Preparing the next generation for climate change: parental effects on larval thermal tolerance in the Pacific blue mussel (*Mytilus trossulus*)

Heidi R. Waite[1]

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[1]University of California Irvine, Irvine, CA 92697

Contact Information:
Heidi R. Waite
Department of Ecology and Evolutionary Biology
University of California, Irvine
Irvine, CA 92697
hwaite@uci.edu

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Abstract

As ocean and atmospheric temperatures increase globally, marine organisms will need to cope with or adapt to changing temperatures in order to persist. This is especially important in sessile species which cannot move to avoid stress. Parental effects, where offspring responses to environmental change are influenced by environments experienced by parents, may include mechanisms of coping with thermal stress. This study evaluated the degree to which thermal tolerance of larvae of the mussel, *Mytilus trossulus*, is influenced by parental environments. Adult mussels were collected from two sites of varying thermal stress on San Juan Island: Friday Harbor Laboratories dock (low stress) and False Bay (high stress). Air, water, and body temperatures were recorded in the field, and thermal tolerance (LT_{50}) trials were conducted on adults and on their veliger larvae raised in the laboratory. Thermal tolerance was higher in adults from the high stress environment than those from the low stress site. For mussels from the FHL site, larval thermal tolerance was ~2°C higher than that of the adults. The opposite was found for mussels and their larvae from FB. Larval tolerances were higher for mussels from the low stress environment. This study concluded that thermal acclimation may have occurred in the two study populations. In addition, larvae of parents from the high-stress environment may be more susceptible to thermal stress than those from low-stress parents. Finally, larval tolerances were similar to or higher than adult thermal tolerances which suggests larvae may not be as vulnerable as suspected.

Introduction

Marine invertebrates are threatened by the rapidly changing environment as a result of climate change and other anthropogenic activities (Hoegh-Guldberg & Bruno, 2010; Thomas et al., 2004). Changes in abiotic environmental conditions are especially important for marine invertebrates, whose physiology relies on environmental conditions (Angilletta et al., 2004). Environmental temperature is closely tied to metabolism, and consequently life span, growth, development, survival, and ultimately population dynamics (Chown et al., 2010). Thermal tolerance is often used as an indicator of a species ability to cope with temperature changes and a population's ability to persist as temperature increases (Sorte et al., 2011). In the face of ocean warming, marine organisms will need to adapt to changing temperatures in order to persist (Berg et al., 2010).

Parental effects are one possible category of mechanisms that species may use to respond to rapid temperature increases such as those under climate change. Parental effects are a type of phenotypic plasticity where offspring responses to the environment are influenced by environments experienced by parents (Fox & Mousseau, 1998). Such coping mechanisms could potentially be faster, reversible and/or more flexible than adaptation or individual plasticity (Jensen et al., 2014). Evidence of parental effects has been found in several taxa from insects to fish (e.g. Jablonka & Raz, 2009). In marine fish, parental effects have been found to mediate ocean acidification (Miller et al., 2012) and temperature increases (Donelson et al., 2012; Salinas & Munch, 2012). Several studies have also found positive effects of parental effects on temperature and salinity tolerance in marine tubeworms (Chirgwin et al., 2018; Guillaume et al., 2016; Jensen et al., 2014) and other polychaetes (Massamba-N'Siala et al., 2014). Parental effects among marine invertebrates are only now beginning to be explored as potential coping mechanisms under climate change. Little is known about the presence or absence of parental effects in mussels.

Given the knowledge gaps and importance of understanding how climate change will impact species with complex life cycles, I evaluated the degree to which responses of *Mytilus trossulus* larvae to thermal change are influenced by the parental environments as a means of coping with climate change. *M. trossulus* is a dominant competitor and native in protected intertidal and subtidal habitats in the West Coast of North America that inhabit areas of temperature extremes (Dowd & Somero, 2013). Extremes in air and mussel body temperatures are expected to increase in frequency and intensity in the next 100 years (Diffenbaugh & Ashfaq, 2010; Parry et al., 2007). Parental effects may be one option for mussels. *M. trossulus* is an ideal study species as it is present in both low thermal stress (subtidal) and high thermal stress (intertidal) environments in the San Juan Islands, WA.

