Thermal Tolerance Limits and Cardiac Acclimation Potential of Sablefish (*Anoplopoma fimbria*) Embryos and Yolk-Sac Larvae Incubated at Different Temperatures

by

Chrissy Schellenberg
B.Sc., Vancouver Island University, 2018

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Abstract

Average global ocean temperatures and the frequency and intensity of marine heat waves have been increasing over the last century. Temperature plays a critical role in defining the geographical range of the majority of marine species. Some species may respond to ocean warming trends by shifting their latitudinal and depth ranges, while others may be able to cope with changes in temperature through phenotypic plasticity and local adaptations. If a species is unable to shift its distribution or has limited thermal plasticity, it may face severe population declines or local extinction. Therefore, describing thermal tolerance limits is a useful tool for predicting how a given species will respond to ocean warming.

Due to its commercial importance, sablefish (*Anoplopoma fimbria)* is a fish species of particular interest in British Columbia. Sablefish are semi-demersal and spawn along the continental slopes of the Pacific coast from California to Alaska. Their various life history stages occupy different depth strata and thus experience substantially different environments with respect to temperature (as well as salinity, oxygen, etc.). Adult sablefish spawn at depths that exceed 300 m and embryos sink to depths of ~1,000 m after fertilization. Embryos hatch into yolk-sac larvae until they become mobile at the post-yolk-sac larvae stage. The latter migrate to near-surface waters (<3 m) at which temperatures are approximately 12-15°C in the late spring.

Heart rate is a temperature-dependent performance measure and has been used to gain insight into the thermal tolerance of many adult fishes. However, few studies have used this approach with the early life stages of fishes such as embryos and yolk-sac larvae (YSL). The purpose of this study was to assess whether sablefish embryos and YSL have the potential for cardiac acclimation by examining changes in their thermal tolerance limits when incubated at temperatures outside of what they experience in a natural setting (~5°C). Cardiac performance was assessed during an acute temperature challenge from 2.0° to 12.0°C in increments of 1.0°C (at a rate of 1°C 40 min^{-1}) for individuals incubated at 3.0°C, 5.0°C (control), and 7.0°C. Embryos were video recorded at each 1.0°C increment and videos were viewed at a later date to determine heart rate at each temperature. This study attempted to use temperature breakpoint analysis, commonly used in studies of adults, on these early life stages to assess cardiac performance. It was hypothesized that sablefish embryos and yolk-sac larvae incubated at warmer temperatures would have a higher thermal tolerance than sablefish embryos and YSL incubated at colder temperatures, as seen in other fish species.

There was some degree of thermal compensation of cardiac function with temperature in sablefish embryos and YSL as mean heart rate increased with incubation temperature throughout acute warming. YSL had consistently higher mean heart rate values at any given temperature of the acute temperature challenge when compared to embryos incubated at the same temperature. T_{AR} is the temperature at which the heart first becomes arrhythmic is considered a sub-lethal index because the organism is expected to experience cardiac collapse soon after. T_{AR} was reached for 100% of embryos incubated at 3.0°C at an average temperature of 8.6 ± 1.0°C. In contrast, only 18% and 33% of embryos incubated at 5.0° and 7.0°C exhibited arrhythmia (mean T_{AR} were 9.0 ± 3.0 and 8.5± 1.5°C, respectively). The lower thermal limit for embryos incubated at 7.0°C was likely near 1.0°C, which was determined during preliminary testing. Neither the upper or lower limits were reached for YSL during the acute temperature challenge. No mortalities were observed during any acute temperature challenges. Overall percent mortality throughout the entirety of the experiment could not be determined due to limitations in the experimental setup and reduced staff working on this project due to COVID-19 safety protocols.

This study is also the first to investigate whether transporting sablefish embryos from a hatchery to a research facility at different stages of development had an effect on their cardiac performance during acute warming. Embryos were transported in a cooler from the sablefish hatchery on Salt Spring Island to UVic via ferry and vehicular transport. Time of transportation did not significantly change the temperature at which heart rate reaches its maximum or T_{AR}. There was also consistent overlap in mean heart rate ± standard error at each temperature of the acute temperature challenge between these two groups. Therefore, there appears to be no indication that transportation affected the heart rate response of sablefish embryos when incubated at the same temperature. However, future studies may want to confirm this by identifying and comparing other breakpoint temperatures that characterize physiological performance. Determining whether transportation has effects on cardiac performance may be of interest for other researchers who need to transport fish embryos from the field to the laboratory.

Heart rate measurements during an acute temperature challenge of sablefish embryos and yolk-sac larvae (YSL) incubated at various temperatures provided initial insight to their overall success in a warming climate. Currently, it is projected that waters at depths of 1,000 m will warm on average by less than a degree by the end of the 21st century. The results of this study suggest that the early life stages of sablefish may not be exposed to critical temperatures in the near future, but future impacts on overall physiological decline remain unknown. The novel data presented here lay the groundwork for future researchers to continue to characterize the thermal tolerances of the early life stages of sablefish, and the likely response of this important species to ocean warming.

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List of Abbreviations

The following is a list of abbreviations that are commonly used throughout the thesis. They are presented in the order in which they appear.

