# HEAT WAVE IMPACTS ACROSS SCALES IN THE SPLASH POOL COPEPOD, *TIGRIOPUS CALIFORNICUS*

by

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# Abstract

Temperature influences biological processes at all levels of biological organization. As such, temperature is a fundamental abiotic variable affecting ectotherm fitness. As human induced climate change persists, the need to understand biological responses to temperature has never been more pressing. One consequence of rapid climate change is an increase in the frequency and intensity of heat waves. Despite substantial interest in the physiological effects of heat stress, it is less clear how individual responses to extreme heat events influence population-level responses such as persistence and population growth.

Energy balance is a unifying concept that integrates the effect of temperature across scales. The Energy Limited Tolerance to Stress (ELTOS) framework links physiological models of temperature and oxygen availability to dynamic energy budgets in order to predict the effect of stress on individual reproduction and population growth.

Drawing on the ELTOS framework, I tested three critical predictions about individual thermal experience, and how the influence of heat stress scales up from physiological to individual to populations, utilizing the splash pool copepod, *Tigriopus californicus*. In Chapter 2, I tested the prediction that aerobic energy production declines with increasing heat wave intensity and duration, and that individual reproduction declines similarly. In Chapter 3, I tested the assumption that short-term impacts of heat waves on individual reproduction have persistent effects on longer-term population dynamics. In Chapter 4, I tested for an effect of spatial variation in thermal history on subsequent heat wave survival.

Together, these tests provide a comprehensive examination of ELTOS predictions of biological responses to heat waves across scales. My data are consistent with ELTOS predictions that heat wave intensity affects energy balance and subsequent individual reproductive effort. However, I did not find consistent patterns of population dynamics over both short and longer-term time scales. The opposing effect of temperature on different life-history traits that occur over different time-scales likely underlies differences in the effect of short-term heat wave effects on population dynamics over short and longer time periods. Lastly, I found that spatial variation in thermal history, particularly recent heat accumulation, explains reduced survivorship during experimental heat waves.

# Lay Summary

More intense heat waves are increasing in frequency due to climate change. An unanswered question is how the effects of heat stress at one level scale up to affect biological processes at another – that is, do biochemical changes affect individuals, and do effects on individuals scale up to affect populations. Here, I examined the effect of heat waves in the copepod, *Tigriopus californicus*. I have utilized physiological models of temperature and energy to investigate the effects of heat waves at multiple scales. I found that changes in energy utilization during periods of heat stress during heat waves are consistent with reduced individual reproductive effort. Over longer time scales, however, impacts on individual reproductive effort do not consistently lead to impacts on population growth. Furthermore, I found spatial differences in heat wave survival, and a higher amount of heat accumulation in the field was consistent with reduced heat wave survival.

# Preface

This dissertation is based on original, unpublished, independent work by the author. The ideas were developed with substantial guidance from the author's advisors, Dr. Mary O'Connor and Dr. Eric Taylor, and the supervisory committee (Dr. Patricia Schulte and Dr. Christopher Harley).

In Chapter 2, undergraduate research assistant, Baldwin Huang, collected a substantial amount of the nauplii count data.

My study area maps were made by John Cristiani.

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*For my mom,*
*who made everything possible.*

# CHAPTER 1

## General introduction

Temperature exerts a major influence on biological processes at all levels of biological organization (Hochachka & Somero, 2002). As such, temperature is a fundamental abiotic variable affecting ectotherm fitness (Huey, 1982; Angilletta et al., 2002; Trudgill et al., 2005; Angilletta, 2009; Schulte, 2015). As human induced climate change persists, the need to understand biological responses to temperature and temperature change has never been more pressing. One consequence of rapid climate change is an increase in the frequency and intensity of heat waves (i.e., an extended period of unusually high temperature; Robinson, 2000) (Frich et al., 2002; Meehl & Tebaldi, 2004; Rahmstorf & Coumou, 2011). Despite substantial interest in the physiological effects of heat stress, it is less clear how individual responses to extreme heat events, such as heat waves, influence population-level responses such as persistence and population growth.

Energy is a unifying currency that integrates effects of temperature stress across multiple levels of biological organization. An individual's energy budget reflects the rate of energy production and consumption. Energy allocation is a dynamic process responding to changes in energy availability and the prioritization of homeostatic maintenance (Kooijman, 2010). Mismatches in organismal production and consumption rates can limit energy available for growth, reproduction, and activity, because organisms maximize the physiological processes that ensure survival under stressful conditions (e.g., physiological stress responses and maintaining basal metabolism). The energy-limited tolerance to stress framework (ELTOS, Sokolova et al., 2012; Sokolova, 2013) links mechanistic physiological models of temperature, oxygen, and energy (Pörtner & Knust, 2007; Pörtner, 2010) to concepts of energy allocation and trade-off developed in dynamic energy budget models (Kooijman, 2010). Such linkage results in a mechanistic framework that links the effects of temperature from individuals to populations through the lens of energy balance.

## 1.1 Research goals

The goal of my dissertation was to examine the effect of heat waves at multiple levels of biological organization. Drawing on the ELTOS framework, I tested three critical predictions about individual thermal experience, and how the influence of heat stress scales up from physiological to individual to populations. In Chapter 2, I tested the prediction that aerobic energy production declines with increasing heat wave intensity and duration, and that individual reproduction declines similarly. In Chapter 3, I tested the assumption that short-term impacts of heat waves on individual reproduction have persistent effects on longer-term population dynamics. In Chapter 4, I tested for an effect of spatial variation in thermal history on subsequent heat wave survival. Together, these tests provide a comprehensive examination of ELTOS predictions of biological responses to heat waves across scales.

## 1.2 The *Tigriopus calfornicus* system

The splash pool copepod, *Tigriopus californicus* (Copepoda: Harpacticoida; Baker) is distributed from Baja California, Mexico up to southeastern Alaska (Detheir, 1980; Ganz & Burton, 1995). It occupies splash pools in the supralittoral zone of the high intertidal. Splash pools may only be infrequently refreshed with seawater during the highest tides during the tidal cycle or during storm events (Dybdahl, 1994; Powlik, 1998). As such, these pools maybe more influenced by atmospheric rather than oceanic conditions. Moreover, given the relatively small splash pool volume and position in the high intertidal, organisms inhabiting these pools are often subjected to large fluctuations in temperature and salinity, and are prone to desiccation (Powlik, 1998). In the rocky intertidal, competition for limited space is a dominant biological interaction (Connell, 1961), whereas the effects of respiration, photosynthesis and bacterial activity likely underlie splash pool microsite population and community dynamics.

The reproductive life-history of *Tigriopus californicus* makes it a tractable system for addressing my questions regarding energy balance, individual fitness, population growth, and spatial variation in temperature tolerance. Individuals hatch from egg sacs and develop into mature adults in 3-4 weeks. Adult males will clasp immature females and inseminate them upon reaching sexual maturity. The males will then release the female to try and procure another mate, but the female will only mate that one time (Burton 1985). Females are capable of producing upwards of 12 clutches (Haderlie et al., 1980) of approximately 30 nauplii each (Powlik, 1997). Clasping pairs are easily identifiable, reproductive females are easily tracked, and it is relatively easy to count the number of offspring produced by females. Thus, directly estimating fitness in this species is achievable.

The range of *T. californicus* spans a pronounced climatic gradient, where high levels of genetic differentiation among populations have been detected (Burton et al., 1979; Edmands, 2001; Willett & Ladner, 2009). In *Tigriopus* spp., the extensive geographic variation and high levels of genetic differentiation suggest that temperature adaptation is an important trait. Thus, *T. californicus* likely functions as a model system to investigate molecular, ecological and evolutionary responses to temperature in other taxa broadly distributed across climatic gradients.

## 1.3 Chapter 2 background:
## How does heat wave intensity and duration impact energy balance?

The ELTOS framework links physiological models of temperature and oxygen availability to individual energy budgets, integrating the effects of environmental stress to higher order effects on individuals and populations. High temperatures induce simultaneous changes to energy allocation, energy production, and energy availability. In ectotherms, basal metabolic rate increases with increasing environmental temperature. This increase in basal metabolic rate reduces aerobic scope (the difference between maximum and basal metabolic rates), and reflects energy allocation trade-offs that prioritize processes maintaining basal metabolic functions over other activities. Physiologically stressful temperatures exacerbate allocation trade-offs by initiating energetically expensive stress responses. Stress-induced induction of heat shock proteins, for example, monopolizes cellular ATP, requiring high RNA and protein turnover (Feder & Hofmann, 1999; Gracey et al., 2008; Bragg & Wagner, 2009). Concurrently, high temperatures impact energy production by limiting oxygen availability through decreased efficacy of ventilation and circulatory systems (Pörtner, 2010). As the cell becomes hypoxic, ATP production shifts away from aerobic pathways to less efficient anaerobic pathways (Verberk & Calosi, 2012). Sustained exposure to stressful temperatures impacts individual energy budgets by increasing the costs of basal metabolic functioning exacerbated by expensive stress responses and by decreasing energy production through changes from aerobic to anaerobic production pathways.

These changes reduce pools of cellular ATP, and can lead to mismatches between energy needed available and energy needed to maintain homeostasis. In Chapter 2, I tested the ELTOS prediction that aerobic energy production decreases with increasing heat wave intensity and heat wave duration.

## 1.4 Do impacts on energy balance influence individual reproductive effort?

Temperature intensity and exposure duration can result in mortality. However, the ELTOS framework predicts that even moderate stress can impact individual reproduction via energetic allocation trade-offs, as energy is diverted towards maintaining basal metabolic functions and cellular repair that would otherwise be put towards growth and/or reproduction. However, the extent to which heat waves impact immediate individual reproduction is not well characterized. In Chapter 2, I tested the prediction that immediate individual reproductive effort declines with increasing heat wave intensity and duration. I tracked female reproductive effort over a twenty day period by measuring the timing of egg clutch production and counting the number of offspring produced during and post-heat wave treatments.

## 1.5 Chapter 3 background:

## Do impacts of temperature stress on individual reproductive effort influence population dynamics over longer periods of time

Temperature stress may affect population growth through both lethal and sublethal means. The ELTOS framework predicts that sublethal stress that reduces fecundity should also affect population abundances over longer periods of time. However, temperature may have opposing effects on different processes that occur at different levels of biological organization which contribute to fitness, leading to mismatches between the effects of temperature stress across different time-scales. For example, population growth rate can be calculated from life-time offspring production, which is scaled to generation, while other measures of population growth rate are scaled to time. Thus, sublethal temperature stress can simultaneously reduce short-term offspring reduction, leading to lower within generation population growth rates. Across generations, however, warmer temperatures can accelerate development time (Cossins & Bowler, 1987), which shortens generation time and leads to higher estimates of population growth rates (Cole 1954; Huey & Berrigan 2001). It is crucial to evaluate the effect of short-term stress on population dynamics over different time-scales to understand how sublethal stress can impact population persistence.

In Chapter 3, I tested how heat wave intensity and duration affect short-term population growth rates, and if these effects persist to influence population abundances over longer periods of time. I founded laboratory populations of *Tigriopus californicus* with an equal number of gravid females, and measured abundance six times over the course of 100 days. I calculated population growth rates over short-term (less than 2 weeks) time periods that encompass the heat wave treatments, and compare the pattern of short-term population growth rates to abundance measures (peak abundance, final abundance) that occur over longer time periods.

## 1.6 Chapter 4 background: How does thermal history influence survival during heat waves?

Thermal tolerance is an organismal-level trait that is driven by hundreds, if not thousands, of cellular and biochemical traits and reactions. The polygenic nature of organismal thermal tolerance entails that thermal tolerance is highly plastic, and shaped by an individual’s thermal history. Hypotheses based on physiological responses to sublethal temperature suggest competing predictions result in different effects of sublethal temperature exposure on future tolerance to heat events. Processes associated with acclimatization and heat hardening can increase heat tolerance, whereas stressful temperature exposure may restrict thermal tolerance via energetic constraints. Understanding the context in which sublethal temperature exposure bolsters or hinders future heat tolerance is important for predicting heat wave impacts on population demography and persistence. In Chapter 4, I tested how spatial variation in thermal history correlates with variation in heat wave survival. I used eight temperature variables to characterize thermal history, tested for spatial differences in thermal history, and identified the aspects of thermal history that predict heat wave survival.

# CHAPTER 2

# Heat wave intensity drives fitness trade-offs through impacts on energy balance

## 2.1 Summary

Moderate stress may impact fitness through energy balance, leading to trade-offs between survival and reproduction. In addition to lethal effects, environmental stressors may result in sublethal effects on vital rates, linking stress events such as heat waves to population demography. Here, I test the hypothesis that heat wave intensity and consecutive days of exposure reduce aerobic energy production, which impacts survival and individual reproductive success. I subjected groups of the marine harpacticoid copepod, *Tigriopus californicus,* to six heat wave regimes that differed in maximum exposure temperature (26°C or 32°C) and number of consecutive exposure days (1, 2, or 7). During the heat wave and the two weeks following the last day of exposure, I measured aerobic energy production (oxygen consumption) during each period of daily maximum temperature, and demographic rates: individual survival and offspring production. Despite similar survivorship between the two maximum temperature treatments, sublethal effects of heat wave intensity were apparent. Individuals that experienced high maximum temperature (32°C) heat waves produced fewer offspring overall than those that experienced the 26°C heat wave. Furthermore, the number of nauplii per clutch was lower in the 32°C group for egg clutches produced immediately after the final day of exposure. I did not detect effects of heat wave intensity or duration on the rate of oxygen consumption between temperature groups, despite the expectation that Arrhenius

effects should result in increases in oxygen consumption with temperature. Instead, these patterns are more consistent with entry into an inactive state or period of metabolic depression (i.e., direct suppression of metabolism) in response to high temperature exposure. My results are consistent with the hypothesis that increasing thermal stress exacerbates trade-offs between survival and reproduction, and that these effects can be traced to changes in energy homeostasis. The development of conceptual frameworks that mechanistically integrate the effects of physiological stress on short-term demographic rates are needed to fully address the effects of global climate change on population persistence.

## 2.2 Introduction

Temperature is one of the most fundamental abiotic variables affecting fitness, especially in ectotherms (Huey, 1982; Angilletta et al., 2002: Hochachka & Somero, 2002; Trudgill et al., 2005; Schulte, 2015). As climate change accelerates, understanding the biological responses to extreme temperature events has never been more pressing. However, despite climate models predicting a warmer world that includes an increase in the frequency, duration, and severity of temperature events (Frich et al., 2002; Meehl & Tebaldi, 2004; Rahmstorf & Coumou, 2011), the effect of increasing thermal variation on fitness has received less attention than the effect of increasing average temperatures (Bozinovic et al., 2011; Folguera et al., 2011; Estay et al., 2013), which may, in some cases, have exacerbated effects on fitness (Vasseur et al., 2014; Buckley & Huey, 2016). Links between physiological responses and individual fitness are often assumed but less frequently demonstrated. Testing this assumption is critical for understanding how physiological stress might influence demography and population persistence.

Heat waves may impact fitness through lethal and sublethal components. Exposure to high temperatures may result in large-scale mortality events (Tsuchiya, 1983; Garrabou et al., 2009; McKechnie & Wolf, 2009), but may also reduce growth and/or reproduction. The relationship between heat stress and subsequent survival and reproduction is not well understood, but is likely mediated by consequences of energetic trade-offs between surviving a heat stress event and allocation to later reproduction. Under a period of heat stress, energy normally allocated to growth and reproduction under non-stressful conditions may be re-allocated to the maintenance of energetic balance (homeostasis) in response to the increasing thermal stress (Somero, 2002; Helmuth et al., 2010; Sokolova et al., 2012; Sokolova, 2013).

Extreme heat events may further exacerbate the effect of temperature on energy balance through changes in energy production and reductions in total energy availability. At higher temperatures, aerobic scope (i.e., the total energy available beyond maintaining basal metabolic functions) declines as oxygen concentration decreases (Pörtner & Knust, 2007). As oxygen concentration decreases, energy production shifts away from higher yielding aerobic pathways to lower yielding anaerobic ones resulting in lower total energy production (Verberk & Calosi, 2012). Additionally, energetically expensive physiological stress responses may be activated in response to cellular damage (Bell et al., 1988; Feder & Hofmann, 1999; Bragg & Wagner, 2009; Hoekstra & Montooth, 2013). These concurrent changes in energy allocation, energy production and energy availability extend an individual's survival time in stressful conditions. However, these changes in individual physiology can impact individual fitness by reducing reproductive effort. Advancing frameworks that link models of energy and temperature (Pörtner, 2010, 2012) to ecological models of energy budgets (Kooijman, 2010) is critical for mechanistically understanding the sublethal effects of physiological stress to ecologically relevant demographic rates (Sokolova et al., 2012; Sokolova, 2013).

Individual fitness links physiological processes to the demographic vital rates that underlie population persistence in the face of environmental stress. The impact to individual fitness, however, depends upon on identity of the stressor, the intensity of the stressor, and the duration of the exposure period (Rohmer et al., 2004; McNamara & Buchanan, 2005). The effects of temperature stress on individual survival and reproductive effort may be hard to predict, due to the non-linearity with which many metabolic processes scale with temperature, and also because thermotolerance is plastic, and resistance to stressful temperatures reflects both long-term evolutionary adaptation and short-term effects of thermal history (Angilleta, 2009; Schulte, 2015). Moreover, while the physiological responses to heat stress (e.g., the heat shock response) are well studied (Lindquist & Craig, 1988; Schlesinger, 1990; Hightower, 1991), organismal responses may not scale linearly with repeated heat stress events (Smith, 2011), and may instead change rapidly leading to population level-effects that exceed a threshold and have long-lasting effects on population demography (Harley & Paine, 2009).

The objective of this study is to test: i. the hypothesis that disruptions to energy balance increase with heat wave temperature intensity and exposure duration, and ii. that these energetic impacts negatively affect individual fitness via increasing mortality or decreasing reproductive output. I tested for the effect of heat waves on a survival/reproduction trade-off in the splash pool copepod, *Tigriopus californicus* (Copepoda: Harpacticidae). I subjected individuals to one of six experimental heat wave regimes varying in heat wave intensity (daily maximum temperature: 26°C or 32°C) and exposure duration (consecutive days of exposure: 1, 2, or 7). I measured metabolic rate (i.e., rate of oxygen consumption) during the period of daily maximum temperatures at day 1, 2, and 7 of the experimental heat wave, and tracked survival and reproduction to assess individual fitness of individuals.

## 2.3 Materials and methods

### 2.3.1 Study system, site description and field collection

The splash pool copepod, *Tigriopus californicus,* is a well-suited organism in which to investigate the effect of heat stress on energy balance and fitness. This copepod species is distributed from Baja California to Southeast Alaska. Populations occupy splash pools in the supralittoral zone of the high intertidal – a zone characterized by daily temperature fluctuations and highly variable salinity conditions. The replenishment of splash pools with seawater may only occur during the highest tides each tidal cycle or with storm events, one or two days per month (Egloff, 1966; Dybdahl, 1994).

The reproductive life history of *T. californicus* makes it highly amenable to experimental manipulation and quantification of fitness metrics. Over approximately 3-4 weeks individuals develop through 12 life stages: six naupliar stages (N1-N6) and five copepodid stages (C1-C5) to reach the adult stage. Adult males clasp immature females, and immediately after a female's final molt into adulthood, a male will inseminate and then release the female. Males may mate more than once, but females generally mate only once. However, females are capable of producing multiple broods from the same insemination (Burton, 1985). Eggs are brooded on the ventral side of the urosome where the egg clutches are readily apparent. Females typically produce ~30 nauplii per clutch (Powlick et al., 1997). The clasping behavior of mating pairs, visibility of gravid females, and moderate number of nauplii produced per clutch make tracking reproductive effort relatively straightforward.

I collected individuals from splash pools located within the rocky shores of Botany Bay (48° 31.7'N 124° 27.1'W) in Juan de Fuca Provincial Park, located on southwestern Vancouver Island near Port Renfrew, BC (Figure 2.1). Botany Bay is located at the westernmost entrance to the Strait of Juan de Fuca, and is characterized by cool maritime weather due to fog, cloud cover, and high precipitation (annual mean precipitation ~2-3 m). Summer air temperatures range from a mean low of 12°C to a mean high of 23°C. Individuals were collected on two different occasions for two separate experiments. Individuals used to measure aerobic respiration were collected in May 2016, while individuals for the fitness assay were collected in November 2015. Both collections were composed of individuals from ~5 splash pools, that were brought back to The University of British Columbia, where they were combined and housed in 500 mL jars and 10 L aquaria. Water temperature in the lab ranged from 19-21°C, consistent with small fluctuations in room temperature, and salinity was maintained at 30-34ppt. I fed *Tigriopus* cultures ground *Spirulina* algae (Max Pro brand fish food) *ad libitum*.

### 2.3.2 Heat wave experiments

To achieve the objectives of this study, I subjected groups of copepods to one of six experimental heat wave scenarios. The simulated heat waves reflect both realistic daily temperature fluctuations as well as realistic heat wave durations (Figure 2.2). Daily fluctuations of experimental heat waves included a six-hour gradual increase from the minimum temperature (20°C), one hour at the maximum temperature (26°C or 32°C), and a six-hour decline back to the minimum temperature (Figure 2.3a). The six heat wave treatments included heat wave durations of 1, 2, or 7 consecutive days of exposure at either maximum temperature. The maximum temperatures were selected to span a range of high temperatures encountered in the field. Field summer temperature data show that 26°C is in the 50th percentile of daily maximum temperature and 32°C is in the 5th percentile of daily maximum temperature (Table 2.1). I recorded summer splash pool hourly temperatures by epoxying ibutton data loggers (Embedded Data Systems) into 6-12 splash pools during the summers of 2014 and 2015. The thermal environments in the lab were created with a Panasonic M1R-154 programmable incubator.

To meet objective i, I tested the hypothesis that increasing heat wave intensity and exposure duration increasingly disrupts energy balance. For the six experimental heat wave groups, I estimated aerobic metabolic rate during maximum heat exposure by measuring oxygen consumption at maximum daily temperature on days 1, 2, and 7 of the simulated heat waves (Figure 2.3a). Oxygen concentrations were measured using optical fluorescence with a 24-channel microplate oxygen system (Loligo Systems, Denmark). Five to twelve replicates per heat wave group, each containing ~15 copepods, were placed in a 200 µl chamber on the microplate. The microplate was placed in a water bath preheated to the maximum temperature (26°C or 32°C) to minimize temperature fluctuations. Copepods acclimated in the chambers for ~15 minutes and then oxygen concentrations were recorded every 15 seconds for 1-1.5 hours. Concurrently, I tested three controls to account for oxygen flux in the water: water from the *Tigriopus* cultures to account for background microbial respiration, and aerated distilled water and anoxic water (a super-saturated solution of sodium sulfite) were used to monitor for sensor drift. To calculate oxygen uptake rates, I used data from 20-30 minutes of the first 40 minutes of each respiration assay.

To estimate metabolic mass, I removed copepods from the respiration chamber, put them in 300 µl of distilled water, and dried them to estimate their dry weight. Diluting the sample in distilled water helped reduce amounts of salts, algae and other non-copepod material that would subsequently be weighed along with the dried copepod samples. Copepod samples, along with three 300 µl samples of the diluted seawater were dried at 50°C for 16 hours. To estimate final dry weight of the copepod sample, I subtracted the mean value of the three diluted seawater samples from each copepod sample weight. Weights were obtained with a Mettler Toledo XP2U microbalance to the nearest 0.1 µg. Rates of oxygen uptake are expressed as µl $O_2$ hour$^{-1}$ mg dry weight$^{-1}$, in which dry weights represented approximately 15 copepods.

To meet objective ii, I tested the hypothesis that energetic impacts of heat wave intensity and duration cascade to impact individual fitness. In this separate experiment, I subjected mated females to heat wave treatments and tracked female survival and reproduction for 20 days following the first day of the experimental heat waves (Figure 2.3b). I isolated mate-clasping pairs in 6mL wells of 12-well plate. Each well was filled with 4-5ml of sterilized seawater (28-32 ppt). By isolating clasping pairs, I ensured that I tracked females at the beginning their reproductive life-stage, rather than females that had already produced multiple broods or were post-reproductive (Burton, 1985). Pairs were held at 20°C for 12 days, then subjected to the heat wave treatments (the sample size of clasping pairs was 29-32 for each heat wave treatment). After each heat wave, individuals were held at 20˚C with a 16:8 hour light:dark cycle for the remainder of the experiment. Each pair received ~0.05mg of ground *Spirulina* algae (Nutrifin Max brand) at day 1, 4, 8, and 16 of the experiment. This addition, in conjunction with some natural algae growth provided ample food without excessive bacterial growth. I periodically conducted 50% water changes, and added distilled water to maintain salinity. Feeding and water changes occurred on the same day for all treatments.

