THE EARLY BENTHIC PHASE IN A WARMING CLIMATE: CONSEQUENCES OF EXTENDED THERMAL EXPOSURE AND CARRY-OVER EFFECTS FOR INTERTIDAL MARINE INVERTEBRATE PERFORMANCE

by

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ABSTRACT

Intertidal marine invertebrates naturally experience strong environmental variability, but climate change is increasing both the frequency of short-term extreme heat events and the duration of periods with elevated temperatures, including marine heatwaves. The early benthic phase of marine invertebrates is characterized by rapid development and high mortality; therefore, understanding how elevated temperatures influence performance during this critical life stage is essential for predicting population persistence under future climate change. However, thermal tolerance has traditionally been estimated using short-term exposure experiments and adult organisms, whereas comparatively little attention has been given to the effects of extended exposure or to whether previous thermal experiences influence subsequent performance.

This study examined the effects of elevated sublethal temperatures on the performance of early benthic phase intertidal marine invertebrates from the west coast of Vancouver Island, British Columbia. First, the effects of extended exposure (25–30 d) to elevated water temperatures on survivorship, growth, and thermal tolerance were evaluated in the barnacles *Chthamalus dalli* and *Balanus glandula*, the predatory gastropod *Nucella ostrina*, and the mussel *Mytilus trossulus*. Subsequently, the potential for initial acute (6 h, air) and extended (5 d, water) exposure to elevated temperatures to generate carry-over effects on subsequent survivorship, growth, and thermal tolerance was investigated in *C. dalli*, *B. glandula*, and *N. ostrina*.

Extended exposure to elevated water temperatures substantially reduced survivorship, altered growth, and resulted in thermal tolerance thresholds more than 10 °C lower than estimates derived from acute exposure experiments. Responses varied among species, with *C. dalli* exhibiting the greatest tolerance to prolonged warming. Thermal tolerance thresholds estimated after extended exposure also predicted that these species may reach their current

thermal limits sooner under future ocean warming than previously suggested by acute exposure experiments.

Initial thermal exposure during the early benthic phase also produced carry-over effects that persisted after individuals returned to ambient conditions, although their magnitude and direction depended on species and exposure duration. Acute exposure to elevated air temperatures enhanced subsequent survivorship and thermal tolerance in the two barnacle species but reduced subsequent thermal tolerance in *N. ostrina*. In contrast, extended exposure to elevated water temperatures reduced subsequent survivorship in *N. ostrina* but had no detectable effect on later growth or thermal tolerance.

Overall, this study demonstrates that the consequences of climate warming for early benthic phase intertidal marine invertebrates cannot be adequately predicted from short-term exposure experiments alone. Both the duration of thermal exposure and the potential for carry-over effects influence performance during the early benthic phase and should therefore be considered when evaluating species vulnerability to climate change. By incorporating both immediate and delayed responses to elevated temperature, this research provides a more realistic basis for predicting the resilience and persistence of rocky intertidal populations under future climate change.

Keywords: Early benthic phase, thermal tolerance thresholds, exposure duration, prolonged warming, carry-over effects, climate change, species persistence.

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CHAPTER 1: General Introduction

Climate change and the future of rocky intertidal communities

Climate change is altering marine ecosystems at an unprecedented rate, with increasing ocean temperatures representing one of the most prevalent environmental stressors affecting coastal habitats worldwide (IPCC 2021, Guild et al. 2025). Over recent decades, sea surface temperature (SST) has increased across all ocean basins and is projected to continue rising throughout the twenty-first century (IPCC 2021). In addition to this gradual warming trend, marine environments are experiencing an increase in the frequency, intensity, and duration of extreme thermal events, including marine heatwaves, with record-breaking events documented globally during the past decade (Smale et al. 2019, Smith et al. 2021, Zhang et al. 2024). These changes are expected to alter the structure and functioning of marine ecosystems by modifying species distributions and interactions, ultimately affecting the persistence of coastal populations and communities (IPCC 2022).

Among coastal habitats, rocky intertidal ecosystems are considered particularly vulnerable to increasing thermal stress because they occur at the interface between marine and terrestrial environments. Organisms inhabiting these ecosystems experience highly dynamic environmental conditions driven by tidal cycles, solar radiation, wind exposure, wave action, and shore elevation, resulting in pronounced thermal variability over relatively short temporal and spatial scales (Stephenson & Stephenson 1949, Helmuth et al. 2006, Leeuwis & Gamperl 2022). During low tide, the temperature of exposed rock surfaces may greatly exceed seawater temperatures; as a result, many intertidal species already function near their upper physiological limits, leaving little capacity to tolerate additional thermal stress (Tomanek & Helmuth 2002, Somero 2002, Helmuth et al. 2006). As climate warming intensifies, these natural thermal challenges are expected to become more severe. Accordingly, the Intergovernmental Panel on Climate Change projects that the ecological risk faced by rocky intertidal ecosystems will increase from moderate at present to very high by 2100 (IPCC 2021).

Rocky intertidal ecosystems support a diversity of algae and marine invertebrates that provide valuable ecosystem services. These communities supply habitat and food resources for a wide range of marine and terrestrial species while also supporting coastal fisheries, aquaculture, tourism, and cultural connections for human communities worldwide (Martínez et al. 2007, Liquete et al. 2013). However, increasing environmental stress associated with climate change, particularly heatwaves, has already contributed to mass mortality events, shifts in species distributions and community composition, and declines in the abundance of ecologically and commercially important species (Smale et al. 2019). Such changes have important ecological and socioeconomic consequences by altering ecosystem functioning and reducing the services provided by coastal ecosystems (Liquete et al. 2013, Smale et al. 2019). Consequently, predicting the future of rocky intertidal communities requires understanding how populations respond to increasing thermal stress and identifying the factors that determine their capacity to persist under continued climate warming.

Population persistence depends on successful recruitment

The future persistence of rocky intertidal communities depends not only on the capacity of individual species to tolerate environmental change but also on the demographic processes that sustain their populations (Caley et al. 1996). Among these processes, recruitment plays a central role by determining whether enough juveniles survive to replenish adult populations (Underwood & Fairweather 1989). In marine invertebrates, recruitment represents the successful transition from planktonic larval or benthic embryonic development to the benthic population through a sequence of processes that includes larval settlement or emergence from benthic eggs, metamorphosis when present, and early benthic survival (Thorson 1950, Rodriguez et al. 1993, Hunt & Scheibling 1997). Species with planktonic larvae enter benthic habitats through settlement, which has been defined as the process beginning with the behavioural search for a suitable substrate and ending with metamorphosis, the transition from the larval to the benthic juvenile form (Rodriguez et al. 1993), whereas direct-developing species lack a free-swimming larval stage and enter benthic habitats upon hatching from benthic egg masses or following release from a brooding parent (Thorson 1950). Recruitment refers to those newly settled or newly emerged individuals that

survive long enough to reach a proper size or developmental stage (Connell 1985, Rodriguez et al. 1993). Consequently, processes acting immediately after settlement or emergence may pose a greater influence on population persistence than settlement itself (Hunt & Scheibling 1997).

Marine invertebrates often exhibit complex life cycles in which individuals transition through multiple developmental stages characterized by distinct ecological and physiological requirements (Rodriguez et al. 1993, Hunt & Scheibling 1997). Among these stages, the early benthic phase (EBP) encompasses the first days to weeks of benthic life following larval settlement or emergence from benthic eggs (Gosselin & Qian 1997). During this transition, juveniles experience direct exposure to benthic environmental conditions for the first time while simultaneously undergoing rapid morphological, physiological, and ecological changes. Accordingly, Gosselin (1997) proposed that the EBP represents an ecologically distinct life stage, during which juveniles differ from older individuals in their use of microhabitats and food resources as well as in their susceptibility to environmental stressors and other sources of mortality.

The EBP has long been recognized as a demographic bottleneck because mortality during this period is frequently extremely high. Mortality frequently exceeds 60% and may surpass 90% in some species and cohorts, with a large proportion of mortality occurring within the first days after settlement or emergence from benthic eggs or from brooding adults (Gosselin & Qian 1997). Multiple biotic and abiotic factors contribute to these losses, including predation, competition, pathogens, food availability, desiccation, salinity, and temperature (Gosselin & Qian 1997, Hamilton & Gosselin 2020). At the same time, juveniles typically exhibit rapid growth, and attaining a larger body size substantially reduces vulnerability to predators and environmental stress (Gosselin & Qian 1997, Moran 1999, Moran & Emlet 2001). Consequently, environmental conditions experienced during the EBP influence not only immediate survivorship and growth but also recruitment success and population dynamics. Predicting the resilience of rocky intertidal communities under climate change therefore requires understanding how environmental stress affects the performance of the EBP individuals responsible for replenishing future populations.

Temperature as a determinant of EBP performance

Marine invertebrates are ectothermic, making their biological performance highly dependent on environmental temperature (Somero 2002, Schulte et al. 2011). During the EBP, this dependence is expected to be particularly strong because individuals are undergoing rapid development while becoming established in the benthic environment (Byrne 2011, Hamilton & Gosselin 2020). Exposure to elevated temperatures may alter key components of EBP performance, including survivorship, growth, and thermal tolerance, although the magnitude and direction of these responses vary among species and developmental stages (Hamilton & Gosselin 2020).

Importantly, biological responses to warming depend not only on the intensity of thermal stress but also on its duration (Schulte et al. 2011, Prieto et al. 2020). The same temperature may produce different outcomes depending on whether organisms experience it for a few hours or for several consecutive days or weeks (Somero 2010, Nguyen et al. 2011). Elevated temperatures experienced over short periods may trigger rapid physiological or behavioural responses that temporarily maintain performance. In contrast, prolonged exposure often requires sustained physiological adjustments and may increase energetic costs associated with maintenance, repair, and homeostasis (Somero 2010, 2020). Under climate change, intertidal organisms are expected to encounter both brief episodes of extreme heat during low tide, likely repeated at intervals throughout the spring and summer, as well as occasional prolonged periods of elevated temperatures associated with seasonal warming and marine heatwaves (Masanja et al. 2023, Zhang et al. 2024). Understanding how juvenile performance changes across these different exposure durations is therefore fundamental to predicting recruitment success and the persistence of rocky intertidal populations under future climate change.

The effects of both acute and extended thermal stress do not necessarily end once temperatures return to normal. Environmental conditions experienced early in development may continue to influence subsequent performance, producing delayed responses commonly referred to as carry-over effects (Harrison et al. 2011, O'Connor et al. 2014, Moore & Martin 2019). Broadly, carry-over effects occur when previous environmental conditions influence

later performance after the original conditions have changed (O'Connor et al. 2014). Although this concept has traditionally been applied to relationships among different life stages, particularly between larval development and post-settlement performance (Pechenik 2006), previous environmental experiences may also influence performance within a single developmental stage. This broader perspective is particularly relevant during the EBP, when juveniles continue to grow and develop while experiencing highly variable thermal environments.

Previous thermal exposure can have different effects on subsequent performance. Early exposure to elevated temperatures may reduce later performance, enhance tolerance to later stress through acclimation, or produce no measurable effect, depending on species characteristics, developmental stage, and the intensity and duration of the initial exposure (Pechenik 2006, O'Connor et al. 2014, Donelan et al. 2021, Glass et al. 2023). Therefore, predicting the resilience of rocky intertidal populations under climate change requires understanding both the direct effects of elevated temperatures during exposure and the delayed consequences that may influence EBP performance long after the initial thermal event has ended.

Knowledge gaps and thesis objectives

Studies evaluating thermal responses have most often focused on the adult stage and have involved short-term experimental exposures (Jenewein & Gosselin 2013, Hamilton & Gosselin 2020, Iwabuchi & Gosselin 2020, Raby et al. 2025). Few studies have examined the consequences of prolonged exposure, despite evidence that thermal tolerance thresholds may decline with increasing exposure duration (Nguyen et al. 2011). Moreover, although carry-over effects have been documented across a variety of marine taxa and life stages (Pechenik 2006, O'Connor et al. 2014), relatively little attention has been given to the EBP.

These knowledge gaps limit our ability to predict how intertidal populations will respond to ongoing climate warming and increasing thermal variability. Responses to elevated temperature are likely shaped not only by the conditions organisms experience at a given time but also by the lasting consequences of previous exposure, particularly during

vulnerable developmental stages. Evaluating both the immediate and subsequent effects of sublethal temperatures during acute and extended exposure to thermal stress is therefore necessary to improve our understanding of juvenile performance and, ultimately, the persistence of intertidal populations under future climate scenarios.

This thesis addresses these questions by investigating how elevated sublethal temperatures influence the performance of juvenile marine invertebrates during the EBP. Specifically, it examines both the effects of prolonged thermal exposure and the potential for early thermal experiences to influence subsequent performance in species commonly found on the Pacific coast of Canada, including the barnacles *Chthamalus dalli* and *Balanus glandula*, the predatory gastropod *Nucella ostrina*, and the mussel *Mytilus trossulus*.

Chapter 2 investigates the effects of extended exposure to elevated temperatures on survivorship, growth, and thermal tolerance during the EBP of four species of intertidal marine invertebrates. Individuals were exposed to four seawater temperatures, including a control (16 °C) and three elevated temperature treatments previously shown to be below each species' acute LT_{50}. This chapter examines whether thermal tolerance thresholds under extended exposure differ from those previously determined by short-term experiments and explores the implications of prolonged warming for the persistence of intertidal species under sea surface temperatures projected for the west coast of Vancouver Island.

Chapter 3 examines whether previous exposure to elevated sublethal temperatures generates carry-over effects that influence subsequent juvenile performance and thermal tolerance. Specifically, EBP individuals from three species of intertidal marine invertebrates were initially exposed to either acute (6 h, aerial) or extended (5 d, in seawater) sublethal heat stress before being reared under ambient conditions for one month. Subsequent survivorship, growth, and thermal tolerance (LT_{50}) were then evaluated to determine whether previous thermal experience influences later performance and whether the magnitude and direction of these carry-over effects differ among species.

Chapter 4 synthesizes the principal findings of the thesis and discusses their implications for understanding how elevated temperatures influence the resilience of juvenile

intertidal invertebrates under climate change. It concludes by highlighting the relevance of these findings for the management of rocky intertidal ecosystems and explores future research directions.

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CHAPTER 2: Responses of early benthic phase intertidal invertebrates to prolonged warming: implications for species persistence under climate change

INTRODUCTION

Coastal marine habitats are among the most economically and ecologically important ecosystems worldwide, supporting biodiversity while providing essential services such as food resources, coastal protection, and tourism opportunities (Martínez et al. 2007, You-Ji 2014). However, these ecosystems are increasingly threatened by rapid environmental change, particularly ocean warming associated with global climate change (IPCC 2021, Guild et al. 2025). Among coastal habitats, intertidal rocky shores represent one of the most physically challenging environments on Earth. These ecosystems occur at the interface of marine and terrestrial conditions, where organisms are alternately exposed to aquatic and aerial environments (Helmuth et al. 2006). As a result, they experience pronounced and rapid temperature fluctuations driven by tidal cycles and solar exposure, creating strong environmental gradients from low to high intertidal zones that impose constraints on species distributions and result in well-defined vertical zonation patterns (Stephenson & Stephenson 1949, Murray et al. 2006, Helmuth et al. 2006). Intertidal communities are dominated by algae, seagrasses, and a wide range of slow-moving or sessile invertebrates (Murray et al. 2006), many of which rely on physiological, morphological, and behavioural adaptations to cope with environmental stress (Somero 2002, Leeuwis & Gamperl 2022). The pronounced thermal variability in this habitat makes intertidal organisms particularly vulnerable to climate change, as many species already operate near their physiological thermal limits (Somero 2002, Stillman 2003, Helmuth et al. 2006).

Globally, sea surface temperature (SST) has increased by approximately 0.60 °C between 1980 and 2020, and is projected to rise by an additional 0.86–2.89 °C by 2100 (IPCC 2021). Coastal and shallow-water habitats, however, tend to warm faster than the open ocean (Guild et al. 2025). On the west coast of Vancouver Island, peak summertime SSTs have also increased, at rates of approximately 0.81–1.13 °C per century (Iwabuchi & Gosselin 2019). Importantly, ocean temperatures are not the only thermal stress experienced by intertidal organisms, as rock surface temperatures can fluctuate by more than 20 °C within

a single tidal cycle during summer days (Iwabuchi & Gosselin 2019), such that present-day conditions are already exposing intertidal organisms to substantial thermal stress.

The biological consequences of a warming environment on the performance of an organism depend not only on temperature intensity but also on the duration of exposure (Schulte et al. 2011). In addition to gradual ocean warming, extreme thermal events such as marine heatwaves are expected to increase in frequency and intensity (Marx et al. 2021; Zhang et al. 2024), potentially exposing marine organisms to elevated temperatures more frequently over longer periods. Elevated temperature experienced over a very brief (acute) period of up to a day or two may trigger rapid physiological or behavioural adjustments, whereas a longer exposure requires sustained physiological responses and may impose cumulative energetic costs associated with maintenance and repair processes (Somero 2002, 2010). Consequently, prolonged exposure to elevated temperatures may increase energetic demands and reduce energy available for growth and survival. Since elevated summer seawater temperatures on the coasts of Vancouver Island can persist over several days or even weeks (Jenewein & Gosselin 2013, Iwabuchi & Gosselin 2019), understanding organismal responses to extended exposure would provide a more realistic assessment of the responses of organisms under future warming scenarios.

The early benthic phase (EBP) in marine invertebrates corresponds to the first days to weeks of benthic life immediately following larval settlement or emergence from benthic eggs (Gosselin & Qian 1997). This EBP is characterized by substantial physiological and developmental changes while individuals experience direct exposure to benthic environmental conditions for the first time (Rodriguez et al. 1993, Gosselin & Qian 1997, Hunt & Scheibling 1997). Mortality during the EBP is often extremely high, with rates exceeding 90% in some species and a large proportion of individuals dying within the first few days after settlement or emergence (Gosselin & Qian 1997). Survivorship can vary widely among cohorts, reflecting the sensitivity of this stage to both biotic (predation, competition) and abiotic factors (temperature, desiccation, salinity) (Gosselin & Qian 1997, Hamilton & Gosselin 2020).

Temperature plays a key role in shaping survivorship and growth, and therefore recruitment success, during the EBP (Hamilton & Gosselin 2020). Growth during this stage is typically rapid, and reaching a proper size substantially reduces vulnerability to predation and abiotic stressors (Gosselin & Qian 1996, 1997). Consequently, any reduction in physiological performance may have disproportionate effects on survival, recruitment success, and ultimately population dynamics (Rodriguez et al. 1993, Moran & Emlet 2001). Existing evidence also suggests substantial variation in thermal tolerance across developmental stages and species. For example, in their study of co-occurring rocky intertidal species from Barkley Sound, Hamilton and Gosselin (2020) identified changes in tolerance across developmental stages, with adults generally exhibiting higher resistance to heat and desiccation than juveniles, as well as differences among species in acute tolerance thresholds.