MHWs	Marine heat waves
TPCs	Thermal performance curves
AS	Aerobic scope
MMR	Maximum metabolic rate
SMR	Standard metabolic rate
$\mathbf{T_{crit}}$	Critical temperature
$\mathbf{T_{AB}}$	Arrhenius breakpoint temperature
$\mathbf{T_{QB}}$	Q_{10} breakpoint temperature
$\mathbf{T_{max}}$	Temperature when heart rate reaches its maximum
$\mathbf{T_{AR}}$	Arrhythmic temperature
SDMs	Species distribution models
ESMs	Earth-system models
YSL	Yolk-sac larvae
f_H	Heart rate
UVic	University of Victoria
TE	Sablefish embryos that were transported later in their development
UVic E	Sablefish embryos reared at UVic since 0 days-post-fertilization
dpf	Days-post-fertilization
b min^{-1}	Beats-per-minute

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I am very grateful to my supervisors, Dr. John Dower and Dr. Nadja Steiner, as well as my other committee members, Dr. Chris Pearce and Dr. Helen Drost, for their continued support throughout my graduate studies. I was presented with a very unique set of challenges throughout grad school, especially with the COVID-19 pandemic impeding my collaborative research plans. Despite all of these challenges, all of you made me feel extremely supported, both academically and from a mental health perspective. I am fortunate to have worked with such respected scholars that all specialize in different areas, providing me with a breadth of tools and scientific insight.

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Chapter 1: Introduction

1.1 Impacts of Ocean Warming Trends and Marine Heat Waves on Fish Distribution and Mortality

The ocean plays a crucial role in regulating earth's climate by acting as the primary heat reservoir of the climate system due to its high specific heat capacity (Singh et al. 2022). Average global ocean temperatures are currently 0.76°C higher than the average temperature for the past one hundred years, due to anthropogenically-driven rising global atmospheric temperatures (NOAA, 2021). Globally, annual sea-surface temperatures have increased at an average rate of 0.08°C per decade since 1880 and +0.18°C since 1981 (NOAA, 2021). In addition, extreme weather events such as marine heat-waves (MHWs) have been increasing in frequency, intensity, and duration over recent decades (Gruber et al. 2020). It is projected that abnormal MHWs that historically occurred once every hundred to thousands of years will become decadal to centennial events if global average temperatures rise by 1.5°C, and annual to decadal events if a 3°C increase is reached (Laufkötter et al. 2020). Compound events (multiple extreme events that occur simultaneously or in close sequence) are also a growing concern as their individual effects may interact synergistically (Gruber et al. 2020).

Temperature plays a critical role in defining the geographical range of the majority of marine species (Hutchins, 1947). Many marine species may respond to ocean warming trends by shifting their latitudinal and depth ranges (Campana et al. 2020; Dulvy et al. 2008; Jung et al. 2013; Simpson et al. 2011; Sunday et al. 2015). Several boreal marine fish species have already begun to expand their poleward range boundaries and contract their equatorial range boundaries over the last several decades (Fossheim et al. 2015; Kortsch et al. 2015). Northeast Atlantic mackerel have been occupying increasingly larger portions of the Nordic Seas in the summer during feeding (Gullestad et al. 2020). Arcto-Norwegian cod have shifted their spawning habitat to include the coast of East Finnmark (Sundby & Nakken, 2008). Atlantic cod and haddock are now being frequently caught with polar cod in Svalbard coastal and fjord waters (Renaud et al. 2012). The projected global median rate of range shifting for marine fish and invertebrates is 45-59 km per decade (Cheung et al. 2009). Some species are expected to shift their distribution >159 km per decade (Fossheim et al. 2015). However, some species may have difficulties overwintering successfully if they shift their distributions to higher latitudes, resulting in population declines (Booth et al. 2018; Sunday et al. 2012). Overwintering mortalities may be due to one or more life stages of a species being exposed to temperatures below their cold tolerance limit, or experiencing a reduction in the quality of prey (i.e., larger zooplankton) due to a reduction in sea ice (Mueter et al. 2021). Increased mortalities during overwintering have been observed in several fish populations such as the common roach, Dolly Varden, and walleye Pollock (Graham & Harrod, 2009; Mueter et al. 2021; Reist et al. 2006).

MHWs can have more dramatic effects on marine ecosystems than longer warming trends because the unusual and extreme temperatures that occur during MHWs are felt in a fraction of the time (NOAA, 2022). MHWs can cause more sudden latitudinal displacements of populations, severe declines in population numbers, and increased instances of local extinctions (Deguette et al. 2022; Husson et al. 2022). For example, the North Pacific marine heatwave of 2014-2016 (also known as “The Blob”) caused significant declines of several populations such as walleye Pollock and Pacific cod (Laurel & Rogers, 2020; Rogers et al. 2020). A MHW off the Western Australian coast in 2011 resulted in significant declines of habitat-forming seaweeds and a sudden-appearance of warm-water species in Jurien Bay, such as western scalyfin, west

Australian butterflyfish, and lined dottyback (Wernberg et al. 2013). Pile Bay saw localized extinction of bull kelp (*Durvillaea spp.*) and resulting recruitment of invasive kelp (*Undaria pinnatifida*) due to MHWs in the summers of 2017/18 (Thomsen et al. 2019). When exposed to a simulated marine heatwave, a temperate sponge (*Crella incrustans*) had a shorter recruitment period, lower recruitment rate, and higher post-settlement mortality (Strano et al. 2022).