To reduce the possibility of cannibalism (Gallucci & Ólafsson, 2007), males were removed and discarded when the first egg clutch appeared. After nauplii hatched or egg sacs degraded, I transferred the female to a new well. This process was repeated for each subsequent egg clutch. I checked each individual daily for clasping status (still clasped vs. unclasped), appearance of an egg clutch, mortality, and nauplii hatching. Nauplii were collected 2-3 days after hatching, and preserved in 75% ethanol. Egg clutches that had not hatched by the end of the experiment were not used in any subsequent analyses on the rate of nauplii production. A Leica M165C stereoscope was used to count the number of nauplii produced from each egg clutch.

### 2.3.3 Statistical Analyses

*Oxygen consumption rates*

Oxygen concentration change over time patterns were linear. I quantified oxygen consumption rate (ln($O_2$) ug/L/min) by estimating the slope of corrected oxygen concentration using linear regression. I log transformed the rate of oxygen consumption data to meet assumptions of normality (Shapiro-Wilk test: $W = 0.98$, $p = 0.47$), and performed a two-factor ANOVA to test for main and interactive effects of temperature intensity and exposure duration. To account for unbalanced data (5-12 replicates for each experimental group) I performed both a Type III and Type II ANOVA with the *car* package (Fox & Weisberg, 2011) in R v3.1.3 (R Core Team, 2015).

### *Fitness assays*

#### Female survival

I estimated female survival probabilities during the experimental heat waves and for two-weeks post heat wave using the standard non-parametric Kaplan-Meier (KM) survivorship function (with right-censored data) (Kaplan & Meier, 1958). The probability of survival (S) depends upon the number of deaths divided by the number at risk (the hazard function; $\lambda$) at a particular time point ($t$). The KM method updates the information at each censoring event (i.e., each death).

$$S(t) = S(t-1) * (1 - \lambda(t))$$

I first used the Gehan-Breslow (modified Wilcoxon) test to test for differences in the survival curves between the 26˚C and 32˚C groups. This test weights earlier time points, and because the heat waves occurred at the beginning of the experiment I expected most mortality to occur during this time. Next, I performed a Log-rank test (giving equal weight to all time points) to test for differences in the survival curves between the two temperature groups. The Gehan-Breslow and Log-rank tests were performed with the *survMisc* package in R (Dardis, 2015).

#### *Female reproduction*

Total lifetime reproductive output can be impacted by shortening a female's lifetime and by reducing the number of offspring she produces while reproductive. To distinguish the two, and isolate the effect of heat wave intensity and duration on the rate of offspring production, I standardized the total number of nauplii produced by a female by the total number of days she persisted in the experiment. This entailed that I only measured the direct of effect of heat wave intensity and duration on reproduction, and did not include the indirect effect of a shortened lifespan on total reproduction. I performed a two-way ANOVA to investigate the effect of heat wave intensity and duration on the standardized number of offspring produced per female. I performed a Wilcoxon Rank Sum test in the *coin* package (Hothorn et al., 2008) in R to test for an effect of temperature intensity on the number of nauplii per clutch. To further test my hypothesis that energy allocated to reproduction diminishes with increasing heat wave intensity, I restricted the analysis of temperature intensity on the number of nauplii per clutch to only those clutches produced immediately after the last day of the heat wave.

## 2.4 Results

The mean rate of oxygen consumption varied from 0.74 – 1.99 µl $O_2$ hour$^{-1}$ mg dry weight$^{-1}$ (Figure 2.4, Table 2.2). However, I did not detect a significant effect of temperature intensity or exposure duration (or a significant interaction) (Table 2.3).

Survivorship did not differ between the two temperature intensity groups (Figure 2.5) (Log-rank test: $\chi^2 = 0.385$, df = 1, p = 0.53 and Gehan-Breslow test: $\chi^2 = 0.165$, df = 1, p = 0.68). Mortality rates were highest in the first 2-3 days of the heat waves, and declined thereafter.

Of the 151 egg clutches produced, 35 were produced 17 days after the first heat wave day and these subsequent nauplii were not counted. That left 116 egg clutches containing 2199 nauplii. Zero to 75 nauplii were produced per clutch, while 9.59% (7/73) and 18.6% (8/43) of egg clutches yielded no nauplii in the 26˚C and 32˚C groups, respectively. Per female total reproductive output declined in the high-intensity (32˚C) group compared to the low intensity group (t = -2.313, df = 61, p = 0.024) (Figure 2.6). I did not detect an effect of temperature intensity on the number of nauplii per clutch for all 116 clutches (mean nauplii per clutch: 21.01 and 15.47 for the 26˚C and 32˚C groups, respectively; Z = 1.63, p = 0.103) (Figure 2.7a). However, with the analysis restricted to the 15 egg clutches (n = 9 and 6 for the 26˚C and 32˚C groups, respectively) produced immediately after the last day of the heat wave, increasing temperature intensity reduced the number of nauplii per clutch (mean nauplii per clutch: 26.1 and 11.0 for the 26˚C and 32˚C groups, respectively; Z = 2.24, p = 0.025) (Figure 2.7b). There was a slight trend of increasing heat wave duration negatively affecting offspring production, despite not being statistically significant (Day 2: t = -1.765, p = 0.083; Day 7: t = -0.044, p = 0.965) (Figure 2.6).

## 2.5 Discussion

Linking the effects of environmental stress and subsequent physiological responses to individual fitness is critical for understanding changes to population demography and population persistence. Here, I tested the hypothesis that disruptions to energy balance increase with heat wave intensity and heat wave duration, and subsequently impact individual survival and reproduction similarly. I found that aerobic metabolic rate neither varied between temperature intensity groups nor with increasing heat wave exposure duration. Likewise, survivorship did not differ between individuals in the different temperature intensity groups. However, females in the high intensity (32˚C) heat wave group produced fewer overall offspring than those in the 26˚C heat wave group, and the number of nauplii per clutch was lower in the 32˚C group than the 26˚C group for clutches produced immediately after the last day of heat wave exposure. Thus, survivorship in the 32˚C group was maintained at a similar rate as the 26˚C group, but individuals exhibited reduced reproductive output. My results are consistent with the hypothesis that increasing thermal stress exacerbates trade-offs between survival and reproduction, and that these trade-offs can be linked to energetic trade-offs that maintain homeostasis.

### 2.5.1 Rates of oxygen consumption

Contrary to my predictions, I did not observe significant differences in oxygen consumption rates between heat wave treatments, nor did I see significant decreases in oxygen consumption as heat waves persisted. A signature of deteriorating physiological condition is the switch from aerobic to anaerobic respiration as oxygen becomes less available. Two mechanisms may help explain the pattern of oxygen consumption I observed: an Arrhenius breakpoint temperature (ABT) between 26˚C and 32˚C, or metabolic rate depression.

Metabolic rate has been shown to increase curvilinearly as temperature increases (Ikeda, 1985; Clarke & Johnson, 1999), following the standard shape of a thermal performance curve (TPC; Figure 2.8a). I predicted that metabolic rate would be higher at 32˚C than at 26˚C on the first day of exposure, corresponding to the curvilinear portion of the TPC under non-stressful conditions (Figure 2.8b). It is possible that 26˚C and 32˚C spans the Arrhenius breakpoint temperature (Figure 2.8c), which is the first point corresponding with reduced biochemical or physiological functioning (Schulte 2011). In this scenario, the metabolic rates at 26˚C and 32˚C may be similar because they fall on the increasing and decreasing parts of the TPC, respectively.

However, data on copepod metabolic rate from other studies suggest that this potential explanation for my observation is unlikely. McAllen et al. (1999) compiled data on respiration rates from several studies of copepod respiration that showed greater rates of oxygen consumption at temperatures lower than those in this study. In three other studies on respiration rates in *Tigriopus spp.*, mean rates of oxygen consumption at 20˚C varied from 2.9 – 13.9 µl $O_2$ hour$^{-1}$ mg dry weight$^{-1}$, compared to a mean of 1.25 µl $O_2$ hour$^{-1}$ mg dry weight$^{-1}$at 26˚C in this study. The rates I observed are considerably lower than what would be expected in non-stressful conditions, and, therefore, other mechanisms underlying metabolic rate should be invoked to explain the low and similar rates of oxygen consumption I observed at 26˚C and 32˚C.

One alternative is that *Tigriopus californicus* may employ a strategy of metabolic depression, where a coordinated reduction in ATP turnover and energy demanding processes (e.g., protein synthesis, Hochachka et al., 1996) reduces basal maintenance costs (Storey & Storey, 1990, 2004; Guppy & Withers, 1999). Conserving energy reserves extends survival under increasingly stressful physiological condition, but this comes at the expense of growth and/or reproduction as energy production is suppressed (Sokolova, 2013). Metabolic depression has been observed in *Tigriopus brevicornis* (McAllen et al., 1999) and other intertidal organisms that regularly experience anaerobiosis and elevated temperatures during periods of emersion at low tide (Storey & Storey, 1990; Hand & Hardewig, 1996; Sokolova et al., 2000; Sokolova & Pörtner, 2001). *Tigriopus* copepods routinely experience widely fluctuating temperatures, which characterize the suppralittoral zone. However, despite remaining fully immersed in splash pools, the high daily temperatures may result in oxygen limitations, leading to conditions favoring metabolic depression.

### 2.5.2 Effects on fitness

I observed declines in reproduction within the 32˚C treatment despite similar survival between the two heat wave intensity treatments. Based on summer field temperatures, I predicted that survivorship would decrease more rapidly in the 32˚C treatments than in the 26˚C treatments, as individuals rarely experience 32˚C. At Botany Bay, daily splash pool temperature reaches 26˚C approximately 50% of summer days, while 32˚C is reached on less than 5% summer days. However, it is important to note that my experimental heat waves were intended to reflect summer thermal regimes, but the copepods used for the fitness assays were collected in November. Mean daily maximum temperatures for October-November are closer to 16˚C, and reach 20˚C less than 20% of the time. Combined with a laboratory acclimation at 20˚C for 12 days prior to the experiment, seasonal acclimatization to November conditions may have led to lower thermal tolerances than would be exhibited during the summer. However, the higher than expected mortality rates in the 26˚C groups demonstrate that the experimental heat wave treatments were sufficiently stressful to study both lethal and sub-lethal effects on fitness. Moreover, while reduced thermal tolerance due to seasonal acclimatization may explain a higher than expected mortality rate, it is not sufficient to account for the similar patterns of survivorship between the 26˚C and 32˚C temperature intensity groups.

Most mortality occurred early in the experiment before heat waves were finished. It is possible that the stress of clasping contributed to high female mortality rates. Adult males exhibit pre-copulatory mate-guarding behaviour by clasping immature females to ensure that a potential mate has not been previously fertilized (Burton, 1985). Males will clasp developing females anywhere in the C2-C5 stage. Thus, the clasping phase can last anywhere from 1-7 days. Pre-copulatory mate-guarding behaviour is commonly observed in crustaceans, and likely evolved due to intersexual conflict over precopula duration (Parker, 1974; Jormalainen, 1998). Females consistently resist male clasping, and the mate-guarding is characterized by repeated female escapes and re-capture events (personal observations). The high activity levels needed to resist male clasping likely require large energy expenditures and also cost the female in terms of reduced feeding (Jormalainen et al., 1994). It is possible that the interaction between clasping stress and thermal stress resulted in equal mortality rates between the 26˚C and 32˚C despite the prediction that mortality increases with thermal stress.

As predicted, offspring production decreased with increasing temperature intensity. Several factors possibly contributed to this result: a decrease in the number of eggs produced by females in the 32˚C treatment, differential mortality rates of N1-N2 nauplii, a decline in hatching success, and a delay in egg sac production. I found that females in the 32˚C treatment slightly delayed egg sac production relative to females in the 26˚C treatment (Figure 2.9); however, these differences were not statistically significant (Log-rank test: $\chi^2 = 1.84$, $p = 0.18$). I let the nauplii grow for 2-3 days post hatching in order to attain a large enough size to visualize and count effectively under the microscope. As such, I am not able to differentiate the contributions of a decline in egg number, a decline in hatching success, and a disproportionately higher mortality rate of N1-N2 nauplii in the 32˚C treatment to the overall decline in offspring production, although similar mechanisms involving maternal stress and provisioning underlie all three (Koski & Kuosa, 1999; Holste & Peck, 2006).

In line with my predictions, I observed a slight trend of increasing heat wave duration negatively impacting offspring production. Due to the experimental design, most females did not begin producing egg clutches until they were four or five days into the heat wave. It is likely that if the heat wave treatments were shifted to have a greater overlap with the period of egg clutch production that a stronger signal of an effect of heat wave duration would have been detected. Furthermore, while my data are consistent with a trade-off between survival and reproduction, direct evidence of an energetic trade-off is beyond the scope of my thesis. It is possible that less energy for reproduction in the hotter heat wave treatment was available, which lead to the observed decline in reproduction, rather than a re-allocation of energy away from reproduction to processes prioritizing survival. However, food was made available throughout the 20-day fitness experiment, and it is unlikely that energy was limiting. Additionally, given the extensive literature on the energetic cost of heat stress and associated physiological responses (Bell et al., 1988; Feder & Hofmann, 1999; Bragg & Wagner, 2009; Hoekstra & Montooth, 2013) it is realistic to assume that an energetic trade-off between survival and reproduction did underlie the decline in reproduction I observed in the hotter heat wave intensity treatment.

### 2.5.3 Heat waves in the intertidal

Extreme heat events (heat waves) are a major source of disturbance in intertidal systems (Dethier, 1984). Eurythermal organisms in the intertidal routinely experience dramatic fluctuations in temperature, and are predicted to be more robust to climatic extremes than organisms from stable environments (Hoffmann et al., 2003). However, they routinely encounter temperatures close to their lethal limit, and may actually be more susceptible to increasing temperatures (Somero, 2010). Rapid climate change is leading to an increase in the frequency and severity of heat waves. As such, they will likely become an even more important factor structuring intertidal communities.

I manipulated the heat wave thermal regime along two axes: daily maximum temperature and duration of consecutive days of exposure. In reality, heat wave structure may vary in different but equally important ways not examined here (e.g., ramping speed; length of the daily maximum temperature exposure; increasing daily minimum temperatures). The interactive effects of multiple axes of heat wave structure on fitness are not well understood. For example, mortality may increase with increasing daily maximum temperatures, or through an increase in the number of degree warming hours, which do not necessarily entail higher daily maximum temperatures. Additionally, increases in daily minimum temperatures may reduce the availability of recovery time as the daily temperature recedes following afternoon highs. This recovery time allows for the repair of cellular components, and reduction in concentration of heat shock proteins, which may have deleterious effects if they persist at too high a concentration for too long (Feder & Hofmann, 1999).

### 2.5.4 Conclusion

My results showed that temperature *per se*, but not the number of consecutive days of heat wave exposure, negatively impacted reproduction but not survival. This study contributes to a small but growing literature on the impact of heat waves on demography, and shows that sublethal effects of heat waves may impact population vital rates even if mortality rates are comparable between thermal regimes of varying temperature intensity. My data show that metabolic rate is consistently low and similar between my temperature intensity groups, and likely reflects a ‘quiescent state’ consistent with metabolic depression. Metabolic depression extends survival time but at the cost of immediate growth and reproduction, a pattern consistent with the results of my fitness assay. Follow-up studies should investigate how manipulating other aspects heat wave structure, such as increasing the daily maximum temperature exposure time, affects individual survival and reproduction. Elucidating how heat wave structure influences the survival/reproduction trade-off may be critical for predicting population persistence.

## 2.6 Tables and figures

### 2.6.1 Tables

**Table 2.1.** Daily maximum splash pool water temperatures from Botany Bay, British Columbia. The percentage of days where daily maximum temperatures reached 26°C, 30°C, or 32°C for each annual group of splash pools is shown. Daily maximum temperatures reached 26°C ~50% of days whereas temperatures reached 32°C less than 5% of the time.

| Year | # pools | Date range | # days | Freq. 26° | Freq. 30° | Freq. 32° |
|---|---|---|---|---|---|---|
| 2015 | 3 | July-Sept | 82 | 34 – 55% | 0 – 10.9% | 0 – 4.9% |
| 2016 | 5 | May-Aug | 86 | 46.5 – 53.5% | 7 – 15.1% | 0 – 4.7% |

**Table 2.2.** Mean rate of oxygen uptake and standard deviation (SD) for each experimental group. Rates are reported as µl $O_2$ hour$^{-1}$ mg dry weight$^{-1}$. N = the number of traces per group.

| Temperature | Duration | N | Mean | SD |
|---|---|---|---|---|
| 26˚C | Day 1 | 12 | 1.14 | 0.66 |
| - | Day 2 | 5 | 1.10 | 0.46 |
| - | Day 7 | 12 | 1.51 | 1.22 |
| 32˚C | Day 1 | 12 | 0.74 | 0.26 |
| - | Day 2 | 8 | 1.99 | 1.48 |
| - | Day 7 | 8 | 1.27 | 0.71 |

**Table 2.3.** Type II ANOVA results for the model testing the effect of temperature and duration on the log of the rate of oxygen consumption. No significant interaction was detected with the Type III ANOVA, so the Type II was used. DF = degrees of freedom.

| Effect | DF | F-value | P-value |
|---|---|---|---|
| Temperature | 1 | 0.108 | 0.743 |
| Duration | 2 | 2.797 | 0.070 |
| Temp*Dur | 2 | 1.569 | 0.218 |

### 2.6.2 Figures

**Figure 2.1.** Map of the *Tigriopus californicus* collection site: Botany Bay in Botanical Beach Provincial Park, BC, Canada.

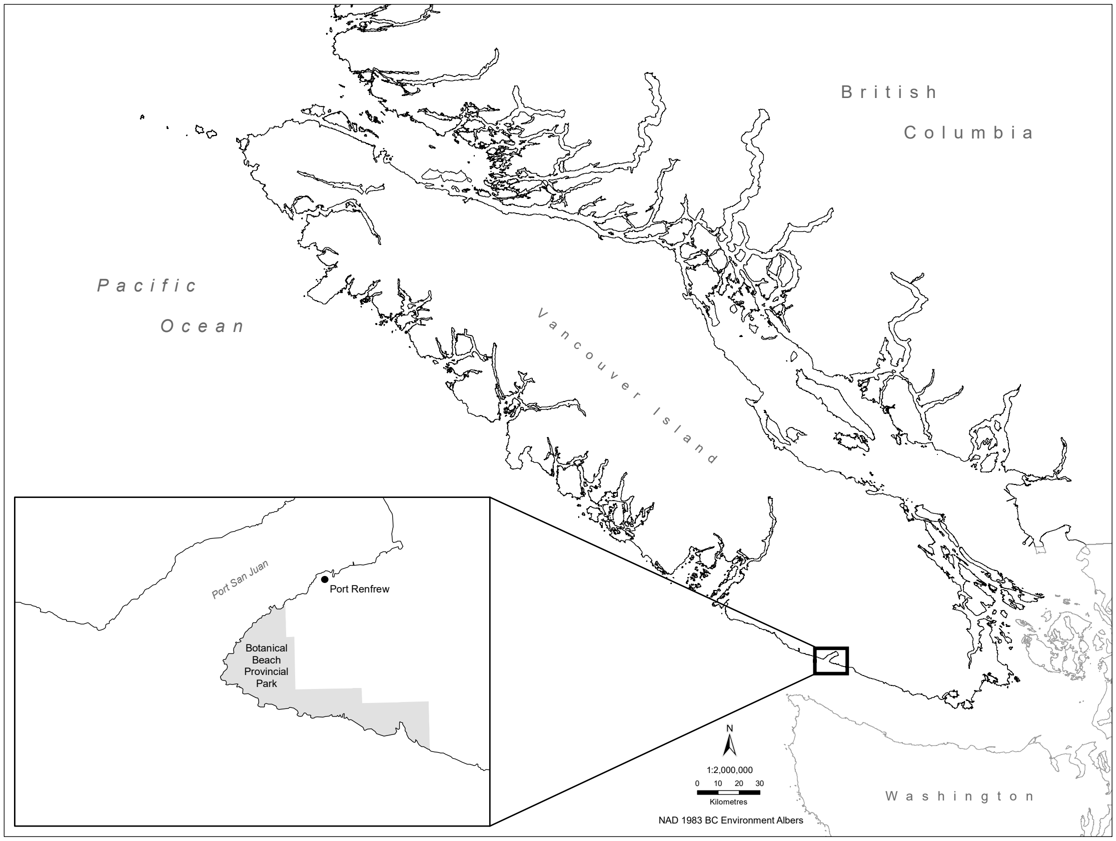


**Figure 2.2.** Typical daily temperature profiles for six splash pools in Botany Bay (BY), BC, from 2-July-2016 through 27-June-2016. Each panel represents one splash pool. Horizontal lines are shown at 20°C, 26°C and 32°C.

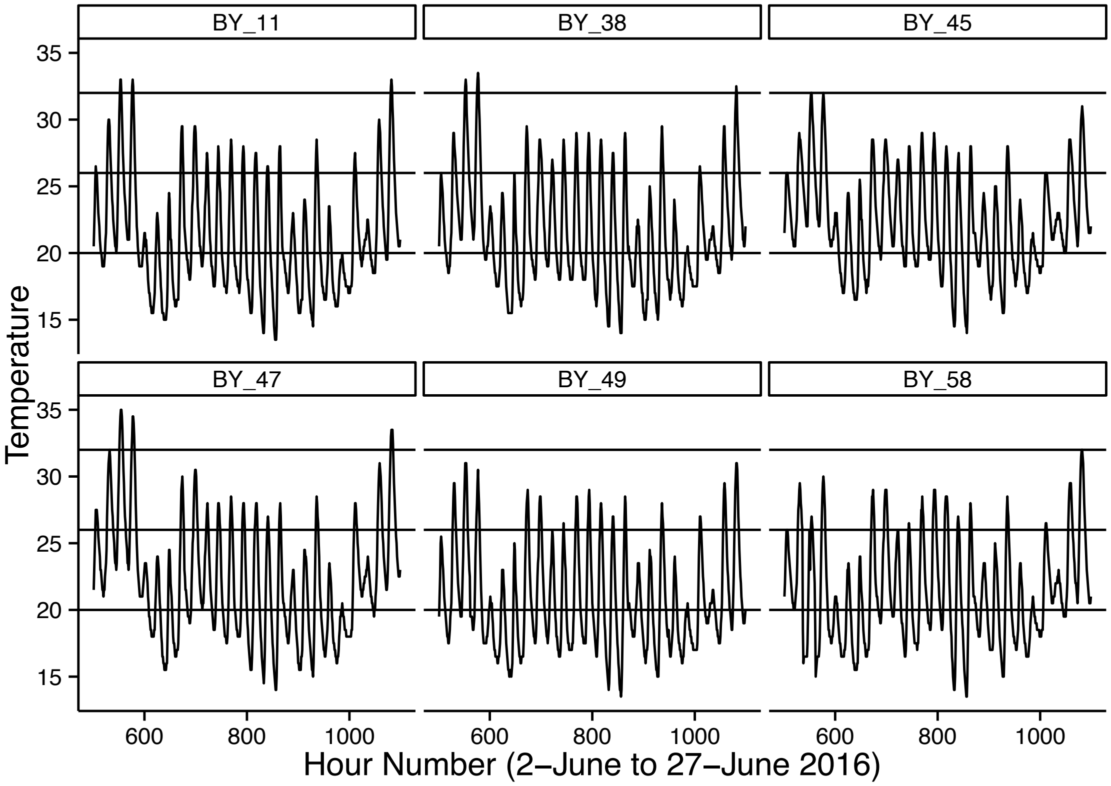
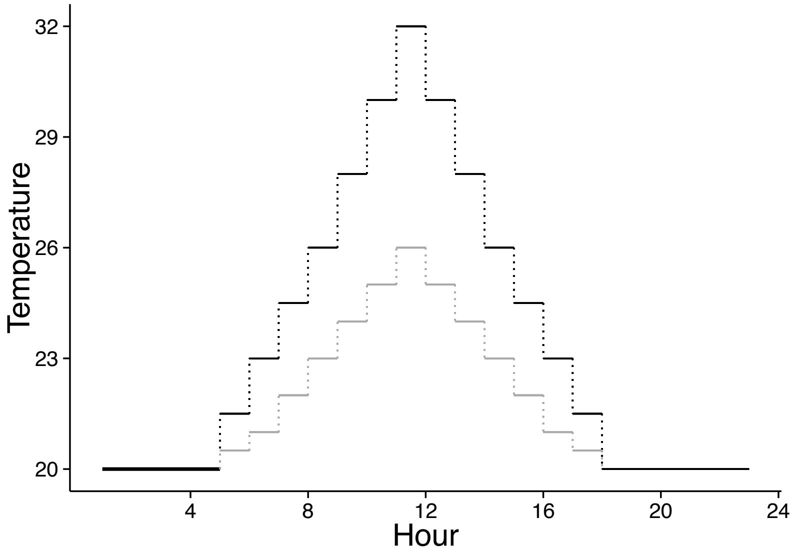


**Figure 2.3.** (*a*) Daily temperature regime for the experimental heat waves to which the groups of *T. californicus* copepods were exposed. Solid lines are the 1 hour time slots at a given temperature, dotted lines indicate the stepping stone temperature ramp-up. Black lines are for the 32°C heat waves and grey lines are for the 26°C heat waves. (*b*) The full thermal profiles for the one, two, and seven day/26°C heat waves are shown. The 32°C heat waves include the same pre and post-heat wave thermal profile.



