Thermal history, heatwaves, and the costs and benefits to physiological plasticity in the California mussel (*Mytilus californianus*)

by

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

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Abstract

Temperature, due to its influence on biochemical reaction rates, is one of the fundamental drivers of physiology for life on Earth. Because of this relationship, one of the predominant challenges species face in persisting in a changing climate is responding to shifts and perturbations in their thermal environment. While evolutionary processes can favourably shift phenotype distributions in response to altered thermal histories and demographic processes can improve genetic diversity and support population size and growth, possessing rapid-response mechanisms at the organismal level can assist the immediate persistence of a population facing extreme temperatures. Physiological plasticity is a process that can allow species to respond to a changing environment and improve their survival or maintain ecological performance. However, not all species are able to increase their upper thermal tolerance when facing more stressful temperatures, and while higher tolerance limits may improve survival in the short-term, there can be important and underappreciated impacts in sublethal responses like reproductive capacity. The primary goal of my thesis was to test the physiological plasticity of upper thermal tolerance, cardiac thermal tolerance, and reproductive capacity in an important rocky intertidal zone foundation species, Mytilus californianus (Conrad, 1837) following long-term aerial acclimation. Additionally, I tested the seasonal variation in upper thermal tolerance M. californianus expressed during the summer of 2021 and documented the effects of the Pacific Northwest heat dome on mussel survival in wave-exposed and wave-sheltered beds. I found that M. californianus substantially increases its upper thermal tolerance and maximum heart rate following acclimation to increasing aerial temperatures (22°C, 28°C, 34°C), but reproductive capacity is reduced at higher temperatures. M. californianus’ upper thermal tolerance increased over the summer of 2021, but to a lesser degree than the shifts produced at higher acclimation temperatures. Finally, I found minimal mortality in wave-exposed mussel beds following the heat dome, but substantial mortality in wave-sheltered beds. While plasticity in the upper thermal tolerance of M. californianus can improve persistence following short-term warming and heatwaves, declining reproductive capacity may have important population-level consequences on longer timescales that require further investigation to better understand the costs and benefits of physiological plasticity.
Lay Summary

The ability of an organism to shift its physiology in response to variations in temperature, and the consequences of elevated thermal stress, can play important roles in determining immediate the survival and longer-term persistence of a species or population facing climate warming or heatwaves. I tested the impacts of long-term exposure to increasing aerial temperatures on the physiology of an important rocky intertidal zone species, the California mussel (*Mytilus californianus*). I found that lethal thermal limits and cardiac function positively respond when mussels are exposed to higher temperatures, but reproductive capacity declines. I also found that lethal thermal limits of *M. californianus* vary seasonally, and that thermal tolerances mussels express in the field are insufficient in fully protecting populations from mass-mortality due to heatwaves. While shifts in tolerance can improve survival in the short-term, longer-term impacts of declining reproduction may also affect the persistence of vulnerable mussel populations.
Preface

This thesis is original, unpublished work. My experimental designs for my data chapter were produced in collaboration with Chris Harley. Madeleine Abbott, Jessica Li, and Ryan Ju all assisted in the preparation for and assessment of mussel survival in thermal tolerance trials. I implemented the acclimation treatments, conducted all physiological experiments, collected all specimens, conducted all mortality surveys, analyzed all data, and wrote the manuscript. Chris Harley assisted in interpreting the results and editing the manuscript.
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1: Introduction

Temperature is a fundamental driver of rates of biological systems across all levels of organization. Within an organism, temperature alters molecular kinetics which determine the rates of physiological processes ranging from simple to highly complex systems (Brown, Gillooly, Allen, Savage, & West, 2004). These temperature-dependent physiological rates regulate the ecological performance of an organism and can affect a species’ interactions, abundances, and distributions (O’Connor, 2009; Sunday, Bates, & Dulvy, 2012). Environmental temperature can be highly variable through space and time due to the variety of biogeochemical processes and feedbacks that influence it; thus, organisms need to possess mechanisms to respond to fast and slow thermal change in order to cope. Demographic and evolutionary processes can assist species in responding to thermal change on longer timescales, while physiological plasticity can provide more immediate responses to rapid shifts in temperature. Additionally, these processes interact and feed back on one another and are strongly influenced by the thermal history and life history strategies of species (Fox, Donelson, Schunter, Ravasi, & Gaitán-Espitia, 2019; Ghalambor, McKay, Carroll, & Reznick, 2007; Somero, 2010). Due to its near-universal importance for organisms, studying how temperature affects biological systems, how physiological processes dictate short-term responses to changes in temperature, and how these responses influence and are influenced by ecological and evolutionary processes helps to build our foundational knowledge of what determines the past, present, and future abundances and distributions of species.

1.1 The importance of temperature in biological systems

In biochemical reactions, higher temperatures increase the kinetic energy of molecules, increasing the frequency of molecule collisions and the proportion of molecules reaching activation energy. Within a population of molecules, most do not possess enough energy to reach or surpass activation energy (Ea), but as temperatures rise the proportion of molecules that reach this threshold increases at a greater rate than the median energy state of the population (Hochachka & Somero, 2002). This relationship allows for small increases in temperature to have disproportionately large effects on the number of molecules reaching Ea, which dictates rates of chemical reactivity (Gillooly, Brown, West, Savage, & Charnov, 2001). In addition to
temperature, enzymes play a key role in determining reaction rates. Enzymes greatly lower activation energy values, which can greatly increase the number of molecules reaching $E_a$ at a given temperature. These decreases in $E_a$ play a crucial role in increasing reaction rates at lower and more frequently experienced temperatures by a population of molecules (Somero, 1978). The relationship between temperature, the distributions of kinetic energy in molecules, and the role of enzymes in lowering activation energies plays a fundamental role in determining rates of almost every chemical reaction within a cell. Therefore, almost every biological rate, from the most basic and fundamental to those for highly complex processes, are highly temperature dependent.

Temperature’s role in altering reaction rates within temperature-dependent biological systems form some of the foundational relationships that dictate the physiology of an organism. Metabolic rates – the fundamental biological rates of life that determine the uptake, transformation, and distribution of energy within an organism – are dependent on molecular kinetics dictated by temperature. Higher levels of biological function are driven by these rates and the relationships between their own systems and temperature (Brown et al., 2004). The heat-shock response is a cosmopolitan system where proteins known as molecular chaperones prevent denatured proteins from aggregating and assist unfolded proteins in returning to their natural and productive states. Heat-shock protein induction, peak production, and downregulation are all temperature-dependent parameters whose values and rates play an important role in the response of an organism to thermal stress (Hochachka & Somero, 2002; Somero, 1995). Cardiac function contributes to aerobic metabolism and is highly temperature-dependent; thermal stress and the impairment of cardiac function can hinder aerobic metabolism and increase rates of energetically costly anaerobic ATP production (Dong, Liao, Han, & Somero, 2021). These biological systems and their thermal performance are determined via physiological and evolutionary processes, which can in turn be supported or hindered by ecological and demographic processes. Having multiple pathways by which biological systems can respond to shifts in temperature plays an important role in supporting the persistence of a species in a changing environment.
1.2 How can species respond to rapid thermal change?

Changes in thermal regimes, on both local and global scales, can occur at greatly different rates depending on the earth systems and feedbacks driving the change. In response to these variable rates of thermal change, there are multiple processes that can help species persist and adapt when facing novel conditions. In response to longer-term warming or cooling events, evolutionary and demographic processes can facilitate persistence. If temperatures become unfavourable for a population in their current location, they can be supported by an influx of individuals from a nearby source in two ways: through demographic rescue, they can increase population sizes and subsequently reduce Allee effects or the risk of stochastic fluctuations; and through genetic rescue, where incoming individuals can facilitate adaptation and reduce inbreeding risk through the introduction of genetic diversity – however, it is not a forgone conclusion these immigrants are providing novel or adaptive alleles to the at-risk population (Hufbauer et al., 2015). Evolutionary rescue, where a population adapts to a changing environment using the genetic variation found within that population, is another important process by which species can respond to change. Evolutionary rescue in practice can also interact with genetic or demographic rescue to further support persistence (Carlson, Cunningham, & Westley, 2014; Hufbauer et al., 2015). Ultimately, the potential for adaptation in temperature-dependent biological systems to a new thermal regime is one of the primary determinants of a species’ viability. While these longer-term, generational processes are crucial for the persistence of a species and their ability to adapt to new environments, possessing mechanisms that can respond to rapid change at the organism-level are important for maintaining ecological performance and fitness on shorter timescales.

Phenotypic plasticity is an important mechanism for shifting an organism’s response to a fluctuating environment. By altering morphology, phenology, behaviour, or physiology when exposed to variability in environmental drivers, organisms can improve their survival and continue to perform important ecological functions. Plants and corals can alter their physical structures to improve resource acquisition in response to local environmental conditions or competition (Hutchings & de Kroon, 1994; Todd, 2008). The timing of leaf unfolding and senescence can track with shifts in temperature in trees, and warmer springtime temperatures can lead to birds laying eggs earlier in the year (Nussey, Postma, Gienapp, & Visser, 2005; Vitasse,
Organisms can use a variety of strategies to alter behaviour to thermoregulate their body temperatures and avoid stressful conditions (Abram, Boivin, Moiroux, & Brodeur, 2017). Physiological plasticity, the focus of this thesis, is a crucial process for species facing unstable thermal conditions given the temperature-dependence of biochemical reaction rates and the upstream consequences to higher-order processes and ecological performance. Changing body temperatures within an organism can alter the structure of proteins and their stability (Chao, Merritt, Schaefferkoetter, & Evans, 2020). To counteract protein denaturation and repair unfolded proteins, heat-shock response induction and peak production temperatures can shift in response to acclimation or acclimatization (Hochachka & Somero, 2002; Tomanek, 2002; Tomanek & Somero, 2002). Cardiac function, which plays an important role in aerobic metabolism for many species, can shift critical thresholds in response to altered thermal histories (Braby & Somero, 2006; Dong et al., 2021). Plasticity can shift thermal reaction norms, performance curves, and upper and lower thermal limits, lessening the impact of changing temperatures on physiological rates – which can be particularly important in avoiding thresholds or nonlinearities that can dramatically affect organismal performance (Seebacher, White, & Franklin, 2015; Vasseur et al., 2014). The utility of physiological plasticity in improving the performance or survival of a species in response to variable or novel thermal conditions has been well-studied. In recent decades, as human-driven climate warming has progressed alongside our understanding of physiological plasticity and its potential benefits to organisms, studying plastic responses – and their potential to assist the persistence of populations – when faced with unprecedented thermal change has become a topic of increasing import.