Overall, the effects of ocean warming trends and MHWs can have consequences for the biological diversity, community composition, productivity, and stability of marine ecosystems (Cheung et al. 2009; Strano et al. 2022; Talukder et al. 2022; Thomsen et al. 2019). The severity of impacts caused by ocean warming and extreme temperature events will vary substantially among taxa, even within similar regions, and even within the same species with differences between populations (Chambers et al. 2014; Eliason et al. 2011; Sunday et al. 2015; Tang, 2020).

1.2 Implications of Temperature for Fish Physiology

Here, physiology is defined as the branch of biology that deals with the normal functions of living organisms and their parts, such as biochemical reactions and metabolic rates. The physiology of ectothermic fish is directly influenced by temperature, as they typically lack the ability to to self-regulate their internal body temperatures (Wieser, 1985). Variation and changes to biochemical reactions and metabolic rates has direct impacts on the performance and fitness of organisms in their environments (Brown et al. 2004; Payne et al. 2016).

Thermal tolerance is limited by the cardiorespiratory system to meet tissue oxygen demand, which is referred to as the oxygen and capacity limited thermal tolerance (OCLTT) hypothesis (Pörtner, 2010; Blasco et al. 2020). During acute (quick and severe) warming, cardiac output increases in order to compensate for the increase in tissue-oxygen demand (Gilbert & Farrell, 2021). Cardiac output is the product of stroke volume (amount of blood pumped from the left ventricle per beat) and heart rate, f_H (number of times the heart beats per minute) (Farrell, 1993). Stroke volume does not tend to change with acute warming; therefore, increasing f_H is the primary mechanism that regulates tissue oxygen supply of fish (Farrell, 2016; Gilbert & Farrell, 2021). Heart rate only increases with temperature to a certain temperature, beyond which the heart loses its ability to thermally compensate and f_H eventually decreases to a point where the organism experiences cardiac collapse. As f_H begins to decline, meeting tissue oxygen demands becomes increasingly difficult.

The maximum amount of oxygen that is available for any aerobic activity above routine metabolism is referred to as aerobic scope (AS) (i.e., the difference between maximum metabolic rate (MMR) and standard metabolic rate (SMR)) (Farrell, 2013). As temperatures increase, the difference between MMR and SMR increases to a maximum and AS is the greatest. Beyond this, MMR loses it ability to thermally compensate and the difference between MMR and SMR eventually becomes zero.

Because fish require oxygen to execute essential activities such as swimming, feeding, and reproducing, inhabiting temperatures that result in reduced oxygen availability affects overall organismal performance (Farrell, 2013). Therefore, f_H and AS are temperature-dependent performance measures that ultimately limit an organism’s thermal tolerance. When f_H and AS of an organism are plotted against temperature, it depicts the range of temperatures at which an organism can perform activities and is referred to as a thermal performance curve (TPC) (Schulte et al. 2011) (Figure 1). A typical TPC that includes the full thermal tolerance limits of an organism will show a performance measure increasing with temperature to a maximum value and then decreasing at temperatures beyond that (Figure 1).

Some fish species are able to cope with changes in temperature through phenotypic plasticity and local adaptations, often revealed through changes in metabolic rates (Brown et al. 2004; Eliason et al. 2011). For example, metabolic plasticity may be reflected in reductions or increases to SMR (Guillaume et al. 2020). Such changes thereby alter an organism's performance capacity within a given temperature range and can also change the maximum and minimum temperatures which its cardiorespiratory system can tolerate (Guillaume et al. 2020; Schulte et al. 2011). The degree to which a species is able to express physiological plasticity reflects its acclimation potential to various temperatures and, thus, its overall ability to cope with changes within its environment (Leeuwis et al. 2021).

