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Impacts of Natural Temperature Variation and Heatwaves on Coastal Marine Invertebrates

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Impacts of Natural Temperature Variation and Heatwaves on Coastal Marine Invertebrates

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requirements for the degree Doctor of Philosophy
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by

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Strader, M.E., Kozal, L.C., Leach, T.S., Wong, J.M., **Chamorro, J.D.**, Housh, M.J., & Hofmann, G.E. (2020) Examining the role of DNA Methylation in transcriptomic plasticity of early-stage sea urchins: Developmental and maternal effects in kelp forest herbivore. *Frontiers in Marine Science* 7:205. doi:10.3389/fmars.2020.00205

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ABSTRACT

Impacts of Natural Temperature Variation and Heatwaves on Coastal Marine Invertebrates

by

Jannine D. Chamorro

The rapid pace of global climate change is challenging marine species worldwide. Increases in average seawater temperatures, combined with extreme climatic events such as marine heatwaves (MHWs), are pushing organisms near or past their physiological tolerance limits. To predict how these stressors will impact species and ecosystems, knowledge of thermal limits and the capacity to adjust these limits via acclimation is critical. This is especially true for species living near their thermal limits or species that vary in sensitivity to thermal stress across their life history.

In my dissertation I examined how changes in ocean temperature are impacting two ecologically important species in coastal waters of the Northeast Pacific, the California mussel (*Mytilus californianus*) and the purple sea urchin (*Strongylocentrotus purpuratus*). For both study organisms, I began by examining how the thermal experience of adult animals was altered during MHW events using data collected by long-term monitoring programs. Here, I found that across the biogeographic range of *M. californianus,* mussels differed in thermal exposure during MHW events where Oregon and California populations differed in the onset of elevated temperatures and the proximity to optimal performance thresholds.

Regarding *S. purpuratus,* I found that MHWs coincide with reproductive life history events that may have implications for gametogenesis, spawning, and larval development.

For early life history events in my study organisms, I examined transgenerational and intragenerational plasticity as an acclimatory response mechanism to warming oceans. In a field-to-laboratory experiment using intertidal mussels, I investigated transgenerational plasticity in nature by examining how parental exposure to differing thermal regimes that exist across tidal height and season impact offspring phenotype. To further examine the influence of developmental temperature on larval performance, offspring were reared at two developmental temperatures. At the veliger stage, physiological performance of offspring was assessed via measurements of larval body size, respiration rate, and thermal tolerance. Results from this study show that seasonal acclimatization of adult *M. californianus* and larval developmental temperature had prevailing effects on offspring performance.

Lastly, to study whether MHWs might induce a transgenerational response in *S. purpuratus,* adult urchins were acclimated to MHW and non-MHW conditions in the laboratory during their period of gametogenesis. Following the adult acclimation, offspring from each parental treatment were reared in reciprocal conditions. To assess for transgenerational and intragenerational effects throughout ontogeny, thermal tolerance and body size of each offspring treatment was measured at critical developmental stages. Embryos from MHW-acclimated females were more thermally tolerant with higher LT_{50} values compared to progeny from non-MHW-acclimated females. Additionally, there was an effect of female acclimation state on offspring body size at two stages of development - early gastrula and prism. To examine maternal provisioning as a means to also alter embryo performance, I examined egg size and biochemical composition. Here I found that eggs from MHW-acclimated female had higher total protein concentrations.

Overall results from my dissertation research highlighted natural temperature variation experienced by *M. californianus* and *S. purpuratus*, and how this thermal exposure was influenced during heatwave events. Further, using a combination of field and laboratory experiments I provided evidence for transgenerational and intragenerational acclimation to alter performance of early developmental stages.

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I. Introduction

Statement of the problem

Living organisms have adapted to operate within a thermal range shaped by the temperature conditions experienced over evolutionary time. Organismal performance declines when temperatures exceed thermal limits and if behavioral or physiological adjustments are not made, this could lead to mortality (Sinclair et al., 2016). Warming associated with anthropogenic global climate change is more frequently pushing species closer or past their upper thermal limits, leading to population level changes that can subsequently impact ecosystems and the humans that depend on them (Smale et al., 2019; Smith et al., 2023).

In marine systems, a multi-decadal trend of warming ocean temperature has been documented due to the increased uptake of heat by the ocean trapped in the atmosphere by greenhouse gases (IPCC, 2014). In addition to this gradual increase, episodic warm water anomalies, known as marine heatwaves (MHWs) have increased in frequency and intensity (Perkins et al., 2012; Oliver et al., 2018). MHWs, widely defined as periods of time where the seawater temperature is above the 90th percentile for a period of five days or longer (Hobday et al., 2016), have recently gained the attention of the marine science community due to the far-reaching impacts on marine ecosystems. Around the globe MHW events have had organism level effects such as changes in growth (Short et al., 2015) and reproduction (Wild et al., 2019; Piatt et al., 2020) as well as ecosystem level effects such as mass mortality events (Cavole et al., 2016; Hughes et al., 2017; Wernberg, 2021), range expansions (Sanford et al., 2019), and species invasions (Verdura et al., 2019).

One of the longest and largest documented MHWs, known as ‘the Blob’, occurred along the northeastern Pacific from January 2014 – August 2016 (Gentemann et al., 2017). During this time sea surface temperature (SST) increased an average of 1-4°C (Cavole et al., 2016). Subtidal and intertidal algal species were some of them most drastically impacted species. Lethal effects on these foundation species, associated with increased temperature and decreased nutrient availability, led to restructuring of benthic invertebrate communities along the Northeastern Pacific coast (Michaud et al., 2022; Whalen et al., 2023). Numerous fisheries were also impacted during this time (Free et al., 2023). The loss of kelp as a food source eventually led to the collapse of the red sea urchin (*Mesocentrotus franciscanus*) and red abalone (*Haliotis rufescens*) fishery (Rogers-Bennett, 2013; Free et al., 2023). Pacific cod also showed a steep decline in biomass following the heatwave. Recent studies predicted that increased metabolic demands and decreased prey availability (Piatt et al., 2020), in addition to reduced egg survival and recruitment may have contributed to this decline (Laurel and Rogers, 2020). However, not all impacts were negative. For example, the MHW promoted the range expansion of the California market squid (*Doryteuthis opalescens*). Before, the fishery existed predominantly in California, however due to the northward expansion up to the Gulf of Alaska there has been growing development of the squid fishery in Oregon and Alaska (Free et al., 2023). Differences in population level response indicates that some species may be better adapted to coping with these thermal events, thus knowledge on thermal limits of species or mechanisms that increase tolerance will allow us to predict which species will be “winners” or “losers” in a warming world (Somero, 2010).

For intertidal organisms living at the intersection of the marine and terrestrial realm, the increased occurrence of aerial heatwaves (AHWs) is yet another stressor associated with global climate change. The prolonged periods of excessive heat have documented lethal impacts on plants, animals, and humans alike (White et al., 2023). Sessile marine species habiting the intertidal region are some of the species most vulnerable to heatwaves, as many are currently living near their thermal limit and have limited ability to cope behaviorally or physiologically (Somero, 2002). Together the increased occurrence of marine and aerial heatwaves present new challenges for marine species. As long-term averages in SST and these extreme climatic events are expected to increase in frequency, intensity, and duration, it is crucial that we advance our understanding of organismal capacity to tolerate rapidly warming environments (Oliver et al., 2018; Perkins-Kirkpatrick and Lewis, 2020).

Organismal response to environmental warming

In the face of rapid environmental change, species have few options to avoid extinction: disperse to a more hospitable habitat, adapt through genetic change, or acclimate via phenotypic plasticity (Hofmann and Todgham, 2010). These processes are not mutually exclusive, and it is likely that species will employ them all in tandem (Donelson et al., 2019). However, with the rapid rate of ocean warming, acclimation via phenotypic plasticity is likely the first line of defense (Somero, 2010). Mechanistically, organisms can induce an acclimatory response through processes such as intragenerational plasticity (IGP) and transgenerational plasticity (TGP). Plasticity within a generation, IGP, refers to changes in phenotype induced by signals occurring within an organism’s lifetime. Environmental drivers can alter tolerance and performance of individuals through biochemical, molecular, or physiological processes (Hofmann and Todgham, 2010). For example, in response to MHW stress, organisms can upregulate production of molecular chaperones critical to protein homeostasis, heat shock proteins, as seen in the pearl oyster (*Pinctada maximal*) and the short-spined urchin (*Heliocidaris erythrogramma*) (Harianto et al., 2021; Xu et al., 2022).

Developmental plasticity, which refers to modifications to phenotype induced by environmental signals experienced during early ontogeny is another form of IGP. Regarding warming environmental conditions, numerous studies have examined the effects of developmental temperature on thermal tolerance (Pottier et al., 2022). A meta-analysis by Pottier and colleagues found a modest increase in thermal tolerance is associated with increased developmental temperature (Pottier et al., 2022). Thus, exploring developmental plasticity as an acclimatory mechanism to ocean warming warrants further research.

Transgenerational plasticity, on the other hand, refers to modification of offspring phenotype induced by signals experienced by the parents. Through mechanisms of TGP such as nutritional provisioning, transmission of hormones and proteins, and epigenetic inheritance, parental generations can prime offspring with better suited phenotypes to deal with environmental conditions (Munday, 2014). Given that numerous marine species are broadcast spawners, including marine invertebrates, maternal provisioning of eggs represents the entire parental investment (McEdward and Miner, 2006). These initial investments are critical for early development, and thus changes attributed to environmental temperature experienced by adults during gametogenesis may later lead to population level effects. Overall, it is likely that organisms will employ a combination of all these mechanisms to cope with novel thermal conditions. Importantly, all these non-genetic mechanisms work at shorter timescales than evolutionary processes, thus can buffer populations allowing genetic adaptation to ‘catch up’ (Donelson et al., 2016).

The objective

The core objective of my thesis was to 1) investigate how heatwaves alter thermal regimes experienced by ecologically important intertidal and subtidal invertebrate species, and 2) examine transgenerational and intragenerational plasticity as an acclimatory response mechanism to warming oceans. My investigation covers two systems 1) intertidal mussels, *Mytilus californianus*, inhabiting the rocky intertidal shores along the Pacific coast of the United States and 2) purple sea urchins, *Strongylocentrotus purpuratus*, kelp forest herbivores in the Santa Barbara Channel.

Study System #1: Mytilus californianus inhabiting the rocky intertidal shores along the Pacific coast

Along the Northeastern Pacific coast, California mussels (*M. californianus*) are a dominate foundation species found along rocky intertidal shores that form large aggregations (aka mussel beds) in the mid intertidal zone. Across vertical height in intertidal zone, and subsequently mussel beds, thermal gradients exist due to the tide cycle, where organisms at the upper and lower edge of the rocky intertidal zone can vary greatly in their thermal exposure due to differences in immersion and emersion time (Helmuth et al., 2016). Because the timing of low tides changes throughout the year, thermal exposure of intertidal organisms is additionally influenced by seasonal changes in aerial and seawater temperature. Differences in timing of low tides and shoreline characteristics across latitude further contribute to the thermal variability experienced by mussels, leading to a mosaic of temperature regimes along their biogeographic range (Helmuth et al., 2006).

The rocky intertidal zone is often considered to be one of the most thermally challenging places to inhabit. Here, *M. californianus* act as ecosystem engineers altering rocky habitat. Three-dimensional mussel beds alleviate these thermally stressful conditions by increasing the retention of moisture during low tide, thus decreasing temperature relative to bare rock (Jurgens and Gaylord, 2016). In doing so, mussel beds provide habitat for newly settled and adult organisms, greatly increasing biodiversity of the rocky intertidal zone (Seed and Suchanek, 1992). Thus, abiotic and/or biotic factors that contribute to changes in *M. californianus* abundance can alter community composition of the rocky intertidal zone.

Apart from their ecological importance, mussel beds are natural laboratories for studying thermal biology (Tomanek and Helmuth, 2002). Natural differences in thermal exposure at varying proximities have allowed researchers to gain insight into physiological and molecular mechanisms organisms employ to generate an acclimatory response (Place et al., 2012; Moyen et al., 2020). While most studies on acclimation mechanisms of *M. californianus* have focused on the post-settlement or adult stages (Gleason et al., 2018; Moyen et al., 2019), differences in thermal acclimatization of adults across tidal height and season make them ideal candidates for studying transgenerational acclimation mechanisms.

As with most marine invertebrates, *M. californianus,* have a complex life history. Adult mussels inhabit the benthos of the rocky intertidal zone, while embryos and larvae develop in the water column following synchronous broadcast spawning events (Strathmann, 1987; Gosselin, 2004). Since early developmental stages are often the most sensitive to environmental stress (Pandori and Sorte, 2018), examining the impact of ocean warming on these stages can provide critical insight into future population response. This is critical as numerous studies have shown latent effects of MHWs that appear years after the onset of the event (Free et al., 2023).

Study System #2: Kelp forest herbivores, Strongylocentrotus purpuratus, in the Santa Barbara Channel

A dominant herbivorous grazer, purple urchins (*S. purpuratus*) can have dramatic effects on kelp forest ecosystems, with the ability to transform kelp canopies into urchin barrens when population densities are high (Pearse, 2006). While urchins have long been studied with regard to their vulnerability to ocean acidification (Yu et al., 2011), less is known about how they will be affected by increased temperature. Furthermore, the purple urchin has long been used as a model organism in developmental biology. Its well documented development allows for an in-depth investigation on the effect of temperature during ontogeny. Lastly, from an ecological development perspective (Sultan, 2007), a large portion of the purple urchin's reproductive cycle take place when MHWs occur in the SBC. MHWs have tended to occur in the late summer to early winter, a time period that coincides with the period of gametogenesis in purple sea urchins (Cochran and Engelmann, 1975; Strathmann, 1987).

Using a combination of approaches from timeseries analysis of long-term data to field and laboratory experiments, my thesis investigated how warming associated with natural variation in temperature and episodic thermal anomalies impact coastal benthic marine invertebrates, *M. californianus* and *S. purpuratus*. This body of work has been organized in to three chapters:

Chapter II: Heatwave impacts on Mytilus californianus body temperature across latitude

For this chapter I conducted a time series analysis investigating the effects of natural temperature variability and heatwaves on *M. californianus* body temperature. Temperature data was collected with biomimetic temperature loggers, robomussels, at six sites in Oregon and California. I found that monthly averaged mussel body temperatures in Oregon and California showed distinctly different patterns. In terms of MHWs, I observed that temperature differences were influenced by site and mussel immersion/emersion during the 2014-2016 Northeastern Pacific MHW. Increased body temperature was associated with immersion rather than emersion. Moreover, temperature increases were higher and occurred earlier in California than Oregon. Lastly, I examined the impact of the Pacific Heat Dome, on the most Northern sites in Oregon and found that recorded temperatures were within the naturally occurring range. Results from this study provide key information relating to *M. californianus* with regard to natural temperature variability and during heatwave events, which can help to explain ecosystem level impacts of heatwaves observed in the rocky intertidal zone.

Chapter III: Field acclimatization of adults influences offspring performance in the rocky intertidal mussel, Mytilus californianus

In this field-to-laboratory experiment, I investigated transgenerational plasticity in nature by examining how parental exposure to differing thermal regimes that exist across tidal height and season impact offspring phenotype. Here, *M. californianus*, were collected from mussel beds in the high and low zone of the rocky intertidal following the summer and winter season. Adults were spawned in the laboratory and sets of offspring were then reared at cool (16°C) and warm (20°C) temperatures to further examine the influence of developmental temperature on larval performance. At the veliger stage, physiological performance of offspring was assessed. Results from this study show that seasonal acclimatization and larval developmental temperature had prevailing effects on offspring performance. Larvae from high and low mussels collected after winter were generally larger, had higher thermal tolerance, and lower respiration rates than those collected after summer when reared at the same temperature. In terms of developmental temperature, larvae reared in the warm treatment we larger, had higher metabolic rates, and lower thermal tolerance when compared to those reared in the cool treatment. These results support the idea that parental acclimatization can impact offspring performance, however, differences in adult thermal exposure at the microhabitat scale may not be the main driver. While laboratory studies have increased our understanding of these transgenerational acclimation processes, field studies such as this provide insight into how these processes function in natural populations.

Chapter IV: Transgenerational plasticity as a mechanism of response to marine heatwaves in the purple sea urchin, Strongylocentrotus purpuratus

To study the effect of thermal stress from MHWs during gametogenesis in *S. purpuratus* and further, whether MHWs might induce transgenerational plasticity (TGP) in thermal tolerance of progeny, adult urchins were acclimated to two conditions in the laboratory – a MHW temperature of 18°C and a non-MHW temperature of 13°C. Following a four-month long acclimation period (October–January), adults were spawned and offspring from each parental condition were reared at MHW (18°C) and non-MHW temperatures (13°C), creating a total of four embryo treatment groups. To assess transgenerational effects for each of the four groups, I measured thermal tolerance of hatched blastula embryos in acute thermal tolerance trials. Embryos from MHW-acclimated females were more thermally tolerant with higher LT_{50} values compared to progeny from non- MHW-acclimated females. Additionally, there was an effect of female acclimation state on offspring body size at two stages of development - early gastrula and prism. To assess maternal provisioning as means to also alter embryo performance, I examined gamete traits from the differentially acclimated females, by measuring size and biochemical composition of eggs. MHW-acclimated females had eggs with higher protein concentrations, while egg size and lipid content showed no differences. These results indicate that TGP plays a role in altering the performance of progeny as a function of the thermal history of the female, especially when thermal stress coincides with gametogenesis.

II. Heatwave impacts on *Mytilus californianus* body temperature across latitude

Introduction

Heatwave impacts on intertidal organisms

Organisms inhabiting the rocky intertidal zone live at the intersection of terrestrial and marine environments and thus are exposed to complex thermal regimes. Here, thermal environments are highly variable, influenced by the tidal cycle, seasonal fluctuations in air and seawater temperature, and solar radiation. In addition to these temporal variables, spatial characteristics such as latitude and shoreline profiles can further increase variability (Helmuth et al., 2006). While intertidal organisms have adapted to withstand these thermally stressful conditions, many are currently living near their thermal limits (Somero, 2002). Thus, increases in temperature associated with heatwaves are of great concern for intertidal ecosystems. The overarching goal of this thesis chapter is to examine how thermal anomalies associated with marine and aerial heatwaves (hereafter referred to as MHWs and AHWs) are influencing the thermal experience of a foundation species in the rocky intertidal zone of the Pacific coast, *Mytilus californianus*.

dIn early studies of MHWs many focused on examining the impacts of these anomalously high seawater temperatures on subtidal and pelagic ecosystems (Smith et al., 2023). In recent years, however, studies investigating the effects of MHWs on intertidal ecosystems have emerged. These studies have predominantly focused on algal foundation species, as changes in their abundance can alter community structure. For example, in a study examining the effects of the 2014-2016 MHW on algae species along the Oregon coast, researchers found a decline in performance related to elevated temperatures (Spiecker and Menge, 2022). Similarly, in a study conducted as part of the Gulf Watch Alaska Long-Term

Monitoring program, researchers found a shift in community structure following the same heatwave. Where the intertidal ecosystem shifted from autotrophic macroalgae dominated system to a heterotrophic filter-feeder dominated system (Weitzman et al., 2021).

In addition to MHWs, AHWs can have similar if not greater impacts on intertidal species. Since intertidal organisms are exposed to aerial temperatures during emersion, AHWs where temperatures surpass thermal maxima of species have led to mass mortality events (Harley, 2008; Seuront et al., 2019). These mass mortality events have been documented when heatwaves coincided with midday low tides, often occurring at higher latitude sites along the Pacific coast (Helmuth et al., 2002). However mass motility events have been documented at lower latitudes as well (Harley, 2008). More recently, the unprecedented Pacific Northwest aerial heatwave, known as the Pacific Heat Dome, which occurred from June 25 to July 2, 2021, from British Colombia to Oregon had devastating impacts on rocky intertidal ecosystems of the Salish Sea. Here, temperatures surpassed 50°C, which is above the physiological tolerance limit of species, such as mussels, barnacles, oysters, inhabiting the area (Hesketh and Harley, 2023; White et al., 2023).

Long-term monitoring

As heatwaves are predicted to increase in intensity, frequency, and duration, (Oliver et al., 2019) understanding their impacts on critical species will be important to determining ecosystem response. To determine if periods of elevated temperature are in fact thermal anomalies for ecosystems, long-term data is required to make meaningful comparisons of temperature across time. Along the west coast of the United States (US), specifically, a collaborative effort between the Partnership for Interdisciplinary Studies in Coastal Ocean

Studies (PISCO), the Multi-Agency Rocky Intertidal Network (MARINe) and the Helmuth Lab at Northeastern University, has monitored important physical and biological parameters in the intertidal zone over the past two decades, and continues to this day. This substantial repository of long-term data allows us to not only determine how averages are changing over time but see the impacts of extreme climatic events.

Due to its role as a foundation species, scientists have long been interested in assessing temperature dynamics experienced by the California mussel, *Mytilus californianus*. Early studies employed traditional temperature loggers to record thermal regimes experienced by mussels, however these devices often did not match the body temperatures of live mussels. Thus, biomimetic temperature loggers, robomussels, were designed (Fitzhenry et al., 2004). These robomussels have been critical in aiding in our understanding of how climate patterns influence mussel body temperature and how these patterns change over time and space (Helmuth et al., 2002; Helmuth et al., 2006; Helmuth et al., 2016). Further these measurements allow us to make better predictions on the physiological performance of mussels in their natural environment (Seuront et al., 2019).

Summary of the study

Early studies conducted by Helmuth and colleagues examined temperature profiles experienced by *M. californianus* along their biogeographic rage (Helmuth et al., 2002; Helmuth et al., 2006; Helmuth et al., 2016). In this thesis chapter I revisit this now expanded dataset for sites along the US west coast (i.e., Oregon and California) and measure the impacts of heatwaves on body temperatures of *M. californianus.* The specific goals of this chapter were to 1) compare temperature profiles of mussel body temperature across ten degrees of latitude from northern Oregon to southern California, 2) determine the impact of MHWs on mussel body temperature, and 3) examine the impacts of an AHW, the Pacific Heat Dome, on mussel temperature.

As a brief summary of the results, I observed temperature differences influenced by site location and immersion/emersion conditions in relation to the occurrence of a MHW. Specifically, I found that monthly averaged mussel body temperatures across different sites showed distinct patterns. In terms of MHW effects, unsurprisingly, mussel body temperature was higher during MHW years. Increases in body temperature were related to increased temperature during immersion rather than emersion (i.e., exposure to seawater rather than air temperature). Temperature increases were higher for California sites than Oregon sites, and the onset of these elevated temperatures occurred earlier in California than in Oregon. Lastly, I examined the impact of the Pacific Heat Dome on the most northern sites in Oregon and found that recorded temperatures were within the naturally occurring range. Overall, these results provide meaningful insight into the thermal experience of a critical species in the rocky intertidal ecosystem. While prior studies have focused on heatwave impacts at the community level, it is rare that we are able to examine how these thermal events impact organism level factors such as body temperature. In all, these findings can help to explain ecosystem level impacts of heatwaves observed in the rocky intertidal zone.

Materials and Methods

History of data collected

Temperature data analyzed in this chapter was collected through a collaborative effort between, me (Hofmann Lab), the Helmuth lab, and MARINe. Data prior to 2015 was collected by the Helmuth Lab at Northeastern University. Following 2015, numerous sites were decommissioned, leading to a gap in the dataset until loggers were redeployed in 2021 (some sites in California were maintained between this period). In 2021 due to the production of a new robomussel design, and a collaboration between myself and MARINe, robomussels were redeployed at Oregon sites. When loggers were redeployed at each site, special attention was taken to place loggers in the same locations as the original, however exact replacements were not often feasible (see Table 1). Thus, to not confound differences in temperature that may be related to different placements in the mussel bed, when examining heatwave impacts on mussel body temperature, I analyzed data collected prior to 2015 separate from data collected after when making temporal comparisons.