1.3 How might physiological plasticity improve species’ responses to human-driven climate change?

Human-driven climate warming has led to shifting thermal regimes around the globe. Mean atmospheric temperatures are rising worldwide and are increasing at greater rates at higher latitudes (IPCC, 2021). In addition to overall warming, climate change is increasing both the frequency and severity of extreme weather events. Atmospheric and marine regional heatwaves are becoming more frequent, lasting longer, and reaching higher temperatures than those in recent history (IPCC, 2019; Oliver et al., 2019; Perkins-Kirkpatrick & Lewis, 2020). Climate warming and intensifying heatwaves are dramatically altering ecosystem stability and function,
the abundances and distributions of species, and many natural resources (Palacios-Abrantes, Rashid Sumaila, & Cheung, 2020; Ruthrof et al., 2018; Sunday et al., 2012). Thus, identifying mechanisms that can support the resilience of species or populations, better manage current ecosystems, or improve our ability to predict responses to future conditions are critical objectives for ecophysicists.

Physiological plasticity, due to its capacity to act as a rapid-response mechanism when facing shifting or extreme thermal conditions, has been the subject of numerous studies and debates on whether it can help species cope with climate change impacts. Developmental plasticity and bet-hedging (diversifying phenotypes to improve the chances of one surviving) have been found benefit population growth rates under variable environmental conditions (Xue & Leibler, 2018). The capacity for rapid and substantial adaptation and acclimatization have both been considered crucial components in determining winners and losers under climate change (Somero, 2010). Animals from more stable environments were found to have greater capacity to acclimate to new conditions, and that acclimation decreases the sensitivity of ecotherms to climate warming effects (Seebacher et al., 2015). However, ectotherms have been often found to be limited in their capacity to increase their acute upper thermal tolerance, and those with higher thermal tolerances may be unable to shift these limits further (Barley et al., 2021; Gunderson & Stillman, 2015; van Heerwaarden & Kellermann, 2020; van Heerwaarden, Kellermann, & Sgrò, 2016). While the effects of physiological plasticity on acute metrics like upper thermal tolerance often receive attention, sublethal consequences to more stressful thermal histories also play an important role in determining persistence. Exposure to more stressful thermal conditions can limit reproduction at temperatures far below a species’ lethal threshold (van Heerwaarden & Sgrò, 2021; Walsh et al., 2019). Even if plasticity can improve the survival of populations in the short-term, these sublethal consequences and declines in fitness in response to thermal stress may have important and often overlooked implications for persistence. Many studies look at the effects of thermal history on plastic shifts in lethal or sublethal responses, but often not both concurrently. Thus, there remains a large gap in research that examines lethal and sublethal plastic responses together to obtain a holistic understanding of the costs and benefits to acclimation or acclimatization to more stressful temperatures.
1.4 Temperature and physiological plasticity in the rocky intertidal zone

While the effects of temperature on plastic responses in ectotherms have been studied on organisms from a diverse range of ecosystems, rocky intertidal zones – due to the thermal physiology of many species and the harsh conditions of these habitats – are a unique and important system where these relationships have been explored in-depth. Body temperatures of organisms can vary dramatically over short timespans as tidal cycles interact with summertime heat (Elvin & Gonor, 1979). Topography, wave exposure, and cloud cover can all play important roles dictating the relative thermal stress species face and their abundances and distributions (Harley, 2008; Helmuth & Denny, 2003; Szathmary, Helmuth, & Wetley, 2009). Many key intertidal zone species are sessile and highly thermally tolerant due to the degree of thermal stress they face in their habitats and their inability to behaviourally thermoregulate; however, because of their life history strategies and their dependence on the environment on control their body temperatures, they have become a key subject of research as climate change, their physiology, and the implications of warming on dependent ecological communities have become increasingly relevant (Bertness & Leonard, 1997; Feder & Hofmann, 1999; Harley, 2011).

Due to the influence of aerial and seawater temperatures on the physiology and ecology of many rocky intertidal invertebrates, and their relative ease of collection and experimentation, a variety of species have been subjects for experiments on the effects of thermal exposure and acclimation on plastic responses (Bjelde & Todgham, 2013; Roberts, Hofmann, & Somero, 1997; Tomanek & Somero, 2002). In particular, the California mussel, Mytilus californianus (Conrad, 1837), is an important foundation species commonly found on wave-exposed shores on the west coast of North America and has been the subject of numerous physiological experiments (Jurgens & Gaylord, 2016). Plasticity in lethal and sublethal response to temperature have been tested in a variety of systems on both field-acclimatized and lab-acclimated mussels (Gleason, Strand, Hizon, & Dowd, 2018; Logan, Kost, & Somero, 2012; Moyen, Crane, Somero, & Denny, 2020; Moyen, Somero, & Denny, 2019; Roberts, Hofmann, & Somero, 1997). However, there is a key research gap regarding the effects of elevated thermal histories on both lethal and sublethal plastic responses within the same cohort of individuals; addressing this gap will not only provide a greater mechanistic understanding of how upper thermal limits can change with warming, but also how fitness and reproductive capacity may be altered alongside these potential shifts in
lethal responses. Further exploring the capacity for plastic shifts in lethal and sublethal responses to temperature in *M. californianus*, and the overall costs and benefits to acclimation and acclimatization in the near- and medium-term, will help to address this gap and provide improved clarity on how the species may fare when facing hotter summers with more frequent and intense heatwaves.

### 1.5 Thesis overview and research questions

Testing the impacts of rising thermal stress in lethal responses is necessary for accurately predicting how species may cope with heatwaves and acute temperatures in the immediate. However, examining sublethal responses and consequences to fitness will also be important in understanding how these near-term effects may contribute to larger population-level consequences in the future. The primary objective of my thesis was to address this important research gap in *M. californianus*, an ecologically important rocky intertidal species. The first experiment of my data chapter explores plasticity in the upper thermal tolerance and cardiac thermal tolerance of *M. californianus* following long-term aerial acclimation to varying degrees of thermal stress and quantifies the effects of varied thermal histories on the reproductive capacity of mussels. My second experiment in this chapter examines the seasonal variation in upper thermal tolerance that *M. californianus* may currently express in the field and compares it to the shifts in tolerance produced following laboratory acclimation. Finally, the 2021 heat dome in the Pacific Northwest had devastating yet uneven impacts on mussel mortality within British Columbia. Following this heatwave, I quantified survival at sites in British Columbia with varying degrees of wave exposure to document the impacts of present-day thermal events on *M. californianus* populations. This thesis unifies our understanding of the near-term implications of physiological plasticity in lethal and sublethal systems, contributes to our mechanistic understanding of how these responses may interact with one another, and provides a roadmap for future experimental work that can better reflect the consequences of elevated thermal histories.
2: Acclimation improves cardiac and upper thermal tolerance at the expense of reproductive capacity in the California mussel (*Mytilus californianus*)

2.1 Introduction

Global temperatures are expected to rise as human activity continues to increase levels of atmospheric carbon. In addition to climate warming, climatic variability and the frequency and intensity of extreme weather events are predicted to increase (IPCC, 2019, 2021). One form these damaging weather events can take are heatwaves, which can occur in either the atmosphere or the ocean (Oliver et al., 2018; Perkins-Kirkpatrick & Lewis, 2020). The impacts of these heatwaves serve as stark reminders of the critical importance of the thermal environment to ecological communities. The 2011 heatwave in western Australia devastated terrestrial and marine systems across trophic levels and altered species distributions in favour of more tropical species (Pearce & Feng, 2013; Ruthrof et al., 2018; Wernberg et al., 2013). More recently, the heat dome that occurred in the Pacific Northwest of North America from late June to early July of 2021 harmed natural and human systems from rocky intertidal zones to fruit tree orchards and forestry operations, though the full scale of these impacts are still being determined. As atmospheric and oceanic heatwave events continue to increase in frequency and intensity alongside more gradual climate warming, vulnerable species or populations will need to respond via physiological, behavioural, or evolutionary mechanisms to remain viable.

Shifts in a species’ thermal environment can drive evolutionary or ecological responses, and the magnitude and rate of thermal change can interact with these responses to dictate persistence. Species can adapt to new thermal conditions, shift their ranges to track suitable temperatures, or face extinction. However, the rapid rate of human-driven warming and the intensification of heatwaves may be temporally misaligned with the life history strategies of species and these longer-term evolutionary or demographic processes (Burrows et al., 2014; Fox et al., 2019; Hughes et al., 2019; Román-Palacios & Wiens, 2020). In birds, for example, not all species show adaptive changes in phenotype as a response to climate change, and those that do may not be doing so fast enough to remain viable (Radchuk et al., 2019). In addition, while range shifts occasionally track gradual temperature shifts and more so in the marine realm, more
prevalent and intense heatwaves can lead to key physiological thresholds being crossed earlier and more often than previously anticipated (Harvey, Marshall, Harley, & Russell, 2021; Sunday et al., 2012; Vasseur et al., 2014). If these longer-term processes fail to keep pace, rapid changes in thermal conditions may require species to respond on shorter, more physiologically relevant timescales to persist in a given location.

Phenotypic plasticity within physiological systems is an important mechanism for buffering species against rapid changes in their thermal environment. At the organism-level, phenotypic plasticity within sublethal responses can improve physiological and ecological performance at stressful temperatures, alter the timing of phenological events in response to environmental cues and ultimately affect the ability of the organism to perform associated ecological functions (Duputié, Rutschmann, Ronce, & Chuine, 2015; Körner & Basler, 2010; Schulte, Healy, & Fangue, 2011). In rocky intertidal zone gastropods, heat-shock protein induction temperatures can shift with acclimation to hotter thermal regimes (Tomanek, 2002; Tomanek & Somero, 1999). Shifts in cardiac function following altered thermal histories, which may reflect changes in systems that limit cumulative cellular damage, have been documented in a number of species (Braby & Somero, 2006; Stenseng, Braby, & Somero, 2005). Acclimatization to colder temperatures also correlates with greater cold tolerance in the mussel *Mytilus trossulus*, likely through increased intracellular osmolyte concentrations (Kennedy, Harley, & Marshall, 2020). Plasticity within these mechanisms can support organisms by shifting thermal performance to minimize the deleterious effects of stressful yet sublethal temperatures. However, these plastic responses do not always contribute to higher acute thermal limits and the associated costs to their expression can have underappreciated and often obscured effects on fitness and persistence.