Despite the importance of temperature during early life stages, the effects of prolonged exposure to elevated temperatures on EBP performance remain poorly understood. Studies evaluating thermal tolerance have predominantly focused on adult organisms, which are generally easier to sample and manipulate; in addition, estimates of thermal tolerance thresholds have most commonly been derived from short-term exposure experiments, whereas studies of extended exposure remain limited in comparison (Jenewein & Gosselin 2013, Hamilton & Gosselin 2020, Iwabuchi & Gosselin 2020, Mendt & Gosselin 2022, Raby et al. 2025). Evidence suggests that tolerance thresholds estimated under acute conditions may not accurately reflect performance under prolonged thermal stress. For example, Nguyen et al. (2011) documented marked differences in upper thermal limits among adults of subtidal and intertidal marine invertebrates across seven phyla in Singapore and reported that these thermal tolerance thresholds decreased under chronic warming relative to short-term exposure experiments. This distinction may be especially important during the EBP, when individuals are simultaneously allocating energy to growth, development, and maintenance. Accordingly, the importance of evaluating responses to extended or chronic sublethal temperatures has been proposed by different authors (Iwabuchi & Gosselin 2020, Falkenberg et al. 2021, Mardones et al. 2021), particularly for early life stages of marine invertebrates.

This research therefore aims to determine the impacts of extended exposure to elevated water temperature on the performance and thermal tolerance of four species of intertidal invertebrates during their EBP. Specifically, EBP individuals were exposed to temperatures known to be sublethal under short-term (<48 h) exposure conditions in order to (1) determine the effects of extended exposure durations (6–30 d) on survivorship, growth, and thermal tolerance, (2) characterize interspecific variation in responses to extended exposure to elevated temperature, (3) determine whether thermal tolerance thresholds under extended exposure, measured as LT_{50} estimates, are comparable to thresholds estimated under short-term exposure, and (4) explore the significance of these findings in terms of the future persistence of these species under the temperatures predicted to occur on the coast of British Columbia. We hypothesized that temperatures considered sublethal during short-term exposure would reduce survivorship, alter growth, and lower thermal tolerance when exposure duration was extended. We further predicted that the magnitude of these responses would vary among species, with the mid-intertidal species expected to be more strongly affected than the upper-intertidal species because of the lower reported thermal tolerance of mid-intertidal species under acute exposure (Hamilton & Gosselin, 2020)

METHODS

Study species

To understand the implications of extended exposure to elevated temperature on the EBP of intertidal invertebrates, four species with different modes of feeding and motility, and also readily available and abundant in the study area, were selected: the barnacles *Balanus glandula* Darwin, 1854 and *Chthamalus dalli* Pilsbry, 1916, the mussel *Mytilus trossulus* Gould, 1850 and the snail *Nucella ostrina* Gould, 1852. These mid- to high intertidal species are all widely distributed along the northwest coast of North America, ranging from Alaska to California for *C. dalli* (Wares & Castañeda 2005) and *M. trossulus* (Martel et al. 2000), and to Baja California for *B. glandula* and *N. ostrina* (Morris et al. 1980, Bering et al. 2017). *B. glandula* and *C. dalli* are sessile filter-feeders (Morris et al. 1980), whereas *M. trossulus* is

also a filter-feeder but is motile during the first weeks of juvenile life (Bayne 1964), and *N. ostrina* is a motile predator (Gosselin & Chia 1994).

Study site and collection of organisms

Collection and controlled experiments with barnacles and snails were carried out from June to July 2024, and experimentation with mussels was carried out in July 2025. EBP individuals of these species were collected from the rocky intertidal zone of two field sites in Barkley Sound on the west coast of Vancouver Island, Canada. Newly settled barnacles and mussels were collected on a rocky beach in Kelp Bay (48°51'02.0"N 125°07'06.1"W). To obtain EBP *N. ostrina,* ripe egg capsules of this species were collected at First Beach (48°49'06.6"N 125°09'49.4"W) near Prasiola Point. Experimental work was carried out at the Bamfield Marine Sciences Center (BMSC).

Newly settled *C. dalli* and *B. glandula* were obtained following the methodology described by Sandee et al. (2016). Briefly, small rocks (5-10 cm in diameter) already colonized by barnacles were collected from the mid-intertidal zone and brought to BMSC, where all recently settled juvenile barnacles were removed. The rocks were then returned to the field for 72 h to allow new settlers to attach, after which the rocks were collected again and returned to the laboratory. The rocks bearing newly settled barnacles were placed in filtered seawater at 15-17 °C for an acclimation period of 24 to 36 h; this temperature range approximated the average summertime temperature in Barkley Sound (Iwabuchi & Gosselin 2019).

Juvenile *M. trossulus* were obtained from the mid-intertidal zone in Kelp Bay by collecting strings of filamentous algae of the genus *Acrosiphonia* to which newly settled mussels had attached. Mussels measuring 275-1000 µm were carefully removed from the algae using a fine paintbrush.

Newly hatched *N. ostrina* were obtained according to the methodology described by Gosselin & Chia (1995), in which ripe egg capsules are gently dislodged from the substrate using tweezers. Egg capsules were brought to the laboratory, placed in meshed 50 ml plastic

containers, and held in aerated seawater at 10-11 °C. Individuals hatching during the following 72 h were retained for experiments.

Water temperature treatments

In this study, we define acute exposure as thermal stress lasting less than 48 h, and extended exposure as sustained thermal stress exceeding this threshold, which could last for days, weeks or months. We focus on LT_{50} (lethal temperature causing 50% mortality) as a metric of thermal tolerance, as it provides a practical and statistically robust measure of survivorship that is well-suited to EBP organisms, whose small body size makes other types of measurements logistically challenging.

To simulate extended exposure to elevated temperatures, EBP individuals of each species were subjected to one of four water temperature treatments under controlled laboratory conditions for 25-30 d. These treatments included a control water temperature (15–17°C), representing the average highest summertime seawater temperature in Barkley Sound (Iwabuchi & Gosselin 2019), and three warmer experimental temperature treatments. The experimental treatments consisted of a high sublethal temperature, 5–10°C below their acute LT_{50} , and two intermediate temperatures (Table 2.1). Based on the LT_{50} of each species, the selected water temperature treatments for *C. dalli* and *B. glandula* were therefore: 16 °C (control), 25 °C, 30 °C and 34 °C. For *N. ostrina* and *M. trossulus*, the temperature treatments were 16 °C (control), 20 °C, 24 °C and 28 °C. The control temperature used in this study (~16 °C) was slightly warmer than the current two-month average summertime SST in this region (~14.8 °C) and was comparable to the warmest summertime temperatures occurring in the region (15.5 °C) as reported by Iwabuchi & Gosselin (2019). The actual average water temperature and corresponding standard deviations obtained over the duration of the experiments are presented in Table 2.1. The laboratory setting for these experiments is described in Appendix A.

Table 2.1. LT_{50} values in air under acute (6 h) exposure reported by Hamilton & Gosselin (2020), as well as sublethal water temperature treatments used in the present extended exposure experiments on four species of EBP intertidal invertebrates. Water temperature values represent average ± SD.

Species and duration of exposure	LT_{50} in air (Hamilton & Gosselin 2020)	Sublethal water temperature treatments			
		Control	Intermed. 1	Intermed. 2	Highest
C. dalli (25 d)	45.8 °C (EBP)	16.2 ± 0.9 °C	24.5 ± 0.5 °C	29.7 ± 0.5 °C	33.2 ± 1.7 °C
B. glandula (25 d)	42.8 °C (Adults)	16.2 ± 0.9 °C	24.5 ± 0.5 °C	29.7 ± 0.5 °C	33.2 ± 1.7 °C
N. ostrina (30 d)	32.3 °C (EBP)	15.9. ± 0.5 °C	19.8 ± 0.6 °C	23.9 ± 0.2 °C	27.9 ± 0.2 °C
M. trossulus (30 d)	35.6 °C (EBP)	15.0 ± 0.8 °C	19.8 ± 0.3 °C	23.5 ± 0.6 °C	28.2 ± 0.3 °C

Rearing and monitoring EBP marine invertebrates under extended elevated water temperature treatments

To ensure consistency across treatments, barnacles were divided into replicate groups of 10 individuals of each species per rock, additional individuals being removed with a dissecting needle. To keep track of each EBP barnacle, a small dot of yellow nail polish was placed on the rock surface next to each individual, and a photograph was taken of the rock showing all barnacles and dots. The shell of *N. ostrina* hatchlings was marked with a small dot of nail polish following the method described by Gosselin (1993) to facilitate repeatedly finding them throughout the experiment. The markings were made the day before starting the experiment and, once the nail polish was dry, the snails were put back in a petri dish with seawater to confirm their survival. After marking, hatchlings were divided into 10 snails per replicate in modified plastic containers with a 600 µm mesh window and placed in their corresponding water temperature treatment (Table 2.1). EBP mussels were also divided into

replicates of 10 individuals and placed in modified 1 mL microcentrifuge tubes with a 102 µm mesh in the bottom.

Survivorship and growth responses to each of the temperature treatments were recorded at intervals during the experiment. Survivorship was assessed by observing each individual for movements under a dissecting microscope for a maximum of 5 min. Growth during the trials was determined by measuring their body size using an eyepiece scale at 40X magnification; the rostro-carinal diameter (RCD) of barnacles and the maximum shell length (SL) of snails and mussels were recorded. The trials were carried out over a 25 d period for *C. dalli* and *B. glandula*. Survivorship and individual RCD were measured on days 0 (start of experiment), 10, 20 and 25. For both mollusc species, the trials were conducted for a 30 d period and survivorship and SL were recorded on days 0, 6, 12, 18, 24, and 30. The feeding procedures for each species throughout the experiment are detailed in Appendix A

The experimental design for *C. dalli* and *M. trossulus* was: 4 water temperature treatments X 5 replicates per treatment X 10 individuals per replicate = 200 individuals of each species. For *B. glandula* and *N. ostrina*, the experimental design was: 4 water temperature treatments per species X 4 replicates per treatment X 10 individuals per replicate = 160 individuals of each species.

Data analysis

All statistical analyses were carried out using R Statistical Software (v.4.5.0) (R Core Team 2025)**.**

Survivorship of EBP individuals under extended exposure to elevated temperature

To analyze the EBP survivorship responses of the four invertebrate species under temperature stress, a survival analysis was conducted using the survival package in R (Therneau et al. 2026). Time to mortality was the event of interest, and individuals still alive

at the end of the 25–30 d experimental period were treated as right-censored observations, meaning their time to mortality was unknown by the end of the experiment.

Kaplan-Meier survival curves were constructed to visualize survivorship patterns and estimate survival probabilities over time for each temperature treatment. Survival objects were created using the Surv() function, and survival curves were fitted using the survfit() function. To test whether survivorship differed significantly among temperature treatments, pairwise log-rank tests were performed separately for each species using the survdiff() function. Resulting p-values were adjusted for multiple comparisons using the Benjamini-Hochberg method (Benjamini & Hochberg 1995).

To quantify temperature effects on mortality relative to the control treatment (reference), Cox proportional hazards regression models were fitted using the coxph() function, with the 16 °C treatment as the reference level. Hazard ratios (HR) were calculated to express mortality risk at elevated temperatures relative to the reference. The proportional hazards assumption was verified graphically using complementary log-log plots, which showed approximately parallel curves across treatments.

Growth responses of EBP individuals under extended exposure to thermal stress

To assess differences in initial sizes of a species across temperature treatments, the RCD (for barnacles) and SL (for snails and mussels) before the experiment (day 0) were tested for normality using Shapiro-Wilk tests, and homogeneity of variances using Levene's test. Depending on whether parametric assumptions were met, one-way ANOVAs or Kruskal-Wallis tests were performed. RCD and SL at each time point were then used to calculate the daily growth rates using the formula:

$$\text{Growth rate (\%)} = \left(\frac{Current\ size - Previous\ size}{Previous\ size\ \times \#\ Days\ since\ the\ previous\ measurement}\right) \times 100$$

For barnacles, the daily growth rate was tracked for each individual, while for snails and mussels an average daily growth rate was calculated at the replicate group level due to the difficulty of marking and identifying individuals. To model daily growth rates over time,

Linear Mixed Effects models (LME) were fitted using the lmer() function from the lme4 package (Bates et al. 2025). Only individuals that survived to each sampling day were included in the growth analysis. The candidate models included temperature treatment and time (Day) as fixed effects, a temperature × day interaction term, and a random intercept for the individual (barnacles) or the replicate (snails and mussels). Models differed in whether time was modelled as a linear or quadratic term. The best-fitting model for each species was selected based on the lowest Akaike Information Criterion (AIC) value.

Pairwise comparisons among temperature treatments were conducted using estimated marginal means with the emmeans package (Lenth et al. 2025). Model diagnostics included inspection of residuals plotted against fitted values and the pseudo-R^2 values were checked using the rsquared function from the piecewiseSEM package (Lefcheck et al. 2025), following the approach of Nakagawa and Schielzeth (2013), to quantify the Marginal R^2 (R^2m = proportion of variance explained by fixed effects alone) and Conditional R^2 (R^2c= variance explained by the entire model including random effects).

Thermal tolerance thresholds under extended exposure

To estimate the extended lethal temperature causing 50% mortality (LT_{50}) for each species, survivorship data at each time point were used to fit Generalized Linear Models (GLM) with a binomial distribution and a logit link with temperature as the predictor and survival (alive/dead) as the binary response. The LT_{50} values and their standard errors were extracted from the models using the dose.p() function from the MASS package (Ripley et al. 2025).

Model diagnostics were assessed by testing whether residual dispersion differed from that expected under the fitted model and whether the frequency of zeros differed from model expectations, using the testDispersion() and testZeroInflation() functions from the DHARMa package (Hartig et al. 2024). Pseudo-R^2 values were calculated using the rsquared() function from the piecewiseSEM package (Lefcheck et al. 2025), to quantify the proportion of variation explained by the models.

Future persistence of four invertebrate species under extended exposure to thermal stress

To estimate when sea surface temperatures (SST) in Barkley Sound may reach each species' thermal tolerance threshold, the extended LT_{50} values obtained after 10 d for barnacles and 12 d for molluscs were compared with predicted future SST trends reported for the west coast of Vancouver Island by Iwabuchi and Gosselin (2019). Specifically, their regression describing the historical trend of the warmest SST of the year for the period 1935–2016 was rearranged to solve for year:

$$Year = 1935 + \frac{SST - 14.80}{0.0081}$$

SST in the equation was then replaced by the LT_{50} value estimated for each species to determine the year at which SST would reach the corresponding thermal tolerance threshold. These projections assume that the warmest SST continues to increase at a linear rate of approximately 0.81 °C per century, as it has over the period of 1935-2016 (Iwabuchi & Gosselin 2019).

RESULTS

Survivorship of EBP individuals under extended exposure to elevated temperatures

Survivorship of all four species in ambient 16 °C (control) seawater remained high (>86 %) throughout the experiment, as expected, but survivorship was significantly affected by some of the warmer temperature treatments (Log-rank tests, $p<0.001$) (Fig. 2.1 and Fig. B.1 in Appendix B). Survivorship of the two upper intertidal species (*C. dalli* and *B. glandula*) declined sharply in the 30 °C and 34 °C treatments, with survivorship in these two elevated temperatures being significantly lower than at 16 °C (Table 2.2.). In the mid-intertidal species (*N. ostrina* and *M. trossulus*), survivorship was impacted at lower temperatures: survivorship in *M. trossulus* was negatively affected by the 28 °C treatment, and in *N. ostrina,* survivorship declined even in the 24 °C treatment compared to those kept at 16 °C (Table 2.2.). In all species, the negative effects of these elevated temperatures

mostly occurred within the first 6-10 d of exposure (Fig. 2.1.); the only exception was *N. ostrina* at 24 °C, in which the decline in survivorship began after 18 d. The Cox proportional hazards model (Table 2.3.) revealed that extended exposure to temperatures ≤25 °C in barnacles and ≤20 °C in snails did not significantly increase mortality risk relative to the control, as expected, but warmer temperature treatments did result in a significant increase in mortality risk. The Cox model analysis for *M. trossulus*, however, could not be interpreted because survivorship remained very high in three of the four treatments, leading to near-complete separation in the model.

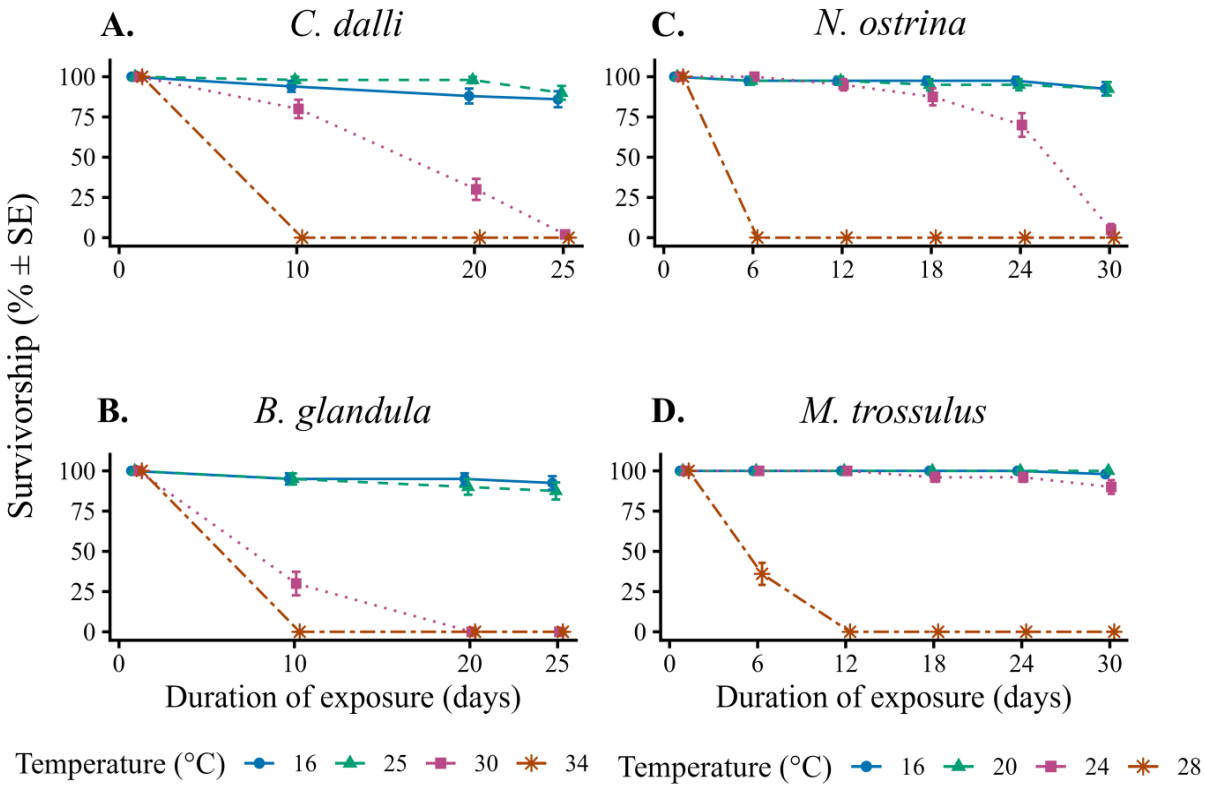

Figure 2.1 Mean observed survivorship (± SE) of EBP marine invertebrates exposed to extended elevated temperature treatments over 25–30 d. A. *Chthamalus dalli* (n = 50 per temperature treatment), B. *Balanus glandula* (n = 40 per temperature treatment), C. *Nucella ostrina* (n = 40 per temperature treatment). and D. *Mytilus trossulus* (n = 50 per temperature treatment).