I compared the thermal tolerance (LT_{50}) of adults and their larvae from two thermally distinct sites on San Juan Island, Washington: Friday Harbor Laboratories dock (low stress) and False Bay (high stress) – on San Juan Island, Washington. I addressed the following questions: (1) does thermal tolerance of adults vary between those in thermally high and low stress environments?; (2) how do thermal tolerances of larvae and adults compare within study sites?; (3) do thermal tolerances of larvae vary between parents from thermally high and low stress environments? I expected higher tolerance to occur in the high stress site adult population due to exposure to more thermally stressful environments. I also predicted that larval thermal tolerances will be lower than adult thermal tolerances because larval stages are considered to be a more vulnerable life stage especially to temperature (Pandori & Sorte, 2019). Finally, I predict that more thermally tolerant adults will yield more thermally tolerant larvae.

Methods

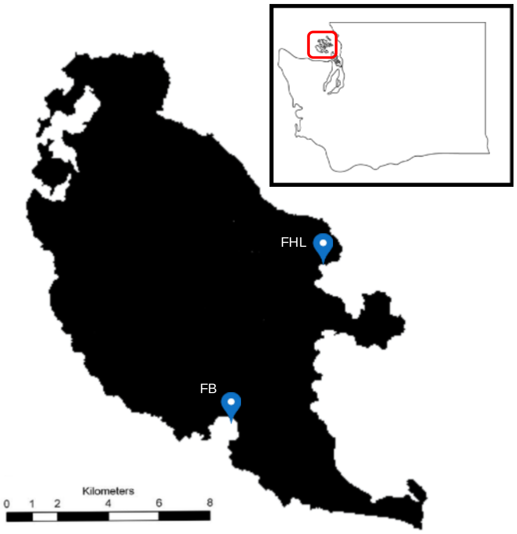

Figure 1: Study Sites. Mussels were collected from False Bay (FB) and Friday Harbor Labs dock (FHL) on the San Juan Island, Washington.

Study Sites and Collections

Mytilus trossulus individuals (N=20 per site) were collected from two sites: False Bay (FB) and the Friday Harbor Labs dock (FHL) (Fig. 1). FB was classified as a high thermal site and FHL as a low thermal stress environment. Mussels at FHL were collected from tires hanging off the dock. These mussels were subtidal and experience only water temperatures. The average FHL water temperature during study period from mid-June to early August in 2019 was 11.5°C (NOAA National Data Buoy Center). FB was classified as a high stress environment. FB is a ~1km^2 sand flat where mussels are found on boulders exposed during low tide. Mussels were collected near the head of the bay where water depth reaches about 1 m at high tide (Price & Hylleberg 1982). Water temperatures range from 11.2°C at night to 18.9°C during the day at high tide and may exceed 23°C after low tide (Price & Hylleberg 1982). However, thermal stress is highest during low tide when mussels are exposed to air temperatures.

To determine thermal stress experienced by mussels, I deployed iButtons (Maxim Integrated) were deployed at each site for at least 1 hour while I collected adults. Air temperature and rock/substrate temperature were recorded for FB and water temperature for FHL using multilogger thermometers (Omega Engineering HH506RA). Multilogger thermometers were also used to record individual mussel body temperatures of at least 3 mussels at each site by inserting a sensor inside of the shell for at least 1 hour. Mussels were transported back to Friday Harbor Labs and immediately cleaned, measured (length, width, depth, and weight), and induced to spawn (Fig. 2).