Previously, plastic responses to thermal environments have been considered an important mechanism that allow a species to increase their upper thermal limits, avoid acute thresholds, and reduce mortality resulting from an extreme heat event (Chevin & Lande, 2010; Somero, 2010). While the benefits to possessing higher thermal tolerances in surviving acute thermal events may be apparent, plasticity in upper thermal tolerance is not universal, and both the species-specific scope for plasticity and associated underlying consequences to more stressful thermal histories
are important to consider when testing plastic responses to temperature. There so far has been inconsistent support for the capacity of many species to plastically shift their upper thermal tolerances, and the tolerance-plasticity tradeoff hypothesis predicts that species adapted to more stressful areas are more limited in their plastic responses in thermal tolerance (Gleason et al., 2018; Gunderson & Stillman, 2015; Moyen et al., 2020; van Heerwaarden & Kellermann, 2020; van Heerwaarden et al., 2016). Additionally, focusing on acute upper thermal limits and their plasticity may divert attention from other sublethal consequences that can affect fitness and persistence; for example, the reproductive capacity of ectotherms can become limited at temperatures below a species’ lethal threshold (Roberts, Hoffman & Somero, 1997; van Heerwaarden & Sgrò, 2021). Obtaining a more holistic understanding of whether ecologically important species express plasticity in their upper thermal tolerance, the associated costs and benefits to that expression, and the implications for both the immediate- and longer-term persistence of a population are crucial objectives in a time of unprecedented thermal change.

While processes like range shifts or adaptation may prove too slow to respond given the rates of thermal change many species are facing, phenotypic plasticity may prove valuable in supporting populations exposed to variable or extreme thermal conditions now and in the near future. Many ectothermic species already experience temperatures at or near key physiological thresholds or critical limits; therefore, possessing mechanisms that can improve their thermal tolerance, faster than evolutionary or demographic processes can, may be important for their immediate viability (Fox et al., 2019; Román-Palacios & Wiens, 2020; Vasseur et al., 2014). Additionally, as anthropogenic climate warming continues and vulnerable populations approach or surpass their thermal limits more regularly, plasticity may prove increasingly important as a rapid-response mechanism that supports that a species’ functions within an ecological community (Fox et al., 2019; Harvey et al., 2021; Jurgens & Gaylord, 2016). Conducting experiments on key species that test the plasticity of lethal and sublethal responses to more stressful thermal histories is an important step in more accurately predicting how these species, and their associated dependents, will respond to climate change.

Given the species-specific variability in plasticity of lethal and sublethal responses when exposed to higher temperatures and the importance of testing these responses in conjunction with
one another, I first examined lethal and sublethal responses to long-term thermal acclimation by simulating low tide exposure to different peak aerial temperatures (22°C, 28°C, and 34°C) in the rocky intertidal mussel species *Mytilus californianus* (Conrad, 1837). Foundation species like *M. californianus* play a key role in rocky intertidal systems by providing biogenic habitat for less thermally tolerant juveniles or infaunal species to avoid thermal stress and desiccation (Bruno, Stachowicz, & Bertness, 2003; Jurgens & Gaylord, 2016; Stephens & Bertness, 1991). While foundation species are often some of the most thermally tolerant intertidal zone inhabitants, their inability to behaviourally thermoregulate by retreating to cooler microhabitats can lead to high levels of mortality when heatwaves push temperatures beyond their physiological limits (Harley, 2008, 2011).

Despite the documented importance of *M. californianus* to intertidal zone communities and the number of ecological and physiological studies conducted on the species, a number of key connections between thermal history and plastic responses have yet to be made. Past field experiments have been unable to identify plasticity in upper tolerance in adult mussels in the field, relate changes in sublethal responses specifically to their thermal environmental, or identify consequences to expressing plasticity in their upper thermal tolerance within sublethal systems (Gleason et al., 2018; Moyen et al., 2020; Petes, Menge, & Harris, 2008). Additionally, lab-based acclimation experiments have often only examined the effects of short-term exposure on thermal tolerance or acclimation to long-term immersion under a range of thermal treatments, rather than long-term exposure to stressful aerial temperatures (Braby & Somero, 2006; Moyen et al., 2020; Roberts, Hofmann, & Somero, 1997). In my lab acclimation experiment, I looked for plasticity in upper thermal tolerance, cardiac thermal tolerance, and any resulting changes to reproductive capacity in the form of gonadosomatic index (GSI) values to gain a more complete understanding of the costs and benefits of expressing plasticity when facing hotter thermal regimes. I predicted that if peak acclimation aerial temperatures were sufficiently stressful to induce physiological defences, such as above 23-25°C where heat-shock protein production is induced in *M. californianus*, but remained below lethal thresholds, then upper thermal tolerance and cardiac thermal tolerance would increase. I also predicted a resulting decline in GSI following acclimation due to the prolonged stress and energy requirements of maintaining
underlying physiological systems (Braby & Somero, 2006; Moyen et al., 2020; Petes et al., 2008; Roberts, Hofmann, & Somero, 1997).

In addition to my thermal acclimation experiment, I tested the seasonal variation in upper thermal tolerance expressed by *M. californianus* at a site far from their southern range boundary during the summer of 2021. My goal was to document any seasonal shifts in upper thermal tolerance currently expressed over the summer and qualitatively compare those shifts to those from my acclimation experiment to identify whether *M. californianus* may be approaching the realistic limit of plasticity it could express in the field. Like my acclimation experiment, I predicted that if thermal conditions produced sufficient stress responses in field-acclimatized individuals without reaching acutely lethal temperatures, I would see increases in upper thermal tolerance over the course the summer as higher aerial temperatures continue to coincide with daytime low tides. Finally, I also quantified *M. californianus* mortality in mussel beds on wave-exposed and wave-sheltered shores following the record-breaking heat dome. I conducted these surveys to document the degree of mortality mussel beds experienced in sites far from where would typically be considered the most thermally stressful areas within their range. *M. californianus* is a widely distributed and ecologically important species on the west coast of North America; exploring the relationship between thermal histories, plasticity, and mass-mortality events in the face of intensifying heatwaves is essential in understanding where vulnerable populations may exist, what tools they have to respond to their thermal environment, and the implications climate warming may have on their persistence and ecological function.

2.2 Methods

2.2.1 Thermal acclimation experiment

2.2.1.1 Animal collection and maintenance

I collected specimens of *M. californianus* on September 1, 2020, from a southwest-facing, wave-exposed shoreline in Bamfield, British Columbia, Canada (Prasiola, lat = 48.815400°, long = -125.174905°). Mussels were collected near the upper edge of the bed but not directly from it to avoid potential edge effects. I exclusively selected mussels between 4–6cm – a
representative size class for this high intertidal site – to avoid additional confounding factors that may affect thermal tolerance (e.g. thermal inertia). After collection, I kept the mussels in a recirculating seawater system (~12°C) prior to the experiment, during the thermal acclimation period, and during the assessment period following upper thermal tolerance trials. Prior to the acclimation experiment and throughout the acclimation period, I fed the mussels in each treatment group (see below) phytoplankton shellfish mix (PhytoGold-M, Brightwell Aquatics, Fort Payne, AL, USA) once per week in an isolated flood table with a recirculating pump for two hours.

**2.2.1.2 Thermal acclimation treatment design**

I randomly assigned 864 *M. californianus* to three aerial thermal acclimation treatment groups (n = 288 for each group). There were no statistically significant differences in mussel size between treatment groups. The three temperature treatments, 22°C, 28°C, and 34°C, represented a range of peak summer temperatures experienced by high intertidal mussels at Bamfield, but did not exceed their lethal threshold of thermal tolerance (G.R.P. Brownlee, pilot study). I divided the mussels within each treatment group into four 72-cell plant propagation trays, with one mussel confined to each cell for the duration of the experiment.

Thermal acclimation treatments were applied using three separate incubators (Panasonic MIR-154, Mississauga, ON, Canada). I began thermal acclimation treatments on October 14, 2020, and continued to apply treatments until all experiments were completed on December 20, 2020. I randomly assigned acclimation treatments (22°C, 28°C, 34°C) to each incubator daily and randomly assigned trays to rack locations within each incubator. I applied the thermal acclimation treatments six times per week and fed mussels on the remaining day. Thermal acclimation treatments were designed as 12-step programs beginning at 13°C and increasing in temperature every 30 minutes until peak temperatures (22°C, 28°C, 34°C) were reached, after which individuals were held at that temperature for an additional 30 minutes for a total duration of 360 minutes (Fig. A1). Each day, I removed all mussel trays from the sea tables and immediately placed all trays in their appropriate location in the incubators set on standby at 13°C. I started each incubator program simultaneously and immediately returned the mussels to the sea tables following their completion.
2.2.2 Experimental design

2.2.2.1 Upper thermal tolerance

I conducted upper thermal tolerances assays in ten incubators on December 4, 2020. Incubators were randomly assigned peak temperature programs. Each program began at 13°C and ramped up to a peak temperature between 34°C and 43°C in 1°C intervals to capture any differences in upper thermal tolerance between thermal acclimation treatment groups. The ramping period from 13°C to the final temperature for each incubator occurred over 5 hours, increasing in temperature every 10 minutes, after which individuals were held at the final peak temperature for 1 hour before immediately being returned to the sea table. Because the duration of the trial remained constant regardless of peak temperature for each incubator, ramping rates increased with increasing peak temperatures (Fig. A2). I designed these exposures to mimic a summer low tide series in British Columbia, where emersion generally occurs at or near the hottest part of the day.