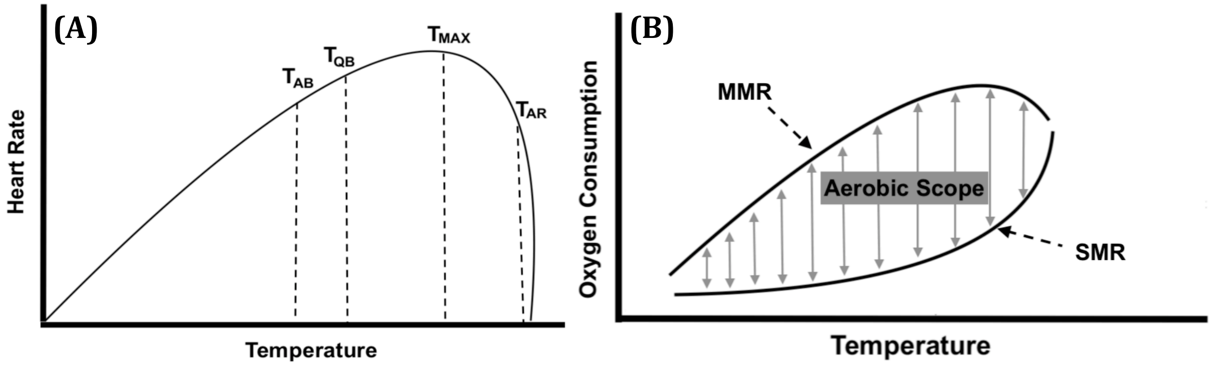

Figure 1. Idealized performance curves **(A)** Represents the thermal dependency of heart rate (f_H). T_{AB} is the Arrhenius Breakpoint Temperature and is the temperature at which f_H changes significantly. T_{QB}, the Q_{10} breakpoint temperature, is the temperature at which the incremental Q_{10} first decreases and remains below 2.0. T_{max} is the temperature at which f_H reaches its maximum. T_{AR} is the temperature at which the heart first becomes arrhythmic. **(B)** A typical aerobic scope (AS) curve. AS represents the maximum amount of oxygen that is available for any aerobic activity above routine and is the difference between standard metabolic rate (SMR) and maximum metabolic rate (MMR).

1.3 Physiological Methods to Determine Thermal Tolerances and Acclimation

Ideally, both f_H and AS would be measured to provide a more confident prediction of how an organism responds to a range of temperatures. However, measuring both f_H and AS may not be experimentally feasible. Measuring f_H is typically more cost-effective and provides a faster output than measuring AS and may be more achievable for some researchers (Anttila et al. 2014; Casselman et al. 2012). Measuring f_H during acute warming has shown to be a viable method in defining the thermal tolerances of adult and larval (beyond the yolk-sac stage) marine and freshwater fishes (Anttila et al. 2014; Casselman et al. 2012; Drost et al. 2014 and 2016; Ferreira et al. 2014; Gilbert et al. 2020; Joyce et al. 2018).

Temperature breakpoint analysis is used to indicate changes in cardiac performance of fish during acute warming. The following breakpoint temperature indices are commonly used:

1. Arrhenius breakpoint temperature (T_{AB})
2. Q_{10} breakpoint temperature (T_{QB})
3. Temperature when f_H reaches its maximum (T_{max}), and
4. Arrhythmic temperature (T_{AR})

The point at which the slope in heart rate changes significantly is termed the Arrhenius break point temperature (Figure 2, and Anttila et al. 2013; Farrell, 2016).

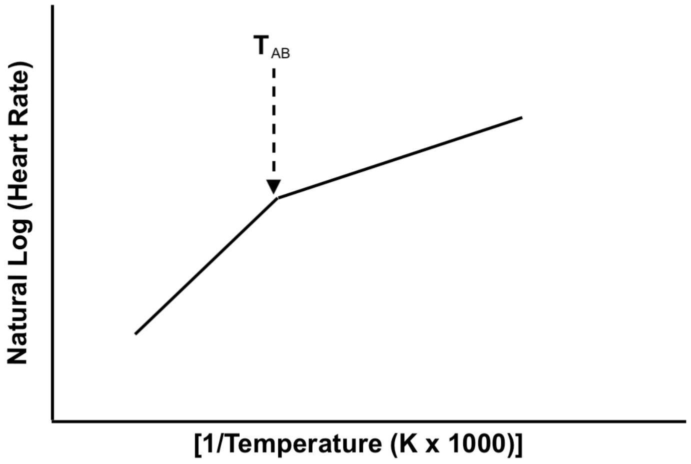

Figure 2. An example of a typical Arrhenius plot. The Arrhenius breakpoint temperature (T_{AB}) can often be difficult to detect on a typical performance curve. An Arrhenius plot is made by plotting the natural log of a rate against the inverse of temperature (1000 K-1). T_{AB} is calculated by running piecewise regression models and represents where the two lines intersect. This reflects where heart rate first fails to increase exponentially with temperature.

Q_{10} is a temperature coefficient that is a measure of temperature sensitivity of a chemical reaction to a 10°C rise in temperature (Aisami et al. 2017). In general, most biological rates double for every 10°C rise in temperature resulting in Q_{10} values ~2.0 (Aisami et al. 2017). The Q_{10} breakpoint temperature, T_{QB}, refers to the temperature at which the incremental Q_{10} first decreases and remains below 2.0 (Farrell, 2016). A Q_{10} value of ~2.0 is expected during acute warming (Clark et al. 2008; Gollock et al. 2006). A Q_{10} value of ~2.0 has also been consistent with other fish species at the embryonic stage, such as brook charr, Atlantic salmon, and zebrafish (Benfey and Bennett, 2009; Fisher, 1942; Klinkhardt et al. 1987; Pelster, 1999). The temperature at which the f_H reaches its maximum value is termed T_{max} (Farrell, 2016). Arrhythmic temperature (T_{AR}) is the lowest temperature at which f_H first becomes arrhythmic and typically occurs after T_{max} has been reached (Farrell, 2016). Once the organism's heart becomes arrhythmic, it is thought that they are near cardiac collapse and their life becomes time limited (Casselman, 2012; Drost et al. 2014). Therefore, T_{AR} is considered an ecologically relevant index of overall organismal performance.