Table 2.2. Pairwise comparisons following Log-Rank tests on the survival times among temperature treatments of EBP marine invertebrates exposed to extended elevated temperature treatments over 25–30 d. ($\alpha = 0.05$).

Species	Treatments	χ^2	df	p-value
C. dalli	16 °C vs 25 °C	0.470	1	0.493
	16 °C vs 30 °C	68.100	1	**<0.001**
	16 °C vs 34 °C	87.790	1	**<0.001**
	25 °C vs 30 °C	84.310	1	**<0.001**
	25 °C vs 34 °C	95.120	1	**<0.001**
	30 °C vs 34 °C	66.000	1	**<0.001**
B. glandula	16 °C vs 25 °C	0.540	1	0.462
	16 °C vs 30 °C	75.930	1	**<0.001**
	16 °C vs 34 °C	71.480	1	**<0.001**
	25 °C vs 30 °C	70.710	1	**<0.001**
	25 °C vs 34 °C	71.480	1	**<0.001**
	30 °C vs 34 °C	13.940	1	**<0.001**
N. ostrina	16 °C vs 20 °C	0.000	1	0.987
	16 °C vs 24 °C	56.31	1	**<0.001**
	16 °C vs 28 °C	75.000	1	**<0.001**
	20 °C vs 24 °C	53.45	1	**<0.001**
	20 °C vs 28 °C	75.150	1	**<0.001**
	24 °C vs 28 °C	79.000	1	**<0.001**
M. trossulus	16 °C vs 20 °C	0.000	1	1.000
	16 °C vs 24 °C	5.210	1	**0.025**
	16 °C vs 28 °C	105.40	1	**<0.001**
	20 °C vs 24 °C	5.210	1	**0.025**
	20 °C vs 28 °C	105.40	1	**<0.001**
	24 °C vs 28 °C	105.40	1	**<0.001**

Table 2.3. Summary table of the Cox proportional hazards model for EBP marine invertebrates exposed to extended elevated temperature treatments over 25–30 d, comparing mortality in each elevated temperature treatment with mortality in the control treatment (16 °C). Sample sizes: *Chthamalus dalli,* n = 50 per temperature treatment*; Balanus glandula,* n = 40 per temperature treatment*; Nucella ostrina,* n= 40 per temperature treatment. (α = 0.05).

Species	Temperature (°C)	Coefficient	Hazard Ratio	SE	p-value
C. dalli	25	-0.406	0.666	0.59	0.488
	30	2.937	18.865	0.42	**<0.001**
	34	5.302	200.817	0.51	**<0.001**
B. glandula	25	0.539	1.715	0.73	0.460
	30	4.124	61.814	0.65	**<0.001**
	34	4.885	132.336	0.68	**<0.001**
N. ostrina	20	1,09E+01	1.011	0.82	0.989
	24	0.034	31.111	0.61	**<0.001**
	28	0.078	2,56E+06	0.96	**<0.001**

Growth responses of EBP individuals under extended exposure to thermal stress

At the start of the experiment (Day 0), before exposure to elevated temperatures, initial body sizes (RCD or SL) of *C. dalli* and *M. trossulus* did not differ significantly among temperature treatments (Table 2.4.). In *B. glandula* and *N. ostrina*, however, initial body sizes differed slightly but significantly, with individuals in the 16 °C treatment in these species being smaller than in warmer treatments, despite individuals having been haphazardly assigned to treatments. Specifically, *B. glandula* individuals at 16 °C were 5.28-6.34% smaller than those in the 30 °C and 34 °C treatments, while *N. ostrina* individuals at 16 °C were 9.32% smaller than those at 28 °C. Accordingly, growth responses are examined as proportional growth relative to the initial (Day 0) body size.

Table 2.4. Descriptive statistics and results of one-way ANOVAs and Kruskal-Wallis tests comparing initial (Day 0) body size (RCD and SL) of EBP marine invertebrates among the four temperature treatments. Sample sizes: *Chthamalus dalli,* n = 50 per temperature treatment*; Balanus glandula,* n = 40 per temperature treatment*; Nucella ostrina,* n = 40 per temperature treatment. ($\alpha = 0.05$).

Species	Variable	Mean (mm)	SD (mm)	Test	Result	Post-Hoc
C. dalli	RCD	0.486	0.034	Kruskal-Wallis	$\chi^2 = 3.496$, df = 3, p = 0.321	N/A
B. glandula	RCD	0.613	0.050	Kruskal-Wallis	$\chi^2 = 14.59$, df = 3, **p = 0.002**	**16 °C vs 30 °C:** Z = -2.788, p = 0.016 **16 °C vs 34°C:** Z = -3.495, p = 0.003
N. ostrina	SL	1.187	0.190	ANOVA	F = 3.037, df = 3, **p = 0.031**	**16 °C vs 28 °C:** t = -2.820, p = 0.023
M. trossulus	SL	0.660	0.152	Kruskal-Wallis	$\chi^2 = 5.079$, df = 3, p = 0.166	N/A

In the analysis of growth rates, the mortality of all *B. glandula* at 30 °C beyond day 10, and all *M. trossulus* at 28 °C beyond day 6, were such that interval growth rates could not be calculated for subsequent time intervals; therefore, the 30 °C treatment in *B. glandula* and the 28 °C treatment in *M. trossulus* were dropped from all analyses. Within each of the four species, marginal and conditional R^2 values were identical among temperature treatments (Table 2.5.), indicating that variation among individuals, or among replicate groups, of the

same species was minimal, with all variation explained by the fixed effects of temperature, time, and their interaction.

During the experiment, interval growth rates in all four species were significantly affected by temperature and time (all $p < 0.05$; Table 2.5.), particularly at 30 °C in *C. dalli* and at 24 °C in snails and mussels, with growth generally declining as temperature and exposure time increased ($p < 0.05$ compared to the 16 ° C, Table 2.6.). Interestingly, the growth rate patterns differed across species, with polynomial models best describing growth patterns in *C. dalli*, *N. ostrina*, and *M. trossulus*. *C. dalli* and *M. trossulus* exhibited inverted-U patterns peaking mid-experiment, while *N. ostrina* showed U-shaped trends with minimal growth around 18-24 d, whereas *B. glandula* exhibited a linear decline in growth over time, with the highest growth rates observed after 10 d of exposure (Table 2.5, Fig. 2.2).

Table 2.5. Results of linear mixed-effects models analyzing the effects of temperature and time on daily growth rates (% day^{-1} ± SE) of EBP marine invertebrates exposed to extended elevated temperature treatments over 25–30 d. ($\alpha = 0.05$).

Species	Effect	F-statistic	df1, df2	p-value	R^2
C. dalli (polynomial)	Temperature	4.238	2, 326	**0.015**	$R^2_M = 0.29$
	poly(Day, 2)	29.007	2, 326	**<0.001**	$R^2_C = 0.29$
	Temperature x poly(Day, 2)	5.404	4, 326	**<0.001**	
B. glandula (linear)	Temperature	19.779	2, 235	<0.001	$R^2_M = 0.32$
	Day	79.474	1, 235	<0.001	$R^2_C = 0.32$
	Temperature x Day	0.026	1, 235	0.8728	
N. ostrina (polynomial)	Temperature	51.107	2, 9.722	**<0.001**	$R^2_M = 0.83$
	poly(Day, 2)	84.118	2, 41.780	**<0.001**	$R^2_C = 0.83$
	Temperature x poly(Day, 2)	1.352	4, 41.624	0.2671	
M. trossulus (polynomial)	Temperature	6.878	3, 70	**<0.001**	$R^2_M = 0.48$
	poly(Day, 2)	22.874	2, 70	**<0.001**	$R^2_C = 0.48$
	Temperature x poly(Day, 2)	2.697	4, 70	**0.038**	

Table 2.6. Pairwise comparisons of mean daily growth rates (% day^{-1} ± SE) among temperature treatments of EBP marine invertebrates exposed to extended elevated temperature treatments over 25–30 d. ($\alpha = 0.05$).

Species	Treatments	Estimate	SE	df	p-value
C. dalli	16°C vs 25°C	-0.298	0.218	313	0.358
	16°C vs 30°C	0.855	0.336	310	**0.030**
	25°C vs 30°C	1.154	0.332	310	**0.002**
B. glandula	16°C vs 25°C	-0.616	0.165	78.5	**<0.001**
N. ostrina	16°C vs 20°C	0.149	0.406	34.9	0.929
	16°C vs 24°C	3.101	0.408	35	**<0.001**
	20°C vs 24°C	2.952	0.408	35	**<0.001**
M. trossulus	16°C vs 20°C	1.37	0.628	50.7	0.084
	16°C vs 24°C	2.83	0.628	50.7	**<0.001**
	20°C vs 24°C	1.46	0.628	50.7	0.062

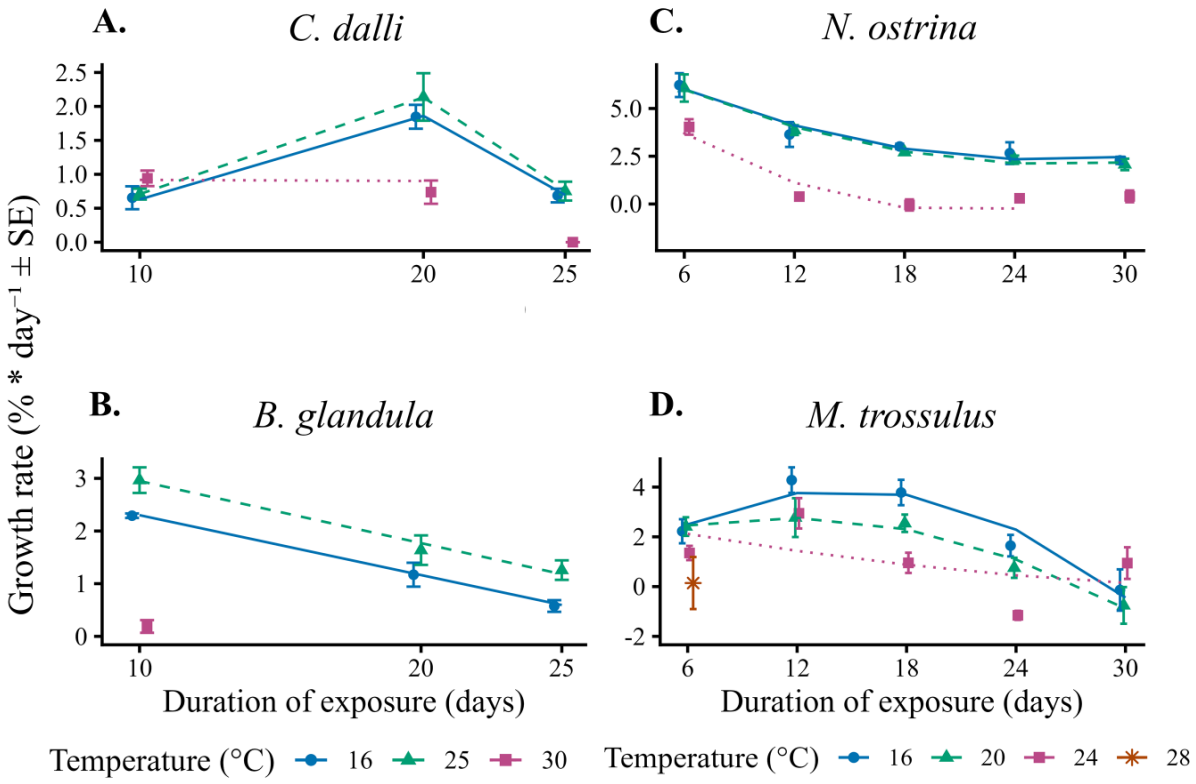

Figure 2.2. Interval growth rates (mean per day ± SE) of EBP marine invertebrates exposed to extended elevated temperature treatments over 25–30 d. Points represent observed means per replicate (± SE), and lines show fitted trends from linear mixed models (LMM). Only individuals who survived to each time point were included in the models. Fitted trends do not extend to time points excluded from the models because the number of surviving individuals was insufficient for analysis.

Thermal tolerance thresholds under extended exposure

Thermal tolerance thresholds (LT_{50} values) of EBP individuals determined in this study were substantially lower than those reported under short-term (acute) air exposure for the same four species (Hamilton & Gosselin 2020), being consistently >10 °C lower than acute LT_{50} estimates (Fig. 2.3.; Table C.1. in Appendix C). In the two barnacle species, LT_{50} values were below 32 °C for all extended durations of exposure, whereas in the two mollusc species they were consistently below 26 °C. In addition, LT_{50} values declined with increasing duration of exposure to elevated temperatures (Fig. 2.3).

Model diagnostics indicated some limitations in the LT_{50} calculations. Under-dispersion was detected in *C. dalli* at 10 d, *B. glandula* at 25 d, and *N. ostrina* at 6 d, indicating reduced variability in the data relative to model expectations. For *M. trossulus*, reliable LT_{50} estimates were only obtained for the 30 d duration (SE = 0.29 °C), as high survivorship at the three lowest temperature treatments between days 6 and 24 resulted in large uncertainty (SE > 10 °C) at earlier time points. Despite this, LT_{50} estimates were generally well resolved for most species and time points, and a consistent decline in thermal tolerance with increasing exposure time was observed across species. A complete summary of LT_{50} estimates and model diagnostics is provided in Appendix C (Table C.1.).

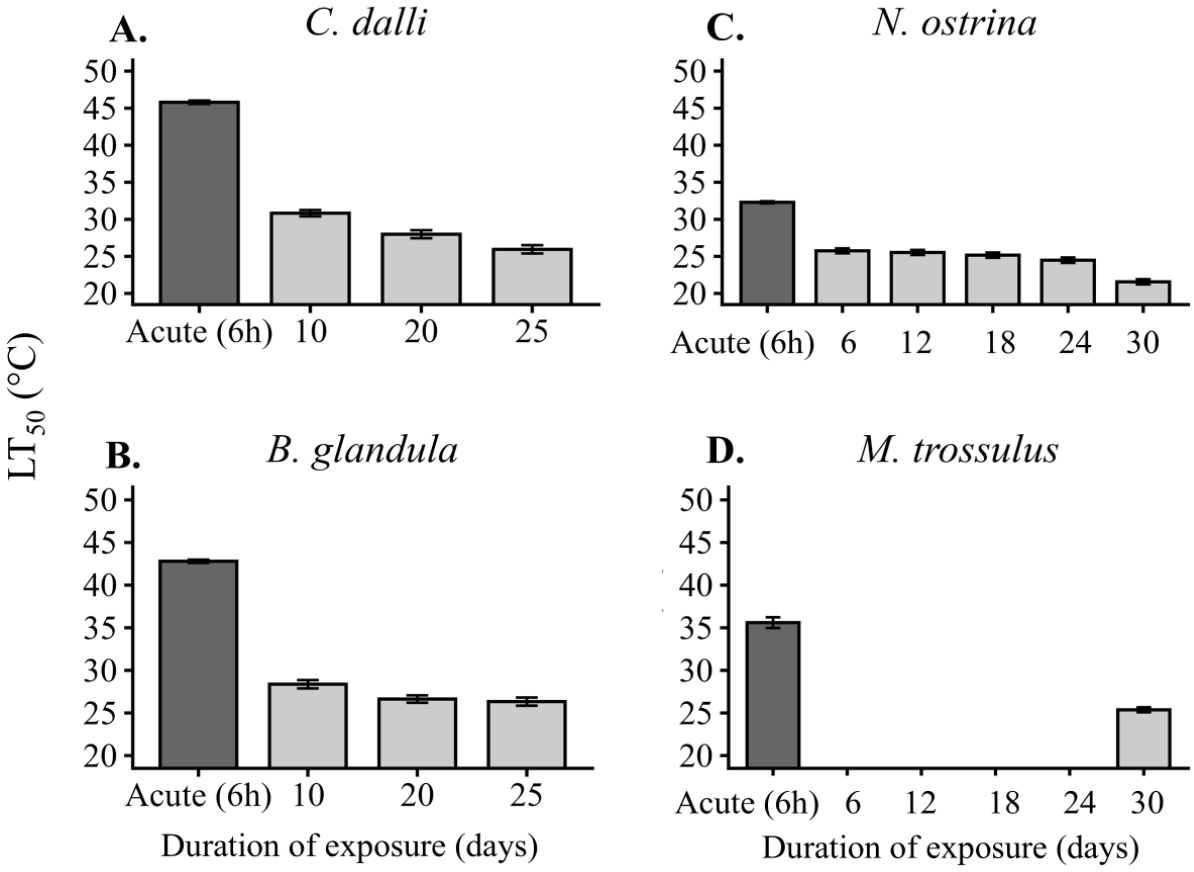

Figure 2.3. LT_{50} estimates of the EBP of four marine invertebrate species under extended (10–30 d) exposure to elevated water temperature. Dark bars indicate the LT_{50} values of these species under acute (6 h) exposure to air temperature, reported by Hamilton & Gosselin (2020), for comparison. Error bars represent the standard error.

Future persistence of four EBP invertebrate species under extended exposure to thermal stress

Based on the current SST warming trend on the west coast of Vancouver Island reported by Iwabuchi & Gosselin (2019) (SST = 14.80 + 0.0081[Year − 1935]), the thermal tolerance thresholds under extended exposure estimated in this study are projected to be reached within approximately 1200–1800 y. The current tolerance thresholds of EBP *N. ostrina* and *M. trossulus* are projected to be reached first, followed by the tolerance thresholds of *B. glandula* and *C. dalli* approximately 300–700 y later (Fig. 2.4). In comparison, the threshold estimated for adult *B. glandula* under acute exposure by Iwabuchi & Gosselin (2020) is projected to be reached ~700 y later than the threshold under extended temperatures estimated here for EBP *B. glandula*.