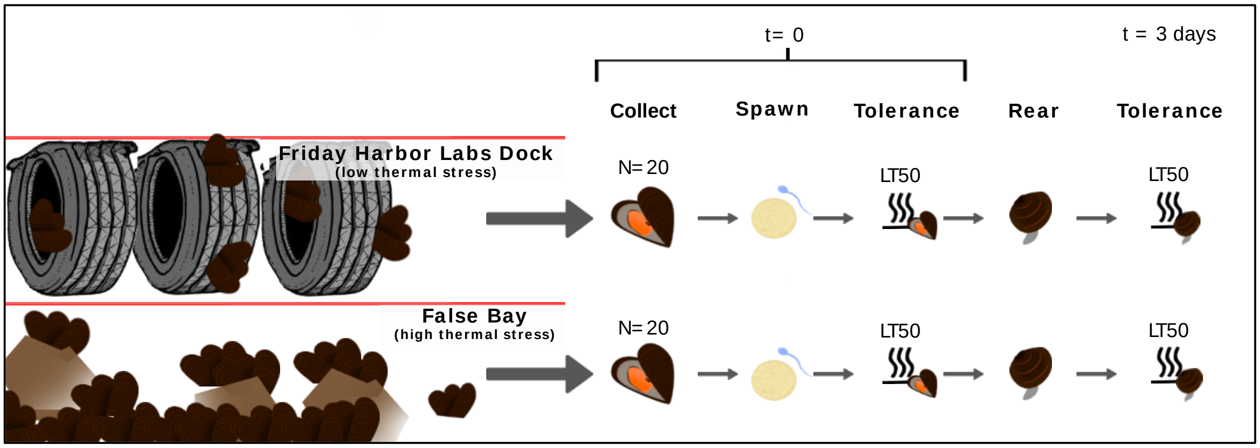

Figure 2: Methodology overview. Mussels (N=20) were collected from two locations (FHL & FB) and spawned at t=0 (Day 1). Adult thermal tolerance was evaluated immediately after spawning using an LT_{50} design. Eggs and diluted sperm were combined and larvae were reared for 3 days (t=3) after which thermal tolerance of larval LT_{50} was measured.

Spawning

To induce spawning, mussels were injected with 0.4-0.5 mL of a 2mM solution of serotonin (5-hydroxytryptamine, sulfate complex) into the abductor muscle and/or mechanically stimulated to release gametes (Strathmann, 1987). Mussels were kept in separate fingerbowls with filtered seawater (0.45 micron). Eggs from 2 females were pooled and fertilized with diluted sperm from 3 males for FHL. Eggs from 1 female and 1 male were combined for FB. Fertilization success as indicated by formation of a polar body (Strathmann,1987) was qualitatively evaluated under the microscope. The diluted sperm solution was added until ~ 90% of the sampled eggs were fertilized. Fertilized eggs were then split into 3 replicate jars (~480 per 800 mL glass jar). This was done for each site for a total of 3 replicate jars per site.

Adult LT_{50}

After spawning, adults were immediately tested for thermal tolerance in a LT_{50} (temperature lethal to 50% of larvae) experiment. Five mussels were randomly assigned to 1 of 4 temperature water bath treatments: 12°C (ambient/control), 24°C, 32°C, and 38°C. Mussels were then placed in tubes with perforated caps and a seawater-soaked chamois cloth. Temperature was recorded using multilogger thermometers (Omega Engineering HH506RA) with sensors in an empty "dummy" tube for each water bath. Mussels were exposed to treatment temperatures for 6 hours with an 18-hour recovery period in ambient temperature water in a seawater table. After recovery period, mussels were assessed for survival – those that were gaping or that did not respond to touch were considered dead.