(*b*)

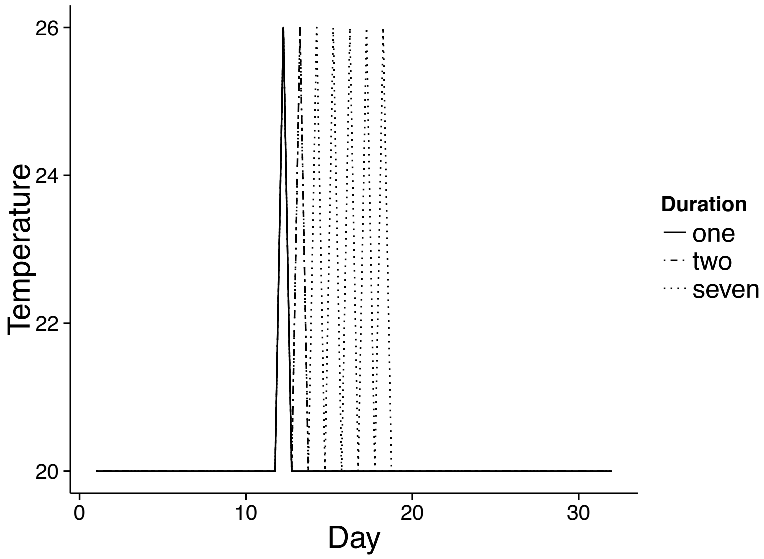


**Figure 2.4.** Oxygen consumption rates (µl $O_2$ hour$^{-1}$ mg dry weight$^{-1}$) for the six experimental groups of copepods corresponding to the two temperature treatments (26°C and 32°C) and three levels of exposure period (1, 2 or 7 consecutive days of exposure). No significant difference in consumption rate was detected for either main effect or an interaction. Boxplots show median values (horizontal lines) and 1st and 3rd quartiles (upper and lower edges of boxes). Whiskers encompass all values (+/-) 1.5 times the distance between the 1st and 3rd quartiles. Outliers are shown as dots.

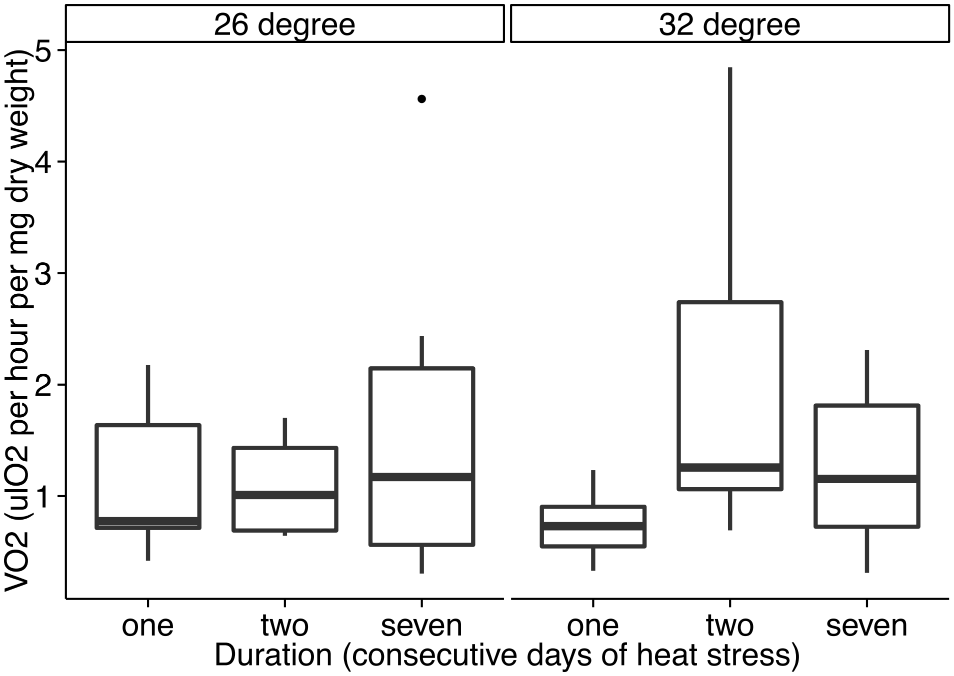


**Figure 2.5.** Estimated daily probability of *T. californicus* survival for the 26˚C (dark grey) and 32˚C (light grey) maximum temperature groups. Data are pooled across all exposure duration levels. No difference in survival probabilities (+ 95% CI) was observed between the two temperature groups.

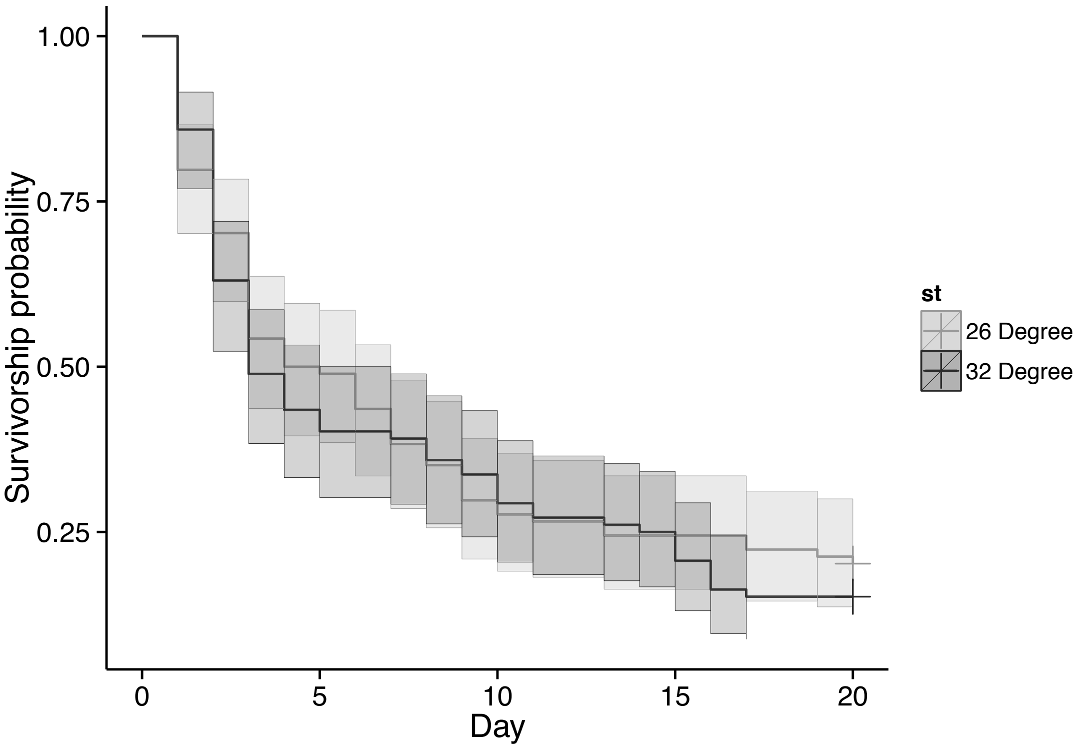


**Figure 2.6.** Total nauplii produced per female standardized by the number of days each female lived during the experiment. Overall offspring production decreases with increasing temperature. However, this effect is not observed within the 26˚C/2-day duration group. Boxplots show median values (horizontal lines) and 1st and 3rd quartiles (upper and lower edges of boxes). Whiskers encompass all values (+/-) 1.5 times the distance between the 1st and 3rd quartiles. Outliers are shown as dots.

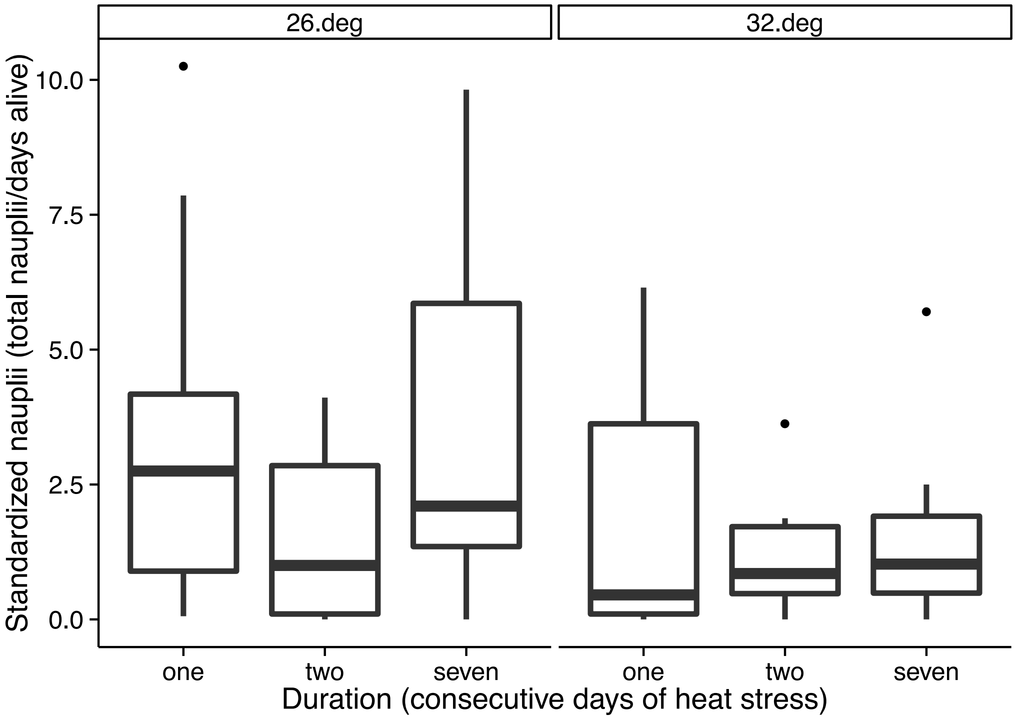


**Figure 2.7.** Number of *T. californicus* nauplii per clutch. The number of nauplii per clutch for each temperature intensity group is shown for all 116 clutches over the entirety of the experiment (*a*), and for the 15 clutches produced immediately after the last day of exposure (n = 9 and 6 for the 26˚C and 32˚C groups, respectively) (*b*). No significant difference was found when considering all 116 clutches. However, increasing temperature reduced the number of nauplii per clutch for the restricted 15 egg clutches. Boxplots show median values (horizontal lines) and 1st and 3rd quartiles (upper and lower edges of boxes). Whiskers encompass all values (+/-) 1.5 times the distance between the 1st and 3rd quartiles. Outliers are shown as dots.

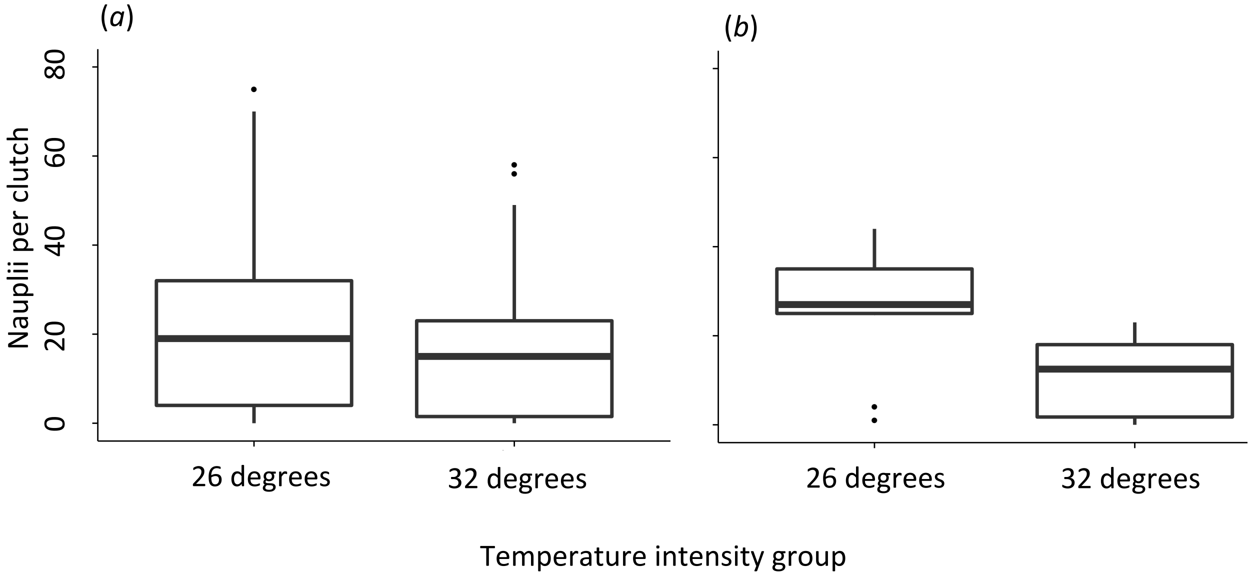


**Figure 2.8.** Thermal performance curves. (*a*) The shape of a standard, hypothetical performance curve. (*b*) Predicted metabolic rates at 26 and 32 degrees under non-stressful conditions. (*c*) A situation showing how metabolic rates at 26 and 32 degrees could be equal if they span the Arrhenius breakpoint temperature.

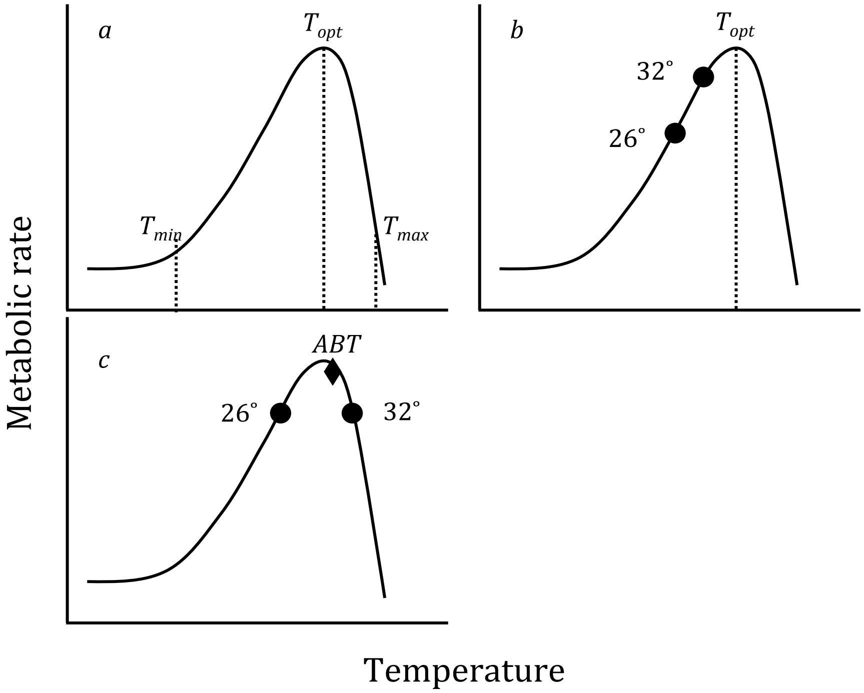
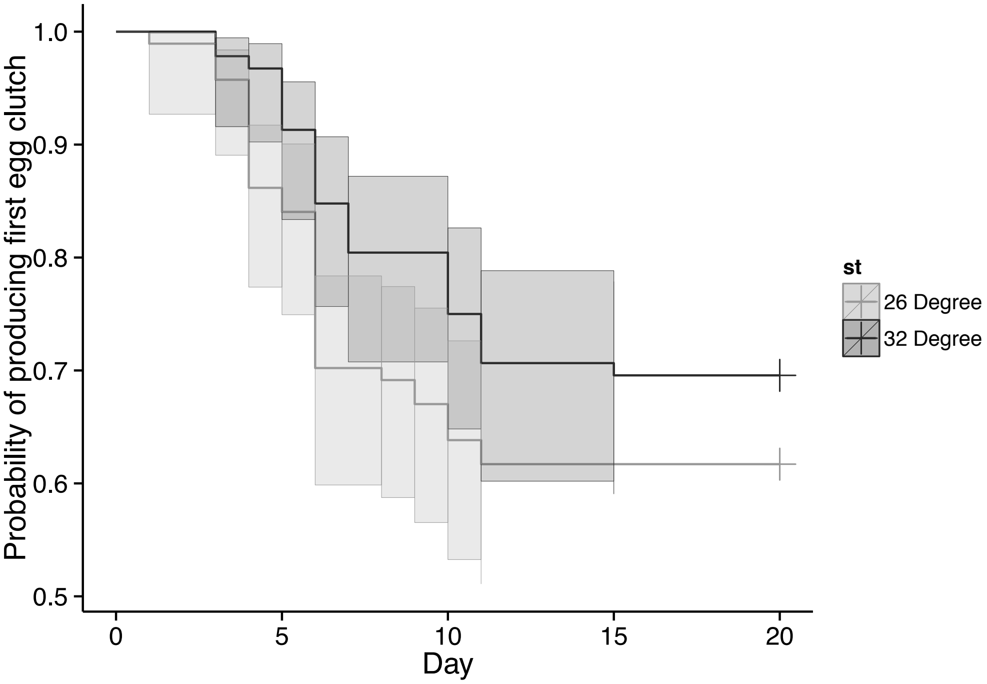


**Figure 2.9.** These curves show the probability of time to first egg clutch for *T. californicus* females. The proportion of individuals that have produced their first egg clutch at each time step are shown for both the 26˚C and 32˚C groups, pooled across all exposure duration levels. No statistical difference was found between the two temperature groups, however there was a slight trend towards the higher temperature group delaying production of their first egg clutch (Log-rank test: $\chi^2 = 1.84$, p = 0.18). Green lines are for the 26˚C temperature group and the red line corresponds to the 32˚C temperature group. The lines represent the estimated probabilities and the shaded regions represent the 95% CIs.



# CHAPTER 3

# Short-term population growth rates influenced by experimental heat waves are decoupled from longer-term population dynamics

## 3.1 Summary

Temperature has profound effects at all levels of biological organization. Bioenergetic frameworks use the concept of energy homeostasis to link the effects of sublethal temperature stress to higher order fitness-related processes, such as individual reproduction and population growth. However, temperature can have variable effects on different life-history traits that contribute to population-fitness, and the net affect of short-term heat accumulation and sublethal temperature stress may lead to different population responses over different time-scales. Here, I test the hypothesis that: i. short-term population growth rate declines with increasing heat wave intensity and duration, and ii. that these effects persist on longer-term population dynamics. I tracked the abundance of laboratory populations of the splash pool copepod, *Tigriopus californicus* over 100 days. Within this period, populations were subjected to one of six experimental heat wave treatments, varying in both daily maximum temperature (heat wave intensity: 26°C or 32°C) and number of consecutive exposure days (heat wave duration: 1, 2 or 7 days). I calculated short-term (11 day) population growth rates during the period encompassing the heat wave, and tested the effect of short-term growth rates to predict long-term abundance. The results do not conform to bioenergetics-based predictions that short-term population growth rate declines with increasing heat wave intensity and duration, and that short-term population growth rate predicts long-term population abundance. Population

growth rates in the 26°C heat wave groups declined with increasing heat wave duration. However, an overall net benefit of heat accumulation during heat waves was apparent at longer time-scales via higher peak and final abundances. In contrast, higher short-term population growth rates for the 32°C heat wave treatment group were observed for the 2-day and 7-day groups relative to the 1-day group. However, peak and final abundances were significantly lower in the 7-day group than the 1 and 2-day groups. Overall, my results show that the effect of heat wave intensity and duration on short-term population growth rate depends upon the interaction between intensity and duration, and that short-term growth rates do not predict longer-term population abundances. The multifaceted effect temperature can have life-history traits underlying population vital rates should be considered when extrapolating the effect of short-term temperature stress on longer-term population dynamics.

## 3.2 Introduction

Temperature stress may negatively impact population persistence through both lethal and sublethal mechanisms (Calow, 1989; Pörtner & Farrell, 2008). Sublethal effects, such as reduced fecundity (Krebs & Loeschke 1994; Petes et al., 2007; 2008), arise as energy is directed away from reproduction to processes that promote survival (Sokolova et al., 2012) . However, the variable effect of temperature on different life-history traits that contribute to population fitness may obscure our ability to extrapolate short-term population dynamics to longer-term trends. Here, I test how heat wave intensity (daily maximum temperature) and heat wave duration (consecutive number of heat wave days) affect short-term population growth rates, and if these short-term effects influence longer-term population dynamics. As climate change accelerates, heat waves are expected to increase in severity and frequency (Frich et al., 2002), and experiments are needed to specifically test how biological responses to heat waves vary with specific heat wave structural properties. Additionally, testing the expectation that sublethal effects of heat waves on individual fitness influences population dynamics over longer time periods is crucial for understanding how heat waves impact population demography and long-term persistence.

Population growth rate is commonly used to estimate population fitness. However, different estimates of population growth rate can exhibit different responses to temperature. The intrinsic rate of increase ($r$) and the net reproductive rate ($R_o$) are two common measures of population growth rate. The intrinsic rate of increase is the birth rate minus the death rate, and the net reproductive rate ($R_o$) is the average *per capita* rate of lifetime female offspring production. Both of these measures estimate population growth rate, however $r$ is scaled to time while $R_o$ is scaled per generation. This difference has important implications for the predicted affect temperature has on population growth (Huey & Berrigan, 2001).

Development accelerates with increasing temperature (Cossins & Bowler, 1987; Gillooly et al., 2002), which shortens generation time and leads to an elevated intrinsic rate of growth. Thus, $r$ increases with temperature relative to $R_o$ if population growth is measured across multiple generations. Within a generation, high temperatures may negatively impact $R_o$ through decreases in lifespan or fecundity (Boyce, 1977). However, the impact of these within generation effects of sublethal temperature stress are typically not large enough in magnitude to counter the positive effects of shortened generation times on long term population growth that spans multiple generations (Cole, 1954; Huey & Berrigan, 2001).

A host of factors may affect intrapopulation variation in thermal tolerance, leading to disparate effects of sublethal temperature stress on populations across different time scales. Differences in thermal tolerance can vary with life-stage and age (Krebs & Loeschcke, 1995; Bowler & Terblanche, 2008; Tangwancharoen & Burton, 2015), sex (Huey et al., 1991; Loeschcke et al., 1994; Sørenson et al., 2005), and body size (Loeschcke et al., 1994; Kelly et al., 2013). Moreover, temperature can also affect sex ratio (Kvarnemo & Ahnesjö, 1996; Voordouw & Anholt, 2002), which has an enormous potential influence on population dynamics (Steifetten & Dale, 2006).

Bioenergetic models predict that energetic constraints due to sublethal temperature stress should impact individual reproductive ability, and consequently, population fitness via impacts to population growth rate (Pörtner & Farrell, 2008;

Sokolova et al., 2012). However, intrapopulation variation in thermal tolerance may result in opposing effects of temperature variable effects of heat accumulation between individuals. This individual variation in temperature responses may lead to an overall net benefit in population-level fitness, where short-term impacts to population growth do not reflect longer-term population dynamics. In this study, I test the hypotheses that i. short-term population growth rate declines with increasing heat wave intensity and duration, and ii. these effects persist over longer time scales, negatively affecting long-term population dynamics.