I randomly selected five individuals from each of the four propagation trays per thermal acclimation treatment to be exposed to each peak temperature (n = 20 per thermal acclimation treatment per peak temperature). *M. californianus* from each thermal acclimation treatment group were randomly assigned locations within a new propagation tray the day prior to the tolerance assay. On the day of the assay, I removed the mussel trays from the sea table and placed them in their assigned incubators set on standby at 13°C for ~30 minutes prior to the start of the experiment to alleviate potential handling stress. The ramping programs were then started, and mussel trays were immediately returned to the sea table following their completion. I assessed overall survival at the end of a seven-day period, removing dead mussels daily to maintain seawater quality. Mussel survival was assessed by physically opening or closing the shell; mussels that did not close themselves or ended up fully open due to stimulation were scored as deceased (Gleason et al., 2018).
2.2.2.2 Cardiac thermal tolerance

For my cardiac thermal tolerance experiment, I randomly selected additional mussels and measured their heart rates and body temperatures for the duration of each trial. To record mussel heart rate, I attached an infrared sensor to each *M. californianus* shell over the pericardial sac using mounting putty, which was then connected to an amplifier (Newshift AMP03, Newshift, Leiria, Portugal) and data logger (Picoscope 2204A, Pico Technology, Tyler, TX, USA) connected to a processing software (Picolog 6, v. 6.13.6.3775, 2018). Mussel heart rates were sampled at 4Hz with a low-pass filter of 10Hz (Burnett et al., 2013). To measure mussel body temperature, I drilled a ∼1.5 mm hole at the anterior end of the shell, wiped down the shell to remove dust and water, and inserted a type K thermocouple into the shell cavity and secured it with mounting putty to facilitate reuse (Pico Technology, Tyler, TX, USA; Moyen, Somero, & Denny, 2019).

I conducted cardiac thermal tolerance trials in the same incubators as the thermal acclimation treatments and upper thermal tolerance experiment. Eight *M. californianus* were tested each day of the trial and were randomly and evenly assigned to sensors throughout the experiment (e.g., day 1: 3 22°C-acclimated mussels, 2 34°C-acclimated mussels, 3 28°C-acclimated mussels). After the thermocouples and IR sensors were attached to each mussel, I placed mussels in the incubators on a Styrofoam platform where they were held at 22°C for 40 minutes to equilibrate their body temperatures and alleviate any potential handling stress before the experiment began.

The incubators used in the trials were programmed to increase by 1°C on 10-minute intervals beginning at 22°C. At the 7-minute mark of each interval, mussel body temperatures were recorded for each mussel and at the end of each 10-minute ramping interval all recording software was paused and files of heartbeat signals (from minute 5 to minute 10 of the interval) were saved. Incubator ramps continued to increase every 10 minutes until all individuals in the trial had flatlined (i.e., no distinct heartbeat signal). I ran cardiac thermal tolerance trials for eight consecutive days (Dec 13–20, 2020); only individuals with clear heartbeat signals throughout the entire duration of a trial were included in analyses (22°C-acclimated n = 12, 28°C-acclimated n = 13, 34°C-acclimated n = 14).
To quantify heart rates for each temperature interval, beats were counted from the first complete beat after eight minutes to the first complete beat after nine minutes, then divided by the total duration of this period (in minutes); this method allowed for a more precise estimate of heart rate by avoiding an artificial cut-off time that may inconveniently coincide with the periodicity of heartbeats, particularly when they became irregular at high temperatures (Fig. 1c). There was a high degree of variability in heart rates at lower incubator temperatures, which may have been due to residual handling stress. For analyses, I only considered heart rates including and beyond the 26°C incubator interval, though mussel body temperatures may still fall below that temperature due to thermal lag (Fig. 1; Moyen et al., 2019). Maximum heart rate ($f_{\text{max}}$) and flatline temperature (FLT, the temperature at which the last heartbeat occurred) were both recorded as responses to heat stress (Moyen et al., 2019; Stenseng et al., 2005). I also recorded when critical temperature ($T_{\text{crit}}$), the body temperature beyond which a precipitous decline in heart rate is observed, occurred for each individual (Braby & Somero, 2006; Moyen et al., 2019).
Figure 1. Heartbeat traces for a single *M. californianus* specimen at body temperatures including: a.) 24.5°C, at the 60-minute mark where datapoints were first included for heart rate metric calculations; b.) 33.8°C, the temperature at which \( f_{\text{max}} \) for this individual occurred; and c.) 38°C, the last incubator temperature interval before flatlining (FLT). These body temperatures occurred at 26°C, 37°C, and 44°C incubator temperature intervals respectively. These panels highlight the i.) degree of variation in heartbeat magnitude observable in a single individual across body temperatures, ii.) differences in the periodicity of heart rates at stressful body temperatures prior to \( T_{\text{crit}} \), and iii.) the irregular beat patterns that can be observed beyond \( T_{\text{crit}} \). d.) Time series data for *M. californianus* heart rate versus body temperature. Curves represent smoothed conditional means for each thermal acclimation treatment group (22°C, 28°C, and 34°C) beyond the 50-minute 26°C incubator interval, and points represent an individual mussel’s heart rate at a given 10-minute interval. The framework of this plot was not used for any statistical analyses, but rather highlights the broader pattern of *M. californianus* heart rates with increasing body temperatures, where heart rates track with higher body temperatures to a point where they plateau, and beyond which heart rates steeply decline until all individuals flatlined.
2.2.2.3 Gonadosomatic Index (GSI)

All acclimated M. californianus individuals that were not used for lethal or cardiac thermal tolerance experiments (22°C-acclimated n = 42, 28°C-acclimated n = 54, 34°C-acclimated n = 66) were dissected for their gonadosomatic index (GSI). I dissected each mussel, separated their somatic and gonad tissue, dried each component in a drying oven for seven days, then weighed both tissues separately. Additionally, mussel shells were dried and weighed to examine the effect of shell weight in addition to acclimation treatment. GSI was calculated by dividing the gonadal tissue weight by the sum of the gonadal and somatic tissues, then multiplied by 100 to improve the readability of figures (Logan et al., 2012).

2.2.3 Seasonal variation in upper thermal tolerance

2.2.3.1 Animal collection and experimental design

To examine seasonal variation in M. californianus upper thermal tolerance, I collected mussels between 4–6 cm in length from my previously-sampled location in Bamfield, British Columbia on April 14, June 14, and August 12, 2021. Immediately following collection, I transferred mussels to recirculating sea tables (12°C) and the next day randomly assigned them to incubators following the same 1°C intervals as the acclimation experiment. Due to limited incubator availability and time and space constraints relating to the COVID-19 pandemic, a reduced design was used for some trials (June 15, 35°C to 43°C; Aug 13, 36°C to 43°C). In these cases, the lowest peak temperatures were removed first; because there was no mortality until 38°C in the April 15 trial, when I expected upper thermal tolerance to be lowest, peak temperatures below this were unlikely to produce any mortality throughout the remainder of the summer. I conducted these upper thermal tolerance trials following the same incubator ramps as the acclimation experiment trials (Fig. A2), and mussels were assessed for final survival after 7 days in the same manner.
2.2.4 Post-heatwave mortality surveys

2.2.4.1 Survey location and design

After the June 2021 heat dome in the Pacific Northwest, I conducted transect surveys of *M. californianus* mortality at Bamfield, British Columbia, Canada. Surveys were conducted on August 11, 2021 at four sites with varying degrees of wave exposure (from most to least exposed: Prasiola, 48.817299°, -125.16947°; Bluestone exposed, 48.820561°, -125.16503°; Bluestone sheltered, 48.8203°, -125.16472°; Nudibranch, 48.815462°, -125.17354°). For each site, I laid as many horizontal 10m transects across the bed at the approximate vertical mid-point that the site would fit. Along each transect, I took overhead photos of the transect every 1m to quantify mortality at a later time (Nudibranch n = 40; Bluestone sheltered n = 20; Prasiola n = 30; Bluestone exposed n = 20).

To assess *M. californianus* mortality, I uploaded each transect photo into ImageJ (v. 2.1.0; Schneider, Rasband & Eliceiri, 2012) and used the transect tape as a reference to draw a 25x25cm quadrat on a haphazardly-selected location of the mussel bed within each photo. Within each quadrat, I then counted the number of mussels where survival — or lack thereof — was clearly observable (i.e. I did not count mussels whose orientation made accurate assessments unlikely). I then calculated the percentage of surviving individuals within each quadrat to obtain an overall estimate of mortality for each site.

2.2.5 Statistical analyses

I completed all statistical analyses using R (v. 4.0.2; R Core team, 2020). I ran ANOVAs for all experiments using the ‘car’ package (v. 3.0-8; Fox & Weisberg, 2019) and used the ‘emmeans’ package for all post-hoc comparisons (v. 1.5.2-1; Russell, 2020). All estimates of LT50 and LT90 were obtained from GLMs using the ‘MASS’ package (v. 7.3-53; Venables & Ripley, 2002). All figures were produced using “ggplot2” (v. 3.3.3; Wickham, 2016).
2.2.5.1 Upper thermal tolerance

For upper thermal tolerance, I fit a binomial generalized linear model with a logit link function to the survival data and tested the fit with ANOVA, with incubator interval temperature and thermal acclimation treatment group as numeric and categorical explanatory variables respectively. The interaction between thermal acclimation treatment group and incubator interval temperature was nonsignificant and subsequently dropped from the model. I then conducted post-hoc comparisons of thermal acclimation treatment groups. Finally, I produced estimates of LT50 and LT90 (temperatures that led to 50% and 90% mortality, respectively) for each acclimation treatment group.

2.2.5.2 Cardiac thermal tolerance

To analyze cardiac thermal tolerance, I compared the flatline temperatures (FLT), maximum heart rates ($f_{\text{max}}$), and critical temperatures ($T_{\text{crit}}$) between thermal acclimation treatment groups with one-way ANOVAs and Tukey post-hoc comparisons.

2.2.5.3 Gonadosomatic index (GSI)

To compare GSI values between thermal acclimation treatment groups, data were analyzed two-way ANOVAs and Tukey post-hoc tests with acclimation treatment group as a categorical variable and shell length as a numerical variable to explore potential interactions between mussel size, acclimation treatment group, and reproductive output.