1.4 Study Organism: Sablefish (*Anoplopoma fimbria*)

Sablefish (*Anoplopoma fimbria*), also known as "black cod" (Figure 3), is a semi-demersal fish species that spawns along the continental slopes of the Pacific coast from California to Alaska (Figure 4, and Cook et al. 2018; Kendall & Matarese, 1987; Mason et al. 1983). Adult sablefish are found near bottom over soft substrate and commonly occur at depths between 200 to 1,000 m but have been observed as deep as 2,700 m (DFO, 2018; Kreiger, 1997; Wolotira et al. 1993). Spawning begins in January at depths that exceed 300 m (Guzman et al. 2017; McFarlane & Beamish, 1992). After fertilization, the specific gravity of embryos increases and they sink to depths of ~1,000 m (McFarlane & Beamish, 1992). Embryos hatch at depth at temperatures near 5°C in the early spring, remain at that level as YSL, and then migrate to near-surface waters (<3 m) by late spring, where water temperatures reach between 12-15°C (Deary et al. 2019; Mason et al. 1983). By the time sablefish reach near-surface waters, up to two months have passed and larvae are more developed and have depleted their yolk-sac. Post-yolk-sac larvae are therefore exposed to a large range of temperatures during their migration from deep to near-surface waters. Juveniles migrate inshore and rear in nearshore and shelf habitats and only migrate offshore as adults (DFO, 2018).

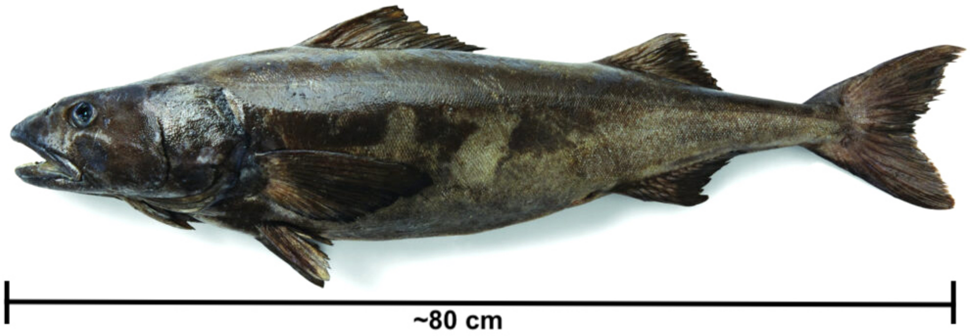

Figure 3. Photo of an adult sablefish (Anoplopoma fimbria), also commonly known as "black cod" (source: www.alaskaseafood.org).

As a result of occupying different depth strata, the various life history stages of sablefish experience substantially different environments with respect to temperature (as well as salinity, oxygen, etc.). Therefore, each life stage may be impacted differently by climate change. It is known that embryos and larvae of marine fish tend to be more sensitive to temperature change than adults (Brett, 1970; Burggren & Reyna, 2011; Drost, 2016). However, embryos and larvae are much less mobile than adults and are unable to make significant changes in their distribution patterns to remain within suitable temperatures. Therefore, the success of the species as a whole is more reliant on the adults to distribute to waters that provide suitable conditions to the early life stages. If adult sablefish shift their distribution to higher latitudes, embryos and larvae will need to be able to cope with colder temperatures, especially since spawning begins in the winter. However, if this species is unable to shift its distribution due to restrictions in predator/prey relationships, or other environmental limitations, they must possess the required metabolic plasticity to cope with a changing environment. Understanding the thermal tolerance limits of sablefish is therefore important to predict how the species will respond to a changing climate.

Sablefish is a highly valuable commercial fish species that is caught year round in the North Pacific, with an approximate landed annual value of $160 million CAD (DFO, 2021). In addition to its importance as a capture fishery, sablefish is regarded as an emerging aquaculture species because of its high growth rate and market value, as well as the increase in consumer demand for this species (Gores & Prentice, 1984; Guzmán et al. 2017; Leeuwis et al. 2019; Sonu, 2014). For the purposes of this work, sablefish was also a practical species to study due to UVic’s proximity to the Golden Eagle Sablefish Hatchery on Saltspring Island – one of the only two sablefish hatcheries in all of B.C. Therefore, the hatchery was able to conveniently provide us with multiple batches of sablefish embryos at staggered times.