Field sites

To assess temperature dynamics experienced by mussels the along west coast of the United States, temperature data was collected and analyzed from six sites in Oregon and California which included: Fogarty Creek (FC), Boiler Bay (BB), Strawberry Hill (SH), Bodega Marine Reserve (BD), Lompoc Landing (LL), and Coal Oil Point (CP) (Figure 1). These sites were selected not only for their coverage *M. californianus'* biogeographic range, but because many are historically important for experimental intertidal biology. Temperature data collection began in 2000 for most sites excluding FC which had intermittent sampling from 2006-2023.

Robomussel design and instrument deployment

Temperature data was collected from two equally functional designs of the biomimetic logger: (1) the original robomussel design, and (2) an updated robomussel design which was produced in 2019. The original design made by the Helmuth lab, contained a TidbiT logger (TB132-20+50 and UTBI-011; Onset Computer Corporation, Pocasset, MA) or a HOBO TidbiT v2 Water Temperature Water Temperature Data Logger (UTBI-001; Onset Computer Corporation, Pocasset, MA) encased polyester resin (Evercoat Premium Marine Resin, Illinois Tool Works, Inc.). The updated robomussel design, contained a T7.3 EnvLogger in acrylic resin and is produced by the biotechnology company Electric Blue (Portugal). The updated version facilitated collection of data since loggers could now be downloaded in the field with Near Field Communication technology. Whereas with the original design, loggers needed to be collected and downloaded in the laboratory. From 2000-2019 the original design was employed, following 2019 all original robomussels were replaced with the updated version.

Robomussels were be deployed as described in Helmuth et al. (2016) with minor modifications. Each site was equipped with approximately 6 loggers. Two loggers were adhered to the rocky substrate in the high, mid, and low zone of the mussel bed. Placement at the different locations would account for differences in temperature based on tidal height. Special care was taken to ensure that loggers were surrounded by mussels, since solitary loggers have shown to have anomalously high temperature readings. All loggers were adhered to rock substrate using Z-SPAR Splash Zone Epoxy.

Approach to data analysis

It is important to note that some sites vary in completeness of long-term data (i.e., some have larger gaps than others), therefore special consideration was taken to make sure temperature comparisons between sites and through time were made accurately. With this in mind, analyses in this thesis chapter focused on temperature collected in the mid zone of the mussel bed, as this dataset was the most complete and is the most representative of temperature experienced by mussels across the entire mussel bed. Placement of mid zone loggers ranged from 0.65 – 2.75 above mean lower low water (MLLW) level (Table 1).

Annual patterns across latitude

General observations of temperature data were made across sites, which included mean and maximum daily temperature. To further compare temperature profiles across sites, annual cycles were determined by calculating monthly averaged mean and maximum daily temperatures for the entire time series (i.e., 2000-2023 when data was available). Each daily average included 5-17 years of data. To measure similarity or dissimilarity across sites, a cross-correlation analysis on monthly averaged mean and maximum daily temperature was conducted.

Analysis of MHW impacts on temperature

To determine the influence of MHWs on mussel body temperature, average daily temperature recorded during MHW years (i.e., 2014 and 2015 when data was available) was compared to the climatological mean of data (i.e., averaged daily temperature from 2000-2013). To reduce excess variation in the data, daily temperature data was smoothed using a moving average window of 7. To examine how mean daily temperatures during MHW years compared to highest recorded daily averages prior to the MHW, days with a mean temperature in the 90th percentile were counted for each site and the percentage of these days occurring in 2014 and/or 2015 was calculated. Since data is only available for 2014 and 2015, for the remainder of this chapter I will refer to the heatwave as the 2014-2015 MHW when discussing results, even though the MHW extended into 2016.

Since mussel body temperature is influenced by seawater and air temperature, which can lead to differences in physiological responses, I sought to investigate if any observed differences during MHW years were due to changes in seawater or air temperature. Body temperature during immersion (i.e., seawater temperature) and emersion (i.e., air temperature), was separated using tide height measurements of robomussels and comparing those to predictions of tidal height collected at stations closest to each site (http://tbone.biol.sc.edu/tide/). Specifically, robomussels were determined to be immersed if predicted tide height exceeded the measured tide height for the highest deployed logger. Robomussels were determined to be emersed if predicted tide height was below the measured tide height for the lowest deployed logger.

Analysis of AHW impacts on temperature

In addition to examining the influence of a MHW, the impacts of Pacific Heat Dome (i.e., an AHW) on mussel body temperature was assessed. Here, temperature recorded during the eight-day heatwave (June 25 - July 2, 2021) was compared to the thermal maxima documented for mussels at each site. Since this heatwave occurred during the later deployment of robomussels, temperature comparisons were made for data collected only after April 2021. Only FC and BB were investigated since, these sites were in closest proximity to the area affected by the heatwave.

Results

Annual patterns across sites

Distinct differences in annual temperature patterns were observed across latitude (Figure 2). General observations showed an inverse relationship between mean daily temperature and latitude, a pattern that was not as evident for measurements of maximum daily temperature. To further compare temperature profiles across sites, annual cycles were determined by calculating monthly averaged mean and maximum daily temperatures. (Figure 3). Annual patterns of monthly averaged mean daily temperatures showed clear difference between Oregon (i.e., FC, BB, SH) and California sites (i.e., BD, LL, CP), where mussels from California experienced higher mean daily temperature than mussels from Oregon. In addition, there was a difference in the time of year that the peak temperatures were detected by the robomussels. For Oregon sites peak temperatures occurred between May and July, whereas for California sites peak temperatures occurred between July and October (Figure 3a).

In a comparison of monthly averaged mean daily temperature with monthly averaged maximum daily temperature, annual temperature patterns differed in their grouping. Annual patterns for BD were more similar to the Oregon sites than those recorded at LL or CP. For Oregon sites maximum daily temperatures where highest during June and July, whereas for LL and CP sites April and May were the months with the highest daily temperatures. A cross correlation analysis revealed that sites exhibited high correlations with regard to state, supporting general observations (Figure 4). Specifically, the three Oregon sites were highly correlated with one another regarding monthly average mean daily temperature. Similar patterns were observed for the three California sites. However, for monthly averaged maximum daily temperature, BD exhibited a higher correlation with Oregon sites than California sites.

MHW impacts on mussel body temperature

For each site, mean daily temperature during MHW years (i.e., 2014 and 2015) was compared to the climatological mean (2000-2013). Here, average daily temperature experienced by mussels was generally higher during MHW years than the climatological mean (Figure 5; Table 2). Across sites this increase in temperature ranged from 0.88 – 2.36°C. In a comparison of across latitude, California sites (i.e., BD, LL, and CP) experienced a greater increase in temperature than Oregon sites (i.e., FC, BB, SH) in 2014. Due to the lack of data for 2015, cross site comparisons were not conducted for BB, SH, and BD. A seasonal pattern was detected in the time series of robomussel temperature data. Elevated temperature was documented earlier for California sites, beginning in July whereas Oregon sites showed an increase in temperature in September (Figure 5). For all available data prior to 2016, days with a mean temperature in the 90th percentile were counted for each site. For BB, 16% of the counted days occurred in 2014. For SH, 11% of the counted days occurred in 2014. For BD, 23% of the counted days occurred in 2014. For LL, 23% and 20% of the counted days occurred in 2014 and 2015, respectively. For CP, 21% and 26% of the counted days occurred in 2014 and 2015, respectively.

The 2014-2015 MHW influenced mussel body temperature during immersion and emersion, however immersed robomussels showed greater variation from the climatological mean. Immersion temperature was isolated and mean daily temperature during 2014 and 2015 was calculated and compared to the climatological mean. Temperature patterns were similar to those observed in the combined dataset (i.e., immersion and emersion together), however average daily temperature was approximately 1-2 standard deviations above the climatological mean from July to December depending on the site (Figure 6). Contrastingly, emersion temperature fell mostly within one standard deviation of the mean or was slightly higher from July to December (Figure 7).

AHW impacts on mussel body temperature

Temperatures recorded during the Pacific Heat Dome were within the naturally occurring range for Oregon sites. Maximum daily temperature ranged from 19.4 - 28.0°C at FC and 17.7 - 35.9°C at BB during the AHW (Figure 8). These temperature values fell within the normal range recorded at FC and BB which are 8.9 - 37.3°C and 8.9 - 37.8°C, respectively.

Discussion

Summary of the study

In this thesis chapter I examined spatiotemporal patterns of *M. californianus* body temperature using long-term data collected with biomimetic loggers called robomussels, devices that have been used extensively in intertidal marine ecology (Helmuth et al., 2006; Seuront et al., 2019). Recent studies have reported changes in intertidal community composition following marine and aerial heatwaves along the Northeastern Pacific coast, changes that have been attributed in part to altered abundance of *M. californianus* itself (Weitzman et al., 2021). Using temperature data collected by robomussels, we can ascertain how thermal exposure of mussels was influenced by these heatwave events. This information can provide critical insight into mussel physiological performance during these thermal anomalies, supplementing studies examining ecosystem response. Thus, the overarching goals of this thesis chapter were to 1) examine annual temperature patterns of mussel body temperature across latitude, 2) investigate the impact of MHWs on mussel body temperature, and 3) determine the extent to which a recent AHW in the Pacific Northwest was captured by robomussels deployed on the Oregon coast.

Annual patterns across latitude

Results from the present analysis of annual patterns were similar to those previously documented in the literature (Helmuth et al., 2006). Here, I found an inverse relationship between mean daily temperature and latitude, a pattern that was not as evident for measurements of maximum daily temperature (Figure 2 and 3). These results support those reported in Helmuth et al. (2006), a comprehensive analysis of mussel body temperature across the west coast of the United States. While this may seem counterintuitive, because often temperature is predicted to increase with latitude, the timing of midday low tides for northern sites leads to higher daily maxima experienced by *M. californianus* (Helmuth et al., 2002).

This study further examined annual patterns summarized as monthly averaged mean and maximum daily temperature (Figure 3). For each site, available data to calculate these summary statistics ranged from 5-17 years, providing a robust analysis of temporal patterns.

While these results may not provide specific information on lethal thermal anomalies experienced by *M. californianus* (i.e., specific instances when temperature exceeds maximum thermal thresholds), they can provide insight into how thermal patterns influence mussels on long-term scales. This information can be useful in determining how temperature may be related to the phenology of important life history traits such as gametogenesis, spawning, and recruitment. For example, previous studies have documented differences in timing of spawning and recruitment between mussels in Oregon and California (Broitman et al., 2008), where spawning is highest from June-July in Oregon and occurs year-round in California (Strathmann, 1987; Broitman et al., 2008). Similarly, results from this study showed distinct differences in annual temperature patterns across Oregon and California (Figure 3 and 4). Together these studies may suggest that temporal patterns experienced by mussels may influence reproductive capacity or timing of spawning, however further research investigating this potential relationship is needed. Lastly if temperature does influence the phenology of important life history events in *M. californianus*, as with other species (Scranton and Amarasekare, 2017), knowledge of these natural patterns is critical if we are to understand whether prolonged climactic events such as the 2014-2015 MHW have the potential to shift the timing of these events in natural populations.

Impacts of MHWs on M. californianus body temperature

With this in mind, the second goal of this study was to investigate the impact of the 2014-2015 MHW on *M. californianus* body temperature. Here I found that *M. californianus* experienced higher temperatures relative to the climatological mean during 2014 and 2015. The average increase ranged from 0.75 - 2.66 °C in 2014 and 2015 (Table 2). However, due to missing data, predominantly for 2015, these values may not be entirely representative of the impact of heatwave events. Temperature increases were higher for California sites than Oregon sites, and the onset of these elevated temperature occurred earlier in California than in Oregon. Examination of the 90th percentile of mean daily temperature across the dataset revealed that during MHW years California sites experienced a higher percentage of its warmest days compared to Oregon sites. To further determine how thermal profiles were altered during the MHW, periods during robomussel immersion and emersion were separated and analyzed independently. Here I found that deviation from the mean was higher for mussels during immersion than emersion (Figure 6 and 7). Together these results provide useful information to better understand how MHWs could influence *M. californianus* physiological conditions.

Temperature effects on *M. californianus* performance have been the center of numerous studies (Zippay and Helmuth, 2012). Such studies have examined changes in metabolic rate, filtration rate, growth, and reproductive capacity (Bayne et al., 1976; Blanchette et al., 2007; Petes et al., 2008). Generally, elevated temperatures during immersion increases filtration rate leading to mussel growth when food is available (Bayne et al., 1976; Blanchette et al., 2007). However, growth has been documented to decrease when temperature exceeds 20°C for some populations (Coe and Fox, 1942). In this regard, if food remains available, increased temperature associated with MHWs may not negatively impact populations that do not exceed their thermal limits. This idea would support the increased mussel cover observed following the 2014-2016 MHW in the Gulf of Alaska and British Columbia (Weitzman et al., 2021; Whalen et al., 2023). However, it is important to note that these changes in abundance may also be due to other factors such as increased recruitment, a decrease in competition for space, and reduced predation rates (Weitzman et al., 2021; Traiger et al., 2022; Whalen et al., 2023). While elevated temperatures associated with MHWs may positively impact sites where *M. californianus* are exposed to temperatures well below their upper thermal limits, this may not be the case for those inhabiting warmer regions. Of all the sites examined in this study, mussels at the most southern site in California, CP, experienced prolonged temperatures above 20°C during MHW years. Conditions such as these may have contributed to the decrease in mussel cover at sites along the southern California coast (Miner et al., 2021). Thus, susceptibility to MHW conditions may depend on whether *M. californianus* are inhabiting areas where conditions are currently near physiological limits.

Impacts of AHWs on M. californianus body temperature

The final objective of this study was to determine the extent to which a recent AHW, the Pacific Heat Dome, was captured by robomussels deployed at the Oregon sites. Here I found that recorded temperatures were within the naturally occurring range for the study sites. For FC and BB that is 8.9 - 37.3°C and 8.9 - 37.8°C, respectively (Figure 8). Interestingly, temperatures regularly documented at both FC and BB, were above the lethal limits reported for *M. californianus* (36-38°C) (Denny et al., 2011). My initial thoughts were that these observed temperatures may not be representative of the actual temperatures occurring in the mussel bed as they were recorded during early deployment of the robomussels. However, these patterns were also observed during the initial deployment of robomussels (2000-2014). The discrepancy between the reported lethal limit value and the persistence of live mussels, may be due to fact that this LT_{50} value was derived from mussels located in Pacific Grove, California. As mentioned previously, mussels at higher latitude sites are regularly exposed to temperature extremes, as summer low tides occur during midday. This difference in exposure could lead to differences in thermal acclimatization or local adaptation that could result in higher upper tolerance limits (Howells et al., 2012).

Conclusions

In this thesis chapter I provided new information on natural fluctuations of *M. californianus* body temperature across their biogeographic range and how they were influenced by recent marine and aerial heatwaves. Overall, my results show distinct differences in thermal patterns experienced by *M. californianus* across latitude. Moreover, results suggest that across latitude *M. californianus* vary in their susceptibility to MHW and AHW events. As heatwaves are predicted to increase in intensity, frequency, and duration (Oliver et al., 2021), understanding their impacts on the performance of critical species such as *M. californianus* will be important in determining ecosystem response (Miner et al., 2021).

Temperature data from biomimetic temperature loggers, robomussels, provided critical insight into organism thermal experience that is not accurately captured by conventional temperature sensors (Helmuth et al., 2016). Thus, I was able to make physiologically meaningful comparisons between *M. californianus* body temperature and measurements of abundance documented in prior studies. For other calcifying foundation species such as coral, this approach to physiological monitoring may be useful. As their morphological complexity may lead to body temperatures that are not accurately captured by conventional temperature sensors. Lastly, recognition of natural patterns and anomalies was possible due to the long-term nature of the dataset which highlights the importance of long-term data for assessing the impacts of climate change on species response.

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I would like to thank my collaborator Dr. Gretchen Hofmann. I am thankful for Avery Parsons-Field, Dasun Hemachandra, Aida Loza, Adriane McDonald, Amelia Ritger, Alexis Reynoso, Leeza-Marie Rodriguez, and the Menge Lab at Oregon State University who assisted in fieldwork and collection of the data. Finally, I thank Drs. Erika Eliason and Nick Nidzieko for feedback provided in the analysis of the data and writing of this chapter. During this study, I was supported by an NSF Graduate Research Fellowship and the UCSB Graduate Division Gradate Opportunity Fellowship. This research was supported by funding from the Schmidt Family Foundation.

Figures & Tables

Table 1. Average tide height (reported as height above mean lower low water [MLLW]) for mid zone robomussel deployments. Original robomussels are those deployed prior to 2015, new robomussels are those deployed in 2019 or later.

Site	Original	New
Fogarty Creek	1.48 m	2.75 m
Boiler Bay	1.20 m	2.28 m
Strawberry Hill	1.08 m	3.38 m
Cape Blanco	-	2.43 m
Bodega Marine Reserve	1.22 m	-
Lompoc Landing	0.65 m	1.32 m
Coal Oil Point	0.66 m	1.32 m

Table 2. Average ± standard deviation increase in temperature recorded during MHW years (i.e., 2014 and 2015) compared to climatological mean for each site.

Site	2014	2015
Fogarty Creek	1.34 ± 1.47	1.78 ± 1.41
Boiler Bay	1.18 ± 1.53	-
Strawberry Hill	0.75 ± 1.59	-
Bodega Marine Reserve	1.36 ± 1.30	-
Lompoc Landing	1.61 ± 1.43	1.54 ± 1.66
Coal Oil Point	1.38 ± 1.30	2.66 ± 1.60

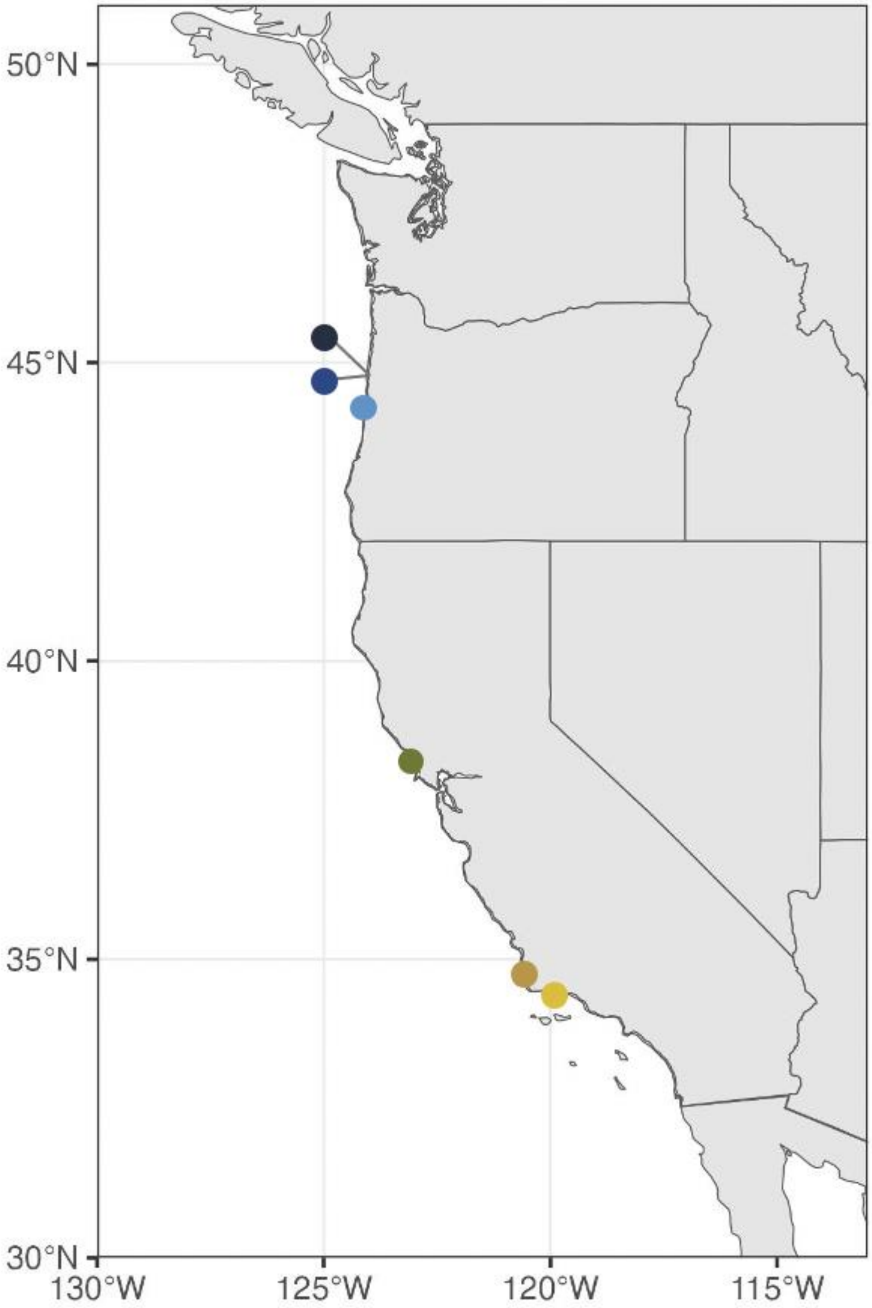

Figure 1. Map of rocky intertidal sites used in temperature analysis. From north to south, Fogarty Creek (FC), Boiler Bay (BB), Strawberry Hill (SH), Bodega Marine Reserve (BD), Lompoc Landing (LL), and Coal Oil Point (CP).

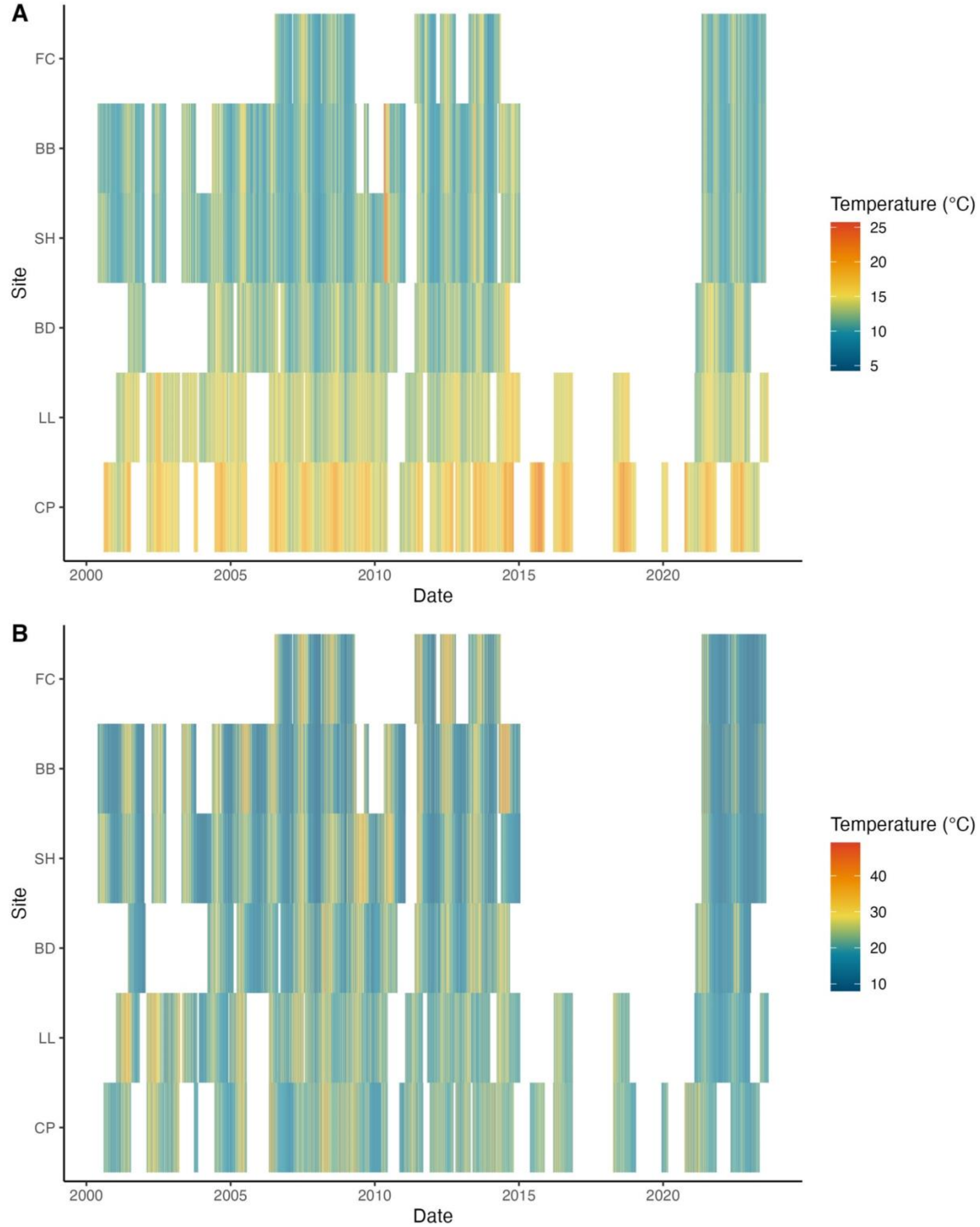

Figure 2. Heat map displaying A) mean and B) maximum daily temperature for all sites. Temperature was collected using robomussels in the mid zone of the mussel bed. White regions are periods when temperature data was not collected.