2.2.5.4 Seasonal variation in upper thermal tolerance

Due to quasi-complete separation in survival data for all three time periods (one single incubator interval temperature where survival was not either 100% or 0%), two separate approaches were taken to analyze these data. To compare upper thermal tolerance across seasons, I first analyzed the survival data of the three time periods using a binomial GLM (logit link) and ANOVA in the same manner as the upper thermal tolerance trials from my thermal acclimation experiment. However, since these models did not reliably estimate LT50 and LT90 estimates due to issues with maximum likelihood estimates, I also fit a binomial GLM with maximum penalized likelihood estimates using the ‘brglm’ package (v. 0.7.2, Kosmidis, 2021) then calculated these parameters. Since ‘brglm’ is not currently recommended for model
comparisons, I have provided both model outputs in the results below to provide some means of comparing time periods while maintaining more accurate estimates of LT50 and LT90 (Kosmidis, 2021).

2.2.5.5 Post-heatwave mortality surveys

To analyze the relative degree of *M. californianus* mortality between the four sites, I ran a one-way ANOVA and Tukey post-hoc comparisons on percent mussel survival with site as a categorical variable.

2.3 Results

2.3.1 Thermal acclimation experiment

2.3.1.1 Upper thermal tolerance

As expected, increasing temperature had a strong and negative effect on *M. californianus* survival (Fig. 2; Fig. 3; ANOVA, df = 566, LR $X^2 = 539.10, P < 0.001$). I found that acclimation at higher temperatures significantly increased upper thermal tolerance (Fig. 2; Fig 3; ANOVA, df = 566, LR $X^2 = 50.92, P < 0.001$). The 34°C acclimation treatment group was significantly more tolerant than both the 28°C (pairwise z = -3.602, $P < 0.001$) and 22°C groups (Fig. 3; pairwise z = -5.984, $P < 0.001$) respectively; the 28°C group had intermediate thermal tolerance which was significantly higher than the 22°C group (pairwise z = -3.467, $P < 0.001$).
Figure 2. Binomial GLM fits for survival probability of *M. californianus* thermal acclimation treatment groups with increasing incubator interval temperatures. Points represent survival status for an individual mussel after 7 days (0 = dead, 1 = alive), curves represent GLM fits, and bars denote standard error.
Figure 3. Summary of upper thermal tolerance parameters (LT50 and LT90) for M. californianus thermal acclimation treatment groups obtained from GLM fits. Points represent temperature estimates for each parameter while bars denote standard error. Lowercase letters indicate significant differences between thermal acclimation treatment groups (Tukey’s HSD, $P < 0.05$).

2.3.1.2 Gonadosomatic Index (GSI)

In examining the reproductive capacity of M. californianus in response to differing thermal acclimation regimes, I found that the GSI of mussel specimens significantly differed between treatment groups (Fig. 4; ANOVA, $F_{2,159} = 26.171$, $P < 0.001$). Mussels from the 22°C group had significantly higher GSI values than both the 28°C (pairwise $t = 5.571$, $P < 0.001$) and 34°C (pairwise $t = 7.006$, $P < 0.001$) groups, which did not significantly differ from one another (pairwise $t = 1.290$, $P = 0.403$). Shell dry weight was also a significant positive predictor of GSI (ANOVA, $P < 0.001$); however, the interaction between thermal acclimation treatment and shell dry weight was nonsignificant so the term was dropped from the model (ANOVA, $P = 0.56$).
2.3.1.3 Cardiac thermal tolerance

I found that flatline temperatures (FLT; the temperature at which heartbeats cease for an individual) did not differ between thermal acclimation treatment groups (Fig. 5a.; ANOVA, $F_{2,37} = 0.6487, \ P = 0.53$). Critical temperatures ($T_{\text{crit}}$; the temperature beyond which a precipitous drop in heart rate occurs) also did not significantly differ between treatment groups (Fig. 5b.; ANOVA, $F_{2,37} = 1.874, \ P = 0.1678$). Finally, I found that maximum heart rate ($f_{\text{max}}$) increased with higher temperature thermal acclimation (Fig. 5c.; ANOVA; $F_{2,37} = 5.5034, \ P = 0.0081$). The 28°C group did not significantly differ from the 22°C (pairwise $t = -0.797, \ P = 0.71$).
group and marginally differed from the 34C group (pairwise $t = -2.366, P = 0.0592$), while the 34C group had a significantly higher $f_{\text{max}}$ than the 22C group (pairwise $t = -3.178, P = 0.0082$).
Figure 5. *M. californianus* thermal acclimation treatment group values for a.) Flatline temperature (FLT), b.) Critical temperatures ($T_{\text{crit}}$), and c.) Maximum heart rate ($f_{\text{max}}$). Centre lines denote median values, boxes first and third quartiles, and whiskers visualize largest and smallest values within 1.5 times above and below the 75th and 25th percentiles, respectively. Jittered points represent individual *M. californianus* values and are added for transparency. Lowercase letters indicate significant differences between thermal acclimation treatment groups (Tukey’s HSD, $P < 0.05$).
2.3.2 Seasonal variation in upper thermal tolerance

For the following summaries of my seasonal variation in upper thermal tolerance experiment, ANOVA results and pairwise comparisons are obtained from non-penalized MLE GLMs, with LT50 and LT90 values obtained from Firth logistic regressions due to quasi-complete separation between incubator temperature intervals. Increasing assigned incubator temperatures had a strong negative effect on *M. californianus* survival (Fig. 6; ANOVA, df = 575, LR $X^2 = 63.31$, $P < 0.001$). I found that time period also had a significant effect on survival (Fig. 6; ANOVA, df = 575, LR $X^2 = 63.31$, $P < 0.001$). Individuals from the August 13 trial had significantly higher upper thermal tolerance than individuals from both the June 14 (pairwise $z = 0.928$, $P < 0.001$) and April 14 (pairwise $z = -3.563$, $P < 0.001$) trials. Mussels from the June 14 trial were intermediate in their thermal tolerance, being significantly higher than the April 14 trials (pairwise $z = -5.031$, $P < 0.001$).
Figure 6. Summary of seasonal variation in upper thermal tolerance parameters (right; LT50 and LT90) for M. californianus obtained from Firth GLM fits, with upper thermal tolerance metrics from the thermal acclimation experiment (left) included for visual comparison. Points represent temperature estimates for each parameter while bars denote standard error. Lowercase letters indicate significant differences between thermal acclimation treatment groups (left) and upper thermal tolerance dates throughout the summer (right; Tukey’s HSD, $P < 0.05$).

2.3.3 Post-heatwave mortality surveys

Following the 2021 heat dome, M. californianus mortality in Bamfield BC was highly site dependent. Site had a significant effect on mussel survival (Fig. 7; ANOVA, $F_{3,106} = 54.822$, $P < 0.001$). I found that wave-sheltered sites (Bluestone sheltered and Nudibranch) did not significantly differ in survival from one another (pairwise $t = -0.773$, $P = 0.867$), but both had significantly lower survival than the wave-exposed sites of Prasiola (Bluestone sheltered pairwise $t = -3.563$, $P < 0.001$; Nudibranch pairwise $t = -10.845$, $P < 0.001$) or Bluestone exposed (Bluestone sheltered pairwise $t = 6.719$, $P < 0.001$; Nudibranch pairwise $t = 6.986$, $P < 0.001$).
Prasiola had the highest survival of all sites, with little observed mortality overall and marginally higher survival than Bluestone exposed (pairwise t = -2.446, *P* = 0.0747).

**Figure 7.** *M. californianus* survival post mid-summer heatwave in Bamfield, British Columbia, Canada, from four sites decreasing in wave exposure from left (Prasiola, most wave-exposed) to right (Nudibranch, least wave-exposed). Centre lines denote median values, boxes first and third quartiles, and whiskers visualize largest and smallest values within 1.5 times above and below the 75th and 25th percentiles, respectively. Lowercase letters indicate significant differences between survey sites (Tukey’s HSD, *P* < 0.05).
2.4 Discussion

Rapid human-driven climate warming, accompanied by more intense and frequent heatwave events, may increase the relevance of phenotypic plasticity in ectotherms responding to near- or medium-term thermal change. Experiments that measure phenotypic plasticity within both lethal and sublethal responses of foundation species can provide a greater understanding of the consequences of shifting thermal histories and identify potential vulnerabilities that may be obscured when testing these responses independently. Here, I examined the lethal and sublethal effects of acclimation in an important intertidal zone species, *Mytilus californianus*. Additionally, I examined the seasonal variation in upper thermal tolerance that *M. californianus* expressed during the summer of 2021 and documented the impacts of the Pacific Northwest heat dome on mussel mortality.

2.4.1 Thermal acclimation experiment

2.4.1.1 Upper thermal tolerance

Following long-term thermal acclimation, I found that upper thermal tolerance increased with higher acclimation temperatures, corresponding well with my prediction of greater tolerance following acclimation at higher yet sublethal temperatures. These findings only partly agree with those of other recent studies on *M. californianus* upper thermal tolerance. *M. californianus* produced prolonged acclimation responses in their upper thermal tolerance following a single stressful thermal exposure, with greater shifts in thermal tolerance when the period between the acclimation and acute thermal event was shorter (Moyen et al., 2020). However, these results do not align with a recent study on acclimatization and shifting thermal tolerance in adult *M. californianus* that found evidence of developmental plasticity in upper thermal tolerance between individuals acclimatized in wave exposed and protected sites, but no plastic responses in adult individuals (Gleason et al., 2018).

