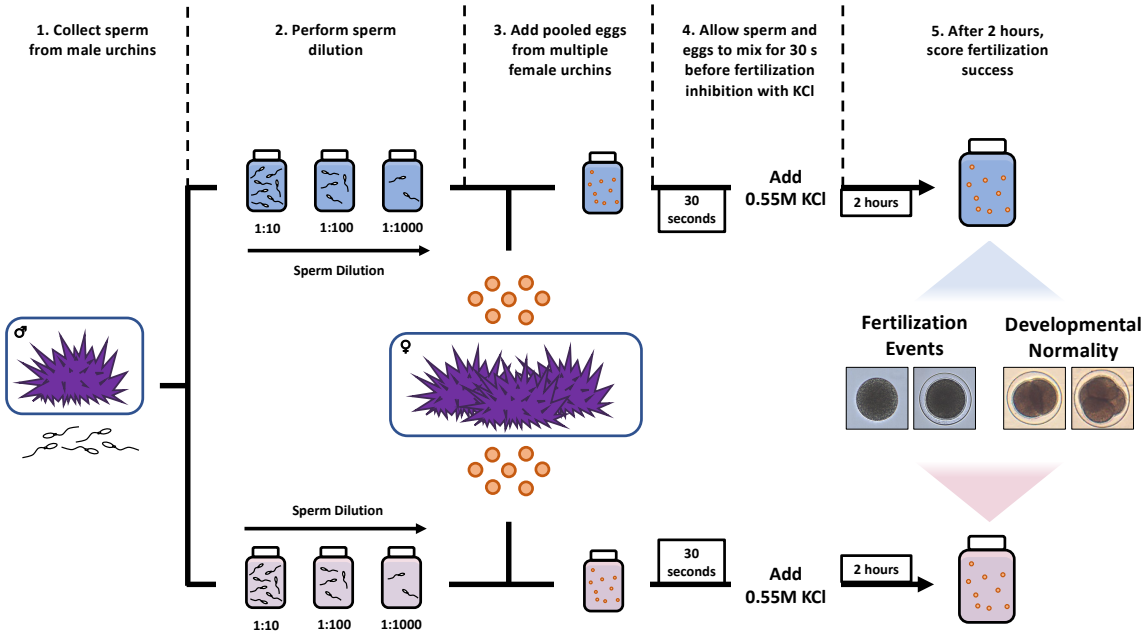


Figure 3. Conceptual figure showing the protocol for measuring fertilization success.

Protocol for measuring fertilization success

Quantification of fertilization success was adapted from protocols outlined in (Crean et al. 2013, Kapsenberg et al. 2017) (Fig. 3). First, sperm concentration was standardized across sires by centrifuging a sperm sample from each individual at 10,000 rpm for 1 minute in a microfuge and then removing the supernatant. Each sire's concentrated sperm was then activated by diluting the sperm 1:200 in 2 mL of FSW. This active sperm could then be observed under the microscope to check for satisfactory motility under the microscope. Then, the sperm were diluted again in FSW representing the environmental conditions to be tested during the experiment (pH, temperature). Diluted sperm was re-checked for motility before use in the experiment. Sperm concentrations were estimated from these sperm stock solutions by preserving a subsample of each stock in 2% formaldehyde-seawater and then counting sperm cells via a hemocytometer after the trials were completed (Hauser Scientific) (Sewell et al. 2014). Depending on the goal of the experiment, a single dilution or serial dilutions of the sperm stock solution were performed in 25-mL glass vials to create triplicate 20-mL solutions representing specific sperm concentrations. This process involved pipetting 2 mL of sperm (either from the stock solution or from a more concentrated vial within a serial dilution) into 20 mL of treated FSW. All vials were kept in either in a temperature-controlled aluminum block or a cold room to regulate temperature within vials during each trial. A thermocouple (OMEGA) was used before and after each trial to monitor temperatures across trials. Sperm within each vial were then mixed gently to homogenize contents before the addition of eggs.

For the fertilization trials, 1,000 eggs (in 2 mL of treated FSW) were added to each vial containing diluted sperm. Each vial was homogenized by inversion immediately after egg

addition. Egg and sperm were given 30 seconds of contact time within each vial before 1 mL of 0.55M KCl was added to inhibit further fertilization events (Farley and Levitan 2001). This addition of KCl serves to mimic the fast block to polyspermy naturally used by sea urchins, where an altered electric potential at the egg surface prevents sperm from fusing with the eggs now possessing a positive membrane potential (Jaffe 1976). As this process does not inhibit embryonic development, vials were then moved to an incubator where fertilized eggs were allowed to develop for at least 2 hours. The processes of egg and KCl addition were also repeated for one control vial, containing 18 mL of treated FSW with no sperm, during each trial. Any observation of fertilization within control vials invalidated that whole trial and led to its removal from analysis.

Fertilization success was quantified by sampling >75 eggs from each vial and assessing the presence of a fertilization envelope (FE) and normal post-fertilization development (see Table 1 in Chapter III). Successfully fertilized eggs that demonstrated normal development were characterized by a smooth, uniformly elevated FE as well as equal, holoblastic cleavage. Unsuccessful fertilizations within eggs were represented by one of two categories – unfertilized eggs (which possessed no FE) and abnormal embryos (where tight FEs or delayed/abnormal cleavage or development was observed) (Sewell et al. 2014, Kapsenberg et al. 2017). Scoring eggs and embryos in this manner allowed for an assessment of the two important aspects of the fertilization process: (1) the fertilization event, or how well sperm was able to find and fuse with egg under the 30 second time limit, and (2) the subsequent embryonic development following fertilization. The proportion of successful fertilization events was calculated by the following equation:

Proportion Fertilization Events = (Total eggs – Unfertilized eggs)/Total eggs

The proportion of normally and abnormally developed embryos were then calculated from the eggs that were fertilized:

$$\textit{Proportion Normal Development} = \textit{Normally developing embryos/Fertilized eggs}$$

$$\textit{Proportion Abnormal Development} = \textit{Abnormally developing embryos/Fertilized eggs}$$

Discussion

Fertilization success is an important metric to consider in assessments of reproductive success, but research has highlighted how dependent results can be on the methodology used to quantify the fertilization process (Lera et al. 2006; Byrne 2011). The above procedure allows for robust quantifications of fertilization success within sea urchins under a number of different environmental conditions and can be adapted to answer a variety of questions focused on the impact of environmental stress on fertilization. Across this dissertation, I used this protocol to explore the influence of paternal environment on fertilization (Chapters III) as well as the impact that thermal conditions during fertilization can have on later development (Chapter V). Below I compare this protocol to that of other studies and discuss the potential considerations for future scientists seeking to utilize/develop quantifications of fertilization success in their own research.

The biggest influences on fertilization success methodology appear to revolve around decisions made about crossing design, gamete concentrations, and scoring procedure. Across the literature, studies can be divided based on their use of pooled gametes vs. individual male-female pairings in fertilization success trials. The decision made has heavy influence on results and interpretation (Byrne 2011). The use of pooled sperm and eggs can be thought to better mimic natural conditions, reducing the effects of individual male-female

incompatibilities (Byrne et al. 2009, Eads et al. 2016b). Studies utilizing this design have also demonstrated a positive influence of polyandry on fertilization success, with researchers commonly finding less pronounced effects of the environment on fertilization as compared to when individual male-female pairings are utilized (Evans and Marshall 2005). These individual pairings instead highlight intra-individual variability that could allow for speculation about the fitness of individuals in the population (Foo et al. 2014, Eads et al. 2016a). The protocol used in this dissertation has been utilized in the pooled gamete format as well as the less frequent split-clutch design outlined above. In Chapters III and V, this split-clutch design involved fertilizing eggs from the same pool of females with sperm from individual males (Crean et al. 2013). This approach can serve somewhat as a middle ground between the population-level and individual-level designs. Assessment of individual male's fitness is possible but the method also serves to limit the influence of pair-specific incompatibility. This approach made the most sense for the dissertation experiments given the focus on paternal-specific effects on fertilization. Given the variety in results and interpretations between crossing designs, researchers should take great care in what methodology they choose to answer a specific question.

Another important consideration for the implementation of fertilization success trials is the sperm concentration utilized. The number of fertilization events positively correlates with sperm concentration, with a higher likelihood of gametic collisions (Kupriyanova and Havenhand 2002). To some degree, this translates to higher fertilization success when higher sperm concentrations are used, but the relationship is bell-shaped given potential polyspermy at high ratios of sperm to egg (Sewell et al. 2014, Kapsenberg et al. 2017). During early iterations of this protocol (Chapter III), fertilization success was assayed at

various sperm concentrations to explore the relationship between sperm concentration and fertilization success. Environmental influences on fertilization success were most visible at lower concentrations (concentrations that led to <100% fertilization), supporting the view that use of these concentrations more accurately assess the effect of environmental stress (Marshall 2015).

In response to Chapter III's findings, following iterations of the protocol (Chapters V) involved use of one sperm concentration, targeting that at which ~75% of eggs were fertilized under ambient conditions. It is important to note that use of higher sperm concentrations is not as ecologically relevant, but this also depends on study species. Purple urchins, for example, congregate before spawning, increasing the likelihood that eggs will be exposed to higher concentrations of sperm (Levitan et al. 1992). Knowledge of the natural history of the study organism, paired with whether the goal of the study is more mechanistic or ecological should be considered in decisions about sperm concentration.

Lastly, the scoring procedure implemented during fertilization success trials can provide varying information about what mechanisms influence results. Here, a scoring procedure was developed to minimize subjectivity between scorers. Scoring in all chapters assessed two components of successful fertilization, (1) general fertilization kinetics, or how well sperm was able to find egg and fertilize it in a given time period (30 seconds), and (2) the developmental success of embryos post-fertilization. While many studies present fertilization success as just one of these metrics or by lumping them together, analyzing them separately can shed light onto what component of the fertilization process is most affected by the environmental conditions. Decreased number of fertilization events under environmental stress can point toward mechanistic deficits in gamete performance, such as

altered sperm motility, acrosomal functionality, or morphology (Immler 2018). Quantifications of fertilization events alone though can provide misleading interpretations of fertilization success (Fig. 4).

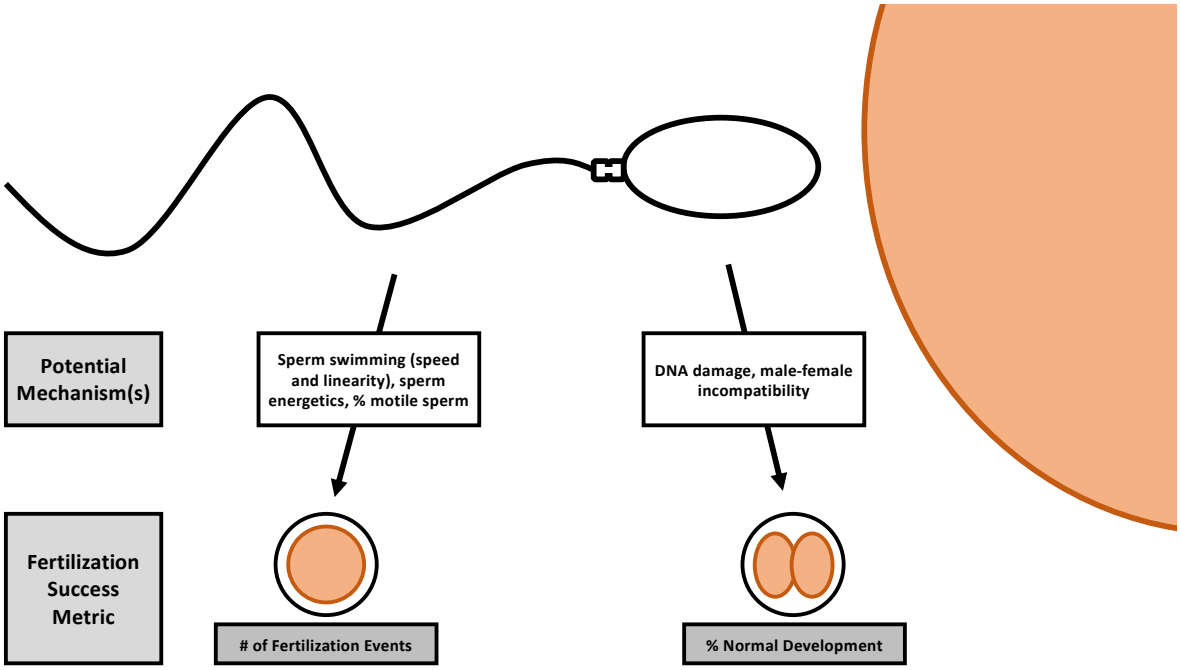


Figure 4. Conceptual diagram showing different potential interpretations when fertilization success is based on measuring successful fertilization events versus normal post-fertilization development

Looking at the subsequent development of fertilized eggs can shed light on whether those fertilization events will translate to viable offspring. Observations of early cleavage events, as was utilized in Chapter III, can detect potential incompatibilities and polyspermy (Sewell et al. 2014, Kapsenberg et al. 2017). There is speculation whether the influence of environmental stress during fertilization or by the parents on development would manifest during these early of stages, so many studies wait to later developmental stages to score developmental success (Byrne 2011). This was the process utilized in the Chapter V. This

experiment revealed that developmental success is influenced by the interaction of fertilization and developmental environmental conditions, so scoring at later developmental stages may require additional logistics to account for such dynamics (factorial design, close monitoring of environmental conditions during development).

Conclusion

Fertilization success is a crucial element for the reproductive output of marine organisms, where declines in fertilization could have drastic impacts on the larval output and eventual recruitment of populations as well as increased selection for particular genotypes (Campbell et al. 2016). Climate change assessments thus require more research on how the fertilization process is influenced by the environment itself as well as by other factors, like parental environmental history. Current literature about fertilization dynamics under climate change scenarios has inconsistent results, partially attributed to differences in the methodology used. Here, I provide a detailed outline of a fertilization success protocol that is robust and adaptable. I advise that researchers take deep consideration into what the goal of their study is and how decisions about crossing design, gamete ratios, and the scoring procedure before implementing a similar protocol in their own research.

III. Exploring Impacts of Marine Heatwaves: Paternal Heat Exposure Diminishes Fertilization Success in the Purple Sea Urchin, *S. purpuratus*

Overview

In this chapter, I explore the effects of paternal exposure to MHW-like temperatures on fertilization success in S. purpuratus. Here, we acclimated groups of male adult urchins for 28 days at 2 temperature conditions - a non-MHW treatment (14 ºC) and a MHW treatment (20 ºC), based off temperature data collected within the Santa Barbara Channel during multiple MHW events. After paternal acclimation, sperm from individual males was used in the fertilization success trials (outlined in Chapter II) with eggs pooled from females kept at the non-MHW temperature. Paternal temperature, but not fertilization temperature, was observed to have a significant influence on fertilization success, with sperm from sires acclimated to MHW-like temperatures possessing reduced fertilization success. Results from this chapter highlight the importance of considering both male and female environmental history in projections of reproduction under climate change scenarios and suggest that MHW events may have a negative impact on fertilization in situ for *S. purpuratus* populations. The following work is published in *Marine Biology* (Leach et al. 2021).

Materials and Methods

Animal collection

Urchins were hand-collected on SCUBA in December 2019 from two separate temperate reef study sites in the Santa Barbara Channel; these locations are also Santa Barbara Coastal

Long-Term Ecological Research (LTER; https://sbclter.msi.ucsb.edu/) sites: Mohawk Reef (34.394°N, 119.730°W) and Arroyo Quemado Reef (34.468°N, 120.119°W). Urchins in these locations, which are ~37 km apart, generally experience similar water temperature conditions and there is little evidence to suggest a difference in the timing of gravidity or reproduction between locations. Collections were conducted within a week of one another using California Scientific Collection permit SC-1223 (to G. Hofmann). Urchins from Mohawk Reef and Arroyo Quemado Reef were collected at depths of 8 ft. and 10 ft., respectively. Adult urchins were transported to the Marine Science Institute's seawater research facility and placed in flow-through seawater aquaria held at 13 ºC. Urchins from each location were kept separate and their location identity was tracked for the entirety of the study.

Determination of urchin sex and adult acclimation

In the days following collection, urchins were sexed following the procedure outlined in Chapter 2. From this process, 74 urchins (representing ~67% of the total urchins attempted to be spawned) were successfully identified as either male (n = 31 urchins) or female (n = 43 urchins) and separated into appropriately labeled tanks. Over the time spanning both recovery and the subsequent experimental acclimation, only 10 out of the original 111 urchins exposed to this spawning induction method were removed for signs of death or disease.

After the recovery period, male urchins were acclimated to either a high (20 ºC) or low (14 ºC) temperature treatment for 28 days (Fig. 5). The 28-day acclimation period was used to both reflect a timeframe that is within the duration of past MHW events as well as overlap

with the production of new sperm cells within the regularly fed male sea urchins (personal communication). This would increase the likelihood that the sperm used in this study included those produced under experimental conditions mimicking either ambient or ecologically relevant heat stress conditions. Male urchins were randomly selected and placed in one of the three acclimation tanks in either the high temperature (H) or low temperature treatment (L). Test diameters for each urchin were measured to ensure that no significant size distinction existed between tanks or treatments.

Acclimation tanks consisted of a 20-gallon tank equipped with an aquarium pump (Aqua-Supreme) and supplied with UV-sterilized, filtered seawater at a rate of 12 L/hr through irrigation button drippers (DIG Corporation). Tanks from each treatment were half-way submerged in a large water bath where temperatures could be controlled. Seawater temperature was controlled using a Delta Star heat pump with a Nema 4x digital temperature controller (AquaLogic). Temperatures in each tank were monitored through point measurements with a wire thermocouple (Thermolyne PM 20700 / Series 1218) and for the entirety of the acclimation, every 5 minutes, by Hobo temperature loggers (Onset Computer Corporation). Once per week, each acclimation tank received one kelp blade per urchin present. Each weekly feeding interval lasted three days before any uneaten kelp material was removed. Female urchins were kept in multiple 10-gallon flow-through tanks maintained at ambient temperatures, approximately 13 ^{o}C and fed kelp *ad libitum* for the entirety of the male acclimation.

Due to the limited resources and time available for this study, adult acclimations and the following spawning were conducted in three blocks staggered by 96 hours from one another. To facilitate these staggered adult acclimations, each male acclimation tank was separated

into three subdivisions representing each of the three blocks. For clarity, the first block of adult acclimations involved the random selection of 12 male urchins from the recovery tanks that were then placed into one subdivided section of 6 different tanks (n = 2 urchins per tank section), 3 replicate high temperature acclimation tanks and 3 replicate low temperature tanks. After 96 hours, the second block was initiated by selecting 12 more male urchins and placing them into a separate, subdivided section for each of the same 6 acclimation tanks previously mentioned. This process was repeated one last time 96 hours after that for the third block of this experiment. Temperatures, pCO_2 levels, and urchin behavior were closely monitored throughout to ensure that there were no significant disturbances to the urchins already present in the acclimation tanks when urchins were added or removed. This method was implemented to allow increased replication while ensuring that each block's male urchins endured exactly 28 days of acclimation before spawning without a significant amount of time between spawning events.

Urchin spawning and experimental design

During each block, male and female urchins were randomly selected to be spawned. Males from different replicate acclimation tanks were represented within each block. Urchin spawning, gamete collection, and measures of gamete quantity and quality followed that outlined in Chapter 2. For this experiment, sperm were used within 6 hours of collection. Urchins within each block were crossing utilizing a modified split-clutch crossing design for measuring fertilization success: involving the fertilization of pooled eggs (n = 6 females) by three males from each high and low treatments, resulting in 6 unique crosses. This crossing design with pooled eggs was utilized to minimize the effects of male-female interactions on

fertilization and subsequent development. Across the three blocks, 9 males from each treatment and 3 pools of females were used to proxy this question over a total of 18 crosses.

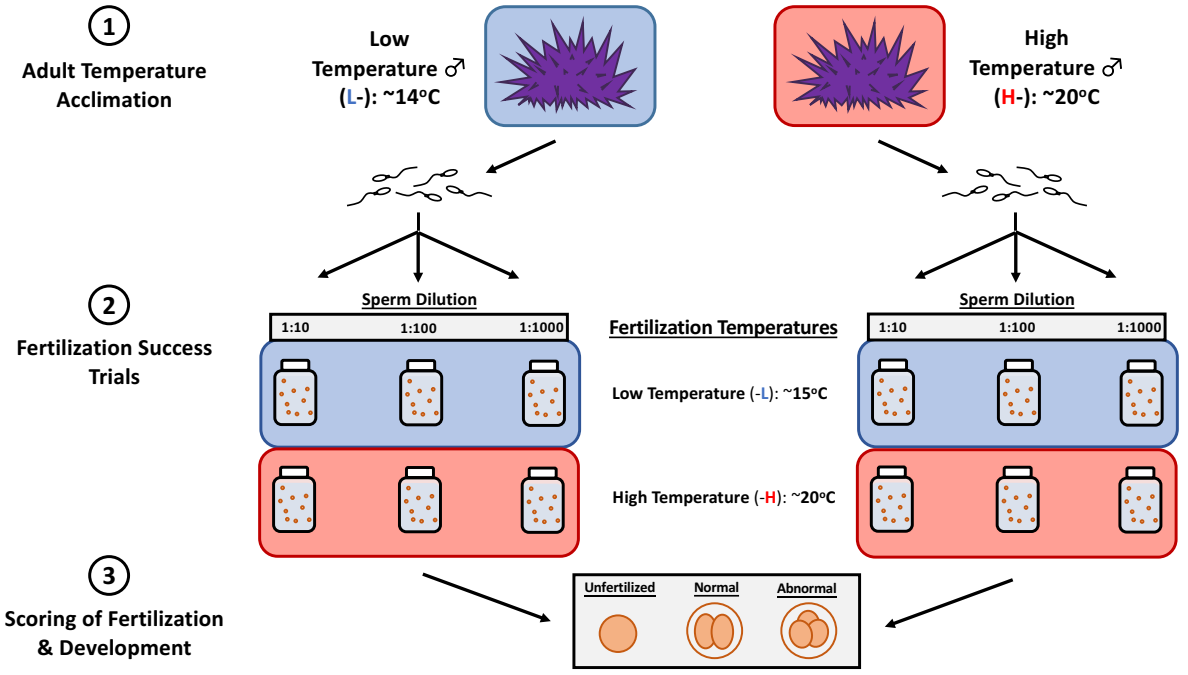


Figure 5. Diagram of the experimental design, highlighting three main aspects of the process. (1) Adult male urchins were acclimated for 28 days at either a low (L-; 14 °C) or high (H-; 20 °C) temperature treatment. (2) Urchins were then spawned to conduct a fertilization success assay for individual males from each treatment (n = 9 male urchins/treatment). Fertilization assays, performed at both a low (-L;14 °C) and high (-H; 20 °C) temperature, included serial dilution of each male's sperm to three different concentrations (1:10, 1:100, 1:1000) and then a 30 s exposure to pooled eggs (portrayed here by orange circles within each vial) from multiple female urchins. (3) After a 2-h incubation at 14 °C, eggs were scored for successful fertilization and subsequent development

Statistical Analysis

The influence of paternal temperature on the proportion of fertilization events observed was analyzed as a binomial response with a generalized linear mixed-effects model. The binary data (fertilized vs unfertilized) was fitted using a logit link function. The model included paternal temperature treatment, fertilization temperature treatment, and sperm

concentration as fixed effects in addition to both experimental round and male identity as random effects. Non-significant random terms, such as site location and some of the interactions between fixed factors, were removed from the analysis following standard model reduction procedures outlined in (Quinn and Keough 2002). A Wald chi-square test was employed to further determine the significance of each factor on the number of fertilization events observed. To determine if the sperm concentrations referenced in the model were themselves influenced by paternal temperature acclimation, the relationship between paternal temperature group and sperm concentration was examined using a Welch two-sample t-test. Additionally, the relationship between fertilization success and the sperm concentrations corresponding to dilutions (1:10, 1:100, 1:1000) for each individual male were analyzed by linear regression and found no significant correlation ($R^2 < 0.1$), so concentrations were binned into three categories (HIGH for 1:10, MID for 1:100, LOW for 1:1000) for use in the previously described model.

Analysis of how paternal temperature affected the proportion of fertilized eggs that developed normally was also analyzed with a generalized linear mixed-effects model. This dataset, again modeled as a binomial response fitted with the logit link function, excluded datapoints corresponding to eggs fertilized under the LOW sperm concentration as all replicates had < 10 eggs that had even been fertilized to start with. As such, sperm concentration, now containing only two categories (MID and HIGH), was modeled as a fixed effect in addition to paternal temperature treatment and fertilization temperature treatment. Male identity and round were included as random effects once again. Again, non-significant random terms were removed following standard model reduction procedures and a Wald chi-square test was utilized to determine whether each factor had a significant effect

on normal development (Quinn and Keough 2002). All statistical analysis was performed in R (version 3.6.3) with models created using the lmer4 package (Bates et al. 2015).

Table 1. Scoring protocol for fertilization success assay.

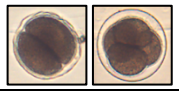
Fertilization Success	Description	Image
Unfertilized	No fertilization membrane	
Normal Embryo	Normal fertilization membrane; symmetrical cleavage	
Abnormal Embryo	Tight fertilization membrane or asymmetrical cleavage	

Table 2. Temperatures measured during adult acclimations and fertilization success trials. All values displayed as mean ± standard deviation.

Acclimation	Duration	Treatment	Temperature (ºC)
Adult	28 days	High Temp (H-)	19.6 ± 0.23
		Low Temp (L-)	13.5 ± 0.42
Fertilization	5 min	High Temp (-H)	19.8 ± 0.65
		Low Temp (-L)	14.9 ± 0.94

Results

Fertilization Events

In general, male thermal history significantly impacted the number of successful fertilization events observed *in vitro* at all sperm concentrations (Table 3; $p = 0.035$). In addition, both sperm concentration alone, and the interaction between sperm concentration and the temperature at which fertilization occurred, had significant effects in the model (Table 3). Within each sperm concentration, a lower proportion of eggs were fertilized by

sperm from high temperature males (HIGH: 97.2 ± 0.7%, MID: 44.3 ± 2.4%, LOW: 6.2 ± 0.6%, presented as mean ± standard error) as compared to sperm from low temperature males (HIGH: 98.0 ± 0.7%, MID: 53.7 ± 3.1%, LOW: 9.2 ± 0.8%) (Fig. 6). The effect of paternal temperature was most pronounced under the middle (MID) sperm concentration, where high temperature males produced sperm that resulted in ~9% less fertilization than that of low temperature males. The other sperm concentrations exhibited a similar pattern but to a lesser degree, with high temperature males having ~3% and ~1% less fertilization under the most dilute (LOW) and concentrated (HIGH) sperm conditions, respectively.

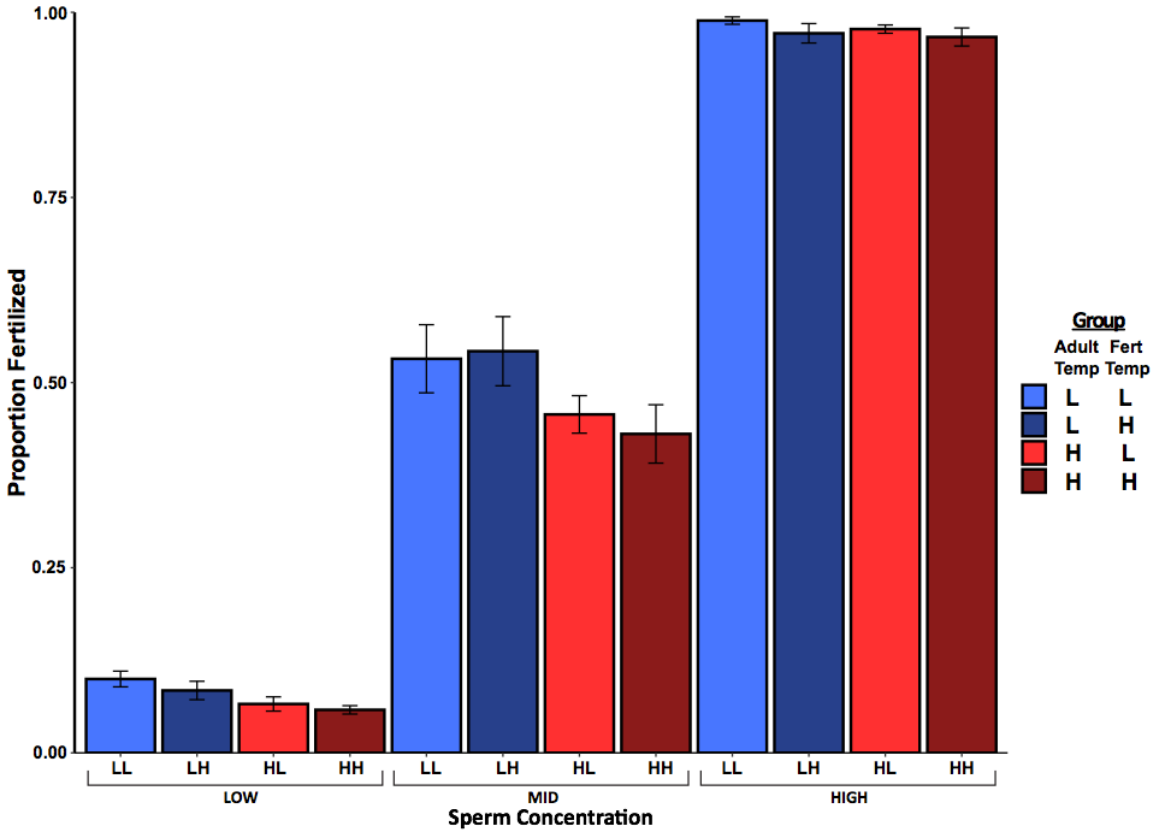


Figure 6. Effects of sperm concentration (low, mid, high), paternal temperature experience (L-, 14 °C; H-, 20 °C) and fertilization temperature treatment (-L, 15 °C; -H, 20 °C) on the percentage of fertilization events in the urchin *S. purpuratus*. Error bars represent mean ± standard error.