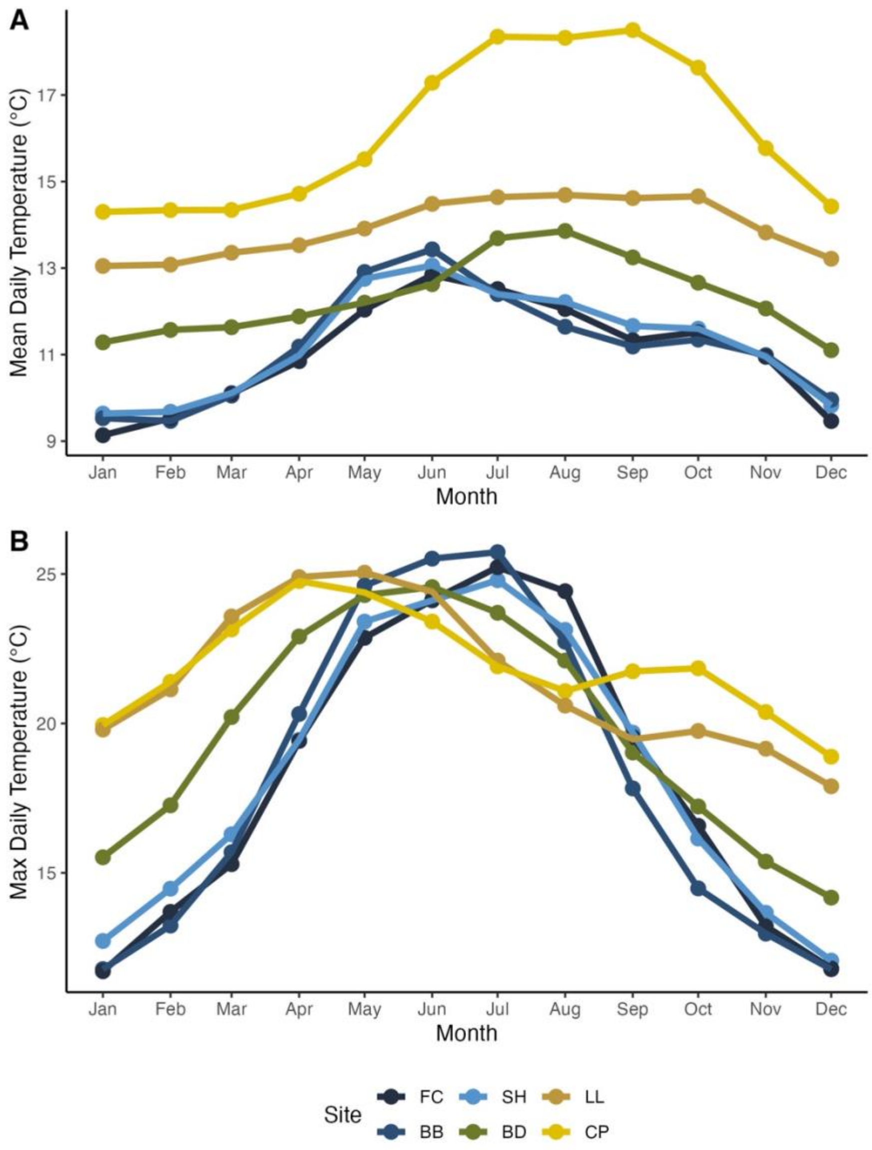

Figure 3. Monthly averaged A) mean and B) maximum daily temperature, for 2000-2023 when data was available.

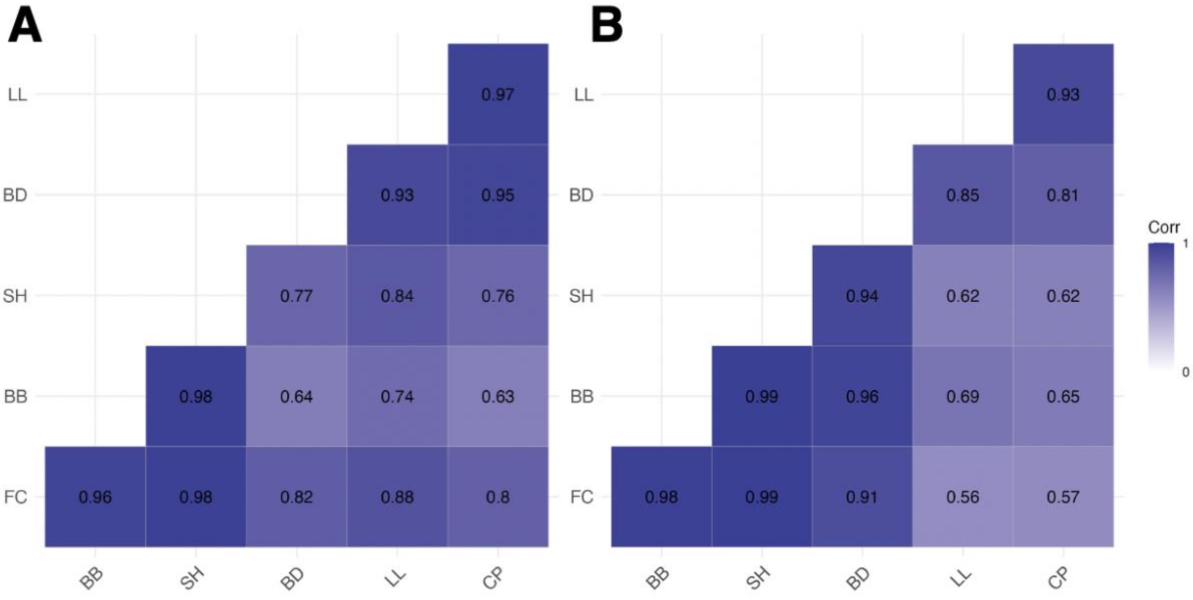

Figure 4. Cross-correlation matrix displaying the relationship between each site for monthly averaged A) mean and B) maximum daily temperature.

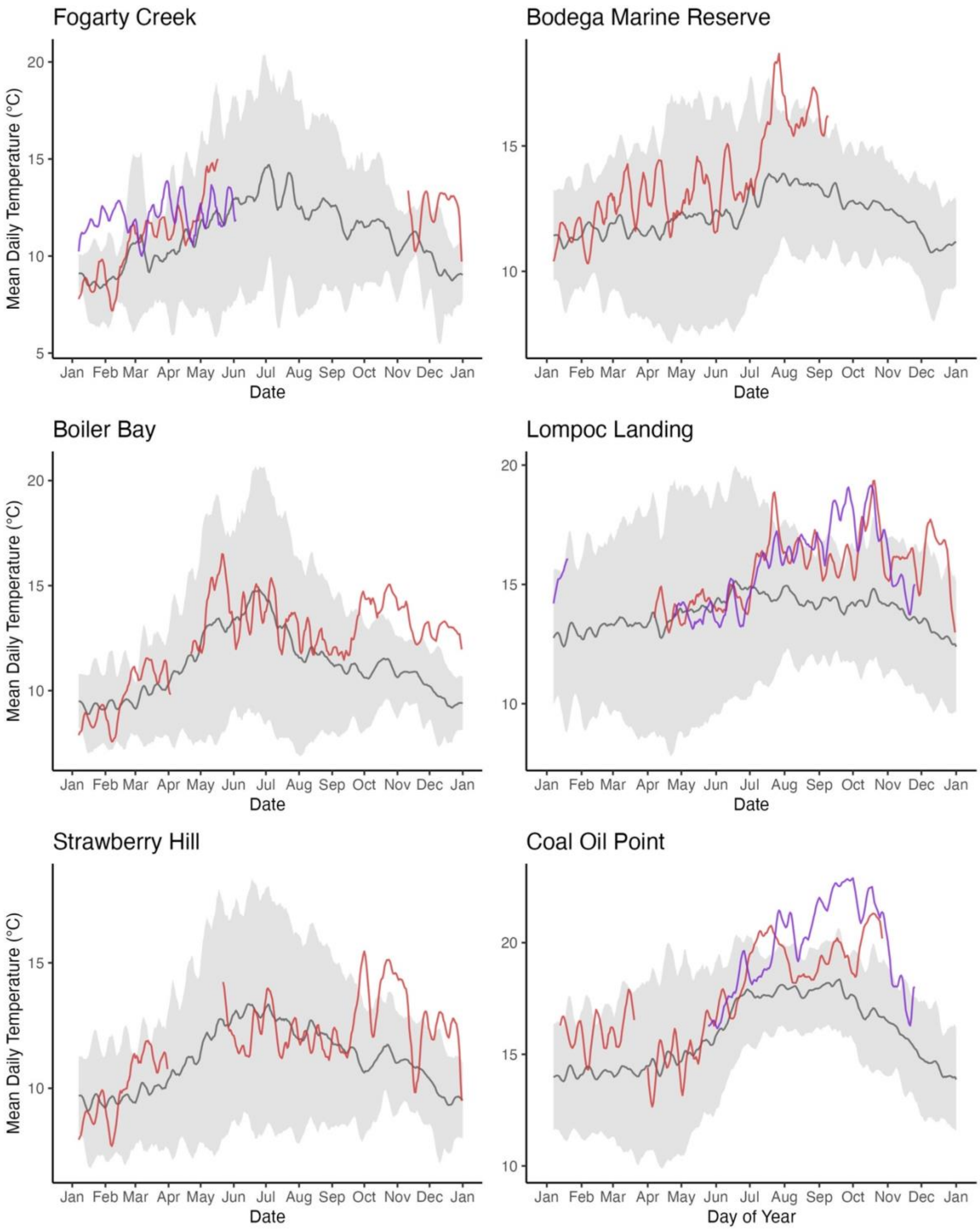

Figure 5. Temperature recorded by robomussels prior and during MHW years for each intertidal site. The red and purple lines represent 2014 and 2015, respectively; the grey line represents the climatological mean (i.e., 2000-2013 when data is available). Shaded area represents ± standard deviation.

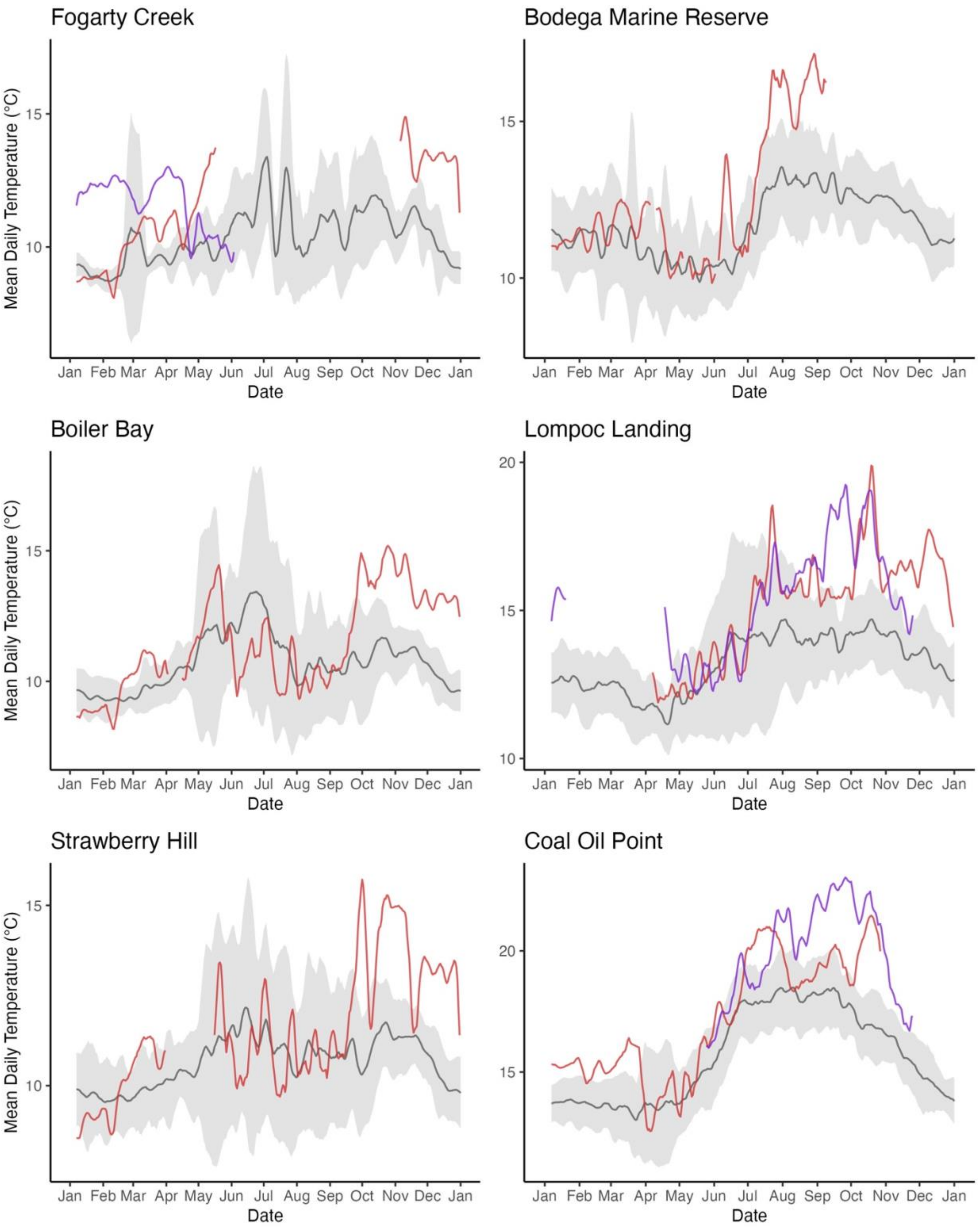

Figure 6. Temperature recorded by *immersed* robomussels prior and during MHW years for each intertidal site. The red and purple lines represent 2014 and 2015, respectively; the grey line represents the climatological mean (i.e., 2000-2013 when data is available). Shaded area represents ± standard deviation.

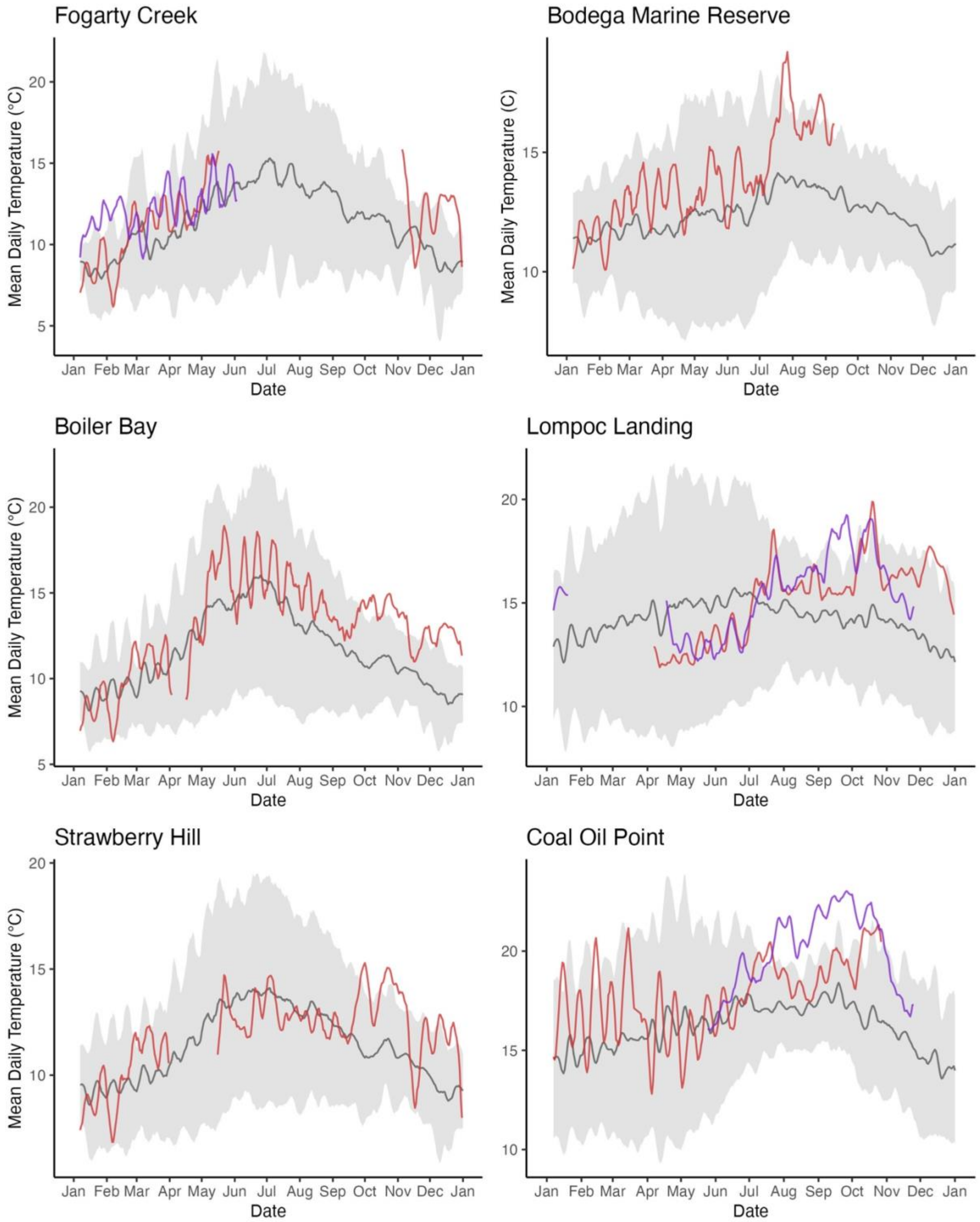

Figure 7. Temperature recorded by *emersed* robomussels prior and during MHW years for each intertidal site. The red and purple lines represent 2014 and 2015, respectively; the grey line represents the climatological mean (i.e., 2000-2013 when data is available). Shaded area represents ± standard deviation.

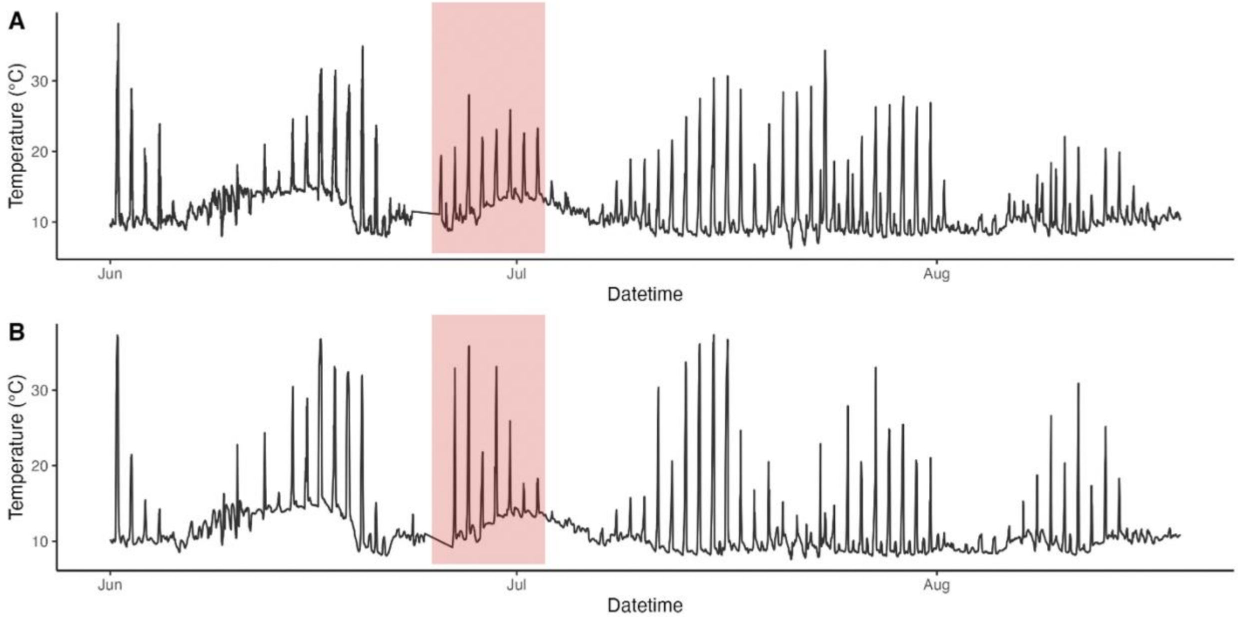

Figure 8. Temperature recorded at A) FC and B) BB, from June 2021- September 2021. The is Pacific Heat Dome highlighted in red.

III. Field acclimatization of adults influences offspring performance in the rocky intertidal mussel, *Mytilus californianus*

Introduction

While the number of transgenerational plasticity (TGP) studies on marine species has steadily increased and improved our understanding of the role these processes may play in response to anthropogenic climate change, most studies have been conducted in the laboratory (Byrne et al., 2020). TGP studies conducted in controlled laboratory settings are critical as they allow researchers to narrow in on specific drivers of interest (i.e., temperature, pCO_2, food availability), however, information gained from these studies is not a complete representation of how these processes function in nature. Natural populations experience a mosaic of environmental variables that change over time and space (Helmuth et al., 2006; Hoshijima and Hofmann, 2019). Thus, if we are to accurately predict how TGP may influence organismal response to climate change, field-based studies are needed. In this chapter, I conducted a field-to-laboratory experiment to investigate how thermal exposure to natural varying conditions influences TGP in field acclimatized *Mytilus californianus*.