Larval Rearing and Larval LT_{50}

Embryos were left undisturbed for 48 hours to allow larvae to develop to D-stage veligers. After 48 hours, larvae were fed *Isochrysis galbana* at 30,000 cells/mL/day. 72 hours after fertilization, larval thermal tolerances were evaluated with a LT_{50} experiment. Using heat blocks, larvae were exposed to treatment temperatures (12°C control/ambient, 21°C, 24°C, 28°C, 38°C) in 1.5mL microcentrifuges (one microcentrifuge for each of the 3 replicate culturing jars) with 20-30 larvae for 1 hour with 1-hour recovery. After the recovery period, survival was assessed. Mussel larvae were considered dead if (1) no movement was detected, (2) they had dull opaque shells, and/or (3) their shells gaped and did not move.

Data analysis

Thermal tolerance (LT_{50}) was calculated using binomial regressions between assay temperature and proportional survival in the MASS package (Venables & Ripley, 2002). T-tests or Mann-Whitney Wilcoxon tests were used to compare adult mussel sizes (length, width, & depth) and wet weights between sites.

Results

Environmental Parameters and Mussel Measurements

Average *in situ* body, air, water, and/or rock temperatures were calculated for each site (Table 1). Mussels at FHL were on average 1.1°C warmer than the water temperatures they were submerged in. Mussels at FB were 3.3°C warmer than the average air temperature, but 2.0°C cooler than the rock surface to which they were attached to.

Table 1: Average temperatures for water, air, rock, and body temperatures at both sites. Only, water and body temperature were taken at FHL because mussels were subtidal. N=4 mussels were used for body average temperatures at FB, and N=3 mussels at FHL.

Site	Average Water Temperature (°C)	Average Air Temperature (°C)	Average Rock Temperature (°C)	Average Body Temperature (°C)
FHL	13.2 ± 1.4	-	-	14.3 ± 0.8
FB	-	23.3 ± 5.2	28.6 ± 2.0	26.6 ± 0.3

Mussels from FHL were greater in length (Mann-Whitney; $p<0.01$), width (T-Test; $p<0.001$), and wet weight (T-Test; $p<0.01$) (Table 2) than those from FB. Shell depth was not significantly different between sites (Mann-Whitney; $p=0.4407$).

Table 2: Means (± standard deviation) for length, width, depths, and wet weights are listed below for 20 mussels at each site. *significantly different between sites

Site	Shell length (mm)*	Shell width (mm)*	Shell depth (mm)	Wet Weight (g)*
FHL	48.0 ± 9.9	25.4 ± 3.9	18.5 ± 4.3	13.2 ± 7.4
FB	38.8 ± 4.3	20.7 ± 2.4	17.3 ± 2.2	7.6 ± 3.5

Spawning

Using serotonin or mechanical stress (pulling byssal threads, shaking, etc.), 25% of mussels at FHL (3 males and 2 females) and 10% (1 male and 1 female) from FB spawned. On average, egg diameters from mussels at FHL were 68 ± 9.0 µm (N=15 eggs). Egg diameters from mussels from FB were on average 66 ± 8.2 µm (N=15 eggs). Egg diameter were not significantly different between sites (Mann-Whitney; $p=0.8044$).

Adult Thermal Tolerances (LT_{50}) Between Sites

Thermal tolerance was ~ 1°C lower for the mussel population from FHL to those from FB (Figure 3). Mussels at FHL had an LT_{50} of 32.5 ± 1.3 °C while FB mussel had an LT_{50} of 33.5 ± 2.1°C.