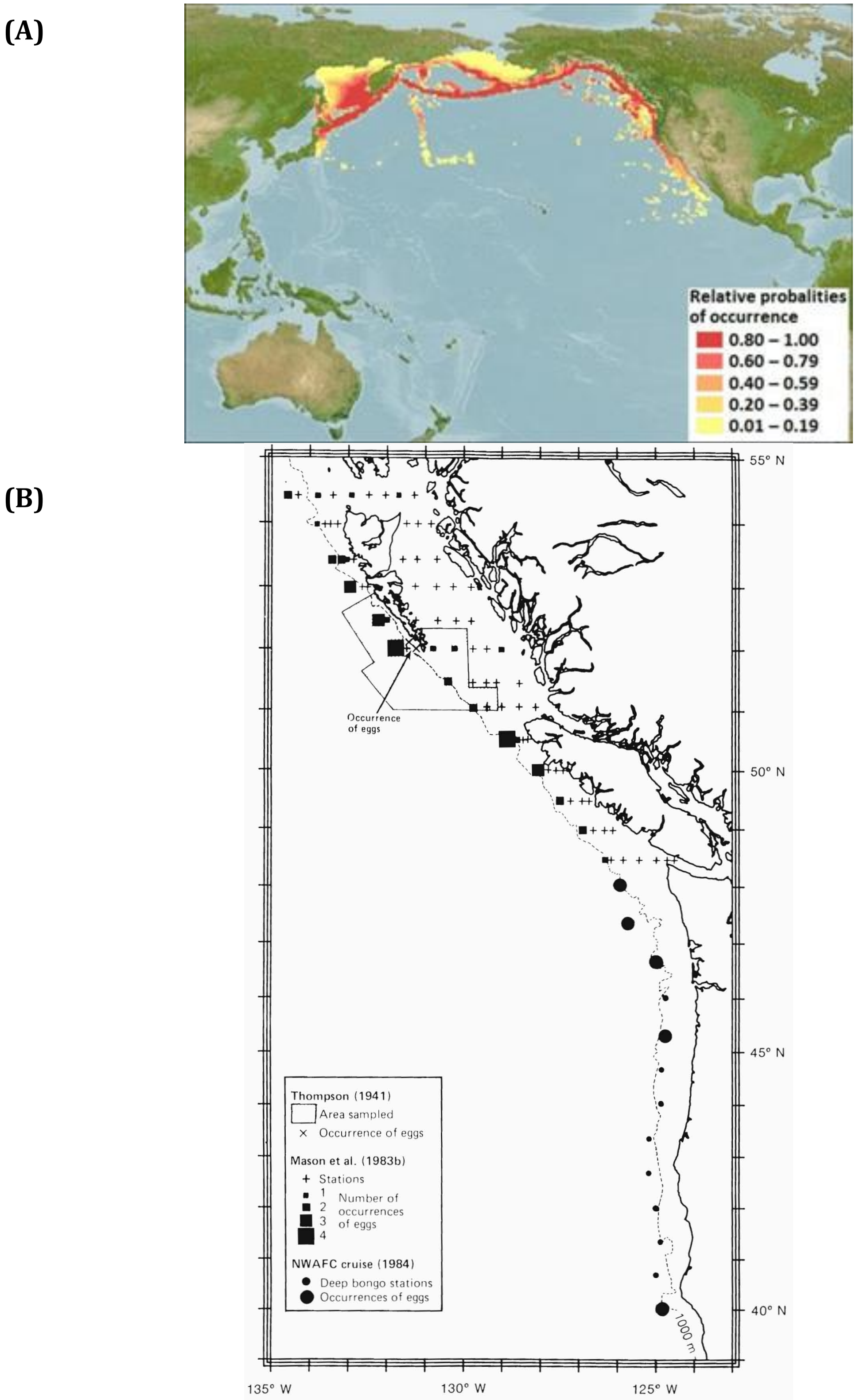

Figure 4. (A) Currently known global distribution (source: www.fishbase.org) and **(B)** occurrences of sablefish eggs along the Eastern Pacific coast based on Thompson (1941), Mason et al. (1983), and NWAFC (source: Kendall & Matarese, 1987).

1.5 Thesis Objectives

Describing thermal tolerance limits of fishes is useful for predicting how species will respond to ocean warming and how the structure of marine food webs may be altered due to changes in distribution patterns. Species distribution models (SDMs) are important for furthering the application and analysis of earth-system models (ESMs) by helping define impact-relevant metrics that ESMs should produce and also by validating simulated impacts (Bonan & Doney 2018; McKenzie et al. 2016). In addition, because phenotypic plasticity has the capacity to alter the position, height, breadth, and shape of acute TPCs, it is important to include acclimation responses when generating SDMs to provide more accurate depictions of acute responses to temperature (Schulte et al. 2011). Furthermore, conservation physiology research enables the development of evidence-based fisheries management practices and climate policies (Cooke et al. 2012; McKenzie et al. 2016).

To date, although there has been extensive research on the reproductive and developmental biology and life history of sablefish (Alderdice et al. 1988; Cook et al. 2018; Deary et al. 2019; Guzmán et al. 2017; Jensen & Damon, 2002; Kendall & Matarese, 1987; Krieger et al. 2019; Mason et al. 1983), research on how the heart responds to acute warming has only been examined in adults (Leeuwis et al. 2020). Leeuwis et al. (2020) examined the cardiorespiratory response to warming under acute hypoxia and found that adult sablefish were unable to increase heart rate or cardiac output while hypoxic. This was further associated with reductions in maximum O_2 consumption and thermal tolerance (CT_{max}). To date, no studies have quantified heart rates of the early life stages of Sablefish under any condition. The current study also measured heart rate to investigate thermal tolerances of sablefish embryos and yolk-sac-larvae, although hypoxia was not included as a variable.