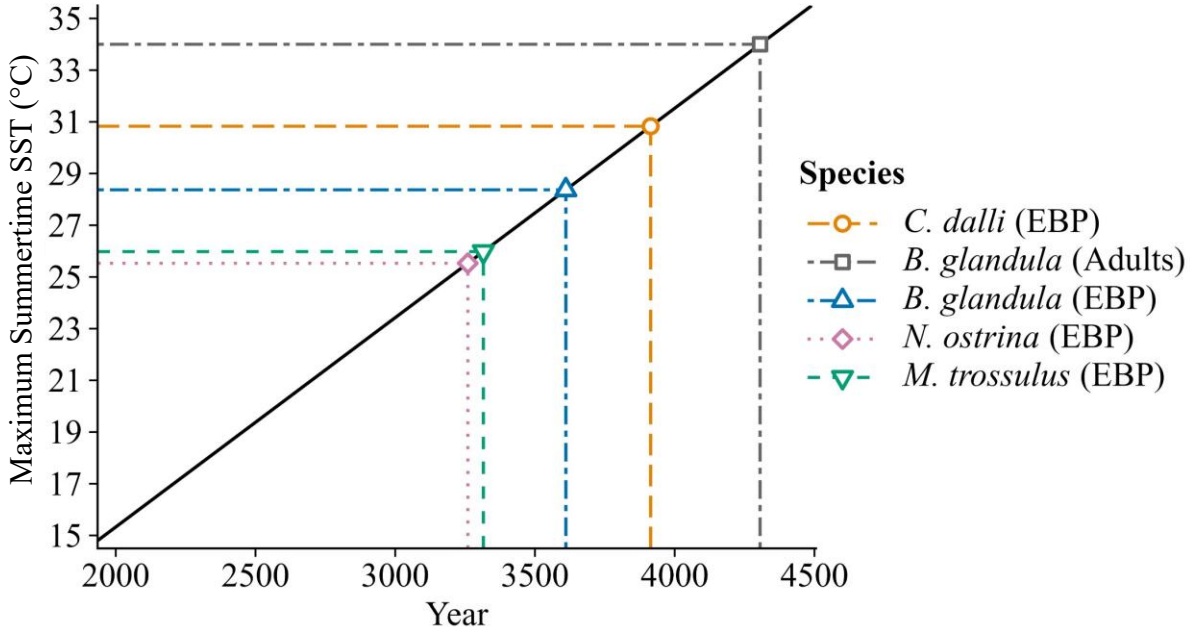

Figure 2.4. Projected year in which the maximum summertime sea surface temperature (SST) is expected to reach the LT_{50} of the EBP of four marine invertebrate species, based on the SST warming trend reported by Iwabuchi & Gosselin (2019): SST= 14.80 + 0.0081*(Year - 1935). LT_{50} values reflect extended thermal exposure of 10 d for barnacles (*C. dalli*, *B. glandula*) and 12 d for molluscs (*N. ostrina*, *M. trossulus*). LT_{50} for *B. glandula* adults from Iwabuchi & Gosselin (2020).

DISCUSSION

Climate change is causing coastal marine organisms to be exposed to increasingly elevated summertime temperatures, but also to experience these elevated temperatures for extended periods lasting days to weeks (Marx et al. 2021, Zhang et al. 2024). The present study reveals that thermal tolerance thresholds in EBP intertidal invertebrates vary as a function of exposure duration. Across the four species studied, the tolerance thresholds under extended exposure were substantially lower than those documented under acute conditions. Tolerance thresholds measured after only 6-10 d of exposure were >10 °C lower than those reported by Hamilton & Gosselin (2020) under acute (6 h) exposure. These findings indicate that past studies characterizing thermal limits under short-term exposures are unlikely to accurately represent how EBP invertebrates will respond to the prolonged warming events associated with marine heatwaves and seasonal temperature increases, conditions that are already occurring on the coast of British Columbia (Iwabuchi & Gosselin 2019) and are projected to intensify over the coming decades (IPCC 2021).

Influence of extended exposure to elevated temperatures on the survivorship and growth of EBP marine invertebrates

The reduced survivorship observed under extended exposure suggests that temperatures considered non-lethal during short-term exposure can impose cumulative lethal physiological stress when sustained over longer periods. Under extended or chronic thermal stress, organisms must continuously allocate energy toward maintenance processes such as cellular repair, protein stabilization, immune function and physiological acclimatization (Somero 2002, Pörtner & Farrell 2008). Over time, these demands may reduce the energy available for growth and survival, eventually leading to mortality once physiological compensation is no longer possible. This pattern was consistent across all species, with survivorship declining both with increasing temperature and increasing duration of exposure.

Despite the overall negative effects of prolonged warming on survivorship, moderate increases in temperature that caused little or partial mortality did not consistently reduce growth. In some cases, elevated temperatures resulted in growth rates comparable to, or greater than, those observed under control conditions. For example, *B. glandula* exhibited

greater growth at 25 °C than at 16 °C, while *C. dalli* maintained similar growth across the 16–25 °C treatments, indicating a broad thermal optimum for growth. Among molluscs, both *N. ostrina* and *M. trossulus* exhibited optimal growth at temperatures ranging from 16 - 20 °C. However, the benefits of moderate warming were not sustained beyond species-specific tolerance thresholds. Extended exposure to temperatures approximately 4–5 °C above the apparent optimum resulted in rapid mortality and reduced growth across all species, indicating an upper thermal limit for prolonged exposure. These findings support the idea that marine invertebrate performance can improve with increasing temperature when conditions remain within a species' thermal optimum. Similar patterns have been reported in other marine invertebrates, including juveniles of Atlantic surfclams (*Spisula solidissima*) (Acquafredda et al. 2019), mussels (*Mytilus galloprovincialis*) (Prieto et al. 2020), and dwarf surf clams (*Mulinia lateralis*) (Yang et al. 2022), as well as in *Nucella spp*., where moderate warming enhances foraging activity and growth (Sanford 2002, Yamane & Gilman 2009).

This trade-off between survival and growth was particularly evident in *N. ostrina*. At 24 °C, survivorship remained relatively high (>70%) after 24 d; growth, however, was only sustained during the first 6 d and declined afterwards. Although feeding activity appeared to continue throughout the experiment, as indicated by drill holes on barnacles provided *ad libitum* (pers. obs.), this did not translate into sustained growth. By day 30, survivorship dropped sharply to approximately 5%. This pattern suggests that prolonged thermal stress imposes either cumulative energetic costs or tissue damage that cannot be fully compensated by food intake, eventually leading to metabolic failure and mortality. Similar trade-offs have been documented in adults of *Mytilus californianus* under extended thermal stress (Fitzgerald-Dehoog et al. 2012) and may reflect the physiological challenges experienced during marine heatwaves, where extended exposure leads to cumulative negative effects (Olabarria et al. 2016, Masanja et al. 2023).

Interspecific variation in the responses of EBP marine invertebrates under extended exposure to elevated temperatures

The effects of temperature on survivorship were strongly species-dependent. *B. glandula* experienced a steep decline at 30 °C after only 10 d (~30% survivorship), whereas

C. dalli experienced much higher survivorship (~80%) under the same conditions. Among the two mollusc species, *N. ostrina* reached ~5% survivorship at 24 °C after 30 d, while *M. trossulus* maintained high survivorship (~90%) at the same temperature and duration. Growth responses also differed among species, with inverted U-shaped relationships between growth rate and duration of exposure in *C. dalli* and *M. trossulus*, a linear decline in *B. glandula*, and a U-shaped response in *N. ostrina*. These patterns highlight species-specific differences in the magnitude, timing, and pattern of responses to extended thermal exposure.

The observed interspecific variation in survivorship and growth likely reflects differences in life history traits, physiological capacity, and ecological strategies typical of intertidal species (Somero 2002, Dong et al. 2022, Leeuwis & Gamperl 2022). In the case of barnacles, *B. glandula* experienced rapid mortality at temperatures that *C. dalli* tolerated well, *C. dalli* maintaining growth and survivorship above 50% at 30 °C. This difference may relate to vertical zonation and ecological strategies: although both species occupy the mid- to upper-intertidal zone, *C. dalli* extends slightly higher in the intertidal zone (Miner et al. 2005), where thermal and desiccation stress are more severe. The higher thermal tolerance observed in *C. dalli* is consistent with the general pattern that species from higher intertidal zones exhibit greater tolerance to environmental extremes than species occupying lower levels in the intertidal zone (Stephenson & Stephenson 1949, Helmuth et al. 2006, Leeuwis & Gamperl 2022).

Among the two molluscs, *N. ostrina* and *M. trossulus*, differences in motility and habitat use may also contribute to variation in survivorship and thermal tolerance. *N. ostrina* is a motile gastropod capable of seeking refuge in cooler microhabitats in natural settings, thereby reducing direct exposure to thermal stress (Gosselin & Chia 1995). In contrast, *M. trossulus* is largely sessile as an adult, remaining attached to the substrate by byssal threads. Although juvenile mussels are moderately motile during the first weeks after settlement, prior to becoming sessile (Bayne 1964), their capacity to behaviourally avoid prolonged thermal stress is limited compared to fully motile species. As a result, EBP *M. trossulus* may rely more strongly on physiological tolerance to withstand elevated temperatures, whereas *N. ostrina* can partially reduce thermal stress through behavioural avoidance during the EBP and

throughout all juvenile life. This difference may also help explain why the thermal tolerance thresholds of both mollusc species were lower than those of the barnacles. These two barnacle species occupy higher positions in the intertidal and experience more frequent exposure to heat and desiccation during low tide than *N. ostrina* and *M. trossulus*, such that greater physiological tolerance is likely favoured in these barnacles compared to species that experience less extreme thermal conditions through lower intertidal distribution or movement.

Overall, these findings reinforce the understanding that thermal tolerance is highly species-specific and that physiological differences are likely associated with differences in vertical distribution and in ecological traits such as motility (Somero 2002, 2005, Dong et al. 2022, McIntire & Miller 2025). Tolerance may also differ between atmospheric and marine heatwaves, particularly among species occupying different intertidal zones. Consequently, responses to thermal stress cannot be generalized across species, even species living in the same ecosystem, emphasizing the importance of multi-species approaches when predicting community-level responses to climate change.

Acute vs. extended thermal tolerance thresholds

As mentioned above, a key finding of this study is that thermal tolerance thresholds of EBP individuals, measured as LT_{50}, under extended exposure (6–30 d) were substantially lower than those reported from acute aerial thermal tolerance tests of the same life stage and species (Hamilton & Gosselin 2020). Temperatures previously considered sublethal under short-term aerial exposure caused significant mortality and reduced growth when sustained over days or weeks in seawater. This pattern is consistent with previous evidence from tropical intertidal and subtidal adult marine invertebrates from seven phyla, including crustaceans and molluscs, showing that upper thermal limits decline under prolonged exposure, although the magnitude of this reduction is species-specific (Nguyen et al. 2011).

The lower thermal tolerance thresholds observed under extended exposure likely reflect the distinct physiological demands imposed by short- versus long-term thermal stress, as thermal performance depends strongly on the time scale of temperature exposure (Schulte

et al. 2011). Acute exposure typically triggers rapid, short-term responses, such as increased metabolic rate or behavioural avoidance (Leeuwis & Gamperl 2022, Masanja et al. 2023) that may not be sustainable in the longer term. In contrast, extended or chronic exposure requires sustained physiological adjustments, including energy reallocation, cellular repair, and maintenance processes (Tomanek 2010, Masanja et al. 2023); over time, these processes impose increasing energetic costs that constrain growth and reduce survivorship (Pörtner & Farrell 2008). In addition to exposure duration, differences in exposure medium may have contributed to the differences between acute and extended thermal tolerance estimates, as the same temperature can have different effects on organisms in air and water. When temperatures exceed critical thresholds, compensatory mechanisms are no longer sufficient to maintain cellular homeostasis. This can lead to protein denaturation, mitochondrial dysfunction, and irreversible cellular damage (Somero 2010, Leeuwis & Gamperl 2022, Jørgensen et al. 2022, Masanja et al. 2023). This breakdown was evident at the highest temperatures in the present study, where mortality occurred early in the exposure period, suggesting that these conditions exceeded the species' capacity for thermal compensation.

Overall, these findings demonstrate that thermal tolerance is not fixed but depends strongly on the duration of exposure. As marine heatwaves become more frequent and prolonged under climate change, organisms are increasingly likely to experience extended periods of thermal stress, which may lead to greater impacts on survival, growth, and population persistence than predicted from acute tolerance estimates alone.

Implications for the long-term persistence of intertidal invertebrate species on the BC coast

Projections based on present-day thermal tolerance thresholds estimated in this study suggest these species may persist for several centuries under current rates of warming. However, projected persistence timelines varied among species by hundreds of years and were considerably shorter than projections based on previously reported acute tolerance thresholds for adults. Maximum summertime seawater temperatures off the BC coast would reach the thermal tolerance limits of the two mollusc species approximately 300 y earlier than for the two barnacle species. Likewise, the projected thermal limit for EBP *B. glandula*

under extended exposure (present study) would be reached approximately 700 y earlier than projections based on acute exposure estimates (air) for adults reported by Iwabuchi & Gosselin (2020). These differences suggest that estimates based exclusively on adult stages or short-term exposure experiments may overestimate how much time these populations have before increasing temperatures exceed their current tolerance thresholds. Persistence, however, will likely depend not only on current thermal tolerance thresholds but also on the ability of individuals to acclimate and populations to adapt to increasingly warm and variable thermal environments. The shorter timelines reported in this study indicate the populations have less time to evolve increased tolerance thresholds than previously reported. As for the exact timeline for each species, that will depend on whether the warmest summertime sea surface temperatures (SST) along the west coast of Vancouver Island continue to increase at a rate of ~0.81 °C per century as projected by Iwabuchi & Gosselin (2019). Therefore, these timelines should be considered estimates based on the assumption that the current rate of warming will continue, rather than exact predictions of future conditions.

Finally, it should be noted that SST measurements and projections likely underestimate the thermal conditions experienced by intertidal organisms. During summer, air and rock temperatures in intertidal zones of Vancouver Island can vary by more than 20 °C over a 12-hour tidal cycle (Jenewein & Gosselin 2013, Iwabuchi & Gosselin 2019), often exceeding SST, particularly during low tide under solar radiation. Future persistence will therefore depend not only on gradual increases in average SST and air temperature, but also on the frequency, intensity, and duration of extreme atmospheric heat events. The 2021 Pacific Northwest heatwave, which coincided with low tides, resulted in rock surface temperatures during low tide exposure reaching up to 50 °C in some locations and caused considerable mortality of intertidal species such as *B. glandula* and *M. trossulus*, with losses estimated in the billions of individuals (White et al. 2023). Such extreme events can exceed physiological limits and override projections based on gradual warming trends. Because early life stages are generally more sensitive to thermal and desiccation stress than larger juveniles and adults (Jenewein & Gosselin 2013, Hamilton & Gosselin 2020), even if average SST remains below adult tolerance thresholds, repeated or prolonged exposure to elevated

temperatures may reduce recruitment and ultimately alter community structure, potentially contributing to shifts in species distributions under climate change (Sunday et al. 2012).

In conclusion, EBP marine invertebrates are more vulnerable to extended thermal stress than suggested by acute tolerance tests. While short-term exposure studies indicate that the EBP of these organisms can withstand high temperatures, their responses under extended exposure reveal significantly lower thermal tolerance thresholds. This finding leads to substantially altered projections of when current thermal tolerance thresholds will be reached by warming oceans, bringing the timeline earlier by hundreds of years. The present study also indicates that reliance on acute exposure tests, or on tolerance thresholds of adults, substantially overestimates the resilience of intertidal invertebrate species to climate change. Going forward, reaching a more complete understanding of how marine invertebrates will respond to climate change will require studies exploring the responses of the EBP to fluctuating thermal regimes or to multiple co-occurring environmental stressors, and documenting the extent of seasonal variation in EBP thermal tolerance thresholds.

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CHAPTER 3: Carry-over effects of initial acute and extended thermal exposure on the performance of early benthic phase intertidal marine invertebrates

INTRODUCTION

Increasing air and surface seawater temperatures associated with climate change are altering environmental conditions in coastal ecosystems, potentially affecting the performance and persistence of marine organisms (Harley et al. 2006, Guild et al. 2025). Among coastal ecosystems, rocky intertidal shores are particularly vulnerable because organisms alternate between immersion in seawater and exposure to air; during low tides, body temperatures may substantially exceed those of the surrounding seawater (Iwabuchi & Gosselin 2019, IPCC 2022). In addition, the frequency, intensity, and duration of marine heatwaves and other extreme temperature events are increasing (Smith et al. 2021, Zhang et al. 2024). Consequently, intertidal organisms are expected to experience repeated episodes of elevated temperatures and prolonged periods of thermal stress over the course of a single summer. Under these conditions, the biological consequences of warming may depend not only on the intensity and duration of individual thermal events but also on whether previous exposure to elevated temperature alters responses to subsequent episodes of thermal stress (Ishida et al. 2023, Haskett et al. 2024).

Physiological changes induced during an initial thermal event may influence how organisms respond to subsequent episodes of thermal stress, ultimately affecting later performance (O'Connor et al. 2014, Moore & Martin 2019). These lasting influences are referred to as carry-over effects, whereby previous environmental conditions contribute to the current performance of the individual (O'Connor et al. 2014). In marine invertebrates, studies of carry-over effects also referred to as latent effects) have mostly focussed on the effects of environmental conditions experienced by larvae on their post-settlement performance (Pechenik 2006); however, carry-over effects may also arise following sublethal thermal stress experienced within a single life stage. Since sublethal exposure to elevated temperatures can alter physiological condition without causing immediate mortality (Sanford 2002, O'Connor et al. 2014), individuals that survive an initial thermal event may subsequently exhibit improved, reduced, or unchanged performance depending on the

intensity and duration of exposure, species, and developmental stage (Pechenik 2006, Donelan et al. 2021, Rebolledo et al. 2023).

Intertidal organisms have evolved a range of behavioural and physiological strategies that allow them to cope with elevated temperatures and mitigate the effects of thermal stress under variable environmental conditions (Tomanek & Helmuth 2002, Leeuwis & Gamperl 2022). Behavioural responses include seeking shaded microhabitats, occupying lower shore positions, reducing activity during periods of elevated temperatures, and modifying body orientation to reduce heat (Leeuwis & Gamperl 2022, Masanja et al. 2023). Physiological responses include acclimation, metabolic adjustments, modifications in membrane properties, and the production of heat shock proteins that help maintain cellular function during thermal stress (Somero 2010, 2020, Tomanek 2010). Because the capacity to employ these strategies varies among species, body sizes, and developmental stages, organisms exposed to similar thermal conditions may exhibit different carry-over effects.

Among developmental stages, the early benthic phase (EBP) is considered one of the most critical periods in the life cycle of marine invertebrates (Gosselin & Qian 1997, Gosselin 1997). The EBP encompasses the first days of benthic life following settlement from the plankton or emergence from benthic egg capsules (Gosselin & Qian 1997). During this period, individuals undergo major morphological, physiological, and ecological changes while transitioning to benthic conditions (Rodriguez et al. 1993, Gosselin & Qian 1997, Hunt & Scheibling 1997, Gosselin 1997). At the same time, juveniles exhibit rapid growth and development while being exposed to strong selective pressures arising from both biotic and abiotic factors (Gosselin & Qian 1996, Gosselin 1997). Mortality during the EBP is often extremely high, and individuals that survive this period contribute directly to recruitment because larger juveniles are generally less vulnerable to predation and environmental stress than newly settled individuals (Gosselin & Qian 1996, Gosselin 1997, Moran & Emlet 2001). Understanding how elevated temperatures experienced during the EBP affect subsequent juvenile performance is therefore important for predicting recruitment success and the persistence of marine invertebrate populations under future climate change scenarios.