Table 3. Statistical output (Wald chi-square test) from generalized linear mixed effects model used to analyze the effect of paternal temperature and fertilization temperature on the proportion of fertilization events seen across three sperm concentrations

Source	χ^2	*df*	*p*
Sperm Concentration Group	2747.66	2	*<0.001
Paternal Temperature	4.46	1	*0.035
Fertilization Temperature	2.78	1	0.096
Sperm Concentration Group * Fertilization Temperature	17.65	2	*0.002

Not surprisingly, sperm concentration was a highly influential parameter in the experiment, displaying a positive relationship with the proportion of fertilized eggs observed (Table 3; $p < 0.001$). Measured sperm concentrations, calculated from a sample taken from each male's original sperm stock solution, averaged $1.46 \times 10^6 \pm 7.32 \times 10^4$ sperm.mL^{-1}. There was no observed difference between the sperm concentrations produced by differentially acclimated males (t (16) = 0.043, p = 0.9663), with sperm concentrations from high temperature males averaging $1.48 \times 10^6 \pm 1.11 \times 10^5$ sperm.mL^{-1} compared to $1.44 \times 10^6 \pm 9.81 \times 10^4$ sperm.mL^{-1} in low temperature males. Based off of these calculations and estimations from conducted serial dilutions, we assume that the concentrations were roughly 10^5, 10^4, and 10^3 sperm.mL^{-1} for the HIGH, MID, and LOW sperm concentration groups, respectively. The proportion of fertilized eggs increased positively with these estimated sperm concentrations (Fig. 6), with a majority of eggs remaining unfertilized in the most dilute sperm conditions (LOW: 7.7 ± 0.54%) to nearly all eggs exhibiting fertilization under the most concentrated sperm conditions (HIGH: 97.6 ± 0.49%). The MID sperm concentration exhibited an almost even split between the fertilized and unfertilized eggs observed (48.9 ± 2.1%). These concentrations also exhibited an interactive effect with

fertilization temperature (Table 3; $p = 0.002$). Note that there was no significant effect of fertilization temperature alone on the proportion of fertilized events observed (Table 3; $p = 0.096$).

Development of Embryos

There was not a detectable effect of paternal thermal history on the proportion of fertilized eggs that cleaved normally (Table 4; $p = 0.35$). Alternatively, there were significant effects of sperm concentration and the interaction between sperm concentration and paternal thermal history (Table 4). As the trials associated with the LOW sperm concentration all yielded < 10 fertilized eggs, they were not included in this analysis due to low sample sizes. In comparing the normal development of fertilized eggs between the MID and HIGH sperm groups, we observed a large significant effect of sperm concentration (Table 4; $p < 0.001$). The proportion of normally developed fertilized eggs was diminished with higher sperm concentration, with only 32.5 ± 1.7% of fertilized eggs developing normally under high concentrations as opposed to 75.4 ± 1.9% for the middle concentrations (Fig. 7). While there was no significant effect of either paternal or fertilization temperature on the proportion of normal development (Table 4; $p = 0.18$), under the HIGH sperm concentration, embryos from high temperature males that experienced high temperatures during fertilization exhibited more normal development (37.5 ± 2.9%) than any other combination of paternal and fertilization temperatures (HL: 31.3 ± 2.7%, LH: 28.7 ± 4.4%, LL: 31.4 ± 3.5%). This pattern was not observed under the MID sperm concentration, driving the significant interactive effect of concentration and paternal temperature seen in the model (Table 4; $p = 0.01$).

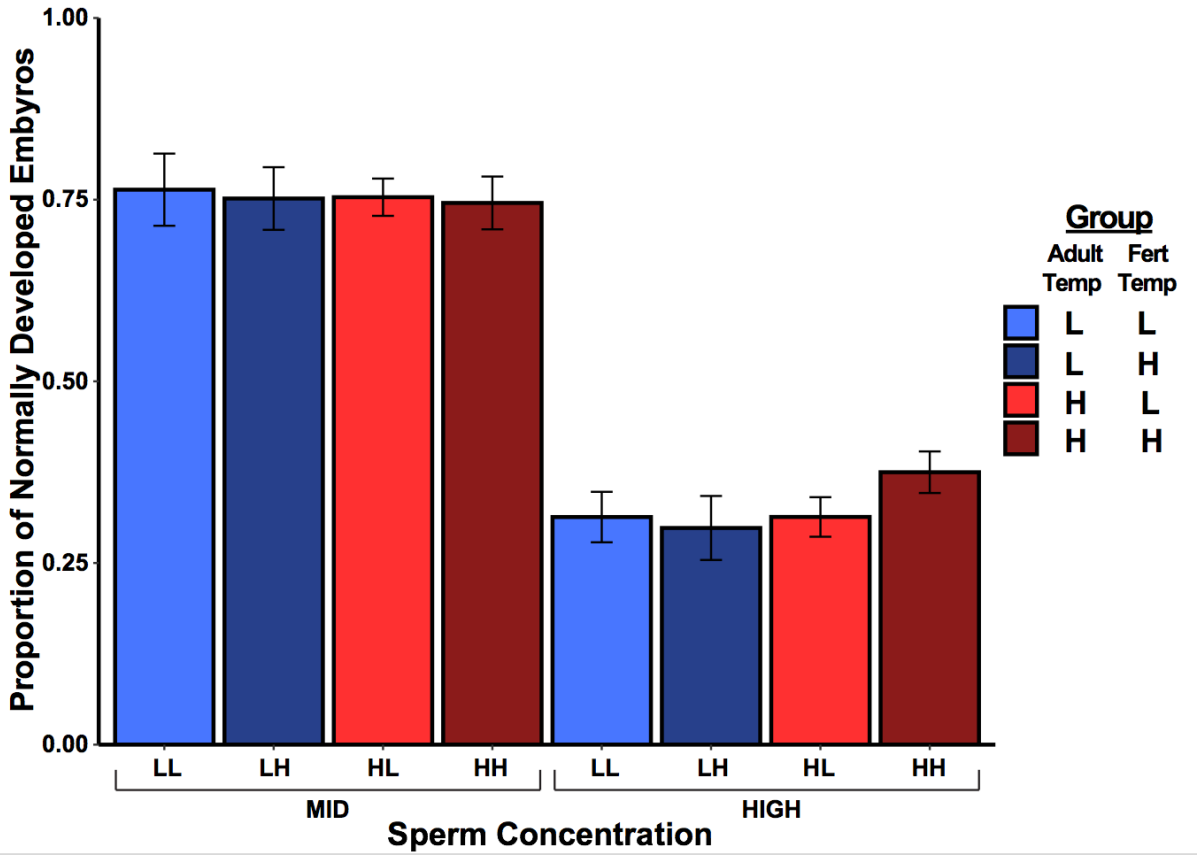


Figure 7. Effects of sperm concentration (mid and high), paternal temperature experience, and fertilization temperature treatment on developmental success in the urchin *S. purpuratus*. Representative of the proportion of normally developed embryos from eggs that were fertilized. Error bars represent mean ± standard error.

Table 4. Statistical output (Wald chi-square test) from generalized linear mixed effects model used to analyze the effect of paternal temperature and fertilization temperature on the proportion of normally developed embryos seen across two sperm concentrations.

Source	χ^2	*df*	*p*
Sperm Concentration Group	1027.99	1	*<0.001
Paternal Temperature	0.86	1	0.353
Fertilization Temperature	1.79	1	0.181
Sperm Concentration Group * Paternal Temperature	6.68	1	*0.010

s

Discussion

In this study, we examined the fertilization success of the purple sea urchin, *Strongylocentrotus purpuratus,* under the elevated temperatures associated with marine heatwave (MHW) scenarios. Our experimental goal was to determine how MHW-like temperatures experienced during spermatogenesis and fertilization would influence the fertilization process. To meet this goal, adult male sea urchins were exposed to two temperatures, 14 ºC or 20 ºC, for 28 days before their sperm was assessed via fertilization success trials under the same temperature treatments. In examining the role of paternal thermal history on fertilization dynamics under these temperatures, we discovered two central findings: (1) the temperature at which fertilization occurred had no significant effect on fertilization itself, and (2) paternal exposure to a MHW-like temperature had a negative impact on fertilization success as compared to the non-MHW treatment. Underlying the second finding is the observation that these patterns in fertilization success were driven mainly by a reduced ability of the sperm to reach the egg and fertilize it as opposed to abnormal development seen post-fertilization. Below we discuss each aspect of our study and relate our results to the phenology of the sea urchin life cycle relative to the timing of MHWs in our area.

Fertilization robust to high temperature

In our study, fertilization in *S. purpuratus*, regardless of paternal thermal history, was not significantly altered by high temperature. Successful fertilization under a high, MHW-like temperature (20 ºC) was observed at similar proportions to fertilization occurring under a low, non-MHW temperature (15 ºC). Although we did not directly measure any sperm

traits in this study, a scoring design was utilized that allowed for more insight into what elements of the fertilization process could be most influenced by temperature. The presence of a fertilization membrane alone was used to calculate the proportion of fertilization events observed, which could serve as a proxy for general fertilization kinetics involved in the sperm reaching the egg and fertilizing it in a defined time frame. We then measured how many of those fertilized eggs demonstrated normal cleavage after 2 hours at 14 ºC, allowing for the assessment of abnormality caused by polyspermy, male-female incompatibility, or sperm DNA damage. Here, neither the proportion of fertilization events or normally cleaving embryos was significantly altered by fertilization temperature, but the effect was just non-significant ($p = 0.095$) for the number of fertilization events observed. Together, these results align with the prevailing view that fertilization in sea urchins displays a great deal of plasticity and is generally robust under ocean warming scenarios (Byrne 2011).

In terms of species comparisons, studies focused on how temperature affects sea urchin fertilization biology report some contrasting and species-dependent results. Most tropical and temperate sea urchins demonstrate successful fertilization over a wide range of temperatures, even under temperature challenges much higher than what the urchin populations experience *in situ* (Rupp 1973; Byrne et al. 2010b; Delorme and Sewell 2014; Foo et al. 2014). Several echinoid species across coastal Australia exhibit high fertilization success (>80%) when challenged with temperatures 2–6 ºC higher than that experienced locally (Byrne et al. 2010b; Foo et al. 2014). Alternatively, the fertilization of a few temperate and polar species exhibits a lower thermal tolerance threshold (Farmanfarmaian and Giese 1963; Byrne et al. 2009; Ericson et al. 2012; Gianguzza et al. 2014). Such vulnerabilities have renewed relevance when considering the anomalously high temperatures

associated with current MHW events. For example, while the Mediterranean sea urchin species, *Arbicula lixula*, exhibited successful fertilization at temperatures above that predicted under near-future warming scenarios, the complete disruption of fertilization (< 1%) at temperatures +7 ºC than ambient conditions leave *A. lixula* vulnerable to MHW events experienced in the area over the past decade (Olita et al. 2007; Gianguzza et al. 2014). Circumstances such as this are only becoming more common with increased MHW prevalence across global oceans (Oliver et al. 2018), potentially leaving species with narrow fertilization thermal tolerance windows, like the Antarctic sea urchin, *Sterechinus neumayeri* (Ericson et al. 2012; Ho et al. 2013), at risk sooner than projected under ocean warming scenarios.

This species-dependent thermal tolerance of fertilization is observed across other marine invertebrate taxa as well, including corals (Negri et al. 2007; Albright and Mason 2013) and mollusks (Parker et al. 2010; Armstrong et al. 2019; Enricuso et al. 2019). There is also evidence that a great deal of intraspecies variability in fertilization success exists, with contrasting results observed in the mussel, *Mytilus edilus* (Eads et al. 2016a; Eads et al. 2016b), the sea urchin, *Heliocidaris erthyogramma* (Byrne et al. 2009; Byrne et al. 2010a; Byrne et al. 2010b), and the polychaete, *Galeolaria caespitosa* (Kupriyanova and Havenhand 2005; Chirgwin et al. 2020). This variation highlights the need to consider other factors, both biological (e.g., environmental history of study populations) and methodological (e.g., experimental design), that could influence fertilization dynamics under altered temperatures.

Methodological differences in fertilization success assays are common across studies, an issue prompting the creation of a more standardized approach for environmental monitoring

(Lera et al. 2006). The type of crossing design used, for example, can heavily influence results, with the use of pooled gametes reducing the effects of male-female incompatibilities (Byrne et al. 2009; Eads et al. 2016b) and benefitting from the positive contributions of polyandry (Evans and Marshall 2005), while individual male-female pairings instead illuminate the extent of intra-individual variability within fertilization (Foo et al. 2014; Eads et al. 2016a). Our study employed the less-frequently used split-clutch design where sperm from individual males were used to fertilize eggs from the same pool of females (Crean et al. 2013). While some variability between males (within each paternal temperature treatment) did exist, this method appeared to conform to results seen in population-level approaches with no general effect of temperature on fertilization. Alternatively, fertilization success was significantly altered by the sperm concentration used, providing more evidence that these concentrations are an important factor to consider in the design of fertilization success assays (Marshall 2015). Here, the proportion of fertilization events increased positively with sperm concentration, most likely a result of more gamete collisions at higher ratios of sperm to egg (Kupriyanova and Havenhand 2002). The highest sperm concentration, estimated to be 10^5 sperm.mL^{-1}, led to significant amounts of abnormality (~70%). This perceived polyspermy could have been induced by the combination of sperm concentration, contact time between gametes, and vessel size utilized in this study (Byrne 2011).

Studies aimed at uncovering the mechanistic basis of how temperature influences fertilization success could provide more standardized results in addition to accounting for individual variability. Research on fertilization under temperature stress attributes varied tolerance to differential maternal investment of heat shock factors to eggs (Yamada and Mihashi 1998) in addition to altered sperm kinetics and energy metabolism (Mita et al.

1984; Johnson and Yund 2004). For sperm specifically, elevated temperatures can increase the cell's metabolic rate, visualized through diminished sperm longevity and increased respiration (Kupriyanova and Havenhand 2005; Rahman et al. 2009; Binet and Doyle 2013). As such, the inclusion of varied contact times between egg and sperm in addition to quantifications of sperm longevity, motility and respiration could provide useful insight for future studies quantifying fertilization under MHW scenarios.

Overall, the varying thermal tolerance of fertilization within and across marine taxa may support the idea that there is not one general effect of the environment on sperm cells but instead certain conditions that favor specific sperm phenotypes, affecting the fertilization success landscape (Marshall 2015). For example, the optimal head size of sperm cells of the tubeworm, *Galeolaria gemineoa*, was found to differ depending on the concentration and age of the sperm, with longer heads favored under both low concentrations and amongst aged sperm, as opposed to fertilization under high sperm concentrations where smaller heads (with longer tails) possessed increased success (Johnson et al. 2013). If this selection for specific sperm morphology holds true for other environmental conditions, the increased variation in sperm phenotypes associated with pooled sperm samples could heavily influence fertilization success results, especially when compared to studies using sperm from single males. Therefore, it is prudent for future studies of fertilization success to consider measures of sperm phenotypes in addition to what factors could influence the production of these specific phenotypes, such as paternal environmental history.

Paternal environment alters fertilization success

Based upon previous research on temperature effects in marine invertebrates (O'Connor et al. 2007; Zippay and Helmuth 2012), we hypothesized that paternal heat exposure would have a negative impact on fertilization success. Our results support this hypothesis as we observed diminished proportions of successfully fertilized eggs in male urchins exposed to 20 ºC as opposed to 14 ºC for 28 days. Similar, if not pronounced, results were seen in the tubeworm, *Galeolaria caespitosa* following a 2-week exposure of male and female worms to varied temperatures (Guillaume et al. 2016). For *G. caespitosa*, fertilization success in warm acclimated (21.5 ºC) males was significantly lower than that of cool acclimated males (15.5 ºC) across multiple sperm concentrations. To date, these are the only two studies that have explored the effect of paternal thermal acclimation on fertilization success in marine systems.

One pathway by which temperature could influence paternal function is via direct effects on gametogenesis. Generally, elevated temperatures can alter the gametogenesis process for both male and female organisms, but can be particularly detrimental to sperm production (Rogers-Bennett et al. 2010; Uthicke et al. 2014). Even when sperm are successfully produced under temperature stress, thermal history can be reflected in the sperm quality and performance (Boni et al. 2016; Johnstone et al. 2019). In this study, all male *S. purpuratus* were successfully spawned after a 28-day acclimation to 20 ºC, but the sperm produced during this period had diminished performance regarding fertilization. Paternal exposure to high temperature exhibited a significant negative effect on the number of fertilization events observed across different sperm concentrations (LOW: -3%, MID: -9%, LOW: -1%). These

patterns could potentially reflect compromised fertilization kinetics resulting from paternal temperature stress via alterations of sperm morphology, motility, or processes associated with sperm chemotaxis or acrosomal function (Immler 2018). Mussels exposed to 28 ^{o}C for a similar period of time (30 days) exhibited both decreased sperm motility and increased abnormality (Boni et al. 2016). Here, sperm from both paternal temperature treatments demonstrated nearly 100% fertilization at the highest sperm concentration, providing little evidence to suggest high-temperature males produced sperm that was unable to contact or fertilize eggs when given the opportunity (Marshall 2006). Environmental stress can additionally affect sperm quality in the form of DNA damage within the sperm cells themselves, leading to developmental abnormalities in embryos and larvae (Lewis and Galloway 2009; Johnstone et al. 2019). For *S. purpuratus* used in this study, measures of post-fertilization development (cleavage) varied only with sperm concentration, not with fertilization or paternal temperature alone.

While the presence of environmentally induced maternal effects has been acknowledged for years (Mousseau and Fox 1998), the potential for non-genetic paternal effects is just beginning to gain traction (Crean and Bonduriansky 2014; Marshall 2015). Depending on the predictability of the environment, adaptive paternal effects could exist where males under stressful conditions can confer tolerance to offspring experiencing similar environments (Marshall 2015). Positive impacts on larval survival, metamorphosis, and growth have been observed in response to perceived sperm competition (Crean et al. 2013) and salinity (Jensen et al. 2014), but not pH (Lane et al. 2015). Jensen et al. (2014) found that fertilization success in the tubeworm, *Hydroides diramphus*, was at its highest when the salinity during fertilization matched that experienced by both parents. In this study, we

found that the exposure of male *S. purpuratus* to elevated temperatures did not confer any positive effects onto the fertilization process even when fertilization temperatures matched that experienced by the sires. All eggs used in fertilization trials came from dams kept at ambient conditions so we were unable to determine if these fertilization success results changed when both parents experienced a similar stress.

Alternatively, the extent of adaptive paternal effects observed during fertilization may depend on the duration of the adult acclimation to stressful conditions (Stillman 2003; Seebacher et al. 2014). As such, longer (or shorter) exposure of male urchins to the temperatures used in this study could result in additional shifts in the overall fertilization success seen. Guillaume et al. (2016) observed more dramatic decreases in fertilization success when male tubeworms were kept at elevated temperatures for 14 days, half the time we acclimated urchins for in this study. In that same study, larval survival was also measured at 14 days and exhibited a similarly negative response to temperature, but when authors assessed survival again after 28 days, this result had been ameliorated. The research question in this study was fueled by the growing threat of marine heat waves, which can vary significantly in duration and intensity. As such, these questions would be aided by future experiments directed toward elucidating the extent that paternal (and maternal) effects change in response to temperature over different timescales.

Summary & Implications in MHW-context

The results of our study suggest MHWs will have a significant impact on marine invertebrate reproduction through alterations to the quality and performance of produced gametes. Other investigators have shown that MHW events can disrupt marine ecosystem

structure and function through increased mortality events (Harvell et al. 2019; Seuront et al. 2019), diminished recruitment success (Okamoto et al. 2020; Shanks et al. 2020), and altered ecological interactions (Sanford et al. 2019). In our work, an empirical approach using MHW-like conditions (20 ºC) in the lab revealed another potential negative outcome for marine invertebrate populations, altered reproductive output through diminished fertilization success. Paternal acclimation and fertilization treatments in this study were selected based on temperature data taken over the past 6 years at the study sites in which we collect *S. purpuratus* (Fig. 8). MHW events from 2013–2020 were defined using this temperature data and a baseline climatology created from temperatures recorded over the past ~17 years (Hobday et al. 2016). During this time, populations of urchins and other marine invertebrates in the Santa Barbara Channel (SBC) have been exposed to multiple MHW events of varying lengths and intensities, with temperatures seen exceeding the 20 ºC treatment used here.

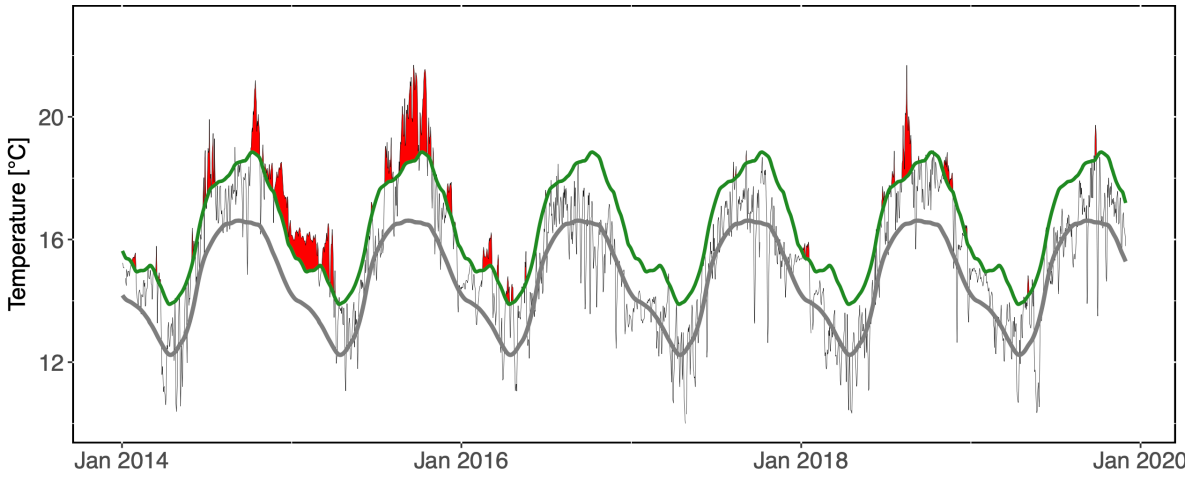


Figure 8. Marine heatwave events in the Santa Barbara Channel (SBC) over a 6-year period. From January 2014 to January 2020, marine heatwave events were calculated at an SBC LTER site, Arroyo Quemado. Temperature data were collected at the benthos and on a LTER mooring using ONSET Tidbit loggers. MHW events were calculate using heatwaveR,

where the long-term climatology seasonal cycle (grey line), seasonal variation threshold at the 90th percentile (green line), and MHW events occurring for at least 5 days (red areas) were determined from average daily temperature data taken at this site from 2003 to 2012.

Additionally, observations of MHWs in the SBC add more evidence of these events' seasonality (Sen Gupta et al. 2020). MHWs seen in 2014, 2015, and 2018 all occurred within late summer to Fall, times in which temperatures were already at some of their highest. The timing of these MHWs threatens the overlapping seasonality of *S. purpuratus* reproduction, where the bulk of gametogenesis occurs from October to January (Strathmann 1987). This places our results in an ecologically relevant context. MHW events can last anywhere from 5 days to multiple months and are only expected to become more frequent and longer in duration over the next decade (Frolicher et al. 2018; Oliver et al. 2018; Smale et al. 2019). Results from this study indicate that current and future MHW events lasting around one month can negatively impact the fertilization success of individual male urchins. While fertilization itself was unaltered by the MHW-like temperatures, pre-spawning exposure of males to elevated temperatures was harmful. If similar effects were seen in nature, the reproductive output of sea urchin populations could be significantly altered by lower numbers of larvae produced or increased selection on particular genotypes from males who are the most competitive under these environments (Campbell et al. 2016). While this study only acknowledges the effect of temperature stress on an early developmental bottleneck, fertilization, negative responses to MHW events within later developmental stages of sea urchins have also been observed (Martino et al. 2021). Additionally, one must also consider the potential of significant carryover effects not explored here, where the effects of this exposure of parents and/or gametes to MHW temperatures could appear much later in development (Byrne and Hernandez 2020). Past MHW events in the SBC already

coincided with decreased *S. purpuratus* biomass and recruitment (Reed et al. 2016; Okamoto et al. 2020). Together these data point toward MHW events as a potentially significant factor in the persistence and structure of sea urchin populations across the CCLME.

Acknowledgements

This research was supported by NSF award IOS-1656262 to G.E.H. This work was also supported by resources from Santa Barbara Coastal Long Term Ecological Research program (NSF award OCE-1831937; Director: Dr. Robert Miller), an award for which GEH is a co-PI. T.S.L. was supported by a Ford Predoctoral Fellowship. The Gorman Scholars Program provided support for B.B. Specimens were collected in the Santa Barbara Channel under a California Scientific Collecting Permit to G.E.H. (SC-1223). Our appreciation goes out to Clint Nelson for collection of animals. We are also extremely grateful for the assistance provided by Logan Kozal, Jannine Chamorro, Xochitl Clare, Samuel Bogan, Amelia Ritger, Adriane McDonald, Cailan Sugano, Avery DeSantis, and Alix Martin during the experiment. We also would like to thank Dr. Kathy Foltz and Dr. Debora Iglesias-Rodriguez for the feedback provided during the writing of this manuscript.

IV. Paternal Thermal History Influences Offspring Thermal Tolerance, but not Larval Size, in the California Purple Sea Urchin

Overview

In this chapter, I explored the impact of paternal thermal history on offspring performance within the context of marine heatwave (MHW) events. Environmentally mediated paternal effects are a relatively understudied area within climate change biology, but these effects could represent a relevant component of an organism's adaptive capacity. Given the growing evidence for such paternal effects in marine taxa, I hypothesized that paternal environment influences offspring performance in the California purple sea urchin, *Strongylocentrotus purpuratus*. To test this hypothesis, I used the same urchins acclimated for work outlined in Chapter III, where males were acclimated during a period in which spermatogenesis would occur under different one of two temperature conditions. Here, sperm from individual males was used to produce embryos that were subsequently raised under varying thermal conditions. Once offspring reached an early larval stage, the impact of paternal and offspring environments were assessed on two aspects of offspring performance: larval size and thermal tolerance. The results represent one of the first examples of an adaptive paternal effect in a benthic marine invertebrate (see also Crean et al. 2013 and Jensen et al. 2014). In the Discussion, I address the results in the context of MHW events in the Santa Barbara Channel and further connect observations in this Chapter to the results in Chapter III.

Materials and Methods

Urchin collection & sex identification

Collection and sex determination of purple sea urchins were conducted as described in Chapter II and in (Leach et al. 2021). In brief, sea urchins were hand-collected via SCUBA from two kelp forest sites in the Santa Barbara Channel: Mohawk Reef (34.394°N, 119.730°W) and Arroyo Quemado Reef (34.468°N, 120.119°W). Collections occurred on December 13th and December 18th, 2019, respectively, using California Scientific Collection permit SC-1223 (to G. Hofmann). Following collection, sea urchins were housed in UCSB Marine Science Institute seawater facilities maintained at 13 ºC and then sexed by a physical induction of spawning shortly after. Both male (n = 31) and female (n = 43) sea urchins were successfully identified through this method and subsequently separated into different recovery tanks, allowing for the tracking of sex and collection location identities throughout the study. Sea urchins recovered for 2 weeks at 13 ºC while being fed kelp (*Macrocystis pyrifera*) *ad libitum.* Over this time, there was minimal disease and/or death observed across both male and female sea urchins.

Adult acclimation

After the recovery period concluded, male urchins were acclimated for 28 days to one of two temperature treatments mimicking the conditions experienced in the Santa Barbara Channel either: (1) during a MHW event (20 ºC) or (2) during ambient conditions (14 ºC) (Fig. 9). The design and structure of how treatment conditions were maintained are detailed

in Chapter III and (Leach et al. 2021). For brevity, each treatment was represented by three acclimation tanks where temperature was tightly regulated and monitored over the month-long acclimation. Each tank was further subdivided to support different rounds (n = 3 rounds) of male sea urchin acclimation, allowing for increased replication. During each round, two male urchins were placed in a subdivided portion of each acclimation tank and maintained for 28 days with a weekly feeding of kelp. Rounds were staggered by 96 hours to ensure that each male used experienced exactly 28 days of temperature acclimation. Alternatively, female sea urchins were maintained at ambient conditions, ~13 ^{o}C, and fed kelp *ad libitum* in multiple tanks for the entirety of male acclimations.

Urchin spawning & crossing design

At the completion of each round's acclimation, male and female urchins were induced to spawn following the process outlined in Chapter II. In summary, a combination of intercoelomic injections of 0.53M KCl and physical perturbation was utilized to induce spawning. Male and female urchins were randomly selected from their tanks, with males from different replicate acclimation tanks represented within each round. Spawned sperm was collected dry and kept on ice while eggs were collected by inverting female urchins over beakers filled with FSW as described in Chapter II (Strathmann 1987). Gamete quantity and quality were observed under a light microscope. The compatibility of gametes for every combination of male and female urchin spawned was assessed by conducting test fertilizations. Only male and female urchins demonstrating healthy gametes (good sperm motility/mature, normal looking eggs and >90% fertilization success during test fertilizations) were used for the offspring rearing portion of the experiment (see Chapter II).

Gametes were crossed in a modified split-clutch design: involving individual fertilizations of pooled eggs (n = 5 females) by sperm of three males from both the MHW-like and ambient temperature treatments. This resulted in 6 unique crosses (n = 3 males per treatment x 2 treatments), or families per round (Fig. 9). The split-clutch design was utilized to minimize the effects of male x female interactions on fertilization and subsequent development. Across the three rounds, 9 males from each treatment and 3 pools of females were used for a total of 18 families. During each round, individual fertilizations by each male were conducted by titrating sperm into a 1L-beaker containing pooled eggs (concentration of eggs) until >90% of the eggs were fertilized. Fertilizations for males acclimated to MHW-like or ambient temperatures were initiated 1 hour apart from one another to ensure consistent sampling from the culture vessels (described below).