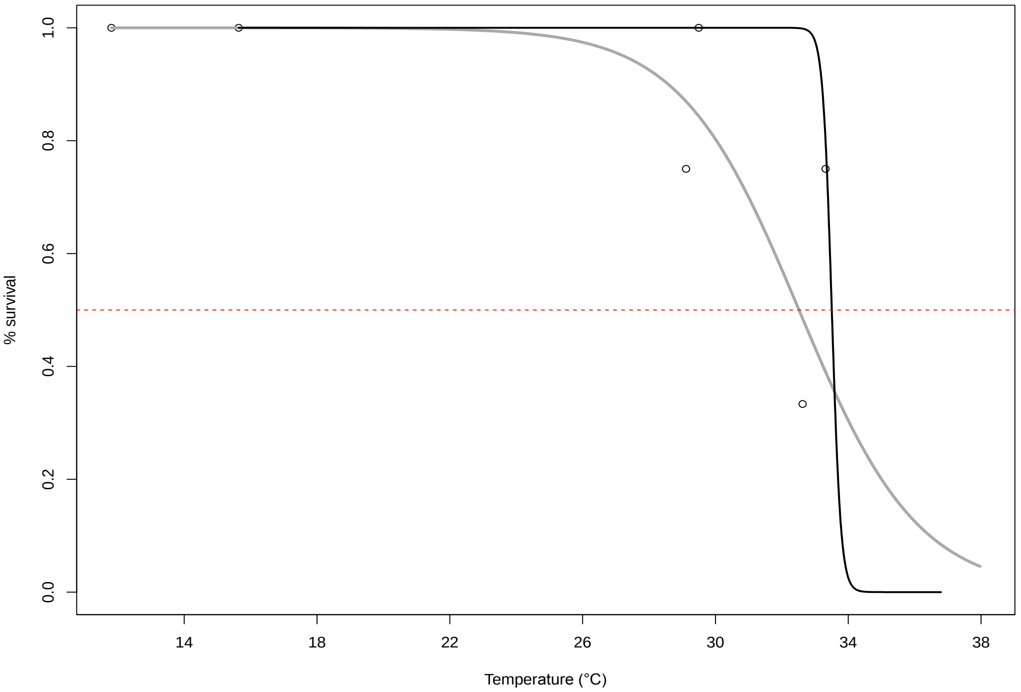

Figure 3: Thermal tolerances of adult mussel populations at FHL (grey) and FB (black). The mussel population at FB had a slightly higher LT_{50} thermal tolerance (33.5 ± 2.1°C) than those at FHL (32.5 ± 1.3 °C). The red line shows 50% survival.

Parental Effects: Comparing Adult and Larval Thermal Tolerances

Adult mussels from FHL had a lower thermal tolerance than their larvae (Figure 4). Adult mussels had a LT_{50} of 32.5 ± 1.3 °C while the combined larval LT_{50} was 34.7 ± 0.8°C.