TGP studies in the field and laboratory

Field studies examining TGP under naturally occurring abiotic conditions that differ across time and space have provided critical insight into how parental exposure may impact offspring response to climate change associated stressors. For example, in a study on Atlantic silverside (*Menidia menidia*) researchers found that early developing offspring were more sensitive to high pCO_2 conditions when spawned earlier in the spawning season than those spawned later, which became increasingly tolerant to elevated pCO_2 conditions. Increased tolerance to elevated pCO_2 conditions by offspring corresponded to pCO_2 experienced by adults in the wild, suggesting that parents could seasonally condition offspring to acidified conditions (Murray et al., 2014). Spatial variation in acclimation environments can also impact TGP, for example in a study on *Strongylocentrotus purpuratus,* researchers found that offspring from urchins held outside of a kelp forest had higher thermal tolerance than those from parents held inside a kelp forest during their period of gametogenesis (Kozal and Hofmann, in revision). Since physiochemical properties of seawater differ among these sites (i.e., inside and outside of the kelp forests), it is predicted that adult exposure to these differing conditions could have induced a transgenerational effect altering offspring performance. Field-based studies such as these providing evidence for the occurrence of TGP in nature, together with laboratory studies examining its underlying mechanisms are critical areas of marine science research as this will help determine the role that TGP will play in species response to global climate change.

For bivalve species, numerous studies have examined TGP with most investigating the effects of ocean acidification in a laboratory setting. Several studies have reported positive effects where progeny from adults conditioned to acidified conditions show lower sensitivity to acidified conditions than progeny from adults held at control conditions (Parker et al., 2015; Spencer et al., 2020). However, in a recent meta-analysis looking at the effects of pH, researchers found that overall parental conditioning to acidified conditions produced offspring that were still negatively affected by acidification (Byrne et al., 2020). But, for some traits such as growth, the degree to which progeny were negatively affected was reduced when adults were conditioned to low pH conditions (Byrne et al., 2020). In terms of field-based studies, researchers examining TGP in *M. californianus* found that offspring thermal tolerance decreased in relation to parental exposure to thermal stress associated with tide height and season (Waite and Sorte, 2022). While the quantity of studies examining TGP in response to acidification has allowed us to make generalizations, studies on other climate change stressors (i.e., changes in temperature, dissolved oxygen, and salinity) and/or in field conditions remain understudied.

In this study I investigated TGP driven by field acclimatization in natural populations of rocky intertidal mussels, *M. californianus*. Across tidal height in the intertidal zone, and subsequently mussel beds, thermal gradients exist due to the tide cycle (Denny et al., 2011; Zippay and Helmuth, 2012). At the upper edge, organisms are emerged during low tide exposing them to air temperature, solar radiation, and wind, often leading to higher and more variable temperatures. At the lower edge, organisms spend more time immersed, thus experiencing lower and less variable temperatures (Helmuth et al., 2016). Because the timing of low tides changes throughout the year, thermal exposure of intertidal organisms is additionally affected by seasonal changes in aerial and seawater temperature. In *M. californianus* temperature exposure across tidal height and season has been shown to impact within generation acclimatory responses at the organismal to molecular level (Roberts et al., 1997; Place et al., 2012; Moyen et al., 2019), thus, for this thesis chapter, I was interested in examining if this acclimatory response could extend to the next generation.

Summary of the study

Using the rocky intertidal ecosystem as the study system not only allowed me to investigate TGP in natural populations, but as deleterious heatwaves are becoming more prominent in the rocky intertidal zone (Seuront et al., 2019), differences in thermal exposure provide a space-for-time approach to understanding TGP under climate warming. Here, I narrowed in on temperature acclimatization, with regard to season and tidal height, as a diver of TGP in *M. californianus.* To characterize temperature profiles experienced by adult *M. californianus* I collected high frequency *in situ* measurements using biomimetic temperature loggers located at high and low zones of the mussel bed over the course of the adult acclimatization (Helmuth et al., 2016). Using temperature data to inform collection dates, adult *M. californianus* were collected following the summer and winter season. Because seawater temperatures are warming (Oliver et al., 2021), I was further interested in investigating how temperature experienced by developing larvae impact performance. Thus, offspring from each adult acclimatization treatment (i.e., season-zone) were reared in cool (16°C) and warm (20°C) temperature conditions, allowing me to examine the influence of parental and developmental conditions on larval performance. At the veliger larvae stage, physiological performance of offspring was assessed via measurements of body size, respiration rate, and thermal tolerance. Lastly, to gain insight as to whether differences in maternal provisioning may be a contributing factor to offspring performance, egg size was measured for spawned eggs from females in each acclimatization treatment. Based on findings from prior studies on TGP and IGP on *M. californianus*, my general hypotheses were that 1) adults acclimatized to higher temperatures (i.e., high zone and/or summer acclimatized) would produce less thermally tolerant progeny, 2) larvae reared at warmer developmental temperature would be more thermally tolerant those reared at cool temperatures and 3) eggs from adults acclimatized to higher temperatures would be smaller in size.

Materials and Methods

Measurements of adult mussel temperature

To accurately characterize environmental temperature experienced by intertidal mussels across tidal height and season, robomussels (ElecticBlue, Portugal) were deployed at the upper and lower edge of the mussel bed (henceforth known as the high (H) and low (L) zone) at the rocky intertidal field site, Lompoc Landing, CA. High and low zone loggers were deployed at approximately 2.74 m and 1.13 m above mean lower low water (MLLW), respectively. Temperature loggers were programmed to record temperature at 30 min intervals at a resolution of 0.1°C for a year from April 01, 2021 – March 30, 2022.

To assess differences in temperature exposure across tidal height over the course of the year, daily mean, daily maximum, and daily range in temperature were averaged monthly for high and low zone robomussels. Additionally, overall mean temperature and range in temperature of each adult acclimatization treatment (i.e., season-zone) was also calculated. Each season was defined as the three months prior to each adult collection. Heat stress across tidal height and season was also estimated. Using temperature values documented in the literature, heat exposure was classified as either “moderate” or “severe” heat stress. Moderate heat stress was defined as temperature exposure between 23-30°C, as this temperature range coincides with the onset of a heat shock response in *M. californianus* (Roberts et al., 1997). Exposure above 30°C was defined as severe heat stress as these temperatures have induced death in *M. californianus* (Denny et al., 2011; Waite and Sorte, 2022).

In terms of reproductive timing, *M. californianus* inhabiting the shores of California are good candidates for examining how environmental conditions experienced during gametogenesis influence gametes and offspring performance. In California, mussels have been documented to spawn year-round, producing gametes in relatively short time intervals (i.e., within a month) (Coe and Fox, 1942; Young, 1942; Phillips, 2007a). Thus, I am confident that gametogenesis occurred during the adult acclimatization window (i.e., three months prior to collection) described in this study.

Mussel collection and spawning

To determine how adult acclimatization associated with tidal height and seasonal temperatures impacts gametes and offspring performance, adult *M. californianus* from the high and low zone were collected during a low tide series on August 19, 2021, and March 30, 2022 (Figure 1). Hereafter, these collection dates will be referred to as summer and winter, as they correspond to the seasonal acclimatization experienced by adults prior to collection. For each collection date, forty adult mussels were collected from each zone then transported to the University of California, Santa Barbara, where mussels were held overnight in a flow-through seawater table at ambient temperature. Following the 24 hr acclimation adults were spawned. Mussels were collected under the California Department of Fish and Wildlife Scientific Collecting permit #SC-210480009.

Mussels were induced to spawn via “mechanical stimulation”, which involved scraping and removing all epifauna attached to the shells, then being placed in a bucket, and shaken for approximately 2 min. Mussels were then rinsed and placed communally in plastic containers containing UV sterilized 0.35micron filtered seawater (FSW). Mussels collected from each zone (i.e., H and L) were maintained in separate plastic containers. Once a mussel began to release gametes it was promptly removed, rinsed with fresh and FSW, and placed in an individual beaker. Males were placed in a dry beaker to prevent sperm from becoming active until time of fertilization. Spawning females were placed in beakers containing FSW. As spawning terminated, individual mussels were removed from each beaker.

Larval culturing and sampling

For analysis of egg traits and larval culturing, five females with the highest quality eggs were selected from each adult acclimatization treatment. Adult acclimatization treatment refers to the conditions experienced by the parents *in situ*, that is, whether mussels were collected following the summer or winter season from the high or low zone. In total, eggs from twenty females were supplied over the course to experiment (Figure 1). Quality of eggs and sperm were assessed visually using a compound microscope. For each larval culturing run (i.e., summer and winter), eggs from high and low females were pooled and fertilized by a single low zone male. Since this experiment focused on the influence of maternal acclimatization on TGP, only one sire was chosen to reduce differences in offspring phenotype that may be attributed to genetic diversity. Pooled eggs from each acclimatization treatment were gently mixed with dilute activated sperm. When 80% fertilization was observed, embryos were deposited in larval culturing containers stocked at 90,000 embryos per container.

Larval culturing containers were maintained at a cool (C: ~16°C) or warm temperature (W: ~ 20°C), creating a total for four treatment groups for each seasonal collection. The treatment groups are denoted as HW, HC, LW, LC, where the first letter indicating the collection zone (i.e., high vs low zone) and the second letter indicating the developmental rearing temperature (i.e., C vs W). Each of the four groups were raised in three replicate culturing containers (Figure 1). The culturing container consisted of two nested 19L buckets. The inner bucket, which held the developing embryos, had mesh (35micon) covered cutouts which allowed incoming sea water to leave the bucket without losing the embryos. Culturing containers were held in a seawater table, acting as a water bath, to maintain temperature. FSW, which was first supplied to a reservoir tank to come to temperature, was supplied to each container via a drip irrigation system at a flow rate of 6L/hr. The temperature of the seawater table was controlled using a Delta Star® heat pump with a Nema 4x digital temperature controller (AquaLogic, San Diego, CA, USA).

Embryos developed at experimental temperatures until they exhibited distinguishing characteristics of a D-stage veliger. That is, the body became laterally compressed, with a bivalved shell and a straight hinge (Strathmann, 1987). At this stage, larvae were sampled for analysis of body size, respiration rate, and thermal tolerance. Sampling occurred at approximately 34 and 46 hours post fertilization (hpf) for warm and cool acclimated larvae, respectively.

Egg and larval morphometrics

Promptly following spawning, 500 eggs from each female (N=20) that contributed to larval cultures were collected for analysis of egg size. Eggs were preserved in 2% formalin in FSW, which was achieved by adding an equal volume of 4% formalin to the sample volume. Preserved eggs were promptly photographed for morphometric analysis using a compound microscope (Olympus BX50) with an attached digital camera (Motic 10 MP and Motic Image Plus software). Images were measured using ImageJ (National Institutes of Health, USA) and calibrated with stage micrometer. Between 30-35 eggs were measured from each contributing female. Eggs size was assessed by measuring average egg diameter and two-dimensional (2D) area. Diameter was calculated by averaging the length of three diameters for each egg. Area was measured and calculated by tracing around the perimeter of each egg. For veliger larvae, 500 larvae from each culturing container were collected for analysis of body size. Larvae were preserved and photographed using the same protocol used for eggs. Photographs were taken of larvae positioned laterally. As a proxy for body size, hinge length and 2D area was measured for approximately 30-35 veliger larvae from each culturing container.

Respirometry

To examine metabolic differences amongst larvae with differing thermal histories, oxygen consumption rates for each treatment were measured. Respiration rate was measured using methods described in Marsh and Manahan (1999), with some modification. For each culturing container (*N*=24), a subset of larvae were placed in microscale Biological Oxygen Demand (µBOD) vials (684–795 µL) containing FSW, at a range of concentrations (approximately 150, 300, 450, 600, 750). All vials were incubated at temperatures corresponding to larval developmental rearing temperature for each treatment (i.e., 20°C or 16°C) for 4.5-7 hrs. In addition, a “blank” vial for each of the container replicates per treatment was filled with only FSW and used as a control to account for any background respiration. Oxygen concentrations of each vial were measured following the incubation time. At the end of the incubation, each µBOD vial was briefly spun down, and seawater was transferred using a gastight syringe (Hamilton Company, USA) to an optode containing a fiber-optic oxygen meter (Micro TX3; PreSens, Germany). Prior to taking measurements the optode was calibrated using sodium sulfate (Na_2SO_3) and aerated FSW. For each culturing container a standard curve was generated from which oxygen consumption rate per individual was estimated. Additionally, for each adult acclimatization treatment, the temperature coefficient (Q_{10}) was calculated for respiration rate, using the formula

$$Q_{10} = \left(\frac{R_2}{R_1}\right)^{\frac{10}{T_2 - T_1}}$$

which represents the factor by which the rate (R) of a reaction increases when temperature is raised by ten degrees.

Thermal tolerance, LT_{50}

Upper thermal limit of veliger larvae was determined via an acute thermal tolerance trial. The thermal tolerance trail was conducted following methods described in Wong and Hofmann (2020), with some modification. Briefly, using a gradient heat block, vials containing 3.5mL of FSW were brought to nine temperatures (~28.9, 29.7, 31.1, 32.0, 33.0, 33.9, 35.0, 36.0, 37.4 °C). One thousand larvae in 0.5mL of FSW, from each treatment, were added to each vial. Larvae from replicate culturing containers in each treatment were pooled so that each container contributed equally to the thermal tolerance trial. Vials containing larvae were held at temperature for 1 hr. Following the 1 hr exposure, the first 100 larvae sampled from each vial were scored for survival. Larvae were scored as alive if any swimming or cilia movement was observed using a compound microscope. LT_{50} (the temperature at which the population experiences 50% mortality) was calculated for each treatment.

Statistical analysis

All statistical analyses were conducted in R studio (vers. 2022.12.0+353). The influence of adult acclimatization (i.e., zone and season) and larval developmental rearing temperature on gamete and offspring phenotype were examined with linear mixed effect models or generalized linear mixed effects models dependent on the data structure, using the *lme4* package (Bates et al., 2015). Model selection between full and reduced models, was performed using Schwarz information criterion (SIC, also called BIC), where the model with the lowest SIC value was assigned the best fit model. However, all models with ΔSIC < 7 were considered (Jerde et al., 2019). To ensure that models fit, model diagnostics were conducted using the *DHARMa* package.

A linear mixed effect model was used to determine the effect of adult acclimatization and larval developmental temperature on veliger body size, where zone, season, and developmental temperature were set as fixed effects and culturing container replicate was set as a random effect. A linear mixed effect model was used to determine the influence of adult acclimatization and larval developmental temperature on veliger larvae respiration rate where, zone, season, and developmental temperature were set as fixed effects and culturing container replicate was set as a random effect. A generalized linear mixed effect model was used to determine the interaction between temperature, adult acclimatization, and larval developmental temperature on larval survivorship, where zone, season, and developmental temperature were set at fixed effects and vial identity was set at a random effect. The binary data (alive vs. dead) were fit using a logit link. The LT_{50} values were calculated using a logistic regression (*MASS* package) for each treatment. Lastly, a linear mixed effect model was used to determine the influence of adult acclimatization on egg size, where collection zone and season were set as fixed effects and maternal identity was set as a random effect.

Results

Temperature data from the high and low collection sites

Using temperature data collected by robomussels deployed in the high and low zone of the mussel bed, I was able to characterize temperature experienced by adult mussels throughout the course of the acclimatization period. In general, the high site had a more extreme range of temperatures as compared to the low site. Recorded temperatures ranged from 3.7 - 41.1°C across the mussel bed over the course of the experimental period in the high zone, whereas the range in temperature recorded by low zone loggers was 6.6 - 27.8°C. In addition, the monthly averaged daily mean and daily maximum were greater following the summer acclimatization than the winter for high zone mussels. In contrast, daily range in temperature was similar following the summer and winter season (Figure 2). For low zone mussels, monthly averaged daily mean, daily maximum, and range in daily temperature did not differ following the summer and winter season (Figure 2). Mean, standard deviation, and range in temperature for each adult acclimatization treatment are presented in Table 1. In sum, the robomussel data indicated that the high and low adult mussels had different thermal histories during the acclimatization period, but the degree of variation was dependent on the season.

To capture this, I sought to express heat stress experienced high and low zone mussels via a heat stress index (Figure 3). For high zone mussels, cumulative exposure to moderate heat stress was 87.3% higher in summer than the winter while cumulative exposure to severe heat stress was 310.5% higher in the summer than winter. For low zone mussels, heat stress exposure was relatively low (i.e., less than 6 hrs). In the summer, mussels from the low zone experienced 4.5 hrs of moderate heat stress, whereas in the winter these mussels only experienced 1 hr of moderate heat stress. Low zone mussels did not experience severe heat stress in the summer or winter season.

Veliger body size

To examine the effects of adult and larval thermal history on offspring traits, body size was measured for D-stage veliger larvae. I found that larval body size was influenced by seasonal acclimatization of the adults and by larval developmental temperature, but not tide height. Hinge length and 2D area were measured as proxy for body size for larvae from each treatment (Figure 4; Table 2). Generally, larvae were larger following the winter season than the summer season at both developmental rearing temperatures (Figure 4; Table 2). Additionally, average body size was generally larger for veliger larvae reared at the warm (20°C) developmental temperature than those reared at the cool (16°C) temperature. The best fitting model for veliger hinge length included season and developmental temperature as fixed effects and container replicate as a random effect, where season (seasonWinter df=776.00, t=6.56, $p < 0.001$) and developmental temperature (developmentalTempW df=776.00, t=5.30, $p < 0.001$) had a significant effect on veliger length. The best fitting model for veliger area included season, developmental temperature, and their interaction as fixed effects and treatment replicates as a random effect, where developmental temperature (developmentalTempW df=776.00, t=2.84, $p = 0.005$) and interaction between season and developmental temperature had a significant effect on veliger area (seasonWinter:developmentalTempW *df*=776.00, *t*=4.08, $p < 0.001$).

Respiration rate

Seasonal acclimatization of the adults interacted with developmental temperature to affect respiration rate. To determine differences in metabolic performance based on adult acclimatization and developmental temperature, oxygen consumption rates were measured for larvae from each treatment. Due to a possible error in vial loading or O_2 measurement, one replicate from the winter-HW treatment was removed. Average individual respiration rate ranged from 5.94 – 11.63 (pmol O_2 h^{-1} individual^{-1}). Generally, larvae reared in the warm developmental temperature had higher respiration rates than those reared at the cool temperature (Figure 5). The highest respirations rate was recorded for larvae from summer-L acclimatized females reared at the warm developmental temperature. The best fitting model included season, developmental temperature, and their interaction as fixed effects and container replicates as a random effect, where only developmental temperature (developmentalTempW *df*=23.00, *t*=5.00, $p < 0.001$) and the interaction between season and developmental temperature (seasonWinter:developmentalTempW *df*=23.00, *t*=-2.48, $p = 0.021$) had a significant effect on respiration rate. For offspring from summer acclimatized mussels from high and low zone, calculated Q_{10} values were 3.97 and 3.31, respectively. For offspring from winter acclimatized mussels from high and low zone, calculated Q_{10} values were 1.98 and 1.86, respectively.

Thermal tolerance, LT_{50}

Larval LT_{50} was influenced by season, tide height, larval developmental temperature, and their interaction. Calculated LT_{50} values for all treatments ranged from 34.4 – 36.5°C (Figure 6; Table 3). Notably, veliger larvae reared in the cool developmental temperature, had higher LT_{50} values than those reared in the warm temperature, irrespective of adult history in the field. There was greater variation in LT_{50} values amongst progeny from summer-acclimatized than winter-acclimatized mussels. For each treatment offspring from winter-acclimatized mussels tended to have higher thermal tolerance than summer-acclimatized mussels. The best fit generalized linear mixed effect model included temperature, temperature:season, temperature:zone, temperature:developmental temperature, temperature:season:developmental temperature, as fixed effects and individual vial identity as a random effect, all of which had a significant effect on thermal tolerance (Table 4).

Egg size

Adult acclimatization did not influence egg size. Eggs from high zone females collected after the summer acclimatization (summer-H) period trended larger in size (i.e., average diameter and area) compared to the three other acclimatization treatments (summer-L, winter-H, winter-L) (Figure 7). However, there was not a significant effect of season or zone on egg size. The best fit model for both egg average diameter and area only included maternal identity as a predictor variable.

Discussion

The overarching goal of this chapter was 1) to determine if adult acclimatization *in situ* to differing thermal profiles could induce a transgenerational response in larval offspring, and 2) to investigate how temperature experienced by developing mussel larvae may interact with the adult thermal history to affect larval physiological performance. The bigger picture results were that thermal history of adult mussels differed with time and space, moreover, laboratory experiments detected transgenerational effects of adult seasonal acclimatization and larval developmental temperature on larval performance.

Using biomimetic temperature loggers, thermal exposure was estimated for natural populations of adult *M. californianus* across time (i.e., season) and space (i.e., tidal height). Robomussel-collected temperature data showed that mussels in the high intertidal zone experienced higher, more variable temperatures than mussels in the low intertidal zone. However, the magnitude of these differences changed with the season. These data were a critical foundation to the overall study – as the difference in adult thermal history was needed to form hypotheses and inform adult collections for laboratory experiments.

My general hypotheses were that 1) adults acclimatized to higher temperatures (i.e., high zone and/or summer acclimatized) would produce less thermally tolerant progeny, and 2) larvae reared at warmer developmental temperature would be more thermally tolerant those reared at cool temperatures. With regard to the effect of adult thermal history, there was a distinct difference between summer and winter-sourced larvae. Larvae from both high and low winter-exposed mussels were generally larger, had lower respiration rates, and a higher LT_{50} than larvae from summer-exposed adults when reared at the same temperature. Generally, tide height did not influence offspring performance. In terms of the effects of developmental temperature, larvae reared in the warm temperature were larger, had higher metabolic rates, and a lower LT_{50} than those reared in at the cool temperature.

Together these results show that adults acclimatized to summer temperatures, had less thermally tolerant larvae, indicative of a negative carry-over effect. Moreover, larvae reared at the warm developmental temperature were less thermally tolerant than their cool counterparts. While these results suggest that thermal histories, both trans- and intra-generational, can influence larval performance, the direction in which larval performance was shifted regarding temperature partially support my initial hypotheses for transgenerational effects but not intragenerational effects. Lastly, in an examination of gamete size in assessment of maternal investment as a mechanism of TGP, differences in egg size were not observed.

Adult temperature experience as a function of season and tide height

A critical component of this study was to collect high frequency *in situ* measurements of temperature experienced by *M. californianus* at the microhabitat level to provide evidence of varying thermal acclimatization patterns that exist across time and space in the mussel bed. Temperature profiles along tidal height were similar to those documented in the literature, where high zone mussels experience higher and more variable temperatures than low zone mussels (Miller and Dowd, 2017). The range in temperatures observed at my field site spanned 3.7 - 41.1°C degrees in the high zone and 6.6 - 27.8°C degrees in the low zone. Monthly patterns observed in our experiment were also similar to past studies examining seasonal temperatures in *M. californianus* beds in California, where peak temperatures occurred between the months of May and June, then proceeded to decline reaching the lowest temperatures during the month of December (Helmuth and Hofmann, 2002). While temperature differences between the high and low zone were evident at shorter time scales (i.e., days and months), seasonal averages in the high and low zone were relatively similar (Table 1).

In an examination of heat stress, where the cumulative time exposed to temperatures that have been documented to induce moderate (i.e., a heat shock response) or severe (i.e., death measured via LT_{50}) stress, results show that high zone mussels overall experienced higher stress than low mussels. Specifically, following the summer acclimatization, mussels in the high zone experienced the highest heat stress (moderate and severe). Differences in temperature patterns and heat stress experienced by high and low mussels following the two seasons supported my efforts to collect adult *M. californianus* acclimatized to different thermal regimes.

Differences in temporal and spatial temperature profiles that exist at the microhabitat scale (i.e., tide height) have been shown to impact adult thermal physiology from the molecular to organismal level. For example, in a study by Roberts and colleagues, synthesis of heat shock proteins varied with season but not tidal height in *M. californianus* (Roberts et al., 1997). However, differential gene expression in high vs. low mussels has been observed (Place et al., 2012). At the whole organism level, Moyen and colleagues found that upper thermal tolerance, measured via critical temperature for cardiac performance, was higher for high zone mussels (Moyen et al., 2019), but this pattern is not consistent in measurements of LT_{50} (see Waite and Sorte (2022)). Such studies, together with the observed differences in thermal histories documented in this study provided initial motivation to further investigate if acclimatory responses experienced by adults could be passed onto the next generation.