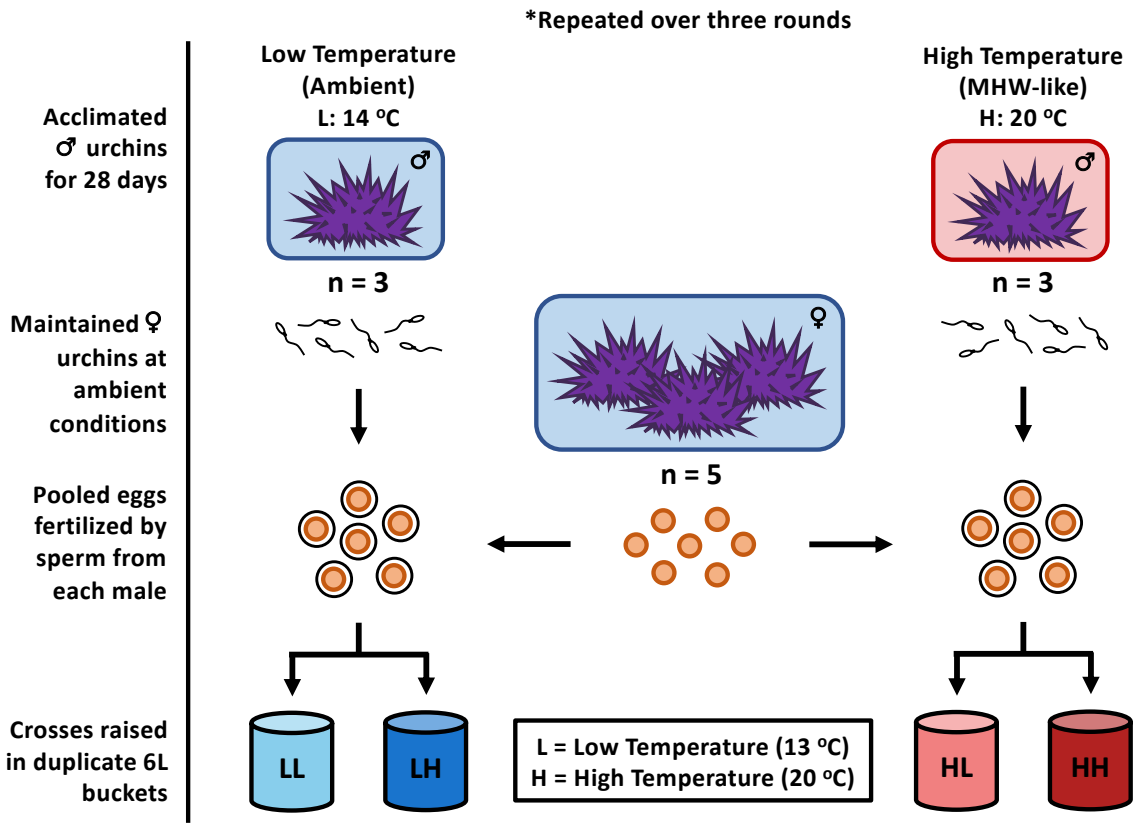


Figure 9. Experimental Design. Across three rounds, male purple sea urchins (*S. purpuratus*) were acclimated to either a low (L-; 14 ºC) or high (H-; 20 ºC) temperature treatment for 28 days. In each round, sperm from individual males (n = 3 male urchins/temperature treatment) were collected and crossed with pooled eggs from five female urchins in a split-clutch breeding design. Fertilizations by each male and the resulting embryos were then raised under the same low (-L; 13 ºC) and high (-H; 20 ºC) temperature conditions experienced during adult acclimation. Offspring were raised under these conditions, in duplicate culture vessels, until being sampled at the echinopluteus larval stage for various performance metrics.

Larval Culturing

Fertilized eggs produced by each cross (n = 3 crosses per paternal treatment per round) were subsequently used to stock four, flow-through culture vessels kept at either the same MHW-like or ambient temperature conditions used for the adult acclimation (Fig. 9). This resulted in a total of 12 cultures (n = 6 cultures per larval rearing treatment) per round.

Culture vessels consisted of two, nested 2.5-gallon buckets capable of holding 6 L total. The inner bucket, where developing urchins were held, contained eight, 5.5cm holes covered with 64-micron mesh to permit water flow without the loss of embryos. The lid of each vessel served as a platform for a 12-V motor that powered an 8-cm by 10-cm acrylic paddle, which gently mixed cultures. Filtered, UV sterilized seawater (FSW) flowed into each culture vessel from a reservoir tank at a rate of 4L/hr via irrigation button drippers (DIG Corporation). Each culture vessel was half-way submerged in a large water bath where target temperatures were controlled using a Delta Star heat pump with a Nema 4x digital temperature controller (AquaLogic). To maintain a favorable larval density of 10 embryos/mL (Strathmann 1987), each 6-L culturing vessel was loaded with 60,000 embryos. Once loaded with sea urchin embryos, the temperature of each culture was monitored by regular point measurements using a wire thermocouple (Thermolyne PM 20700 / Series 1218).

Cultures were monitored frequently to assess developmental progression. Sampling occurred once offspring reached an early echinopluteus stage, where larvae possessed a differentiated gut, skeletal rods, and the early formation of feeding arms (~49 hpf at 20 ^{o}C; ~60 hpf at 13 ^{o}C). Larvae were siphoned from culture vessels onto a submerged 35-micon mesh filter before being transferred, using a plastic transfer pipette, to a 15-mL Falcon tube. This step in the sampling process resulted in larval concentrations that were easier to handle in down-stream applications. Larval concentrations within the Falcon tubes were estimated by counting the number of individuals within three small aliquots, where a coefficient of variance (CV) of <10% was reached. The average number of larvae across aliquots was used to represent the new concentration, or the number of larvae per mL of FSW. Subsamples

were then collected for various downstream phenotypic quantifications based off these concentrations.

Thermal tolerance assay

The influence of paternal and offspring rearing temperature on echinopluteus thermal tolerance was assessed following a protocol established by (Hammond and Hofmann 2010). In brief, larvae from each sample were divided and exposed to one of 6 different temperatures for a set period of time and then assessments of survival and normality were conducted using microscopy. Within offspring temperature treatments, larvae from each individual male were pooled across duplicate culture vessels. 6,000 of these pooled larvae were then divided across six, 20-mL glass scintillation vials so that each contained 1,000 larvae in 5mL of FSW (200 larvae/mL). These scintillation vials were placed in an aluminum block possessing a temperature gradient from 16-30 ºC. This temperature gradient was created by attaching two water baths set at a high and low temperature at either end of the aluminum block. Larvae within each scintillation vial were exposed to 6 different assay temperatures (~24.6 ºC, ~27.0 ºC, ~27.9 ºC, ~28.9 ºC, ~30.0 ºC, ~31.2 ºC) for 1 hour before being removed from the temperature block. 100 larvae from each vial were then coded and scored blind (each vial had a random number ID) for mortality and abnormality.

Mortality was scored by the lack of ciliary movement, while abnormality was based upon the presence of ruptured membranes or enlarged cells obstructing the gut lining. As the degree of abnormality did not appear reversible, only healthy-looking larvae with moving cilia were scored as alive in later analysis. Statistical analysis of thermal tolerance followed that outlined in (Waite and Sorte 2022). A binomial regression was performed using binary

mortality data (alive vs. dead) across assay temperatures to calculate LT_{50}, LT_{25}, and LT_{10} values (de Vries et al. 2008, Bates et al. 2015). A generalized mixed effects model with a Gamma distribution was then used to assess how larval thermal tolerance (LT_{50}) was affected by offspring and paternal temperature. The model was produced using the lme4 package (Bates et al. 2015) and included paternal temperature, offspring rearing temperature, and their interaction as fixed factors, while male identity and round were included as random factors.

Morphometric analysis

Larval size was measured to quantify the impact of paternal and offspring rearing temperature on offspring performance. 500 larvae were sampled for each culture vessel and preserved using 4% formalin in 0.01 M phosphate buffered saline (PBS) pH 8.7. This solution was used to minimize dissolution of skeletal rods within echinopluteus larvae. The fixative and larvae were added in equal parts so that the final concentration of formalin was 2%. Preserved larvae were kept at 4 ^{o}C until they were further processed. Larvae were digitally photographed under a compound microscope using an attached digital camera (Infinity Lite) and Infinity Capture software (version 6.2.0). Using Image J (National Institutes of Health, USA), individual larvae from each culture vessel ($n > 30$) were measured. Only echinopluteus larvae falling in the right orientation, where the length of the postoral arm of the plutei, from the spicule tip of the postoral arm to the spicule tip of the aboral point, were photographed and measured (Yu et al. 2011).

Images were taken for all larvae in the above orientation, regardless of which postoral arm, left or right, was in focus. Later analysis showed that at this stage of early pluteus, there

was no asymmetry in spicule length between left or right postoral arms, so all larvae imaged were included in analysis. Differences in arm length, spicule length, and the ratio of the spicule length to body length between treatments were compared using two-way ANOVAs. Here, the linear mixed effects model included paternal acclimation temperature, offspring rearing temperature, male identity, as well as the interactions of offspring rearing temperature with paternal temperature and male identity, separately, as fixed factors. Pooled eggs used for this experiment decreased in size (diameter and area) between each round, the cause remaining unclear. Average diameter decreased from 95.9 +/- 4.8 um in Round 1 to 93.3 +/- 2.5 um and 91.5 +/- 3.1 um in Rounds 2 and 3, respectively. As such, Round was accounted for as a random effect in all statistical analysis of the various morphological features. Statistical analyses were performed in R.

Table 5. Temperatures recorded during adult acclimations and larval rearing. All values displayed as mean ± standard deviation.

Acclimation	Treatment	Duration	Temperature (°C)
Adult	High Temp (H-)	28 days	19.6 ± 0.2
	Low Temp (L-)	28 days	13.5 ± 0.4
Development	High Temp (-H)	48 – 60 hrs	19.5 ± 0.4
	Low Temp (-L)	48 – 60 hrs	13.3 ± 0.3

Results

Adult conditioning and larval culturing

Adult conditioning in the lab was very successful and resulted in healthy, high-quality, gravid animals for the larger experiment. Specifically, no mortality was observed in either

the high or low temperature treatment over the month-long acclimation period. In addition, temperature conditions remained relatively stable across these acclimations with a ~6 ºC separation between high and low temperature treatments (Table 5). During the larval rearing stage, temperature conditions under which the embryos and larvae developed closely matched the temperatures used for the adult acclimations.

The experiment also resulted in high numbers of early stages to sample. Across the three rounds of the experiment, only 2 out of the 72 culturing buckets did not possess enough larvae to obtain samples (due to a malfunction of the culture bucket). Development remained synchronous across rearing temperature treatments; however, there was variation in the degrees of larval loss (either due to mortality or human error). Although each culture vessel was estimated to receive 60,000 fertilized eggs at the outset of the experiment, no vessel contained more than 48,000 larvae by the time of sampling, a decrease in ~20% of the numbers of embryos that were calculated to be stocked into each culture bucket. In the first two rounds, there were significantly fewer larvae in high temperature treatment buckets on average in comparison to the low temperature treatment, but the concentrations of larvae were relatively even during the last round of sampling. Conversely, there were no significant differences in larval loss between paternal temperature treatments across any of the rounds. Overall, despite the apparent loss and/or discrepancy in the number of individuals in each culture vessel, I was able to successfully sample larvae for all the down-stream analyses described below.

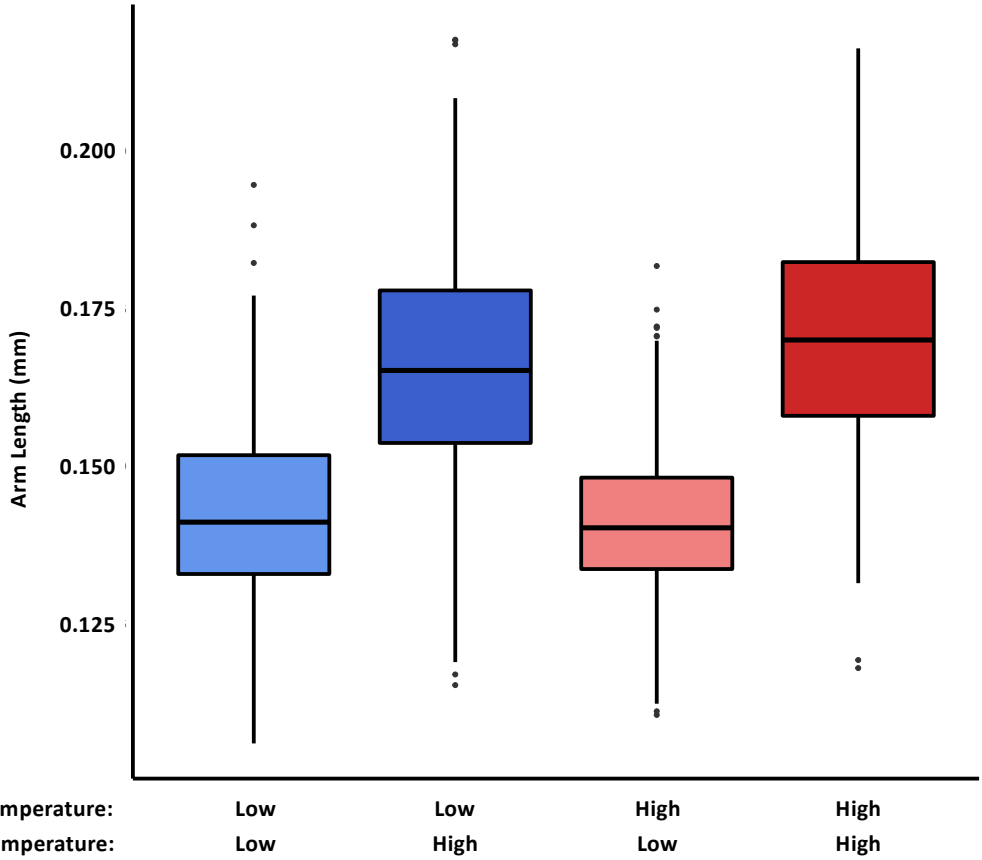


Figure 10. Effect of paternal and offspring temperature treatments on larval arm length in the purple sea urchin, *S. purpuratus*. Bars show standard error of the mean.

Table 6. Body morphometrics for echinopluteus larvae. All values given as mean ± standard deviation.

Adult Temperature Treatment	Developmental Temperature Treatment	Pluteus Spicule Length (mm)	Pluteus Arm Length (mm)	Spicule:Body Length Ratio
Low Temperature (L-)	Low Temperature (-L)	0.142 ± 0.020	0.142 ± 0.014	0.998 ± 0.063
	High Temperature (-H)	0.179 ± 0.021	0.166 ± 0.018	1.078 ± 0.058
High Temperature (H-)	Low Temperature (-L)	0.142 ± 0.018	0.141 ± 0.012	1.004 ± 0.062
	High Temperature (-H)	0.181 ± 0.018	0.170 ± 0.017	1.063 ± 0.045

Morphometrics

In general, as factors that could alter morphometrics, I found that developmental conditions had more influence on larval body form than did the thermal history of the sire. In terms of morphological measurements, arm length, spicule length, and the ratio of spicules to body length were significantly influenced by the temperature at which development occurred ($F_{1,1} = 538.92$, $p = <0.001$), whereas paternal acclimation temperature ($F_{1,1} = 1.65$, $p = 0.208$) and the interaction between paternal and larval temperature treatments ($F_{1,1} = 3.78$, $p = 0.104$) had no observable effect. Regardless of paternal acclimation, pluteus reared under 20 ºC possessed much longer arms (LH: 179.3 +/- 1.6, HH: 182.2 +/- 1.9 um) than those reared under 14 ºC (LL: 143.6 +/- 3.4 um, HL: 141.5 +/- 2.1 um) (Fig. 10). These trends were consistent across the other metrics used to quantify pluteus morphology (Table 6).

Although there was not a significant effect of paternal thermal experience on the size of larval offspring, there was a notable effect as a function of individual males. Here, paternal identity (e.g., specific individuals) ($F_{1,16} = 3.52$, $p = 0.001$) as well as the interactive effect between paternal identity and larval rearing temperature, significantly influenced larval size ($F_{1,16} = 2.528$, $p = 0.011$). Larvae from individual males varied in arm length across developmental and paternal temperature treatments (Fig. 11). Of note is the decreased amount of variability in average arm length between offspring sired by high temperature males but raised under low temperature conditions.

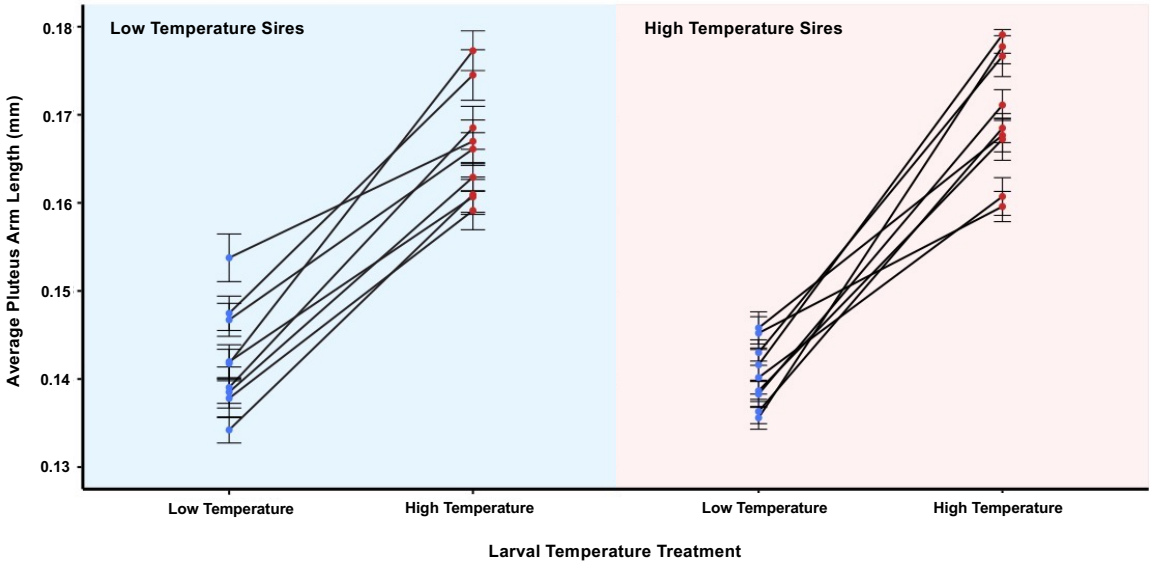


Figure 11. The effect of individual male identity and larval temperature treatment on echinopluteus arm length in the purple sea urchin, *S. purpuratus*. Datapoints represent average arm lengths of offspring produced by individual sires (n = 9 individual male urchins/paternal treatment) and plots are separated by paternal acclimation temperatures. Error bars are representative of mean ± standard error.

Thermal Tolerance

Thermal tolerance assays yielded interesting, but complex results, with LT_{50} values significantly influenced by: (1) developmental temperature, (2) paternal acclimation temperature, and (3) the scoring methodology utilized (abnormality-based or movement-based). When thermal tolerance was based upon scoring of developmental abnormality, LT_{50} values across treatments ranged from 27.2 – 28.1 ºC (Table 7). Developmental temperature significantly influenced larval LT_{50}, with larvae raised under the high temperature treatment (-H; 20 ºC) possessing higher thermal tolerance than those raised under the low temperature treatment (-L; 14 ºC) ($F_{1,16}$ = 12.59, p = 0.001; Fig. 12). Despite the minimal effect of paternal temperature alone on larval thermal tolerance (Table 8; $F_{1,16}$ = 0.006, p = 0.938), LT_{50} values were also significantly influenced by the interaction between paternal and larval

temperatures (Table 8; $F_{1,16} = 12.13$, $p = 0.002$). Here, larval thermal tolerance was greatest when the thermal environments experienced by the sire and their offspring matched (Fig. 12). This interaction can be visualized most clearly by comparing the change in LT_{50} values between larvae from high temperature (H-) and low temperature (L-) sires when they developed at different temperatures; specifically, HL vs. HH, or LL vs. LH. Larvae from H sires exhibited a near 1 ºC increase in LT_{50} when they were raised under the 20 ºC treatment (HH_{LT50} = 28.1 +/- 0.1 ºC) as compared to the 14 ºC treatment (HL_{LT50} = 27.2 +/- 0.1 ºC). Alternatively, there was fairly little variation in the LT_{50} values of larvae produced by L sires when they developed under either rearing temperature (LH_{LT50} = 27.7 +/- 0.2 ºC, LL_{LT50} = 27.8 +/- 0.1 ºC). Finally, the trends outlined above regarding the match of sire exposure and larval thermal tolerance were also observed in calculated LT_{25} and LT_{10} values.

Table 7. LT_{50}, LT_{25}, and LT_{10} values for echinopluteus larvae from various paternal and larval treatments. All values given as mean ± standard deviation.

Scoring Methodology	Temperature Treatment (Sire - Offspring)	LT_{10}	LT_{25}	LT_{50}
Cilia Movement	Low - Low	29.1 ± 0.1	29.4 ± 0.1	29.7 ± 0.1
	Low - High	28.6 ± 0.3	29.4 ± 0.2	30.2 ± 0.1
	High - Low	28.9 ± 0.1	29.2 ± 0.1	29.5 ± 0.2
	High - High	29.3 ± 0.2	29.7 ± 0.2	30.1 ± 0.2
Abnormality	Low - Low	26.6 ± 0.3	27.1 ± 0.2	27.7 ± 0.2
	Low - High	26.9 ± 0.2	27.4 ± 0.1	27.8 ± 0.1
	High - Low	26.7 ± 0.3	27.0 ± 0.2	27.2 ± 0.1
	High - High	27.3 ± 0.2	27.7 ± 0.1	28.1 ± 0.1

Interestingly, overall LT_{50} values and the factors influencing larval thermal tolerance varied when utilizing a scoring procedure that classified mortality solely by cilia movement, with no consideration of abnormality (Hammond and Hofmann 2010). Here, LT_{50} values were ~2 ºC higher, ranging from 29.5 – 30.2 ºC, when larvae were scored using the movement-based procedure as opposed to those scored using the abnormality-based method reported above (Table 7). Despite this increase in general LT_{50} when using the movement-based method, a strong effect of developmental temperature on thermal tolerance was still observed, where pluteus reared under high temperature conditions exhibited increased LT_{50} values as opposed to those experiencing low temperatures (Table 9; $F_{1,16} = 26.38$, $p < 0.001$). However, there was little effect of paternal temperature treatment (Table 9; $F_{1,16} = 0.45$, $p = 0.513$) or the interaction between paternal and larval (Table 9; $F_{1,16} = 0.139$, $p = 0.715$) treatments. As such, larvae raised under the high temperature treatment exhibited LT_{50} values (LH: 30.2 +/- 0.1 ºC, HH: 30.1 +/- 0.2 ºC) around 0.5 ºC higher than those raised under 14 ºC (LL: 29.7 +/- 0.1 ºC, HL: 29.53 +/- 0.2 ºC), with only modest variation between larvae whose sires experienced differing temperature conditions (Table 7). Again, calculated LT_{25} and LT_{10} values followed the same trends.

Table 8. Statistical output from generalized linear mixed-effects model analyzing the effect of paternal and larval temperature treatments on LT_{50} values of echinopluteus, when values were based upon the abnormality scoring method.

	F	*df*	*p*
Paternal Temperature Treatment	0.006	1,16	0.938
Larval Temperature Treatment	12.591	1,16	0.001*

Paternal Temperature Treatment * Larval Temperature Treatment	12.135	1,16	0.002*

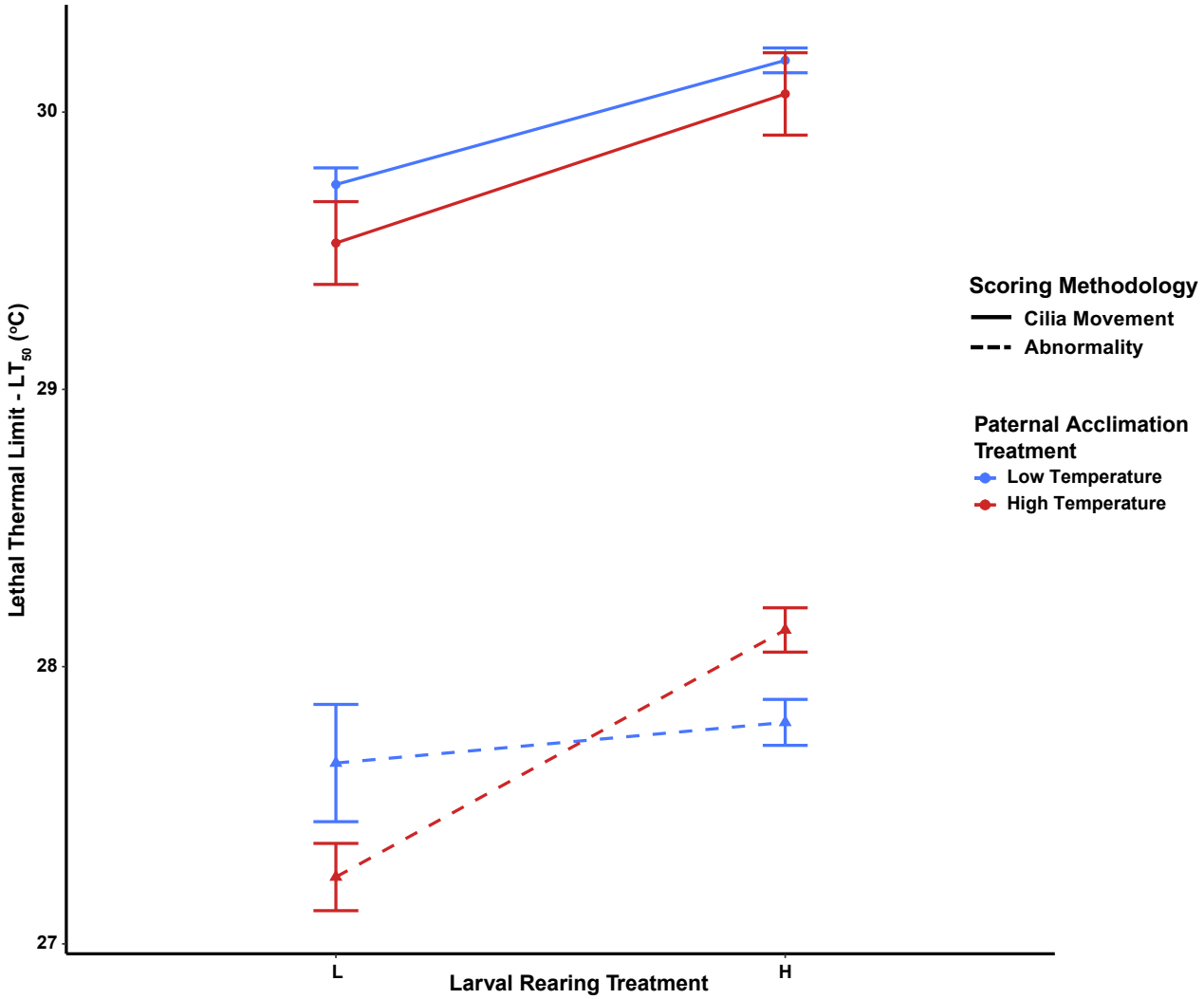


Figure 12. Effect of paternal acclimation (Low, 14 °C; High, 20 °C) and larval rearing temperature (Low, 13 °C; High, 20 °C) treatments on the lethal thermal limit (LT_{50}) of echinopluteus larvae in the purple sea urchin, *S. purpuratus*. Data points represent average LT_{50} across larvae from individual males (n = 9 male urchins/paternal treatment) and error bars represent mean ± standard error. LT_{50} values were calculated with data produced using different scoring methodologies to assess heat-induced mortality, either by cilia movement (solid lines) or significant abnormality (dashed lines).

Table 9. Statistical output from generalized linear mixed-effects model analyzing the effect of paternal and larval temperature treatments on LT_{50} values of echinopluteus, when values were based upon the ciliary movement scoring method.

	F	*df*	*p*
Paternal Temperature Treatment	0.450	1,16	0.513
Larval Temperature Treatment	26.380	1,16	< 0.001*
Paternal Temperature Treatment * Larval Temperature Treatment	0.138	1,16	0.715

Discussion

Summary of study and results

In this study, we investigated whether the thermal experience of adult, male purple sea urchins, *Strongylocentrotus purpuratus*, influenced their offspring's performance under the extreme heat stress associated with marine heatwave (MHW) events. This was achieved by acclimating male urchins to two temperatures, 14 ºC or 20 ºC, for 28 days before collecting each male's sperm to separately fertilize eggs pooled from multiple female urchins. The resulting embryos were then raised under one of the same two temperature conditions, allowing us to examine how paternal thermal history influenced the performance of the offspring as a function of developmental environment. The overarching goal was to examine the role of parental effects on the physiological capacity of their progeny, shedding light on whether such effects under projected climatic changes will aid or further hinder the persistence of future populations.

Two clear findings emerged: (1) offspring were most thermally tolerant under scenarios when the temperature at which development occurred matched that of the paternal

acclimation; and (2) larval size was not influenced by paternal thermal history but was instead overwhelmingly affected by larval rearing temperature, with higher temperatures producing significantly larger offspring. In regard to the former finding, I also demonstrated a potential need to revisit how thermal tolerance is estimated for marine invertebrate larvae, with varying results observed depending on the trait that was scored as an indicator for response to thermal stress. Below I discuss each aspect of the study and conclude by framing the results in the context of MHW events in the Santa Barbara Channel (SBC) occurring now and predicted into the future.