Although carry-over effects have been documented across a variety of taxa and life stages, most studies in marine invertebrates have focused on larval stages, metamorphosis, or adult organisms (Pechenik 2006, O'Connor et al. 2014). Comparatively little attention has been given to the EBP despite its importance as a period of rapid growth and high mortality. Given that intertidal organisms typically experience repeated episodes of elevated temperatures during the spring and summer on rocky shores (Hamilton & Gosselin 2020, Iwabuchi & Gosselin 2020), thermal conditions encountered during the EBP might influence not only immediate performance but also later survivorship, growth, and thermal tolerance through carry-over effects (Pechenik 2006, Moore & Martin 2019). Evaluating these potential carry-over effects is therefore necessary to improve predictions of how intertidal populations respond to increasingly frequent thermal stress associated with climate change and the implications for recruitment success and population persistence in intertidal ecosystems (Harrison et al. 2011, Moore & Martin 2019).

This study aimed to evaluate whether an initial exposure to elevated sublethal temperature produces carry-over effects on the performance of EBP marine invertebrates. Specifically, EBP individuals were exposed to temperatures previously identified as sublethal to: (1) determine whether an initial acute (6 h) exposure to elevated air temperature alters subsequent survivorship, growth, and thermal tolerance thresholds, measured as LT_{50} estimates, later in juvenile life; (2) evaluate whether an initial extended (5 d) exposure to elevated water temperature influences subsequent survivorship, growth, and thermal tolerance thresholds; and (3) characterize interspecific variation in the magnitude and direction of carry-over effects. Based on previous studies, it was predicted that an initial exposure to elevated sublethal temperatures during the EBP would alter subsequent performance, with stronger negative effects expected following an extended exposure than following an acute exposure. Carry-over effects were also expected to differ among species according to their motility, with sessile species predicted to be less negatively affected by thermal exposure than motile species that can behaviourally avoid stressful temperatures.

METHODS

Study species

For the first part of this study, investigating the carry-over effects of an initial acute (6 h) exposure to sublethal air temperatures on EBP marine invertebrates, three common and abundant intertidal species that were consistently available in the study area were selected: the barnacles *Balanus glandula* Darwin, 1854 and *Chthamalus dalli* Pilsbry, 1916, and the snail *Nucella ostrina* Gould, 1852. *C. dalli* and *B. glandula* are commonly found in the high intertidal, while *N. ostrina* typically occupies the mid-intertidal zone. The two barnacle species are sessile filter-feeders, while *N. ostrina* is a motile predator (Morris et al. 1980, Gosselin & Chia 1994). For the second part of the study, evaluating the effects of an initial extended (5 d) exposure to sublethal water temperatures, only hatchlings of *N. ostrina* were tested.

Study site and collection of organisms

Experiments were conducted using EBP individuals collected from rocky intertidal shores in Barkley Sound on the west coast of Vancouver Island. Newly settled juveniles of the barnacles *B. glandula* and *C. dalli* were collected in May 2025 from a rocky shore in Kelp Bay (48°51'02.0"N 125°07'06.1"W). To obtain EBP individuals of *N. ostrina*, ripe egg capsules were collected at First Beach near Prasiola Point (48°49'06.6"N 125°09'49.4"W) in June 2025 for acute exposure trials and again in July 2025 for extended exposure experiments. All laboratory work was conducted at the Bamfield Marine Sciences Centre (BMSC).

Newly settled juveniles of the barnacles *C. dalli* and *B. glandula* were obtained following the methodology described by Sandee et al. (2016) in which small rocks (5–10 cm in diameter) colonized by adult barnacles were collected from the mid-intertidal zone and transported to BMSC, where all adult and juvenile barnacles were removed. The rocks were subsequently returned to the field for 72 h to allow new settlers to attach before being recovered and brought back to the laboratory. Rocks bearing newly settled barnacles were maintained in filtered seawater at 15–17 °C for an acclimation period of 24–36 h,

corresponding to the average summertime seawater temperatures recorded in Barkley Sound (Iwabuchi & Gosselin 2019).

Newly hatched *N. ostrina* were obtained following the methodology described by Gosselin & Chia (1995), in which ripe egg capsules were carefully detached from the substrate using tweezers. Egg capsules were transported to the laboratory, placed in meshed 50 ml plastic containers, and maintained in aerated seawater at 10–11 °C. Hatchlings emerging during the subsequent 72 h were retained for use in the experiments.

Temperature treatments and laboratory setting

Carry-over effects of an initial acute (6 h) exposure to elevated sublethal air temperatures

To evaluate the carry-over effects of an initial acute exposure to elevated sublethal temperatures during the EBP, individuals of each species were subjected to short-term aerial heat exposure under controlled laboratory conditions, followed by a return to average summertime seawater temperatures. The experimental procedure consisted of four consecutive steps: (1) initial acute exposure of EBP individuals to four elevated but sublethal air temperatures for 6 h, (2) rearing individuals in average summertime seawater temperature conditions for one month, (3) at the end of step 2, assessment of survivorship and body size, and (4) second exposure to elevated temperature, this time to determine the LT_{50} (lethal temperature causing 50% mortality in a population) for EBP individuals of each species for each of the initial acute temperature treatments.

During the acute exposure (step 1), rocks bearing newly settled *C. dalli* and *B. glandula*, as well as petri dishes containing newly hatched *N. ostrina*, were placed inside sealed plastic containers lined with a cloth saturated with freshwater to maintain high humidity, ensuring the animals were exposed to thermal stress without desiccation stress. Each container also included a Thermochron iButton temperature logger (model DS1921G-F5) programmed to record temperature every 10 min. Containers were then transferred to incubators, each one set to one of the four sublethal air temperatures for 6 h.

Acute temperature treatments in step 1 included a control (15–17 °C), representing air temperatures that are common in the mid-intertidal zone of Barkley Sound during the summer (Jenewein & Gosselin 2013a), and three warmer experimental temperatures selected based on previously reported acute LT_{50} values under 6 h aerial exposure (Hamilton & Gosselin, 2020) as well as preliminary trials for this study. For *C. dalli* and *B. glandula*, elevated temperature treatments consisted of 25 °C, 30 °C, and 34 °C, whereas elevated temperature treatments for *N. ostrina* were 20 °C, 24 °C, and 28 °C. The actual mean temperatures recorded by the data loggers in the incubators, along with their corresponding standard deviations, are presented in Table 3.1. Survivorship immediately after the exposure period of step 1 was high in all treatments and species, ranging from 90 to 100% across replicates, confirming the initial temperature treatments were sublethal. The experimental design for *C. dalli* and *B. glandula* consisted of four acute air temperature treatments, five replicate groups of individuals per treatment, and 15 individuals per replicate group, for a total of 300 EBP individuals per species. For *N. ostrina*, the design consisted of four acute air temperature treatments, five replicate groups of individuals per treatment, and 12 individuals per replicate group, for a total of 240 EBP individuals.

Table 3.1. LT_{50} values of EBP individuals under acute (6 h) exposure to elevated air temperature reported by Hamilton & Gosselin (2020), and initial acute (6 h) sublethal air temperature treatments employed in the present study (step 1). Air temperature values represent average ± SD.

Species	LT_{50} in air (Hamilton & Gosselin, 2020)	Initial acute sublethal air temperature treatments			
		Control	Intermed. 1	Intermed. 2	Highest
C. dalli	45.8 °C (EBP)	16.0 ± 0.1 °C	24.6 ± 0.9 °C	29.4 ± 1.4 °C	33.9 ± 1.9 °C
B. glandula	42.8 °C (Adults)	16.0 ± 0.1 °C	24.6 ± 0.9 °C	29.4 ± 1.4 °C	33.9 ± 1.9 °C
N. ostrina	32.3 °C (EBP)	16.0 ± 0.1 °C	20.1 ± 0.6 °C	24.5 ± 0.2 °C	28.4 ± 0.2 °C

The second step (2) involved rearing EBP individuals in the laboratory under average summertime seawater temperatures (15–17 °C) (see Appendix A for details of the laboratory setup and feeding procedures). The rearing period lasted 30 d for *N. ostrina* and *B. glandula*, and 35 d for *C. dalli*, the slightly longer duration for *C. dalli* being due to the logistics of running simultaneous experiments with both barnacle species.

In step 3, survivorship was assessed by observing 1-month-old individuals under a dissecting microscope for a maximum of 5 min each. Body size at the end of the rearing period was also measured using an ocular scale at 40× magnification. For barnacles, rostro-carinal diameter (RCD) was measured, whereas shell length (SL) was recorded for snails.

The fourth step (4) involved subjecting EBP individuals to a second exposure to elevated temperatures to estimate their thermal tolerance (LT_{50}) one month after they had been exposed to elevated sublethal temperatures. The procedure to estimate the LT_{50} followed the methods described by Hamilton & Gosselin (2020). For this step of the experiment, all surviving 1-month-old individuals from replicates within the same initial

acute air temperature treatment (step 1) were exposed to a sequence of increasing and eventually lethal air temperature treatments, each treatment lasting 6 h, and with subsequent temperatures increasing in 2 °C intervals (Table 3.2.). Between each temperature exposure, individuals were provided a 6 h recovery period in 16 °C seawater; individuals were checked for mortality during the last 2 h of the recovery period. Dead individuals were removed after each exposure, whereas surviving individuals were transferred to the next higher temperature treatment until 100% mortality was reached. The temperature at death and the number of survivors per replicate after each exposure were recorded to estimate LT_{50} values.

Table 3.2. Air temperature treatments applied to 1-month-old juveniles of three invertebrate species in step 4, to determine the LT_{50} after an initial acute (6 h) exposure to sublethal temperatures.

Species	n	Step 4 air temperature treatments
C. dalli	277	36, 38, 40, 42, 44
B. glandula	252	34, 36, 38, 40, 42
N. ostrina	230	26, 28, 30, 32, 34

Carry-over effects of extended (5 d) exposure to elevated sublethal water temperatures

This objective was addressed using a methodology similar to that described for the initial acute exposure experiment, but using only *N. ostrina* and with a modification to step 1 involving a prolonged initial exposure to elevated seawater temperatures for 5 d instead of a 6 h aerial exposure. Step 1 included a control (16 °C) treatment as well as three elevated sublethal water temperature treatments: 20 °C, 24 °C and 28 °C. The actual mean temperatures recorded by the data loggers during seawater exposure are presented in Table 3.3. Details of the experimental tank setup and feeding procedures are provided in Appendix A. The experimental design consisted of four initial sublethal water temperature treatments, five replicate groups per treatment, and 12 individuals per replicate group, for a total of 240 EBP individuals of *N. ostrina*. However, due to complete mortality of individuals in the 28

°C treatment after the 5 d exposure, this temperature treatment was removed from further analysis.

Table 3.3. Initial sublethal water temperature treatments employed in the experiment examining carry-over effects of extended (5 d) exposure in EBP *N. ostrina.* Water temperature values represent average ± SD. (n = 60 temperature measurements per treatment)

Species	LT_{50} in air (Hamilton, 2020)	Initial sublethal water temperature treatments		
		Control	Interm. 1	Highest
N. ostrina	32.3 °C (EBP)	14.5 ± 0.9 °C	19.8 ± 0.3 °C	23.5 ± 0.4 °C

Following the initial extended exposure period (step 1), all (100%) EBP *N. ostrina* individuals survived across all treatments, confirming the initial treatments were sublethal. These EBP *N. ostrina* were then reared for 30 d in seawater maintained at 15–17 °C (step 2, rearing). Feeding procedures for *N. ostrina* hatchlings during the rearing were the same as those used in the acute exposure experiments (see Appendix A). Survivorship and shell length (SL) were recorded at the end of the rearing period (step 3).

To assess the thermal tolerance (LT_{50}) of EBP *N. ostrina* one month after they had been exposed to sublethal water temperatures for 5 d (step 4), procedures from Iwabuchi & Gosselin (2020) were used with modifications to the duration of the temperature treatments and recovery. Groups of 1-month-old individuals previously exposed to the different initial extended (5 d) water temperature treatments were subjected to successive 24 h periods of seawater temperature exposure, ranging from 26 °C to 32 °C, with temperature increments of 2 °C. Between these temperature exposures, individuals were provided a 24 h recovery period in 16 °C seawater and were checked for mortality during the last 2 h of the recovery period. Dead individuals were removed after each exposure, whereas surviving individuals were transferred to the next higher temperature treatment until 100% mortality was reached. Water temperature at death and number of survivors per replicate following each exposure were recorded to estimate LT_{50} values in water.

Data analysis

The following analyses were conducted separately for each species and for each of the two experiments, assessing the carry-over effects of an initial acute (6 h) and initial extended (5 d) exposures to elevated sublethal air and water temperatures, respectively. All statistical analyses were performed in R Statistical Software (v.4.5.0) (R Core Team 2025).

Survivorship of 1-month-old EBP individuals after initial exposure to elevated sublethal temperatures

Differences in survivorship after the 1-month rearing period among initial temperature treatments were analyzed using generalized linear models (GLMs) with a logit link function and a binomial distribution, fitting temperature as the predictor and survivorship (alive/dead) as the binary response. For all models, the control treatment was the reference level. The overall effect of initial temperature treatment on survivorship was assessed using a likelihood ratio test via the drop1() function from the lmerTest package (Kuznetsova et al. 2026). Model fit and dispersion were assessed using the residual deviance-to-degrees-of-freedom ratio and overdispersion and zero-inflation tests from the DHARMa package (Hartig et al. 2024). Estimated marginal means (EMMs) and 95% confidence intervals were calculated on the response scale using the emmeans package (Lenth et al. 2025) to estimate survivorship probabilities for each treatment and visualize differences among treatments.

For *N. ostrina* under initial extended thermal exposure, fitting a GLM was not possible due to perfect separation in the model. Survivorship was 100% across all replicates in the 16 °C and only one individual died in the 20 °C treatment, which prevented stable parameter estimations in the model. Observed survivorship per treatment is therefore reported as mean ± standard deviation, and the effect of treatment on survivorship was assessed using a Kruskal-Wallis test on replicate-level survivorship proportions, followed by Dunn's post-hoc test for pairwise comparisons. One replicate in the 24 °C treatment was additionally excluded from the analysis, as complete mortality was observed after the 30 d rearing (step 3).

Size of 1-month-old EBP individuals after initial exposure to elevated sublethal temperatures

Differences in body size (RCD or SL) among surviving individuals, one month after initial temperature treatments, were analyzed separately for each species. Data on RCD (barnacles) and SL (*N. ostrina*) were first tested for normality using the Shapiro-Wilk test. Linear mixed models (LMMs) were fitted using the lme4 package (Bates et al. 2025), with treatment as a fixed effect and replicate as a random effect, and the normality of model residuals was assessed using the Shapiro-Wilk test. Where residuals were non-normal, data were log-transformed, and models were refitted. The overall effect of treatment was assessed using F-tests via the drop1() function from the lmerTest package (Kuznetsova et al. 2026). For *B. glandula* and *N. ostrina*, residuals remained non-normal after log-transformation; therefore, Kruskal-Wallis tests were conducted on replicate means, and the results from both approaches were consistent. Pairwise comparisons with a Bonferroni correction using the emmeans package (Lenth et al. 2025) were conducted when the models detected differences in sizes across initial thermal exposure treatments.

Thermal tolerance thresholds of 1-month-old EBP individuals after initial exposure to elevated sublethal temperatures

To calculate the LT_{50} for each initial temperature treatment and species, survivorship recorded at each successive temperature of the thermal tolerance trial (step 4) was summarised as the proportion of survivors per replicate, weighted by the total number of individuals per replicate at the start of each temperature step, which varied among replicates due to mortality during the 1-month rearing period. For each species, a single GLM with a binomial distribution and logit link function was fitted to all initial sublethal treatments simultaneously, including an interaction term between recorded lethal temperature and initial sublethal temperature treatment to allow survivorship curves to vary among initial temperature treatments. Where perfect separation prevented stable parameter estimation, a Bayesian modification of the binomial GLM was applied using the bayesglm() function from the arm package (Gelman et al. 2026), following the approach of Hamilton & Gosselin (2020). This approach was used for all three species. Model fit was assessed using the

residual deviance-to-degrees-of-freedom ratio and visual inspection of Pearson residuals plotted against fitted values. The influence of Bayesian priors was evaluated by comparing coefficients from the Bayesian and standard binomial GLMs.

LT_{50} values and standard errors were extracted using the dose.p() function from the MASS package (Ripley et al. 2025), refitting the model with each treatment set as the reference level in turn. Confidence intervals (95%) were calculated as $LT_{50} \pm 1.96 \times SE$. Differences in survivorship curves among initial sublethal treatments were assessed using pairwise comparisons with Bonferroni correction via the emmeans package (Lenth et al. 2025). Where substantial prior dependence was detected for specific initial sublethal treatments, LT_{50} values were additionally estimated using the non-parametric Spearman-Karber method (Hamilton et al. 1977). In all cases, Spearman-Karber estimates differed from the Bayesian GLM estimates by less than 0.3 °C, supporting the reliability of the LT_{50} values extracted from Bayesian GLMs.

RESULTS

Carry-over effects of an initial acute (6 h) exposure to elevated sublethal air temperatures

Survivorship of 1-month-old EBP individuals after initial acute exposure to elevated sublethal temperatures

Survivorship of the three species after the 30-35 d rearing period under ambient conditions was generally high, being >80% across all treatments. Initial exposure to sublethal acute temperature treatments nevertheless significantly affected survivorship in the two species of barnacles, but not in *N. ostrina*. (Table 3.4.). In *C. dalli*, survivorship significantly increased with higher initial temperatures (LRT: $\chi^2 = 13.29$, df = 3, p = 0.004), with estimated survival probabilities ranging from 0.85 (95% CI: 0.754–0.917) in the 16 °C control to 0.987 (95% CI: 0.911–0.998) at 34 °C (Fig. 3.1. A.). The 30 °C and 34 °C treatments showed the highest survival probabilities, with coefficients significantly higher

than the control (Table 3.4). In *B. glandula*, a similar trend was observed, but only the 34 °C treatment differed significantly from the control (Table 3.4.); the overall effect of treatment was also significant (LRT: $\chi^2 = 15.50$, df = 3, p = 0.001). In contrast, survivorship of *N. ostrina* was consistently high across all initial acute temperature treatments, with no significant effect of initial temperature treatment on survivorship to day 30 (LRT: $\chi^2 = 4.46$, df = 3, p = 0.216), and estimated survival probabilities ranging narrowly from 0.917 (95% CI: 0.790–0.969) at 16 °C to 0.976 (95% CI: 0.851–0.996) at 28 °C (Fig. 1. C.).