I hypothesize that the differences in plastic shifts in upper thermal tolerance among these experiments can be attributed to differences in the timing and magnitude of thermal stress experienced by *M. californianus* prior to each tolerance trial. Heat-shock protein production is induced at temperatures between 23–25°C for *M. californianus*, and other notable physiological
consequences occur at higher temperatures. Further, acclimation to seawater at a range of temperatures below 20°C produced no meaningful differences in heat-shock responses between treatment levels (Roberts, Hofmann, & Somero, 1997). Acclimation to 28C and 32C seawater altered cellular signaling and repair processes associated with thermal stress in *M. californianus* and led to declining concentrations of hemocytes — cells that perform a diverse range of essential functions — with high levels of single strand DNA breaks at both temperatures and high levels of serious double stranded breaks at 32C (Yao & Somero, 2012, 2013). During the Gleason et al. experiment, from mid-June to mid-July where temperature data were available (see Gleason et al. supplement S3), there were approximately seven occasions where aerial temperatures surpassed 25°C and depending when collections occurred it may have been a number of days prior to collection that mussels experienced any stressful aerial temperatures (Gleason et al., 2018). In contrast, mussels from my experiment were exposed to their peak aerial temperatures (22°C, 28°C, and 34°C) daily for 51 days, including the day prior to any experiment, and Moyen et al. (2020) tested the differences in shifts in thermal tolerance following single-day acclimations to 30°C or 35°C between 1-28 days prior to their upper thermal tolerance trials. Given the differences in thermal histories and ultimate findings between ours and other studies, it appears that in order to induce a plastic response in upper thermal tolerance, the recent thermal history of *M. californianus*, either through short- or long-term acclimation periods, must be sufficiently stressful to induce physiological defences. Nuances in thermal histories have been shown to affect critical thermal limits of other ectotherm species; for example, CT_{max} in Antarctic marine fishes and invertebrates are affected by the duration of acclimation and the ramping rate of exposures (Peck, Morley, Richard, & Clark, 2014). The mussels that Gleason et al. used likely did not experience enough thermal stress at either site to produce an acclimatization response that shifted upper thermal tolerance, unlike the single-day acclimation done by Moyen et al. or the long-term acclimation of my experiment (Gleason et al., 2018; Moyen et al., 2020).

While these findings provide further support for *M. californianus* being highly plastic in their upper thermal tolerance, a number of uncertainties remain regarding the kinds of thermal histories that may be required to produce these shifts in tolerance. We do not know how high upper thermal tolerance can be shifted with long-term acclimation following more realistic tidal
cycles. Furthermore, the duration of thermal stress I exposed *M. californianus* to may be longer than necessary to produce the shifts in upper thermal tolerance I documented in light of the meaningful shifts in tolerance observed after a single thermal event (Moyen et al., 2020). We also do not know how other aspects of organism performance (e.g., cardiac thermal tolerance or gonad development) may differ when comparing the effects of long- and short-term exposure to stressful thermal conditions. From field-collected temperature data, Moyen et al. found that almost two thirds of consecutive heat events occur 24–48 hours prior to the first, with the other approximate third occurring up to 22 days later (Moyen et al., 2020). Both rapid and prolonged acclimation responses to thermal stress will be important for improving the persistence of *M. californianus* populations following concurrent or sporadic heat events. Exploring the potential differences between long- and short-term thermal stress on lethal and sublethal responses will help elucidate potential differences in and associated consequences of plastic responses of *M. californianus* to gradual warming and the effects of highly stressful heatwaves.

**2.4.1.2 Cardiac thermal tolerance**

My results partially supported my prediction that increased aerial acclimation temperatures would produce resultant shifts in cardiac thermal tolerance. I observed increased maximum heart rates with higher acclimation treatment temperatures but no difference in FLT or Tcrit values between treatment groups. These results align with other studies on the cardiac thermal tolerance of adult *M. californianus*. In mussels collected from Baja California to Tatoosh island, Tcrit was not found to be phenotypically plastic in adults; long-term common garden acclimation did not nullify site-level differences, which were attributed to developmental plasticity or local adaptation (Logan et al., 2012). In another study, mussels from the high intertidal had higher critical temperatures than low zone mussels from a single site, and that high zone mussels increased their Tcrit values with faster aerial heating rates. FLT was minimally affected by heating rate and did not differ between high and low zone mussels. However, these mussels were not acclimated to common garden conditions prior to their trials, so it is not clear whether these differences are due to developmental plasticity or acclimatization in adults (Moyen et al., 2019). While I found increasing fmax values with increasing acclimation temperatures, the physiological implications of that parameter are not clear. Tcrit reflects a critical failure of cardiac function and may indicate underlying cellular damage, while FLT often indicates impending
death of an organism (Logan et al., 2012; Moyen et al., 2019; Stenseng et al., 2005). As \( f_{\text{max}} \) and upper thermal tolerance both increased with higher acclimation temperatures, while acute cardiac metrics did not change, more studies that explore the connection or lack thereof between cardiac thermal tolerance and upper thermal tolerance are required to better understand the role of cardiac function in determining upper thermal limits.

These parameters of cardiac thermal tolerance, two of which were not affected by acclimation to very different thermal histories, do not follow the same patterns of change in other marine invertebrate species. In three conegers of the genus Tegula, thermal acclimation to higher seawater temperatures increased Arrhenius breakpoint temperatures and FLT values, and that the magnitude of this effect was dependent upon the vertical zonation of the species in question (Stenseng et al., 2005). Three blue mussel congeners were found to have species-specific \( T_{\text{crit}} \) values and acclimation to higher seawater temperatures increased \( T_{\text{crit}} \), though there was no significant interaction between species and acclimation temperatures (Braby & Somero, 2006). These shifts, and lack thereof, in \( M. \text{californianus} \) cardiac function provide further support that these cardiac parameters may be genetically fixed in adults, and that developmental plasticity or local adaptation may be more important mechanisms for altering cardiac functions as a response to the thermal environment (Logan et al., 2012). The interspecific differences in plasticity within cardiac thermal tolerance parameters, even between species that are closely related to one another, highlight the potential case-dependent utility of cardiac thermal tolerance in detecting acclimation or acclimatization responses in adult organisms. While cardiac thermal tolerance may be a useful system for obtaining instantaneous responses to temperature in a non-invasive manner, it may not be a panacea for detecting sublethal acclimation responses across all species (Dong et al., 2021). For \( M. \text{californianus} \), teasing apart the roles of developmental plasticity and local adaptation could provide valuable insight into how cardiac thermal tolerance is determined. Additionally, thermal acclimation experiments that pair cardiac function with other physiological mechanisms, such as the heat-shock response, could provide more explicit links between these sublethal responses and their effects on the upper thermal tolerance of an organism.
2.4.1.3 Gonadosomatic Index (GSI)

My results supported my prediction that thermal acclimation treatments significantly affected the GSI values of *M. californianus*, with GSI values decreasing with increasing acclimation temperatures. While a number of environmental stressors have been linked to shifts in GSI values and indirectly to the allocation of energy to other physiological systems away from reproductive development in *M. californianus* and its congeners, my study is the first I am aware of to identify long-term consequences of thermal acclimation, independent of other factors, on the reproductive capacity of *M. californianus*. Both seasonal and site-dependent patterns of GSI with intertidal zonation have been found in *M. californianus*, with mussels from the less stressful low intertidal possessing greater GSI values than those from the more stressful high intertidal (Petes et al., 2008; Waite & Sorte, 2022). *M. californianus* can also possess different reproductive strategies between sites, in terms of spawning timing and velocity, likely owing to differences in food availability or desiccation or thermal stress (Petes et al., 2008). Ocean acidification negatively affects sex steroid production and the regulation of genes related to gonad development in *M. coruscus*, contributing to overall declines in GSI values (Wang et al., 2021). During my acclimation period, differences in thermal histories between treatment groups could have led to differential spawning events in the lab that could potentially affect GSI values, like those observed in Petes, Menge, and Harris (2008), but this remains unlikely given that I frequently monitored mussels but observed no spawning events.

Studying the effects of temperature and warming on fertility in addition to lethal thermal limits will provide important context for accurately predicting the effects of climate change. Fertility and reproductive success can be affected by temperature in a variety of ways and can strongly interact with the life history strategy of the species in question to affect the consequences of stressful temperatures (Porcelli, Gaston, Butlin, & Snook, 2017; van Heerwaarden & Sgrò, 2021; Walsh et al., 2019). GSI values, larval growth, and the timing and magnitude of spawning events in *M. californianus* have all been found to be impacted by temperature; studies that test these parameters following acclimation to future thermal regimes will provide a clearer understanding of how reproductive capacity and success for *M. californianus* may respond to greater thermal stress going forward (Petes et al., 2008; Trevelyan
& Chang, 1983). I also recommend that future experiments use more realistic low tide cycles during a long-term thermal acclimation experiment and explore other underlying parameters that can contribute to GSI values like sex steroid production. Obtaining a greater mechanistic understanding of how acclimation states affect underlying systems that subsequently shift reproductive capacity will provide more clarity on the overall costs and benefits of plastic responses to stressful thermal histories in *M. californianus*.

2.4.2 Seasonal variation in upper thermal tolerance

I found that *M. californianus* shifted its upper thermal limit over the course of the summer to a lesser degree than was observed following my lab acclimation experiment. On April 14, field-acclimatized mussels had a lower upper thermal tolerance than mussels from the 22°C acclimation treatment, and by August 13 mussels from Bamfield had a similar upper thermal tolerance to mussels from the 28°C treatment. Like my thermal acclimation experiment, the differences in observed shifts in upper thermal tolerance between ours and other studies are likely due to differences in thermal history and the relative degree of stress experienced by individuals between these two experiments (Gleason et al., 2018; Moyen et al., 2020). I cannot rule out the possibility that recent hardening or short-term acclimation events prior to each trial may contribute to, or be the primary force behind, observed shifts in upper thermal tolerance, such as those in Moyen et al 2020. However, the consistent increases in upper thermal tolerance observed over the summer indicate that seasonality produces a cumulative acclimatization response in *M. californianus*. Seasonal plasticity in cold tolerance occurs in both *M. trossulus* and *M. edulis* (Bourget, 1983; Kennedy et al., 2020). In *M. californianus*, heat-shock protein induction temperatures, as well as the magnitude of synthesis, both shifted seasonally with higher relative levels of heat-shock protein induction in the spring than the summer (Roberts, Hofmann, & Somero, 1997). The shifts in the upper thermal limit of *M. californianus* observed in this study following field acclimatization likely reflect plasticity in underlying physiological mechanism; future experiments which take a mechanistic approach and measure both upper thermal tolerance and physiological metrics such as heat-shock protection induction will help explicitly connect shifts in this lethal threshold to these systems.
The differences in shifts in upper thermal tolerance between field-acclimatized and lab-acclimated individuals leaves a number of questions that require further investigation. Meaningfully higher tolerance in the 34°C acclimation treatment group indicates that mussels from Bamfield do possess the capacity to increase their upper thermal tolerance further than what was expressed during the summer of 2021, but a number of caveats may limit these increases. During the summers in British Columbia, mussels get reprieve from stressful and prolonged summertime low tides during neap tides, which limits both the magnitude and duration of thermal stress mussels can receive. Shifts in upper thermal tolerance diminish over time following exposure to high temperatures; therefore, *M. californianus* from the field may not be able to reach the upper thermal limits produced by daily acclimation to stressful temperatures in the lab (Moyen et al., 2020). Additionally, as field-acclimatized individuals approached the upper thermal tolerance produced by the 28°C acclimation treatment group without following strict acclimation regimes, shorter-term exposure to stressful temperatures may be sufficient to produce substantial shifts in tolerance, though differences in the effects to underlying sublethal responses are unknown. Testing plasticity in upper thermal tolerance following realistic summertime tidal conditions following short- or longer-term, in conjunction with sublethal responses like GSI or cardiac thermal tolerance, can improve my understanding of the overall costs and benefits to prolonged or brief exposure to stressful thermal regimes.