Influences on larval thermal tolerance: paternal acclimation and larval rearing temperature

In this study, we observed one of the first documented cases of an adaptive paternal effect in sea urchins, where thermal tolerance of the larvae was increased when the thermal environment during development matched that of their sire's acclimation temperature. More specifically, when urchin larvae were raised at 20 ºC, offspring sired by males acclimated at 20 ºC had significantly higher thermal tolerance (represented by measuring the degree of abnormality-based mortality following a 1-hour heat exposure to varying temperatures) than offspring produced by males acclimated to 14 ºC. This trend was flipped when larvae were raised under ambient temperature conditions of 14 ºC, where offspring from 14 ºC - acclimated males now had the higher thermal tolerance compared to those from sires held at 20 ºC. This pattern appeared to be largely driven by the larvae of high temperature males, which exhibited a ~1 ºC difference in LT_{50} values (the temperature in which 50% of larval mortality was observed) depending on their rearing temperature (14 ºC or 20 ºC).

These results join a rich literature in larval ecology and the role of parental effects in the ecological development of marine organisms. Parental effects have been widely studied in marine organisms, ranging from fish to benthic invertebrates (Salinas et al. 2013, Munday 2014, Ross et al. 2016, Donelson et al. 2018). The broader literature indicates that parental effects, in general, can influence offspring success both positively or negatively, but the extent of these effects depends on the stressor itself (duration, intensity, variability) as well as the contributions made by both dams and sires (Donelson et al. 2018, Yin et al. 2019). Our results add to a small, but growing body of evidence that the paternal environment is relevant and thus perhaps merits additional research in climate change contexts (Crean and Bonduriansky 2014, Marshall 2015).

In general, one of our results, that of matched tolerances, supports the theory behind anticipatory maternal effects, where positive benefits to offspring are passed from their mother if their parent can predict what environment their offspring will encounter (Marshall and Uller 2007). This scenario posits a cost for inaccurate predictions from the parent, which was witnessed in this study addressing paternal effects. In our analysis, the lowest LT_{50} values observed in this study across all treatments were in larvae that were sired by 20 ^{o}C - acclimated males but developed at 14 ^{o}C. Mechanistically, understanding how the males were predicting the physical environment is outside the scope of this project. One issue to note that that the male urchins used in this experiment were removed from all normal *in situ* cues and experienced static temperatures during the laboratory acclimation, not reflecting the natural variability they likely encounter in nature. As such, the ability of our males to "predict" what their offspring might face could be driven by this month of unchanging

thermal conditions, although there is much to be learned about what timescales and fluctuations influence these predictive effects.

Adaptive paternal effects have also been observed in both marine fish and invertebrate taxa under varying environmental conditions including competition, salinity, and temperature (Crean et al. 2013, Jensen et al. 2014, Yin et al. 2019, Chang et al. 2021). For example, sheepshead minnows possessed increased growth rates when their thermal environment matched that of their sires (Chang et al. 2021). Jensen et al. 2014 similarly found a positive impact of both paternal and maternal hyposalinity exposure, individually, on fertilization success under similar conditions in the tubeworm, *Hydroides diramphus* (Jensen et al. 2014). Both studies found that these adaptive effects were further maximized when offspring experienced the same environment, in regard to temperature or salinity, faced by both parents as opposed to just one. As I sought to isolate the sole effect of paternal environment on offspring performance, all female urchins used in this study were exposed to the same ambient temperature conditions. In nature, both male and female sea urchins are found living near one another and thus are likely to experience similar environmental regimes. If similar dynamics to that observed in the above studies are operating in urchins, one might expect additional benefits for offspring thermal tolerance under heat stress scenarios to that seen here.

While this is the first explicit example of paternal environment alone influencing larval thermal tolerance, parental influences on offspring thermal physiology are common across invertebrate taxa. Our observations of a positive, adaptive effect of paternal temperature on larval thermal tolerance agree with findings in parthenogenic *Artemia* that experienced a heat shock event as adults (Norouzitallab et al. 2014). There, the adaptive maternal effects

on offspring tolerance lasted for three successive generations, with each generation lasting on the scale of months (as opposed to 1-2 years it takes urchins to reach sexual maturity). Positive effects on offspring thermal tolerance are also seen in other urchin species, where early developmental stages from adults that inhabit warmer regions display increased thermal tolerance (Byrne 2011, Pecorino et al. 2013). Alternatively, decreased thermal tolerance is also observed in offspring produced by environmentally stressed adults in various marine invertebrate taxa, including sea urchin (*Strongylocentrotus intermedius*), mussel (*Mytilus galloprovincalis*), and nematode (*Caenorhabditis remanei*) species (Sikkink et al. 2014, Shi et al. 2020, Waite and Sorte 2022). The negative carry-over effects seen in these studies highlight the trade-offs that exist between adult organisms prioritizing their own survival and investing in their offspring (Kooijman 2009).

In the sea urchin, *Paracentrotus lividus*, parental temperature stress over 15 months led to decreased expression of *hsp70*, a well-studied gene associated with the heat shock response in animal taxa (Shi et al. 2020). Given the clear increase in thermal tolerance seen in our study, measurements of heat shock protein expression or abundance could shed light onto the mechanism behind the observed intergenerational plasticity of thermal tolerance. Changes in offspring transcriptome due to parental environment, specifically paternal environment, may be particularly relevant in light of recent studies of epigenetics (Eirin-Lopez and Putnam 2019). The observed adaptive effects in *Artemia* mentioned above were underpinned by changes in DNA methylation and histone acetylation patterns in offspring (Norouzitallab et al. 2014). Similar maternally derived epigenetic patterns have already been observed in purple urchins and could be at play here (Strader et al. 2019, Strader et al. 2020). Although not to the same degree as eggs, sperm are known to contain

environmentally-dependent amounts of various non-genetic, elements such as DNA methylation, small RNAs, and histone modifications that affect gene expression (Immler 2018). Differential expression of such elements, like small RNAs, in sperm has been observed to influence offspring phenotype and performance in invertebrate taxa (Lymbery et al. 2020, Lymbery et al. 2021).

Another significant observation is that results regarding larval thermal tolerance were heavily dependent on the traits used to characterize heat-induced mortality. Specifically, mortality scored using either larval movement or abnormality influenced possible interpretations about how paternal environment impacts offspring thermal tolerance as well as how close sea urchin larvae are to their physiological thermal limits. LT_{50} values for pluteus larvae were 2-3 ^{o}C higher, and not influenced by paternal temperature, when we utilized the scoring procedure outlined in past studies of purple urchins (Hammond and Hofmann 2010). This procedure bases larval mortality on the observation of moving cilia. When using this procedure, LT_{50} values observed here, 29.5 – 30.2 ^{o}C, fall in the range of values previously reported for *S. purpuratus* larvae (Hammond and Hofmann 2010). Slightly lower LT_{50} values, 29.1 – 29.4 ^{o}C, were also seen in pluteus larvae of the red sea urchin, *Mesocentrotus franscicanus*, a species that occupies similar habitats as *S. purpuratus* (Wong and Hofmann 2020). In our study, though, we also separately scored for the presence of highly abnormal individuals (due to lysed cells or broken membranes) to visualize thermal tolerance trends under an expanded definition of mortality. These abnormal larvae, despite possessing the ability to move, were considered functionally dead given the inability of individuals to recover from such a significant degree of abnormality. Abnormality-based mortality produced significantly lower LT_{50} values, ranging from 27.2 – 28.1 ^{o}C across

treatments. Differences in LT_{50} between scoring metrics likely highlight the varying thermal sensitivities of diverse molecular pathways operating within individual larvae. Future studies on thermal tolerance in marine invertebrate larvae should consider using abnormality within their metrics of tolerance as the ~2 ºC decrease in LT_{50} observed here may mean that *S. purpuratus* larvae are living closer to their physiological thermal limits than previously thought.

Regardless of what scoring methodology was used, pluteus larvae in this study had higher thermal tolerance when they were reared under elevated temperatures. A similar trend was seen in the red sea urchin (*Mesocentrotus franciscanus*), where a +4 ºC increase in rearing temperature led to moderately more tolerant larvae (Wong and Hofmann 2020). Similar to the mechanisms that could underly the intergenerational plasticity of thermal tolerance, offspring rearing temperature may affect the abundance of molecular chaperones, like hsp70, produced by urchin larvae. Expression of *hsp70* and other genes related to the heat response pathway are upregulated when urchin embryos and larvae develop under elevated temperatures (Sconzo et al. 1995, Runcie et al. 2012, Shi et al. 2020). If the above-mentioned mechanisms of paternal non-genetic inheritance are also at play, offspring from heat-acclimated males and developing under stressful thermal conditions themselves may receive additive increases in the proteins related to the heat response pathway (Immler 2018). Alternatively, the maintenance of protein integrity and production of molecular chaperones that underly a heat shock response have large energetic requirements (Hochachka 2002). For males unable to accurately predict their offspring's environment, paternal priming of this cellular machinery in offspring when unnecessary could prove costly. In the Mediterranean mussel, *Mytilus galloprovincialis*, decreased expression of the

heat shock protein, *hsp90*, in heat-acclimated sperm was correlated with increased developmental success in larvae raised under ambient conditions but appeared to have a maladaptive effect on development for the same larvae under elevated temperatures (Lymbery et al. 2020, Lymbery et al. 2021). Such effects could be due to increased apoptosis, or cell death, triggered by prolonged expression of hsp proteins (Takayama et al. 2003). Again, further exploration of the presence of hsp70 or other molecular chaperones within larvae, and sperm, from this study could shed more light into the mechanisms behind both the inter- and intragenerational plasticity in thermal tolerance observed here.

Effects of developmental and adult acclimation temperatures on larval size

Across marine invertebrate taxa, the temperature experienced during development is a dominant variable determining the size of embryos and larvae. In this study, larvae that developed at higher temperatures (+7 ºC) were significantly larger than those in the control temperature treatment. This result is consistent with studies from various marine invertebrates, including other echinoderm, arthropod, and mollusk species (reviewed in (Byrne 2011). For sea urchins, specifically, longer larval arms under higher temperatures appear in species found in tropical, temperate, and polar regions (Sheppard Brennand et al. 2010, Byrne 2013, Wong and Hofmann 2020). This trend may be stage-specific in urchins though, as similar temperature increases (+4 ºC) did not elicit any influence on the size of early developmental stages in the red sea urchin, *M. fransicanus*, until they became larvae (Wong and Hofmann 2020). Positive temperature effects on the larval size of marine invertebrates may also confer additional benefits in multistressor scenarios. For example, the negative effects of pH on larval growth, especially for taxa possessing calcifying structures

during early development, are mitigated when raised under the high temperatures predicted to be experienced by benthic marine invertebrates in future oceans (Byrne 2011).

Interestingly, one of few studies where results contradict the generally positive relationship between temperature and size in marine invertebrates involves our study species, *S. purpuratus* (Padilla-Gamino et al. 2013). In that study, pH was the dominant variable influencing larval size, with little influence of temperature on pluteus arm length. A number of factors may have contributed to the difference in results found here, including the age of the pluteus sampled, crossing design, and the pre-conditioning of adults. Padilla-Gamiño et al. (2013) assessed much older larvae produced from individual male-female pairs taken directly from the field, while we employed a split-clutch crossing design (one male and multiple females) using urchins that were conditioned, and fed *ad libitum*, in the lab for 28 days.

The larger body sizes conferred by elevated temperatures can have a myriad of positive impacts on an individual's survival and performance during early development. During the planktonic portion of many marine invertebrate life cycles, smaller individuals are more likely to be predated upon (Allen et al. 2008). Furthermore, reduced energy reserves associated with smaller body sizes can prolong the amount of time spent in the water column before settlement (Byrne et al. 2009, Ross et al. 2011). Here, we measured the length of early feeding arms in echinopluteus larvae, with those reared at 20 ^{o}C possessing longer arms than those at 14 ^{o}C. In echinoderm larvae, ciliated arms provide an effective means to capture algae for consumption. As expected, the increased surface area associated with longer arms translates to increased feeding success (Chan et al. 2011). The larvae raised at higher temperatures in this study also demonstrated faster development, with offspring at 20

ºC reaching the echinopluteus stage ~11-12 hours before those raised at 14 ºC. Taken together, the projected increases in feeding associated with obtaining longer arms much earlier may exacerbate the advantages of larger body sizes for larvae developing under high temperature scenarios (i.e., less time before settlement, or increased energy reserves).

Parental environment can also be an important driver of offspring size, but there is little evidence for a role of paternal environment alone. This lack of evidence stems generally from an absence of studies exploring the sole impact of paternal environment on offspring phenotype in marine invertebrates. At this time, the only exception is in the marine polychaete, *Hydroides diamphus*, where males exposed to more acidic conditions sired offspring with reduced juvenile growth rates (Lane et al. 2015). Paternal effect studies in marine invertebrate taxa have instead focused on how paternal environment influences other elements of offspring success such as fertilization success, larval survival, and developmental abnormality (Crean et al. 2013, Jensen et al. 2014, Guillaume et al. 2016, Leach et al. 2021). More often, marine invertebrate studies do not attempt to disentangle the distinct impacts of paternal and maternal environment on offspring phenotype. Such experiments have shown that general parental exposure to various environmental conditions positively influence offspring size and growth rates (Parker et al. 2012).

As compared to studies on invertebrates, there is more literature focused on the individual role of paternal effects on offspring size in fish. Both paternal and maternal thermal environment separately have an influence on the growth rate of various fish species, including sheepshead minnows and stickleback (Shama and Wegner 2014, Chang et al. 2021). Chang et al. (2021) further found that increases in larval growth rate were higher in sheepshead minnows when offspring temperature mimicked maternal temperature as

opposed to paternal temperature, but growth rate was maximized when the temperature conditions of offspring matched that of both parents. In this study, paternal thermal experience had no observable influence on larval size in *S. purpuratus*, but because all females used in this study were kept at ambient conditions, we cannot make any claims about the relative impacts of paternal and maternal environments on offspring size in urchins. Although, the variability in larval size observed between the different rounds of this experiment may shed some light. Across each round, a different pool of eggs was utilized in a split-clutch design to produce offspring. The interpretation here is that within each round, the larvae produced from individual males of both high and low temperature treatments were more likely to experience the same maternal investment. The average egg size differed between clutches used in each round. This size difference, as well potential differences in investment between mothers, may have been strong enough to mask any effects of paternal environment on larval size, although we still observed some influence of paternity within each round.

Given the relationship between egg size and the initial size of urchin larvae, it is generally thought that offspring size is largely driven by maternal provisioning (Moran and McAlister 2009). This hypothesis has been tested and supported in the context of climate change, (elevated temperatures, decreased pH, increased exposure to UV radiation) across many marine taxa (Adams and Shick 2001; Uthicke et al. 2014; Suckling et al. 2015). Mothers experiencing higher temperature, or lower pH conditions, tend to produce smaller offspring, but this response can vary based on the duration of adult exposure (Uthicke et al. 2014, Suckling et al. 2015). The mechanism behind this trend stems not only from the initial size of the eggs, but also the biochemical content (proteins and lipids) of the eggs that

nourish the developing embryos/larvae (Moran and McAlister 2009). For instance, female purple urchins exposed to varying temperature x pH regimes for 4 months produced similarly sized eggs but varied in lipid content (Wong et al. 2019). The mothers acclimated under colder, more acidic conditions produced more lipid-rich eggs, a quality authors suggested could explain the increased size of offspring from these mothers during early development (from hatching to the onset of larval development). Interestingly, Wong et al. 2019 and a follow-up study both observed no influence of maternal environment on the size of echinopluteus larvae (Wong et al. 2019, Strader et al. 2020). This aligns with the results of this study, where paternal environment also exhibited no influence of the size of sea urchin at this larval stage. Echinopluteus arm length may therefore be a metric that is more heavily influenced by the offspring's current environment as opposed to a carry-over effect from their parents' past experiences. Future studies trying to disentangle these dynamics might consider targeting stages earlier in development (or later as juveniles) or choosing another performance metric such as thermal physiology.

Implications in MHW-context

Marine heatwave (MHW) events are becoming an increasingly pervasive threat in oceans worldwide (Smale et al. 2019; Oliver et al. 2019). Recent modeling activities have suggested that MHWs will shift from a decadal phenomenon to an annual stressor at the highest CO2 emission scenarios (IPCC 2020). Given the projected increases in frequency and intensity of such events necessitates research focused on how current populations of marine organisms will respond under these extreme temperature stress events. In the Santa Barbara Channel (SBC), MHW events of varying lengths and intensities have been

frequently observed over the past decade (see Chapter III; Cavanaugh et al. 2019). Importantly the seasonality of SBC MHW events, which have occurred during the summer and fall, overlaps with the phenology of *S. purpuratus* reproductive cycles (see Fig. 1 in Chapter 1). As such, our results shed light onto how current and future MHW events in the area may influence population dynamics for purple urchins within the SBC. Namely, the carry-over effects of the high temperature experience of the sire might result in progeny better able to resist the stress of higher developmental temperatures *in situ* during a MHW event.

We observed generally positive effects of MHW temperatures (~20 ºC) on *S. purpuratus* larvae, indicating greater success under MHW conditions. The traits that were bolstered were significant in terms of larval ecology. Larvae under a MHW-like temperature developed faster, had a larger body size and further, maintained an increased thermal tolerance as compared to those larvae raised under non-MHW conditions (~14 ºC). Paternal exposure to these MHW-like conditions further increased tolerance within their offspring when reared under elevated temperatures. These results, when coupled with the observations in Chapter III, paint a complex picture of how urchin populations might respond to MHW events. Male exposure to MHW-like temperatures elicited gamete plasticity in sperm, but the consequences varied depending on the aspect of development observed. Fertilization success was significantly diminished in MHW-exposed males, but the results presented above appear to show that embryos that were successfully produced from the fertilization process inherit some mechanism of tolerance from their sires.

Overall, the results of this study showed that male urchins can influence the performance of their progeny after only a 28-day temperature acclimation. Although the temperature

conditions used here were static and did not reflect the natural fluctuations expected *in situ*, the general timescales of temperature exposure in this study support the ecological relevance of the data and potential outcomes in the field. There have been many MHWs in the SBC that lasted for varying amounts of time, from days to multiple months. Most notably is the 2014-2016 heatwave, that is referred to as one, 2-year event (74% of the days in that period were classified as anomalously warm). However, this particular MHW was actually composed of multiple MHW events each lasting on the timescale of months (Cavanaugh et al. 2019). Since the process of spermatogenesis occurs more rapidly relative to oogenesis for many organisms, including sea urchins, we hypothesize that the male urchins acclimated at 20 ^{o}C in these trials had sufficient time to alter the biochemical or physiological state of their sperm, supporting the transference of thermal tolerance to their progeny. Under such a scenario *in situ*, MHW events mimicking the timescales experienced in 2014-2016 may confer higher thermal tolerances for sea urchin larvae via paternal inheritance of epigenetic marks that prime offspring thermal physiology (Immler 2018).

The results presented here may be particularly interesting with regard to the massive purple urchin population outbreaks and consequent barrens seen along the California coast (Rogers-Bennett and Catton 2019). Ecologically, purple urchins are vital members of kelp forest communities (Ebert 2010). As grazers of kelp recruits, urchin density influences the shift of their habitat from a rich, kelp forest to a kelp-depleted "barren" and vice versa (Dayton et al. 1992). As such, changes in urchin populations can have significant impacts on kelp forest structure and the communities within them (Dayton et al. 1998, Graham 2004). In northern California, the combination of MHWs and substantial increases in the local *S. purpuratus* population underpinned a massive shift in ecosystem state from dense kelp

forests to sea urchin barrens (Rogers-Bennett and Catton 2019). On the other hand, the response of urchin population and recruitment to MHW events appear to vary between geographic locations across California (Okamoto et al. 2020). Urchin recruitment in the SBC was negatively correlated with sea surface temperatures, results that are in line with decreases in *S. purpuratus* population size following the 2014-2016 MHW event mentioned above (Reed et al. 2016). The effect of paternal exposure to MHW-like temperatures on larval thermal tolerance was context-dependent in this study, with positive and negative effects contingent on whether offspring experienced the same or different thermal conditions, respectively, to their sire. Such dynamics might represent one source of variation between the different responses of urchin populations to extreme events across their biogeographical range, given the temporal and spatial environmental variability characterizing the West Coast of the United States (Hofmann et al. 2014).

MHW events can vary substantially in their duration, from days to months, and are predicted to increase significantly in duration and frequency in future oceans (Frolicher et al. 2018). Given this variation, exploring how different timescales (both short and long) influence parental investment, both maternal and paternal, is crucial (see (Guillaume et al. 2016). The logistics of such experiments may be complicated, but the results presented in the past two chapters highlight specific areas of interest, fertilization and thermal tolerance. Future studies may be additionally aided by research tying a mechanism to these processes, such as altered sperm swimming or expression of heat shock proteins in sperm (Immler 2018). In those scenarios, experiments exploring the timescales of a paternal effect may only need to collect sperm that may serve as a bioindicator for the quality of future offspring under MHW events. Overall, the results of this study highlight paternal effects as a relevant

component of an organism's adaptive capacity and have the potential to influence the response of marine ecosystem function and structure under future climate change.

Acknowledgements

This research was supported by an NSF award IOS-1656262 to Gretchen E. Hofmann. This work was also supported by resources from Santa Barbara Coastal Long Term Ecological Research program (NSF award OCE-1831937; Director: Dr. Robert Miller), an award for which Gretchen Hofmann is a co-PI. I was supported by a Ford Predoctoral Fellowship. The Gorman Scholars Program provided support for undergraduate researchers working on the project. Specimens were collected in the Santa Barbara Channel under a California Scientific Collecting Permit to Gretchen Hofmann (SC-1223). Our appreciation goes out to Clint Nelson for collection of animals. We are also extremely grateful for the assistance provided by Logan Kozal, Jannine Chamorro, Xochitl Clare, Samuel Bogan, Amelia Ritger, Adriane McDonald, Cailan Sugano, Avery DeSantis, and Alix Martin during the experiment. We also would like to thank Dr. Kathy Foltz and Dr. Debora Iglesias-Rodriguez for the feedback provided during the writing of this manuscript.

V. The Developmental Consequences of Heat Stress During Fertilization in the Summer Spawning Sea Urchin, *Lytechinus pictus*

Overview

In this chapter, I explored whether the temperature at which fertilization takes place has an impact on developmental success in the painted sea urchin, *Lytechinus pictus*. This work was conducted within the context of predicted marine heatwave (MHW) events, both present-day and future. While gametes and fertilization in broadcast spawners are typically thought of as being robust to environmental stress, there is evidence to suggest that fertilization under stressful abiotic conditions may induce latent effects not seen until later, more vulnerable developmental stages (Byrne 2011). For summer spawning species, such as *L. pictus*, gametes and embryos are likely to experience seasonally higher temperatures during fertilization and early development as compared to other winter spawning species such as *S. purpuratus* (Strathmann 1987). The anomalous temperatures associated with summer MHW events could further push these species past their physiological limits, making it crucial to understand potential carry-over effects from fertilization under such temperatures. These extreme, short-term events may be important in determining species success across their range, especially at range boundaries (Harvey et al. 2021).

For this part of my thesis research, I hypothesized that fertilization temperature influences the developmental success of *L. pictus*. This hypothesis was tested under two MHW scenarios representing either: (1) present-day thermal conditions in the Santa Barbara Channel (SBC), with one treatment mimicking temperatures associated with non-MHW summer conditions (17 ºC) and the other mimicking temperatures associated with observed

summer MHW events (20 ºC); or (2) future high temperature extremes that could be experienced in the SBC based on projected increases in sea surface temperature over the coming decades (IPCC 2022), again comparing a future non-MHW temperature (20 ºC) to that of a future MHW temperature (24 ºC). For the experimental approach, I used pooled egg and sperm from multiple adults, and then conducted fertilization success trials at each temperature (17 ºC, 20 ºC, or 24 ºC). The resulting embryos from these trials were then split and raised reciprocally under the temperatures associated with each MHW scenario (Scenario #1, present-day: 17 ºC vs. 20 ºC; Scenario #2, future: 20 ºC vs. 24 ºC). Embryo size, developmental success, and abnormality were assessed at two critical stages of development: hatching and gastrulation. The data presented here represent one of the first studies investigating the carry-over effect of fertilization temperature on later developmental success in an echinoderm species, and adds to a small, but growing body of literature focused on such effects in marine metazoans. The results are discussed in the context of other organisms that have been studied for the effect of fertilization temperature, including tubeworms, mollusks (including mussels, scallops, and oysters), and various fish species (Parker et al. 2009; White et al. 2014; Chirgwin et al. 2019; Chirgwin et al. 2021; Lymberry 2021).

In the Discussion, I outline the relevance of my findings, where exposure to both present-day and future MHW temperatures during fertilization reduced fertilization success and increased abnormality in post-fertilization development. These developmental responses were stage-specific and dependent on the MHW scenario, but overall fertilization and development under MHW-like temperatures had a negative influence on developmental success. Comparing the results seen here to the few studies mentioned above, there does

appear to be a larger role of fertilization environment on offspring success than previously acknowledged. These comparisons additionally highlight potential underlying mechanisms and provide the ground for suggested future work on the topic. Putting my results in the context of ocean climate change research, I conclude the Discussion commenting on the use of *L. pictus* as a model for studying the developmental impacts of MHW events in the SBC (Nesbit et al. 2019; Watts et al. 2020).

Materials and Methods

Urchin collection

Painted sea urchins, Lytechinus pictus, were hand-collected for use in this study via SCUBA in June 2021 from Pelican Anchorage (34.021°N, 119.697°W), an inshore ocean study site of the coast of Santa Cruz Island within the Santa Barbara Channel (SBC). This location serves as a Santa Barbara Coastal Long-Term Ecological Research (LTER; https://sbclter.msi.ucsb.edu/) site. Adult L. pictus were collected at a depth of 14m at a temperature of ~17 ºC. Adult sea urchins were transported to the Marine Science Institute's seawater research facility following collection and placed in flow-through seawater aquaria held at 16-17 ºC for ~1 month. During this time, urchins were fed kelp weekly and regularly monitored for injury and death.

Urchin spawning and fertilization trials

Spawning of adult *L. pictus* and the collection of gametes followed the procedures outlined in Chapter II. Following the collection of sperm and egg, fertilization success trials

were conducted using a procedure that was modified from that outlined in Chapter II and in (Leach et al. 2021). In this iteration of the fertilization success assay, three major elements of the original methodology were altered to better address the goals of this research project: (1) pooled sperm was utilized instead of conducting separate trials for sperm from each individual male, (2) trials were conducted using one sperm concentration, and (3) gametes were acclimated to fertilization temperature treatments before the assay was conducted. While the use of pooled sperm and one sperm concentration were utilized due to space and time limitations, the acclimation of gametes before the trial likely represents a more ecologically relevant scenario for the broadcast spawning *L. pictus.*

The effect of temperature on fertilization success in *L. pictus* was assayed at three temperatures, representing that experienced in summers under current ambient (17 ºC), current MHW (20 ºC), or future MHW (24 ºC) conditions observed or predicted within the SBC. Future summer MHW temperatures were estimated based upon projected increases in SST over the coming decades under the high greenhouse emissions scenario outlined in (IPCC 2022). Target temperatures within fertilization vials were reached using an aluminum heating block attached to two recirculating water baths (LAUDA), creating a temperature gradient across the block. Within trials, pooled sperm (n = 5 male urchins) was used to fertilize pooled eggs (n = 7 female urchins) at each temperature. Fertilization trials were conducted under one sperm concentration in order to maximize space within the aluminum heating block. Preliminary assays of fertilization in *L. pictus* using this protocol determined that a sperm concentration of 10^3 cells/mL^{-1} would lead to successful fertilization in >75% of eggs, so this concentration was targeted for the trial via centrifugation and serial dilutions. Before sperm was fully diluted to the target concentrations within fertilization vials, the

pooled sperm was aliquoted into stock vials within the heat block containing FSW at the three experimental temperatures (17 ºC, 20 ºC, or 24 ºC). Subsets of sperm were exposed to each temperature for 5 minutes before a portion was pipetted into triplicate fertilization vials corresponding to the same temperature experienced during sperm acclimation. Eggs were also acclimated in a falcon tube for 5-10 minutes at the same temperature before its addition into experimental vials. From here, fertilization trials followed that outlined in Chapter II.

Following the trials, fertilization vials were moved to incubators corresponding to each experimental temperature and allowed to develop for 1.5 – 2 hours, until >75% of the fertilized eggs had exhibited a cleavage event. A subsample was then taken from each triplicate fertilization vial and preserved with 4% formalin in FSW for later analysis. In this study, fertilization success was calculated using the proportion of successful fertilization events, represented by the possession of a fertilization membrane, in 100 eggs from each subsample (Leach et al. 2021). The effect of fertilization temperature on fertilization success was statistically analyzed as a binomial response with a generalized linear model using a logit link function to assess binary data (fertilized vs. unfertilized). A one-way ANOVA was run to assess the impact of temperature on fertilization success, with a post-hoc Tukey test used to evaluate pair-wise differences between each fertilization temperature treatment. All analysis was conducted in R using the lme4 package (Bates et al. 2015).

Rearing and sampling of embryos post-fertilization

Embryos produced from the above fertilization trials were then raised under varying temperatures to observe the impact of fertilization temperature on subsequent development. For this portion of the experiment, samples were separated in such a way to survey this

question across two scenarios representing: (1) the effect of fertilization temperature under current MHW event temperature conditions - with 17 ºC representing ambient conditions and 20 ºC representing MHW-like temperatures, and (2) such effects under conditions predicted for future MHW events – with 20 ºC now representing future ambient conditions and 24 ºC representing future MHW-like temperatures. For the current MHW scenario, embryos produced from fertilizations at 17 ºC and 20 ºC were each divided and raised under thermal conditions that either mimicked their fertilization environment or was 3 ºC higher/lower. This resulted in four treatments, each replicated in triplicate, to be compared (fertilization temperature – developmental temperature): 17-17, 17-20, 20-17, 20-20. The same process was repeated for samples under a future MHW scenario, producing the following four treatments: 20-20, 20-24, 24-20, 24-24. Of note is that the same individuals were used for the 20-20 treatment across scenarios, meaning they represented both current MHW-like temperatures and future ambient thermal conditions. The process of preparing these developmental treatments is described below and outlined in (Fig. 13).