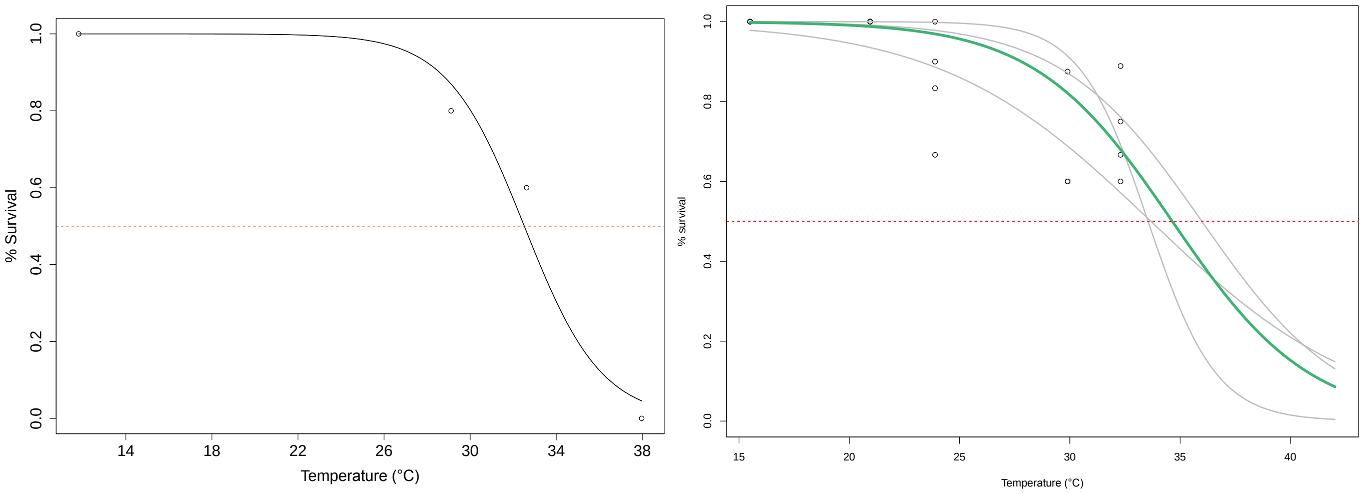

Figure 4: Thermal tolerances of adult and larval mussel populations at FHL. The adult LT_{50} curves is illustrated on the left and the larval LT_{50} curves on the right. Each grey line in the larval curves is a replicate jar. The green line shows the average LT_{50} curve for FHL larvae. Adult LT_{50} was 32.5 ± 1.3°C, while the combined larval LT_{50} was 34.7 ± 0.8°C.

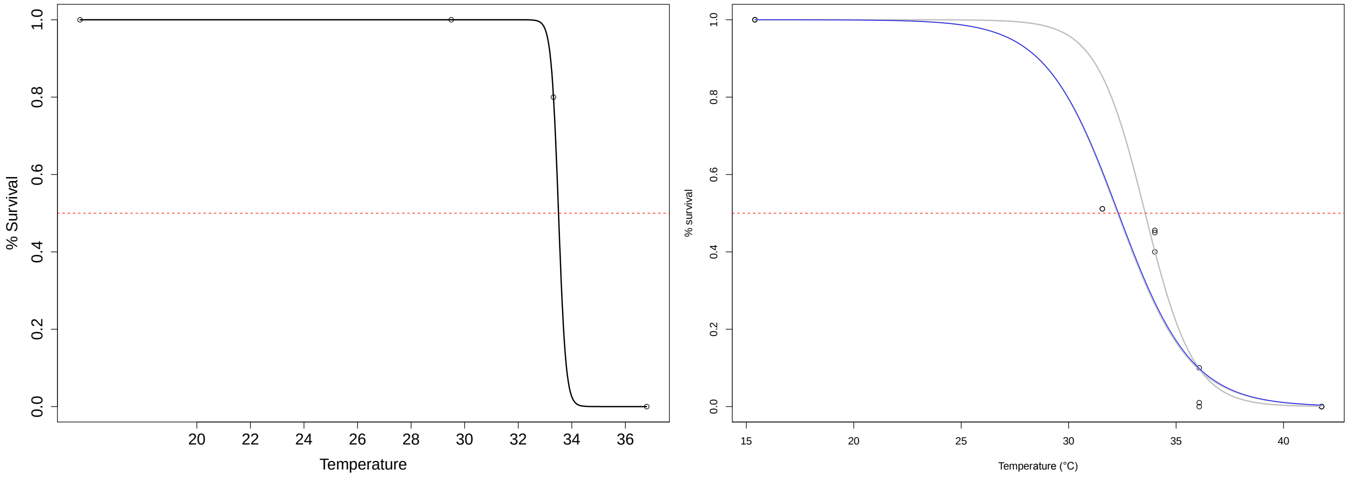

Figure 5: Thermal tolerances of adult and larval mussel populations at FB. The adult LT_{50} curves is illustrated on the left and the larval LT_{50} curves on the right. The two grey lines in the larval curves are replicate jars. The blue line shows the average LT_{50} curve for FHL larvae. Adult LT_{50} was 33.5 ± 2.1°C, while the combined larval LT_{50} was 32.3 ± 0.3°C.

Adult mussels from FB had a slightly higher thermal tolerance than their larvae (Figure 5). Adult mussels had a LT_{50} of 33.5 ± 2.1 °C while the combined larval LT_{50} was 32.7 ± 0.3°C. In addition, LT_{50} temperatures were higher for larvae from FHL adults than those from FB (Table 3).

Table 3: LT_{50} thermal tolerance values for adults and mussels at 2 sites on San Juan Island, WA.