Transgenerational and intragenerational effects on veliger larvae

There is a significant body of literature on TGP and molluscs, with studies showing how environmental history of adults influences larval performance (Ross et al., 2016; Zhao et al., 2020). Carry-over effects have been documented in oysters, mussels, and clams (Byrne et al., 2020) and specifically in *M. Californianus* (Waite and Sorte, 2022). Studies of this nature have indicated that parental exposure influences the performance and traits of progeny, but two key elements remain understudied 1) how seasonal changes in adult history would impact TGP, and 2) how variable developmental temperatures would influence larval physiology and traits. The second point connects directly to climate change where larvae may develop under marine heatwave conditions (warmer) or upwelling conditions (cooler) regardless of adult thermal history.

Analysis of larval body size showed that seasonal acclimatization and larval developmental temperature, but not tidal height, had a significant effect on veliger size. For high and low zone mussels, progeny were larger following the winter season, than the summer. Moreover, larval developmental temperature had the largest effect, where larvae reared at warmer temperatures were larger than those reared in the cool temperature treatment. For larval invertebrates, larger body size is hypothesized to be associated with greater fitness (Strathmann, 1971; Allen and McAlister, 2007), and a pattern of larger body size at warm developmental temperatures has been frequently observed (Byrne, 2011). While development in warmer temperatures can lead to benefits such as increased size, there may be inherent tradeoffs if the energetic demand of growth and metabolism are not met (Clarke, 2017).

To determine differences in metabolic performance based on adult acclimatization and larval developmental temperature, respiration rates (measured as oxygen consumption rates) were assessed for each treatment. Here, higher developmental temperature increased respiration rate in larvae from all adult acclimatization treatments, as expected for ectothermic species (Schulte, 2015). Additionally, statistical analysis revealed significant interactions between developmental temperature and season on respiration rate. Here, the difference in respiration rates between larvae from warm and cool developmental treatments decreased following the winter season. This pattern is most evident when looking at the difference in Q_{10} values for progeny from each adult acclimatization treatment. Progeny from summer-acclimatized mussels had Q_{10} values between 3.97 and 3.31, and winter larvae had Q_{10} values between 1.98 and 1.86. Such differences indicate that progeny from summer-acclimatized mussels may be more sensitive to temperature, with regard to metabolic rate. To our knowledge this is the first assessment of Q_{10} values for *M. californianus* larvae, however, studies on other mollusc larvae have presented similar values (Lough and Gonor, 1973; Lazo and Pita, 2012).

In an assessment of upper thermal tolerance, adult acclimatization (i.e., season and tide height) and larval developmental history significantly influenced LT_{50} values of veliger larvae. LT_{50} values, the temperature at which 50% of the population experiences death, were measured and calculated using an acute thermal tolerance trial for each treatment. Overall, LT_{50} ranged from 34.4 – 36.5°C. Developmental temperature appeared to have the largest influence on larval thermal tolerance, where LT_{50} was lower for larvae reared in the warm temperature treatment. Additionally, offspring from winter-acclimatized mussels generally had higher LT_{50} values than offspring from summer-acclimatized mussels, when reared at the same temperature (i.e., W and C). While statistical analysis revealed a significant effect of tidal height on LT_{50}, this influence was mostly driven by the high LT_{50} of the summer-LC treatment and does not accurately describe observed trends in the data. For all other adult acclimatization treatments, offspring from low zone mussels had lower thermal tolerance than offspring from high zone mussels, which was partially described by the significance interaction between zone and season observed (Table 4). The observation of lower larval LT_{50} following warmer acclimatization of adults *in situ* is consistent with those documented by Waite and Sorte, however unlike their study I did not observe a carry-over effect with relation to tide height (Waite and Sorte, 2022). While few studies have examined the influence of parental thermal history on offspring thermal tolerance in bivalves, studies on other marine invertebrate species are more prevalent (Putnam and Gates, 2015; Morley et al., 2017; Rivera et al., 2021; Chamorro et al., 2023; Leach and Hofmann, 2023). Generally, these studies have shown adult exposure to elevated temperature increased thermal tolerance, however results are species dependent.

In this study it is interesting to note that there was a significant effect of seasonal acclimatization on offspring physiological performance metrics but not tidal height, since temperature differences were most evident between high and low mussels. Average temperatures experienced by mussels following the winter season were similar to those experienced by low mussels following the summer season (Table 1), therefore if adult thermal exposure was the only factor influencing offspring performance, I would predict that offspring from low zone summer mussels would exhibit similar performance to offspring from winter mussels. This leads me to believe that there are other factors that fluctuate with season, than may support the transgenerational effect observed in our study. Such factors may include, but are not limited to food availability, emersion time, and exposure to upwelling. Since each of these factors has been shown to impact adult condition of mussels (Seed, 1968; Menge et al., 1997), it is possible for their influence to extend into the next generation.

Lastly, in the observation that seasonal acclimatization significantly influenced offspring performance, there is one caveat which relates to the experimental design of the study. In order to focus the study on the maternal influence of TGP of offspring performance, sperm from only one male was used to fertilize all eggs for each season. Thus, I cannot say that difference in performance was not attributed to the genetic influence of each male used. However, this experimental design increases the power of the results such that results are more likely due to maternal effects and not the result of a mixed population with variable quality and sperm-egg incompatibilities. Moreover, my results of negative carry-over effects associated with higher seasonal temperatures are supported by those observed in Waite and Sorte which employed multiple males in their experimental design (2022).

In all metrics of physiological performance larval developmental temperature had a dominating influence. Here, larvae reared in the warm developmental temperature treatment were larger, had higher respiration rates, and a lower LT_{50}. The increase in metabolic rate and size, and decrease in LT_{50} suggests an energetic tradeoff, where the elevated temperature treatment is increasing metabolic rate and growth at the cost of thermal tolerance. While thermal tolerance is compromised, it not likely that *M. californianus* larvae would experience temperature close to the LT_{50} values documented in our study while in the water column. Therefore, increased size and growth rate may be more advantages as it could reduce planktonic larval duration, a time at which larvae are most susceptible to predation (Byrne, 2011). The advantage of developing at warm developmental temperature are likely linked with food availability. If there is not sufficient food available to compensate for the elevated metabolic demand associated with the warmer temperature, it maybe that these developmental conditions are more deleterious than developing at cool temperatures.

Maternal investment as a mechanism of TGP

As a measure of maternal investment, size of spawned eggs was compared for females from each acclimatization treatment. While eggs from high zone summer-acclimatized females trended higher in size, there was not a significant influence of season or tide height on egg size. Our results are in line with past studies examining the influence of adult acclimatization on egg traits, where season and collection site did not affect egg size or energetic content (Phillips, 2007b). However, contrary to our results, Waite and Sorte found that females from high tide height produced significantly smaller eggs than low zone females in the fall, but difference amongst tide height was not significant in the spring (2022). While egg size can provide some insight into maternal investment, the relationship is not clear since size is not always correlated with energetic content (further explored in the Chapter IV). Further research on egg traits such as biochemical composition will provide insight into maternal investment.

Conclusions

In this chapter I provided evidence demonstrating that thermal history, both trans- and intra-generational, influenced larval physiological performance. Results from this field-to-laboratory experiment showed that summer acclimatization of adults *in situ* negatively impacted offspring thermal performance. While temperature is likely to have influenced the observed difference in larval performance, the lack of zonal influence indicates that seasonal temperature is perhaps linked with other abiotic and biotic factors (Roberts et al., 1997; Blanchette et al., 2007). Field experiments such as this one provide a more accurate representation of conditions experienced by organisms in nature, however, disentangling the factors influencing observed traits is more difficult. In terms of heatwaves effects, the lack of zonal influence on offspring performance, specifically in the summer-acclimatized mussels may be seen as positive. High zone mussels were exposed to numerous hours of heat stress, but their progeny did not differ significantly from low zone mussels. In this space for time analysis, where high zone mussels are theorized to be experiencing future conditions associated with warming, it would seem as though larvae from mussels currently inhabiting lower shore elevations may not be severely impacted by future warming. Lastly, developmental temperature had a major impact on offspring performance, where larvae reared at warmer temperatures were larger, but it came at the expense of thermal tolerance. In a warming ocean, where the prevalence of MHWs are increasing this may be beneficial for larvae if they are able to reduce their planktonic larval duration, however food availability will play a large role in determining whether or not this will be advantageous. Overall, results from this chapter provide critical insight into how trans- and intra-generational acclimation processes function in nature and their role in determining species response to ongoing and future climate change.

Acknowledgements

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Figures & Tables

Table 1. Mean (± standard deviation) temperature and the range in temperature recorded in the high (H) and low (L) zone following the summer and winter season. Each season is defined as the three months prior to adult collection.

Season	Zone	Mean Temp (°C)	Temp Range (°C)
Summer	H	16.5 ± 5.3	7.8 – 41.1
Summer	L	13.9 ± 1.5	7.9 – 27.8
Winter	H	13.3 ± 4.8	3.7 – 33.0
Winter	L	13.0 ± 1.1	9.0 – 23.6

Table 2. Mean (± standard deviation) body size, measured as hinge length (mm) and area (mm^2) for veliger larvae from each treatment (i.e., season-zone-developmental temperature).

Season	Zone	Developmental Temp	Hinge Length (mm)	Area (mm^2)
Summer	H	C	0.082 ± 0.003	0.0067 ± 0.0003
Summer	H	W	0.082 ± 0.004	0.0068 ± 0.0003
Summer	L	C	0.082 ± 0.003	0.0067 ± 0.0003
Summer	L	W	0.084 ± 0.004	0.0069 ± 0.0003
Winter	H	C	0.084 ± 0.003	0.0068 ± 0.0003
Winter	H	W	0.086 ± 0.004	0.0071 ± 0.0003
Winter	L	C	0.083 ± 0.004	0.0068 ± 0.0003
Winter	L	W	0.084 ± 0.003	0.0070 ± 0.0003

Table 3. Calculated LT_{50} values for veliger larvae following and acute thermal tolerance trial from each treatment.

Season	Zone	Developmental Temp	LT_{50} (°C)
Summer	H	W	34.62
Summer	L	W	34.40
Summer	H	C	35.36
Summer	L	C	36.46
Winter	H	W	35.23
Winter	L	W	35.16
Winter	H	C	35.75
Winter	L	C	35.55

Table 4. Results from generalized linear mixed effect model on veliger thermal tolerance. Bold values indicate factors and interactions which have a significant effect.

	Estimate	**Std. Error**	***z*-value**	***p*-value**
Intercept	75.282	3.736	20.151	**<0.001**
Temp	-2.153	0.108	-19.864	**<0.001**
Temp: Zone (L)	0.071	0.013	5.6	**<0.001**
Temp: Developmental Temp (W)	-0.076	0.021	-3.545	**<0.001**
Temp: Season (Winter)	0.051	0.008	6.34	**<0.001**
Temp: Zone (L): Season (Winter)	-0.075	0.020	-3.736	**<0.001**

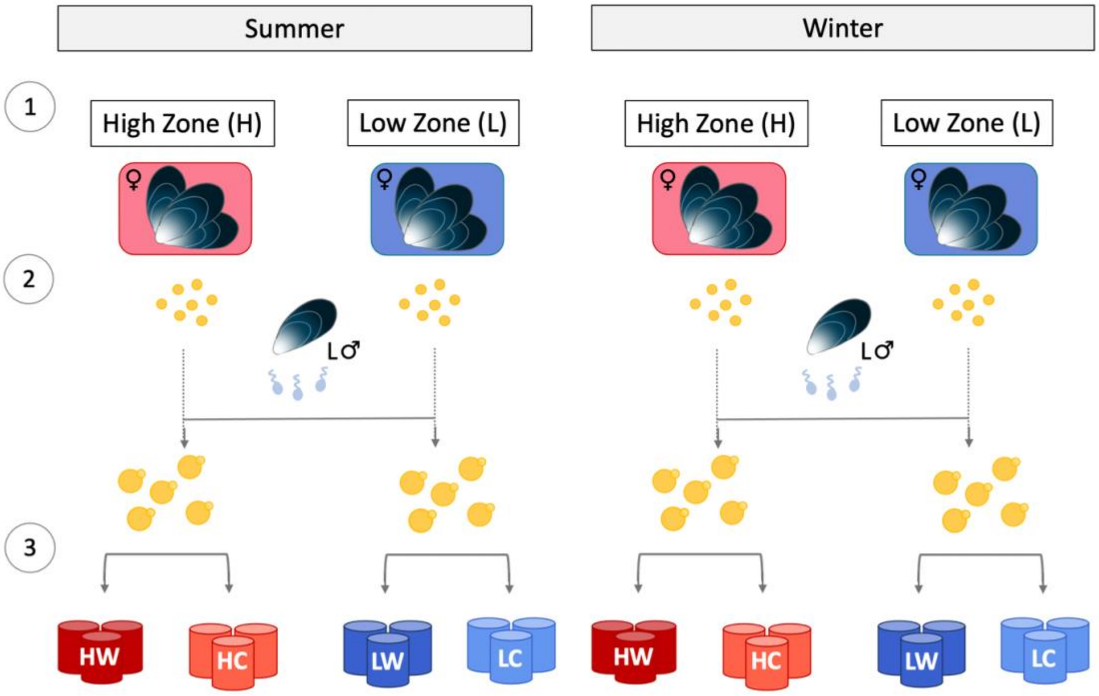

Figure 1. Experimental Design. 1) Adult mussels were collected from the high and low zone following the summer and winter season (*N*=160 total). 2) Mussels were spawned and eggs from five females from each adult acclimatization treatment (i.e., season-zone) were pooled and fertilized with sperm from a single low zone male. 3) Fertilized embryos were reared in warm (W: 20°C) and cool (C: 16°C) temperature treatments until larvae reached the D-shaped veliger stage. Each treatment was raised in triplicate culturing containers.

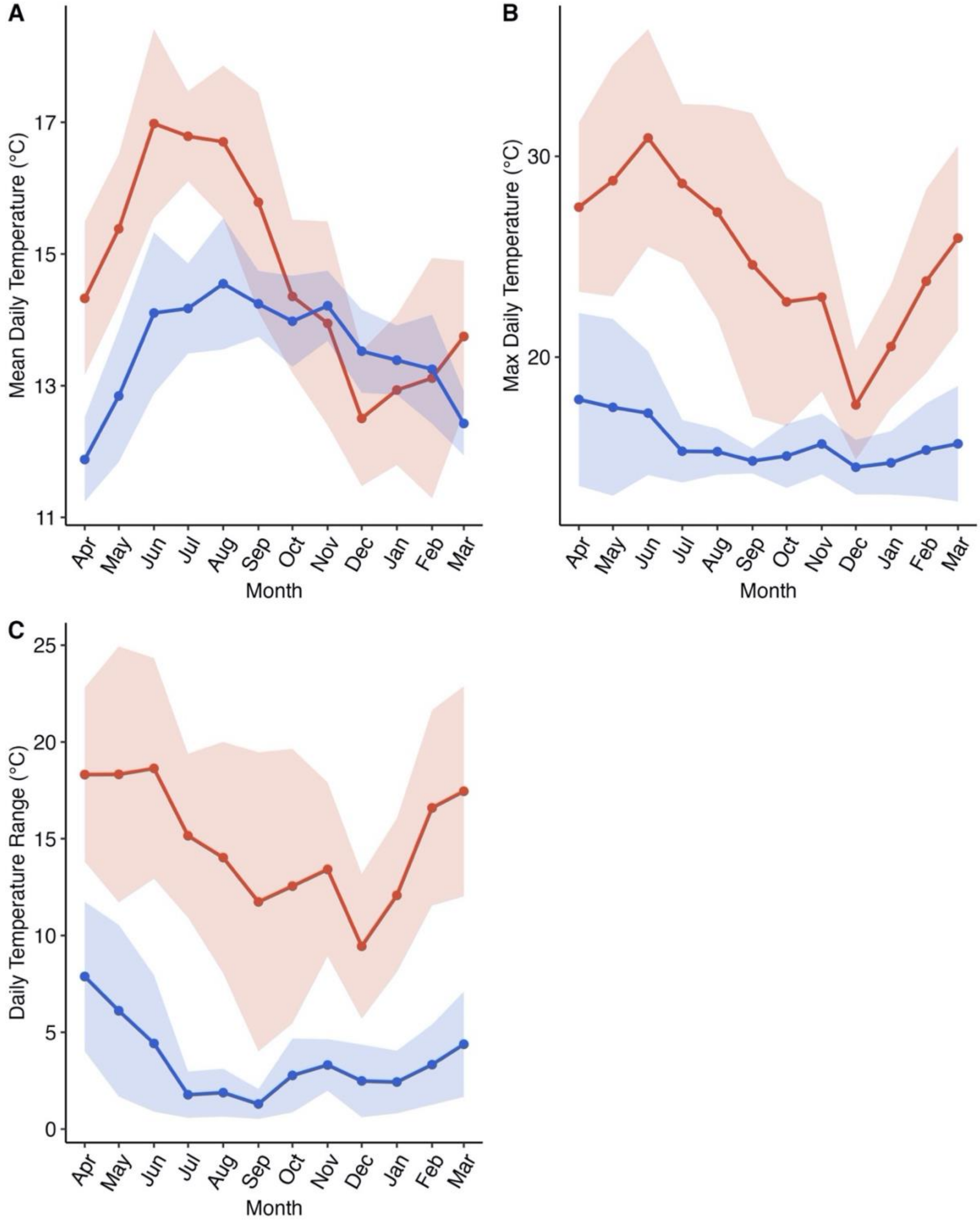

Figure 2. Temperature profiles recorded by robomussels over the course of the field acclimatization, April 1, 2021 – March 30, 2022. A) Monthly averaged mean daily temperature. B) Monthly averaged maximum daily temperature. C) Monthly averaged range in daily temperature. Shaded regions represent ± one standard deviation.

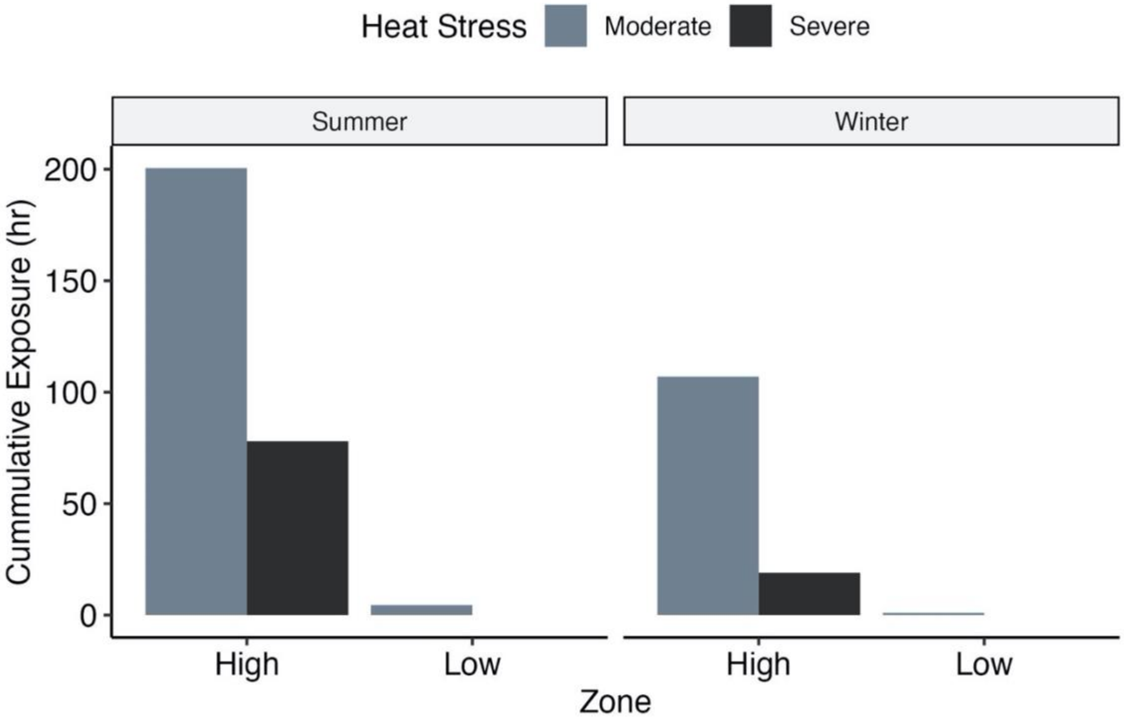

Figure 3. Cumulative exposure to heat stress in hours, record by robomussels for high and low mussels during the summer winter season. Moderate heat stress defined as temperature exposure between 23-30°C. Severe heat stress was defined as exposure above 30°C.

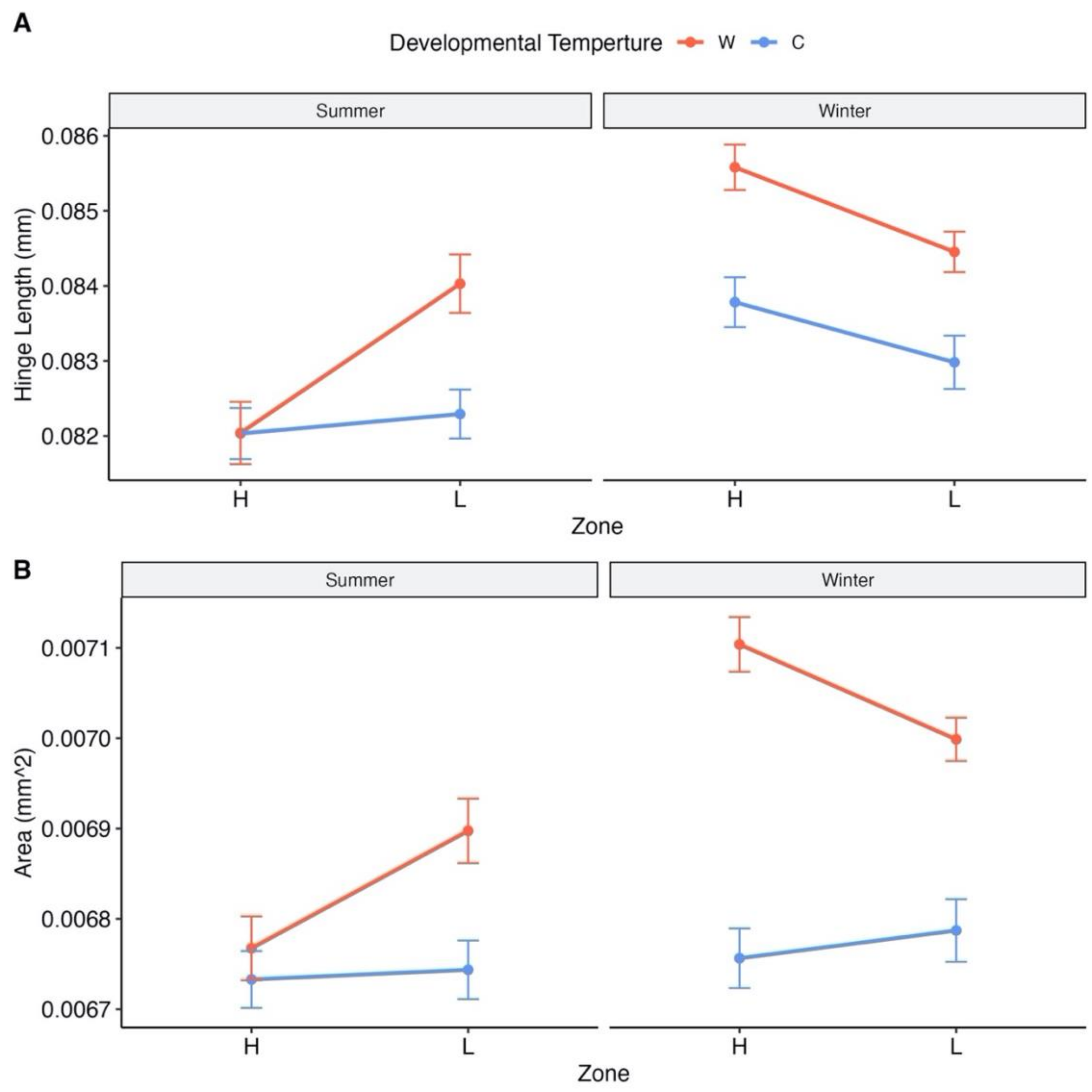

Figure 4. The effect of zone and developmental temperature on D-stage veliger body size. Body size measured as A) hinge length (mm) and B) 2D area (mm^2) for all treatments. Datapoints represent mean values with error bars representing standard error. Panels are separated by season. Developmental temperature is shown by color, W-red and C-blue. High (H) or low (L) parentage is shown on the x-axis.