Once >75% of embryos in each fertilization vial (n = 3 per fertilization temperature) had reached the first cleavage stage, ~200 individuals were pipetted from each replicate vial into four new culturing vials containing 20 mL of FSW at the same temperatures in which fertilization occurred. These vials were then separated so that two (one for measuring hatching success and the other for gastrulation success) were kept at the same temperature experienced during fertilization and the other two were ramped down/up to a new temperature corresponding to one of the other experimental temperature conditions, resulting in 24 total treatment vials (n = 3 replicate vials x 2 fertilization temperatures x 2 developmental temperatures x 2 stages). Development within culturing vials was monitored

closely until embryos reached either the hatched blastula and gastrula stages. During this time, regular water changes were conducted in each vial.

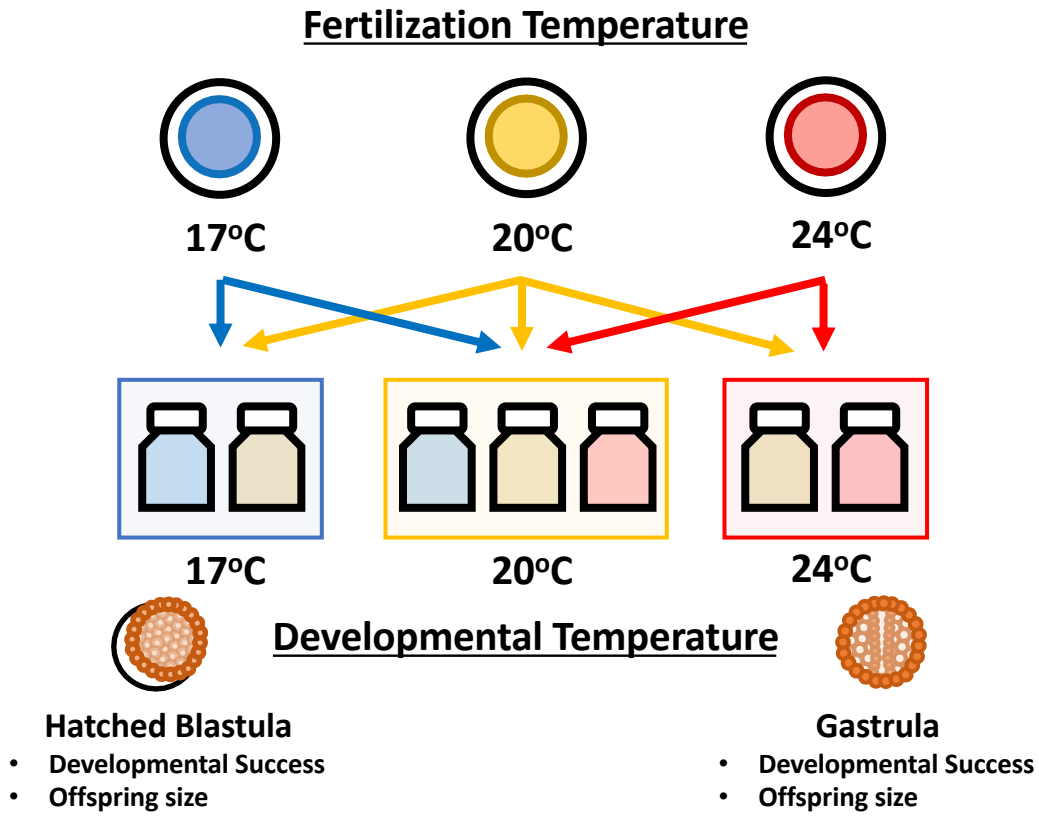


Figure 13. Experimental Design. Fertilization trials were conducted at three temperatures: 17 ºC, 20 ºC, and 24 ºC. The embryos produced from these fertilization events were subsequently split up into varying developmental rearing conditions representing temperatures either 3 ºC above or below the temperature experienced during fertilization. Embryos were raised until the hatching blastula and gastrula stages where they were sampled for developmental success and offspring size.

Once embryos in each culturing vial reached the hatched blastula or gastrula developmental stage, vials were sampled for quantifications of offspring size and developmental success. For hatched blastula, sampling occurred once 50% of individuals had hatched out of their fertilization membrane. This process involved lowering the volume of culture vials to ~5 mL using a serological pipettor with a 35um-mesh filter attached to the

pipette, to avoid removing larvae from the vial. ~60 individuals were then removed from this more concentrated culture and preserved using 2% formalin in FSW for later analysis of embryo size. The volume of culture vials was brought back up to 20 mL using fresh FSW (of the corresponding temperature) and the remaining embryos were allowed to develop for an additional 30 minutes. Once this time had passed, the volume in culture vials was lowered once again before the addition of 4% formalin to preserve the rest of the larvae in that vial (estimated to be ~140 individuals) for measures of developmental success. The same procedure was conducted in vials designated for assessment of gastrulation. At the gastrula stage, individuals were sampled once 50% of individuals demonstrated gastrulation (where the archenteron was at least 25% of the way across the embryo's inner cavity). Here, early gastrula individuals were allowed to develop for an additional hour before preservation for the purpose of measuring gastrulation success.

Quantifications of developmental success

In the days following preservation, ~100 embryos were scored for developmental success based on developmental progression and abnormality. At the hatched blastula stage, individuals were scored based on whether they had hatched from their fertilization membrane and/or exhibited normal development (which included displaying motility within or outside of the membrane). This scoring led to 4 general categories of embryos observed at the hatched blastula stage: (1) hatched and normally developed, (2) hatched but abnormal, (3) unhatched but normal morphology, and (4) unhatched and abnormal. Unfertilized eggs were not incorporated in the scoring. Individuals from each replicate vial scored in category 1 were used to represent successful hatching events while those in the remaining three

categories were deemed as unsuccessful hatching events. The effect of fertilization temperature treatment on hatching success was analyzed as a binomial response with a generalized linear mixed-effects model. Binary data (successful hatching events vs. unsuccessful hatching events) was fitted using a logit link function. The model included fertilization temperature treatment, developmental temperature treatment, and their interaction as fixed factors, while vial replicate was included as a random effect. A Wald chi-square test was employed to further determine the significance of each factor on the number of successful hatching events observed. Hatching success data corresponding to the current MHW (comparisons between 17 ^{o}C and 20 ^{o}C temperature treatments) and future MHW scenarios (for comparisons between 20 ^{o}C and 24 ^{o}C temperature treatments) were analyzed in the same way but using separate models.

For the gastrula stage, individuals were scored for developmental success based upon the degree of gastrulation observed. Here, the four scoring categories were: (1) full gastrulation (archenteron extended fully across the body cavity), (2) partial gastrulation (archenteron had formed but did not reach all the way across body cavity), (3) no gastrulation (complete absence of the archenteron), and (4) significant abnormality (included individuals stalled at earlier developmental stages). Successful gastrulation events in each vial were represented by the number of individuals who demonstrated full or partial gastrulation (categories 1 and 2), while unsuccessful gastrulation was quantified by the number of individuals falling in categories 3 and 4. The effect of fertilization temperature treatment on gastrulation success was analyzed in the same way (using the same factors) that is outlined above, as a binomial response with a generalized linear mixed-effects model. Here, though, binary data in the model represented the number of successful and unsuccessful gastrulation events, fitted

using a logit link function. Again, models were separately created for each MHW scenario. All statistical analysis was completed in R with models created using the lme4 package (Bates et al. 2015).

Morphometric analysis

The size of embryos at hatching and gastrulation were assessed from the ~60 individuals preserved and collected from each replicate vial once cultures had ~50% of individuals hatched or gastrulated. Samples for morphometric analysis were sampled at this timepoint to ensure that assessments were based off individuals who had recently hatched or gastrulated, thus minimizing the influence of asynchronous development within vials and across temperature treatments. At both stages, individual embryos were photographed from each of the 12 treatment vials (i.e., four treatments each with three replicate vials, for both current and future MHW scenarios). Pictures for morphometric analysis were obtained using the same procedure outlined in Chapter IV.

Photographs were taken of individual blastula that had successfully hatched and were oriented so that the vegetal plate, represented by a thicker portion of cells oriented around the vegetal pole, was in focus. The 2-dimensional area of hatched blastula was measured to assess embryonic size at hatching. The gastrula embryos were only photographed when individuals exhibited near full gastrulation (archenteron reached 50-75% across body cavity) and oriented where the side profile of their archenteron was visible and in line with the vegetal plate. Here, both 2-dimensional area and length (distance across the center of the archenteron from anterior to posterior end of the embryo) were measured. Digital photographs of each stage were calibrated for the 20x objective using ImageJ (National

Institutes of Health, USA). Within each MHW scenario, differences in embryo size measured at hatching and gastrulation were analyzed using a two-way ANOVA, with fertilization temperature and developmental temperature set as fixed factors and vial replicate set as a random factor. Statistical analysis was completed in R.

Results

Fertilization success

We observed a significant effect of temperature on fertilization success in *Lytechinus pictus* embryos (Fig. 14; $X^2_{1,2} = 53.345$, $p < 0.001$). Here, the percentage of fertilization events observed at 17 ºC (91.7 ± 1.2%) was significantly higher than that at 20 ºC (73.3 ± 11.2%) ($p < 0.001$), or 24 ºC (70.3 ± 6.1%) ($p < 0.001$). The percentage of successful fertilization events observed when comparing the 20 ºC and the 24 ºC treatments were similar and no statistical difference was found in the analysis ($p = 0.414$).

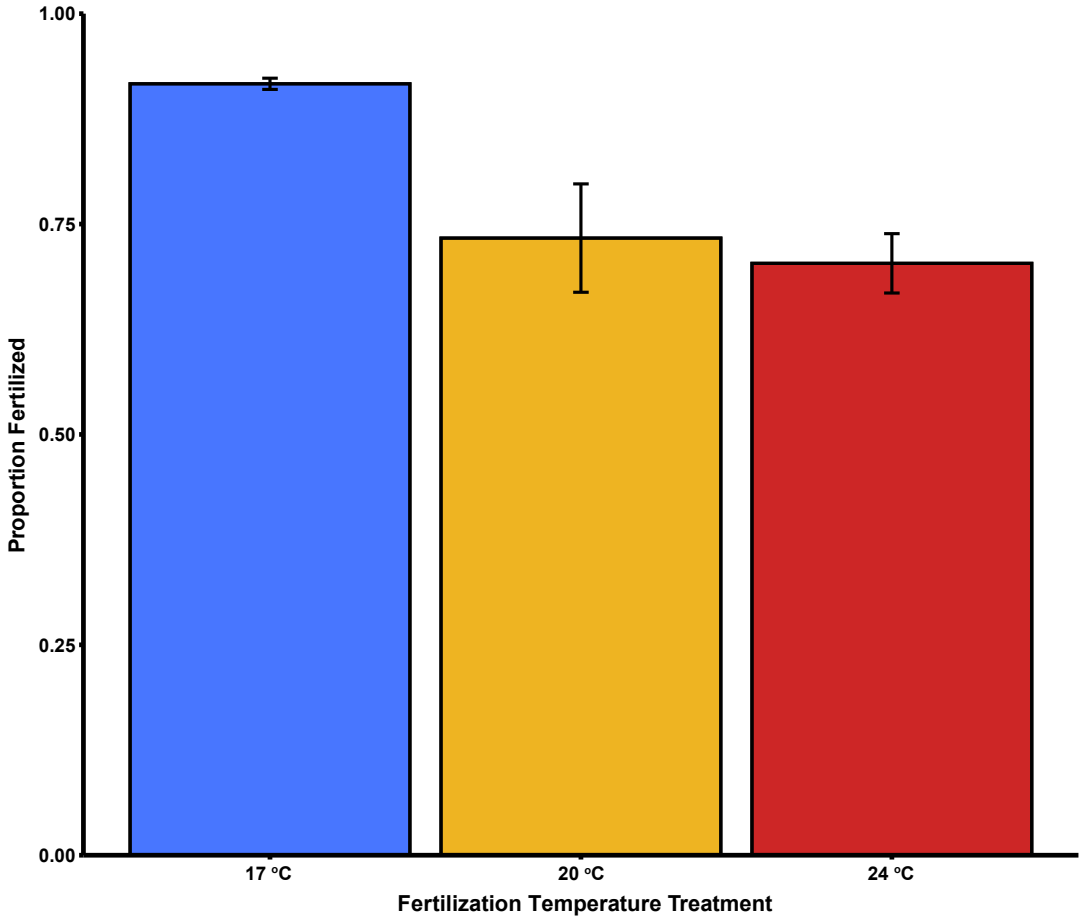


Figure 14. Effect of temperature (17 ºC, 20 ºC, 24 ºC) on fertilization success in *Lytechinus pictus.* Error bars represent mean +/- standard error.

MHW Scenarios

We next evaluated two MHW scenarios, one in which fertilizations and/or development occurred under ambient (17 ºC) or MHW-like (20 ºC) summer temperature conditions currently seen in the SBC (referred to as MHW Scenario #1) and another in which each process experienced temperatures associated with predicted ambient (20 ºC) or MHW-like (24 ºC) conditions in future oceans (MHW Scenario #2). Experimental temperatures experienced during fertilization and development are listed in Table 10.

MHW Scenario #1: Current conditions in SBC

Developmental success and abnormality

Fertilization temperature had a significant effect on developmental success under the present-day MHW scenario (MHW Scenario #1), but the degree of this effect was dependent on the embryonic stage observed (Fig. 15, 16). For the blastula stage, hatching success was significantly influenced by fertilization temperature, with embryos produced from fertilizations that occurred at 17 ºC demonstrating 15.3% more hatching success overall than embryos from 20 ºC fertilizations (Fig. 15a; $p < 0.001$). While there was a minimal effect of developmental temperature on hatching success ($p = 0.232$), the interaction between fertilization and developmental temperatures had a large influence ($p < 0.001$). Here, the hatching success observed in embryos fertilized and raised at 17 ºC was reduced by -7.6% when the same embryos were raised at 20 ºC. Alternatively, embryos produced from 20 ºC-fertilizations exhibited a +8.1% increase in successful hatching when raised under the same 20 ºC conditions as opposed to 17 ºC.

In terms of where these differences were observed, abnormality seen pre- and post-hatching (categories #2 and #4 outlined in methods) appeared to drive many of the patterns observed in hatching success between different fertilization and developmental temperature treatments (Fig. 15b). While a minimal proportion of individuals from 17 ºC-fertilizations exhibited abnormal development at either developmental temperature (~7%), nearly ¼ of individuals from 20 ºC fertilizations (~24%) were abnormal. Of note is the large proportion of individuals from 20 ºC fertilizations that remained unhatched (but did not exhibit any observable signs of morphological abnormality) in the 17 ºC developmental treatment,

12.5% (Fig. 15b). This is nearly double the number of unhatched individuals seen in the next closest culturing treatment.

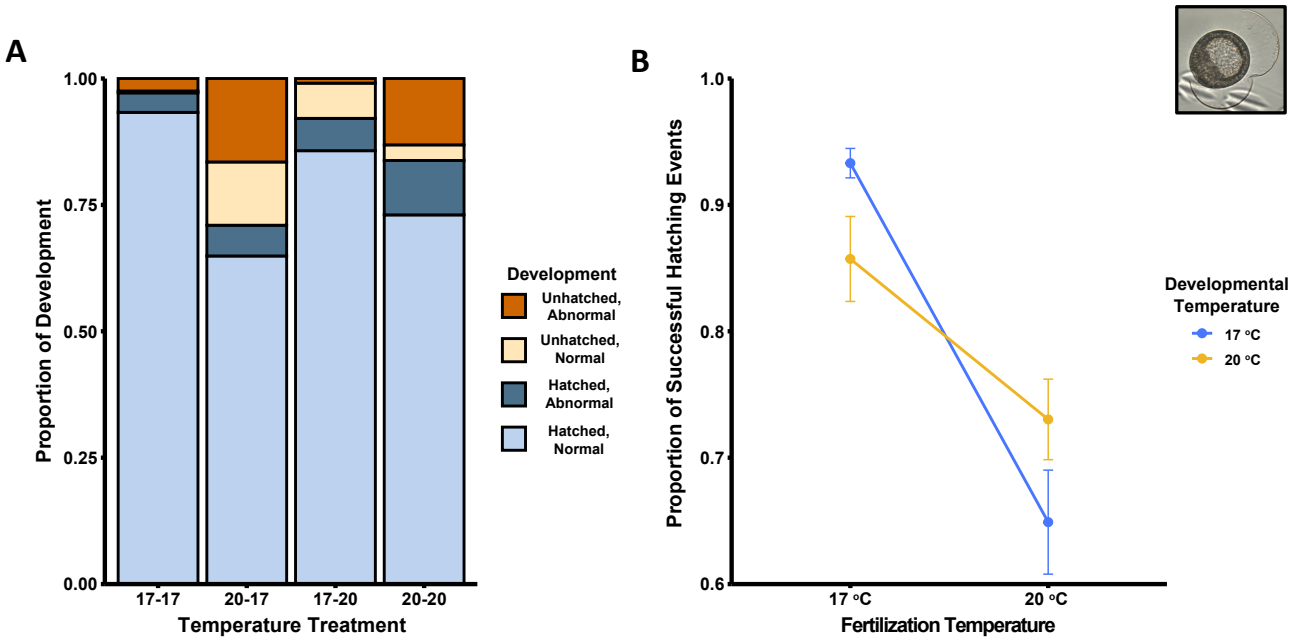


Figure 15. Effect of fertilization temperature (17 ºC or 20 ºC) and developmental temperature (17 ºC or 20 ºC) on hatching success of *L. pictus* embryos under MHW Scenario #1. (A) Representing developmental progression of embryos under various temperature treatments based on normality and hatching. Temperature treatments labeled as follows: fertilization temperature – developmental temperature. (B) Reaction norms representing the interactive effects of fertilization and developmental temperature treatments on hatching success. Error bars represent mean +/- standard error.

At the gastrula stage, the direct influence of fertilization temperature on developmental success was reduced as compared to that observed for the earlier embryonic stage of blastula ($X^2_{1,1}$ = 1.203, p = 0.273). For this later stage embryo, developmental temperature ($X^2_{1,1}$ = 19.878, $p < 0.001$) and the interaction of fertilization and developmental temperatures ($X^2_{1,1}$ = 11.367, p = 0.001) were the more dominant factors influencing gastrulation success (Fig. 16a). Gastrula exhibited the highest degree of developmental success under the 17 ºC developmental treatment. Within this developmental temperature treatment, ~19.0% more

embryos from 17 ºC-fertilizations gastrulated successfully than embryos from 20 ºC fertilizations. For embryos reared at 20 ºC, though, the proportion of gastrulation success was nearly identical between gastrula produced from both 17 ºC and 20 ºC fertilizations.

However, individuals at the gastrula stage exhibited higher proportions of abnormality than at hatched blastula, a trend that held across treatments (Fig. 16b). Exposure to 20 ºC at fertilization or during development resulted in higher numbers of abnormal gastrula in comparison to 17 ºC, although the increase in abnormality was more pronounced between fertilization temperatures, ~14.2% more gastrula were abnormal when fertilization occurred at 20 ºC, than between developmental temperatures, ~5.2% increase in abnormality when development occurred at 20 ºC. Across temperature treatments, ~16% of gastrula did not exhibit significant abnormality but had no presence of an archenteron at all (Fig. 16b).

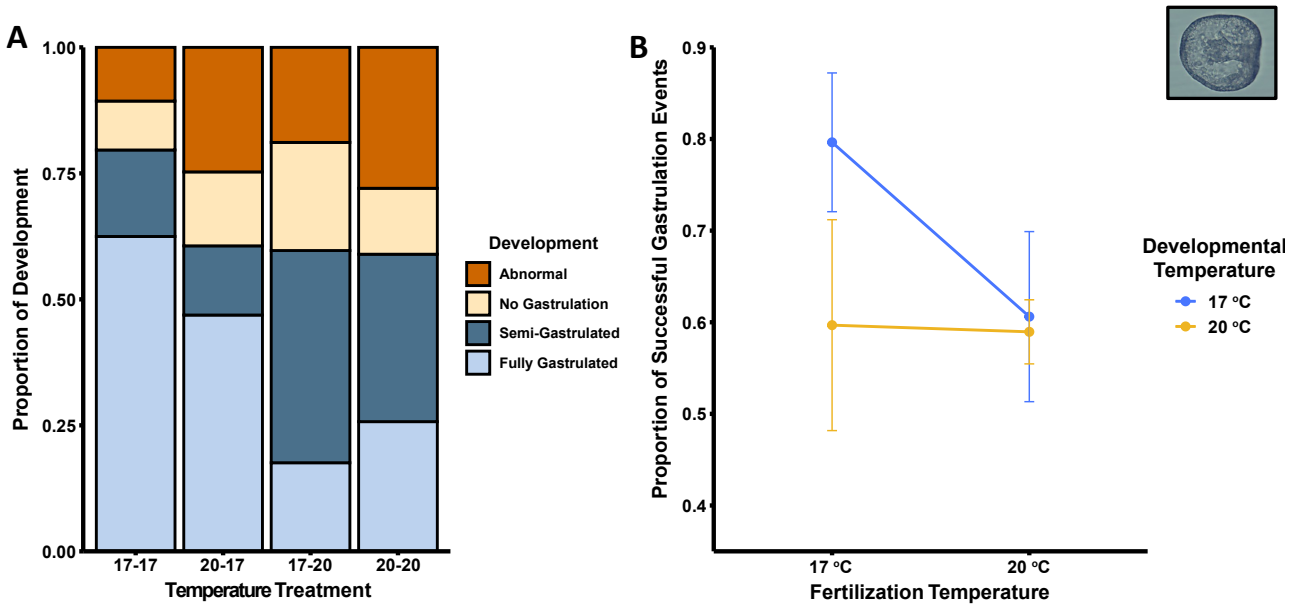


Figure 16. Effect of fertilization temperature (17 ºC or 20 ºC) and developmental temperature (17 ºC or 20 ºC) on gastrulation success of *L. pictus* embryos under MHW Scenario #1. (A) Representing developmental progression of embryos under various temperature treatments based on normality and degree of gastrulation. Semi-gastrulated refers to embryos that possess archenterons > 50% of their body length. Temperature treatments labeled as follows: fertilization temperature – developmental temperature. (B)

Reaction norms representing the interactive effects of fertilization and developmental temperature treatments on gastrulation success. Here, gastrulation success includes proportion of embryos observed to be fully or semi-gastrulated. Error bars represent mean +/- standard error.

Body size at hatching and gastrulation

Neither fertilization (ANOVA, $F = 0.906$, $p = 0.394$) nor developmental temperature (ANOVA, $F = 1.970$, $p = 0.161$) treatments alone influenced the area of hatched blastula under MHW scenario #1, but there was a significant influence of their interaction (Fig. 17a; ANOVA, $F = 7.317$, $p = 0.007$). Hatched blastula from 17 ºC fertilizations were 3.8% larger when raised under 17 ºC as opposed to 20 ºC. Blastula from 20 ºC fertilizations were even closer in size between developmental temperatures, with those raised under 20 ºC just 1.1% larger in size.

Alternatively, developmental temperature alone had a significant effect on gastrula size (ANOVA, $F_{1,8} = 17.422$, $p = 0.003$), with little effect of fertilization temperature alone (ANOVA, $F_{1,8} = 1.053$, $p = 0.335$) or the interaction between fertilization and developmental temperatures observed (ANOVA, $F_{1,8} = 0.028$, $p = 0.886$; Fig. 17b). Gastrula area and length were positively correlated across individuals ($R^2 = 0.78$; $p < 0.001$), therefore similar trends were seen regardless of the metric used. Gastrula raised under 20 ºC conditions had 12% smaller areas than embryos raised under 17 ºC, regardless of fertilization temperature.

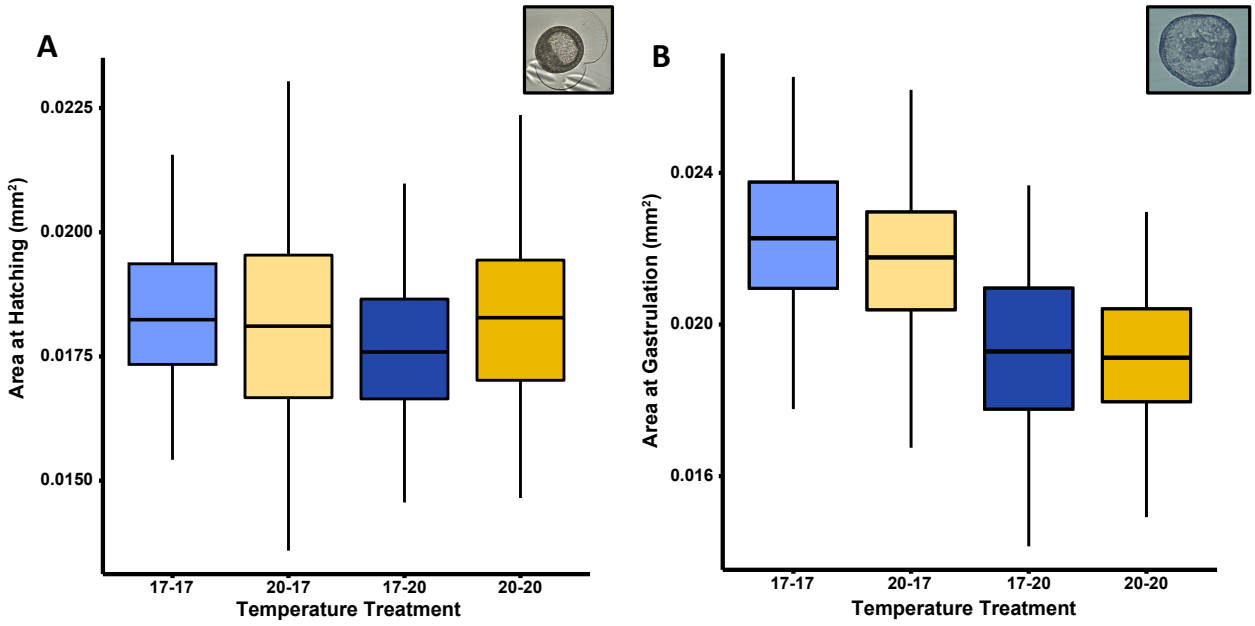


Figure 17. Effect of fertilization temperature (17 ºC or 20 ºC) and developmental temperature (17 ºC or 20 ºC) on *L. pictus* embryonic size at hatching and gastrulation under MHW Scenario #1. Temperature treatments labeled as follows: fertilization temperature – developmental temperature.

MHW Scenario #2: Future MHW conditions in SBC

Developmental success and abnormality

Similar to the results recorded for MHW scenario #1, the effect of fertilization and developmental temperature on developmental success under the future MHW scenario varied between stages (Fig. 18, 19). At the blastula stage, there was no effect of fertilization temperature ($p = 0.321$), developmental temperature ($p = 0.719$), or their interaction on hatching success ($p = 0.664$). Generally, ~71.8% of embryos were able to hatch successfully across treatments (Fig. 18a). Observations of abnormality across treatments was also similar, as ~24.2% of individuals exhibited abnormal development (Fig. 18b).

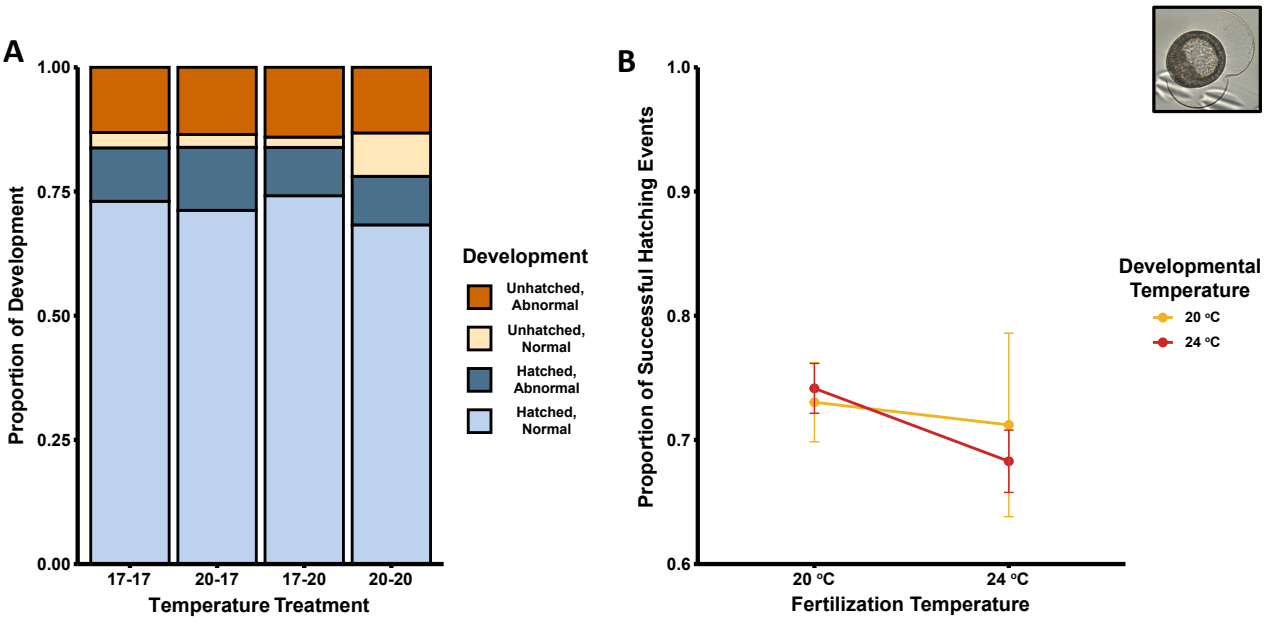


Figure 18. Effect of fertilization temperature (20 ºC or 24 ºC) and developmental temperature (20 ºC or 24 ºC) on hatching success of *L. pictus* embryos under MHW Scenario #2. (A) Representing developmental progression of embryos under various temperature treatments based on normality and hatching. Temperature treatments labeled as follows: fertilization temperature – developmental temperature. (B) Reaction norms representing the interactive effects of fertilization and developmental temperature treatments on hatching success. Error bars represent mean +/- standard errors.