### 2.4.3 Post-heatwave mortality surveys

Following the 2021 heat dome from late June to early July in the Pacific Northwest, I wanted to document any potential mortality in *M. californianus* beds in Bamfield, BC. I also wanted to compare mortality between wave-exposed sites (Bluestone exposed and Prasiola) and wave-sheltered sites (Bluestone sheltered and Nudibranch) to provide additional nuance to my overall documentation on the heat dome impacts at this location far from the southern end of *M. californianus*’ range.

I found little mortality in either wave-exposed site (Bluestone exposed, Prasiola) following the heat dome and an estimated average mortality of approximately 50% in both wave-sheltered sites (Bluestone sheltered, Nudibranch). The influence of site-level topography on differences in thermal history and associated ecological consequences in rocky intertidal zones
has been well documented. *M. californianus* attached to hotter substrata (i.e., with an orientation facing close to direct sun during the hottest part of the day) differentially express heat-shock proteins when compared to mussels from cooler orientations, and substratum orientation strongly affected mussel survival when direct sunlight coincided with a morning low tide (Harley, 2008; B. S. T. Helmuth & Hofmann, 2001). On top of substantial losses of mussel beds that provide biogenic habitat and thermal refugia for many infaunal species, changes to the density and distribution of mussel beds can have important ecological implications for these dependents. Deeper or multi-layered mussel beds provide a high level of thermal protection for infaunal species, while the surfaces or edges of mussel beds generally have much more stressful temperatures (Jurgens & Gaylord, 2016). High levels of mortality within a mussel bed can reduce the amount of suitable space that *M. californianus* provides for infaunal species. Future studies that explore the impacts of heatwave events on mussel bed infaunal communities, as well as the effects of climate warming and heatwave frequency on the long-term persistence of mussel beds in vulnerable areas, will provide a clearer understanding of how *M. californianus* populations may fare on thermally challenging shores and potential implications for their dependent communities.

### 2.4.4 Conclusions

As climate change progresses, warming and the increased frequency and intensity of heatwaves will pressure vulnerable species to respond on physiologically relevant timescales to persist (Harvey et al., 2021; Perkins-Kirkpatrick & Lewis, 2020). Phenotypic plasticity that supports a species’ resilience to rapid thermal changes may prove increasingly important as temporal mismatches between the pace of climate change and evolutionary or demographic processes may be too great to support at-risk populations on shorter timescales (Fox et al., 2019; Gunderson & Stillman, 2015; Somero, 2010). However, associated sublethal responses and their effects on fitness play an important and underappreciated role in determining the ultimate impacts of warming and plasticity. While many studies have focused on upper thermal tolerance when quantifying the effects of warming on species, understanding related sublethal responses to stressful thermal regimes may identify additional and previously unknown consequences that can occur at temperatures well below these lethal thresholds (Sunday et al., 2012; van Heerwaarden & Sgrò, 2021; Walsh et al., 2019). Thus, conducting experiments that explore the plasticity of
both lethal and sublethal responses to temperature can help provide a clearer understanding of the capacity of a species to respond to intensifying thermal environments.

The response of *M. californianus* to changing thermal regimes has broad implications for rocky intertidal zone communities on the west coast of North America. The mortality observed following the 2021 heat dome in British Columbia is an important reminder of the importance of understanding how physiological plasticity may improve mussel survival following acute thermal events. However, the long-term reproductive implications of more thermally stressful summers will also play a key role in determining population persistence. In my study, following long-term aerial thermal acclimation I found that *M. californianus* is highly plastic in its upper thermal tolerance and expresses plasticity in maximum heart rate but not flatline temperature or critical temperatures; however, this acclimation appears to occur at the expense of reproductive capacity with declines in GSI values. This is the first study I am aware of in *M. californianus* that identifies declines in reproductive status as a consequence of thermal acclimation specifically. I also found that *M. californianus*’ upper thermal limit increased over the course of the summer of 2021 at a site well removed from its equatorward range edge where thermal stress would generally be expected to be greater, and that mussel mortality following the 2021 heat dome at wave-exposed sites was minimal when compared to wave-sheltered sites.

These results indicate that plastic shifts in thermal tolerance at a wave-exposed site (Prasiola) were either sufficient or unneeded to protect mussels from the heat dome’s effects, and – assuming a somewhat similar trend in increasing upper thermal tolerance at my wave-sheltered sites – that seasonal acclimatization insufficiently protected many individuals in these topographically unfavourable areas. The shifts in upper thermal limit observed over the summer were less than those produced following long-term acclimation to higher aerial temperatures, which suggests that *M. californianus* may be able to further shift their upper thermal tolerance when acclimatized to hotter summers. The degree of variation in upper thermal tolerance observed also highlights the importance of heatwave timing in determining mussel survival; if heatwaves become more prevalent earlier in the summer, when upper thermal limits are lower, the mussel mortality will likely be higher. Plastic responses in upper thermal tolerance can improve the immediate survival of *M. californianus* and help sustain its ecological functions, and
*M. californianus* has the potential to further shift their tolerance in the face of warmer summers with more heatwaves. However, the consequences of changing thermal histories on the reproductive capacity of the mussels indicate potentially negative longer-term consequences that require further investigation. If populations become increasingly recruitment-limited due to the costs of more stressful acclimatization, reductions in reproductive output could have important and as of yet underappreciated implications for population size and persistence (van Heerwaarden & Sgrò, 2021; Walsh et al., 2019). These limitations could further interact with spatial patterns of recruitment. For example, areas with coastal upwelling are often recruitment-limited; if climate warming restricts larval reproduction, these regions may be challenged further in recovering from heatwave events and persisting on longer timescales (Blanchette & Gaines, 2007; Smith, Fong, & Ambrose, 2009).

Given my findings, there are a number of directions for future work to better understand the relationship between plasticity in lethal and sublethal responses, thermal histories, and ecological consequences in *M. californianus*. Future acclimation or acclimatization studies that pair acute thermal metrics with other relevant sublethal parameters will a.) provide more clarity on whether plasticity in thermal tolerance is sufficient to support the persistence of a population, given the declines in reproduction found in this study and b.) improve our mechanistic understanding of how and why upper thermal tolerance can shift in *M. californianus*. These kinds of experiments could also be conducted in the field to incorporate natural thermal variability and tidal cycles, with some high intertidal zone mussels being shaded to produce different thermal acclimatization treatments (e.g. see Bertness & Leonard, 1997; Bertness, Leonard, Levine, Schmidt, & Ingraham, 1999). Additionally, exploring the potential sublethal implications of recovering from an acute thermal event, like a single- or multi-day heatwave, will help clarify whether gradual climate warming, or the proliferation of heatwave events, could more greatly affect *M. californianus* persistence in the near future.
3: Conclusions

3.1 Summary of results

Physiological plasticity in response to changing thermal environments is an important process for maintaining ecological performance in individuals and increase the likelihood of population persistence. While plastic responses to variable temperatures have been well-studied in a number of systems, testing both lethal and sublethal systems within a single experiment is an important step for understanding the overall costs and benefits to acclimation and its impacts on persistence in the near- and medium-term (Fox et al., 2019; van Heerwaarden & Sgrò, 2021). In chapter 2, I tested the physiological plasticity of an important rocky intertidal zone foundation species, *Mytilus californianus*, following long-term aerial thermal acclimation. I found that acclimation to higher temperatures below acute thresholds increased upper thermal tolerance and maximum heart rate but did not affect critical or flatline temperatures. These acclimation responses to higher temperatures come at a cost to reproductive capacity, however, with declining GSI values in more stressful (28°C, 34°C) acclimation treatments. Additionally, I examined the seasonal variation in upper thermal tolerance *M. californianus* expressed during the summer of 2021 in Bamfield, British Columbia. I found that *M. californianus*’ upper thermal tolerance increases over the summer, approaching a tolerance similar to the 28°C treatment group from my acclimation experiment by the end of the summer. Finally, I quantified mortality at wave-exposed and wave-sheltered sites in Bamfield following the 2021 heat dome to document the present-day impacts of thermal extremes on mussel populations. Even at a location far from their southern range edge where thermal stress would be expected to be greatest, large amounts of mortality in wave-sheltered sites were observed. With warming and heatwaves expected to intensify as climate change progresses, there is an immediate need to better understand *M. californianus* thermal physiology, how it may use plasticity to better survive hotter summers, and how more stressful thermal histories may impact reproduction and the long-term persistence of mussel populations. My data chapter clarifies some of the costs and benefits to plasticity in *M. californianus*. However, the population-level implications of declining reproduction reveals a number of key questions regarding adaptive plasticity – and whether physiological plasticity may help or hinder *M. californianus* persistence beyond its immediate survival.
3.2 Is *M. californianus*’ physiological plasticity adaptive? How might its response to near-term warming impact persistence on longer timescales?

Possessing plasticity in upper thermal tolerance has clear benefits for the near-term survival of *M. californianus*, a rocky intertidal species who can often be rapidly exposed to a broad range of aerial temperatures with little means of behavioural thermoregulation. Higher upper thermal limits can improve survival outcomes for a population of mussels facing extreme temperatures, like the wave-sheltered sites in Bamfield during the summer of 2021. These findings are also particularly noteworthy given that many ectotherm species possess a limited capacity to alter their upper thermal limits (Barley et al., 2021; Gunderson & Stillman, 2015; van Heerwaarden et al., 2016). One of the primary objectives of experiments on phenotypic plasticity is, once a plastic response has been identified, to determine whether the response is adaptive or non-adaptive. Novel environmental conditions can shift the optimal phenotype of a species: adaptive plastic responses move the phenotype of an individual from a disadvantageous state towards this new optima, whereas non-adaptive plastic responses produce a new phenotype that is further from the new optima than their original state (Ghalambor et al., 2007). While higher upper thermal tolerances and altered cardiac function at higher acclimation temperatures may indicate that this plastic response produces a favourable shift in phenotype, the fitness implications of declining reproductive capacity require further investigation to determine whether these plastic responses produce a more optimal phenotype when examining the costs and benefits holistically. Understanding whether phenotypic shifts in response to more stressful thermal acclimation are adaptive or non-adaptive is a particularly important objective when considering how *M. californianus* may persist with climate warming, as plasticity can strongly interact with selection to help or hinder evolutionary adaptation.