I monitored the population abundance of laboratory populations of the splash pool copepod, *Tigriopus californicus* over a period of 100 days (approximately 4-6 generations). During two of the 12 weeks of this experiment, six of the eight experimental groups were subjected to one of six different experimental heat waves varying in both the daily maximum temperature and the number of consecutive exposure days. I calculated short-term (11 day) population growth rates during heat wave phases of the experiment, and related those to peak and final abundances.

## 3.3 Materials and methods

### 3.3.1 Animal collection, laboratory populations and abundance sampling

In May 2016, I collected *Tigriopus californicus* individuals from eight splash pools in Botany Bay, located in Botanical Beach Provincial Park near Port Renfrew, British Columbia (see Figure 2.1). The copepods were transported to The University of British Columbia in 1 L containers and fed ground *Spirulina* algae en route. Forty-eight laboratory populations were established with ten gravid females each in 200 ml glass jars filled with sterilized seawater (28-32 ppt). Every week each jar received 10 mg of ground *Sprirulina* algae, and ~ 40% of the water was refreshed. The jars were held at 20°C for three weeks before the first day of population abundance sampling. To sample population abundance, the water in each jar was mixed around and 10 ml (5%) was removed with a glass baster. Adults and juvenile copepodids were counted under a Leica dissecting microscope, and returned alive to their respective jars. Each jar was sampled six times approximately every two weeks.

### 3.3.2 Temperature treatments

The 48 jars were partitioned into eight experimental groups with six replicates each. Two groups were kept at a constant 20°C or constant 32°C, and six experimental groups were subjected to one of six heat wave treatments. The heat wave treatments varied in both the peak daily temperature (26°C or 32°C) and the number of consecutive exposure days (1, 2 or 7 days). Each heat wave day consisted of a six-hour increase in temperature from 20°C to the maximum temperature, one hour at the maximum temperature, and a six hour decrease back to 20°C (see Chapter 2). The two constant temperature treatments were included to establish a mild (20°C) and severe (32°C) temperature baseline. Each heat wave group was subjected to two heat waves, between the first and second sample day and between the fourth and fifth sample day (Figure 3.1). During non-heat wave days, these groups were kept at a constant 20°C.

### 3.3.3 Statistical analyses

To test hypothesis i, I characterized short-term population dynamics by calculating the population growth rates of the different heat wave treatments during the time period encompassing the first heat wave. To test hypothesis ii, I tested for an effect of heat wave intensity and duration on peak and final abundances (which occur over longer time scales, approximately halfway through the experiment and at the end), on the coefficient of variation in abundance, and compared the pattern of longer-term abundance measures to short-term population growth rates. I calculated population growth rates for each time period by approximating exponential growth using the equation: $r \approx \ln(N_{t+1} / N_t)$. To avoid a confounding effect of multicollinearity between my constant temperature treatments (20°C and 32°C constant temperature), the following analyses were restricted to the 26°C and 32°C heat wave experimental groups.

Hypothesis i: I used a two-way ANOVA to test for heat wave intensity and duration effects on population growth rates. Normality was confirmed with the Shapiro-Wilk test in R (R Core Team, 2015). I also tested the effect of time on population growth rate by constructing a linear mixed-effects model. The model included heat wave intensity, duration, and time-period as main effects and population as a random effect. The model was fit with the *lme4* package (Bates et al., 2014) in R.

Hypothesis ii: To test for an effect of heat wave intensity and duration on peak and final abundances, I initially used a poisson generalized linear model (GLM). However, in both cases the data were overdispersed (tested in the *AER* package in R; Kleiber & Zeileis, 2008). Therefore, I fitted a quasi-poisson and a negative binomial GLM to account for the overdispersed data. To check which GLM better fit the data, I plotted the mean against the variance of the peak and final abundances for each experimental group. This visualization can be used as a diagnostic between the two GLMs; in the quasi-poisson the variance is linearly related to mean, whereas in the negative binomial the relationship is a quadratic function (Ver Hoef & Boveng, 2007). For peak abundance, the relationship between mean and variance showed that the negative binomial model was a better fit. For the final abundance, the relationship between mean and variance showed that the quasi-poisson was a better fit. To examine how heat wave intensity and duration influence variation in abundance I used a two-way type III ANOVA to test for heat wave effects on the coefficient of variation (standard deviation divided by the mean; CV) in population abundance. I also fit a one-way ANOVA to test for an effect of temperature on CV, which included all eight of my experimental groups across three different temperature levels (20°C, 26°C and 32°C).

## 3.4 Results

The two baseline temperature treatments appeared to function as intended. Populations in the mild temperature treatment (constant 20°C) had an overall higher final abundance than populations in the heat wave treatments, while populations in the severe temperature treatment (constant 32°C) went extinct four days into the experiment (Appendix A). Populations in the constant 20°C treatment exhibited logistic population growth, reaching an approximate carrying capacity. The heat wave treatment populations, however, exhibited a general abundance-increasing phase followed by a decline in abundance, which was more pronounced in some heat wave treatments than others. Four of the six replicates in the 32°C/7-day treatment exhibited minimal population growth followed by a slight decrease in abundance. Interestingly, two replicates of the 32°C/7-day duration treatment exhibited a rise in population abundance similar to the less severe heat wave treatments, that was, however, followed by a pronounced decline in abundance (Appendix A).

Average population growth rates during the first heat wave period varied from 0.34 ind/ind/day for the 32°C/7-day group to 0.57 ind/ind/day for the constant 20°C group (Table 3.1). Overall, population growth rates during this time period increased with heat wave intensity and duration (ANOVA: $F = 6.57$, $df = 2$, $p = 0.004$, adj-$R^2 = 0.32$) (Table 3.2, Figure 3.2). This effect, however, was driven by a high population growth rate in the 32°C/2-day duration treatment. The pattern of population growth rates for each duration treatment differed between the two heat wave intensity treatments. Among the less intense (26°C) heat wave treatments, short-term population growth rate declined with increasing heat wave duration. Among the more intense (32°C) heat wave treatments, population growth rates increased with heat wave duration, but did not increase linearly: the 2-day heat wave group exhibited a higher population growth rate than the 7-day heat wave group. Average population growth rates during the time period encompassing the second heat wave were lowest in the 26°C/1-day group (-0.57 ind/ind/day) and highest in the 20°C constant group (0.16 ind/ind/day) (Table 3.1). Among the heat wave treatments, no differences in population growth rate were detected (Table 3.2, Figure 3.2). Population growth rates were significantly higher during the first half of the experiment than during the second half (ANOVA: F-value = 56.4, $df = 5$, p-value $< 0.0001$).

Longer-term population dynamics, characterized by peak and final abundances at day 100, varied between heat wave treatments. Average peak abundances ranged from 96.7 individuals in the constant 32°C treatment to 906.7 individuals in the 26°C/1-day heat wave treatment (Table 3.1). Peak abundance in the 32°C/2-day treatment was significantly higher than the mean peak abundance across all heat wave treatments (GLM: $z = 2.27$, $p = 0.023$; Table 3.3), while peak abundance declined in the 26°C/2-day duration treatment (GLM: $z = -3.121$, $p = 0.0018$; Table 3.3). Within the 26°C heat wave treatment, the 1-day treatment exhibited the highest peak abundance while in the 7-day treatment peak abundance was slightly higher than that of the 2-day group. In contrast, peak abundances within the 32°C heat wave treatments were lowest for the 7-day treatment, and increased in the 1-day and 2-day treatments.

Average final abundances ranged from zero to 329 for the constant 20°C temperature group (Table 3.1). Overall, final abundances were slightly lower in the higher heat wave intensity treatments and lower in the 7-day duration treatments, although these results not significantly significant with alpha set to 0.05 (32°C intensity treatment: $p = 0.0077$, 7-day duration treatment: $p = 0.092$; Table 3.3). Final abundances were higher in the 32°C/2-day treatment relative to mean final abundance (GLM: $z = 2.39$, $p = 0.023$), while overall final abundances were lower in the two-day duration treatments (GLM: $z = -3.22$, $p = 0.003$) (Table 3.3; Figure 3.3).

The coefficient of variation in abundance (CV) was lowest in the constant 20°C group (0.69) and highest in the constant 32°C group (1.69). Excluding the constant 32°C treatment, which went extinct early in the experiment, the 26°C/1-day heat wave treatment exhibited the highest CV of 0.94 (Table 3.1). I did not detect a significant overall effect of heat wave intensity or duration on the CV in abundance (Table 3.2), although the CV for the 32°C/2-day group was higher than the overall mean CV (t-value = 2.14, p-value = 0.041), and the CV declined across both 7-day duration groups relative to the overall mean ($t = -2.15$, p-value = 0.040) (Figure 3.4). If I include all eight of my experimental treatments, and restrict my predictors of CV to temperature alone, CV increases with increasing temperature (ANOVA: F-value = 4.95, df = 2, p-value = 0.01).

## 3.5 Discussion

Ectotherm performance and fitness is profoundly influenced by temperature (Angilletta, 2009). Organisms that occupy the supralittoral zone in the intertidal are routinely subjected to high temperatures. However, as heat wave intensity and frequency increases due to climate change (Calow, 1989; Frich et al., 2002; Meehl & Tebaldi, 2004; Pörtner & Farrell, 2008), mechanistic frameworks that integrate the effects of temperature stress across levels of biological organization are needed to understand the conditions under which population persistence may be compromised (Sokolova et al., 2012).

This study tested the hypotheses that i. short-term population growth rate declines with increasing heat wave intensity and duration, and ii. these short-term effects have persistent effects on longer-term population dynamics. During the first heat wave event, short-term population growth rates did not uniformly decline with increasing heat wave intensity and duration, but depended upon the interaction. These short-term effects on population dynamics, however, were decoupled from peak and final abundances reached over longer time scales. Despite lower population growth rates, peak abundance for the 26°C/7-day treatment was higher than that of the 26°C/2-day treatment, and peak abundance in the 32°C/1-day treatment was higher than the 32°C/7-day treatment. Final abundances conformed to the same pattern. My results contrast with predictions that the effects of sublethal stress on individual reproduction (see Chapter 2) should similarly affect population-level fitness (Sokolova et al., 2012). There are three main contributing factors I think may help explain the disconnect between short-term individual fecundity and longer term population dynamics:

1. The heat waves only occurred over a fraction of the total days of the experiment. *Tigriopus californicus* individual life-span can range between 50 and 80 days (Vittor, 1971), and females are capable of producing up to 12 clutches (Haderlie et al., 1980) of 15-30 nauplii each (Powlik et al., 1997). Thus it is possible that reduced fecundity during heat waves was not substantial enough to impact longer-term abundances. This was observed in *Drosophila melanogaster* by (Dillon et al., 2007), where $R_0$ and $r$ calculated from life tables were not affected by transient temperature exposures despite differences in fecundity during the temperature treatment phase.

2. Heat wave tolerant individuals may contribute a disproportionate amount of offspring to the population. Kelly et al. (2013) found that selecting for high temperature tolerance in lines of *T. californicus*, also selected for larger adult body size and higher fecundity.

3. Temperature and heat stress may have opposing effects on different life-history traits that both contribute to individual and population-fitness. While extreme temperatures may delay development (Roberts et al., 2012), *T. californicus* juveniles

exhibit enhanced heat tolerance (Tangwancharoen & Burton, 2014), and the heat waves likely accelerated development, shortening generation time and increasing intrinsic rates of increase. Additionally, heat exposures can lengthen individual life-span Hercus et al., 2003).

### 3.5.1 General patterns of population growth

The general pattern of population growth over the course of the experiment was influenced by temperature. At a constant 32°C, laboratory populations went extinct in less than one week. The constant 20°C populations appeared to grow logistically, with population abundance hovering around a carrying capacity after a general increasing phase. The majority of the heat wave populations, however, exhibited pronounced declines in abundance after the increasing phase that appears to have overshot a carrying capacity. This trend is reflected in the different coefficient of variation values, which generally show CV increasing with temperature.

Time lags and non-linearity in density-dependent responses can lead to population overshooting carrying capacity, followed by oscillations of abundance around carrying capacity (Belovsky et al., 1999). Short-term resource inputs can contribute to this non-linearity, leading to qualitatively different short-term and long-term population responses (Holt, 2008). In ectotherms, as development time accelerates with increasing temperature (Gillooly et al., 2002), temperature may function as a 'resource' and higher heat accumulation during the heat wave treatments may have facilitated faster population growth. It is unclear how much heat wave treatments overshot carrying capacity, and if the resulting declines in abundance would continue until abundance stayed at low levels or if the declines in abundance solely reflect greater amplitude in the fluctuations. Halbach (1977) observed amplitude of population abundance fluctuations increase with increasing temperatures. However, my abundance time-series is not long enough to differentiate between large periodic fluctuations or a population collapse.

Additionally, it is possible that long-term patterns were influenced by experimental artifacts beyond the effects of temperature and life-history. Water quality issues in my microcosms due to the build-up of metabolic waste products or declines in oxygen concentration may have had an effect on individual reproduction and population growth. However, these effects were most likely minimized by the weekly water changes, where approximately 50% of the microcosm water was replaced with fresh, sterilized water. This volume and frequency of water changes has been used to maintain other laboratory cultures of *Tigriopus californicus* (Kelly et al., 2011).

### 3.5.2 Heat waves and long-term population persistence

Heat waves can dramatically impact population persistence through widespread mortality (Tsuchiya, 1983; Loeschcke et al., 1994; Garrabou et al., 2009; McKechnie & Wolf, 2010; Kelly et al., 2013). Additionally, the carryover effects of sublethal exposure to temperature extremes may also impact population persistence, and incorporating these sublethal effects can improve our understanding of how heat waves compromise long-term population persistence, particularly in systems that regularly encounter extreme temperatures (Williams et al., 2016). Additionally, heat stress can amplify the effect of other stressors, further impacting individual and population-level consequences. Heat stress has been shown to reduce immunocompetence (Roth et. al., 2010; Dittmar et. al., 2014), competitive ability (Willett, 2010; Olabarria et al., 2016), interact non-additively with a wide variety of abiotic stressors (Harley et al., 2006), and influence the strength of species interactions (Sanford, 2002). Thus, the long-term consequences of heat waves on population persistence does not solely depend upon the immediate lethal and sublethal effects of heat stress, but also on the net effect of temperature on a variety of life-history traits. Additionally, evaluating the impact of heat waves in the context of multiple stressors will increase the ecological realism of experimental studies.

Here, I tested a critical prediction of bioenergetics models that declines in individual fitness over short periods of time scale to populations over a longer period of time. My data do not fully support this prediction. I found that peak and final abundances over a longer period of time could not be predicted from short-term population growth rates. Temperature influences a number of life-history traits, which may influence population dynamics over different time scales. For example, declines in fecundity may reduce population abundance in the short-term; however, shortened generation times may result in a net benefit to long-term population growth that would not necessarily be apparent in the short-term. Predicting the effects of heat waves and temperature stress on long-term population persistence from bioenergetics models may be problematic, and the effect of temperature on a variety of life-history traits needs to be considered.

## 3.6 Tables and figures

### 3.6.1 Tables

**Table 3.1.** Variation in average peak and final abundances, population growth rates (pgr) during the heat wave time periods, and the coefficient of variation (CV) of abundnace in laboratory populations of *Tigriopus californicus* from Botany Bay in southwestern Vancouver Island, British Columbia. Laboratory populations were divided into eight experimental groups (six replicates each). Two experimental groups were held at a constant 20°C and 32°C, while six experimental groups were subjected to one of six heat wave treatments varying in daily maximum temperature (26°C or 32°C) and number of consecutive heat wave days (1, 2 or 7 days). Mean values are shown with standard deviation in parentheses.

| Temperature | Duration | Peak abundance | Final abundance | Heat wave 1: pgr | Heat wave 2: pgr | CV |
|---|---|---|---|---|---|---|
| 20°C | constant | 643.3<br>(184.4) | 329.3<br>(125.9) | 0.57<br>(1.05) | 0.16<br>(0.49) | 0.689<br>(0.106) |
| 32°C | constant | 96.7<br>(63.8) | 0<br>(0) | n/a | n/a | 1.69<br>(0.157) |
| 26°C | 1 day | 906.7<br>(208.5) | 216<br>(36.6) | 0.51<br>(1.14) | -0.57<br>(0.58) | 0.943<br>(0.116) |
| 26°C | 2 days | 426.7<br>(102.5) | 160<br>(32.4) | 0.46<br>(1.04) | -0.35<br>(0.72) | 0.775<br>(0.157) |
| 26°C | 7 days | 633.3<br>(171.0) | 194.7<br>(27.9) | 0.49<br>(1.33) | -0.02<br>(0.47) | 0.741<br>(0.114) |
| 32°C | 1 day | 620.0<br>(193.1) | 148<br>(37.8) | 0.45<br>(1.39) | -0.21<br>(0.53) | 0.787<br>(0.105) |
| 32°C | 2 days | 633.3<br>(281.3) | 140<br>(66.2) | 0.42<br>(1.14) | -0.43<br>(0.58) | 0.903<br>(0.174) |
| 32°C | 7 days | 346.7<br>(304.3) | 77.3<br>(48.8) | 0.34<br>(1.26) | 0.02<br>(0.44) | 0.838<br>(0.258) |

**Table 3.2.** ANOVA results for effect of heat wave intensity and duration of the coefficient of variation (CV full) in abundance, the sole effect of temperature on the coefficient of variation in abundance (CV red), and population growth rates during the two heat waves (hw.1 pgr and hw.2 pgr). Significant values are **bolded** with alpha set at 0.05.

| Response | Predictor | DF | F value | P value | Adj-$R^2$ |
|---|---|---|---|---|---|
| CV (full) | Temp | 1 | 0.18 | 0.67 | 0.06 |
| “ | Duration | 2 | 0.67 | 0.52 | |
| “ | Interaction | 2 | 2.75 | 0.08 | |
| CV (red) | Temp | 2 | 4.95 | **0.01** | 0.14 |
| hw.1 pgr | Temp | 1 | 0.30 | 0.59 | 0.32 |
| “ | Duration | 2 | 3.86 | **0.03** | |
| “ | Interaction | 2 | 6.57 | **0.004** | |
| hw.2 pgr | Temp | 1 | 0.25 | 0.62 | 0 |
| “ | Duration | 2 | 1.81 | 0.18 | |
| “ | Temp*Dur | 2 | 0.50 | 0.61 | |

**Table 3.3** Generalize linear model (GLM) results for the effect of heat wave intensity and duration on peak and final abundance in laboratory populations of *Tigriopus californicus*. Due to differences in the relationship between the mean and variance of the two abundance response variables, a quasi-poisson GLM and negative binomial GLM were used to model the different abundance count data. The z ratio is reported for the quasi-poisson GLM, and the t ratio is reported for the negative binomial GLM. DF = degrees of freedom. Significant values are **bolded** with alpha set at 0.05.

| GLM | Response | Predictor | DF | z-value | t-value | p-value |
|---|---|---|---|---|---|---|
| Quasi-poisson | Peak abund. | 32°C | 1 | -1.58 | n/a | 0.115 |
| | | 2-day Duration | 2 | -3.12 | n/a | **0.002** |
| | | 7-day Duration | 2 | -1.49 | n/a | 0.137 |
| | | 32°C/2-day | 2 | 2.27 | n/a | **0.023** |
| | | 32°C/7-day | 2 | -0.65 | n/a | 0.515 |
| Negative binomial | Final abund. | 32°C | 1 | n/a | -1.83 | 0.077 |
| | | 2-day Duration | 2 | n/a | -3.22 | **0.003** |
| | | 7-day Duration | 2 | n/a | -1.74 | 0.092 |
| | | 32°C/2-day | 2 | n/a | 2.39 | **0.023** |
| | | 32°C/7-day | 2 | n/a | -0.66 | 0.515 |

## Figures

**Figure 3.1.** Time-line of events in the 100 day population growth experiment. Forty-eight laboratory populations of *Tigriopus californicus* from Botany Bay, British Columbia were established with 10 gravid females each and held at 20°C for the first 24 days. The six sampling events are denoted with each arrow. In the time-periods between sample day 1 and 2 and sample day 4 and 5, six experimental groups were subjected to one of six heat wave treatments varying in intensity (daily maximum temperature: 26°C or 32°C) and heat wave duration (1, 2 or 7 consecutive days). Abundance was estimated at each sample day by removing 5% of the water volume and counting copepodids and adults, which were returned alive.

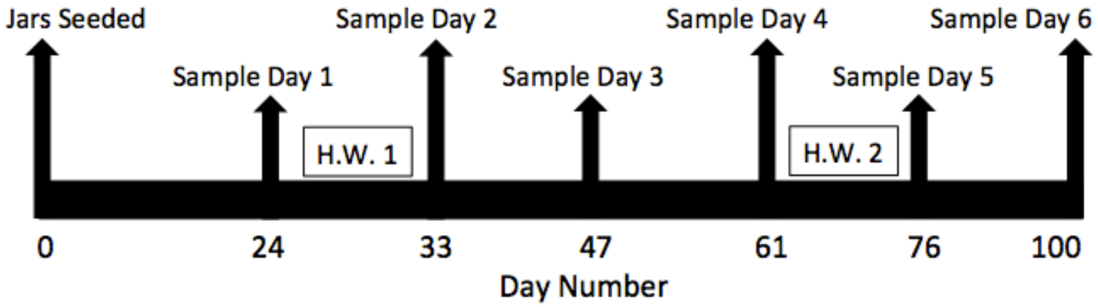


**Figure 3.2.** Population growth rates for the six different time periods between sampling days in laboratory populations of *Tigriopus californicus* from Botany Bay, Vancouver Island, British Columbia. Temperature treatments include a constant 20°C group (20_cons), and six heat wave treatments varying in heat wave intensity (26°C or 32°C) and heat wave duration (1, 2, or 7 days). The heat wave treatments occurred during the two heat wave time periods (denoted heat.wave_1 and heat.wave_2), whereas during the other time periods all laboratory populations were held at a constant 20°C. The horizontal line at zero denotes the transition between negative and positive population growth.

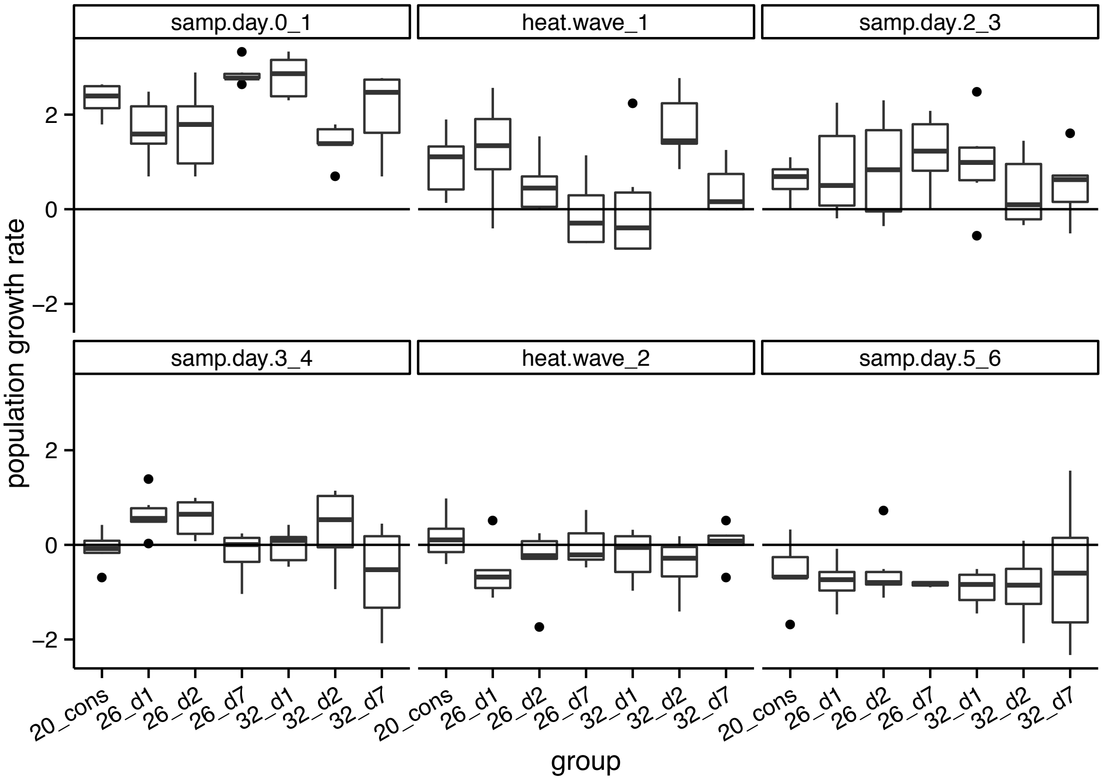


**Figure 3.3.** Peak and final abundances representing the longer-term population dynamics for the eight experimental treatments. The abundances of forty-eight laboratory populations of *Tigriopus californicus* were monitored over 100 days. Peak abundances occurred approximately 50-70 days into the experiment. The eight experimental treatments include a constant 20°C and constant 32°C (20_cons, 32_cons) and six heat wave treatments, corresponding to a daily maximum temperature of 26°C or 32°C and a heat wave duration of 1, 2, or 7 days (d1, d2, and d7, respectively).