Table 3.4. Generalized linear models analysis of the effects of initial acute (6 h) elevated temperature treatments on the survivorship of EBP marine invertebrates through the subsequent rearing period of 30-35 d under ambient conditions. Control treatment (16 °C) was used as the reference level. ($\alpha = 0.05$).

Species	Effect	Estimate	SE	z value	Pr (>|z|)
C. dalli	Intercept	1.761	0.326	5.395	**<0.001**
	Treatment 25 °C	0.878	0.566	1.550	0.122
	Treatment 30 °C	1.836	0.787	2.332	**0.019**
	Treatment 34 °C	2.543	1.058	2.403	**0.016**
B. glandula	Intercept	1.304	0.289	4.802	**<0.001**
	Treatment 25 °C	0.375	0.436	0.860	0.390
	Treatment 30 °C	-2.2e-16	0.408	0.000	1.000
	Treatment 34 °C	2.211	0.772	2.862	**0.004**
N. ostrina	Intercept	2.398	0.467	5.134	**<0.001**
	Treatment 20 °C	1.679	1.111	1.511	0.131
	Treatment 24 °C	0.969	0.857	1.130	0.258
	Treatment 28 °C	1.679	1.111	1.511	0.131

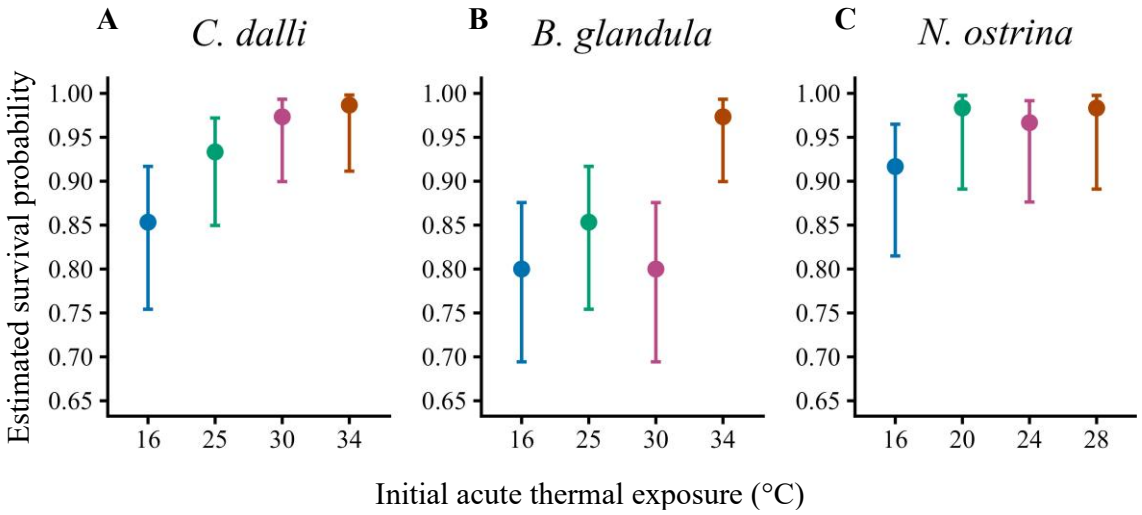

Figure 3.1. Estimated survival probabilities of EBP marine invertebrates after an initial acute (6 h) exposure to sublethal elevated air temperature treatments and a rearing of 30-35 d under ambient conditions. Points represent marginal means estimated from generalized linear models; bars indicate 95% asymptotic confidence intervals.

Size of 1-month-old EBP individuals after initial acute exposure to elevated sublethal temperatures

The initial acute elevated temperature treatments had no significant effect on body size (RCD and SL) reached on day 30 in *B. glandula* or *N. ostrina* under any of the analyses conducted ($p > 0.05$ in Kruskal-Wallis and LMMs) (Fig. 3.2, Table 3.5.). In *C. dalli*, however, size differed significantly among the initial thermal exposure treatments (LMM on log-transformed data: $F = 4.57$, NumDF = 3, DenDF = 15.88, $p = 0.017$), with individuals from the initial 34 °C treatment being significantly larger than those from the 16 °C and 25 °C treatments (pairwise comparison, $p = 0.033$ and $p = 0.034$, respectively), while no significant differences were detected among the remaining treatment comparisons.

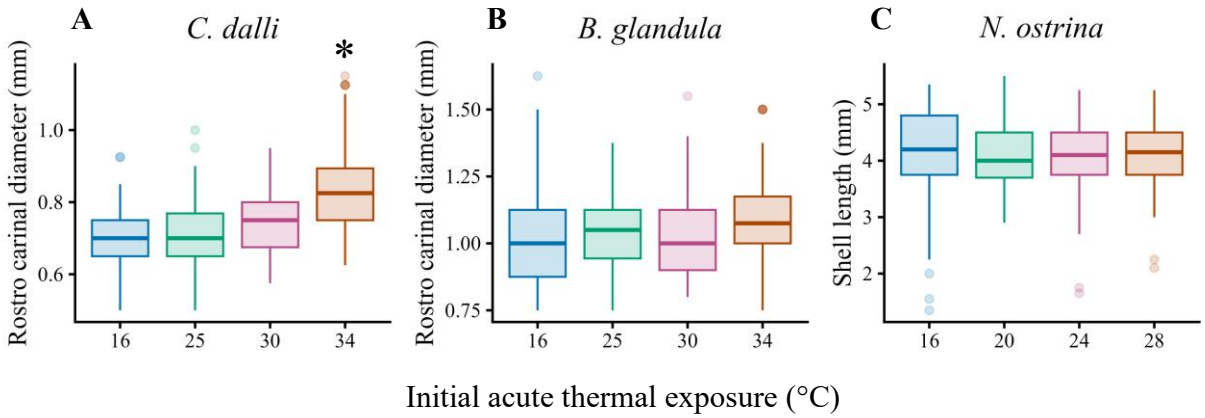

Figure 3.2. Body size of EBP marine invertebrates after an initial acute (6 h) exposure to elevated sublethal air temperatures and a rearing period of 30–35 d under ambient conditions. The boxes show the interquartile range and median; faded points represent observations beyond 1.5 × the interquartile range (outliers). Asterisks (*) indicate treatments that differed significantly from the control ($p < 0.05$).

Table 3.5. Linear mixed models analysis of the effects of initial acute (6 h) elevated temperature treatments on the body size of EBP marine invertebrates through the subsequent rearing period of 30-35 d under ambient conditions. Control treatment (16 °C) was used as the reference level. ($\alpha = 0.05$).

Species	Effect	Estimate	SE	t value	Pr (>|t|)
C. dalli	Intercept	-0.352	0.035	-10.025	**<0.001**
	Treatment 25 °C	0.001	0.049	0.020	0.984
	Treatment 30 °C	0.052	0.049	1.061	0.304
	Treatment 34 °C	0.158	0.049	3.206	**0.005**
B. glandula	Intercept	6.9e-04	3.9e-02	0.018	0.986
	Treatment 25 °C	3.7e-02	5.5e-02	0.674	0.510
	Treatment 30 °C	3.1e-02	5.5e-02	0.551	0.590
	Treatment 34 °C	7.37e-02	5.4e-02	1.347	0.198
N. ostrina	Intercept	1.387	0.040	34.531	**<0.001**
	Treatment 20 °C	0.004	0.056	0.065	0.949
	Treatment 24 °C	-0.009	0.056	-0.174	0.864
	Treatment 28 °C	0.004	0.056	0.076	0.940

Thermal tolerance of 1-month-old EBP marine invertebrates after initial acute exposure to elevated sublethal temperatures

The effect of initial acute thermal exposure on the subsequent thermal tolerance of 1-month-old EBP individuals differed among species in both direction and magnitude (Table 3.6. Fig. 3.4). In the two barnacle species, exposure to the highest acute temperature treatment (34 °C) increased subsequent thermal tolerance, whereas in the snail, *N. ostrina*, initial elevated acute temperatures were associated with lower subsequent thermal tolerance relative to the control treatment. The survivorship curves of the three species, from which the pairwise comparisons were conducted and the LT_{50} were extracted, are provided in Appendix D, Table D. 1. as well as a complete list of LT_{50} estimates, SE and model diagnostics.

In *C. dalli,* individuals exposed to 34 °C exhibited significantly greater thermal tolerance than *C. dalli* individuals from all other initial acute treatments (Figure 3.3, Table 3.6), with LT_{50} values increasing by approximately 2 °C relative to the lower temperature treatments (Fig. 3.4.A.). In *B. glandula*, the positive effect of the 34 °C treatment was weaker, with LT_{50} values differing by less than 1 °C among treatments (Fig. 3.4.B.). Nevertheless, the thermal tolerance curve for the 34 °C treatment differed significantly from those of the 16 °C and 30 °C treatments in *B. glandula* (Table 3.6.).

In contrast, thermal tolerance in *N. ostrina* declined following exposure to elevated acute temperatures. Individuals maintained in the control treatment exhibited significantly greater thermal tolerance than individuals initially exposed to any of the elevated temperature treatments (Table 3.6.). However, differences in LT_{50} values among treatments were relatively small, spanning less than 1 °C across the full range of temperature treatments (Fig. 3.4. C.).

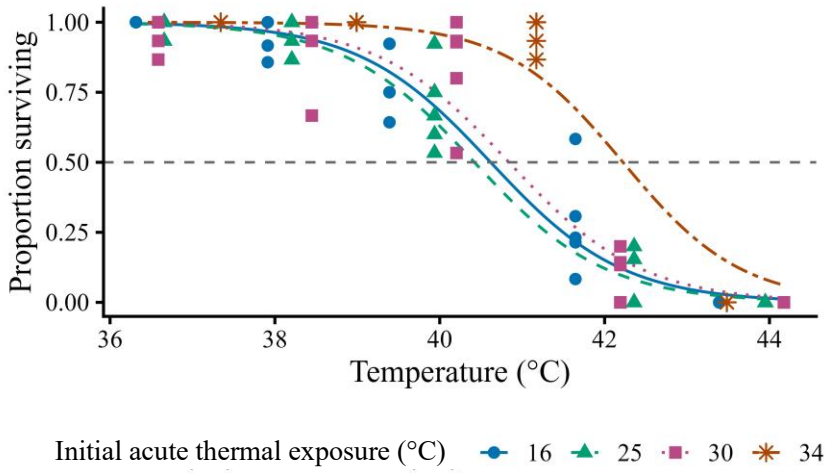

Figure 3.3. Survivorship curves of EBP *Chthamalus dalli* following an initial acute (6 h) exposure to elevated sublethal air temperatures and a 35 d rearing period under ambient conditions. Lines represent fitted Bayesian binomial GLM and points represent observed proportions of individuals surviving in each replicate at each temperature. The dashed horizontal line indicates the LT_{50}.

Table 3.6. Pairwise comparisons of estimated marginal means from Bayesian binomial GLMs, evaluating differences in survivorship curves among initial temperature treatments in EBP marine invertebrates following acute (6 h) exposure to elevated sublethal air temperatures and a 30-35 d rearing under ambient conditions.

Species	Treatments	Estimate	SE	p-value
C. dalli	16 °C vs 25 °C	0.247	0.279	1.000
	16 °C vs 30 °C	-0.276	0.270	1.000
	16 °C vs 34 °C	-5.648	0.860	**<0.001**
	25 °C vs 30 °C	-0.523	0.268	0.307
	25 °C vs 34 °C	-5.895	0.859	**<0.001**
	30 °C vs 34 °C	-5.372	0.856	**<0.001**
B. glandula	16 °C vs 25 °C	-0.221	0.351	1.000
	16 °C vs 30 °C	0.540	0.341	0.679
	16 °C vs 34 °C	-0.904	0.334	**0.040**
	25 °C vs 30 °C	0.762	0.339	0.147
	25 °C vs 34 °C	-0.682	0.333	0.243
	30 °C vs 34 °C	-1.444	0.327	**<0.001**
N. ostrina	16 °C vs 20 °C	1.526	0.403	**<0.001**
	16 °C vs 24 °C	1.709	0.520	**0.006**
	16 °C vs 28 °C	2.801	0.588	**<0.001**
	20 °C vs 24 °C	0.183	0.547	1.000
	20 °C vs 28 °C	1.274	0.603	0.207
	24 °C vs 28 °C	1.091	0.641	0.532

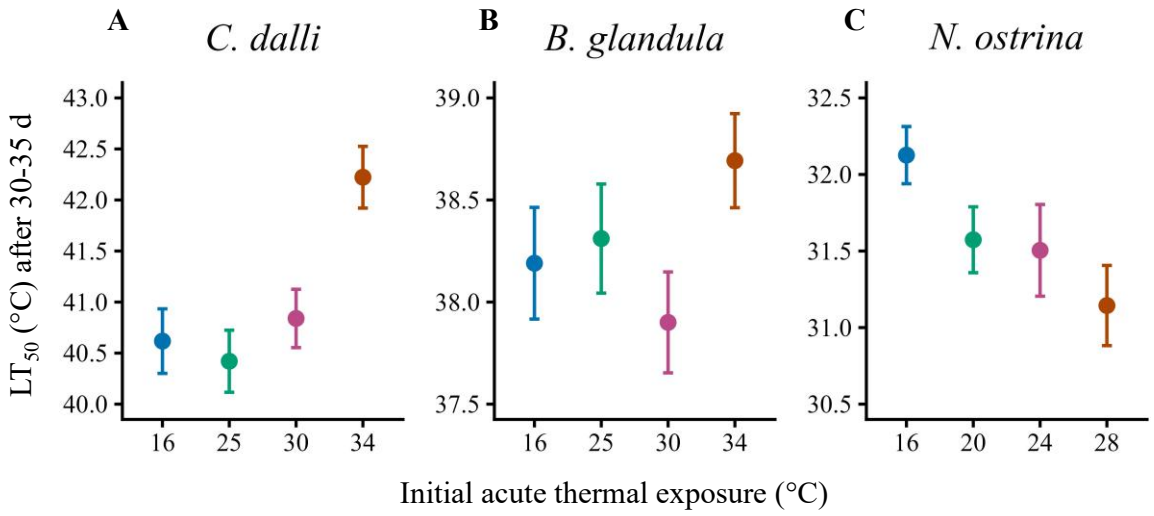

Figure 3.4. Estimated LT_{50} values (°C) of EBP marine invertebrates initially experiencing an acute (6 h) exposure to elevated sublethal air temperatures, followed by 30-35 d rearing under ambient conditions and finally tested for thermal tolerance. Points represent LT_{50} estimates extracted from Bayesian binomial GLMs; bars indicate 95% confidence intervals ($LT_{50} \pm 1.96 \times SE$).

Carry-over effects of an initial extended (5 d) exposure to elevated sublethal water temperatures

Survivorship of 1-month-old EBP individuals after initial extended exposure to elevated sublethal temperatures

Survivorship of *N. ostrina* after the 30 d rearing period remained high in the 16 °C and 20 °C treatments (100 ± 0% and 98.3 ± 3.7%, respectively) but was lower and more variable in the 24 °C treatment. One replicate in the 24 °C treatment experienced complete mortality during the rearing period and was excluded from the analysis, but in the remaining replicates the mean survivorship was 87.5 ± 10.8%, ranging from 75–100% survivors per replicate; Fig. 3.5.). The overall effect of the initial extended temperature exposure on survivorship was significant (Kruskal-Wallis: $\chi^2 = 6.50$, df = 2, p = 0.039), with survivorship in the 24 °C treatment being significantly lower than in the 16 °C control (Dunn's test, p = 0.042), while no significant difference was detected between the 16 °C and 20 °C treatments (p = 0.569).

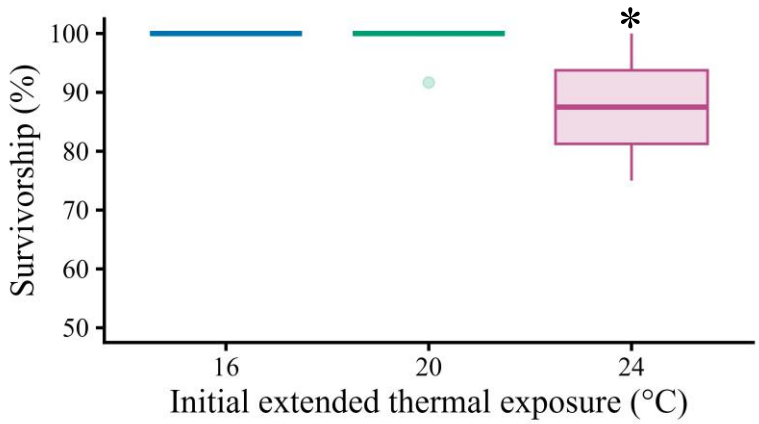

Figure 3.5. Survivorship (%) of *N. ostrina* hatchlings after a 5 d extended exposure to elevated sublethal water temperatures followed by a 30 d rearing period under ambient conditions. Points represent individual replicate values; boxes show the interquartile range and median. Asterisks (*) indicate treatments that differed significantly from the control ($p < 0.05$).

Size of 1-month-old EBP individuals after initial extended exposure to elevated sublethal temperatures

Shell lengths of *N. ostrina* on day 30 of the rearing period did not differ significantly among initial extended water temperature treatments (LMM on log-transformed data: $F = 0.268$, NumDF = 2, DenDF = 11.34, $p = 0.769$; Kruskal-Wallis: $\chi^2 = 1.003$, df = 2, $p = 0.606$; Fig. 3.6.). Although residuals remained non-normal after log-transformation, results were consistent across both statistical approaches.

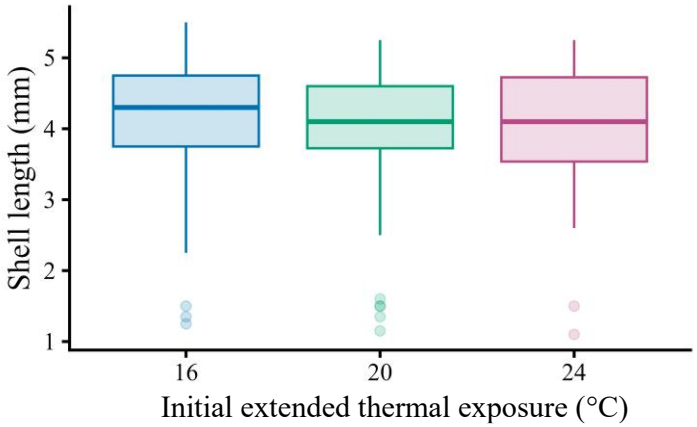

Figure 3.6. Body size of EBP *N. ostrina* after an initial extended (5 d) exposure to elevated sublethal water temperatures and a rearing of 30 d under water ambient conditions. The boxes show the interquartile range and median; faded points represent observations beyond 1.5 × the interquartile range (outliers).

Thermal tolerance of 1-month-old EBP individuals after initial extended exposure to elevated sublethal temperatures

The extended (5 d) initial exposure to elevated sublethal water temperatures had no effect on the thermal tolerance of *N. ostrina* juveniles one month later (Fig. D.2. in Appendix D; Fig. 3.7.). Pairwise comparisons detected no significant differences in survivorship curves among the three initial extended temperature treatments (emmeans pairwise comparisons $p > 0.5$). LT_{50} values differed by less than 0.2 °C among the three temperatures (Fig. 3.7.) and the confidence intervals overlapped broadly, further supporting the absence of an effect of initial extended exposure on subsequent thermal tolerance.