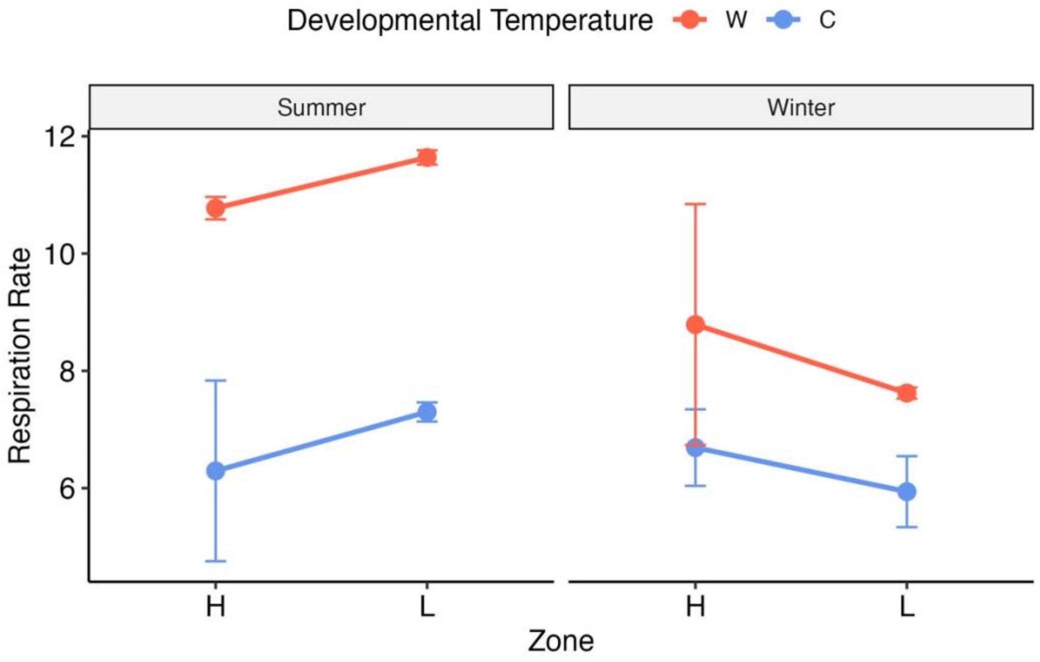

Figure 5. The effect of zone and developmental temperature on larval respiration rate (pmol O_2 h^{-1} individual^{-1}). Datapoints represent mean values with error bars representing standard error. Panels are separated by season. Developmental temperature is shown by color, W-red and C-blue. High (H) or low (L) parentage is shown on the x-axis.

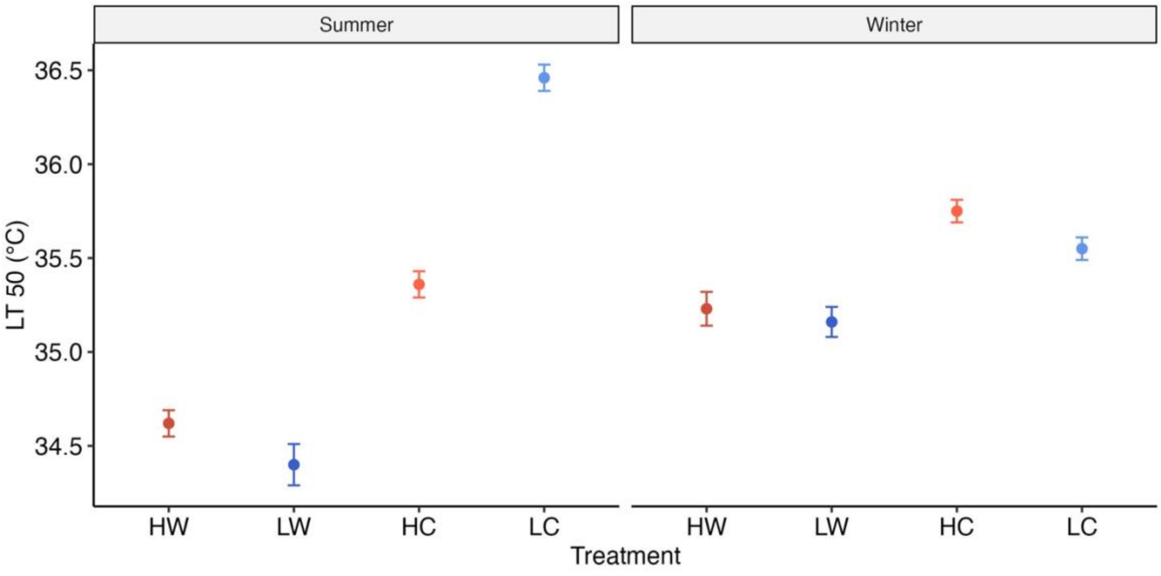

Figure 6. LT_{50} values calculated for veliger larvae following exposure to a 1 hr acute heat shock. Error bars represent standard error. Panels are separated by season. High (H) or low (L) parentage and developmental temperature treatment (W or C) is shown on the x-axis.

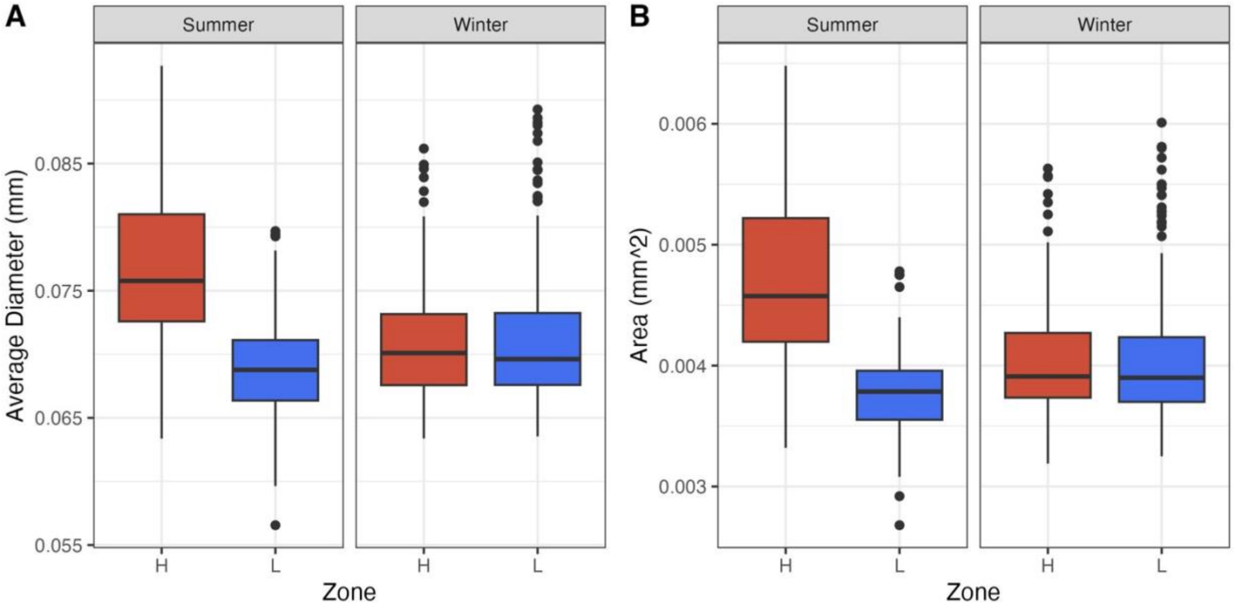

Figure 7. Size distribution of eggs spawned by females from each adult acclimatization treatment (i.e., season-zone). Egg size was measured as A) average diameter (mm) and B) 2D area (mm^2).

IV. Transgenerational plasticity as a mechanism of response to marine heatwaves in the purple sea urchin, *Strongylocentrotus purpuratus*

** This study was conducted based on collaborative effort between me, Adriane McDonald, and Dr. Gretchen Hofmann, results from this study are published in Frontiers in Marine Science.*

Introduction

As recent research indicates that marine heatwaves (MHWs) are expected to increase in frequency and duration in the future (Oliver et al., 2018; Guo et al., 2022; Jacox et al., 2022), understanding how these events will impact species, especially at their most temperature-sensitive stages, will provide critical insight into how MHWs might alter population and community structure. Specifically, research on the timing of MHWs relative to the timing of critical life history events is an important consideration when exploring the physiological mechanisms by which environmental thermal stress might impact organismal performance.

Transgenerational plasticity as a potential acclimation mechanism

Notably, for organisms with complex life histories, such as marine invertebrates, early developmental stages are often the most vulnerable to environmental stress, with early embryos and juveniles showing the most sensitivity across taxa (Pandori and Sorte, 2018). For many benthic marine invertebrates, there are significant knowledge gaps in three areas – how early stages will respond to the acute thermal stress from MHWs, the physiological mechanisms that confer resilience, and lastly, how the timing of MHWs in nature align with the timing of life history events. Mechanistically, the thermal tolerance of embryos and larvae can be influenced via inherent plasticity expressed during development, or alternatively via parentally-sourced mechanisms. Here, maternal provisioning of eggs and/or TGP could transduce tolerance to the embryo as a function of adult environmental history (Donelson et al., 2016). In this chapter I present the results from a laboratory experiment that mimicked MHW patterns observed at a kelp forest study site, and acclimated adult purple sea urchins (*Strongylocentrotus purpuratus*) in the laboratory at a time that coincided with gametogenesis in local natural populations. I then tested the hypothesis that adult thermal experience would influence the tolerance of progeny via TGP. To investigate maternal investment as a specific mechanism of TGP, I examined whether changes in egg provisioning would correlate to the thermal acclimation treatment of the adults.

Laboratory conditions mimicked a major 2014 – 2016 Northeastern Pacific MHW event that occurred in our region. This notable MHW event occurred from January 2014 – August 2016 (Gentemann et al., 2017), and, during this time, sea surface temperature (SST) increased an average of 1- 4°C (Cavole et al., 2016; Di Lorenzo and Mantua, 2016). This event had drastic impacts from the Gulf of Alaska to Baja California that ranged across levels of biological organization (Cavole et al., 2016; Arafeh-Dalmau et al., 2019). Regionally, in the Santa Barbara Channel (SBC) and the local kelp forests, this event has had lasting effects on the kelp forest community, altering local biodiversity (Michaud et al., 2022), interacting with disease (Reed et al., 2016), and changing the quality of kelp as food (Lowman et al., 2022). Since the this heatwave, recurrence of shorter term MHW events are continuing to be documented (Leach et al., 2021) raising concern for the health of these productive coastal ecosystems going into the future.

Timing of MHWs relative to early development of S. purpuratus

Determining how kelp forest ecosystems will be impacted by MHWs will be dependent on our understanding of organismal responses of ecologically important species. In kelp forests, *S. purpuratus* is a dominant herbivorous grazer capable of transforming ecosystems (Pearse, 2006; Smith and Tinker, 2022). Vulnerability to elevated temperatures can vary throughout the sea urchin life cycle, where early developmental stages are often the most sensitive to environmental stress (Byrne, 2011). While increases in environmental temperatures can have a positive effect on early stages by increasing growth and rate of development, thus reducing planktonic larval duration, if temperatures exceed upper thermal thresholds, early forms can experience an increase in abnormal development and/or mortality (Byrne, 2011). Adults are generally less susceptible than embryos to warming in sea urchin species, however heat stress associated with MHWs has shown to have lasting effects on urchin survival, immune response, and reproduction (Shanks et al., 2020; Minuti et al., 2021). From an ecological development perspective (Sultan, 2007), large parts of the purple urchin's reproductive cycle take place when MHWs occur in the SBC (Figure 1). MHWs have tended to occur in the late summer to early winter, a time period that coincides with the period of gametogenesis in purple sea urchins (Cochran and Engelmann, 1975; Strathmann, 1987) (Figure 1). In addition, *S. purpuratus* spawns in this region from December – February (G. Hofmann, pers. observ.). Thus, the overlap between timing of MHWs and gametogenesis in purple urchins makes this species an ideal candidate for examining mechanisms of parental effects in response to local MHWs. From an experimental physiology perspective, this temporal alignment in nature renders parental acclimation in the laboratory during gametogenesis a way to explore whether the thermal history of adults influences the tolerance of their early-stage progeny.

Summary of the study

In this chapter, I examined TGP as a mechanism to increase physiological performance during critical developmental stages of *S. purpuratus* when exposed to prolonged MHW conditions. Specifically, my aim was to investigate maternal effects in a benthic marine invertebrate that routinely experiences MHWs *in situ* and ask whether exposure to MHW temperatures during oogenesis in female urchins induced a transgenerational response, or carry-over effect, when exposed to a simulated prolonged MHW in the laboratory. For this study, adult purple sea urchins were acclimated under two conditions – temperatures that mimicked average MHW conditions typical of the 2014-2016 MHW in the SBC (18°C), and a non-MHW average habitat temperature (13°C) during the period of gametogenesis. Following a four-month acclimation period, adults were spawned and offspring from each maternal acclimation treatment (MHW vs. non-MHW temperature) were reared reciprocally at MHW temperatures (18°C), and non-MHW temperatures (13°C). To test for evidence of TGP in this reciprocal rearing design, I assessed multiple traits throughout early development including thermal tolerance and measurements of body size. To assess the potential for maternal investment as a mechanism or TGP I measured egg traits such as egg size and biochemical composition. The results contribute to our understanding of how ecologically important species of kelp forest ecosystems respond to acute thermal stress from MHWs.

Materials and Methods

Animal collection and adult conditioning

Adult purple sea urchins, *Strongylocentrotus purpuratus* (Stimpson, 1857), were collected on SCUBA subtidally near Goleta Bay, CA, in September 2020. Urchins were collected under the California Department of Fish and Wildlife Scientific Collecting permit #SC-9228 to the Santa Barbara Coastal LTER. Following collection, urchins were held in a flow-through seawater table at ambient temperature (~16.4°C) for 24 hours before being placed in the adult acclimation treatments. During this period urchins were monitored for any disease or premature spawning and removed if needed.

To determine how maternal conditioning influenced provisioning and subsequent offspring performance, adult urchins were acclimated in either a warm (W: ~18.3°C) or cool (C: ~13.1°C) temperature condition representing MHW and non-MHW conditions, respectively. Urchins were held in the acclimation treatments from October 2020 to January 2021, which coincides with the window of gametogenesis for *S. purpuratus* populations in the SBC (Strathmann, 1987). Adult acclimation treatments consisted of three 37L replicate tanks held in a seawater table, acting as a water bath, to maintain temperature. Seawater was supplied to the treatment tanks via a dripper irrigation system with a flow rate of 12L/hr. The temperature of the seawater table was controlled using a Delta Star® heat pump with a Nema 4x digital temperature controller (AquaLogic, San Diego, CA, USA). The temperature of each tank was monitored daily using a wire thermocouple (Omega HH81a). Aquarium pumps (Aqua-Supreme) were installed in each tank to maintain proper water flow. Ten adult urchins were randomly selected and added to each treatment tank at the beginning of the acclimation period (*N*=60 total, 10 urchins per tank replicate for each temperature condition) (Figure 2). Each week seven fronds of kelp (*Macrocystis pyrifera*) were added to each tank and urchins fed *ab libitum*. Urchins were inspected daily, and any individuals that showed signs of disease or death were removed.

Urchin spawning and larval culturing

Following the four-month conditioning period, urchins were induced to spawn by injecting 0.53M KCl into the coelom(Strathmann, 1987). Sperm was collected "dry" via pipette, and then stored on ice in 1.5mL microcentrifuge tubes until activated. Eggs were collected by inverting females over a beaker containing UV sterilized 0.35micron filtered seawater (FSW). Sperm and egg quality were checked under a compound microscope. Females with the highest quality eggs (determined via visual inspection of size, shape, and transparency) from each adult acclimation treatment were selected for test fertilizations with potential sires. To test for gamete compatibility, a sample of eggs from each female were combined with diluted sperm from four different non-MHW males. The male with highest sperm quality and compatibility (e.g., good motility and >95% fertilization) was chosen as the sire. Eggs from five females from each acclimation treatment, with the highest quality eggs and without fertilization incompatibilities, were then pooled in two separate beakers, creating two egg pools (i.e., a pool of eggs from MHW acclimated females, and a pool from non-MHW acclimated females). Each female contributed approximately 72,000 eggs to each pool. Beakers containing pooled eggs were gently mixed while diluted, activated sperm (at approx. 10^5 cells per mL) from the chosen sire was added in small increments to avoid polyspermy. Since this study focused on examining maternal effects, only one sire from the non-MHW group was chosen to reduce any variation in offspring phenotype attributed to paternal genetic variation. Once pooled eggs reached 95% fertilization, embryos were deposited in larval culturing containers for the rearing phase of the experiment.

For the reciprocal culturing experiment embryos were raised at two different developmental temperatures: a MHW temperature (~18.5°C) hereafter denoted as 'W' and a non-MHW temperature (~12.7°C) hereafter denoted as 'C'. This experiment created four offspring combination treatments that are denoted as: WW, WC, CW, CC, where the first letter represents the condition for the adult, and the second letter indicates the temperature at which embryos underwent development. Each of the four groups were raised in three replicate culture containers (Figure 2). A description of culturing containers is found in the "*Larval Culturing and Sampling*" section of Chapter III. Each container contained 12L of FSW and was stocked with 122,000 embryos. Seawater temperature in each culturing container was closely monitored using a wire thermocouple.

Sampling through development

Embryo sampling was conducted at three stages during development: hatched blastula (HB), early gastrula (EG), and an early form of echinopluteus larvae, the prism stage (PR). Each stage was sampled when embryos displayed distinguishing developmental characteristics described in Strathmann (1987). Specifically, hatched blastula stage were characterized by hatching, where the embryo is no longer enclosed in the embryonic envelope; HB were sampled at 16 hours post-fertilization (hpf) for WW and CW treatments and 23hpf for WC and CC treatments. The early gastrula stage was characterized as the embryo displaying invagination at the vegetal plate and was sampled at 21hpf for WW and CW treatments and 34hpf for WC and CC treatments. The prism stage was characterized as the full formation of the tripartite archenteron, in addition to growth of the body rods, and was sampled at 44hpf for WW and CW treatments, 58hpf for WC, and 55hpf for CC treatments.

Thermal tolerance

To determine the effects and interaction of maternal acclimation and developmental temperature on the performance of the four treatments (WW, WC, CC, CW), subsets of hatched blastula embryos from each treatment were tested in an acute thermal tolerance trial. Methods for this trial were as described by Wong and Hofmann (2020) with some modification. Briefly, using a temperature gradient heat block, scintillation vials containing 3.5 mL of FSW were brought to seven temperatures (17.5, 20.5, 26.4, 24.7, 28.0, 29.8, 31.1°C). Embryos from replicate culturing containers in each treatment were pooled so that each container contributed equally to the thermal tolerance trial. For the trials, 1,000 embryos in 500mL of FSW were added to the preheated scintillation vials, and were capped to prevent evaporation. Vials containing the embryos were maintained in the heat block for 1hr, at which point additional cool FSW was added to each vial and the vials were transferred to a 14°C environmental chamber for a recovery phase. During the recovery phase, embryos were allowed to proceed through development to the prism stage, an early pre- feeding larval stage. The timing of the appearance of embryos progressing to the prism stage was assessed visually using microscopy; approximately 43 hours elapsed from the start of the recovery period and to the beginning of the scoring process. Periodic water changes were conducted to make sure water in vials did not become hypoxic. Using approximately 100 individuals from each vial, I screened for advancement to the prism. Larvae were scored as alive if swimming or cilia movement was observed. LT50 (the temperature at which the population experiences 50% mortality) was calculated for each treatment. While previous studies have measured mortality immediately following exposure to an acute thermal stress, for this experiment mortality was measured once the embryos reached the prism stage. This allowed for a more accurate measure of survival by adding a recovery phase to the acute temperature trial.

Embryo and larval morphometric analysis

For HB and EG, 500 embryos from each culturing container were preserved in 2% formalin in FSW. In addition, 500 PR larvae from each culturing container were preserved in 2% formalin in FSW buffered with 100mM sodium perborate to a pH of 8.7, a formulation that reduces degradation of the skeletal rods. For each stage, preservation in 2% formalin was achieved by adding an equal volume of 4% formalin (in FSW or buffered FSW) to the sample volume. Preserved embryos were promptly photographed and measured using methods described in the "*Egg and larval morphometrics*" section of Chapter III. For HB and EG images, individual embryos (*N*=35) from each treatment replicate were oriented so the full lateral view was visible, 2D area was measured and calculated by tracing around the perimeter of the embryo. For PR images, individuals (*N*=35) from each treatment replicate were oriented in a lateral view, so that the side profile of the archenteron was visible, 2D area was similarly measured by tracing around the perimeter of the embryo. At the prism stage, skeletal rod length was also determined by measuring from the tip of the body rod to the tip of the postoral rod.

Analysis of egg traits

To assess maternal investment in females from the different acclimation treatments, I measured egg traits including egg size and biochemical composition. Eggs were preserved and measured using the same methods as described above for embryos. For imaging, 500 eggs from each contributing female (N=10) were collected promptly after spawning, and preserved in 2% formalin in FSW for morphometric analysis. Individual eggs (N=35) from each female were measured by calculating the average of three diameters and two-dimensional (2D) area. For lipid quantification, 2,000 eggs were deposited in a 1.5mL cryovial. Each sample was centrifugated to concentrate eggs (approximately 30s at 16,000 RCF) (Eppendorf 5415C Centrifuge), the water layer was carefully removed, and the sample was flash frozen in liquid nitrogen and stored at -80°C until analysis. Two tubes from each contributing female were used to quantify total lipid concentration. Total lipid was extracted using methods described in Wong et al. (2019), a method based on Sewell (2005) with some modification. The concentrated eggs from each sample were suspended in 250mL of Milli-Q water and sonicated at 2amps for a total of 45s at 15s intervals (Fisher Scientific Sonic Dismembrator 500). Each homogenized sample was then transferred to a 5mL glass V-vial (Wheaton) and combined with 125mL of chloroform (HPLC grade) and 250mL of methanol (HPLC grade). Samples were shaken vigorously and then centrifuged at 2,680 RCF for 5min at 4°C (Beckman Coulter AllegraTM 21R Centrifuge). Using a pulled Pasteur pipette the aqueous and chloroform layers were transferred to a clean V-vial; chloroform and water were then added to the final volume ratio of 4:3:2 of water:chloroform:methanol. Samples were then hand shaken and centrifuged again at 2,680 RCF for 5min at 4°C. After centrifuging, the chloroform layer was carefully transferred to a clean glass vial. All samples were flushed with N_2 and stored at -20°C prior to analysis. Total lipid was quantified spectrophotometrically using modified methods based on Marsh and Weinstein (1966). Briefly, samples were placed under a flow of N_2 to allow chloroform to evaporate (Reacti-VapTM Evaporators TS-18826, Thermo ScientificTM). Once dried, 500mL of sulfuric acid (ACS grade) was added to each sample, covered with aluminum foil, and dried in a furnace at 200°C for 15min. Samples were allowed to cool for 10min prior to the addition of 2.5mL of deionized water to each vial. Samples were allowed to cool for an additional 10min and then absorbance of each sample was measured on a spectrophotometer (Shimadzu UV-1800) at 375nm. A standard curve of a known mass of lipid within a range of 30-300mg was also prepared and run in parallel with each batch of samples ($R^2 = 0.98\text{-}0.99$). The lipid profile used to generate the standard curve consisted of major lipid classes found in *S. purpuratus* eggs which were previously reported in Wong et al. (2019); specifically, the standard was comprised of 51% triacylglycerol (Glyceryl tripalmitate, Sigma-Aldrich, Catalog No. T5888), 38% phospholipid (L-a-Phosphatidylcholine, Sigma- Aldrich, Catalog No. P3556), and 11% sterol (Cholesterol, Sigma- Aldrich, Catalog No. C8667).