Alternatively, patterns in gastrulation success were driven by developmental temperature ($p = 0.008$), with little influence of fertilization temperature ($p = 0.895$) or the interaction between fertilization and developmental temperatures ($p = 0.726$). Gastrula reared at 17 ºC exhibited 11.2% more gastrulation success than those raised under 20 ºC conditions (Fig. 19a). Again, patterns in hatching success were mirrored by similar patterns in abnormality across treatments, with ~18.1% more gastrula exhibiting abnormal development when reared at 24 ºC compared to those raised at 20 ºC (Fig. 19b).

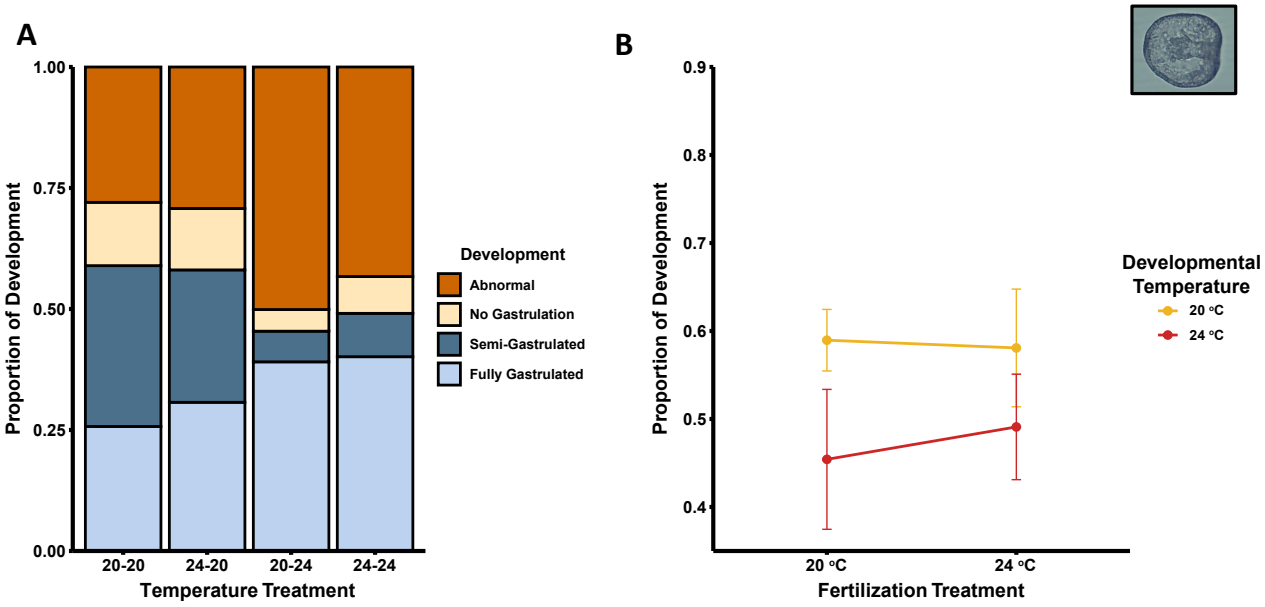


Figure 19. Effect of fertilization temperature (20 °C or 24 °C) and developmental temperature (20 °C or 24 °C) on gastrulation success of *L. pictus* embryos under MHW Scenario #2. (A) Representing developmental progression of embryos under various temperature treatments based on normality and degree of gastrulation. Semi-gastrulated refers to embryos that possess archenterons > 50% of their body length. Temperature treatments labeled as follows: fertilization temperature – developmental temperature. (B) Reaction norms representing the interactive effects of fertilization and developmental temperature treatments on gastrulation success. Here, gastrulation success includes proportion of embryos observed to be fully or semi-gastrulated. Error bars represent mean +/- standard error.

Body size at hatching and gastrulation

Developmental temperature had a significant influence on the size at hatching under the future MHW scenario #2 (ANOVA, $F_{1,8} = 19.833$, $p = 0.002$), with blastula 4.6% smaller when hatching under the 24 °C treatment compared to those who hatched at 20 °C (Fig. 20a). Meanwhile, fertilization temperature alone (ANOVA, $F_{1,8} = 0.001$, $p = 0.977$) and the interaction between fertilization temperature and developmental temperature (ANOVA, $F_{1,8} = 0.020$, $p = 0.883$) had minimal effects on hatched blastula area.

For gastrula, developmental temperature had a similar effect on area (ANOVA, $F_{1,8}$ = 4.868, p = 0.058) and length (ANOVA, $F_{1,8}$ = 5.761, p = 0.044) to that seen in hatched blastula (Fig. 20b). Slight differences in the influence of developmental temperature on gastrula area and length can be explained by the generally positive relationship between the two metrics (R^2 = 0.62; p < 0.001). Interestingly, while developmental temperature was the major influence on embryo size at both gastrulation and hatching, gastrula from the 24 ºC treatment were bigger (+4.8% area, +3.9% length) than those from the 20 ºC, which is the opposite trend seen at hatching (Fig. 20). Fertilization temperature (ANOVA, $F_{1,8}$ = 2.151, p = 0.180) as well as the interaction between fertilization temperature and developmental temperature (ANOVA, $F_{1,8}$ = 0.249, p = 0.631) continued to have very little effect on gastrula area or length (ANOVA, $F_{1,8}$ = 0.686, p = 0.431; ANOVA, $F_{1,8}$ = 0.009, p = 0.962).

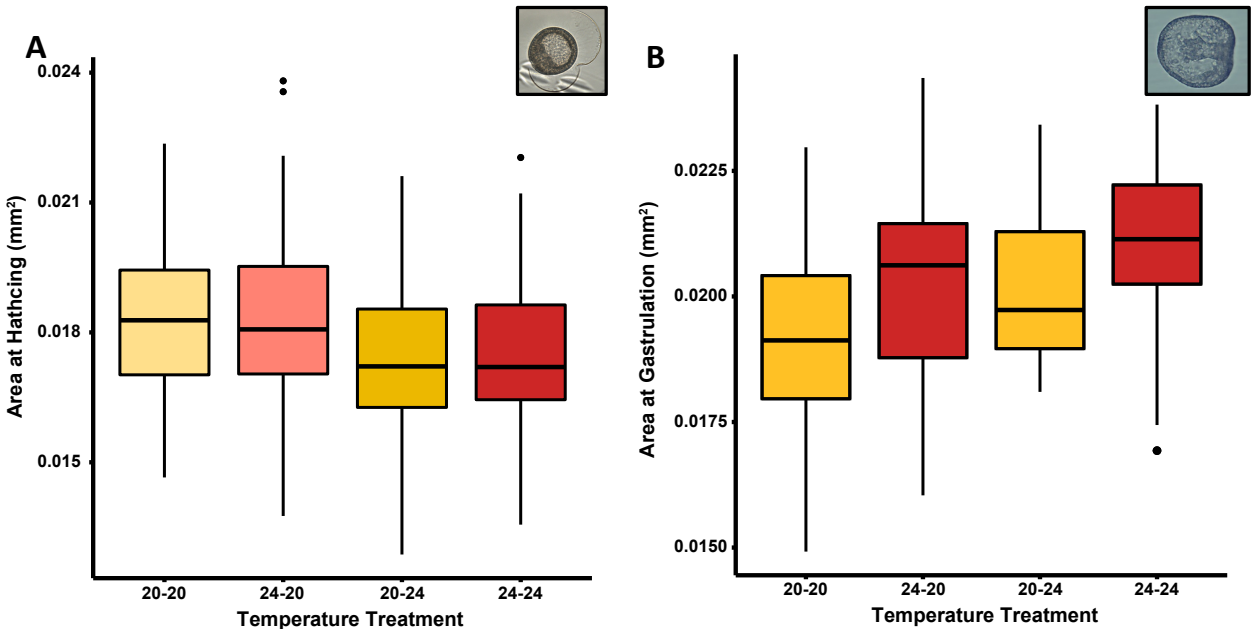


Figure 20. Effect of fertilization temperature (20 ºC or 24 ºC) and developmental temperature (20 ºC or 24 ºC) on *L. pictus* embryonic size at hatching and gastrulation under MHW Scenario #2. Temperature treatments labeled as follows: fertilization temperature – developmental temperature.

Discussion

Summary of study and results

In this study, I investigated whether the thermal environment during fertilization in the painted sea urchin, *Lytechinus pictus*, influenced the developmental success of later embryonic stages. I hypothesized that the consequences of fertilization events under environmental stress extend past the fertilization event itself, influencing the susceptibility of more vulnerable stages under similar conditions. This hypothesis was tested under two scenarios corresponding to temperatures associated with present-day (MHW scenario #1) or future (MHW scenario #2) marine heatwaves events observed or predicted to occur in the SBC, respectively. As a summer-spawning species, both gametes and the early developmental stages of *L. pictus* are likely to experience already elevated temperatures during the summer months, and therefore may be more vulnerable to the additional increase in temperature associated with MHW events (Sen Gupta et al. 2020). Under each MHW scenario, pooled sperm and egg were exposed to one of the two temperature conditions (ambient or MHW-like) before fertilization success trials were conducted. The resulting embryos produced from fertilizations at each temperature were then raised under one of the same two temperature conditions, allowing us to examine how fertilization temperature influenced the developmental success of the embryos as a function of developmental environment. The overarching goal was to examine potential carry-over effects from fertilization that are often overlooked in studies solely considering the influence of fertilization environment on the process itself. Such effects would highlight yet another source of variation that must be considered when predicting an organism's ability to

withstand environmental stress and provide a new perspective of fertilization's role on later development.

I made three clear findings during this study: (1) both present-day and future MHW-like temperatures (20 and 24 ºC, respectively) reduced fertilization success in *L. pictus*, (2) fertilization temperature negatively influenced developmental success and normality, but this response was stage-specific, and (3) developmental temperature, but not fertilization temperature, affected offspring size, with more dramatic effects seen at the gastrula stage. Together, these results demonstrate that the early life history stages of *L. pictus* are vulnerable to the elevated temperatures associated with both present-day and future summer MHW events in the SBC.

Reduced fertilization success in L. pictus *under elevated temperatures*

In this study, exposure of gametes to elevated temperatures corresponding to that of present-day (20 ºC) and future, predicted (24 ºC) MHW events in the SBC had a negative impact on fertilization success in *Lytechinus pictus*. Here, fertilization success was estimated as the proportion of fertilization events observed. When gametes were exposed to 17 ºC during fertilization, I observed nearly ~20% more fertilization events when compared to those observed under either 20 ºC or 24 ºC (which exhibited similar fertilization success to one another). Generally, gametes and fertilization itself are thought of as being robust to thermal stress, but variability across and within species is common (Byrne 2011). Both biological (i.e., environmental history of adult organisms) and methodological (i.e., experimental design) factors could underlie this observed variability (Lera et al. 2006; see Chapters II and III). A related sea urchin species, *Lytechinus variegatus*, while possessing

increased overall tolerance to higher temperatures than what I observed in *L. pictus*, exhibited similar declines in fertilization success when exposed to +4-7 ^{o}C increases in temperature as compared to ambient conditions (Lenz et al. 2019).

In Chapter III, fertilization in the purple sea urchin, *Stronglyocentrotus purpuratus,* a species also found in the SBC, was not significantly influenced by exposure to 20 ^{o}C (Chapter III; Leach et al. 2021). On top of the different responses that may exist between these species, the fertilization success trial methodology differed. Here, the use of pooled sperm, instead of sperm from single males, was used to fertilize pooled eggs. Additionally, sperm and egg were both equilibrated to environmental conditions prior to the fertilizations themselves. While some urchin species exhibit congregating behavior during spawning, it is unclear to what degree this type of spawning behavior exists in *Lytechinus spp.*, although they are typically found in congregations (Levitan 2000; Watts et al. 2020). Regardless, one would expect that gametes from broadcast spawning organisms, like sea urchins, directly experience abiotic conditions (physical, chemical and thermal) of their immediate aquatic environment prior to an actual fertilization event *in situ*. During this exposure, extreme abiotic conditions could influence several aspects of gamete function and performance, including the swimming behavior of sperm, gamete energetics, or the process of chemotaxis that leads sperm to eggs (Boni et al. 2016; Lymberry et al. 2018; Immler 2018). Similar negative temperature effects on fertility were seen in the tubeworm, *Galeolaria caespitosa*, which were underpinned by selection in the thermal environment for specific sperm phenotypes (Chirgwin et al. 2021). As such, future studies should more readily consider pre-fertilization environment of both gametes before conducting fertilization trials in the context of climate change.

Fertilization temperature influences developmental success

Exposure to elevated temperatures during fertilization had negative carry-over effects on the successful development of *L. pictus* embryos at hatching and gastrulation. These effects were more apparent at the hatched blastula stage as embryos produced by fertilizations at 17 ºC had significantly more hatching success than embryos from fertilizations at either of the other temperature conditions. Alternatively, these carry-over effects started to diminish at the gastrula stage, where developmental temperature began to have a heavier influence on developmental success. Under the present-day MHW scenario, gastrula raised under ambient temperature conditions still possessed higher developmental success when produced by 17 ºC fertilizations as opposed to 20 ºC fertilizations, but the proportion of viable gastrula were virtually identical between embryos from either fertilization treatment when raised under the MHW-like temperature. For both hatched blastula and gastrula stages, significantly more abnormality was present in embryos from 20 ºC and 24 ºC fertilizations as compared to those produced from 17 ºC fertilizations.

The hatching and gastrulation of sea urchin embryos are both developmentally relevant and costly processes that can serve as important indicators of later survival (Byrne 2011). These early developmental stages have been considered developmental bottlenecks under climate change scenarios in terms of their decreased tolerance to environmental stress compared to later stages. Our results suggest that vulnerability at these stages may be additionally influenced by the environmental conditions experienced during fertilization. Past studies on fertilization have led to the prevailing view that fertilization and gametes are robust to the environmental stressors associated with ocean warming and acidification, but

these studies have largely ignored the latent effects that fertilization under stressful conditions might have on later development (Byrne 2011). Our work is part of a small body of research that has investigated the influence and carry-over effects of gametic and fertilization environmental conditions on development. Overall, results from this collective work have supported the presence of carry-over effects in marine invertebrate species (Parker et al. 2009; White et al. 2014; Ritchie et al. 2013; Chirgwin et al. 2021; Lymberry et al. 2021). Below, I discuss how this area of research addresses two specific, but very different mechanistic aspects of how the physical environment can drive carry-over effects in broadcast spawning species via the: (1) pre-fertilization conditions experienced by gametes, particularly sperm, or (2) conditions experienced during the fertilization event itself.

First, the influence of the gamete environment on later development has been approached primarily from the view of ejaculate-mediated paternal effects, a theory where the environment experienced by sperm cells, independent of paternal environmental history, serves as an important mediator of offspring success (Evans et al. 2019). Support for such a mechanism has been observed in mussels (Lymberry et al. 2021), tubeworms (Ritchie et al. 2013), and fish species (Kekäläinen et al. 2018), where exposure of mussel and fish sperm to elevated temperatures elicited a negative response on offspring survival, development, and physiological performance (but see Ritchie et al. 2013). Epigenetic marks transmitted via the male gamete as well as selection for genetically different sperm cells within an ejaculate have been suggested as two potential (but non-mutually exclusive) mechanisms that could mediate such outcomes when sperm experience environmental stress directly (Chirgwin et al. 2019). In our study, sperm and eggs were both exposed to elevated temperatures before

fertilization, but there was no way to separate the influences of gamete and fertilization environments. The reduction in fertilization success we observed at elevated temperatures, though, could be in line with a scenario in which the stressful thermal environment during fertilization imposes selection for specific sperm phenotypes within the pooled sperm of *L. pictus*. Future investigations of sperm environment alone could thus prove interesting, although it is very unlikely that sperm and egg are experiencing drastically different temperatures during spawning (Levitan 2000).

Second, as opposed to a focus on the immediate abiotic environment of the gametes, the role of fertilization environment alone has also been explored in a few marine invertebrate species (Parker et al. 2009; White et al. 2014; Chirgwin et al. 2021), with our study representing the first to do so in an echinoderm species. When fertilization in the scallop *Argopecten irradians* and the oyster *Saccostrea glomerata* occurred under varying pH or pH x temperature regimes, larval survival and developmental success were negatively influenced by fertilization environment, with significant mortality and abnormality seen in larvae from low pH and high temperature fertilizations regardless of rearing conditions (Parker et al. 2009; White et al. 2014). Significant abnormality as a product of fertilization environment also drove the observed negative patterns in hatching and gastrulation success in *L. pictus* embryos here. In the tubeworm, *G. caespitosa*, similar reductions in offspring survival following fertilization at elevated temperatures correlated with a decrease in additive genetic variation for survival at the larval stage (Chirgwin et al. 2021). While theory predicts such reductions in variation, or plasticity, during fertilization would likely be coupled with increases in offspring survival (a more adaptive scenario), less well-studied carry-over effects from fertilization related to epigenetics or DNA damage from gametes

may instead underlie the maladaptive effects seen across studies thus far (Lewis and Aitken 2005).

Results on hatching and gastrulation success in this experiment demonstrate that both present-day and future MHW events can have detrimental effects on the persistence of *L. pictus* embryos. Across all fertilization temperatures, embryos demonstrated reduced developmental success when reared under 20 ºC and 24 ºC as compared to 17 ºC. Within ~24 hours, we observed that only 60% and 50% of embryos had successfully developed into gastrula under present-day and future MHW events, respectively. This aligns with literature on the thermal tolerance of early developmental stages in various marine taxa including corals, mollusks, and echinoderms (Byrne et al. 2009; Randall & Szmant 2009). Due to the relevance and energetic costs of gastrulation (formation of the archenteron that becomes the gut in larvae and adults), this stage has long represented a potential bottleneck for marine invertebrate taxa under climate change scenarios (Byrne 2011). In our experiment, fertilization environment did not appear to provide any relief from the detrimental effects of thermal stress during development at this stage. As such, these early developmental stages will be of particular relevance for future assessments of how *L. pictus* will respond to future ocean warming or MHW events.

Developmental, but not fertilization, temperature affects embryonic size

In this study, developmental temperature appeared to be the major determinant of embryonic size at both hatching and gastrulation. Interestingly, the trends in how offspring size changed in response to a +3-4 ºC temperature increase, particularly at the gastrula stage, differed between MHW scenarios. Under the present-day MHW scenario (17 ºC vs. 20 ºC),

L. pictus gastrula were 12% smaller when raised at 20 ºC as opposed to 17 ºC. A 4 ºC increase in the future MHW scenario, on the other hand, led to a ~5% increase in gastrula size. Typically, moderate increases in temperature result in larger offspring in marine invertebrate taxa while more extreme temperature challenges have a more negative influence (Byrne 2011). Deviations from this trend in our study could possibly be explained by the large reductions in viable gastrula present under either MHW temperature. At these elevated temperatures, only ~50% of embryos successfully reached the gastrula stage, so it could be that larger or smaller individuals were overrepresented in this pool of gastrula than under the current ambient thermal treatment. In line with this speculation, the areas within gastrula reared at 24 ºC had less variability than those reared under either 20 ºC or 17 ºC.

At the blastula stage, the 24 ºC rearing treatment led to smaller embryos at hatching. This is in line with accounts of hatching plasticity observed across echinoderm taxa, with environmental conditions being a large factor in how early or late hatching occurs. In the sand dollar, *Echinarachnius parma*, for instance, low salinity caused delays in hatching until after the larval pluteus stage was reached, a nearly 12-hour delay compared to hatching in ambient conditions (Armstrong et al. 2013). In my study, the smaller size of blastula at hatching coupled with visual observations of the embryos under the microscope suggested the embryos from the 24 ºC treatment were hatching at a less-developed stage of blastula compared to the other two temperatures. Early hatching may provide positive benefits to offspring in terms of having the ability to swim vertically away from benthic predators but could also more readily expose embryos to the more elevated pelagic temperatures as opposed to temperatures experienced in benthic environment (Strathmann 2007).

Fertilization temperature did not have an influence on embryonic size at either developmental stage. In the scallop study mentioned above, fertilization environment similarly did not elicit any effect on offspring size, with 1-, 3-, and 7-day old veliger larvae growing to the same length regardless of the pH at fertilization (White et al. 2014). Alternatively, Parker et al. 2009 observed a significant size decrease in 1- and 2-day old veligers produced from fertilizations occurring under low pH and elevated temperature conditions (Parker et al. 2009). For sea urchins, offspring size is typically closely correlated with egg size and maternal provisioning (Moran & McAlister 2009). There is currently no evidence to suggest that maternal provisioning in echinoderms is altered by the exposure of eggs to the thermal increases studied here, so I expect fertilization temperature alone to be of little consequence to offspring size.

Comments on L. pictus as a study organism

Overall, the results of this study indicate that the early developmental stages of *Lytechinus pictus* are susceptible to the temperatures associated with present-day and future summer MHW events in the SBC. Additive reductions in fertilization and developmental success under present-day and future MHW-like temperatures resulted in 25% and 37% less embryos, respectively, successfully making it to the gastrula stage as compared to embryos that were fertilized and raised at present-day non-MHW conditions (17 ^{o}C). As a member of a genus containing mostly tropical species, *L. pictus* has one of the more northern geographic ranges found in *Lytechinus spp.*, extending from the Sea of Cortez to Monterey Bay (Zigler & Lessios 2004). Notably, the local Santa Barbara population used in this study is living more closely to its northern (or cooler) range edge. The early developmental stages

of *L. pictus* in this area, as expected, have lower thermal tolerances than other tropical congenerics and even conspecifics living closer to the species' southern range edge (Lenz et al. 2016; Nesbit et al. 2019). In San Diego populations of *L. pictus*, offspring are raised at 20-22 ^{o}C with little detriment to their development (Nesbit et al. 2019). The embryos of the related tropical species, *L. variegatus*, are found at 28 ^{o}C *in situ* and able to withstand temperature challenges of up to 32 ^{o}C (Lenz et al. 2016; Collin & Chan 2016).

Even with populations of *L. pictus* found subtidally, at depths ranging from 2-300m, SBC populations still maintain similar, if not elevated thermal tolerances compared to other temperate sea urchin species found in shallower habitats (Chapter III; Hammond et al. 2010; O'Donnell et al. Wong et al. 2021). To this point, other temperate species found in the area, like *Mesocentrotus fransicanus* and *S. purpuratus*, have a reproductive cycle where spawning is at its highest from late Fall-early spring (Strathmann 1987). *L. pictus,* on the other hand, has a spawning season from June to September. With the overlap of spawning and the highest temperature conditions in the SBC, it is more likely that *L. pictus* embryos will experience elevated temperatures. In regard to MHW events, the seasonality of which also aligns with these summer and early fall months, our results highlight that the embryos of *L. pictus* might be a species that is pushed past its physiological limits in light of the predicted increased frequency and intensity of MHW events in future oceans.

Given the performance of *L. pictus* under an increasingly important climate change stressor, our results add to a growing body of evidence advocating for the use of the painted sea urchin as study system (Nesbit et al. 2019). Ecologically, *L. pictus* have demonstrated feeding preferences for the early developmental stages of the kelp *Macrocystis pyrifera,* with a potential role in kelp recruitment *in situ* (Sala and Graham 2002). Such community

effects have even been speculated to extend to other sea urchin species, with *L. pictus* demonstrating mobbing/defensive behavior when in contact with other local species, *S. purpuratus* and *Mesocentrotus franscicanus* (Coyer et al. 1987). More commonly, *L. pictus* has served a role as a developmental model species, with the studies using these urchins greatly aiding in our understanding of fertilization and early development (Nesbit et al. 2019). Of recent interest is the capability of raising transgenic lines of *L. pictus,* which have a rapid generational time (4-8 months) compared to other sea urchin species like *S. purpuratus*. This aspect of *L. pictus* biology also is relevant to climate change biology research, as the influence of transgenerational effects in marine organisms is an emerging area of interest (Ross et al. 2016; Donelson et al. 2018). The small size, fast generation time, availability of genomic resources, and ease of husbandry make *L. pictus* an ecologically relevant study system that shares a lot of the qualities of the most common and successful model organisms (e.g., *Drosophila*, *C. elegans*) in biological research. For just the questions outlined in other chapters of this thesis (i.e., paternal effects) alone, use of a species like *L. pictus* instead of *S. purpuratus* could allow for the extent of such parental effects to be more easily explored throughout the echinoderm life cycle (later development, metamorphosis, juveniles) and even into the F_2 or F_3 generations.

Acknowledgements

This research was supported by an NSF award IOS-1656262 to Gretchen E. Hofmann. This work was also supported by resources from Santa Barbara Coastal Long Term Ecological Research program (NSF award OCE-1831937; Director: Dr. Robert Miller), an award for which Gretchen Hofmann is a co-PI. Our appreciation goes out to Christoph

Pierre for collection of sea urchins. A huge thank you to Adriane McDonald and Erin de Leon Sanchez for their help with running fertilization trials and helping prepare the developmental rearing vials. We also would like to thank Dr. Kathy Foltz and Dr. Debora Iglesias-Rodriguez for the feedback provided during the writing of this manuscript.

Chapter VI. Conclusion

Global climate change is an increasingly relevant threat to coastal marine ecosystems. Predictions about how marine populations will respond to the stressors associated with these changing climatic conditions require assessments of an organism's adaptive capacity (Ofori et al. 2017; Thurman et al. 2020). One particularly relevant component of adaptive capacity for marine invertebrate species focuses on the species' reproduction and life history. Given the complex life cycles of many marine invertebrates, there has been a large-scale effort to assess the tolerance of individual stages to various environmental stressors in hopes of finding if/where a "weak link" exists (Byrne 2011; Pandori and Sorte 2019). While the early developmental stages of many species have been identified as the more susceptible parts of the life cycle, there are still significant knowledge gaps about what mechanisms influence the tolerance of these stages.

My exploration of this larger area of research addressed the role of intergenerational, or parental effects on their offspring's performance under climate change stressors. And further, I was specifically interested in paternal effects, once synonymous with additive genetic effects. In comparison to maternal effects, paternal effects have traditionally been understudied in marine invertebrate taxa, but there is evidence to suggest that paternal effects may play a crucial role in shaping an organism's adaptive capacity (Crean and Bonduriansky 2014; Marshall 2015). In this thesis, I sought to explore how the paternal environment influenced offspring development and physiological performance using two sea urchin species that are found in the Santa Barbara Channel (SBC), the purple sea urchin, *Strongylocentrotus purpuratus*, and the painted sea urchin, *Lytechinus pictus*.

This research was conducted in the context of marine heatwave (MHW) events experienced in the SBC, with such events having significant biological consequences currently and projected to become more frequent and intense in the future (Frolicher et al. 2018; Smale et al. 2019). I viewed the anomalously high temperatures of MHWs as an abiotic factor that could influence several steps in the early events of fertilization biology and early development in broadcast spawning sea urchins. Thus, the main research question was: **What role do paternal effects play in species' adaptive capacity?** For the research in my dissertation, I developed three (non-mutually exclusive) hypotheses focused on different elements of sea urchin life history and designed and conducted experiments aimed at addressing my larger research question. These hypotheses are as follows:

H1: Gamete plasticity demonstrated under thermal stress alters fertilization success

H2: Latent temperature effects on fertilization manifest during later development

H3: Paternal thermal history influences offspring performance

The above hypotheses were addressed over three chapters within this thesis. From this general body of research, four main takeaways are highlighted and discussed below.

1. Methodology can significantly influence the interpretation of results

There has been an abundance of research on marine invertebrate reproduction and development over the past decades, particularly in the context of environmental stress (Byrne 2011; Pandori and Sorte 2019). One difficulty in evaluating this research body as a whole, though, stems from the variability between methodologies used to assess conserved

aspects of reproduction and development between taxa. Such methodological variation adds to the already existing biological variation one would expect between species and even different populations of the same species. In this thesis, I took advantage of the wide array of research on marine invertebrate taxa to re-assess common methodologies used on two specific elements of development, fertilization and offspring thermal tolerance.

Fertilization has long been acknowledged as a crucial part of the life cycle, but experimental assessments of fertilization success under stressful conditions vary significantly between and within study systems (Byrne et al. 2010a; Eads et al. 2016a; Chirgwin et al. 2020). Some of this variation has been attributed to methodological differences in fertilization success trials, including crossing design, sperm-egg contact time, gamete concentrations, and the stage at which fertilization success is scored (Lera et al. 2006; Byrne 2011). In this thesis alone, methodological differences used to obtain the results presented in Chapters III and V may have contributed to the distinctive results, and thus interpretations, about how the fertilization process is influenced by temperature stress. Fertilization in *L. pictus* appeared more sensitive to temperature stress (20 ^{o}C) than that in *S. purpuratus*, but these differences could be driven by the use of pooled sperm or the 5-minute pre-trial exposure that *L. pictus* gametes received that *S. purpuratus* gametes did not.

While methodological variation is a necessary product of studies addressing different research questions, I argue that future studies focused on fertilization success should aim to quantify some other element of gamete performance to contextualize results and thus make comparisons easier between studies. In Chapter II, I discussed the utility of separately scoring the proportion of fertilization events and normality at cleavage in providing more context to which part of the fertilization process is being affected by experimental

conditions. In both Chapter III and V, the number of fertilization events were significantly altered by paternal and fertilization thermal environment, respectively. In response, I argue that future studies should focus on quantifying gamete, particularly sperm, physiology that influences egg-sperm collisions, such as sperm swimming speed or linearity, gamete energetics, or the percentage of motile sperm in an ejaculate. When it is not possible to use more mechanistic focused assays, this development of more stringent scoring procedures may prove useful in exploratory work on fertilization biology under environmental stress.

In Chapter IV, I identified that interpretations about thermal tolerance in larval sea urchins could differ drastically depending on how heat-induced mortality was scored. When using the commonly employed scoring metric, cilia movement, thermal tolerance of pluteus larvae was estimated to be 1-2 ^{o}C higher than when larvae were scored for significant abnormality (Hammond and Hofmann 2010; Wong et al. 2020). The implications of such a difference could be that the susceptibility of urchin larvae to temperature stress is currently overestimated, with these vulnerable stages potentially living closer to their physiological limits than previously thought. Differences in methodology here may reflect efforts to save time while also preventing the induction of subjectivity into the scoring process. Determining whether the cilia are moving or not is considerably more straightforward to score as opposed to quantifying the degree of abnormality that represents "functional death" in larvae. To this point, preparation for Chapter IV required all scorers to practice across multiple preliminary runs to ensure scoring was consistent, and even then, measures of abnormality-based mortality were kept hyper-conservative. The identification of this discrepancy between thermal tolerance based upon abnormality and cilia movement provides the means to search for other techniques that may aid in the degree of abnormality

seen. At the very least, trials should be scored blindly, but dyes that indicate cell damage or death coupled with image analysis software could prove useful in future analyses to further limit subjectivity.