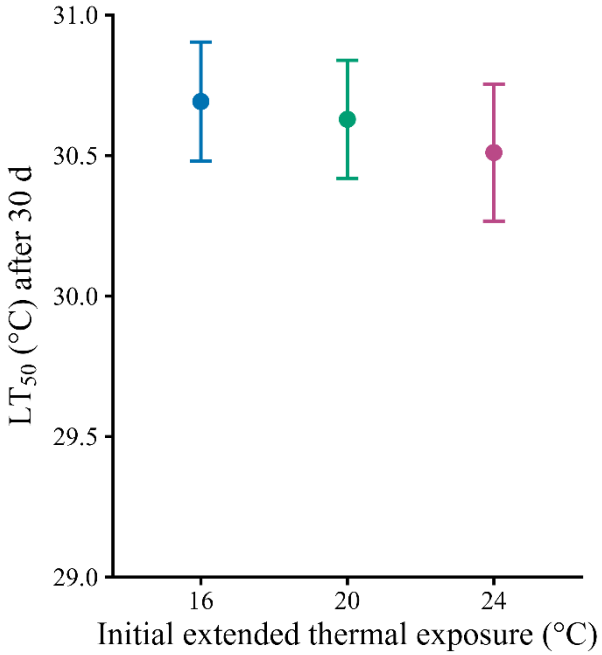

Figure 3.7. LT_{50} values (°C) of EBP *N. ostrina* 30 d after an extended (5 d) exposure to elevated water temperatures. Points represent LT_{50} estimates extracted from Bayesian binomial GLMs; bars indicate 95% confidence intervals ($LT_{50} \pm 1.96 \times SE$).

DISCUSSION

Carry-over effects of an initial acute (6 h) exposure to elevated sublethal air temperatures

Marine invertebrates along the coast of Vancouver Island currently experience summertime temperatures above historical averages (Iwabuchi & Gosselin 2019), and both the intensity and frequency of extreme thermal events are expected to increase under future climate change scenarios (IPCC 2021). The present study demonstrates that a single acute exposure (6 h) to elevated but sublethal temperatures during the EBP of marine invertebrates can produce carry-over effects that persist for at least one month after the initial thermal event. However, the magnitude and direction of these effects differed among species.

The positive carry-over effects observed in the two barnacle species revealed that short-term exposure to elevated temperature enhanced subsequent performance in these

species rather than simply impose physiological costs. Individuals of *C. dalli* and *B. glandula* exposed to 34 °C exhibited higher survivorship and greater thermal tolerance than individuals maintained in the control (16 °C). In *C. dalli*, these benefits were evident even at 30 °C and were accompanied by increased size after one month in the individuals initially exposed to 34 °C. In contrast, an initial acute thermal exposure to 20-28 °C had no effect on survivorship or size over the following month in *N. ostrina* but did result in a reduction in thermal tolerance of approximately 1 °C relative to control individuals. Together, these findings indicate that thermal stress experienced during the EBP can either enhance or impair later performance depending on species-specific responses.

The contrasting responses among these species may reflect adaptive differences related to their typical habitat use, motility, and the thermal environments in the field. Barnacles are sessile throughout their juvenile and adult lives, and both barnacle species occupy high positions in the intertidal zone (Miner et al. 2005) where individuals are regularly exposed to intense aerial heating during summertime low tide emersion (Helmuth et al. 2006, Leeuwis & Gamperl 2022). Repeated exposure to large temperature fluctuations may have favoured the evolution of physiological mechanisms that increase resistance to thermal stress or enhance acclimation capacity (Pörtner & Farrell 2008, Somero 2010, Rohr et al. 2018). In contrast, *N. ostrina* is a motile predator that occupies the mid-intertidal zone, where thermal stress is generally less intense and of shorter duration. Individuals can behaviourally reduce heat exposure by seeking refuge beneath macroalgae, inside crevices, or within shaded microhabitats (Gosselin & Chia 1995, Dahlhoff et al. 2001). Together, these differences in thermal environment and behavioural avoidance of heat may have favoured the evolution of greater physiological acclimation capacity in barnacles while reducing selection for such responses in *N. ostrina*, potentially explaining the contrasting carry-over effects observed in this study.

The enhanced survivorship and thermal tolerance observed in the barnacles following exposure to 34 °C are consistent with the concept of hormesis, or heat priming, where prior exposure to a low dose of stress improves tolerance to subsequent stress events (Costantini et al. 2010, Glass et al. 2023, Stick et al. 2026). Similar responses to sublethal stress have been

described in other marine invertebrates as within-generation acclimatization or environmental memory, and have been documented especially in scleractinian corals and anemones (Brown et al. 2015, Putnam 2021, Hackerott et al. 2021, Brown & Barott 2022, Glass et al. 2023). In general, these responses occur when an initial stress event induces physiological adjustments that persist through time and improve performance under subsequent stressful conditions.

One potential mechanism underlying these responses of the two barnacle species is the induction of heat shock proteins (HSPs), which play an important role in protecting proteins and cellular structures during thermal stress (Somero, 2002, 2020). HSP production is commonly induced in intertidal organisms during periods of aerial exposure and elevated temperature (Helmuth et al. 2006). Although HSP expression was not measured in the present study, activation of these stress responses following the initial thermal exposure could contribute to the enhanced survivorship and elevated LT_{50} values observed in the two species of barnacles. The stronger response observed in *C. dalli* relative to *B. glandula* may further suggest interspecific differences in acclimation capacity, potentially associated with the higher intertidal distribution and greater natural thermal exposure experienced by *C. dalli* (Miner et al. 2005). Similar benefits of non-lethal heat exposure have been reported in aquaculture studies, where induction of HSPs improves thermal tolerance, resistance to oxidative stress, and protection against pathogens in the brine shrimp *Artemia franciscana* (Frankenberg et al. 2000, Sung et al. 2007, 2008, Kumar et al. 2022).

In contrast, the reduced thermal tolerance observed in *N. ostrina* suggests acute thermal exposure imposed physiological costs rather than inducing beneficial acclimation. As a motile species, *N. ostrina* can reduce thermal exposure through behavioural mechanisms such as seeking refuge in crevices, under rocks and beneath *Fucus* spp., which are known to lower rock surface temperatures and increase humidity in the mid-intertidal zones of Barkley Sound (Jenewein & Gosselin 2013a). Consequently, this species may experience weaker selection for energetically costly physiological acclimation mechanisms than sessile species that cannot avoid stressful conditions. Furthermore, exposed rock surface temperatures in the mid-intertidal zone of Barkley Sound can occasionally reach 27–30 °C during summer

(Jenewein & Gosselin 2013b, Iwabuchi & Gosselin 2019), temperatures that approach the acute thermal tolerance thresholds previously reported for juvenile *N. ostrina* (Hamilton & Gosselin 2020). Exposure to temperatures near these limits may impose physiological costs that persist beyond the initial stress event and reduce subsequent performance.

Importantly, the carry-over effects observed in this study persisted for 30–35 d after the initial exposure, indicating that short thermal events experienced after settlement or emergence from egg capsules and early juvenile development can have lasting consequences for juvenile performance. Species capable of increasing thermal tolerance following sublethal heat exposure, such as *C. dalli* and *B. glandula*, may be better equipped to cope with future warming and occasional thermal extremes (Somero 2002, 2010, Rohr et al. 2018). In contrast, species that respond to early thermal stress with subsequent negative effects that persist for weeks or months, such as *N. ostrina,* may be more vulnerable to ongoing climate change as thermal extremes become more frequent and intense (Stillman 2003, Somero 2010).

The present findings also reinforce the current understanding that thermal tolerance is not a fixed trait but a dynamic characteristic that can vary among life stages and be modified by previous thermal experience (Helmuth et al. 2006, Somero 2010, Schulte et al. 2011, Hamilton & Gosselin 2020, Pottier et al. 2022). This is further supported by comparing with the acute thermal tolerance thresholds reported by Hamilton & Gosselin (2020): LT_{50} values obtained here for EBP barnacles were ~5 °C lower than those reported by Hamilton & Gosselin for larger juveniles and adults. Yet, LT_{50} values for EBP *N. ostrina* differed by only ~1 °C from those reported by Hamilton & Gosselin for EBP *N. ostrina*. Overall, the results reveal that acute sublethal thermal events during the EBP can either enhance or reduce later performance depending on species-specific physiology and ecology, highlighting that vulnerability to warming cannot be generalized even among co-occurring intertidal species.

Carry-over effects of an initial extended (5 d) exposure to elevated sublethal water temperatures

In contrast to the negative carry-over effects on thermal tolerance observed following acute (6 h) exposure to elevated air temperature in *N. ostrina*, extended exposure (5 d) to

elevated water temperatures produced relatively weak carry-over effects. An initial exposure to 28 °C water resulted in complete mortality during the initial exposure treatment. This finding is consistent with the results of Chapter 2, examining EBP *N. ostrina* under extended exposure to elevated temperatures, in which the LT_{50} after 6 d of exposure was 25 °C. The present findings reinforce the conclusion from Chapter 2 that temperatures considered sublethal during acute exposure can become lethal when sustained for several days.

Although all EBP *N. ostrina* in this experiment survived the initial 5 d exposure to 24 °C, survivorship over the following 30 d was lower than in the 16 °C and 20 °C treatments, indicating that 24 °C did cause thermal stress, influencing performance beyond the exposure period. In contrast, neither growth during the 30 d period nor the thermal tolerance of EBP individuals one month after the initial treatment differed among treatments. In this case, survivorship appeared to be more sensitive to extended thermal stress than either growth or LT_{50}, demonstrating that delayed effects of warming may not be detected through measurements of growth or thermal tolerance alone. Thus, moderately elevated temperatures can impose physiological costs that may persist long after stressful conditions have ended (O'Connor et al. 2014).

The mechanisms underlying this delayed mortality remain uncertain. One possible explanation is that prolonged thermal stress caused cumulative cellular damage that was not immediately lethal but reduced the probability of surviving during the following month. This is consistent with the fact that prolonged thermal stress requires sustained investment in cellular repair, protein maintenance, and other protective processes (Tomanek 2010, Masanja et al. 2023). Moreover, as temperatures approach physiological thresholds, compensatory mechanisms may become insufficient to maintain cellular homeostasis, leading to protein damage, mitochondrial dysfunction, and cumulative cellular stress (Somero 2002, Leeuwis & Gamperl 2022, Jørgensen et al. 2022, Masanja et al. 2023). At the same time, intraspecific variation in physiological tolerance may have influenced individual responses to this stress (Jacobs & Podolsky 2010), with some juveniles experiencing physiological impairment sufficient to cause death, whereas others survived and showed no measurable effects on growth or thermal tolerance one month after the initial exposure.

Collectively, the results of the acute and extended exposure experiments demonstrate that the biological consequences of thermal stress depend not only on temperature but also on the duration of exposure. Although both experiments evaluated carry-over effects of sublethal temperatures experienced during the EBP, acute exposure to elevated air temperature primarily altered subsequent thermal tolerance in *N. ostrina*, whereas extended exposure to elevated water temperature primarily reduced subsequent survivorship. These contrasting responses suggest that different physiological processes may underlie the effects of short- and long-term thermal stress. Differences in exposure medium may have also contributed to the observed responses, as elevated temperatures in air and water can impose different physiological demands. These findings highlight the importance of considering both exposure intensity and duration when assessing the consequences of warming for EBP marine invertebrates. As marine heatwaves become increasingly frequent, intense, and prolonged, both types of carry-over effects may influence recruitment success and, ultimately, population persistence in intertidal communities (Helmuth et al. 2006, Gómez-Gras et al. 2025).

Implications for the persistence of intertidal invertebrate species on the coasts of Vancouver Island.

The findings of this study suggest that the future persistence of intertidal invertebrates on the coast of British Columbia will depend not only on the temperatures experienced in the future, but also on how species respond to prior exposure to sublethal thermal stress during the early benthic phase. The contrasting carry-over effects observed among the study species indicate that responses to warming are highly species-specific. Consequently, predictions of how climate change will affect recruitment and population persistence should consider not only the direct effects of elevated temperatures, but also how previous thermal exposure influences the performance of juveniles later in life.

Differences among species may also have important ecological consequences. Barnacles constitute an important prey resource for *N. ostrina* (Gosselin & Chia 1994), and species-specific differences in responses to thermal stress could therefore alter predator-prey interactions and ultimately community structure. As climate change progresses and higher

temperatures become more frequent, even relatively small differences in acclimation capacity or sensitivity to previous thermal exposure may influence which species persist under future conditions (Somero 2010, Sunday et al. 2012, Smale et al. 2019).

Although this study demonstrates that carry-over effects can persist for at least one month after the initial exposure, the physiological mechanisms responsible for these responses remain uncertain. Future research should therefore investigate the cellular mechanisms underlying the contrasting responses observed among species, particularly the production of heat shock proteins, metabolic adjustments, and other stress-response pathways. In addition, experiments incorporating repeated thermal events would provide a more realistic representation of the thermal regimes experienced by intertidal organisms under future climate scenarios. Finally, examining parental and transgenerational effects would help determine whether thermal conditions experienced by one generation influence the performance and resilience of subsequent generations under continued warming (Pechenik 2006, Rebolledo et al. 2023). Overall, this study demonstrates that thermal stress experienced during the early benthic phase can have lasting consequences for marine invertebrates, even after individuals are returned to ambient conditions.

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CHAPTER 4: General Conclusion

SUMMARY OF RESULTS

Intertidal organisms naturally experience strong environmental variability (Stephenson & Stephenson 1949, Helmuth et al. 2006); however, climate change is increasing both the frequency of short-term extreme heat events and the duration of periods with elevated temperatures, such as marine heatwaves (Smale et al. 2019, IPCC 2022). Because the early benthic phase (EBP) of the life cycle in marine invertebrates is particularly vulnerable to environmental stress (Gosselin & Qian 1997, Hamilton & Gosselin 2020), this study examined how exposure to elevated temperatures during the EBP influences the performance of juvenile marine invertebrates under conditions relevant to current and future climate change scenarios. Specifically, this research determined (1) how extended exposure (25–30 d) to elevated water temperatures affects immediate survivorship, growth, and thermal tolerance thresholds, and evaluated the implications of these responses for the future persistence of intertidal species under projected ocean warming in the coasts of British Columbia; and (2) whether initial exposure to acute (6 h) elevated air temperatures or extended (5 d) elevated water temperatures generates carry-over effects that alter subsequent survivorship, growth, and thermal tolerance later in juvenile life, and whether these responses vary among species.

The most important findings of objective (1) were that EBP marine invertebrates are more vulnerable to warming than suggested by prior studies that were based on acute (< 48 h) thermal exposure. Although short-term experiments indicated relatively high thermal tolerance, extended exposure to elevated water temperatures, previously considered sublethal, resulted in lower thermal tolerance thresholds, reduced survivorship, and altered growth rates, and the magnitude of these effects was species-specific. These findings suggest that approaches relying only on survivorship, acute exposure experiments, or adult thermal tolerance thresholds may substantially overestimate the resilience of species to climate change. As a result, projections based on thermal tolerance thresholds estimated under prolonged exposure indicate that these species may reach their current thermal limits sooner under future ocean warming than previously predicted from acute exposure experiments.

The most important findings of objective (2) were that thermal stress experienced during the EBP can produce lasting carry-over effects that persist even after organisms return to ambient conditions. However, the magnitude and direction of these effects depended on both the duration and intensity of the initial exposure and differed among species. An acute initial exposure to elevated air temperatures reduced subsequent thermal tolerance in the snail *Nucella ostrina*, but had no effect on their survivorship or size, whereas it enhanced later survivorship and thermal tolerance in two species of barnacles, *Chthamalus dalli* and *Balanus glandula*. In contrast, an extended initial exposure to elevated water temperatures reduced later survivorship in *N. ostrina* but did not affect size and thermal tolerance. Together, these results suggest that motility and life history stages of marine invertebrates are important determinants of their responses to climate warming, and responses cannot be generalized across species. The positive carry-over effects observed in barnacles further indicate that physiological plasticity may increase resilience to future thermal stress in some marine invertebrates.

Overall, this thesis demonstrates that the consequences of climate warming for intertidal species cannot be fully understood through short-term exposure experiments alone. Exposure duration, life history, and possible carry-over effects should be considered when predicting species persistence under future warming conditions.

RELEVANCE OF FINDINGS FOR POLICY AND MANAGEMENT

Rocky intertidal ecosystems provide important ecological, economic, and cultural services, including habitat provision, food resources, and shoreline protection. These ecosystems also support commercial and recreational fisheries, as well as shellfish aquaculture (Martínez et al. 2007, IPCC 2022). The Pacific coast of Canada, with approximately 36,000 km of coastline, is one of the most diverse and productive marine environments in the world (Government of Canada 2014). For thousands of years, these coastal environments have supported Indigenous communities through fishing, harvesting, and trading (Province of British Columbia 2024). In the Bamfield region, the Huu-ay-aht First Nations maintain strong cultural and historical connections to coastal and marine

environments, which continue to play an important role in community wellbeing and stewardship (Huu-ay-aht First Nations 2012).

Current coastal management strategies in British Columbia emphasize protecting coastal ecosystems and biodiversity and improving understanding of climate risks through monitoring, research, modelling, and vulnerability assessments (Province of British Columbia 2024). Similarly, *Canada's Marine Coasts in a Changing Climate* recognizes climate change as an increasing challenge for Canadian coastal ecosystems and communities and highlights the importance of understanding ecological responses to support adaptation planning (Lemmen et al. 2016). In this context, the findings of this thesis contribute to understanding how prolonged and repeated thermal stress may influence species responses under future warming conditions.

Although early life stages are difficult to monitor directly in natural rocky shore systems due to their small size and the logistical challenges of sampling complex and high-energy environments, experimental studies such as this one provide valuable information on the sensitivity of early life stages to elevated temperatures. By demonstrating that prolonged exposure to elevated temperatures can reduce survivorship, growth, and thermal tolerance in juvenile marine invertebrates, this thesis provides new evidence that complements existing knowledge derived largely from short-term exposure experiments. Together, these findings demonstrate that responses of juvenile marine invertebrates to elevated temperatures depend on species, exposure duration, and the lasting effects of previous thermal exposure.

Beyond conservation applications, these findings may also have relevance for aquaculture practices. The positive carry-over effects observed in some species in this thesis suggest that controlled exposure to sublethal temperatures during early development may, under certain conditions, increase resilience to subsequent thermal stress. If similar responses occur in commercially cultured species, the aquaculture industry could investigate whether brief exposure to controlled sublethal temperatures during early development improves juvenile survival during hatchery production, transportation, or predictable periods of elevated summer temperatures. Similar approaches have previously been investigated in

aquaculture species, including the brine shrimp *Artemia franciscana*, suggesting that the responses observed here may have broader applied relevance (Frankenberg et al. 2000, Sung et al. 2007, 2008, Kumar et al. 2022). However, because responses differed among species in this study, exposure protocols would need to be developed and validated individually for each cultured species before implementation.