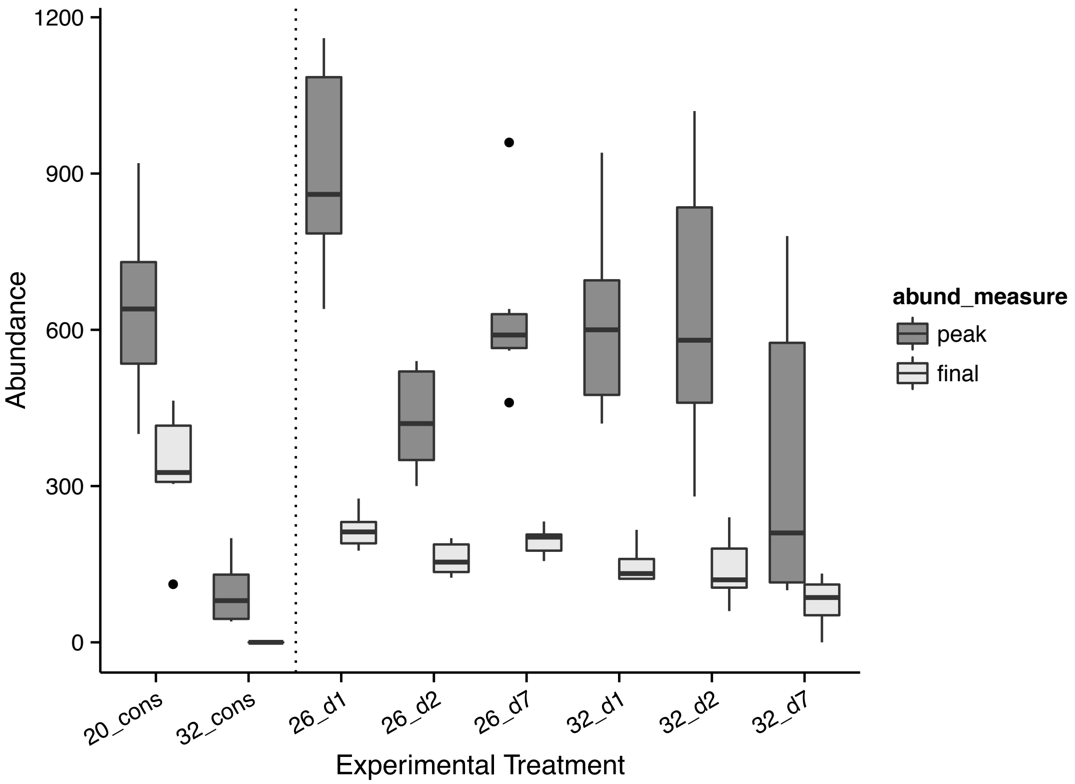
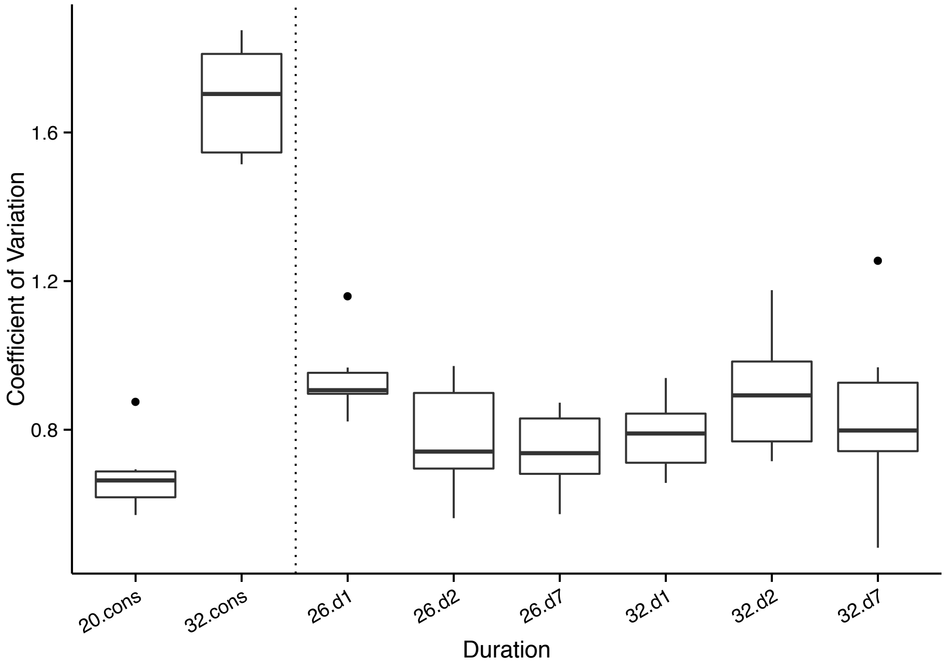


**Figure 3.4** Coefficient of variation (CV) in abundances across eight experimental treatments. The eight experimental treatments include a constant 20°C and constant 32°C (20_cons, 32_cons) and six heat wave treatments, corresponding to a daily maximum temperature of 26°C or 32°C and a heat wave duration of 1, 2, or 7 days (d1, d2, and d7, respectively). The CV did not very across the six heat wave treatments but were qualitatively different from the constant 20°C treatment, where population growth approximated logistic growth, and the constant 32°C treatment that went extinct early in the experiment.



# CHAPTER 4

## Prior heat accumulation reduces survival during subsequent experimental heat waves

### 4.1 Summary

Heat waves are expected to increase in frequency and duration as climate change progresses. Repeated exposure to thermal stress events over a short period of time may affect population dynamics and even population persistence. Understanding whether recent thermal history bolsters or hinders demographic responses such as survival or growth during heat waves is crucial to predicting population persistence in the face of climate change. Here, I tested whether populations of the splash pool copepod, *Tigriopus californicus*, that differ in their recent thermal history experienced differential mortality following an experimental heat wave. I found differences in survivorship correlated with differences in thermal history. Among multiple metrics of thermal history, daily degree-hours (a measure of heat accumulation) best explained heat wave survival, such that higher number of degree-hours correlated with reduced heat wave survival. This finding is consistent with the hypothesis that repeated exposure to sublethal temperatures reduces thermal tolerance. Increasing heat wave intensity and duration, or warmer conditions that increase heat accumulation followed by heat waves may negatively impact population persistence by exacerbating the demographic effects of heat waves.

## 4.2 Introduction

Extreme weather events may strongly affect populations due to the large magnitude of change over short periods of time (Parmesan et al., 2000; Jentsch et al., 2007; Helmuth et al., 2014). Periods of extremely hot weather ("heat waves") are predicted to increase in frequency and duration as climate changes (Frich et al., 2002; Meehl & Tebaldi, 2004). Understanding how heat waves affect population persistence is critical for projecting ecological and evolutionary responses to changing weather patterns associated with climate change. This entails linking the physiology of heat responses to demographic processes that influence population growth and adaptation.

Predicting the effect of heat waves on individual survival and performance, may require more than understanding physiological stress responses to acute or short-term temperature conditions associated with the heat wave event, such as heat wave intensity (how hot did it get) and duration (how long was the heat wave). Heat wave survival may reflect thermal experiences prior to the heat wave event itself (Huber et al., 2010). For example, exposure to sublethal but physiologically stressful temperatures may increase thermal tolerance through mechanisms associated with heat "hardening" or acclimatization (Bowler, 2005; Loeschcke & Sørensen, 2005). In this case, exposure to sublethal temperature stress before a heat wave would be expected to increase survival during and following the heat wave. In contrast, thermally stressful exposures may lower thermal tolerance due to energetic constraints associated with declines in aerobic scope, the amount of energy available beyond the maintenance of basal metabolic costs, under physiologically stressful conditions (Coma et al., 2009; Pörtner, 2010; Sokolova et al., 2012). In this context, heat waves following a series of thermally stressful events would, therefore, be expected to cause higher mortality than heat waves following benign conditions.

The body temperature of ectotherms influences both organismal and biochemical traits that underlie thermal tolerance. As such, an individual's time-course of body temperatures (thermal history) shapes its tolerance to future heat stress events (Ward & Stanford, 1982; Huey & Kingsolver, 1989; Angilletta et al., 2002; Terblanche et al., 2006; Schulte et al., 2011; Dowd et al., 2015; Sinclair et al., 2016). Variation in the physiological responses to different thermal histories may explain why thermal tolerance increases under one set of thermal exposures and declines under another. For example, short exposures to stressful temperatures that initiate the heat shock response can protect cells against subsequent exposures to high temperatures because high concentrations of heat shock proteins are already present (Feder & Hofmann, 1999). Longer-term temperature acclimatization may involve changes at many levels of biological organization, such as biochemical changes (e.g., metabolic reorganization) and structural changes (e.g., changes in cell membrane fluidity) (Somero, 2002). In contrast, exposure to high temperatures that reduce aerobic scope may diminish energy reserves via impacts on energy allocation and production, especially when these temperatures experienced over an extended exposure duration (Pörtner, 2010; Sokolova et al., 2012). These impacts may reduce the efficacy of physiological stress responses (Hand & Hardewig, 1996) and reduce thermal tolerance.

In this study, I investigate how recent thermal history influences mortality during experimental heat waves in the splash pool copepod, *Tigriopus californicus*. *Tigriopus californicus* is fully aquatic and exhibits restricted thermoregulatory behaviours and low thermal inertia (i.e., the rate at which an individual reaches equilibrium body temperature after a change in environmental temperature), making it reasonable to equate *T. californicus* body temperature with the surrounding water temperature (Huey et al., 1999; Fitzgerald & Nelson, 2011). To characterize splash pool thermal history, I used hourly water temperature measurements to estimate average daily maximum temperatures, average daily mean temperatures, average daily minimum temperatures, average daily degree-hours (a time-based integral of daily heat accumulation), as well as each splash pool's standard deviation in daily temperature, the frequency of days with temperature exceeding 26°C and days exceeding 32°C, and the absolute maximum temperature.

I predicted that heat wave survival would decrease with increasing heat wave intensity, but that heat wave survival would vary regionally, and that populations exhibiting higher heat wave survival would be correlated with recent exposure to high temperatures in the field.

## 4.3 Materials and methods

### 4.3.1 Field temperatures and animal collections

I measured splash pool water temperature during the early summer in four regions along the southern coast of Vancouver Island, British Columbia (Figure 4.1). This area spans a regional thermal cline: cooler temperatures are found towards the oceanic end of Straight of Juan de Fuca and temperatures progressively warm as one moves into the eastern parts of the Salish Sea (Harley, 2011). Ibutton temperature loggers (Embedded Data Systems, LLC) were glued using epoxy into 38 splash pools nested within four regions along the thermal cline (N = 11, 7, 10 and 10 for Botany Bay, Fishboat Bay, Saxe Point Park and Neck Point Park, respectively). These were deployed within three days of each other in mid-May 2016 and recorded hourly temperatures for the following 13 weeks. However, I only used temperature data from the final seven days of field conditions to characterize splash pool thermal history. It is likely that over the course of the summer, *T. californicus* populations underwent extinction and recolonization metapopulation dynamics consistent with the evaporation and re-filling of splash pools (Johnson, 2001). As such, it is unlikely that temperature data from the earlier part of the summer matched conditions experienced by individuals collected towards the end of summer. Furthermore, thermal regimes in coastal localities tend to be temporally autocorrelated, leading to a higher probability that the variance in temperature is distributed within extended periods of above or below average conditions (Vasseur & Yodzis, 2004). Thus, even in splash pools that did not recently experience extinction and recolonization, individuals are less likely to be affected by temperature conditions from earlier in the summer relative to the more recent conditions they have experienced.

*Tigriopus californicus* individuals were collected from every splash pool that contained populations with high enough densities for collection. This excluded 19 splash pools, which had completely evaporated or contained too few individuals to collect. Thus, *T. californicus* individuals were collected from 19 out of the 38 splash pools (N = 6, 2, 5, and 6 for Botany Bay, Fishboat Bay, Saxe Point Park and Neck Point Park, respectively). I transported individuals back to The University of British Columbia in 120 mL sample cups. Copepods were fed ground *Spirulina* algae (Max Pro brand fish food), and held at room temperature (20-21°C) for 3-4 days before being subjected to experimental heat waves.

### 4.3.2 Experimental heat waves

To test for an effect of thermal history on survival during an experimental heat wave I subjected individuals to one of two heat wave treatments: daily maximum temperature reaching 26°C or 32°C. The experimental heat waves reflect temperature profiles observed in the field (see Chapter 2). The daily temperature profile of simulated heat waves includes a six-hour increasing phase from the minimum temperature (20°C), one hour at the maximum temperature (26°C or 32°C), and a six-hour decreasing phase back to the minimum temperature. The thermal environments in the lab were created with a Panasonic M1R-154 programmable incubator.

From each source population, 60-104 individuals (mean: 84.5 individuals) were subjected to the 26°C or 32°C heat wave, resulting in survival data for 3,381 individuals. Approximately 3-5 individuals were placed in 6 mL wells of a 12-well plate filled with 4-5 mL of sterilized seawater (28-32ppt). Each well received ~0.05 mg of ground *Spirulina* algae. The copepods were kept at room temperature overnight, and then subjected to the first day of the heat wave. Each individual copepod was inspected under a Leica dissecting scope at day 1, 2, 3, 4, 6, and 7 to assess mortality.

### 4.3.3 Statistical analyses

*Characterizing splash pool thermal history*

I used temperature data from the last seven days of field conditions to construct thermal history profiles for each splash pool. I characterized thermal history with eight temperature variables: average daily maximum temperature, average daily mean temperature, average daily minimum temperature, average daily degree-hours, average daily temperature standard deviation, frequency of days above 26°C, frequency of days above 32°C, and weekly maximum temperature. Daily degree-hours is defined as:

$$dh = \sum (T > v) * time$$

where $dh$ is the degree-hours, $T$ is temperature, and $v$ is a temperature threshold. I used a temperature threshold of 20°C (corresponding to an approximate average daily minimum temperature), where a temperature of 24°C for one hour would contribute +4 degree-hours, 28°C for one hour contributes +8 degree-hours, etc. All temperature variable data were converted to z scores: $z = (X - \mu)/\sigma)$ to set equal means and variances between temperature variables.

To visualize how splash pool thermal history is described by the eight temperature variables, I used principal coordinates analysis (PCA) in the *vegan* package (Wagner, 2015) in R (R Core Team, 2015). I used ANOVA to test for regional differences in each of the eight thermal history temperature variables.

*Survivorship analysis*

I estimated daily heat wave survival probabilities using the standard non-parametric Kaplan-Meier (KM) survivorship function (Kaplan & Meier, 1958) described in Chapter 2. Censoring events occurred on the day before the start of the heat wave and on days 1, 2, 3, 4, 6 and 7 of the heat wave. I tested for regional differences in survival curves with the Gehan-Breslow (modified Wilcoxon) test and a log-rank test. These tests were performed with the *survMisc* package (Dardis, 2015) in R.

*Effect of thermal history and region on experimental heat wave survival*

I constructed 59 mixed-effects models to test for the effect of thermal history on surviving the full heat wave (Day 7 survival probability). These models include the predictors: heat wave treatment (H: 26°C or 32°C), region (R), and different combinations of eight temperature variables (T) as main effects, and individual splash pool $i$ as a random effect (allowing the intercept to vary for each splash pool). The mixed effects models were fit using the *lme4* package (Bates et al., 2014) in R, and heat wave survivorship values were arcsine transformed to improve normality. Each model took the form:

$$Y_i \approx \beta_{oi} + \beta_1 H * \beta_2 R + \beta_3 T_1 + \beta_4 T_2 + \beta_5 T_3 + \beta_6 T_4 + \beta_7 T_5 + \beta_8 T_6 + \beta_9 T_7 + \beta_{10} T_8 \quad \textit{(Eqn 1)}$$

$$\beta_{0i} = \gamma_0 + \gamma_1 * pool + \varepsilon \quad \textit{(Eqn 2)}$$

Multicollinearity between a subset of temperature variables was expected. To control for correlations between predictor variables I calculated variance inflation factors (VIFs) with the *usdm* package (Naimi, 2013) in R. The VIF scores for the full model including all eight temperature variables were high for: average daily maximum (avg.max), mean (avg.mean), minimum temperatures (avg.min), and average daily degree-hours (dh). To minimize collinearity between predictor variables within the same model, each of these four temperature variables were separated into four models. Each of these four models included the predictors: heat wave treatment, region, standard deviation, frequency of days above 26°C, frequency of days above 32°C, weekly maximum temperature, and either average daily maximum, mean, minimum temperature, or average daily degree-hours. I then created four different subsets of models with different combinations of the temperature variables (Table 4.1).

I used two statistical frameworks to infer which predictors best explained variation in heat wave survival within each of the four sets of models (Table 4.1). I first used Akaike Information Criterion (AIC; Akaike, 1974) model selection to compare models with different predictors and identify the most informative set of predictors. I used a change in AICc (AIC corrected for sample size) values (δAICc) of 2.0 to identify the top performing model(s) (Burnham & Anderson, 2002), and 95% confidence intervals to identify significant parameters. After identifying a suite of top performing models, I calculated marginal $R^2$ values for each model according to Nakagawa & Schielzeth (2013) with the *MuMIn* package (Bartoń, 2015) in R.

To test the hypothesis that geographic region in addition to thermal history predicted heat wave survival, I constructed an additional set of the 59 models described above, except without the ‘region’ predictor term. I used AICc to find the top performing models of the total 118 mixed-effects models.

## 4.4 Results

### 4.4.1 Regional differences in thermal history

Across all sites, only three splash pools experienced water temperatures resembling the experimental 32°C heat wave in seven days before the experiment. During the last 28 days of field conditions, two pools in Neck Point Park experienced 13 consecutive days where daily maximum temperature exceeded 30°C, and one pool in Fishboat Bay experienced eleven consecutive days with temperatures exceeding 30°C. Of those three splash pools, one pool in Neck Point Park and one pool in Fishboat Bay experienced more than seven consecutive days where temperatures exceeded 32°C. The nineteen splash pools all experienced daily maximum temperatures above 26°C. However, the number of days out of the previous 28 that exceeded this temperature ranged from 9-25.

Regional differences in thermal history during the last seven days of field conditions were detected for five of the eight temperature variables (Figure 4.2, Table 4.2). In the PCA, two principal coordinates explained 85% of the variation in thermal history. Generally, high temperatures underlie variation along the first principal component, whereas variation along the second component was driven by variation in temperature (standard deviation) and an opposing effect of daily minimum temperature (i.e., high daily minimum temperatures reduced daily standard deviation in temperature) (Figure 4.3).

### 4.4.2 Regional differences in heat wave survivorship

Populations from different regions differed in survival during the heat waves, although these differences depended on the intensity of the heat wave. During the 26°C heat wave, regional differences did not become apparent until five days into the heat wave, when survivorship for Neck Point Park and Saxe Point Park declined relative to Botany Bay and Fishboat Bay (Gehan-Breslow: $\chi^2 = 97.5$, d.f. = 3, $p = < 0.0001$; Log-rank: $\chi^2 = 99.0$, d.f. = 3, $p = < 0.0001$) (Figure 4.4a). During the 32°C heat wave Neck Point Park populations consistently exhibited lower survivorship (Gehan-Breslow: $\chi^2 =$

274.1, d.f. = 3, p = < 0.0001; Log-rank: $\chi^2 = 269.5$, d.f. = 3, p = < 0.0001) than Botany Bay, Fishboat Bay and Saxe Point Park (Figure 4.4b). Intraregional variation in heat wave survival differed considerably between regions, and increased for the 32°C heat wave. For the 26°C heat wave, the range (highest population survival rate minus lowest population survival rate) of final survivorship probabilities for Neck Point Park, Saxe Point Park, Botany Bay and Fishboat Bay was 0.80, 0.80, 0.14 and 0.01, respectively. For the 32°C heat wave, the range of final survivorship probabilities for Neck Point Park, Saxe Point Park, Botany Bay and Fishboat Bay was 0.82, 0.74, 0.45 and 0.14, respectively (Appendix B).

### 4.4.3 Contrasting thermal history and regional effects on heat wave survival

Thermal history had a significant effect on subsequent heat wave survival. Amongst the eight thermal history temperature variables, average daily degree-hours received the strongest support as a negative driver of survivorship (Figure 4.5; t-value = -5.79, p-value < 0.0001). Out of the 118 models compared, the top models are all from the daily degree-hour model subsets, and the top two models included daily degree-hours (dh $R^2$: 0.63 vs. 0.54, 0.53, 0.44 for min, mean, and max temperature; Table 4.3). Despite significant regional differences in heat wave survival, ‘region’ beyond the effect of daily degree-hours was not supported as a predictor of survival (Table 4.3), suggesting that thermal history alone was sufficient to predict heat wave survival across regions. There was less support for the alternative hypothesis that higher average daily maximum temperature would have a positive effect on heat wave survival. Additionally, the result that daily degree-hours and overall variation in temperature had the strongest support for driving heat survival holds across thermal history calculated from temperature data over the previous three, fourteen, and twenty-eight days of field conditions as well (Appendix C).

## 4.5 Discussion

Heat waves affect populations through mortality and sublethal stress (McKechnie & Wolf, 2009; Higgins et al., 2015). However, populations from different thermal environments may not respond uniformly to thermal stress. In this study, I investigated regional differences in experimental heat wave survival, and used field temperature data to evaluate how different aspects of thermal history may be more or less informative for understanding heat wave survival. Unsurprisingly, survival declined with increasing heat wave intensity. While I detected overall regional differences in heat wave survival, there were considerable intraregional differences in survival consistent with differences in thermal history. As such, it appears individual splash pool thermal history alone was sufficient to explain variation in heat wave survival, and larger-scale regional factors were not needed. Among a suite of eight splash pool-specific thermal history characteristics, average daily degree-hours consistently explained a higher amount of variation in heat wave survival than the other temperature variables.

Decreasing survival during heat waves, independent of regional context, is consistent with the hypothesis that accumulated exposure to sublethal temperatures can hinder thermal tolerance via energetic constraints. Accumulated exposure to sublethal temperature can reduce thermal tolerance via impacts on energy balance that occur as aerobic scope declines with increasing temperature (Pörtner, 2010; Sokolova et al., 2012). In this study, potential short-term acclimatization or heat hardening exposures, which may increase thermal tolerance through structural changes of cellular components and induction of heat shock proteins (Somero, 2002), were not sufficient to minimize mortality during the extended seven day heat waves.

Thermal time can have persistent effects on population demography in a number of different ways not captured by estimates of environmental mean, maximum, and minimum temperatures (Sheldon & Dillon, 2016). These latter statistical summaries may not accurately represent the time course of body temperature (Southward, 1958; Helmuth & Hofmann, 2001), whereas thermal time appears to capture a more physiologically meaningful estimate of individual body temperatures (Helmuth et al., 2010). For example, heat accumulation, estimated by growing degree-days, is an important cue for insect growth and emergence time, and plant development and growth (Parry & Carter, 1985; Schwartz, 2003). Accelerated development time increases the number of generations per growing season and population growth rates (Donahue et al., 2015). However, excessive heat accumulation may impact individual energy reserves and fecundity (Klepsatel et al., 2016; Zhang et al., 2016) or increase heat induced mortality as observed in mussels (White et al., 2015), corals (Gleason & Strong, 1995; Berkelmans, 2002; Middlebrook et al., 2008; Anthony et al., 2009), seagrass (Marbà & Duarte, 2009) and flies (Terblanche et al., 2007), reducing population abundances and population growth rates.