Eggs used for protein quantification were prepared using the same process as described for lipid quantification. Three tubes from each contributing female were used to quantify total protein concentration. Protein was extracted from 2,000 eggs from each females using methods described in Wong et al. (2019). Briefly, 100mL of homogenization buffer (20mM Tris-HCl, (pH 7.6); 130mM NaCl; 5mM EDTA) with 1% Triton-X and 1%

Protease Inhibitor Cocktail (Catalog number P8340, Sigma-Aldrich) was added to each sample of concentrated eggs and sonicated on ice for 20s. Samples were then shaken (Thermolyne M71735 Slow Speed Roto Mix) on ice for 15min, followed by centrifugation at 16,000 RCF for 20min (Eppendorf 5415C Centrifuge). The supernatant was transferred to a 1.5mL microcentrifuge tube and stored at -20°C until analysis. Total protein content was quantified using the PierceTM BCA Protein Assay Kit following manufacturer's instructions (Catalog No. 23225, Pierce Biotechnology, Rockford, IL, USA). The standard curve was generated using bovine serum albumin ($R^2 = 0.99$).

Statistical analysis

All statistical analyses were conducted in R Studio (2022.12.0 + 353). Adult survival data was generated using the Kaplan-Meier method (Rich et al., 2010) and analyzed using a log-rank test. For the thermal tolerance trial, the influence of maternal acclimation temperature and offspring developmental temperature on survivorship in response to temperature was analyzed using ageneralized linear mixed effect model (lme4 package) (Bates et al., 2015). The binary data (alive vs. dead) were fitted using a logit link. Models were compared, and the best fit was determined using standard model reduction approach for fixed effects, their interactions, and random effects; where the Akaike information criterion (AIC) was used to determine the best fit model. Maternal and offspring temperature were set as fixed effects and vial number was set as a random effect. A Wald chi-squared test was conducted to determine the significance of each factor on survivorship. The LT50 value was calculated using a logistic regression for each treatment. The influence of maternal condition and the developmental temperature on body size at each stage (i.e., HB, EG, and PR), was similarly analyzed using a linear mixed-effect model. Maternal and offspring temperature were set as fixed effects and individual culturing containers were set as a random effect. For egg traits, the influence of maternal acclimation on egg size (i.e., average diameter and 2D area), total lipid, and total protein content was analyzed using a linear mixed-effect model. For egg size, total lipid, and total protein content, maternal temperature condition was set as a fixed effect while each individual mother was set as a random effect.

Results

Observations following acclimation of adult urchins

Adult *S. purpuratus* were successfully acclimated in the laboratory to two experimental temperature conditions, MHW (18°C) and non-MHW (13°C) conditions, with some mortality. Throughout the four-month acclimation period, mortality was observed in both treatments with a trend of higher survival in the non-MHW treatment (90%), as compared to the MHW treatment (73%). However, the difference in survival probability between treatments was non-significant (Figure 3) (log-rank test: c2 = 2.8, *df*=1, *p*= 0.10). Following the acclimation, surviving urchins from each treatment were spawned, providing ample material to create the embryo families and raise cultures. However, I observed decreased spawning success in MHW-acclimated adults as compared to non-MHW-acclimated adults where 50% of surviving urchins in the MHW treatment spawned as compared to the non-MHW treatment where all adults were induced to spawn.

Thermal tolerance

This study detected a carry-over effect of maternal thermal history to the progeny. In the thermal tolerance trial, progeny from females acclimated to MHW temperatures (denoted as ‘W’) had a higher thermal tolerance to acute thermal stress than progeny from non-MHW-acclimated females (denoted as ‘C’), with higher percentages of larvae alive following the recovery period. Embryos from females acclimated to 18°C, the MHW condition, had higher LT_{50} values than those acclimated to 13°C, the non-MHW condition (Figure 4). Across the experiment, calculated LT_{50} ranged from 27.2 - 28.4°C. For each treatment, LT_{50} values for WW, WC, CW, and CC were 28.2, 28.4, 27.9, 27.2°C, respectively. When comparing the influence of maternal condition, a comparison of WW vs. CW treatments suggested that embryos, when reared at higher temperatures, gained a modest increase in tolerance (+0.3°C) if mothers experienced MHW temperatures during the period of oogenesis. I also found evidence that embryo environmental conditions influenced thermal tolerance. Here, elevated developmental temperature increased thermal tolerance in progeny from females naïve to MHW exposure, where LT_{50} of the CW family was 0.7°C higher than the CC family. A generalized linear mixed-effect model found that maternal acclimation, temperature at which offspring were raised, and their interaction all had a significant effect on embryo thermal tolerance (Table 1). These results indicated that the thermal history of the females and the developmental temperature of the embryo both influenced thermal tolerance of early-stage purple sea urchins in the thermal tolerance trial.

Embryo morphometrics

The influence of maternal acclimation and offspring developmental temperature on body size differed as a function of stage, where maternal acclimation significantly influenced embryo body size at the early gastrula and prism stage, while offspring developmental temperature had a significant influence only at the prism stage (Table 2). Difference in body size amongst treatments were most evident at the prism stage, where on average, offspring from MHW-acclimated females were 7.1% larger than those from non-MHW-acclimated females. Additionally, offspring raised at the MHW temperature were on average 14.3% larger (2D area) than those raised at the non-MHW temperature and had skeletal rod lengths that was on average 53.5% longer than those raised in the non-MHW condition (Figure 5). Results from the best fit linear mixed effect models are summarized in Table 2.

Egg morphometrics and biochemistry

In general, maternal temperature acclimation had no effect on egg size. Although eggs from non-MHW-acclimated females trended larger in size, maternal environmental history during oogenesis did not significantly influence average egg diameter ($F_{1,8} = 0.1272$, $p = 0.7286$) or 2D area ($F_{1,8} = 0.5882$, $p = 0.4608$). Average egg diameter for MHW- and non-MHW-acclimated mothers was 0.0798 ± 0.023 and 0.0801 ± 0.023mm, respectively. The average 2D area of eggs from MHW- and non-MHW- acclimated females was 0.0051 ± 0.003 and 0.0052 ± 0.003mm^2, respectively (Figures 6A, B). In contrast to morphometric traits, the biochemical composition of the eggs did vary in a comparison of females acclimated to MHW vs. non-MHW temperatures. Specifically, protein concentrations were higher in eggs from MHW-acclimated females as compared to eggs from non-MHW-acclimated females ($F_{1,8} = 11.184$, $p<0.01$). Total protein content from MHW and non-MHW acclimated mothers was 40.4 ± 3.38 and 34.6 ± 3.23 ng/egg, respectively (Figure 6C). In contrast, maternal thermal history did not have a significant effect on total lipid content of eggs ($F_{1,8} = 2.3268$, $p= 0.1575$), although trending differences in lipid content were observed. Total lipid content in eggs from MHW- acclimated females was on average 17.6 ± 5.19 as compared to 14.0 ± 2.62 ng/egg in MHW- acclimated females (Figure 6D).

Discussion

The goal of this study was to investigate TGP as a mechanism of resilience to MHW-relevant thermal stress in early developmental stages of the purple sea urchin, *S. purpuratus*. Designing experiments parameterized by local thermal extremes, I found that parental thermal history influenced thermal tolerance of progeny, where females acclimated to MHW temperatures (18°C) during gametogenesis produced more thermally tolerant progeny as compared to their non-MHW acclimated counterparts (13°C). Findings further showed that maternal acclimation significantly influenced offspring body size at various stages in development (i.e., EG and PR), where embryos from MHW-acclimated females were larger. In exploring specific mechanisms of TGP, I hypothesized that differences in maternal provisioning could contribute to the observed differences in offspring phenotype. Here, in an assessment of egg size and the biochemical composition of eggs, I found that eggs from differentially acclimated females differed significantly in total protein content, with eggs from females maintained at higher temperatures having greater total protein. In combination, these results indicated that prolonged exposure to anomalously high environmental temperatures, as would be experienced during MHW events in nature, could influence reproductive success *in situ*.

In general, there are relatively few studies that have examined the effects of MHWs on gamete and offspring traits with an eye to three important elements of an ocean-climate study – here, parental effects that could drive plasticity, ecologically relevant temperatures for that species/populations under study, and species-specific phenology. In this regard, important studies on echinoderms have shown that a New Zealand species, *Evechinus chloroticus*, when acclimated to high summer temperatures decreased reproductive output (Delorme and Sewell, 2016). In this study, operating in an ecologically matched time window for oogenesis, I observed a similar trend were *S. purpuratus* adults acclimated to MHW conditions had lower spawning success when compared to those held at control conditions. In a study of carry-over effects in *Heliocidaris erythrogramma,* Minuti and colleagues found that MHW- acclimated females produced larger, more thermally tolerant progeny at early developmental stages (Minuti et al., 2021). Similar observations were made in this study on the temperate, kelp forest species, *S. purpuratus*. Further, in a study of sperm traits in *S. purpuratus*, male urchins acclimated to local MHW conditions (20°C) had decreased fertilization success when compared to males exposed to non-MHW conditions (15°C) (Leach et al., 2021). Our understanding of MHW effects on reproductive and offspring traits is growing with accumulating evidence noting that early stage and developmental steps are likely influenced by high temperatures typical of MHWs, especially if the thermal stress aligns with critical life history events such as gametogenesis.

Transgenerational effects on offspring thermal tolerance

The main objective of the study was to examine whether adult environmental experience in MHW conditions would influence the performance of their progeny.

Ecologically, this is significant because MHW events can occur throughout the period in which purple urchins are broadcast spawning and thus, early development could occur at anomalously high temperatures *in situ* (Figure 1). Using laboratory experiments, I queried whether adults could “prime” their progeny during periods of anomalously elevated temperatures, or whether MHW stress during gametogenesis would have deleterious effects on offspring performance. Using acute temperature trial assays used previously on early-stage echinoderms (Wong and Hofmann, 2020), I observed that maternal exposure to MHW temperatures increased thermal tolerance in progeny (Figure 4), as demonstrated by higher LT_{50} values.

These results join other studies that examined TGP in marine invertebrates in response to thermal stress, where the offspring response varied with species, timing, and nature of parental conditioning (Putnam and Gates, 2015; Shama, 2015; Morley et al., 2017; Rivera et al., 2021; Bernal et al., 2022; Waite and Sorte, 2022). For example, in a study on the marine polychaetae, *Ophryotrocha labronica*, researchers found that the thermal tolerance of the progeny tracked the timing of the temperature exposure of the female, where tolerance of offspring differed depending on whether maternal acclimation occurred during early or late (Massamba-N'Siala et al., 2014). These results match the outcome of this experiment on *S. purpuratus*. Together, these findings underscore the significance of the timing of the warming events associated with MHWs and their subsequent effects on offspring resilience. Since the nature of MHWs can vary from prolonged to short-term events (Hobday et al., 2016), transgenerational responses may differ depending on duration of maternal exposure.

Transgenerational effects on embryo and larval body size

In addition to measuring upper thermal tolerance as a metric of offspring performance, I examined body size, an important functional trait of echinoderms in larval ecology and physiology (Strathmann, 1971; Allen and Marshall, 2014) that has shown to correlate to performance in studies of local adaptation and plasticity in *S. purpuratus* (Kelly et al., 2013; Strader et al., 2022). In this study using MHW-relevant temperatures, I found that maternal thermal history significantly influenced body size (measured as 2D area) for early gastrula embryos and prism larvae (Figure 5), where offspring from MHW-acclimated females were larger in size, potentially indicating a positive transgenerational effect, since larger size in larvae is often associated with higher fitness (Strathmann, 1971; Allen and McAlister, 2007). Offspring developmental temperature also influenced body size for prism-stage larvae, which was evident in the observed differences in skeletal rod length. An interaction between maternal thermal history and developmental temperature was seen only at the early gastrula stage, where differences in body size within each developmental treatment were dependent on the maternal experience.

Other studies examining transgenerational effects in response to elevated temperature conditions in echinoderms have reported mixed effects on larval size. For example, studies on *Strongylocentrotus intermedius* and *Tripneustes gratilla*, show that adults exposed to long-term elevated temperatures, 15 months and 6 months respectively, produced progeny that were smaller in size compared to progeny from adults conditioned to ambient temperatures (Zhao et al., 2018; Karelitz et al., 2019). In addition, adult long-term acclimation to +2°C temperature conditions did not affect body size of the progeny in *Heliocidaris erythrogamma* (Harianto et al., 2021). Overall, measurement of body size at different stages in development may contribute to the differences seen amongst studies. Furthermore, length of adult exposure, differences in species plasticity, and population-level responses likely contribute to reported differences in transgenerational responses.

Maternal provisioning as a general mechanism of TGP

Mechanistically, maternal contributions to shifts in tolerance of the progeny could manifest in two ways - 1) as maternal energetic investment in the eggs, and/or 2) as maternally-derived egg transcripts and proteins working to reduce cellular damage due to heat stress during early development. Historically maternal energetic investment has been assessed through measurements of egg size and biochemical composition (i.e., lipids and proteins) where studies have shown that egg size and nutritional macromolecules are significant in determining conditions and performance of developing progeny in echinoderms (Jaeckle, 1995; Prowse et al., 2008; Moran et al., 2013; Peters-Didier and Sewell, 2017). Given these data on echinoderm eggs, I hypothesized that observed differences in offspring phenotype would be due to differences in maternal energetic provisioning (i.e., egg size and biochemical composition). In testing this hypothesis, I found that acclimation of females to MHW temperatures during oogenesis resulted in significantly altered egg characteristics. Specifically, although egg size was unaffected by maternal acclimation, eggs from MHW-acclimated females had higher protein content and had total lipid content that trended upward (Figure 6).

For marine invertebrates, egg size is often used a standard measurement of maternal investment as it has been linked to numerous adaptive traits in developing embryos and larvae such as fertilization success, larval development, and post- metamorphosis survival

(Moran and McAlister, 2009; Allen and Marshall, 2014). While differences in egg size were not observed in this study, past research examining the effects of maternal acclimation temperature on egg size report varying outcomes (Foo and Byrne, 2017). In combination, data in the literature suggest that species and the duration of thermal exposure are likely significant determinants of egg size (Skadsheim, 1989; Suckling et al., 2015; Guillaume et al., 2016; Karelitz et al., 2019). However, when examining provisioning of eggs, focusing solely on size may not be the best predictor of energetic content. In echinoid species specifically, while egg size generally scales with energy content, at the intraspecific level this relationship is weakened (McEdward and Morgan, 2001; Moran and McAlister, 2009).

In this light, to further assess differences in egg quality, I measured total protein and lipid concentrations. Protein concentrations were significantly higher in eggs from females with the history of high temperature conditioning (Figure 6C). In addition, although there was a trend indicating higher lipid levels as well, there was not a significant difference between eggs of MHW- and non-MHW-acclimated females. In general, the levels of protein and lipid measured here are consistent with other measurements in *S. purpuratus* (Matson et al., 2012; Wong et al., 2019). Studies examining how these macromolecules are used throughout development show that lipids (specifically triglycerides) are major energy fuels, while structural lipids (e.g., phospholipids) and proteins are used to construct the larval body (Byrne et al., 2008). These patterns of biochemical utilization support the results of this study where larger larvae developed from more protein rich eggs (i.e., eggs from MHW-acclimated females).

While not evaluated in this study, differences in egg protein characteristics may potentially influence thermal tolerance of early-stage embryos via maternal transmission of heat shock proteins. In support of this hypothesis, a study examining gonad tissue of acclimated adult urchins (20 vs 26°C) found higher levels of Hsp70 protein in the warm-acclimated urchin (Harianto et al., 2021). While measurements of gonad tissue do not identify standing stocks of defensome proteins in spawned eggs, this study does highlight the potential for maternal acclimation via changes in egg protein characteristics to alter tolerance of developing embryos. In addition to proteins, maternally sourced transcripts may also contribute to differences in thermal tolerance at early embryonic stages. Together these inherited molecules are necessary for driving early developmental processes, and it is not until the maternal-to- zygotic transition that embryos are able to activate their own genome (Hamdoun and Epel, 2007; Schier, 2007). With this in mind, I chose to study hatched blastula embryos, specifically, as studies have shown that these early-stage embryos still rely on maternal transcripts and are unable to completely activate elements of the embryonic defensome, e.g., genes for molecular chaperones and heat-shock proteins (Sconzo et al., 1995).

To further highlight physiological mechanisms that contribute to TGP, transcriptomic studies on urchins have shown that parental acclimation to different temperatures influence gene expression in offspring during early development (Shi et al., 2020). Similar results have been observed in *S. purpuratus* under variable conditions of temperature and pCO2 (Wong et al., 2018). While transcriptomic techniques have aided our ability to determine the molecular underpinning of TGP, to shed light on maternal provisioning mechanisms, future studies should incorporate long-term acclimation during gametogenic windows followed by examination of maternally sourced RNA and proteins in eggs and early embryos. Assessment of the temperature-related egg changes in a climate-change context could be further investigated by examining the translatome, an approach that has been used in other echinoderms (Chassé et al., 2018).

Effects of developmental temperature on offspring phenotype

In addition to addressing transgenerational processes, the factorial design of the experiment allowed me to examine how the temperature at which embryos developed influenced their phenotypic and functional traits (i.e., body size and thermal tolerance). I found that progeny from non-MHW acclimated females reared in non-MHW conditions had the lowest LT_{50} value. However, thermal tolerance of these progeny increased when reared at the higher MHW temperature (Figure 4). This outcome has been observed in other echinoderm species. In a study on red urchins (*Mesocentrotus franciscanus*), embryos reared at 17°C had an increased thermal tolerance as compared to embryos reared at 13°C (Wong and Hofmann, 2020), indicating that if rearing temperatures do not exceed upper thermal limits there is a potential for a resistance response in early-stage embryos. In this study, developmental temperature also had a significant effect on body size at the latest stage evaluated (i.e., PR), where development in MHW conditions was positively associated with body size and body rod length (Figure 5).

Conclusions

This study provides evidence that TGP may contribute to the capacity to resist the thermal stress associated with MHWs in a benthic marine invertebrate. Findings from the thermal tolerance trials showed that offspring with a history of MHW exposure, whether it be via maternal history or during development, had higher LT_{50} values than those that were naïve to MHW conditions, suggesting that both trans- and intragenerational acclimation were acting to increase tolerance to heat stress. These results suggest that prior exposure to elevated temperature can increase tolerance of early-stage *S. purpuratus*. Since early life history stages are often considered to be the most sensitive to abiotic stressors (Pandori and Sorte, 2018), and are currently being affected by MHWs (Rogers et al., 2021), maternal effects such as those observed in this study may act to ameliorate negative impacts of MHWs.

It is important to note that there are limitations to the interpretation of the results of this laboratory experiment. Specifically, it is unclear how long this resistance will last, either in the lifetime of individuals, or across subsequent generations. While carry-over effects may increase tolerance to thermal stress in a single generation, past TGP studies have shown that acquired tolerances may not persist within or across generations (Byrne et al., 2020). Additionally, due to the variable nature of MHW events it is important to consider the possibility of environmental mismatches between parents and offspring and how this may influence inherited traits (Baker et al., 2019).

From a global change perspective, MHWs are predicted to become a dominant force in many of the world's oceans (Oliver et al., 2021; Smith et al., 2023) and a greater understanding of how ecologically and economically important species respond to the thermal stress associated with MHWs will be critical in predicting the vulnerability of key fisheries (Smith et al., 2023) and on how biodiversity and ecosystem health might be altered in warming coastal oceans (Smale et al., 2019; Michaud et al., 2022). Overall, TGP studies will provide critical insight into mechanisms of acclimation in response to these thermally stressful events that function at ecological timescales, providing time for adaptation to catch up.

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Figures & Tables

Table 1: A Wald chi-squared test was used to determine the significance of each factor: temperature of acute exposure, maternal acclimation temperature, and offspring developmental temperature; and their interaction on survivorship.

	χ^2	*df*	*p*
Temperature	206.264	1	**<0.001**
Temperature x Maternal acclimation	20.412	1	**<0.001**
Temperature x Developmental temperature	57.337	1	**<0.001**
Temperature x Maternal acclimation x Developmental Temperature	38.351	1	**<0.001**

Bold values indicate factors which had a significant effect.

Table 2: Type III Analysis of Variance Table with Satterthwaite's method for the linear mixed effect models showing the effect of maternal acclimation and offspring developmental temperature on body size (i.e., area and skeletal rod length) of early developmental stages of *S. purpuratus.*

		MS	NumDF	DenDF	*F*	*p*
Hatched Blastula						
Area						
	Maternal acclimation	6.12e-07	1	12	3.963	0.07
	Developmental temperature	3.94e-07	1	12	2.552	0.136
	Maternal acclimation x developmental temperature	6.05e-07	1	12	3.918	0.071
Early Gastrula						
Area						
	Maternal acclimation	2.41e-05	1	12	65.612	**3.31e-06**
	Developmental temperature	8.24e-07	1	12	2.242	0.16
	Maternal acclimation x developmental temperature	1.00e-05	1	12	27.265	**2.14e-4**
Prism						
Area						
	Maternal acclimation	5.28e-06	1	12	8.335	**0.014**
	Developmental temperature	4.46e-05	1	12	70.403	**2.95e-06**
Rod length						
	Maternal acclimation	1.64e-04	1	12	0.619	0.447
	Developmental temperature	0.052	1	12	197.586	**8.08e-09**

Bold values indicate factors which had a significant effect.