2. Paternal effects can be a focal part of an organism's ability to withstand environmental stress

For the past decades, the importance of parental effects on offspring performance and survival has been a default interpretation based upon studies focused on maternal investment (Mousseau and Fox 1998). On the other hand, paternal effects were deemed as representations of additive genetic variation, meaning that the influence of paternal environmental history remains relatively unexplored. With the growing body of evidence for non-genetic inheritance mechanisms, such as epigenetic markers, has been a re-assessment of how parental, especially paternal, environment may be reflected in the subsequent generation (Marshall 2015; Eirin-Lopez and Putnam 2019). Observations of these non-genetic inheritance mechanisms might be particularly relevant in broadcast spawning species, as their gametes are released directly into the environment and there is little to no parental care that could compromise interpretation of results. In the purple sea urchin, *S. purpuratus*, maternal environment was a relevant factor influencing offspring size, with the temperature and pH experienced by dams also influencing biochemical investment in eggs and DNA methylation patterns in offspring (Wong et al. 2019; Strader et al. 2019). In this thesis, I explored the influence of paternal thermal environment on *S. purpuratus* development, with both positive and negative impacts seen depending on what developmental process was observed.

In Chapter III, which addressed H1, I observed that paternal exposure to a MHW-like temperature had a negative impact on fertilization success as compared to the non-MHW treatment. Fertilization success was measured based off the number of fertilization events observed, representing how well the sperm across paternal treatments were able to come in contact with and fertilize eggs in a discrete period of time. Thermal stress during spermatogenesis has been observed to limit sperm production in species (Uthicke et al. 2014), but our results add to a smaller body of evidence highlighting that the environmental history of the sires can still be reflected in the quality and performance of their gametes post-spawning (Lewis and Galloway 2009; Guillaume et al. 2016). Further interpretations from this study were limited by the lack of metrics assessing sperm phenotypes directly. As such, it would be beneficial if future research would assess some aspect of the general quality of each sire's ejaculate (concentration, % motility and abnormality) as well as the performance of individual sperm cells (morphology, energetics, swimming speed). Lastly, we observed that paternal temperature, as opposed to fertilization temperature, was the more dominant factor influencing fertilization success (but see Chapter V). This result is of particular significance when thinking about the variability between fertilization success studies, as paternal environment may be a crucial element that needs to be accounted for (Byrne 2011). For example, the coupling of environmental data from collection areas with fertilization success results may better allow for an estimation of parental environment between studies using similar taxa.

Results from Chapter IV, derived from experiments designed to address H3, highlighted a potential adaptive paternal effect on *S. purpuratus* larvae under a MHW scenario. Using the same acclimated male urchins from Chapter III's experiment, pluteus larvae raised under

the same thermal conditions as their father possessed higher thermal tolerances to an acute heat stress event than larvae who experienced a novel thermal environment as compared to their sires. This effect was driven largely by the performance of offspring from sires acclimated to the MHW-like temperature (20 ºC). There, the highest and lowest LT_{50} values across all treatments in the experiment were seen in pluteus from MHW-acclimated sires that underwent development at 20 ºC and 14 ºC, respectively. This trend is supported by the theory of anticipatory parental effects, where positive or negative benefits are conferred to offspring depending on whether the parent can accurately predict what environmental conditions their offspring will encounter (Marshall and Uller 2007). While this is one of few experiments to show such an effect in marine invertebrates, it would be of interest to determine whether these anticipatory benefits to offspring remain if sires were acclimated to experimental conditions that more accurately reflect the natural variability of marine habitats. Furthermore, investigations into the mechanisms behind this intergenerational plasticity is a major area of need in paternal effect research. Coupling phenotypic data with molecular assays (transcriptomics, qPCR, protein assays, DNA methylomics) would greatly improve our understanding of how paternal effects may operate under climate change scenarios.

3. Parental effects potentially mediated by fertilization environment

While fertilization and gametes have been thought of as fairly robust to environmental stressors such as temperature, pH, and salinity, many studies exploring the effect of such stressors on fertilization do not incorporate potential carry-over effects on post-fertilization development (Byrne and Przeslawski 2013). The experiment outlined in Chapter V,

designed to address H2, investigated the effects of fertilization temperature on later developmental success in the painted sea urchin, *Lytechinus pictus*. Here, fertilization was conducted under three different temperatures representing present-day ambient, present-day MHW-like, and future MHW-like conditions in the SBC. When the embryos derived from these fertilizations were raised at various temperatures +/-3 ºC from that at fertilization, we observed negative carry-over effects from fertilizations occurring at either elevated temperature (20 ºC and 24 ºC). Developmental success during hatching and gastrulation was significantly lower in embryos produced from either 20 ºC or 24 ºC fertilizations as opposed to those from 17 ºC fertilizations. These patterns in developmental success were largely driven by increased abnormality in embryos produced from fertilizations at elevated temperatures. While fertilization-mediated differences in abnormality and developmental success patterns between fertilization temperature treatments started to diminish at the gastrula stage, similar negative effects of fertilization environment have been observed at later larval stages in other marine taxa (Parker et al. 2010; White et al. 2014).

Ecologically, such effects have significant consequences for broadcast spawners whose gametes, and thus fertilization, are directly exposed to dynamic environmental conditions. Spawning and early development for many marine invertebrates represents a transition between the benthic (adult) and pelagic (larval) portions of the life cycle, therefore it is not unreasonable to assume that environmental conditions may vary significantly across this short window of time. Therefore, it is of interest to continue studying such carry-over effects during early development in the context of climate change, especially for species that use environmental cues to induce spawning. At the very least, climate change studies focused on reproduction and development in marine invertebrates may be careful to monitor and control

the fertilization environment during experiments, as this may be a contributing source of variability between studies.

4. MHW events in the SBC have the potential to dramatically alter the persistence of marine invertebrate populations via their reproductive and developmental physiology

Overall, the work presented in this thesis supports the idea that MHW events will be particularly relevant climate change stressors in terms of regulating the persistence of marine populations. Past MHW events have significantly altered marine ecosystems through altering biogeographic distributions (Sanford et al. 2019), triggering massive mortality events (Wernberg et al. 2016; Harvell et al. 2019), and impacting the health of ecosystem engineers (Rogers-Bennett and Catton 2019). These dramatic effects have motivated significant research efforts aimed at predicting the responses of different marine taxa to the anomalous temperatures experienced during futures MHW events (Minuti et al. 2021). As MHW events are only expected to become more common, longer, and intense in future oceans, continued shifts toward research exploring their biological consequences in the field of climate change biology are expected (Frolicher et al. 2018; Smale et al. 2019). On top of the lethal effects observed in response to elevated temperatures, it is important to explore the sublethal effects induced by MHW events as they might have similar fitness consequences (i.e., reduced reproduction and recruitment of future populations).

In this thesis, I investigated how the maximum temperatures observed during past MHW events in the SBC would influence the reproduction and development of two ecologically relevant sea urchin species. Here, experimental results identified specific developmental areas of interest, with fertilization being particularly sensitive (in regard to pre- and post-

fertilization thermal conditions; Chapters III and V) but positive effects seen in later, larval stages (Chapter IV). For *S. purpuratus*, in particular, a MHW that occurs during the time of spermatogenesis, spawning, and early development could have complex consequences, where reproductive output (via successful fertilization events) may be reduced but larvae who are successful produced possessing bigger body sizes and increased thermal tolerance. Alternatively, a mismatch between thermal environments experienced across generations (adult vs. fertilization and post-fertilization environments) may be particularly detrimental. Future laboratory studies incorporating thermal variation observed *in situ* during a MHW event into experimental acclimations could provide further insight into how these scenarios might play out in nature. Additionally, experiments in this thesis focused on post-spawning reproductive success, but no data were collected on the adult's physiological experience during acclimation. Energetic trade-offs between maintaining adult physiology and reproduction under extreme events may be a particularly relevant area of future research. If subsets of adult individuals in a population are unable to reproduce at all under stressful environments, extreme weather events may have additional ecological and evolutionary consequences for ecosystems.

References

Albright R, Mason B (2013) Projected near-future levels of temperature and pCO(2) reduce coral fertilization success. Plos One 8 https://doi.org/10.1371/journal.pone.0056468

Allen, R. M., Y. M. Buckley, and D. J. Marshall. 2008. Offspring size plasticity in response to intraspecific competition: An adaptive maternal effect across life-history stages. American Naturalist **171**:225-237.

Armstrong EJ, Dubousquet V, Mills SC, Stillman JH (2019) Elevated temperature, but not acidification, reduces fertilization success in the small giant clam, Tridacna maxima. Marine Biology 167: 8 doi https://doi.org/10.1007/s00227-019-3615-0

Bates D, Machler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. J Stat Softw 67: 1–48 doi DOI 10.18637/jss.v067.i01

Bay RA, Harrigan RJ, Le Underwood V, Gibbs HL, Smith TB, Ruegg K (2018) Genomic signals of selection predict climate-driven population declines in a migratory bird. Science 359: 83-+ doi https://doi.org/10.1126/science.aan4380

Binet MT, Doyle CJ (2013) Effect of near-future seawater temperature rises on sea urchin sperm longevity. Mar Freshwater Res 64: 1–9 doi https://doi.org/10.1071/Mf12121

Bonduriansky, R., and T. Day. 2009. Nongenetic Inheritance and Its Evolutionary Implications. Annual Review of Ecology Evolution and Systematics **40**:103-125.

Boni R, Gallo A, Montanino M, Macina A, Tosti E (2016) Dynamic changes in the sperm quality of Mytilus galloprovincialis under continuous thermal stress. Mol Reprod Dev 83: 162–173 doi https://doi.org/10.1002/mrd.22604

Boyd, P. W., C. E. Cornwall, A. Davison, S. C. Doney, M. Fourquez, C. L. Hurd, I. D. Lima, and A. McMinn. 2016. Biological responses to environmental heterogeneity under future ocean conditions. Global Change Biology **22**:2633-2650.

Branscomb ES, Vedder K, Strathmann RR (2014) Risks for larvae mediated by plasticity in hatching. Invertebrate Biology. doi: 10.1111/ivb.12051

Byers, J. E., and J. M. Pringle. 2006. Going against the flow: retention, range limits and invasions in advective environments. Marine Ecology Progress Series **313**:27-41.

Byrne M (2011) Impact of ocean warming and ocean acidification on marine invertebrate life history stages: Vulnerabilities and potential for persistence in a changing ocean. In: Oceanography and Marine Biology: An Annual Review.

Byrne M, Hernandez JC (2020) Sea urchins in a high CO2 world: Impacts of climate warming and ocean acidification across life history stages. Dev Aquac Fish Sci 43: 281-297 doi 10.1016/B978-0-12-819570-3.00016-0

Byrne M, Ho M, Selvakumaraswamy P, Nguyen HD, Dworjanyn SA, Davis AR (2009) Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. P Roy Soc B-Biol Sci 276: 1883–1888 doi https://doi.org/10.1098/rspb.2008.1935

Byrne M, Ho M, Wong E, Soars NA, Selvakumaraswamy P, Shepard-Brennand H, Dworjanyn SA, Davis AR (2011) Unshelled abalone and corrupted urchins: Development of marine calcifiers in a Changing Ocean. Proceedings of the Royal Society B: Biological Sciences. doi: 10.1098/rspb.2010.2404

Byrne M, Przeslawski R (2013) Multistressor impacts of warming and acidification of the ocean on marine invertebrates' life histories. Integrative and Comparative Biology. doi: 10.1093/icb/ict049

Byrne M, Soars N, Selvakumaraswamy P, Dworjanyn SA, Davis AR (2010a) Sea urchin fertilization in a warm, acidified and high pCO(2) ocean across a range of sperm densities. Mar Environ Res 69: 234–239 doi https://doi.org/10.1016/j.marenvres.2009.10.014

Byrne M, Soars NA, Ho MA, Wong E, McElroy D, Selvakumaraswamy P, Dworjanyn SA, Davis AR (2010b) Fertilization in a suite of coastal marine invertebrates from SE Australia is robust to near-future ocean warming and acidification. Marine Biology 157: 2061–2069 doi https://doi.org/10.1007/s00227-010-1474-9

Byrne, M. 2010. Impact of climate change stressors on marine invertebrate life histories with a focus on the Mollusca and Echinodermata. Climate Alert: Climate Change Monitoring and Strategy:169-212.

Byrne, M. 2013. Impacts of warming and ocean acidification on growth of larval and juvenile sea urchins - from the poles to the tropics. Integrative and Comparative Biology **53**:E27-E27.

Byrne, M., M. Ho, P. Selvakumaraswamy, H. D. Nguyen, S. A. Dworjanyn, and A. R. Davis. 2009. Temperature, but not pH, compromises sea urchin fertilization and early development under near-future climate change scenarios. Proceedings of the Royal Society B-Biological Sciences **276**:1883–1888.

Byrne, M., M. Lamare, D. Winter, S. A. Dworjanyn, and S. Uthicke. 2013. The stunting effect of a high CO2 ocean on calcification and development in sea urchin larvae, a

synthesis from the tropics to the poles. Philosophical Transactions of the Royal Society B-Biological Sciences **368**.

Byrne, M., T. A. A. Prowse, M. A. Sewell, S. Dworjanyn, J. E. Williamson, and D. Vaitilingon. 2008. Maternal provisioning for larvae and larval provisioning for juveniles in the toxopneustid sea urchin Tripneustes gratilla. Marine Biology **155**:473-482.

Campbell AL, Levitan DR, Hosken DJ, Lewis C (2016) Ocean acidification changes the male fitness landscape. Sci Rep-Uk 6 doi https://doi.org/10.1038/srep31250

Cavanaugh, K. C., D. C. Reed, T. W. Bell, M. N. Castorani, and R. Beas-Luna. 2019. Spatial Variability in the Resistance and Resilience of Giant Kelp in Southern and Baja California to a Multiyear Heatwave. Frontiers in Marine Science **6**.

Chan F, Barth JA, Blanchette CA, Byrne RH, Chavez F, Cheriton O, Feely RA, Friederich G, Gaylord B, Gouhier T, Hacker S, Hill T, Hofmann G, McManus MA, Menge BA, Nielsen KJ, Russell A, Sanford E, Sevadjian J, Washburn L (2017) Persistent spatial structuring of coastal ocean acidification in the California Current System. Sci Rep-Uk 7 doi https://doi.org/10.1038/s41598-017-02777-y

Chan, K. Y. K., D. Grunbaum, and M. J. O'Donnell. 2011. Effects of ocean-acidification-induced morphological changes on larval swimming and feeding. Journal of Experimental Biology **214**:3857-3867.

Chang, S. L., W. S. Lee, and S. B. Munch. 2021. Separating Paternal and Maternal Contributions to Thermal Transgenerational Plasticity. Frontiers in Marine Science **8**.

Chirgwin E, Connallon T, Monro K (2021) The thermal environment at fertilization mediates adaptive potential in the sea. Evolution Letters 5:

Chirgwin E, Marshall DJ, Monro K (2020) Physical and physiological impacts of ocean warming alter phenotypic selection on sperm morphology. Funct Ecol 34: 646–657 doi https://doi.org/10.1111/1365-2435.13483

Christensen, A. B., H. D. Nguyen, and M. Byrne. 2011. Thermotolerance and the effects of hypercapnia on the metabolic rate of the ophiuroid Ophionereis schayeri: Inferences for survivorship in a changing ocean. Journal of Experimental Marine Biology and Ecology **403**:31-38.

Cochran RC, Engelmann F (1975) Environmental regulation of annual reproductive season of Strongylocentrotus-Purpuratus (Stimpson). Biol Bull 148: 393–401 doi https://doi.org/10.2307/1540516

Collin R, Chan KYK (2016) The sea urchin Lytechinus variegatus lives close to the upper thermal limit for early development in a tropical lagoon. Ecology and Evolution. doi: 10.1002/ece3.2317

Consortium, S. U. G. S. 2006. Research article - The genome of the sea urchin Strongylocentrotus purpuratus. Science **314**:941-952.

Coyer JA, Engle JM, Ambrose RF, Nelson B v. (1987) Utilization of purple and red sea urchins (Strongylocentrotus purpuratus Stimpson and S. franciscanus Agassiz) as food by the white sea urchin (Lytechinus anamesus Clark) in the field and laboratory. Journal of Experimental Marine Biology and Ecology. doi: 10.1016/S0022-0981(87)80027-8

Crean AJ, Bonduriansky R (2014) What is a paternal effect? Trends Ecol Evol 29: 554–559 doi https://doi.org/10.1016/j.tree.2014.07.009

Crean AJ, Dwyer JM, Marshall DJ (2013) Adaptive paternal effects? Experimental evidence that the paternal environment affects offspring performance. Ecology 94: 2575-2582 doi 10.1890/13-0184.1

Crean, A. J., and D. J. Marshall. 2008. Gamete plasticity in a broadcast spawning marine invertebrate. Proceedings of the National Academy of Sciences of the United States of America **105**:13508-13513.

Dayton PK, Tegner MJ, Parnell PE, Edwards PB (1992) Temporal and Spatial Patterns of Disturbance and Recovery in a Kelp Forest Community. Ecol Monogr 62: 421–445 doi https://doi.org/10.2307/2937118

Dayton, P. K., M. J. Tegner, P. B. Edwards, and K. L. Riser. 1998. Sliding baselines, ghosts, and reduced expectations in kelp forest communities. Ecological Applications **8**:309-322.

de Vries, P., J. E. Tamis, A. J. Murk, and M. G. D. Smit. 2008. Development and Application of a Species Sensitivity Distribution for Temperature-Induced Mortality in the Aquatic Environment. Environmental Toxicology and Chemistry **27**:2591-2598.

Dean TA, Schroeter SC, Dixon JD (1984) Effects of grazing by two species of sea urchins (Strongylocentrotus franciscanus and Lytechinus anamesus) on recruitment and survival of two species of kelp (Macrocystis pyrifera and Pterygophora californica). Marine Biology. doi: 10.1007/BF00393016

Delorme NJ, Sewell MA (2014) Temperature and salinity: two climate change stressors affecting early development of the New Zealand sea urchin Evechinus chloroticus. Marine Biology 161: 1999–2009 doi https://doi.org/10.1007/s00227-014-2480-0

Dickson, A. G., C. Sabine, and J. Christian. 2007. Determination of the pH of seawater using the indicator dye m----cresol purple.

Donelson JM, Salinas S, Munday PL, Shama LNS (2018) Transgenerational plasticity and climate change experiments: Where do we go from here? Global Change Biology 24:

Duxbury, A. E., B. Weathersby, Z. Sanchez, and P. J. Moore. 2018. A study of the transit amplification divisions during spermatogenesis in Oncopetus fasciatus to assess plasticity in sperm numbers or sperm viability under different diets. Ecology and Evolution **8**:10460-10469.

Eads AR, Evans JP, Kennington WJ (2016a) Plasticity of fertilization rates under varying temperature in the broadcast spawning mussel, Mytilus galloprovincialis. Ecol Evol 6: 6578–6585 doi https://doi.org/10.1002/ece3.2375

Eads AR, Kennington WJ, Evans JP (2016b) Interactive effects of ocean warming and acidification on sperm motility and fertilization in the mussel Mytilus galloprovincialis. Mar Ecol Prog Ser 562: 101–111 doi https://doi.org/10.3354/meps11944

Ebert TA (2010) Demographic patterns of the purple sea urchin Strongylocentrotus purpuratus along a latitudinal gradient, 1985-1987. Mar Ecol Prog Ser 406: 105-120 doi 10.3354/meps08547

Eirin-Lopez, J. M., and H. M. Putnam. 2019. Marine Environmental Epigenetics. Annual Review of Marine Science, Vol 11 **11**:335-+.

Enricuso OB, Conaco C, Sayco SLG, Neo ML, Cabaitan PC (2019) Elevated seawater temperatures affect embryonic and larval development in the giant clam Tridacna gigas (Cardiidae: Tridacninae). J Mollus Stud 85: 66–72 doi https://doi.org/10.1093/mollus/eyy051

Ericson JA, Ho MA, Miskelly A, King CK, Virtue P, Tilbrook B, Byrne M (2012) Combined effects of two ocean change stressors, warming and acidification, on fertilization and early development of the Antarctic echinoid Sterechinus neumayeri. Polar Biol 35: 1027–1034 doi https://doi.org/10.1007/s00300-011-1150-7

Evans JP, Marshall DJ (2005) Male-by-female interactions influence fertilization success and mediate the benefits of polyandry in the sea urchin Heliocidaris erythrogramma. Evolution 59: 106–112

Evans JP, Wilson AJ, Pilastro A, Garcia-Gonzalez F (2019) Ejaculate-mediated paternal effects: Evidence, mechanisms and evolutionary implications. Reproduction 153:

Evans, T. G., J. L. Padilla-Gamino, M. W. Kelly, M. H. Pespeni, F. Chan, B. A. Menge, B. Gaylord, T. M. Hill, A. D. Russell, S. R. Palumbi, E. Sanford, and G. E. Hofmann. 2015. Ocean acidification research in the 'post-genomic' era: Roadmaps from the purple sea urchin Strongylocentrotus purpuratus. Comp Biochem Physiol A Mol Integr Physiol **185**:33-42.

Fangue, N. A., M. J. O'Donnell, M. A. Sewell, P. G. Matson, A. C. MacPherson, and G. E. Hofmann. 2010. A laboratory-based, experimental system for the study of ocean acidification effects on marine invertebrate larvae. Limnology and Oceanography-Methods **8**:441-452.

Farley GS, Levitan DR (2001) The role of jelly coats in sperm-egg encounters, fertilization success, and selection on egg size in broadcast spawners. Am Nat 157: 626–636 doi https://doi.org/10.1086/320619

Farmanfarmaian A, Giese AC (1963) Thermal tolerance and acclimation in the western purple sea urchin, Strongylocentrotus purpuratus. Physiological and Biochemical Zoology 36 doi https://doi.org/10.1086/physzool.36.3.30152309

Fitzpatrick, J. L., F. Garcia-Gonzalez, and J. P. Evans. 2010. Linking sperm length and velocity: the importance of intramale variation. Biology Letters **6**:797-799.

Foo SA, Dworjanyn SA, Khatkar MS, Poore AGB, Byrne M (2014) Increased temperature, but not acidification, enhances fertilization and development in a tropical urchin: potential for adaptation to a tropicalized eastern Australia. Evolutionary Applications 7: 1226–1237

Fox RJ, Donelson JM, Schunter C, Ravasi T, Gaitan-Espitia JD (2019) Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. Philos T R Soc B 374 doi https://doi.org/10.1098/rstb.2018.0174

Frances Armstrong A, Blackburn HN, Allen JD (2013) A novel report of hatching plasticity in the phylum echinodermata. American Naturalist 181:

Frieder, C. A., S. H. Nam, T. R. Martz, and L. A. Levin. 2012. High temporal and spatial variability of dissolved oxygen and pH in a nearshore California kelp forest. Biogeosciences **9**:3917-3930.

Frolicher TL, Fischer EM, Gruber N (2018) Marine heatwaves under global warming. Nature 560: 360-+ doi https://doi.org/10.1038/s41586-018-0383-9

Gaitan-Espitia, J. D., J. R. Hancock, J. L. Padilla-Gamino, E. B. Rivest, C. A. Blanchette, D. C. Reed, and G. E. Hofmann. 2014. Interactive effects of elevated temperature and pCO(2) on early-life-history stages of the giant kelp Macrocystis pyrifera. Journal of Experimental Marine Biology and Ecology **457**:51-58.

Gianguzza P, Visconti G, Gianguzza F, Vizzini S, Sara G, Dupont S (2014) Temperature modulates the response of the thermophilous sea urchin Arbacia lixula early life stages to CO2-driven acidification. Mar Environ Res 93: 70–77 doi https://doi.org/10.1016/j.marenvres.2013.07.008

Graham, M. H. 2004. Effects of local deforestation on the diversity and structure of Southern California giant kelp forest food webs. Ecosystems **7**:341-357.

Guillaume AS, Monro K, Marshall DJ (2016) Transgenerational plasticity and environmental stress: do paternal effects act as a conduit or a buffer? Funct Ecol 30: 1175-1184 doi 10.1111/1365-2435.12604

Hamdoun A, Epel D (2007) Embryo stability and vulnerability in an always changing world. P Natl Acad Sci USA 104: 1745–1750 doi https://doi.org/10.1073/pnas.0610108104

Hammond LTM, Hofmann GE (2010) Thermal tolerance of Strongylocentrotus purpuratus early life history stages: Mortality, stress-induced gene expression and biogeographic patterns. Marine Biology. doi: 10.1007/s00227-010-1528-z

Harris RMB, Beaumont LJ, Vance TR, Tozer CR, Remenyi TA, Perkins-Kirkpatrick SE, Mitchell PJ, Nicotra AB, McGregor S, Andrew NR, Letnic M, Kearney MR, Wernberg T, Hutley LB, Chambers LE, Fletcher MS, Keatley MR, Woodward CA, Williamson G, Duke NC, Bowman DMJS (2018) Biological responses to the press

and pulse of climate trends and extreme events (vol 8, pg 579, 2018). Nat Clim Change 8: 840-840 doi https://doi.org/10.1038/s41558-018-0237-3

Harvell CD, Montecino-Latorre D, Caldwell JM, Burt JM, Bosley K, Keller A, Heron SF, Salomon AK, Lee L, Pontier O, Pattengill-Semmens C, Gaydos JK (2019) Disease epidemic and a marine heat wave are associated with the continental-scale collapse of a pivotal predator (Pycnopodia helianthoides). Sci Adv 5 doi https://doi.org/10.1126/sciadv.aau7042

Harvey BP, Marshall KE, Harley CDG, Russell BD (2022) Predicting responses to marine heatwaves using functional traits. Trends in Ecology and Evolution 37:

Havenhand, J. N., F. R. Buttler, M. C. Thorndyke, and J. E. Williamson. 2008. Near-future levels of ocean acidification reduce fertilization success in a sea urchin. Current Biology **18**:R651-R652.

Hepburn, C. D., D. W. Pritchard, C. E. Cornwall, R. J. McLeod, J. Beardall, J. A. Raven, and C. L. Hurd. 2011. Diversity of carbon use strategies in a kelp forest community: implications for a high CO2 ocean. Global Change Biology **17**:2488-2497.

Ho MA, Price C, King CK, Virtue P, Byrne M (2013) Effects of ocean warming and acidification on fertilization in the Antarctic echinoid Sterechinus neumayeri across a range of sperm concentrations. Mar Environ Res 90: 136–141 doi https://doi.org/10.1016/j.marenvres.2013.07.007

Hobday AJ, Alexander LV, Perkins SE, Smale DA, Straub SC, Oliver ECJ, Benthuysen JA, Burrows MT, Donat MG, Peng M, Holbrook NJ, Moore PJ, Scannell HA, Sen Gupta A, Wernberg T (2016) A hierarchical approach to defining marine heatwaves. Prog Oceanogr 141: 227–238 doi https://doi.org/10.1016/j.pocean.2015.12.014

Hochachka, P. W. S., G. N. 2002. Biochemical adaptation: Mechanism and process in physiological evolution., Oxford University Press, New York.

Hoegh-Guldberg, O., P. J. Mumby, A. J. Hooten, R. S. Steneck, P. Greenfield, E. Gomez, C. D. Harvell, P. F. Sale, A. J. Edwards, K. Caldeira, N. Knowlton, C. M. Eakin, R. Iglesias-Prieto, N. Muthiga, R. H. Bradbury, A. Dubi, and M. E. Hatziolos. 2007. Coral reefs under rapid climate change and ocean acidification. Science **318**:1737-1742.

Hoffmann AA, Sgro CM (2011) Climate change and evolutionary adaptation. Nature 470: 479–485 doi https://doi.org/10.1038/nature09670

Hofmann, G. E., and A. E. Todgham. 2010. Living in the Now: Physiological Mechanisms to Tolerate a Rapidly Changing Environment. Annual Review of Physiology **72**:127-145.

Hofmann, G. E., J. E. Smith, K. S. Johnson, U. Send, L. A. Levin, F. Micheli, A. Paytan, N. N. Price, B. Peterson, Y. Takeshita, P. G. Matson, E. D. Crook, K. J. Kroeker, M. C. Gambi, E. B. Rivest, C. A. Frieder, P. C. Yu, and T. R. Martz. 2011. High-Frequency Dynamics of Ocean pH: A Multi-Ecosystem Comparison. Plos One **6**.

Hofmann, G. E., T. G. Evans, M. W. Kelly, J. L. Padilla-Gamino, C. A. Blanchette, L. Washburn, F. Chan, M. A. McManus, B. A. Menge, B. Gaylord, T. M. Hill, E. Sanford, M. LaVigne, J. M. Rose, L. Kapsenberg, and J. M. Dutton. 2014. Exploring local adaptation and the ocean acidification seascape studies - in the California Current Large Marine Ecosystem. Biogeosciences **11**:1053-1064.

Hogan JD, Keenan JL, Luo L, Ibn-Salem J, Lamba A, Schatzberg D, Piacentino ML, Zuch DT, Core AB, Blumberg C, Timmermann B, Grau JH, Speranza E, Andrade-Navarro

MA, Irie N, Poustka AJ, Bradham CA (2020) The developmental transcriptome for Lytechinus variegatus exhibits temporally punctuated gene expression changes. Developmental Biology. doi: 10.1016/j.ydbio.2019.12.002

Hoshijima, U., and G. E. Hofmann. 2019. Variability of Seawater Chemistry in a Kelp Forest Environment Is Linked to in situ Transgenerational Effects in the Purple Sea Urchin, Strongylocentrotus purpuratus. Frontiers in Marine Science **6**.