Site	Adult LT_{50}	Larval LT_{50}
FHL	32.5 ± 1.4 °C	34.7 ± 0.8°C
FB	33.5 ± 2.1°C	32.3 ± 0.3 °C

Discussion

In order to understand how climate change will impact marine species with complex life cycles like mussels, it is essential to look at all stages across development. This study examined the differences in thermal tolerances between mussels, *M. trossulus*, from different sites on San Juan Island, WA and the impact that the thermal environment experienced by adults had on the thermal tolerances of larvae. Thermal tolerances (LT_{50}) of adults and their larvae in two thermally distinct sites were measured. I compared: (1) the thermal tolerance of adult mussels between the two thermally distinct sites, (2) larval and adult thermal tolerances within study sites, and (3) larval thermal tolerances between sites.

Field temperature measurements at the Friday Harbor Laboratory dock and False Bay (Table 1) clearly demonstrate the difference in thermal stress between sites. Mussels at FHL are subtidal and only experience the cooler water temperatures. In contrast, mussels at FB experience both elevated air and rock temperatures at low tide. These heat differences are reflected in the *in situ* body temperatures of selected mussels (Table 1). The highest body temperatures measured for mussels at FB were almost twice as high as those measured at FHL.

Accordingly, this study found that adult thermal tolerance was ~1°C lower in the mussel population from the low stress site (FHL) when compared to the high stress site (FB) with LT_{50} values of 32.52 ± 1.27 °C and 33.50± 2.14°C respectively. However, LT_{50} calculations are sensitive to the treatment temperatures which varied slightly between adult LT_{50} trails from each site. Additionally, the interval around the calculated LT_{50} was a few degrees (partially constrained by the ability to create consistent water baths with aquarium heaters available) which could have impacted the temperature calculated in the binomial regression. Nonetheless, these calculated tolerances were consistent with previous studies on *M. trossulus* which found a thermal behavioral threshold between 25-33°C and observed low survival after short-term exposure to 33°C (Dowd & Somero 2013).

The difference in thermal tolerance is likely a function of thermal history and habitat type (subtidal versus intertidal) of mussel populations. The subtidal mussels from Friday Harbor experiences an average water temperature of 11.5°C and an extreme of 13.9°C in 2018 (NOAA National Bouy Center). It is surprising that the subtidal mussels still had high thermal tolerances despite little temperature stress. The temperatures I collected in the field at FB suggest that mussels experience high air temperatures of up to ~23°C during low tide and up to ~27°C on the rock substrate. Several studies have found phenotypic plasticity in thermal responses and local acclimation in several marine invertebrates including barnacles (Bertness & Gaines 1993), corals (Hellberg 1996), prawns (Berglund & Lagercrantz 1983), mussels (Koehn et al. 1976; McGrorty & Goss-Custard 1993), and *M. trossulus* near Vancouver (Yanick et al. 2003).

In addition, heat shock protein (HSP) could be a potential molecular mechanism used by the False Bay mussel population to mediate thermal stress, resulting in higher thermal tolerances. HSPs are a molecular response to environmental stressors especially heat events which help prevent denaturation of enzymes and are hypothesized to be a mechanism to buffer thermal stress (Buckley et al. 2001). Previous studies found acclimation to occur in mussel populations at