As we learn more about how *M. californianus* responds to thermal acclimation, it is important to place these findings in the context of ongoing research on phenotypic plasticity and how its relationship with evolutionary responses help dictate species persistence on longer timescales. One of the key questions framing this debate is whether phenotypic plasticity hinders or assists evolutionary adaptation; while this remains a context-dependent question with varied empirical evidence, there is a generally accepted framework on what conditions produce...
favourable and unfavourable outcomes between these interacting processes. If the plastic response is adaptive and the new phenotype is close to the new optimum, maintaining high fitness can enable persistence while selection pressure is weakened to the point of evolutionary adaptation being unlikely. If plasticity is adaptive but the new phenotype is further from the optimum, selection pressure may be lessened enough to promote near-term persistence but still strong enough where evolutionary adaptation can still occur. Non-adaptive plasticity that moves phenotypes away from the optimum can act as an additional force that selection pressure must overcome to enable persistence. Finally, some cryptic genetic variation may be expressed following plastic responses to novel conditions; in these cases, this variation may only promote evolutionary adaptation if that new phenotype happens to be favourable (Fox et al., 2019; Ghalambor et al., 2007). Given the plastic responses I observed in *M. californianus* when exposed to a range of temperatures and the case-dependent nature of whether plasticity helps or hinders evolution, exploring this debate with *M. californianus* as a focal species can be an informative exercise. *M. californianus* occupies habitats with widely variable levels of thermal stress and possesses life-history traits that strongly affect gene flow and selection, both of which are factors that could greatly affect the relationship between plasticity and evolution.

For this exercise, I will first focus on two sites I surveyed during the summer of 2021 with different degrees of thermal stress, then consider how the interactions between plasticity and evolution at these sites may scale to broader consequences across *M. californianus*’ range. Prasiola, the site I collected all my individuals for lab experiments from, was relatively thermally benign with minimal mortality following the heat dome. Even so, the upper thermal tolerance of high zone mussels increased over the summer—these shifts either sufficiently protected populations from acute thermal stress or were unnecessary for their survival. Because there was almost no temperature-related morality, the selection pressure towards higher thermal tolerances may be weaker as it only acts through altering reproductive output. In contrast, Nudibranch, one of the wave-sheltered sites with high mortality, experienced high levels of thermal stress and mortality following the heat dome. Assuming that the seasonal variation in upper thermal tolerance for mussels from Nudibranch followed a similar pattern to mussels from Prasiola, there may be stronger selection pressure at this site for mussels with higher upper thermal tolerances, either constitutionally or via plasticity. Beyond the impacts of thermal tolerance and topographic
context on selection, the effects of thermal history on reproductive capacity may also help drive selection, and the life history strategies of *M. californianus* may further impact evolutionary adaptation at larger scales.

How selection acts on a species that can be highly plastic like *M. californianus* may also be strongly affected by site-level variability in thermal stress and gene flow across the species’ range. Across *M. californianus*’ range, there is a high level of genetic homogeneity, indicating broad larval dispersal and large amounts of gene flow between populations (Addison, Ort, Mesa, & Pogson, 2008). In highly wave-exposed sites or north-facing shores on headlands, mussels may experience so little thermal stress that there may almost never be selection pressure for higher upper thermal tolerances (Harley, 2008). Thus, evolutionary adaptation in their upper thermal tolerances may result from net gene flow over time from thermally stressed populations. Populations facing thermally benign conditions can also have greater reproductive capacity than those exposed to thermally stressful conditions, a response documented in my lab-acclimated mussels and in field-acclimatized individuals. Mussels exposed to acute high temperatures will face selection pressure to increase their thermal tolerance, while reductions in reproductive capacity or altered timing and magnitude of spawning events may dampen their gene flow to other populations (Petes et al., 2008). Over time, evolutionary adaptation in response higher temperatures across *M. californianus*’ range may occur if larval dispersal and gene flow from stressed populations remains high enough to support adequate recruitment in non-stressed population, if these adaptations are not too energetically costly to maintain under non-stressful conditions or are useful during extreme heatwaves or abnormally hot summers. This selection occurring in thermally stressed populations may lead to an overall net improvement in upper thermal tolerance across all populations due to high levels of gene flow and may promote the persistence of populations in more locations across its range.

3.3 Study limitations and recommendations for future work

While long-term aerial thermal acclimation that follows realistic emersion times for stressful summertime low tides is a methodological improvement over past experiments on *M. californianus*, which were often done with long-term immersion, more work can be done to better simulate realistic tidal conditions and tease apart the effects of prolonged exposure to
stressful temperatures in lethal and sublethal responses (e.g. see Roberts, Hofmann, & Somero, 1997). During the summers in British Columbia, spring and neap tides produce cycles of emersion and varying degrees of thermal stress. These cycles can provide reprieve for *M. californianus* by limiting emersion time, particularly during the hottest times of the day during certain phases of the fortnightly cycle of spring and neap tides. Therefore, exposure to 6-hour low tides with high peak aerial temperatures daily for approximately two months does not fully reflect the duration of thermal stress mussels would experience over consecutive weeks.

Prolonged daily exposure to stressful aerial temperatures was a useful treatment regime for confirming that *M. californianus* is indeed plastic in its upper thermal tolerance, testing how far its tolerance can shift with acclimation, and the associated impacts on cardiac function and reproductive capacity, there are important biological questions that be better answered following more realistic acclimation regimes. Mussels, including *M. californianus*, can possess responses to acclimatization that accumulate seasonally (Kennedy et al., 2020; Roberts, Hofmann, & Somero, 1997). Additionally, shifts in upper thermal tolerance following single-day acclimations are diminished as the time between acclimation and acute thermal events are increased (Moyen et al., 2020). These cumulative responses to shifting thermal histories and the effects of short-term acclimation reflect a need to better understand potential differences in acclimation responses and associated consequences to short- and long-term thermal stress. We do not know how much shorter our acclimation treatments could have been while producing the same shifts in upper thermal tolerance. We also do not know whether short-term acclimation responses affect cardiac thermal tolerance or reproductive capacity in the same manner as long-term acclimation. These are important questions to address in the context of climate warming, as extremely hot low tides can occur consecutively or with a number of weeks between them (Moyen et al., 2020). Preparing for both scenarios will improve the survival of mussel populations and may have different and important implications for sublethal responses and persistence.

In addition to testing questions about the relationship between acclimation period length and physiological plasticity, examining other sublethal responses to temperature may provide further insight into the mechanisms underlying plastic upper thermal limits and associated sublethal consequences. Critical and flat line temperatures did not shift with acclimation in *M.*
*californianus* while upper thermal tolerance did; therefore, there must be other physiological processes occurring that can produce different responses in acute thermal limits. The heat-shock response is an important defence for almost all ectotherms that face thermal stress, and has been previously demonstrated to shift in response to altered thermal histories in *M. californianus* and other Mytilids (Buckley, Owen, & Hofmann, 2001; Hochachka & Somero, 2002; Roberts, Hofmann, & Somero, 1997). Testing the effects of thermal acclimation on heat-shock response induction temperatures, peak temperatures, and upper limits to heat-shock protein production is a natural next step in exploring how sublethal systems contribute to shifts in lethal limits.

The declines in reproductive capacity I observed in *M. californianus* at higher acclimation temperatures are notable and require further investigation to understand the mechanisms behind these shifts and the implications for reproduction. Sex steroid production declines and genes related to reproductive development are differentially expressed when *M. coruscus* is exposed to acidic seawater, and patterns of carotenoid content in *M. californianus* between high and low zone mussels indicate that environmental stress alters the proportion of energy being devoted to defences against stress versus reproduction (Petes et al., 2008; Wang et al., 2021). These findings indicate that environmental stress can alter these mechanisms that help dictate GSI values; therefore, testing the effects of thermal acclimation on these parameters would be a natural next step in improving our mechanistic understanding of the effects of temperature on reproductive capacity. Beyond the immediate impacts of higher temperature acclimation on adult mussel gonad development, there are important implications of elevated temperature on their larvae that require further investigation. Larvae produced from *M. californianus* from the higher-stress high intertidal possessed lower upper thermal tolerances than those from the lower-stress low intertidal, and *M. californianus* larval growth rates are highly temperature dependent (Trevelyan & Chang, 1983; Waite & Sorte, 2022). More thermally stressful summers will have downstream impacts on larval production and viability, both of which may play an important role in recruitment and persistence that require further investigation.

In summary, I have used field- and lab-based experiments to study the lethal and sublethal effects of thermal acclimation in an ecologically important rocky intertidal species.
While my findings link lethal and sublethal responses in a novel way, continued research into the connections between physiological systems are necessary to improve our mechanistic understanding of plasticity and how it dictates survival, fitness, and ultimately persistence. Temperature, biochemical reaction rates, and organismal responses determine the abundances and distributions of much of life on earth; testing these relationships in the context of climate warming and more frequent and intense heatwaves will be crucial in making accurate predictions for how *M. californianus*, and other species, may respond to anthropogenic climate change now and in the future.
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Appendix: supplementary information for chapter 2

Figure A1. Temperature ramps for low tide thermal acclimation treatments (22°C, 28°C, 34°C) applied in an incubator network 6 days/week for ~9 ½ weeks. Each treatment was applied simultaneously, with all specimens of *M. californianus* being immediately returned to sea tables upon program completion.
Figure A2. Upper thermal tolerance temperature ramps from 13°C to peak temperatures ranging from 34°C to 43°C at 1°C intervals. Each peak temperature occurred simultaneously in separate incubators, with peak temperatures being held for 60 minutes before immediately returning *M. californianus* to sea tables.