The increasing frequency and intensity of marine heatwaves further emphasize the importance of understanding how intertidal species respond to elevated temperatures (Smale et al. 2019, IPCC 2022). The 2021 Pacific Northwest heatwave demonstrated that extreme events can rapidly cause widespread mortality of intertidal organisms over periods of several days or a few weeks (White et al. 2023). Because intertidal organisms experience elevated temperatures both while submerged and during aerial exposure at low tide, their responses to warming will likely depend on both marine and atmospheric heat events. In this context, the extended-exposure thermal tolerance thresholds and performance responses estimated in this thesis may provide useful information for provincial and federal agencies responsible for climate change adaptation and marine resource management when considered alongside long-term environmental monitoring and other ecological studies (Lemmen et al. 2016, Province of British Columbia 2024). More broadly, these results may also be relevant to Indigenous communities and coastal stewardship initiatives seeking to understand the potential effects of climate warming on intertidal resources that are ecologically, culturally, and economically important (IPCC 2022, Province of British Columbia 2024). Collectively, the results of this thesis can support future climate risk assessments and adaptation planning for rocky intertidal ecosystems under ongoing climate warming.

DIRECTIONS FOR FUTURE STUDY

Future studies should include more taxa and additional life stages to determine whether the patterns observed here are broadly representative across intertidal species and throughout development. Longer-term experiments tracking responses of individuals over several months and across developmental stages would help determine whether the effects of

extended thermal exposure and the carry-over effects documented in this thesis persist into adulthood and influence growth, reproduction, or long-term survival. Experiments including repeated thermal exposures are also needed, as organisms in nature are more likely to experience multiple thermal events during the summer rather than single isolated exposures.

Further research should deepen our understanding of the physiological mechanisms underlying species responses to thermal stress and the processes involved in the activation and direction of carry-over effects. Studies examining heat shock protein (HSP) expression, metabolic performance, and other indicators of cellular stress of EBP individuals could help explain the trade-offs between survivorship and growth observed under prolonged exposure and clarify why prior thermal exposure reduced performance in some species but enhanced tolerance in others. Future work should also evaluate the role of parental effects and transgenerational plasticity, as thermal conditions experienced by adults may influence offspring performance and resilience under future warming scenarios (Rebolledo et al. 2023).

Future studies should incorporate greater environmental realism by considering multiple interacting stressors. In particular, experiments combining elevated water temperatures with periods of aerial heat exposure could better represent the conditions experienced by intertidal organisms during marine and atmospheric heatwaves. Intertidal organisms are exposed simultaneously to environmental factors such as temperature, salinity, pH and desiccation, and these interactions may alter physiological responses beyond predictions based on temperature alone. Research should also examine temporal and geographic variation in thermal tolerance thresholds by expanding sampling across seasons and geographic ranges to improve understanding of local thermal adaptation and identify populations that may be more vulnerable or resilient to climate change.

Finally, future studies should move beyond individual-level responses to evaluate ecological consequences at the community level. Changes in juvenile survivorship, growth, and thermal performance may alter species interactions, including predator–prey dynamics, recruitment success, and competitive relationships, potentially leading to shifts in community composition and ecosystem functioning under future warming conditions.

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APPENDIX A

Laboratory setting for experiments on seawater temperature treatments

The setting consisted of 40L tanks containing filtered seawater at the designated water temperature treatment. Each tank contained a water pump to circulate water, a sponge filter with constant airflow an aquarium heater EHEIM Thermocontrol of 75 W (model 3612010) rating for >30°C and 50W (model 3612010) rating for <30°C temperature treatments, an InkBird WiFi Aquarium Temperature Controller (ITC-306A), and a Thermochron iButton (model DS1921G-F5) temperature logger registering temperature every 30 min. Tanks with the lowest temperatures (14-17 °C) did not have a heater, but a water line running constantly on the outside of the walls of the tanks to keep them cooler than the temperature of the laboratory (20°C approx.).

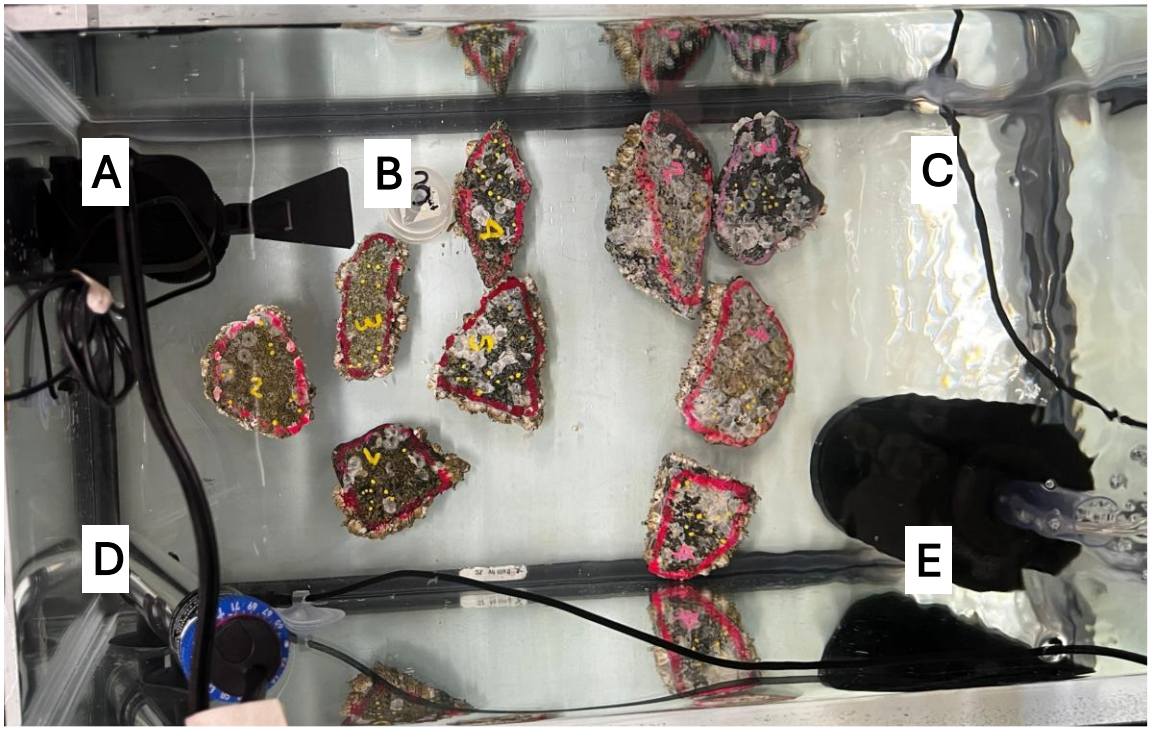

Figure A.1. Tank setting for experiments on seawater for EBP marine invertebrates. A. Water pump; B. Thermochron iButton temperature logger; C. Probe of Aquarium Temperature Controller; D. Aquarium heater; E. Sponge filter.

Feeding of animals during the experiments

EBP barnacles and mussels were fed daily by adding a mixture of diatoms (*Chaetoceros muelleri* and *Chaetoceros gracili*) and flagellates (*Tetraselmis spp.*) to the tanks at an approximate concentration of $45x10^3$ cells/mL. Diatoms and flagellates were reared and provided by Nova Harvest Ltd. *N. ostrina* is a predator, and barnacles *C. dalli* and *B. glandula* are among their preferred prey (Gosselin & Chia 1995). To feed the hatchlings during the experiment, rocks containing barnacles of all sizes were collected and held in the cages with the newly hatched *N. ostrina,* which were put with the operculum down on each rock (replicate group), then carefully submerged in their corresponding experimental tanks.

APPENDIX B

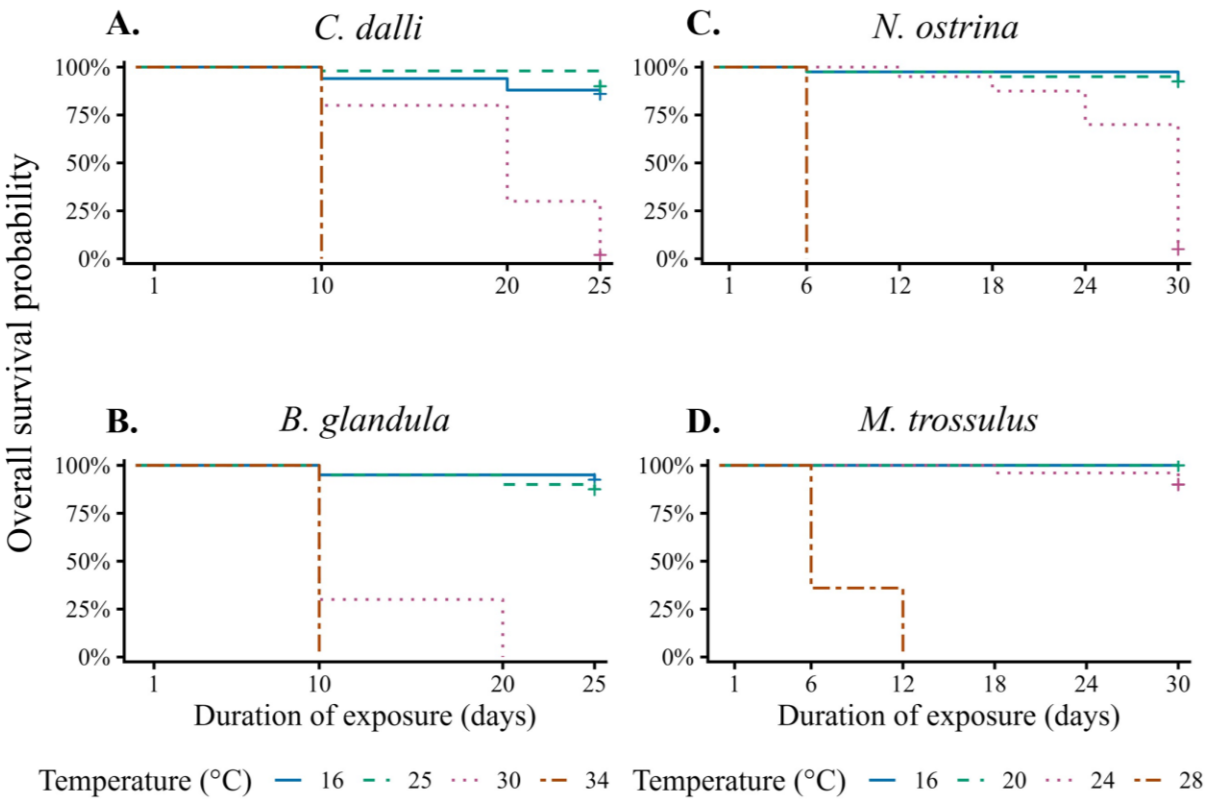

Figure B.1. Kaplan-Meier survival curves for four EBP marine invertebrates exposed to extended elevated temperature treatments over 25–30 d. A. *Chthamalus dalli* (n= 50 per temperature treatment), B. *Balanus glandula* (n= 40 per temperature treatment), C. *Nucella ostrina* (n= 40 per temperature treatment). and D. *Mytilus trossulus* (n= 50 per temperature treatment).

APPENDIX C

Table C.1. Lethal temperature (LT_{50}) values under extended exposure to temperature for four EBP marine invertebrates with model fit diagnostics. Acute LT_{50} values were obtained from Hamilton & Gosselin (2020). LT_{50} values represent water exposure over species-specific durations obtained from Generalized Linear Models with a binomial distribution. Bolded values denote statistically significant differences from model assumptions ($p < 0.05$). Values highlighted in grey are estimates with large uncertainty (SE > 10 °C).

Species	Acute LT_{50} in air (Hamilton & Gosselin, 2020)		Duration of exposure (d)	Extended LT_{50} in water		R^2	Dispersion (p-value)	Zero-inflation (p-value)
	LT_{50} (°C)	SE		LT_{50} (°C)	SE			
C. dalli	45.8	0.23	10	30.83	0.43	0.62	**< 0.001**	0.99
			20	27.99	0.54	0.60	0.080	1
			25	25.95	0.56	0.66	0.146	1
B. glandula	42.8	0.19	10	28.37	0.49	0.71	0.278	1
			20	26.63	0.43	0.82	0.042	1
			25	26.33	0.48	0.76	**0.006**	1
N. ostrina	32.3	0.14	6	25.75	0.33	0.82	**0.028**	1
			12	25.53	0.34	0.80	0.062	1
			18	25.17	0.35	0.75	0.106	1
			24	24.49	0.36	0.73	0.450	1
			30	21.57	0.35	0.80	0.282	1
M. trossulus	35.6	0.63	6	27.89	11.19	0.72	1	1
			12	25.98	923.60	1	1	1
			18	24.51	83.62	1	1	1
			24	24.51	83.62	0.95	1	1
			30	25.37	0.29	0.84	0.230	1

APPENDIX D

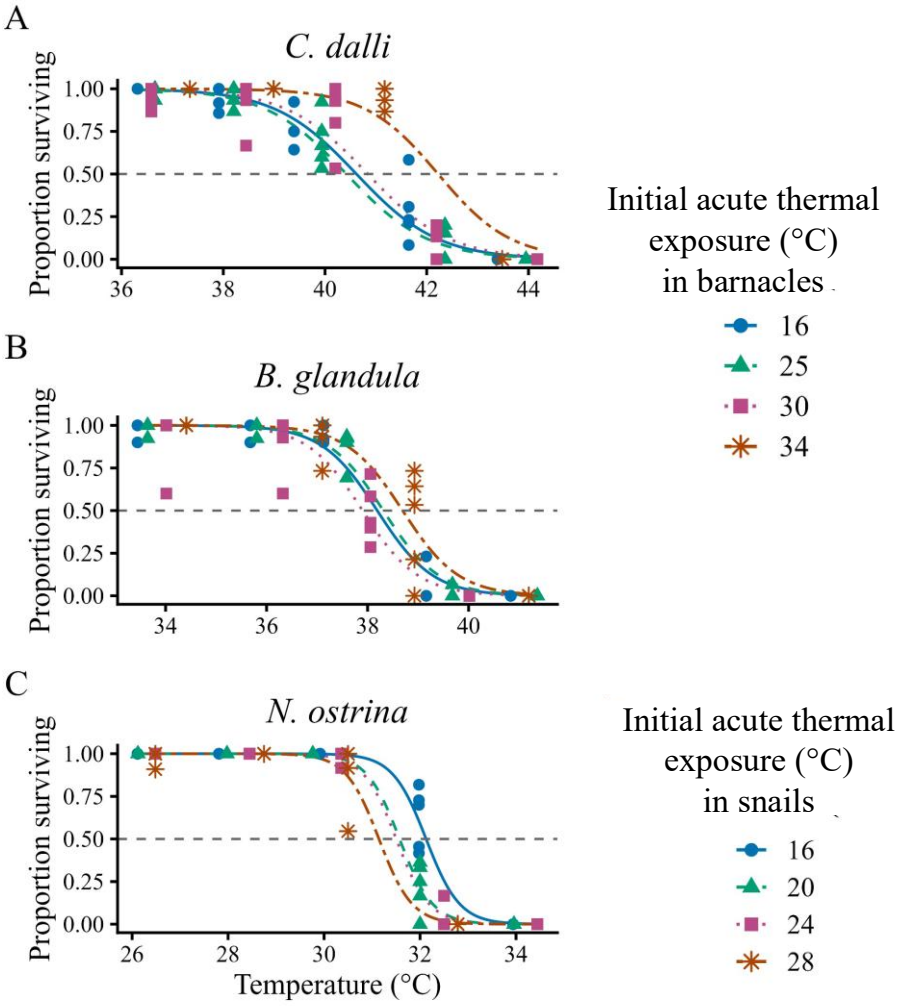

Figure D.1. Survivorship curves of EBP marine invertebrates following an initial acute (6 h) exposure to elevated sublethal air temperatures and a 30-35 d rearing under ambient conditions. Lines represent fitted Bayesian binomial GLM and points represent observed proportions of individuals surviving in each replicate at each temperature. The dashed horizontal line indicates the LT_{50}.

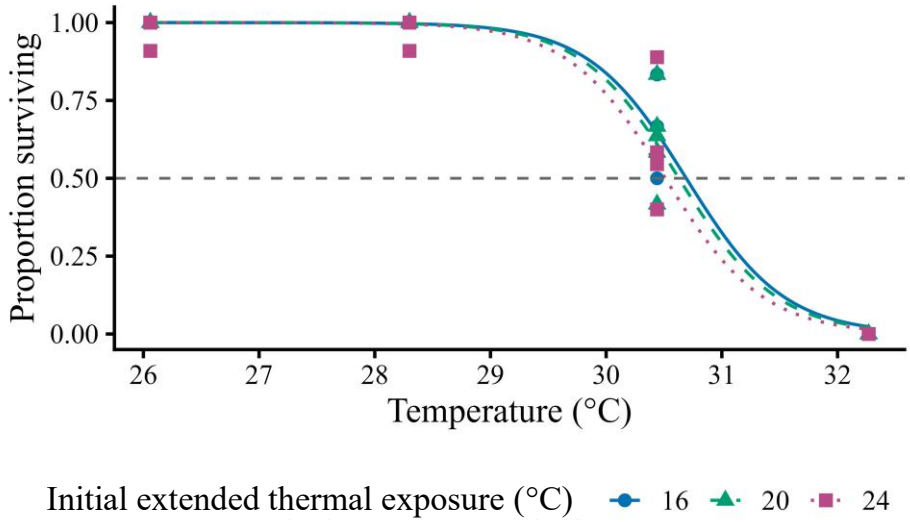

Figure D.2. Survivorship curves of EBP *Nucella ostrina* during thermal tolerance experiments in water following an extended (5 d) exposure to elevated sublethal water temperatures and a 30 d rearing period under ambient conditions. Lines represent fitted Bayesian binomial GLM and points represent observed proportions of individuals surviving in each replicate at each temperature. The dashed horizontal line indicates the LT_{50}.

Table D.1. Lethal temperature (LT_{50}) of three EBP marine invertebrates following acute (6 h) exposure to elevated sublethal air temperatures and a 30-35 d rearing under ambient conditions. LT_{50} values were obtained from Bayesian Generalized Linear Models with a binomial distribution.

Species	Acute temperature treatment	LT_{50} (°C)	SE	Deviance/df
C. dalli	16	40.62	0.16	1.45
	25	40.42	0.15	
	30	40.84	0.14	
	34	42.22	0.15	
B. glandula	16	38.19	0.13	1.78
	25	38.31	0.13	
	30	37.90	0.12	
	34	38.69	0.11	
N. ostrina	16	32.12	0.09	0.72
	20	31.57	0.10	
	24	31.50	0.15	
	28	31.14	0.13	