British Camp in Garrison Bay (Buckley et al. 2001). Buckley et al. (2001) documented thermal acclimation both during different seasons and when reared to different temperatures in the lab where those in higher temperatures (either experimentally or naturally in the summer) had higher levels of HSP set points, suggesting higher thermal tolerance (e.g. Feder et al., 1997; Tomanek and Somero, 1999). Accumulation of HSPs, however, are costly (Williams et al. 2016). Production of HSPs can conflict with other protein synthesis (Heckathorn et al., 1996b), though these could be mediated by resources, energy or nutrient storage from high-resources episodes (Krebs et al. 1998; Kreeger et al., 1995; Widdows & Hawkins, 1989), or depression of non-HSP protein production during high thermal stress episodes (Solomon et al., 1991). But these tradeoffs can have an effect on fitness or other characteristics including growth rates (Krebs et al. 1998). It is possible that this tradeoff between growth, HSP production (molecular response), and other physiological response may explain the smaller sizes of mussels at FB (Table 2). Regardless, *Mytilus edulis* populations exposed to air (e.g. intertidal) tend to have higher tolerances (5°C difference) than those in water (Jones et al. 2009) – implying that intertidal populations would have higher tolerances than subtidal populations which coincides with this study's results. Therefore, local acclimation, phenotypic plasticity, and thermal histories could explain the difference in thermal tolerance found between studied populations.

Interestingly, the larval LT_{50} was higher than the adult LT_{50} at FHL, but the opposite was occurred with mussels from FB. Larvae from FB had a thermal tolerance slightly lower than adults from this site. Most studies have found the larval phase to be one of the most vulnerable and sensitive life stages to thermal stress (Hayhurst & Rawson, 2009; Rayssac et al. 2010) along with embryos (Pandori & Sorte 2019) across marine invertebrates. In fact, the effect of temperature has been suggested to have the most effect on growth and survival on early ontogeny for mussels (Rayssac et al. 2010) and other marine species (Bayne 1965). *M. trossulus* D-stage larvae were found to have higher levels of HSP70 and higher mortality in temperatures higher than 16°C (Hofmann & Somero, 1995) or 20°C (Hayhurst & Rawson, 2009) respectively. It is possible that adults have a lower tolerance at FHL because they experience a relatively consistent environment while larvae disperse to potentially more thermally variable environments, which could select for higher tolerances in larvae. Alternatively, the small size of larvae may make them more susceptible to temperatures as predicted, however, develop mechanisms to be more resilient as a result. Although temperature may be an important factor, other abiotic parameters could be more stressful for larvae or have synergistic effects. Additionally, tradeoffs occurring in the larval stage can have effects on growth, planktonic duration, as well as latent effects (Pechenik, 2006).

In contrast to my predictions, larvae from the low stress site (FHL) had a higher thermal tolerance than that of the high stress site (FB) (Table 3). Instead of imparting an advantage to their offspring, adults from the high stress environments may experience stress that could impede their reproduction, such as in some coral species (Jones & Berkelmans, 2011). The high stress mussels may be investing in mechanisms to prevent thermal damage, such as costly production of HSPs (Krebs et al. 1998) which could impede their ability to create robust gametes. Therefore, larvae from the low stress parents would have an advantage over the high stress parents. Alternatively, spawning for *M. trossulus* populations may occur earlier or later in the season and thus were not as gravid, exasperated by high temperature stress (Lowe et al., 1994). Kreeger (1993), however, suggests that at least part of the portions of the populations are gravid all year. Temperature and food – one of which I have shown varies and the other which is assumed to vary in my two study sites – are considered main factors in controlling reproduction cycles (Blanchard & Feder 1997). Therefore, the low spawning rate (1 male & 1 female) in FB may be a result of these factors. This low spawning rate could skew the larval tolerances observed for FB. The thermal tolerance observed in FB larvae could be a product of these two individual parents' phenotypes rather than a representation of the FB population.

This study found local populations can have different thermal tolerances and may be due to acclimation to the local thermal environment. Adult mussels in higher thermal stress environments tend to have a higher thermal tolerance possibly due to mechanisms such as HSP accumulation. Despite the widely accepted idea that larvae are more vulnerable to thermal change, these data suggest that larvae actually have similar or higher thermal tolerances than their parents. Larvae from the low stress site, however, had higher tolerances than larvae from the high stress site. This is possibly due to tradeoffs and a lower investment in reproduction. Understanding how tolerances develop through stages of species' life cycles is rarely attempted but is critical for predicting persistence for a plethora of Earth's species with complex life.

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