The primary objective of this study was to assess whether sablefish embryos and yolk-sac larvae (YSL) have the potential for cardiac acclimation to warmer and colder temperatures than what they currently experience within their natural setting (~5.0°C) by quantifying changes in thermal tolerance limits. It was hypothesized that sablefish embryos and yolk-sac larvae incubated at warmer temperatures would have a higher thermal tolerance than sablefish embryos and YSL incubated at colder temperatures, as seen in other fish species (Bilyk & DeVries, 2011; Eliason et al. 2011; Pörtner & Lannig, 2009; Windisch et al. 2011). To test this hypothesis, cardiovascular performance during an acute temperature challenge was assessed in sablefish embryos and YSL that were previously incubated at different temperatures. This study also aimed to investigate whether the transportation of embryos (i.e., by humans) at different stages of development affected the response of heart rate (f_H) to acute warming. The effect of transport on embryos is of interest for other researchers who may wish to transport embryos from remote areas for research purposes.

Note: This study had initially intended to focus on Arctic cod (*Boreogadus saida*), a keystone fish species in Arctic ecosystems, and aimed to measure both f_H and AS during acute warming. Unfortunately, the associated experiments (conducted in collaboration with researchers from NOAA and Sheluqun Environmental Consulting at the Hatfield Marine Science Center in Newport, OR) had to be interrupted due to the COVID-19 pandemic. Consequently, I chose to shift my focus to sablefish due to (i) their economic value and (ii) UVic's proximity to a hatchery that could provide embryos. For the sablefish component, I was only able to measure f_H during acute warming and not AS. Preliminary data from the Arctic cod study were insufficient for a conclusive analysis but are included in Appendix A for use in potential future studies on Arctic cod.

Chapter 2: Methods

2.1 Embryo Collection and Transportation

Sablefish embryos were provided by a commercial supplier (Golden Eagle Sable Fish, Salt Spring Island, B.C., Canada). Adult sablefish were held at ~5.3°C and 30 ppt salinity at the commercial hatchery. Five batches (B#) of embryos were collected at different time periods from the commercial supplier (Figure 5). Fertilization occurred the afternoon before embryos were collected on the following morning (<24 hours). The morning of collection, approximately 100 ml of embryos were transferred into 8 L of 30 ppt seawater contained in double-bagged clear plastic. The plastic bags were placed in a cooler filled with Styrofoam packing peanuts and ice packs. Ice packs were not in direct contact with the bagged embryos, as the packing peanuts covered them.

After embryos were collected and packaged, the cooler was transported from Salt Spring Island to Vancouver Island via ferry and driven to the University of Victoria's (UVic). The total transport time from when embryos were collected and packaged to when they arrived to UVic was approximately four hours each time. During transport, the cooler remained sealed. Once at UVic, embryos were evenly distributed into three buckets corresponding to three different temperature treatments (~33 ml = ~20,000 embryos in each). The duration of time that the cooler was open and the bag of embryos was exposed to ambient air was approximately 30 minutes.

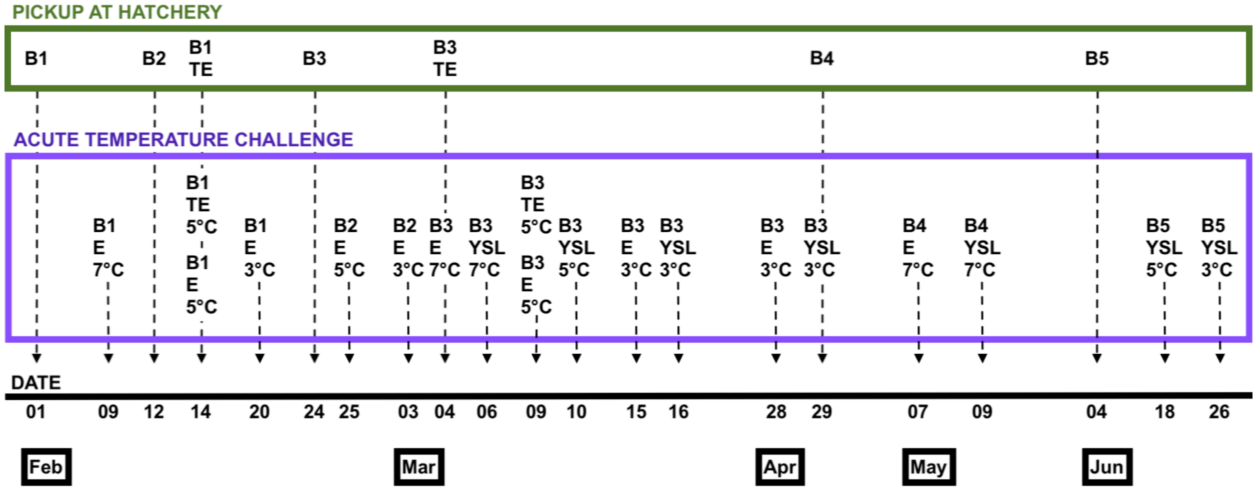

Figure 5. Experimental timeline of when sablefish embryos were picked up from a commercial supplier (Golden Eagle Sablefish Hatchery) and transported to the University of Victoria (UVic). A total of five batches (B#) of embryos (~100 ml or 60,000 embryos / batch) were picked up on five separate occasions (green box) at 0 days-post-fertilization. Once at UVic, embryos (E) were evenly distributed into 3 different temperature treatment groups (~33 ml or 20,000 embryos / temperature treatment): 3.0°, 5.0°, and 7.0°C. Once embryo heartbeats could be detected, embryos were acutely warmed in a testing chamber from 2° to 12°C (+1°C / 40 min-1). Heartbeats of individuals were video recorded one at a time at each +1°C increment and reviewed at a later date to determine f_H (b min-1). Embryos were allowed to hatch to yolk-sac larvae (YSL) and remained in the same bucket and incubation temperature as their initial holding setup. TE were embryos transported later in their development, immediately prior to when heartbeat could be detected. YSL and TE were exposed to the same acute temperature challenge as all other embryos. The various batches, treatment temperatures, and life stages were acutely warmed on separate occasions (purple box). Each temperature challenge took approximately eight hours to complete.