Evolutionary or physiological differences between populations in addition to the effects of thermal history may underlie differences in heat wave survival. High genetic differentiation in *T. californicus* occurs on geographic scales as small as a few kilometers (Burton et al., 1979; Burton, 1986; Edmands, 2001), and there is a potential that local adaptation may underlie variation in thermal tolerance and confound my interpretation of the importance of thermal history. However, despite considerable interpopulation variation in thermal tolerance (Willet, 2010; Kelly et al., 2011), the relative contributions of additive genetic variation (Kelly et al., 2011; Schoville et al., 2012) or plasticity in gene expression (Schoville et al., 2012) that underlie variation in thermal tolerance is less clear. Future studies that incorporate physiological data on energy status, heat stress or other biochemical markers of thermal tolerance would help clarify this potentially confounding effect.

I have shown that differences in experimental heat wave survivorship were correlated with exposure to high degree-hours in the week prior to the heat wave. This is consistent with physiological models (that implicitly incorporate thermal time) linking increasing temperature with declines in aerobic scope to subsequent impacts on energy balance and thermal tolerance. For aquatic species with low thermal inertia, it appears that thermal time may adequately capture the time-course of body temperature, and provide a means to investigate the effect of thermal history on thermal tolerance and mortality in the field.

As climate change persists, heat waves are expected to become more severe and more frequent (Frich et al., 2002; Meehl & Tebaldi, 2004). As such, the extent to which heat waves have the potential to rapidly alter population demography, leading to ecological tipping points where large changes in ecological processes occur over a short period of time (Harley & Paine, 2009; Seifert et al., 2015) is also increasing. While increasing heat wave intensity alone is predicted to increase mortality, populations that more regularly experience high temperatures appear to be more vulnerable to heat waves. Thus, it is the combined effects of a warm recent thermal history and subsequent heat wave intensity that lead to high mortality during heat waves (Denny et al., 2009; Garrabou et al., 2009; Dowd et al., 2015; Roitberg & Mangel, 2016).

My results are consistent with other studies that have examined heat stress in the intertidal and found that mass mortality events caused by heat waves are exacerbated by energetic limitations underlying reduced thermal tolerance due to prior sublethal stress (Denny et al., 2006; Coma et al., 2009). Rather than focussing exclusively on statistical extremes (e.g., Siepielski et al., 2017), studies of heat stress in the field should test specific hypotheses rooted in organismal physiology that incorporate thermal history and sublethal stress. Utilizing statistical summaries of thermal time to better approximate the time-course of body temperature may be one solution for incorporating thermal history into field studies of temperature stress. As climate change accelerates, there is a greater need to integrate mechanistic physiology within the realm of population ecology to bolster our ability to predict population-level responses. Accounting for the effects of thermal history puts predictive physiological models into the realm of population ecology, and may be one fruitful path forward to broaden our understanding of ecological patterns.

## 4.6 Tables and figures

### 4.6.1 Tables

**Table 4.1.** Possible relationships between thermal history and heat wave survival for 19 *Tigriopus californicus* populations across four regions in southern Vancouver Island, British Columbia. A total of 59 candidate mixed-effects models with different combinations of the eight temperature variables were constructed. Due to high variance inflation factor scores for the average daily average daily degree-hours (dh), average daily maximum temperature (avg.max), average daily mean temperature (avg.mean), and average daily minimum temperature (avg.min) variables, these were not evaluated within the same model, but rather separated and used in combination with the other four temperature variables (average daily standard deviation (sd), frequency of days above 26°C (freq.26), frequency of days above 32°C (freq.32), and weekly maximum temperature (weekly.max)), to create four distinct model suites. The full models for each model suite are shown, as well as examples of the reduced models for the dh model suite.

| Model suite | Model subset | Temperature variable predictors |
|---|---|---|
| Degree-hours | Full model | dh, sd, freq.26, freq.32, weekly.max |
| | Reduced model | dh, sd, freq.26, freq.32, |
| | “ | dh, sd, freq.26 |
| | “ | dh, sd |
| | “ | dh, freq.26, freq.32, weekly.max |
| | “ | dh, freq.26, freq.32 |
| | “ | dh, freq.26, weekly.max |
| | “ | dh, freq.32, weekly.max |
| | “ | dh, freq.26 |
| | “ | dh, freq.32 |
| | “ | dh, weekly.max |
| | “ | dh |
| Average max | Full model | avg.max, sd, freq.26, freq.32, weekly.max |
| Average mean | Full model | avg.mean, sd, freq.26, freq.32, weekly.max |
| Average min | Full model | avg.min, sd, freq.26, freq.32, weekly.max |

**Table 4.2.** Differences in thermal regimes of splash pools in four regions (Botany Bay, n = 6; Fishboat Bay n = 2; Saxe Point Park, n = 5; Neck Point Park, n = 6) in southern Vancouver Island, British Columbia. Hourly splash pool water temperature data were used to characterize eight temperature variables (Temp var), compared among regions (n = 4) using ANOVA: average daily degree-hours (dh), standard deviation (sd), frequency of days above 26°C (freq.26), frequency of days above 32°C (freq.32), weekly maximum temperature (weekly.max), average daily maximum temperature (avg.max), average daily mean temperature (avg.mean), average daily minimum temperature (avg.min). Significance codes: < 0.05 = *; < 0.01 = **, < 0.001 = ***, < 0.0001 = ****. Removing the Fishboat Bay (FB) region from the analysis (due to much lower sample size than the other regions) changed the magnitude of the F-values for seven of the eight temperature variables, but did not alter the interpretation. However, removing FB did change the interpretation of regional differences in weekly maximum temperature. The model excluding FB from this analysis is listed in the last row of the table as "weekly.max-2".

| Temp var | DF | F-value | p-value | Adj-$R^2$ |
|---|---|---|---|---|
| dh | 3 | 12.1 | *** | 0.65 |
| avg.max | 3 | 5.6 | ** | 0.43 |
| avg.mean | 3 | 21.0 | **** | 0.77 |
| avg.min | 3 | 17.5 | **** | 0.73 |
| sd | 3 | 2.9 | 0.071 | 0.24 |
| freq.26 | 3 | 3.1 | 0.061 | 0.25 |
| freq.32 | 3 | 2.6 | 0.093 | 0.21 |
| weekly.max | 3 | 3.3 | * | 0.28 |
| weekly.max-2 | 2 | 2.7 | 0.10 | 0.17 |

**Table 4.3.** Experimental heat wave survival in *Tigriopus californicus* declined with increasing heat wave intensity, increasing daily-degree hours, and decreasing standard deviation in splash pool water temperature experienced by that population in the week prior to the experiment. The five top performing models in the AICc analysis are shown, as well as the highest-ranking model that includes average daily minimum, mean, and maximum temperature as predictors of experimental heat wave survival. For each model from the AICc analysis the degrees of freedom (df), log likelihood (loglik), AICc values, δAICc, and Akaike weights (weight) are shown. Significant predictors (95% CI does not overlap zero) are bolded. The marginal $R^2$ values are also shown. Parameters: heat wave treatment (H), region, average daily degree-hours (dh), standard deviation (sd), frequency of days above 26°C (freq.26), frequency of days above 32°C (freq.32), weekly maximum temperature (weekly.max), average daily maximum temperature (avg.max), average daily mean temperature (avg.mean), average daily minimum temperature (avg.min).

| Model Rank | Parameters | df | loglik | AICc | δAICc | weight | $R^2$ |
|---|---|---|---|---|---|---|---|
| 1 | **H, dh, sd, weekly.max** | 7 | 3.72 | 10.3 | --- | 0.285 | 0.63 |
| 2 | **H, dh, sd**, freq.32, **weekly.max** | 8 | 4.88 | 11.2 | 0.90 | 0.182 | 0.67 |
| 3 | **H, region, dh, sd, freq.26, weekly.max** | 11 | 9.58 | 13.0 | 2.69 | 0.074 | 0.76 |
| 4 | **H, dh, sd,** freq.26, **weekly.max** | 8 | 3.92 | 13.1 | 2.81 | 0.070 | 0.64 |
| 5 | **H**, region, **dh, sd, weekly.max** | 10 | 6.92 | 14.3 | 4.00 | 0.039 | 0.71 |
| 11 | **H, avg.min, sd, weekly.max** | 7 | 0.83 | 16.1 | 5.78 | 0.016 | 0.54 |
| 14 | **H, avg.mean, sd, weekly.max** | 7 | 0.63 | 16.5 | 6.18 | 0.013 | 0.53 |
| 22 | **H**, **avg.max**, weekly.max | 6 | -1.56 | 17.9 | 7.56 | 0.007 | 0.44 |

### 4.6.2 Figures

**Figure 4.1.** The four study regions in the southern part of Vancouver Island, British Columbia from which 19 individual splash pool *Tigripous californicus* populations were collected from. The four study regions and number of intraregional populations include Neck Point Park (NP, n = 6), Saxe Point Park (SX, n = 5), Fishboat Bay (FB, n = 2) and Botany Bay (BY, n = 6).

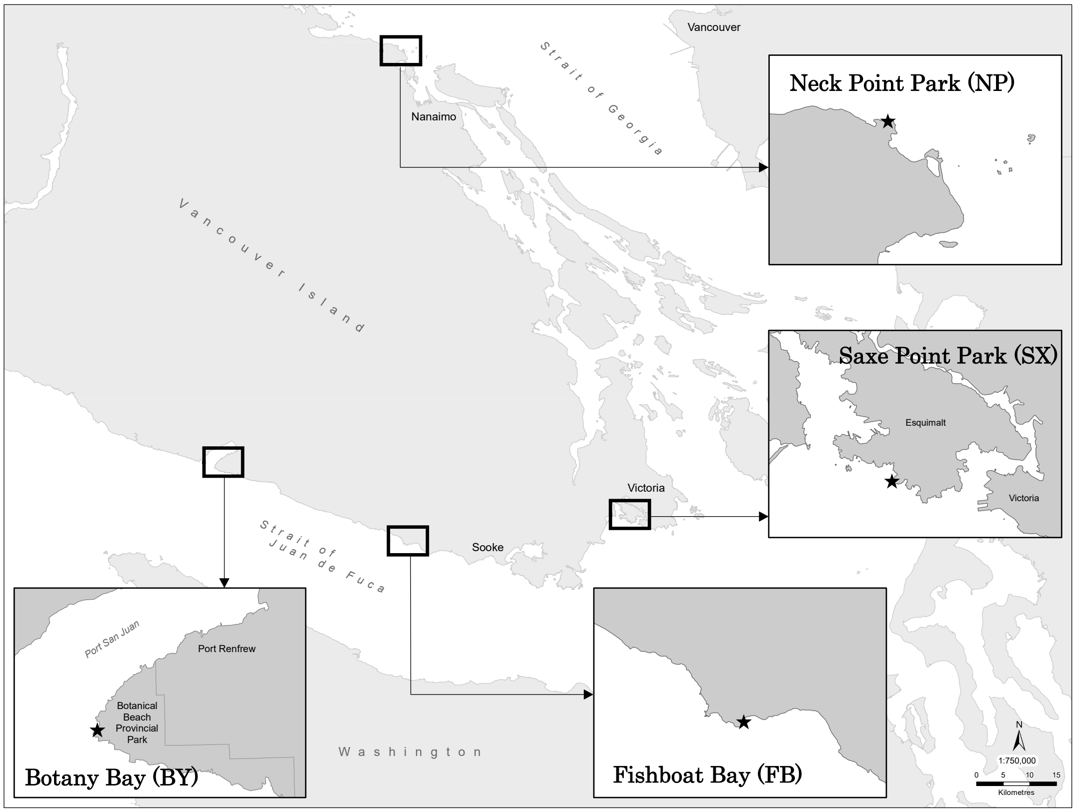


**Figure 4.2.** Regional differences in thermal history among splash pools from southern Vancouver Island, British Columbia. Each splash pool temperature variable is shown as a gray point, and the black 'X' represents the regional means. The temperature variables are shown as converted z-scores. ANOVA shows significant regional differences for average daily maximum (Avg daily max), average daily mean, average daily minimum, average daily degree-hour (Avg deg-hour), and weekly maximum temperature (weekly.max). The four regions and number of splash pools per region include: Botany Bay (BY, n = 6), Fishboat Bay (FB, n = 2), Neck Point Park (NP, n = 6) and Saxe Point Park (SX, n = 5).

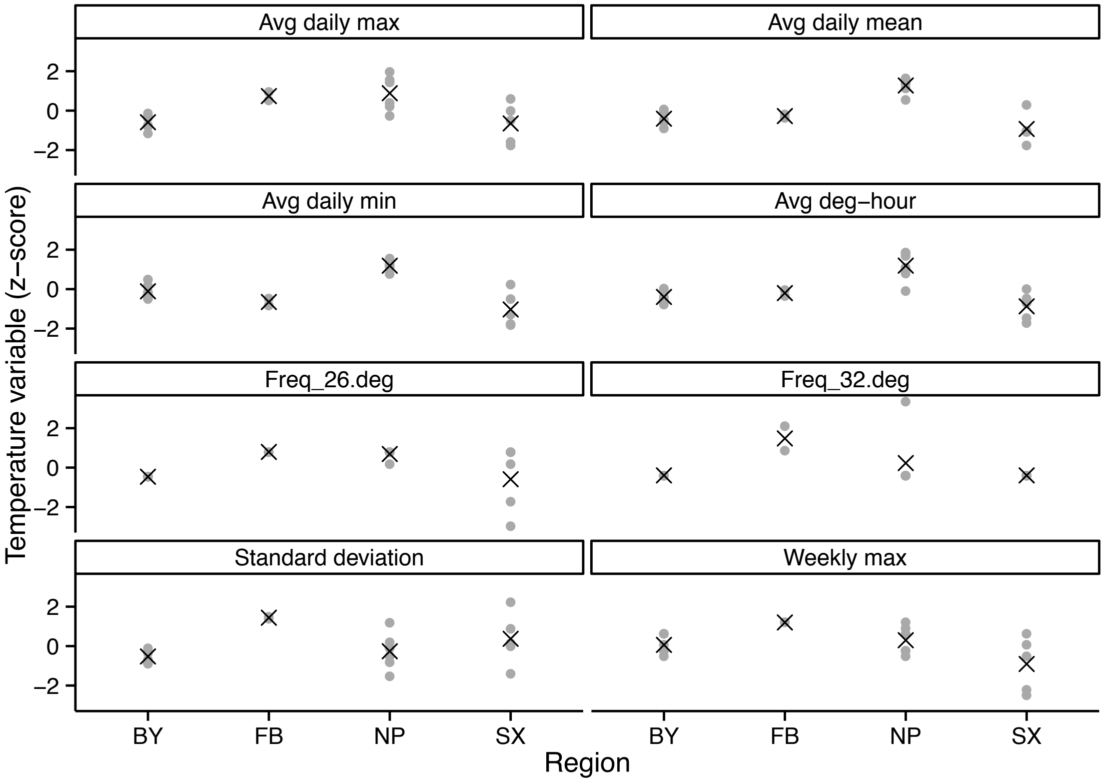


**Figure 4.3.** Correlation among the eight temperature variables used to characterize splash pool thermal history based on a principal components analysis. PC1 explained 60% of variation in the temperature variables, and PC2 explained a further 25%. Generally, PC1 is driven by high temperatures, and PC2 is driven by variation in temperature, which is reduced with higher daily minimum temperatures. Temperature variables are converted to z scores. Standard deviation (sd), frequency of days above 26°C (freq.26), frequency of days above 32°C (freq.32), weekly maximum temperature (week.max), average daily degree-hours (avg.dh), average daily mean temperature (avg.mean), average daily minimum temperature (avg.min), average daily maximum temperature (avg.max).

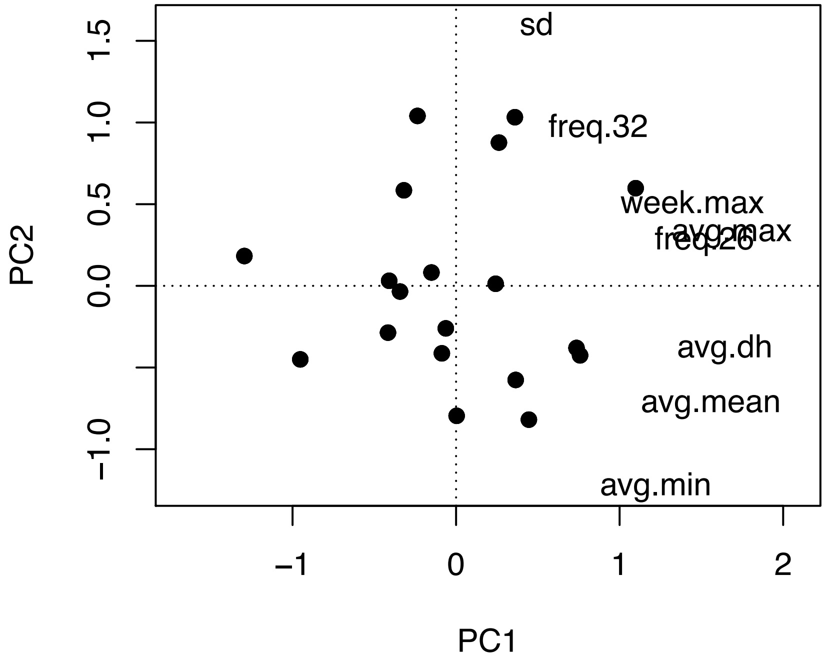
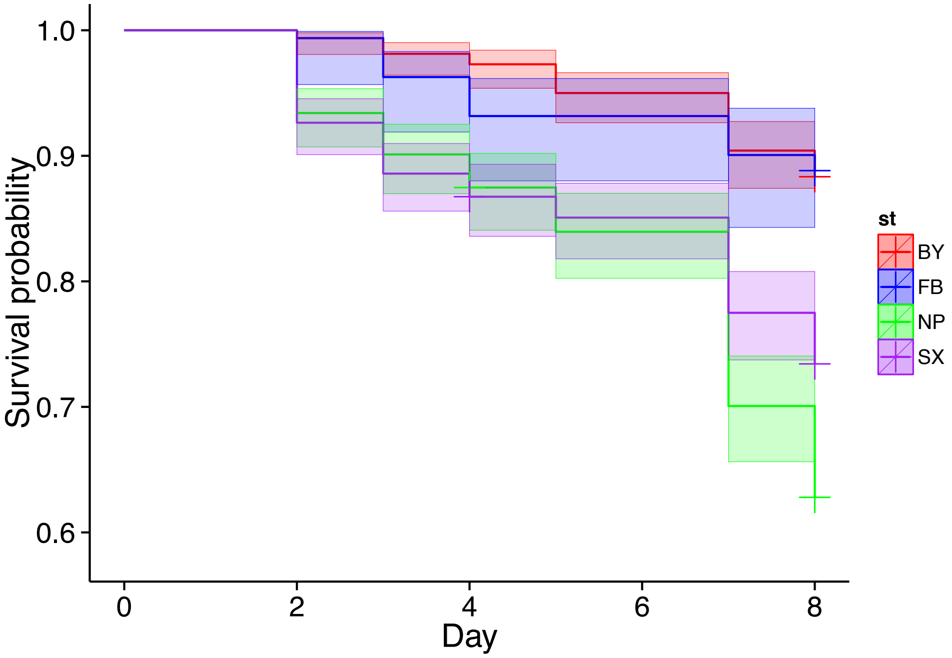


**Figure 4.4.** Regional heat wave survivorship for *Tigriopus californicus* exposed to different heat waves varying in daily maximum temperature. Individuals are pooled across splash pools within regions. The seven-day heat wave begins on day 2 and continues through day 8. Overall survivorship was greater for the 26°C heat wave (*a*) than the 32°C heat wave (*b*). Neck Point Park (NP) and Saxe Point Park (SX) exhibited similar and decreased survivorship relative to Botany Bay (BY) and Fishboat Bay (FB) during the 26°C heat wave, while NP exhibited greatly reduced survivorship during the 32°C heat wave relative to the other regions. Each regional survivorship curve is plotted within its 95% CI (shaded area). Note the differences in the y-axis scale between *a* and *b*.

(*a*)



(*b*)

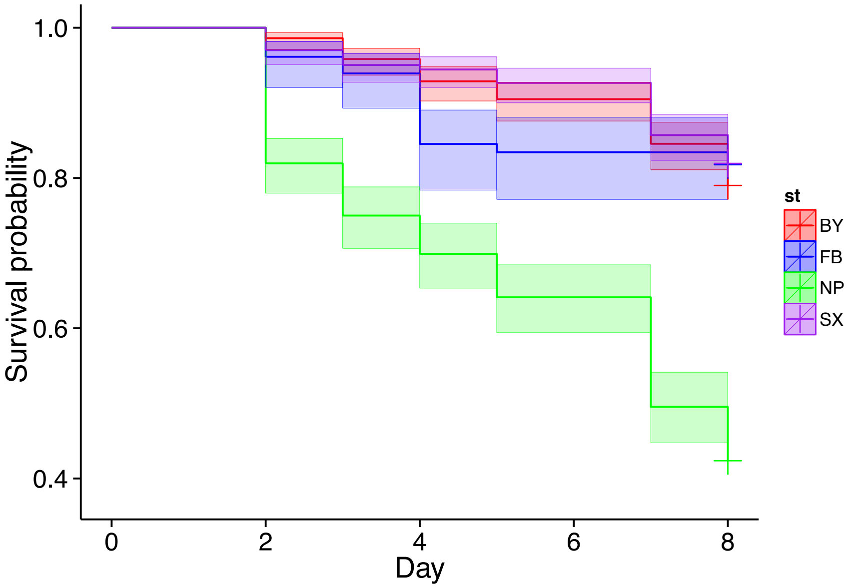
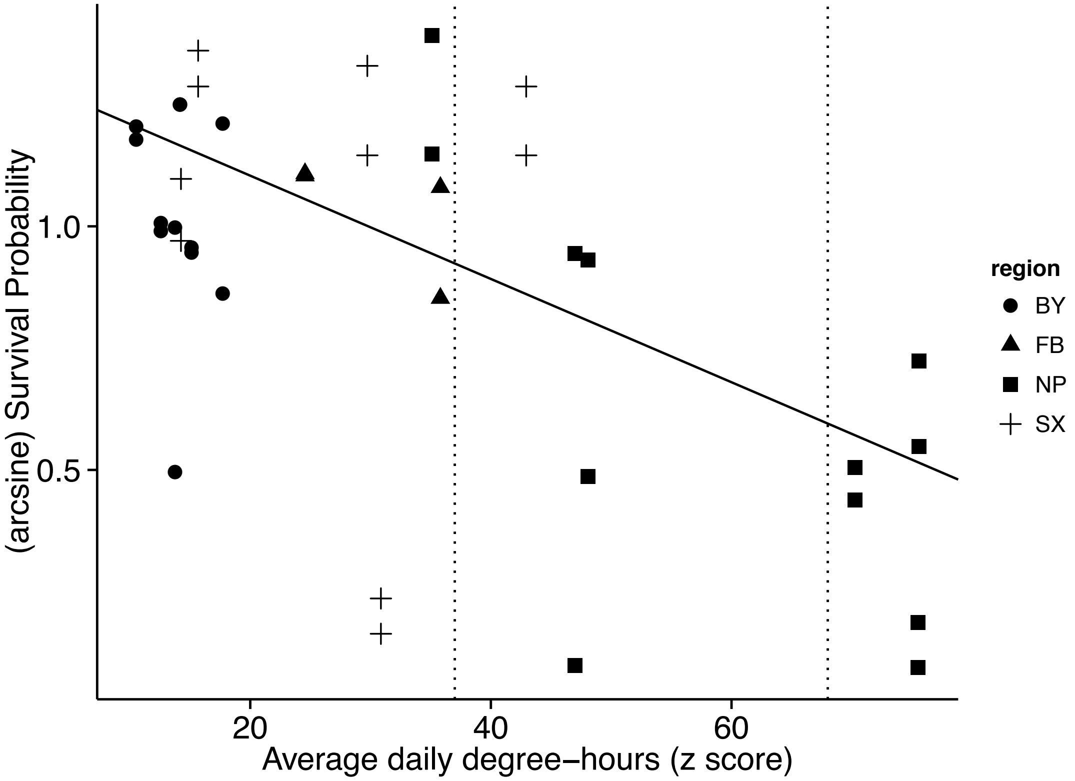


**Figure 4.5.** Heat wave survival declines in *Tigriopus californicus* with higher daily degree-hours recently experienced in the field. Each point represents the average daily degree-hours for each of the 19 splash pools from four regions across southern Vancouver Island, British Columbia. The two vertical dashed lines indicate the daily degree-hours for the 26°C and 32°C experimental heat waves (37 and 68 daily degree-hours, respectively). BY = Botany Bay, NP = Neck Point Park, FB = Fishboat Bay, SX = Saxe Point Park.