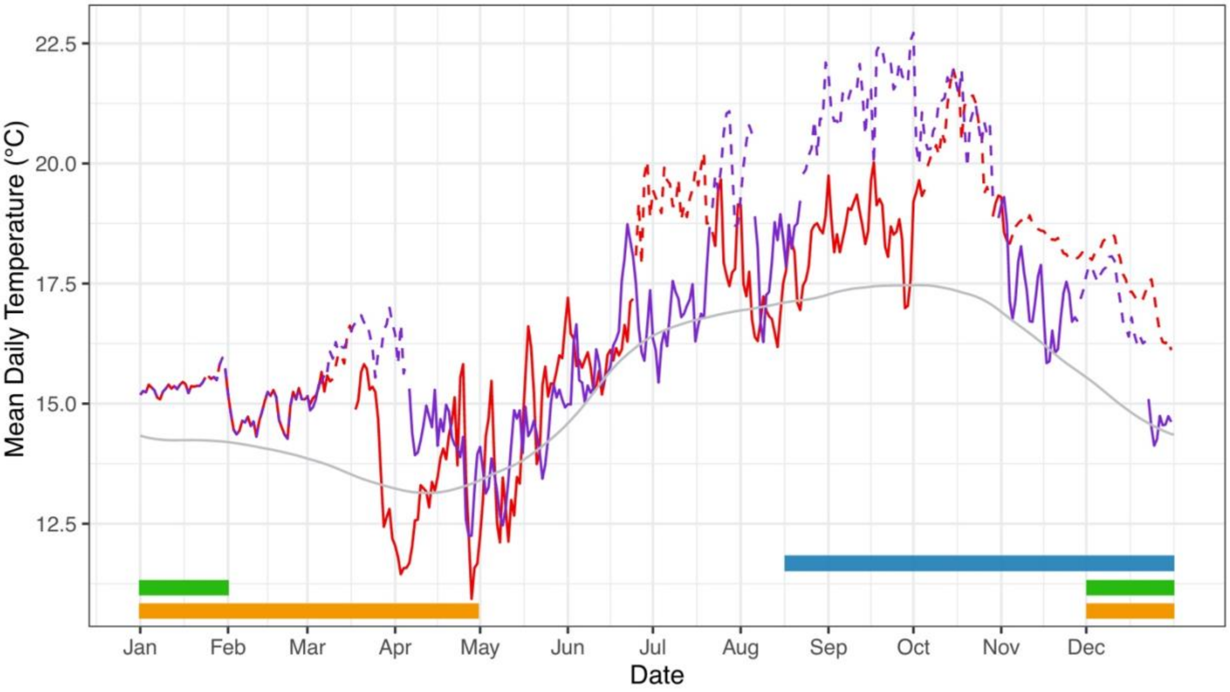

Figure 1. Mean daily temperature experienced by adult purple sea urchins during the 2014-2015 MHW. Temperature data were collected on the benthos using ONSET HOBO temperature loggers by the Santa Barbara Coastal LTER. The red and purple lines represent 2014 and 2015, respectively; the grey line represents the climatological mean (2001-2021). The occurrence of a MHW event, identified using metrics established by Hobday et al. (2018), is indicated by a dashed line for both 2014 (red) and 2015 (purple). Timing of life history events for S. purpuratus are represented by colored bars: gametogenesis (blue), spawning (green), and planktonic larval duration (yellow) (Rogers-Bennett, 2013; Okamoto et al., 2020).

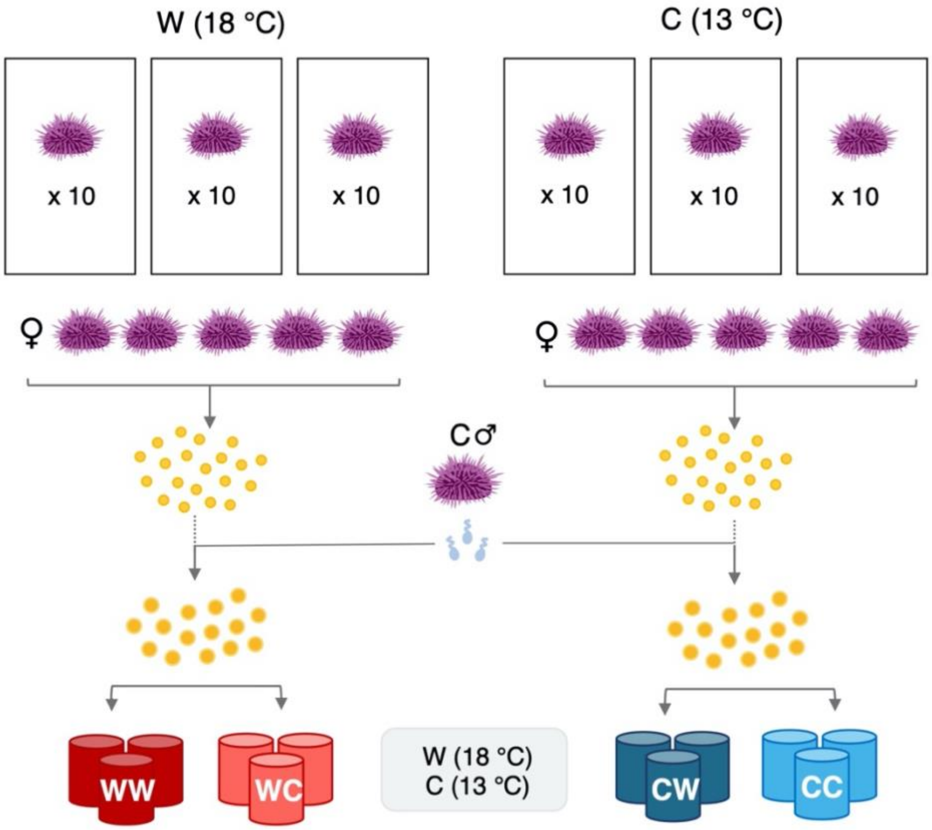

Figure 2. Experimental design. Adult *S. purpuratus* (*N*=60) were acclimated in either a MHW (W: 18°C) or a non-MHW (C: 13°C) condition, for a four-month period, during gametogenesis. Eggs from each maternal treatment were pooled (*N*=5 females per treatment) and fertilized using sperm from a single non-MHW- acclimated male. Embryos from each cross were reared at either a MHW or non-MHW temperature, resulting in four groups: WW, WC, CW, and CC. Each group was raised in three replicate culture vessels.

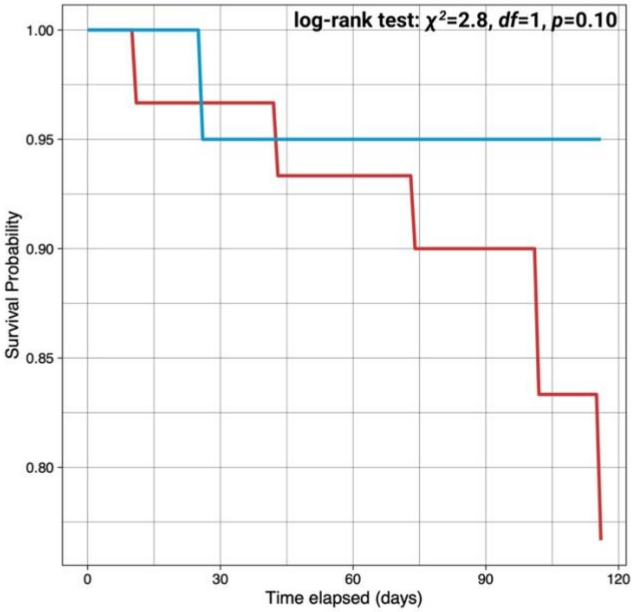

Figure 3. Survivorship in *S. purpuratus* adults over 4 months of acclimation at marine heatwave (18°C) and non-marine heatwave (13°C) temperatures. Adult urchins were acclimated to either a MHW temperature of 18°C or non-MHW temperature of 13°C for a four-month period from October to January. The red and blue lines represent survival probability for MHW or non-MHW condition, respectively.

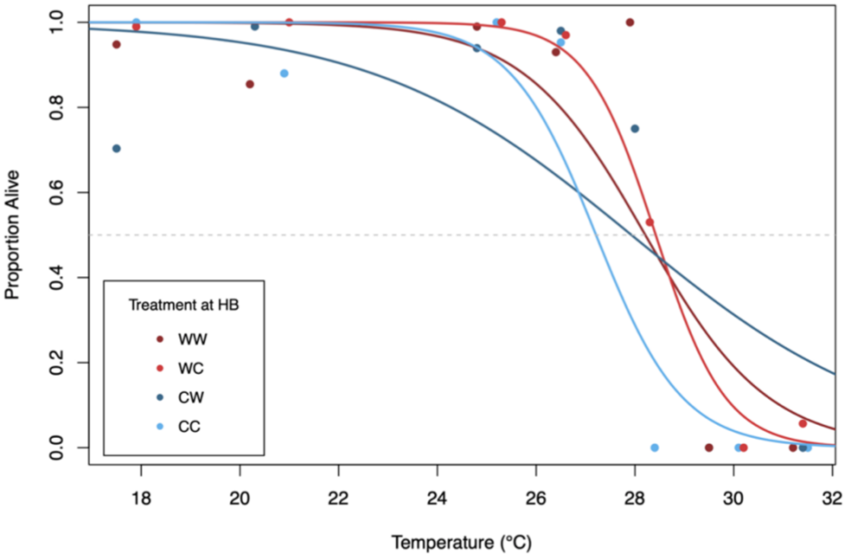

Figure 4. Survivorship of early-stage *S. purpuratus* from the four treatments. Hatched blastula from each of the four treatments were exposed to a 1hr acute temperature trial and then allowed to recover at 14°C. Following recovery and development to prism the proportion of live prism larvae were scored. Colored lines represent a logistic regression for each treatment. LT_{50} is shown at the intersection of the dashed grey line with the color-coded line for each group with different parentage and different developmental temperature.

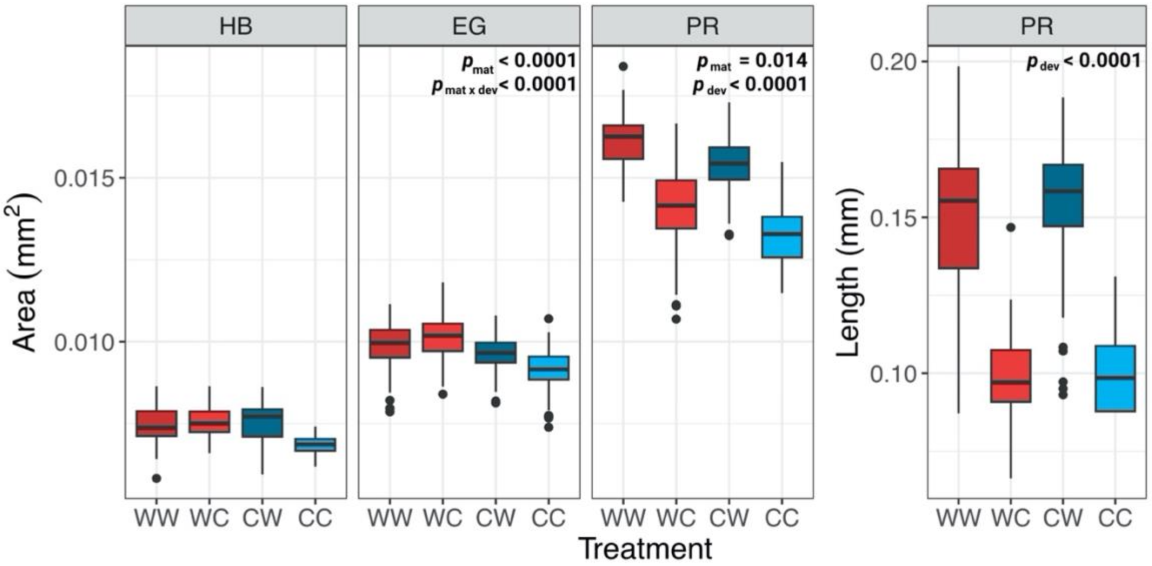

Figure 5. Morphometric analysis of embryo and prism stage *S. purpuratus*. Body size of embryos and larvae at three developmental stages: hatched blastula (HB), early gastrula (EG), and prism (PR), for each family combination (WW, WC, CW, & CC). Only results with significant *p*-values are displayed on each graph.

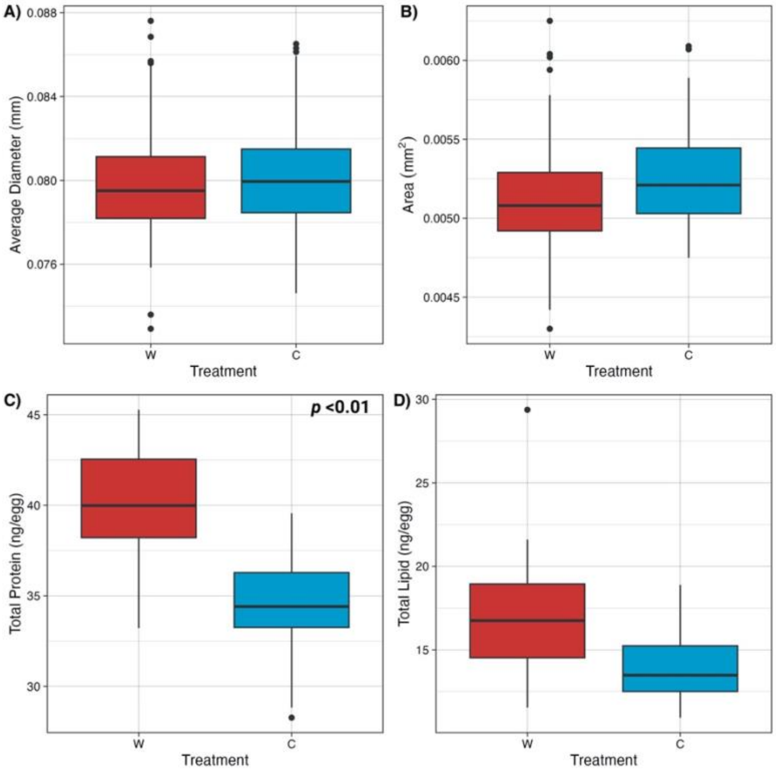

Figure 6. Egg morphometrics and assessment of biochemical storage biomolecules. A) Diameter and B) 2D area of eggs from females acclimated to MHW (W) and non-MHW (C) conditions. C) Total protein (ng/egg) and D) total lipid content (ng/egg) of eggs from females acclimated to MHW (W) and non-MHW (C) conditions. Only results with significant p-values are displayed on each graph.

V. Conclusion

Climactic extremes such as marine heatwaves are becoming a dominant force in the world's oceans, while average seawater temperatures also continue to increase (Oliver et al., 2018). This rapid pace of global change is posing a challenge to species, as organisms are pushed to or past their physiological limits (Smith et al., 2023). To predict how these stressors will impact species and ecosystems, knowledge of thermal limits and the capacity to adjust these limits via acclimation is critical. This is especially true for species living near their thermal limits or species that vary in sensitivity to thermal stress across their life history (Sinclair et al., 2016). In this dissertation I examined how changes in ocean temperature are impacting two ecologically important species in coastal waters of the Northeast Pacific, the California mussel (*Mytilus californianus*) and the purple sea urchin (*Strongylocentrotus purpuratus*). Specifically, I examined how heatwaves alter their thermal experience and their potential to cope with this thermal stress via transgenerational and intragenerational (developmental) plasticity. From this general body of research, three main takeaways are highlighted and discussed below.

1. Long-term monitoring of environmental parameters provides key biological insights

Each chapter presented in this dissertation relied on long-term temperature data. This was made possible through efforts of long-term monitoring programs such as the Partnership for Interdisciplinary Studies in Costal Ocean (PISCO), the Multi-Agency Rocky Intertidal Network (MARINe), and the Santa Barbara Coastal Long Term Ecological (SBC LTER) Research Program.

In Chapter II, I examined patterns of *M. californianus* body temperature across latitude using biomimetic temperature loggers, robomussels. While traditional temperature sensors provide useful environmental data, for ectotherms that experience large variations in temperature in short timeframes, these bio mimics provide key insight into organism physiology (Fitzhenry et al., 2004; Helmuth et al., 2016). This early realization motivated scientists to maintain this dataset for *Mytilus* species, as thermal events that influence mussel populations could drastically alter intertidal biodiversity (Miner et al., 2021). Using this data, I was able to discern annual temperature patterns experienced by mussels across their biogeographic range in Oregon and California. Dependent on the site, annual patterns were compiled using 5–17 years worth of data which increased confidence in the observed trends. Moreover, this long-term data allowed me to assess how body temperatures were impacted by anomalous events such as the 2014-2016 Northeastern Pacific MHW. Here, I found that during MHW years, previously determined annual patterns were disrupted and certain populations may have been pushed past optimal thermal performance windows (Coe and Fox, 1942). The work presented in Chapter III of this dissertation also relied on this long-term dataset from the biomimetic robomussels. Here, temperature data collected at the microhabitat scale provided information on thermal histories of adult *M. californianus* that may have contributed to TGP. Lastly, in Chapter IV subtidal benthic temperature data collected by the SBC LTER was used to inform temperature conditions used in the laboratory experiments on *S. purpuratus.*

While long term research programs are often costly and difficult to maintain, they are of critical importance for understanding how the environment is changing over time. This data allows scientists to identify trends and patterns over extended periods. Moreover, since the marine environment is inherently variable, long-term data allows researchers to distinguish between natural variation and sustained trends. A common approach for examining changes in ocean temperatures has relied on temperature data collected using satellites (Hobday et al., 2016). This data is also critical as it can help discern global patterns by providing more complete spatio-temporal sampling (Merchant et al., 2019). However, it is not representative of temperature profiles at the benthos. Data collected *in situ* by the previously mentioned monitoring programs has allowed for an accurate examination of changes in temperature experienced by benthic marine invertebrates in intertidal and subtidal ecosystems.

Moreover, environmental data paired with biological monitoring has allowed scientists to examine climate change impacts on ecosystem structure and identify ecosystems that may be more or less resilient (Miner et al., 2021; Ziegler et al., 2023). While efforts have been made to measure many biological indices such as organism growth, abundance, and diversity, long-term measurements that target other life history stages can provide a more comprehensive understanding of how organisms are being impacted (Miner et al., 2021; Michaud et al., 2022). For example, using recruitment data, Okamoto and colleagues were able to show how *S. purpuratus* recruitment is differentially impacted by warming across latitude (Okamoto et al., 2020). To support this, long-term measurements of adult gonadal index can provide further insight into identifying how warming is impacting *S. purpuratus* populations.

2. Transgenerational and intragenerational plasticity varies with acclimation state and offspring developmental stage

Transgenerational plasticity, which refers to modification of offspring phenotype induced by signals experienced by parents, is well documented in marine taxa (Donelson et al., 2016; Zhao et al., 2020; Truong et al., 2022). In general, TGP has been studied extensively in marine invertebrates with mixed results depending on taxa and the context of the environmental factor in question (Byrne et al., 2020). At present, TGP studies in wild populations or in a MHW context are few, but emerging data is providing important insight into how thermal stress interacts with the phenology of life history events (Gall et al., 2021; Minuti et al., 2021; Waite and Sorte, 2022).

In Chapter III I found evidence of transgenerational effects with regard to seasonal acclimatization in wild populations of *M. californianus.* Larvae from winter acclimatized mussels exhibited traits related to higher performance (i.e., larvae were larger, had lower metabolic rates, and higher thermal tolerance), than those from summer acclimatized parents. My initial hypothesis was that temperature would be the driving force of any observed transgenerational effects, but the lack of difference seen between high and low zone mussels challenged this idea. Thus, I predict that differences seen across season may be attributed to other factors that also vary across season, such as access to food or exposure to upwelling conditions. Further, in Chapter IV I documented positive transgenerational effects of temperature in *S. purpuratus*. Adults acclimated to warm temperature conditions produced larger more thermally tolerant larvae. In combination both studies provide evidence of TGP in both laboratory and wild populations.

Evidence of developmental plasticity was observed for both *M. californianus* (Chapter III) and *S. purpuratus* (Chapter IV). In Chapter III, developmental temperature influenced body size, metabolic rate, and thermal tolerance. Larvae reared in the warm developmental treatment (20°C) were larger, had higher respiration rate, and lower thermal tolerance than those reared in the cool developmental treatment (16°C). In Chapter IV, however, embryos had higher thermal tolerance when reared at the warm developmental temperature (18°C) when compared to the cool developmental temperature (13°C). I theorize thermal responses differed due to differences in developmental rearing temperatures and the stage at which offspring were assessed. For both studies, developmental rearing temperatures were selected based on temperatures developing embryos experience in the water column. For *S. purpuratus,* spawning occurs from December-February (*G. Hofmann, pers. comm.*), so larvae will likely experience cooler temperatures than *M. californianus* which spawn year-round (Coe and Fox, 1942). Higher developmental temperatures used for *M. californianus* may have exceeded optimal performance windows, thus exhibiting an energetic tradeoff between growth and thermal tolerance.

Moreover, the stage at which offspring were exposed to the acute thermal tolerance may have also contributed to these differences. In both studies, offspring were unfed prior to all assays as I was interested in assessing how initial maternal investment influenced physiological performance during early development. *M. californianus* were exposed at an early larval stage (D-stage veliger), while *S. purpuratus* were exposed at an early embryonic stage (hatched blastula). Thus, depletion of energy stores may have been greater for *M. californianus* larvae than *S. purpuratus* embryos at the time of the thermal tolerance trial.

While both transgenerational and developmental plasticity were observed in this study, it is important to note that there are limitations to the interpretations of these results. It is unclear how long this documented resistance will last, either in the lifetime of individuals, or across subsequent generations. While carry-over effects may increase tolerance to thermal stress in a single generation, past TGP studies have shown that acquired tolerances may not persist within or across generations (Byrne et al., 2020). This can also be said for changes in thermal tolerance due to developmental plasticity (Pottier et al., 2022).

3. Nutritional availability will play a critical role in organismal response to warming environments

For ectotherms, body temperature and thus metabolic processes are dictated by the surrounding temperature. Increased metabolic rate due to elevated temperatures can lead to decreased organismal performance if energetic demands are not met (Brown et al., 2004). In Chapter II of this dissertation, I examined the effects of MHWs on adult *M. californianus* body temperature. I found that for the southernmost population, Coal Oil Point, temperature exposure during the MHWs often exceeded reported thermal thresholds for optimal performance in adults (Coe and Fox, 1942). Here, I hypothesized that this exposure may have contributed to the decreased abundance in mussel cover in southern populations (Miner et al., 2021). In terms of nutritional availability, studies have reported changes in phytoplankton abundance and composition during MHW events which may have further contributed to changes in performance for filter feeders (Michaud et al., 2022; Arteaga and Rousseaux, 2023). Further, changes in macroalgae have the potential to impact adult performance of *S. purpuratus*. Following the four-month lab acclimation described in Chapter IV, I observed increased mortality and lower spawning rates in adults acclimated to warm temperature conditions. Both the MHW and the non-MHW-acclimated adults were fed the same quantity of kelp, however it is possible that this amount was not enough to meet the energetic demands of urchins in the MHW treatment.

Energy limitations will not only impact the adult stages, but larval stages as well. In Chapter III, I observed that *M. californianus* larvae reared in warm temperature conditions were larger, likely due to the increased metabolic rate increasing the rate of growth. However, this came at the cost of thermal tolerance. Faster growth rates and larger sizes are often seen to increase fitness of larvae by reducing planktonic larval duration and predation rates (Strathmann, 1971; Allen and McAlister, 2007). Thus, exposure to elevated temperatures may not decrease fitness if energetic demands are met via access to food.

MHW events have been linked with changes phytoplankton and macroalgae (Michaud et al., 2022; Arteaga and Rousseaux, 2023). These primary producers are a critical food source for benthic invertebrates, and thus increases in temperature and decreases nutritional availability can have compounding effects on key consumers (Michaud et al., 2022). Apart from changes in abundance, change in nutritional compositions in algal communities can extend up the food chain. For example, Lowman and colleagues have reported lower nutritional quality of kelp exposed to high temperatures (Lowman et al., 2022), which may subsequently impact urchin growth and reproduction (McBride et al., 2004). Thus, information on how MHWs will continue to alter marine primary producers will aid in our understanding of ecosystem response.

Future Directions

MHW have far-reaching impacts from organismal physiology to changes in ecosystem structure (Smith et al., 2023). Future research that incorporates the spatio-temporal patterns of heatwaves at the local level and further that matches these events to the phenology of critical life history events will improve our ability to forecast impacts of heatwaves on coastal marine ecosystems. Variation in thermal patterns are likely to have significantly different impacts on important life history events depending on the species and life history strategies in question, this perspective that has been noted in the ocean global change biology (Bautista and Crespel, 2021). Lastly, food quality and availability during MHW conditions will also influence adult and larval condition *in situ*.

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