Hughes, T. P., A. H. Baird, D. R. Bellwood, M. Card, S. R. Connolly, C. Folke, R. Grosberg, O. Hoegh-Guldberg, J. B. C. Jackson, J. Kleypas, J. M. Lough, P. Marshall, M. Nystrom, S. R. Palumbi, J. M. Pandolfi, B. Rosen, and J. Roughgarden. 2003. Climate change, human impacts, and the resilience of coral reefs. Science **301**:929-933.

Immler S (2018) The sperm factor: paternal impact beyond genes. Heredity 121: 239–247 doi https://doi.org/10.1038/s41437-018-0111-0

IPCC (2019) Summary for policymakers. In: IPCC Special Report on the ocean and cryosphere in a changing climate [H.- O. Pörtner, D.C. Roberts, V. Masson-Delmotte, P. Zhai, M. Tignor, E. Poloczanska, K. Mintenbeck, M. Nicolai, A. Okem, J. Petzold, B. Rama, N. Weyer (eds.)]. IPCC, Geneva, Switzerland

IPCC (2022) IPCC, 2022: Summary for Policy Makers.

Jablonka, E., and G. Raz. 2009. Transgenerational Epigenetic Inheritance: Prevalence, Mechanisms, and Implications for the Study of Heredity and Evolution. Quarterly Review of Biology **84**:131-176.

Jaffe, L. A. 1976. Fast Block to Polyspermy in Sea-Urchin Eggs Is Electrically Mediated. Nature **261**:68-71.

Jensen N, Allen RM, Marshall DJ (2014) Adaptive maternal and paternal effects: gamete plasticity in response to parental stress. Funct Ecol 28: 724-733 doi 10.1111/1365-2435.12195

Jiang, L., J. Zhang, J. J. Wang, L. Wang, L. Zhang, G. Q. Li, X. D. Yang, X. Ma, X. Sun, J. Cai, J. Zhang, X. X. Huang, M. Yu, X. G. Wang, F. Liu, C. I. Wu, C. He, B. Zhang, W. M. Ci, and J. Liu. 2013. Sperm, but Not Oocyte, DNA Methylome Is Inherited by Zebrafish Early Embryos. Cell **153**:773-784.

Johnson DW, Monro K, Marshall DJ (2013) The Maintenance of Sperm Variability: Context-Dependent Selection on Sperm Morphology in a Broadcast Spawning Invertebrate. Evolution 67: 1383-1395 doi 10.1111/evo.12022

Johnson SL, Yund PO (2004) Remarkable longevity of dilute sperm in a free-spawning colonial ascidian. Biol Bull 206: 144–151 doi https://doi.org/10.2307/1543638

Johnstone J, Nash S, Hernandez E, Rahman MS (2019) Effects of elevated temperature on gonadal functions, cellular apoptosis, and oxidative stress in Atlantic sea urchin Arbacia punculata. Mar Environ Res 149: 40–49 doi https://doi.org/10.1016/j.marenvres.2019.05.017

Kapsenberg L, Okamoto DK, Dutton JM, Hofmann GE (2017) Sensitivity of sea urchin fertilization to pH varies across a natural pH mosaic. Ecol Evol 7: 1737–1750 doi https://doi.org/10.1002/ece3.2776

Kapsenberg, L., and G. E. Hofmann. 2016. Ocean pH time-series and drivers of variability along the northern Channel Islands, California, USA. Limnology and Oceanography **61**:953-968.

Kelly, M. W., J. L. Padilla-Gamino, and G. E. Hofmann. 2013. Natural variation and the capacity to adapt to ocean acidification in the keystone sea urchin Strongylocentrotus purpuratus. Global Change Biology **19**:2536-2546.

Kelly, P. W., D. W. Pfennig, S. D. Buzon, and K. S. Pfennig. 2019. Male sexual signal predicts phenotypic plasticity in offspring: implications for the evolution of plasticity and local adaptation. Philosophical Transactions of the Royal Society B-Biological Sciences **374**.

Kooijman, S. A. L. M. 2009. Dynamic energy budget theory for metabolic organisation. Third Edition edition, Cambridge University Press, Cambridge, UK.

Kroeker, K. J., E. Sanford, J. M. Rose, C. A. Blanchette, F. Chan, F. P. Chavez, B. Gaylord, B. Helmuth, T. M. Hill, G. E. Hofmann, M. A. McManus, B. A. Menge, K. J. Nielsen, P. T. Raimondi, A. D. Russell, and L. Washburn. 2016. Interacting environmental mosaics drive geographic variation in mussel performance and predation vulnerability. Ecology Letters **19**:771-779.

Kupriyanova E, Havenhand JN (2002) Variation in sperm swimming behaviour and its effect on fertilization success in the serpulid polychaete Galeolaria caespitosa. Invertebr Reprod Dev 41: 21–26 doi https://doi.org/10.1080/07924259.2002.9652731

Kupriyanova EK, Havenhand JN (2005) Effects of temperature on sperm swimming behaviour, respiration and fertilization success in the serpulid polychaete, Galeolaria caespitosa (Annelida : Serpulidae). Invertebr Reprod Dev 48: 7–17 doi https://doi.org/10.1080/07924259.2005.9652166

Läinen JK, Oskoei P, Janhunen M, Koskinen H, Kortet R, Huuskonen H (2018) Sperm pre-fertilization thermal environment shapes offspring phenotype and performance. Journal of Experimental Biology. doi: 10.1242/jeb.181412

Lane A, Campanati C, Dupont S, Thiyagarajan V (2015) Trans-generational responses to low pH depend on parental gender in a calcifying tubeworm. Sci Rep-Uk 5 doi https://doi.org/10.1038/srep10847

Leach TS, BuyanUrt B, Hofmann GE (2021) Exploring impacts of marine heatwaves: paternal heat exposure diminishes fertilization success in the purple sea urchin (Strongylocentrotus purpuratus). Marine Biology. Springer Nature. doi: 10.1007/s00227-021-03915-x

Lenz B, Fogarty ND, Figueiredo J (2019) Effects of ocean warming and acidification on fertilization success and early larval development in the green sea urchin Lytechinus variegatus. Marine Pollution Bulletin. doi: 10.1016/j.marpolbul.2019.02.018

Lera S, Macchia S, Pellegrini D (2006) Standardizing the methodology of sperm cell test with Paracentrotus lividus. Environ Monit Assess 122: 101–109 doi https://doi.org/10.1007/s10661-005-9167-4

Levitan DR (2005) Sex-specific spawning behavior and its consequences in an external fertilizer. American Naturalist. doi: 10.1086/429733

Levitan DR (2006) The relationship between egg size and fertilization success in broadcast-spawning marine invertebrates. Integr Comp Biol 46: 298–311 doi https://doi.org/10.1093/icb/icj025

Levitan, D. R., M. A. Sewell, and F. S. Chia. 1992. How Distribution and Abundance Influence Fertilization Success in the Sea-Urchin Strongylocentrotus-Franciscanus. Ecology **73**:248-254.

Lewis C, Galloway T (2009) Reproductive Consequences of Paternal Genotoxin Exposure in Marine Invertebrates. Environ Sci Technol 43: 928–933 doi https://doi.org/10.1021/es802215d

Lewis SEM, Aitken RJ (2005) DNA damage to spermatozoa has impacts on fertilization and pregnancy. Cell and Tissue Research 322:

Lotterhos KE, Levitan DR (2010) Gamete release and spawning behavior in broadcast spawning marine invertebrates. The evolution of primary sexual characters in animals 99:120.

Lymbery RA, Kennington WJ, Cornwall CE, Evans JP (2019) Ocean acidification during prefertilization chemical communication affects sperm success. Ecology and Evolution. doi: 10.1002/ece3.5720

Lymbery RA, Kennington WJ, Evans JP (2021) The thermal environment of sperm affects offspring success: A test of the anticipatory paternal effects hypothesis in the blue mussel. Biology Letters. doi: 10.1098/rsbl.2021.0213

Lymbery, R. A., J. P. Evans, and W. J. Kennington. 2020. Post-ejaculation thermal stress causes changes to the RNA profile of sperm in an external fertilizer. Proceedings of the Royal Society B-Biological Sciences **287**.

Lynch, M., and B. Walsh. 1998. Genetics and Analysis of Quantitative Traits, Sunderland, MA.

Mak KKY, Chan KYK (2018) Interactive effects of temperature and salinity on early life stages of the sea urchin Heliocidaris crassispina. Marine Biology 165 doi https://doi.org/10.1007/s00227-018-3312-4

Marba N, Duarte CM (2010) Mediterranean warming triggers seagrass (Posidonia oceanica) shoot mortality. Global Change Biol 16: 2366–2375 doi https://doi.org/10.1111/j.1365-2486.2009.02130.x

Marshall DJ (2006) Reliably estimating the effect of toxicants on fertilization success in marine broadcast spawners. Mar Pollut Bull 52: 734–738 doi https://doi.org/10.1016/j.marpolbul.2006.05.005

Marshall DJ (2015) Environmentally induced (co)variance in sperm and offspring phenotypes as a source of epigenetic effects. Journal of Experimental Biology 218: 107-113 doi https://doi.org/10.1242/jeb.106427

Marshall, D. J. 2008. Transgenerational plasticity in the sea: Context-dependent maternal effects across the life history. Ecology **89**:418-427.

Marshall, D. J., and T. Uller. 2007. When is a maternal effect adaptive? Oikos **116**:1957-1963.

Martino C, Byrne M, Roccheri MC, Chiarelli R (2021) Interactive effects of increased temperature and gadolinium pollution in Paracentrotus lividus sea urchin embryos: a climate change perspective. Aquat Toxicol 232 doi https://doi.org/10.1016/j.aquatox.2021.105750

McAlister JS, Moran AL (2012) Relationships among egg size, composition, and energy: A comparative study of geminate sea urchins. PLoS ONE. doi: 10.1371/journal.pone.0041599

Mehrbach, C., C. H. Culberson, J. E. Hawley, and R. M. Pytkowicz. 1973. Measurement of Apparent Dissociation-Constants of Carbonic-Acid in Seawater at Atmospheric-Pressure. Limnology and Oceanography **18**:897-907.

Miles, H., S. Widdicombe, J. I. Spicer, and J. Hall-Spencer. 2007. Effects of anthropogenic seawater acidification on acid-base balance in the sea urchin Psammechinus miliaris. Marine Pollution Bulletin **54**:89-96.

Mita M, Hino A, Yasumasu I (1984) Effect of temperature on interaction between eggs and spermatozoa of sea-urchin. Biol Bull 166: 68–77 doi https://doi.org/10.2307/1541431

Montes-Hugo, M., S. C. Doney, H. W. Ducklow, W. Fraser, D. Martinson, S. E. Stammerjohn, and O. Schofield. 2009. Recent Changes in Phytoplankton Communities Associated with Rapid Regional Climate Change Along the Western Antarctic Peninsula. Science **323**:1470-1473.

Moran AL, McAlister JS (2009) Egg size as a life history character of marine invertebrates: is it all it's cracked up to be? Biological Bulletin. doi: 10.2307/25548157

Mousseau TA, Fox CW (1998) The adaptive significance of maternal effects. Trends Ecol Evol 13: 403–407 doi https://doi.org/10.1016/S0169-5347(98)01472-4

Munday, P. L. 2014. Transgenerational acclimation of fishes to climate change and ocean acidification. F1000Prime Rep **6**:99.

Negri AP, Marshall PA, Heyward AJ (2007) Differing effects of thermal stress on coral fertilization and early embryogenesis in four Indo Pacific species. Coral Reefs 26: 759–763 doi https://doi.org/10.1007/s00338-007-0258-2

Nesbit KT, Fleming T, Batzel G, Pouv A, Rosenblatt HD, Pace DA, Hamdoun A, Lyons DC (2019) The painted sea urchin, Lytechinus pictus, as a genetically-enabled developmental model. In: Methods in Cell Biology.

Nesbit KT, Hamdoun A (2020) Embryo, larval, and juvenile staging of Lytechinus pictus from fertilization through sexual maturation. Developmental Dynamics. doi: 10.1002/dvdy.223

Nielsen, K., Stachowicz, J., Carter, H., Boyer, K., Bracken, M., Chan, F., Chavez, F., Hovel, K., Kent, M., Nickols, K., Ruesink, J., Tyburczy, J., and Wheeler, S. 2018. Emerging understanding of the potential role of seagrass and kelp as an ocean acidification management tool in California. California Ocean Science Trust, Oakland, California, USA.

Norouzitallab, P., K. Baruah, M. Vandegehuchte, G. Van Stappen, F. Catania, J. Vanden Bussche, L. Vanhaecke, P. Sorgeloos, and P. Bossier. 2014. Environmental heat stress induces epigenetic transgenerational inheritance of robustness in parthenogenetic Artemia model. Faseb Journal **28**:3552-3563.

O'Connor MI, Bruno JF, Gaines SD, Halpern BS, Lester SE, Kinlan BP, Weiss JM (2007) Temperature control of larval dispersal and the implications for marine ecology, evolution, and conservation. P Natl Acad Sci USA 104: 1266–1271 doi https://doi.org/10.1073/pnas.0603422104

O'Donnell, M. J., A. E. Todgham, M. A. Sewell, L. M. Hammond, K. Ruggiero, N. A. Fangue, M. L. Zippay, and G. E. Hofmann. 2009. Ocean acidification alters skeletogenisis in larvae of the sea urchin Lytechinus pictus: evidence from

morphometric and microarray data. Integrative and Comparative Biology **49**:E125-E125.

O'Donnell MJ, Hammond LM, Hofmann GE (2009) Predicted impact of ocean acidification on a marine invertebrate: Elevated CO2 alters response to thermal stress in sea urchin larvae. Marine Biology. doi: 10.1007/s00227-008-1097-6

Ofori BY, Stow AJ, Baumgartner JB, Beaumont LJ (2017) Influence of adaptive capacity on the outcome of climate change vulnerability assessment. Sci Rep-Uk 7 doi https://doi.org/10.1038/s41598-017-13245-y

Okamoto DK, Schroeter S, Reed DC (2020) Effects of ocean climate on spatiotemporal variation in sea urchin settlement and recruitment. Limnol Oceanogr 65: 2076–2091 doi https://doi.org/10.1002/lno.11440

Olita A, Sorgente R, Natale S, Gabersek S, Ribotti A, Bonanno A, Patti B (2007) Effects of the 2003 European heatwave on the Central Mediterranean Sea: surface fluxes and the dynamical response. Ocean Sci 3: 273–289 doi https://doi.org/10.5194/os-3-273-2007

Oliver ECJ, Benthuysen JA, Darmaraki S, Donat MG, Hobday AJ, Holbrook NJ, Schlegel RW, sen Gupta A (2021) Marine heatwaves. Ann Rev Mar Sci 13:313–342.

Oliver ECJ, Donat MG, Burrows MT, Moore PJ, Smale DA, Alexander LV, Benthuysen JA, Feng M, Sen Gupta A, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell HA, Straub SC, Wernberg T (2018) Longer and more frequent marine heatwaves over the past century. Nat Commun 9 doi https://doi.org/10.1038/s41467-018-03732-9

Oyarzun FX, Strathmann RR (2011) Plasticity of hatching and the duration of planktonic development in marine invertebrates. In: Integrative and Comparative Biology.

Padilla-Gamino, J. L., M. W. Kelly, T. G. Evans, and G. E. Hofmann. 2013. Temperature and CO2 additively regulate physiology, morphology and genomic responses of larval sea urchins, Strongylocentrotus purpuratus. Proc R Soc B **280**:9.

Pandori LLM, Sorte CJB (2019) The weakest link: sensitivity to climate extremes across life stages of marine invertebrates. Oikos 128: 621–629 doi https://doi.org/10.1111/oik.05886

Parker LM, Ross PM, O'Connor WA (2010) Comparing the effect of elevated pCO(2) and temperature on the fertilization and early development of two species of oysters. Marine Biology 157: 2435–2452 doi https://doi.org/10.1007/s00227-010-1508-3

Parker LM, Ross PM, O'Connor WA (2009) The effect of ocean acidification and temperature on the fertilization and embryonic development of the Sydney rock oyster Saccostrea glomerata (Gould 1850). Global Change Biology. doi: 10.1111/j.1365-2486.2009.01895.x

Parker, L. M., P. M. Ross, W. A. O'Connor, L. Borysko, D. A. Raftos, and H. O. Portner. 2012. Adult exposure influences offspring response to ocean acidification in oysters. Global Change Biology **18**:82-92.

Pearse JS (2006) Perspective - Ecological role of purple sea urchins. Science 314: 940–941 doi 10.1126/science.1131888

Pecorino, D., M. D. Lamare, M. F. Barker, and M. Byrne. 2013. How does embryonic and larval thermal tolerance contribute to the distribution of the sea urchin

Centrostephanus rodgersii (Diadematidae) in New Zealand? Journal of Experimental Marine Biology and Ecology **445**:120-128.

Pineda J, Reyns N, Lentz SJ (2018) Reduced barnacle larval abundance and settlement in response to large-scale oceanic disturbances: Temporal patterns, nearshore thermal stratification, and potential mechanisms. Limnol Oceanogr 63: 2618–2629 doi https://doi.org/10.1002/lno.10964

Pitts KA, Campbell JE, Figueiredo J, Fogarty ND (2020) Ocean acidification partially mitigates the negative effects of warming on the recruitment of the coral, Orbicella faveolata. Coral Reefs 39: 281–292 doi https://doi.org/10.1007/s00338-019-01888-4

Przeslawski R, Byrne M, Mellin C (2015) A review and meta-analysis of the effects of multiple abiotic stressors on marine embryos and larvae. Global Change Biol 21: 2122–2140 doi https://doi.org/10.1111/gcb.12833

Quinn GP, Keough MJ (2002) Experimental design and data analysis for biologists, Cambridge University Press

Rahman MS, Tsuchiya M, Uehara T (2009) Effects of temperature on gamete longevity and fertilization success in two sea urchin species, Echinometra mathaei and Tripneustes gratilla. Zool Sci 26: 1–8 doi https://doi.org/10.2108/zsj.26.1

Randall CJ, Szmant AM (2009) Elevated temperature affects development, survivorship, and settlement of the elkhorn coral, Acropora palmata (Lamarck 1816). Biol Bull 217: 269–282 doi https://doi.org/10.1086/BBLv217n3p269

Reed D, Washburn L, Rassweiler A, Miller R, Bell T, Harrer S (2016) Extreme warming challenges sentinel status of kelp forests as indicators of climate change. Nat Commun 7 doi https://doi.org/10.1038/ncomms13757

Ritchie, H., and D. J. Marshall. 2013. Fertilisation is not a new beginning: sperm environment affects offspring developmental success. Journal of Experimental Biology **216**:3104-3109.

Rogers-Bennett L, Catton CA (2019) Marine heat wave and multiple stressors tip bull kelp forest to sea urchin barrens. Sci Rep-Uk 9 doi https://doi.org/10.1038/s41598-019-51114-y

Rogers-Bennett L, Dondanville RF, Moore JD, Vilchis LI (2010) Response of red abalone reproduction to warm water, starvation, and disease stressors: Implications of ocean warming. J Shellfish Res 29: 599–611 doi https://doi.org/10.2983/035.029.0308

Ross PM, Parker L, Byrne M (2016) Transgenerational responses of molluscs and echinoderms to changing ocean conditions. ICES Journal of Marine Science 73:

Ross, P. M., L. Parker, W. A. O'Connor, and E. A. Bailey. 2011. The Impact of Ocean Acidification on Reproduction, Early Development and Settlement of Marine Organisms. Water **3**:1005-1030.

Runcie, D. E., D. A. Garfield, C. C. Babbitt, J. A. Wygoda, S. Mukherjee, and G. A. Wray. 2012. Genetics of gene expression responses to temperature stress in a sea urchin gene network. Molecular Ecology **21**:4547-4562.

Rupp JH (1973) Effects of temperature on fertilization and early cleavage of some tropical echinoderms, with emphasis on Echinometra-Mathaei. Marine Biology 23: 183–189 doi https://doi.org/10.1007/Bf00389483

Salinas, S., S. C. Brown, M. Mangel, and S. B. Munch. 2013. Non-genetic inheritance and changing environments. Non-Genetic Inheritance **1**.

Sanford E, Sones JL, Garcia-Reyes M, Goddard JHR, Largier JL (2019) Widespread shifts in the coastal biota of northern California during the 2014-2016 marine heatwaves. Sci Rep-Uk 9 doi https://doi.org/10.1038/s41598-019-40784-3

Sconzo, G., M. G. Ferraro, G. Amore, G. Giudice, D. Cascino, and G. Scardina. 1995. Activation by heat shock of HSP70 gene transcription in sea urchin embryos. Biochemical and Biophysical Research Communications **217**:1032-1038.

Seebacher F, Beaman J, Little AG (2014) Regulation of thermal acclimation varies between generations of the short-lived mosquitofish that developed in different environmental conditions. Funct Ecol 28: 137-148 doi 10.1111/1365-2435.12156

Sen Gupta A, Thomsen M, Benthuysen JA, Hobday AJ, Oliver ECJ, Alexander LV, Burrows MT, Donat MG, Feng M, Holbrook NJ, Perkins-Kirkpatrick SE, Moore PJ, Rodrigues RR, Scannell HA, Taschetto AS, Ummenhofer CC, Wernberg T, Smale DA (2020) Drivers and impacts of the most extreme marine heatwaves events. Sci Rep-Uk 10

Seuront L, Nicastro KR, Zardi GI, Goberville E (2019) Decreased thermal tolerance under recurrent heat stress conditions explains summer mass mortality of the blue mussel Mytilus edulis. Sci Rep-Uk 9 doi https://doi.org/10.1038/s41598-019-53580-w

Sewell MA, Millar RB, Yu PC, Kapsenberg L, Hofmann GE (2014) Ocean acidification and fertilization in the antarctic sea urchin Sterechinus neumayeri: The importance of polyspermy. Environ Sci Technol 48: 713–722 doi https://doi.org/10.1021/es402815s

Sewell MA, Young CM (1999) Temperature limits to fertilization and early development in the tropical sea urchin Echinometra lucunter. Journal of Experimental Marine

Biology and Ecology 236: 291–305 doi https://doi.org/10.1016/S0022-0981(98)00210-X

Shama, L. N. S., and K. M. Wegner. 2014. Grandparental effects in marine sticklebacks: transgenerational plasticity across multiple generations. Journal of Evolutionary Biology **27**:2297-2307.

Shanks AL, Rasmuson LK, Valley JR, Jarvis MA, Salant C, Sutherland DA, Lamont EI, Hainey MAH, Emlet RB (2020) Marine heat waves, climate change, and failed spawning by coastal invertebrates. Limnol Oceanogr 65: 627–636 doi https://doi.org/10.1002/lno.11331

Sheppard Brennand H, Soars N, Dworjanyn SA, Davis AR, Byrne M (2010) Impact of ocean warming and ocean acidification on larval development and calcification in the sea urchin Tripneustes gratilla. PLoS ONE. doi: 10.1371/journal.pone.0011372

Shi, D. T., C. Zhao, Y. Chen, J. Y. Ding, L. S. Zhang, and Y. Q. Chang. 2020. Transcriptomes shed light on transgenerational and developmental effects of ocean warming on embryos of the sea urchin Strongylocentrotus intermedius. Scientific Reports **10**.

Sikkink, K. L., C. M. Ituarte, R. Reynolds, W. A. Cresko, and P. C. Phillips. 2014. The transgenerational effects of heat stress in the nematode Caenorhabditis remanei are negative and rapidly eliminated under direct selection for increased stress resistance in larvae. Genomics **104**:438-446.

Smale DA, Wernberg T, Oliver ECJ, Thomsen M, Harvey BP, Straub SC, Burrows MT, Alexander LV, Benthuysen JA, Donat MG, Feng M, Hobday AJ, Holbrook NJ, Perkins-Kirkpatrick SE, Scannell HA, Sen Gupta A, Payne BL, Moore PJ (2019)

Marine heatwaves threaten global biodiversity and the provision of ecosystem services. Nat Clim Change 9: 306-+ doi https://doi.org/10.1038/s41558-019-0412-1

Stillman JH (2003) Acclimation capacity underlies susceptibility to climate change. Science 301: 65–65 doi https://doi.org/10.1126/science.1083073

Strader, M. E., J. M. Wong, L. C. Kozal, T. S. Leach, and G. E. Hofmann. 2019. Parental environments alter DNA methylation in offspring of the purple sea urchin, Strongylocentrotus purpuratus. Journal of Experimental Marine Biology and Ecology **517**:54-64.

Strader, M. E., L. C. Kozal, T. S. Leach, J. M. Wong, J. D. Chamorro, M. J. Housh, and G. E. Hofmann. 2020. Examining the Role of DNA Methylation in Transcriptomic Plasticity of Early Stage Sea Urchins: Developmental and Maternal Effects in a Kelp Forest Herbivore. Frontiers in Marine Science **7**.

Strathmann MF (1987) Reproduction and development of marine invertebrates of the northern Pacific coast: data and methods for the study of eggs, embryos, and larvae. University of Washington Press

Strathmann RR, Strathmann MF, Ruiz-Jones G, Hadfield MG (2010) Effect of plasticity in hatching on duration as a precompetent swimming larva in the nudibranch Phestilla sibogae. Invertebrate Biology. doi: 10.1111/j.1744-7410.2010.00212.x

Suckling CC, Clark MS, Richard J, Morley SA, Thorne MAS, Harper EM, Peck LS (2015) Adult acclimation to combined temperature and pH stressors significantly enhances reproductive outcomes compared to short-term exposures. J Anim Ecol 84: 773–784 doi https://doi.org/10.1111/1365-2656.12316

Thurman LL, Stein BA, Beever EA, Foden W, Geange SR, Green N, Gross JE, Lawrence DJ, LeDee O, Olden JD, Thompson LM, Young BE (2020) Persist in place or shift in space? Evaluating the adaptive capacity of species to climate change. Front Ecol Environ doi https://doi.org/10.1002/fee.2253

Uthicke S, Liddy M, Nguyen HD, Byrne M (2014) Interactive effects of near-future temperature increase and ocean acidification on physiology and gonad development in adult Pacific sea urchin, Echinometra sp. A. Coral Reefs 33: 831–845 doi https://doi.org/10.1007/s00338-014-1165-y

Waite, H. R., and C. J. B. Sorte. 2022. Negative carry-over effects on larval thermal tolerances across a natural thermal gradient. Ecology **103**.

Watanabe, A., H. Kayanne, K. Nozaki, K. Kato, A. Negishi, S. Kudo, H. Kimoto, M. Tsuda, and A. G. Dickson. 2004. A rapid, precise potentiometric determination of total alkalinity in seawater by a newly developed flow-through analyzer designed for coastal regions. Marine Chemistry **85**:75-87.

Watts SA, McClintock JB, Lawrence JM (2020) Chapter 36 - Lytechinus. In: Lawrence JM (ed) Developments in Aquaculture and Fisheries Science. Elsevier, pp 661–680

Wernberg T, Bennett S, Babcock RC, de Bettignies T, Cure K, Depczynski M, Dufois F, Fromont J, Fulton CJ, Hovey RK, Harvey ES, Holmes TH, Kendrick GA, Radford B, Santana-Garcon J, Saunders BJ, Smale DA, Thomsen MS, Tuckett CA, Tuya F, Vanderklift MA, Wilson S (2016) Climate-driven regime shift of a temperate marine ecosystem. Science 353: 169–172 doi https://doi.org/10.1126/science.aad8745

White MM, Mullineaux LS, McCorkle DC, Cohen AL (2014) Elevated pCO2 exposure during fertilization of the bay scallop Argopecten irradians reduces larval survival

but not subsequent shell size. Marine Ecology Progress Series 498:173–186. doi: 10.3354/meps10621

Wong JM, Hofmann GE (2020) The effects of temperature and pCO2 on the size, thermal tolerance and metabolic rate of the red sea urchin (Mesocentrotus franciscanus) during early development. Marine Biology. doi: 10.1007/s00227-019-3633-y

Wong, J. M., K. M. Johnson, M. W. Kelly, and G. Hofmann. 2018. Transcriptomics reveal transgenerational effects in purple sea urchin embryos: Adult acclimation to upwelling conditions alters the response of their progeny to differential pCO(2) levels. Molecular Ecology **27**:1120-1137.

Wong, J. M., L. C. Kozal, T. S. Leach, U. Hoshijima, and G. E. Hofmann. 2019. Transgenerational effects in an ecological context: Conditioning of adult sea urchins to upwelling conditions alters maternal provisioning and progeny phenotype. Journal of Experimental Marine Biology and Ecology **517**:65-77.

Yamada K, Mihashi K (1998) Temperature-independent period immediately after fertilization in sea urchin eggs. Biol Bull 195: 107–111 doi https://doi.org/10.2307/1542817

Yin, J. J., M. Zhou, Z. R. Lin, Q. S. Q. Li, and Y. Y. Zhang. 2019. Transgenerational effects benefit offspring across diverse environments: a meta-analysis in plants and animals. Ecology Letters **22**:1976-1986.

Yorke CE, Page HM, Miller RJ (2019) Sea urchins mediate the availability of kelp detritus to benthic consumers. P Roy Soc B-Biol Sci 286 doi https://doi.org/10.1098/rspb.2019.0846

Yu, P. C., P. G. Matson, T. R. Martz, and G. E. Hofmann. 2011. The ocean acidification seascape and its relationship to the performance of calcifying marine invertebrates: Laboratory experiments on the development of urchin larvae framed by environmentally-relevant pCO(2)/pH. Journal of Experimental Marine Biology and Ecology **400**:288-295.

Zigler KS, Lessios HA (2004) Speciation on the coasts of the new world: Phylogeography and the evolution of bindin in the sea urchin genus Lytechinus. Evolution (N Y). doi: 10.1111/j.0014-3820.2004.tb01702.x

Zippay ML, Helmuth B (2012) Effects of temperature change on mussel, Mytilus. Integr Zool 7: 312–327 doi https://doi.org/10.1111/j.1749-4877.2012.00310.x