# CHAPTER 5

## General discussion

Temperature is perhaps the most fundamental abiotic factor affecting ectotherm performance and fitness, influencing biological processes across all levels of biological organization (Hochachka & Somero, 2002). Less empirical attention has been devoted towards understanding biological responses to variation in temperature than changes in mean temperature (Bozinovic et al., 2011; Folguera et al., 2011; Estay et al., 2013; Vasseur et al., 2014; Buckley & Huey, 2016). This is problematic because many ectotherm performance measures relate nonlinearly to temperature (Denny, 2017), and because thermal tolerance can change with recent thermal history (Schulte et al., 2011). Bioenergetic frameworks act to link mechanistic physiology with impacts on energy balance and subsequent effects on ecological processes. My dissertation focussed on testing predictions from the bioenergetic Energy Limited Tolerance to Stress framework (ELTOS; Sokolova et al., 2012; Sokolova, 2013) in a widespread coastal invertebrate, to relate the effects of heat wave intensity and duration across different levels of biological organization and across spatial scales. I tested how energy production, individual reproductive output, and short-term and long-term population dynamics change with consistent heat wave intensity and duration treatments, as well has how heat wave survival changes with spatial variation in thermal history. I found consistent support for ELTOS predictions on the effect of heat wave intensity and duration on energy production and individual reproductive output. My data, however, do not support the ELTOS prediction that short-term impacts on individual reproduction and population growth rate have persistent effects on longer-term population abundances, and highlight the need for testable hypotheses that integrate different life-history traits that contribute to population-fitness over different time-scales. Additionally, my data indicate that spatial variation and thermal history has a significant effect on heat wave survival, and microsite habitat differences will likely contribute to regional persistence despite the potential for local extirpation.

## 5.1 Major findings

### 5.1.1 Integrating the effect of heat waves across levels of biological organization

High temperatures affect energy balance through changes to energy allocation, energy production, and energy supply. My oxygen consumption rate data (Chapter 2) are consistent with physiological models of temperature and oxygen availability that predict a shift in energy production from high yielding aerobic pathways to less efficient anaerobic metabolism (Pörtner, 2010) as oxygen uptake and transport is limited (Frederich & Pörtner, 2000; Sokolova & Pörtner, 2003). During the experimental heat waves, aerobic metabolic rate during the daily temperature maximum was well below rates predicted by Arrhenius effects under non-stressful conditions. A similar pattern of reduced aerobic respiration at high temperature has been observed in a related species, *Tigriopus brevicornis* (McAllen et al., 1999). The authors suggest that the low rates observed reflect metabolic rate depression, a metabolic adaptation to high temperature that involves a coordinated suppression of ATP demanding processes (Storey & Storey, 1990; Hochachka et al., 1996; Guppy & Withers, 1999).

While my observations are consistent with a quiescent state at these high temperatures (Caceres, 1997), my data can not speak directly to active metabolic rate depression. However, numerous studies have identified metabolic rate depression in organisms from highly fluctuating environments (e.g., the intertidal, deserts, ephemeral water bodies) that regularly experience stressful conditions (Sokolova, 2013). As the temporal frequency of stressful conditions becomes predictable, physiological regulatory changes may evolve to more efficiently match gene expression with distinct environmental conditions (Marshall et al., 2011). Gracey et al. (2008) tracked changes gene expression in mussels across the tidal cycle and identified four distinct physiological states that correspond to environmental conditions favouring metabolism, growth, and stress-responses. I did not observe an effect of heat wave duration on the rate of oxygen consumption, which suggests metabolic strategies to cope with regular and predictable episodes of stress, such as metabolic rate depression, have evolved in *T. californicus*.

Metabolic adaptations to large and predictable fluctuations in temperature suggest that coping with regular temperature stress is a 'cost of living' in the high intertidal (Somero, 2002). As such, increasing daily heat wave intensity or the amount of time spent at high temperatures each day may compromise survival during heat waves, but as long as temperatures decline after the afternoon high to a sufficient recovery temperature the actual number of consecutive heat wave days may be less relevant.

Changes to energy production and allocation can have cascading effects on organismal and population fitness (Pörtner & Farrell, 2008; Sokolova et al., 2012). I did not observe an effect of heat wave intensity on individual survival (Chapter 2). This result is in accordance with the metabolic rate data from Chapter 2 that suggests metabolic adaptations to high temperature, such as metabolic rate depression, extend survival time under stressful conditions. However, I did observe an effect of heat wave intensity on individual reproductive success (Chapter 2). Females subjected to the more intense heat wave (32°C) produced fewer total offspring relative to females in the less intense (26°C) heat wave treatment, demonstrating that while metabolic adaptations can promote survival over a range of stressful temperatures, this may come at the cost of decreased reproduction.

Energetic trade-offs between survival and reproduction have been documented in a wide variety of taxa (Zera & Harshman, 2001). Individual fecundity declines under stressful temperatures, as energy is re-allocated to basal maintenance costs as well as physiological stress responses. This has been experimentally demonstrated in *Drosophila* spp., where overexpression of the heat shock protein *Hsp70* was consistent with reduced fecundity (Krebs & Feder, 1997; Silbermann & Tatar, 2000). The extent to which short-term impacts of sublethal stress on fecundity influences longer-term population persistence, however, is not as well understood.

Under periods of sublethal temperature stress, the re-allocation of energy leading to decreased individual fecundity would also be expected to impact population-level growth and abundance. Temperature, however, may have opposing effects on life-history traits that contribute to population fitness across different time-scales. In Chapter 3, I tested the assumption that observed declines in short-term fecundity (see Chapter 2) have systemic impacts on population dynamics over short and longer-term time scales. While heat wave intensity and duration did affect short-term population growth rate, the effect depended on the interaction between intensity and duration, and short-term population growth rates were decoupled from longer-term abundances. My study, therefore, highlights the need to account for multigenerational effects of stress on long-term population dynamics.

Life-table response experiments and demographic matrix models have been widely used to investigate the effect of sublethal toxicity exposures (Allan & Daniels, 1982; Rao & Sarma, 1986; Bechmann, 1994; Guo et al., 2012) and temperature stress (Nandini et al., 2004; Edmunds, 2005) on demographic vital rates. Huey & Berrigan (2001), however, describe how population growth rate calculated from within generation data (e.g., net reproductive rate, $R_o$) may respond differently to temperature than a measure of population growth rate that is calculated across generations (e.g., intrinsic rate of increase, $r$). The critical difference between these two estimates of population growth rate is that $R_o$ is sensitive to within generation factors that contribute to fitness, such as fecundity and life-span, whereas $r$ is sensitive to factors that span generations, such as generation time. Thus, shortening generation time due to accelerated development may lead to higher intrinsic rates of increase across multiple generations, despite a reduction in fecundity observed over short time scales consistent with short-term exposures to sublethal stress (Cole, 1954; Huey & Berrigan, 2001). This pattern was observed in *Drosophila melanogaster*, where reductions in fecundity during short-term temperature treatments failed to have an observable effect on population growth rate (Dillon et al., 2007).

Intrapopulation variation in thermal tolerance likely contributes to mismatches in the effect of heat stress on population dynamics over short and longer time-scales. Larger body size evolved alongside selection for higher temperature tolerance in several lines of *T. californicus*, and larger individuals exhibited higher fecundity relative to smaller individuals (Kelly et al., 2013). While heat stress may negatively impact total fecundity, it likely disproportionately impacts smaller individuals, and has negligible effects on fecundity and population growth over the long term. Moreover, juvenile *T. californicus* exhibit higher temperature tolerance than adults (Tangwancharoen & Burton, 2014), which disproportionately benefit from higher temperatures that accelerate development and generation time, leading to faster population growth across generations. Bioenergetic frameworks that incorporate energetic trade-offs between survival and reproduction under periods of sublethal temperature stress may not accurately predict subsequent population-level effects over longer time periods. The opposing effects of temperature on different life-history traits may even lead to potential net benefits of transient periods of heat stress.

### 5.1.2 The effect of spatial variation in thermal history on heat wave survival

Populations from different thermal regimes may not respond uniformly to temperature stress. In Chapter 4, I examined how recent thermal history influenced heat wave survival. While heat wave survival generally varied regionally, I detected substantial intraregional variation in survival. Among a suite of eight temperature variables used to characterize thermal history, daily degree-hours (a measure of daily heat accumulation) was sufficient to explain variation in heat wave survival beyond other potential region-wide factors. In addition to heat wave intensity, mortality can be exacerbated by a warm recent thermal history (Denny et al., 2009; Garrabou et al., 2009; Dowd et al., 2015; Roitberg & Mangel, 2016). Body temperatures that govern individual and population responses may differ from the spatial scale of environmental temperatures. Microsite habitat features, such as orientation and shading, can reduce heat accumulation, dampen the impacts of regional heat waves, and maintain populations (Helmuth & Hofmann, 2001; Harley & Helmuth, 2003; Helmuth et al., 2006; see also 5.4 below).

Estimates of thermal time (e.g., daily degree-hours) may more accurately represent body temperature in the field than other statistical measures of temperature (e.g., mean, range), providing a more physiologically meaningful estimate of body temperature (Southward, 1958; Helmuth & Hofmann, 2001). Other studies have identified an energetic limitation to temperature stress due to prior thermal exposures (Denny et al., 2006; Coma et al., 2009).

## 5.2 Heat waves in the high intertidal

Heat waves can impact populations through both lethal and sublethal means. Further, because heat wave severity and frequency are expected to increase with climate change, experimental studies that address the effect of heat waves on population persistence are needed. Mortality during heat waves can be exacerbated by energetic limitations brought about by prior heat accumulation. Organisms in highly variable environments that experience regular and predictable periods of physiological stress may, however, be more resistant to certain heat wave properties than others. The number of consecutive heat wave days did not affect oxygen consumption rates, as the rate of aerobic respiration was low and consistent with metabolic rate depression, a strategy employed to extend survival time under stressful conditions. Changes in the length of heat waves may not affect survival rates to the degree that high daily maximum temperature or the amount of daily heat accumulation does. Energetic trade-offs may, however, underlie sublethal effects of heat waves via reductions in offspring production. The extent to which these short-term effects on fecundity impact longer-term population dynamics, however, depends on the net effect of opposing effects temperature may have on different life-history traits. As heat waves increase in severity and frequency, survival and short-term reproductive success will be limited by energetic constraints. Environmental variation that contributes to variation in body condition will also have important implications for temperature tolerance and heat wave impacts on coastal species.

## 5.3 Future directions

In my dissertation, I tested the effect of heat wave intensity and duration across different levels of biological organization, and the effect of spatial variation in thermal history on experimental heat wave survival. I manipulated heat wave regime in two ways, through changes in the daily maximum temperature and a different number of consecutive heat wave days. Heat wave temperature profiles, however, may vary in ways that I did not address (e.g., ramping speed, length of daily maximum temperature, daily minimum temperature). My results suggest that these other aspects of heat wave structure may be equally important. In Chapter 4, I identified heat accumulation as a significant predictor of heat wave survival, which also negatively affected offspring production (Chapter 2). Moreover, changes in daily minimum temperature may affect recovery time, and further exacerbate both lethal and sublethal effects of heat waves. Future experimental studies of heat waves should consider temperature treatments that alter heat wave structure in these additional ways.

Here, I have not presented any molecular data to help elucidate heat wave effects on energy production or utilization. There are, however, numerous molecular markers underlying heat stress and energy balance that could be used to characterize physiological mechanisms that underlie variation in heat wave survival and reproduction. Heat shock protein production, for example, is energetically expensive and may lead to trade-offs with reproductive effort, but may not explain a substantial amount of variation in heat tolerance (Jensen et al., 2010). Metabolic anaerobic end products, such as lactate, propionate and succinate can be used to mark the transition to full or partial anaerobiosis. Additionally, energy reserves, such as lipids and glycogen, can assess how relative body condition affects energy usage during periods of heat stress. I am currently working on measuring metabolite data to complement my oxygen consumption rate data (Chapter 2), which will help elucidate the role of anaerobic metabolism in maintaining energy balance. These molecular markers can reveal compensatory energetic trade-offs at the cellular level, despite minimal affects of stress treatments observed in organismal-level traits (Pan et al., 2015).

In my dissertation, I used laboratory experiments to test the effect of heat wave treatments on organismal and population-level processes. Laboratory experimentation is a powerful approach to test the effect of temperature across scales. There is, however, always a trade-off between control and realism in laboratory and field approaches, and thermal limits identified in laboratory studies may not accurately predict responses in the field (Terblanche et al., 2011). My heat wave treatments were informed by field temperature data, but my results from Chapter 4 show that thermal history has an important effect on responses to subsequent temperature treatments. Time-series analyses of environmental variation (e.g., noise frequency spectra) can yield important insights into how this variation is temporally distributed (Vasseur & Yodzis, 2004). Pairing spectrum analysis of time-series temperature data with molecular data (Helmuth & Hofmann, 2001) can be a used to understand how thermal history affects heat stress responses, energy balance, and fitness (Dillon et al., 2016).

## 5.4 Integrating research across scales in ecology

A central challenge in ecology is to understand patterns across scales (Levin, 1992). That is, how do we integrate the diversity of processes operating at a variety of spatial and temporal scales to understand the drivers of ecological processes? The problem of scale in ecology has received a great deal of attention (Denny & Benedetti-Cecchi, 2012), and there are a plethora of theories and enormous literatures that seek to address the problem of scale in ecology. Integrating the effects of environmental variation across levels of biological organization is another important dimension of 'scale' in ecology. While we have made enormous advances in understanding the biochemical and molecular mechanisms underlying the capacity and tolerance of species, there is a tremendous need for testable hypotheses that link these effects to higher order ecological responses (Gaston et al., 2009).

## 5.5 Concluding thoughts

My dissertation tested several predictions from the ELTOS framework, and while my data are consistent with predictions that sublethal effects at the biochemical level on energy balance influence individual fitness, my data did not fully support the further prediction that these negative effects on individual fitness also negatively impact populations over longer-time scales. To push the predictive power of the ELTOS framework beyond individuals, we need to incorporate the effects of stress on a variety of life-history traits that contribute to population-level fitness, and account for the different time-scales over which different traits may influence population dynamics and broader ecological trends.

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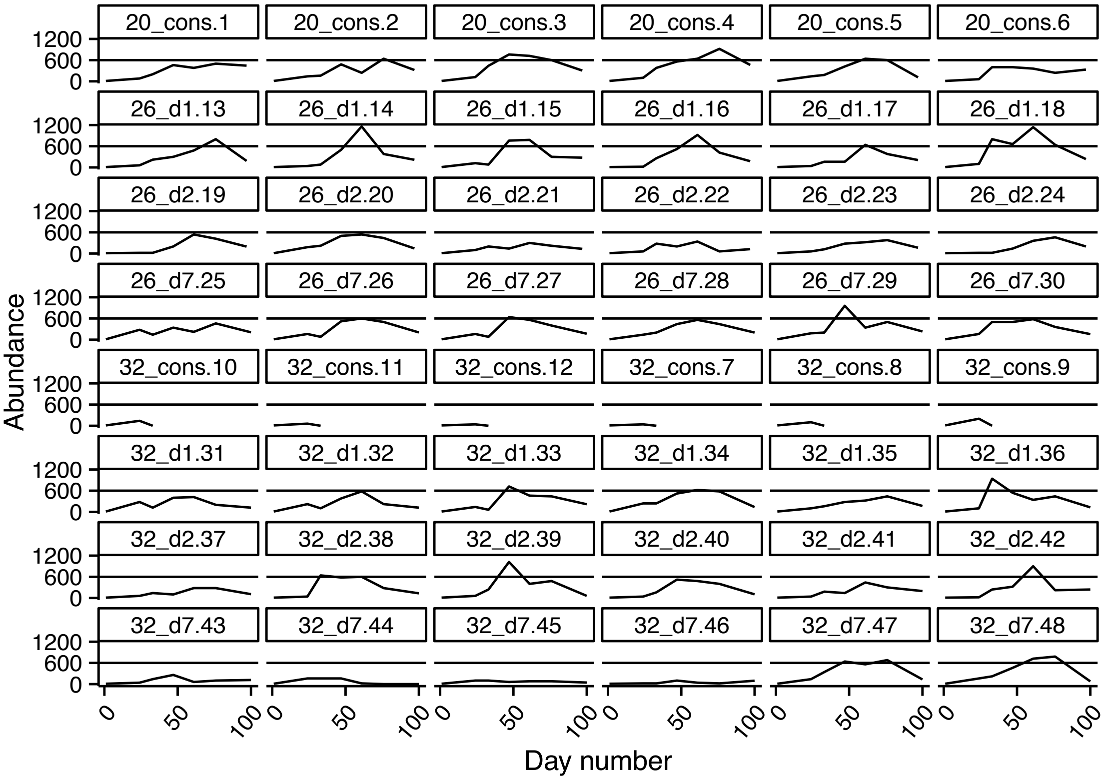
# Appendices

## APPENDIX A

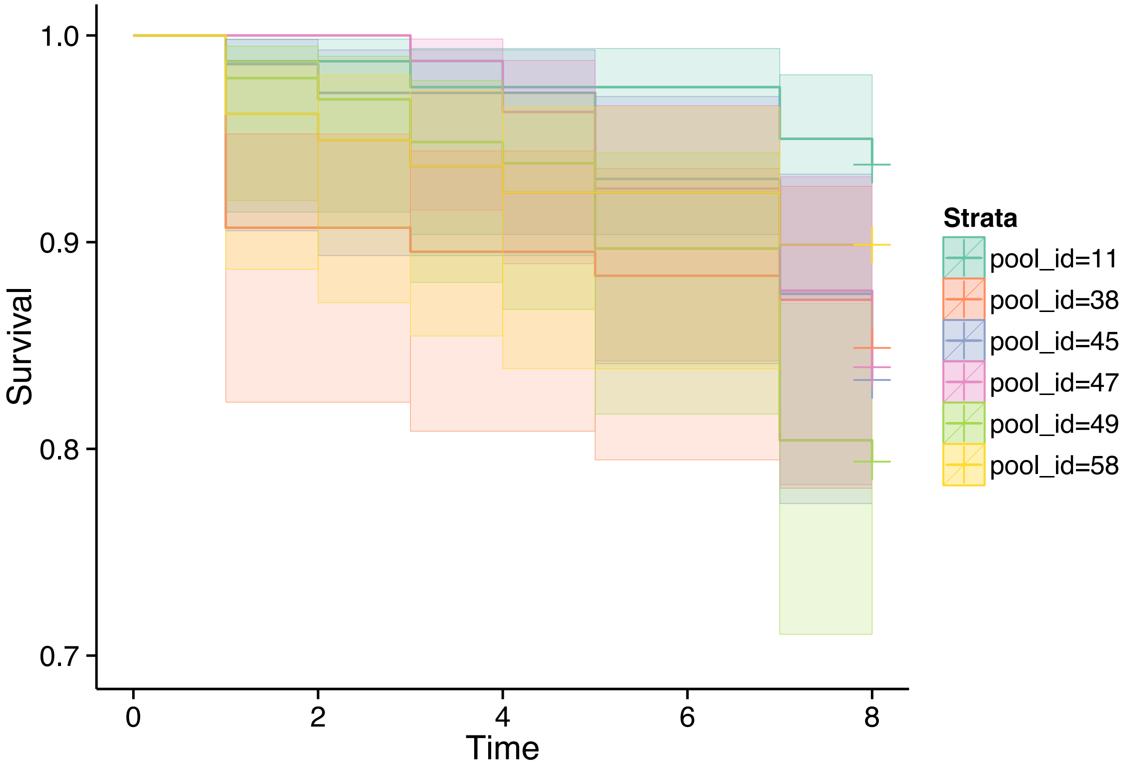
**Chapter 3.** Abundance of each laboratory population of *Tigriopus californicus* over the 100 day experiment. Each row is one of the eight experimental treatments varying in thermal regime. Two experimental treatments were held at a constant 20°C or 32°C, while the other six were subjected to heat waves varying in intensity and duration. The eight experimental treatments are: constant 20°C (20_cons), constant 32°C (32_cons), 26°C/1 day heat wave (26_d1), 26°C/2 day heat wave (26_d2), 26°C/7 day heat wave (26_d7), 32°C/1 day (32_d1), 32°C/2 day (32_d2), and 32°C/7 day (32_d7). The individual population number follows the experimental treatment in the title of each pane.



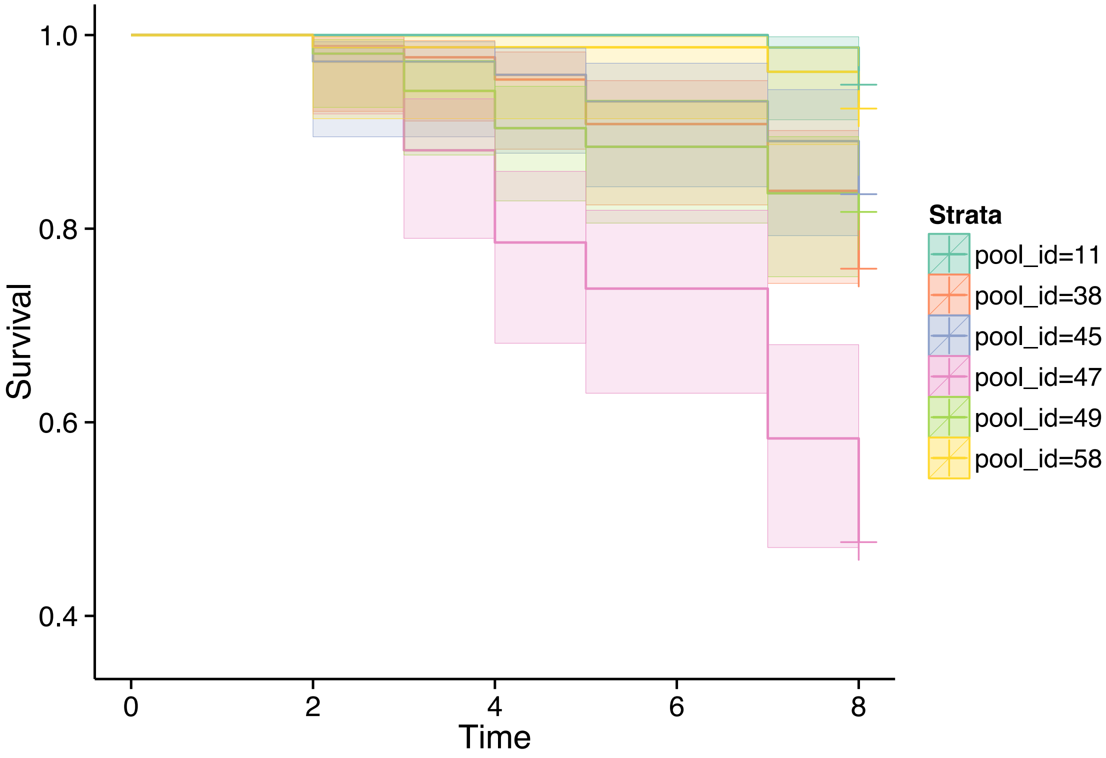
## APPENDIX B

**Chapter 4.** Survival curves for each splash pool population of *Tigriopus californicus* during the 26°C and 32°C experimental heat waves. The survivorship curves are shown for each population within a region. The survival probability for each population is shown by the solid line, and the shaded regions are the 95% CIs. The plots below show intraregional variation in survivorship during the two heat waves.

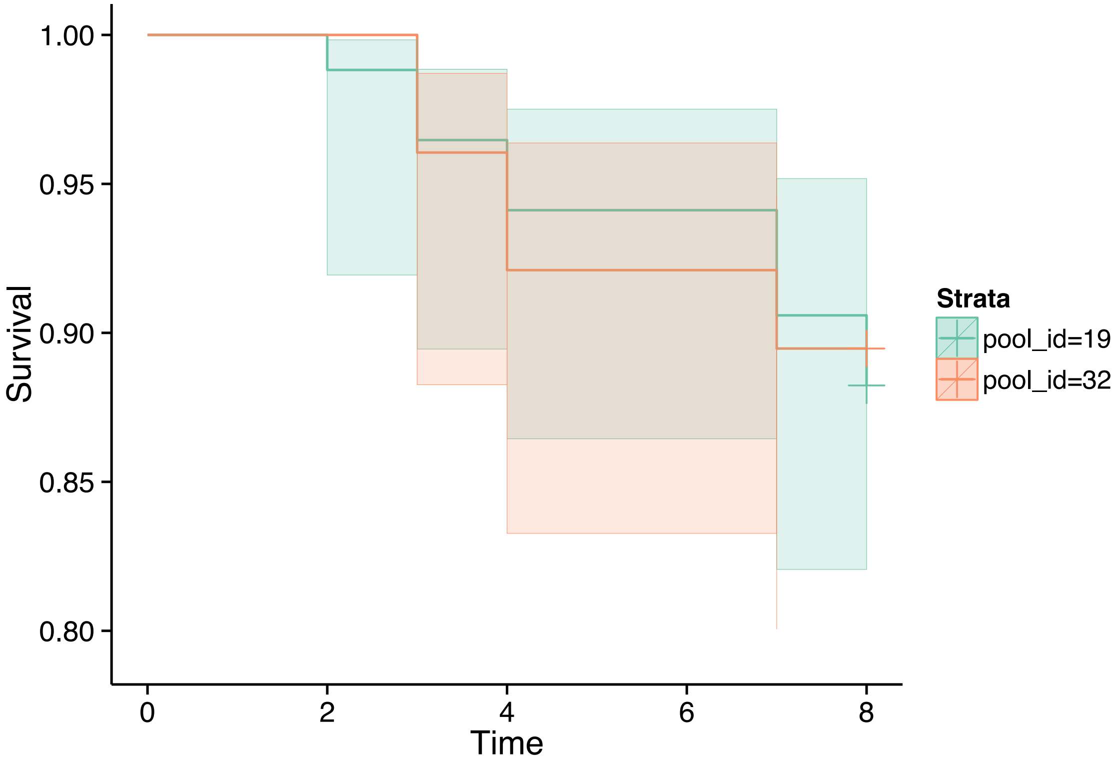
Botany Bay, 26°C heat wave



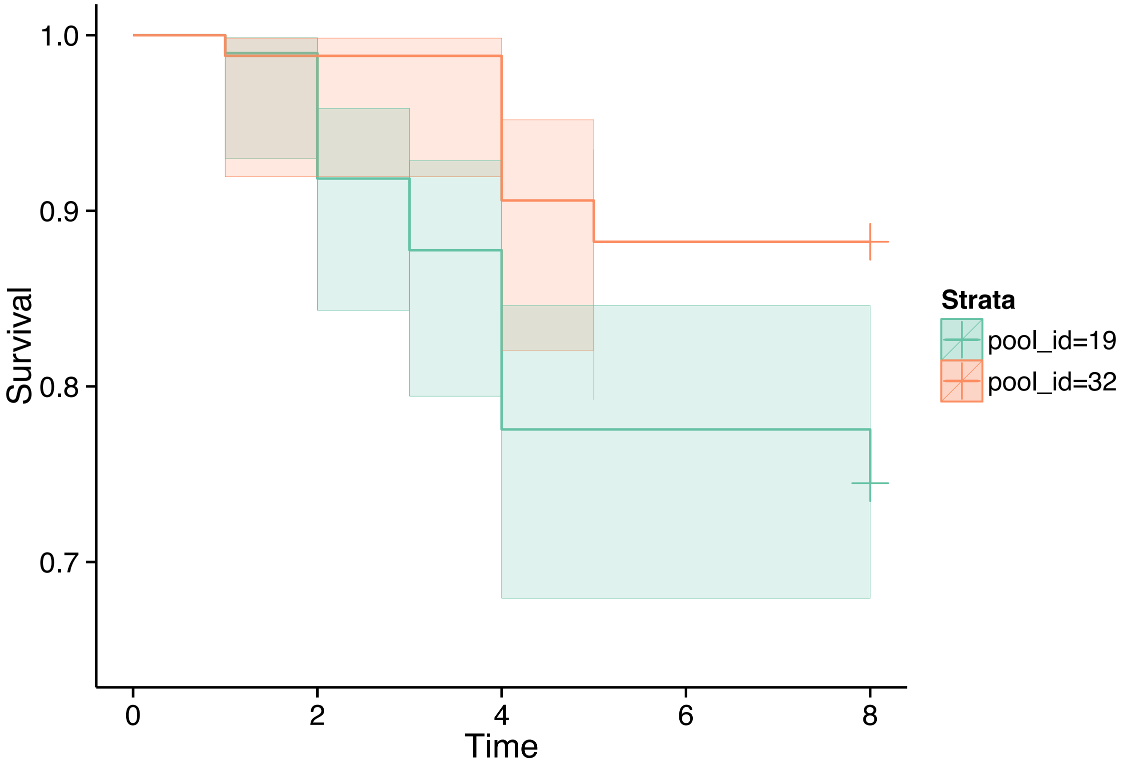
Botany Bay, 32°C heat wave



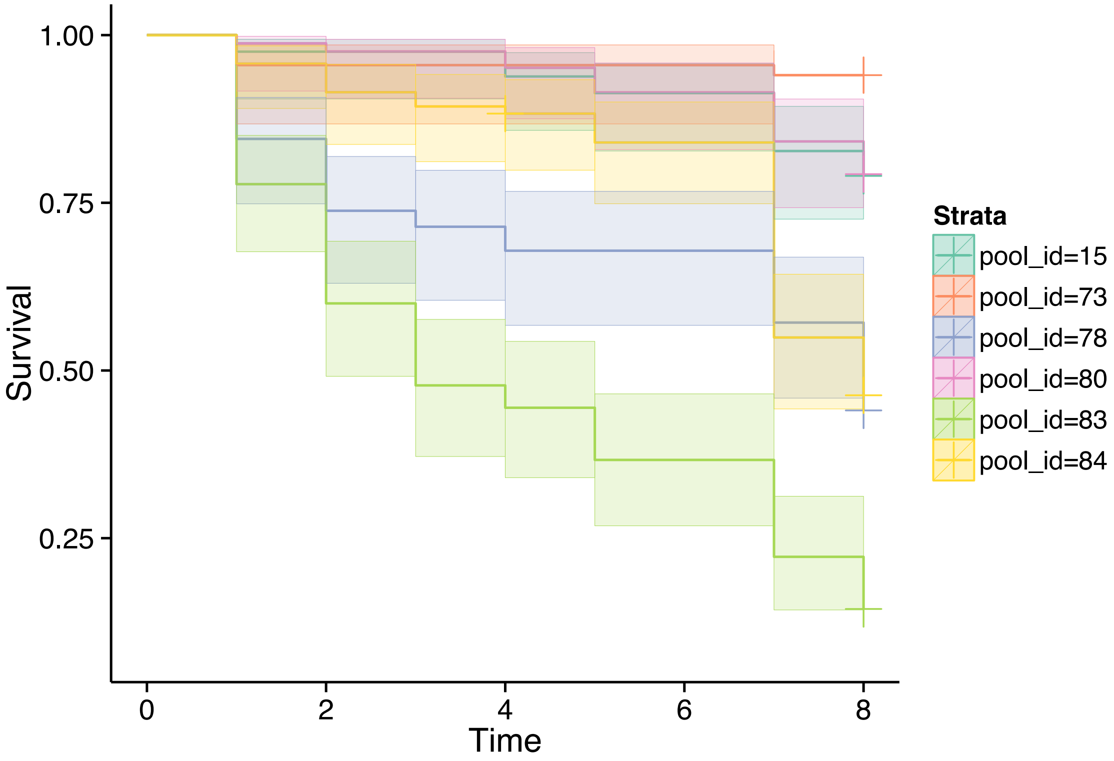
Fishboat Bay, 26°C heat wave



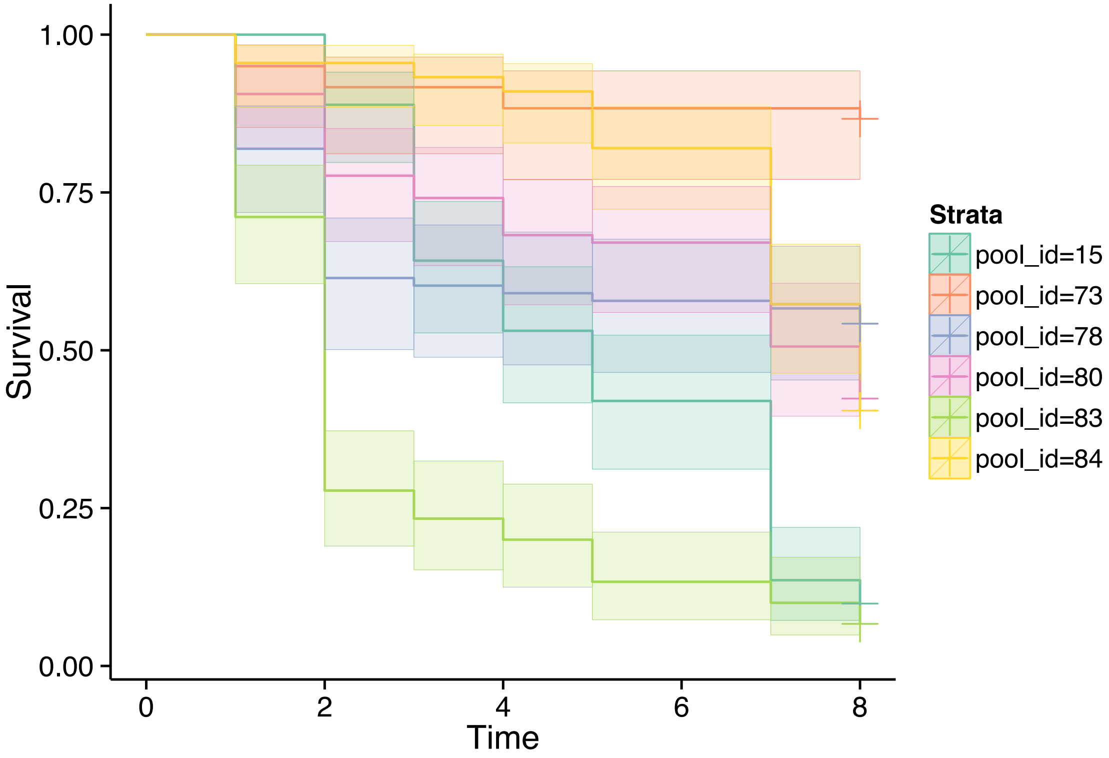
Fishboat Bay, 32°C heat wave



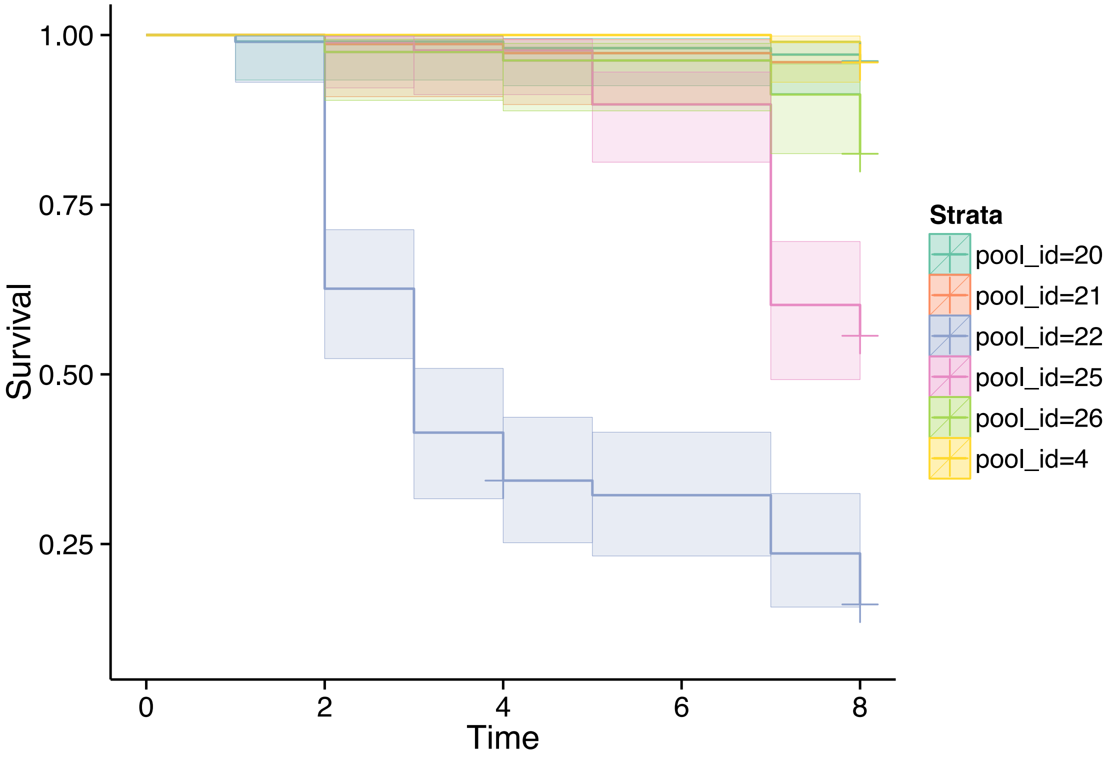
Neck Point Park, 26°C heat wave



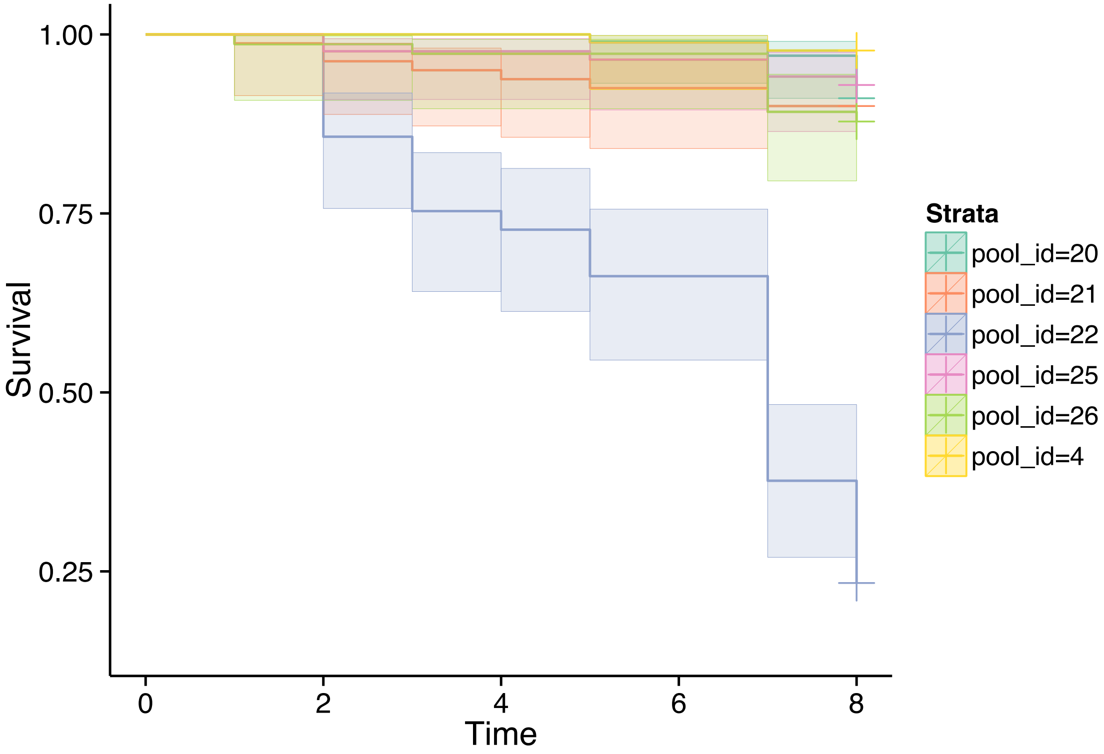
Neck Point Park, 32°C heat wave



Saxe Point Park, 26°C heat wave



Saxe Point Park, 32°C heat wave

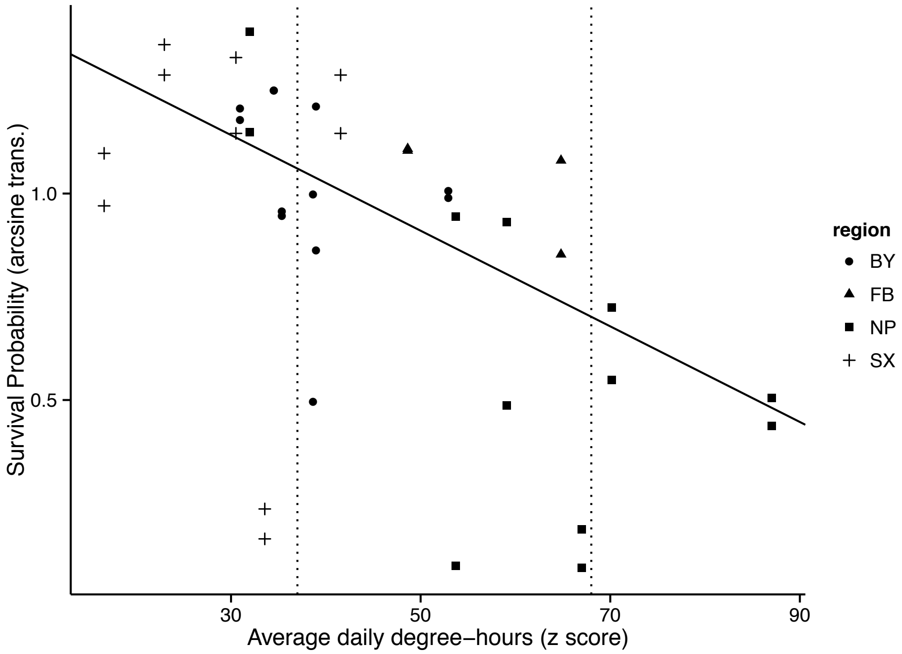


## Appendix C

**Chapter 4.** Analyses of thermal history variables on day 7 heat wave survival using temperature data from different time period durations of thermal history. The following analyses correspond to thermal history data calculated from the previous 3, 14, and 28 days of field conditions. The effect of temperature variation (daily degree-hours, standard deviation) is consistently in the suite of top performing models for all additional thermal history time periods; a result that is consistent with the analyses presented in Chapter 4. Significant model parameters are **bolded**. Degrees of freedom (df), log-likelihood (loglik), change in AICc (δAICc), marginal $R^2$ ($R^2$) calculated from the *MuMIn* package in R. Temperature variables: heat wave intensity (H), region (reg), degree-hours (dh), daily max (avg.max), daily mean (avg.mean), daily min (avg.min) standard deviation (sd), frequency of days above 26°C and 32°C (freq.26 and freq.32).

Number of days of field conditions: 3 days

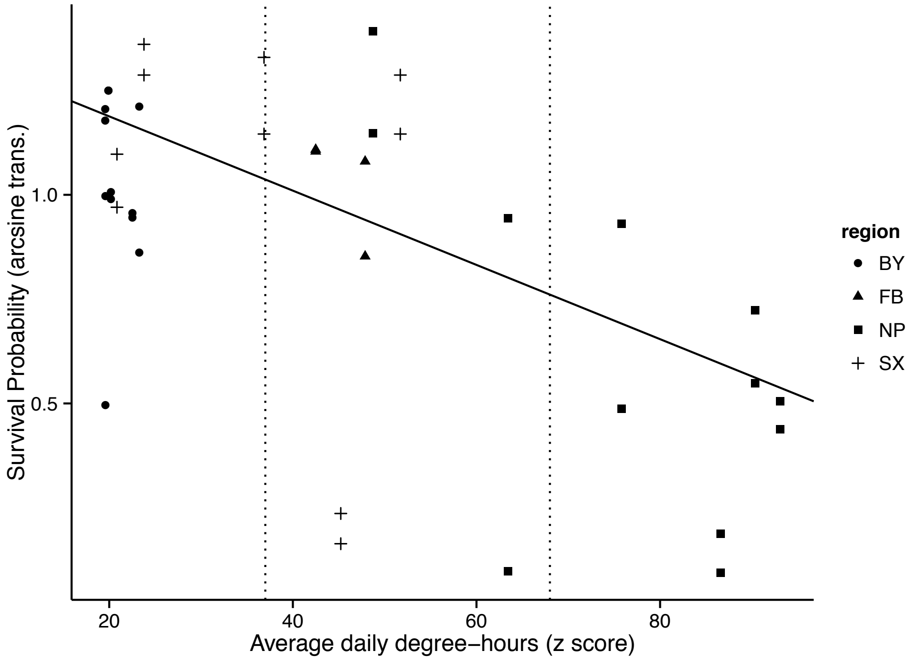
| Model Rank | Parameters | df | loglik | AICc | δAICc | weight | $R^2$ |
|---|---|---|---|---|---|---|---|
| 1 | **H, dh** | 5 | -3.49 | 18.9 | --- | 0.083 | 0.33 |
| 2 | **H, freq.26** | 5 | -3.53 | 18.9 | 0.08 | 0.079 | 0.33 |
| 3 | **H, avg.mean** | 5 | -3.68 | 19.2 | 0.37 | 0.069 | 0.32 |
| 4 | **H,** avg.mean, freq.26 | 6 | -2.57 | 19.9 | 0.99 | 0.050 | 0.38 |
| 5 | **H,** dh, freq.26 | 6 | -2.69 | 20.1 | 1.23 | 0.045 | 0.38 |
| 6 | **H, reg, sd** | 8 | 0.402 | 20.2 | 1.30 | 0.043 | 0.52 |
| 7 | **H, dh,** freq.32 | 6 | -2.79 | 20.3 | 1.42 | 0.041 | 0.38 |
| 8 | **H, avg.max,** | 5 | -4.26 | 20.3 | 1.49 | 0.039 | 0.28 |
| 9 | **H,** avg.min, **freq.26** | 6 | -3.21 | 21.1 | 2.27 | 0.027 | 0.35 |



Survival Probability (arcsine trans.)
1.0
0.5
30
50
70
90
Average daily degree−hours (z score)
region
● BY
▲ FB
■ NP
+ SX

Number of days of field conditions: 14 days

| Model Rank | Parameters | df | loglik | AICc | δAICc | weight | $R^2$ |
|---|---|---|---|---|---|---|---|
| 1 | **H, avg.max, sd, freq.32, max** | 8 | 2.44 | 16.1 | --- | 0.098 | 0.60 |
| 2 | **H, dh** | 5 | -2.34 | 16.7 | 0.58 | 0.073 | 0.39 |
| 3 | **H, dh, sd, max** | 7 | 0.47 | 16.8 | 0.75 | 0.067 | 0.53 |
| 4 | **H, avg.max, sd,** freq.26, **freq.32, max** | 9 | 3.69 | 17.1 | 0.96 | 0.061 | 0.64 |
| 5 | **H, reg, avg.max, sd, freq.32, max** | 11 | 7.51 | 17.1 | 1.05 | 0.058 | 0.72 |
| 6 | **H, dh, sd, freq.32** | 7 | 0.19 | 17.3 | 1.25 | 0.052 | 0.52 |
| 7 | **H, avg.max,** sd**, max** | 7 | 0.04 | 17.7 | 1.57 | 0.045 | 0.51 |
| 8 | **H, avg.mean, sd, max** | 7 | -0.09 | 17.9 | 1.81 | 0.040 | 0.50 |
| 9 | **H, reg, avg.max, sd, max** | 10 | 5.01 | 18.9 | 2.04 | 0.035 | 0.67 |
| 38 | **H,** avg.min**, sd** | 6 | -4.11 | 22.9 | 6.83 | 0.003 | 0.29 |



Number of days of field conditions: 28 days

| Model Rank | | Parameters | df | loglik | AICc | δAICc | weight | $R^2$ |
|---|---|---|---|---|---|---|---|---|
| 1 | **M12r** | **H, dh** | 5 | -1.19 | 14.3 | --- | 0.237 | 0.45 |
| 2 | **M10r** | **H, dh,** freq.32 | 6 | -0.68 | 16.1 | 1.80 | 0.097 | 0.48 |
| 3 | **M11r** | **H, dh,** max | 6 | -0.84 | 16.4 | 2.13 | 0.082 | 0.47 |
| 8 | **M23r** | **H, avg.max, max** | 6 | -1.75 | 18.2 | 3.95 | 0.033 | 0.43 |
| 9 | **M31r** | **H, avg.mean, sd,** max | 7 | -0.46 | 18.7 | 4.39 | 0.026 | 0.49 |
| 33 | **M43** | **H,** reg, avg.min**, sd, max** | 10 | 2.91 | 22.3 | 8.07 | 0.004 | 0.61 |