2.2 Experimental Setup and Holding Conditions

Buckets were filled with 10L of seawater and held in larger rectangular aquaria in which temperature was controlled using recirculating water chillers (Haake GH D8, Julabo F-32, and Fisherbrand™ Isotemp) (Figure 6). Multiple buckets were placed in each aquarium to accommodate more than one batch of embryos at a time. Batches were transported at separate times to stagger testing times and to also provide a variety of embryos (see Appendix B). Opaque buckets were used to block the penetration of light to keep the embryos in a dark environment. Airlines were delivered through a small hole in the lid of each bucket and an airstone was positioned at the bottom of the bucket. Continuous aeration also mimicked an upwelling effect for the embryos, which kept them off the bottom and mitigated fungal overgrowth.

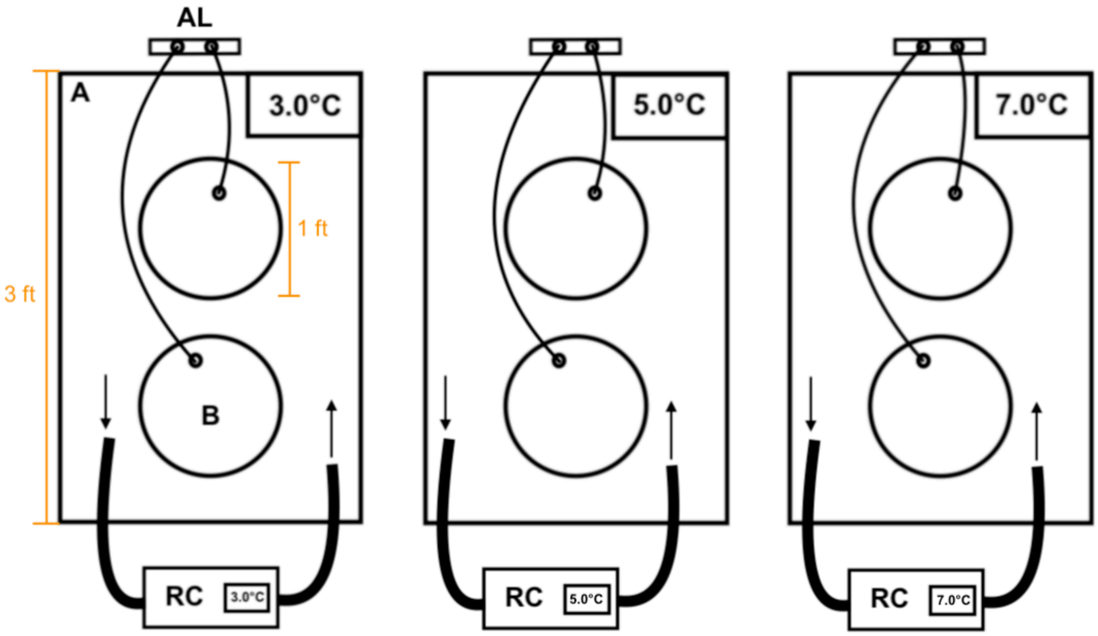

Figure 6. Incubation setup of sablefish (*Anoplopoma fimbria*) embryos and yolk-sac larvae (YSL) incubated at 3.0, 5.0, and 7.0°C. Bucket (B) held in larger aquaria (A) were filled with 10 L of 30 ppt seawater. Aquaria were temperature-controlled with recirculating chillers (RC). Each bucket contained approximately 20,000 (or 33 ml) embryos. Once embryos hatched into YSL, they remained in their respective buckets. Opaque buckets were used to prevent light penetration and were aerated continuously via airlines (AL).

Temperature was monitored twice daily for all buckets that contained embryos. Water changes were performed every other day, during which 5L (50%) of water was manually scooped out of each bucket using a small beaker and then replaced with water that had been previously chilled to the appropriate treatment temperature. This method allowed for minimal disturbance to the embryos.

2.3 Effects of Human Transport on Embryos (Collection, Transportation, and Holding Conditions)

For the transport testing experiment, embryos incubated at 5.0°C at the sablefish hatchery were transported later in their development (TE) and brought to UVic immediately prior to when their heartbeats could be detected. This amount of time was known from previous batches, since the first round of TE came from batch 3. To confirm that their hearts had not begun beating, TE were examined under a microscope the same day as they arrived at UVic. TE transportation occurred during three separate time periods, corresponding to three separate batches. TE were packaged and transported along with the double-bagged embryos that were at 0 days-post-fertilization (dpf). During transport, 5ml of TE were placed in a jar (500 ml) of 5.0°C 30ppt seawater and placed next to the other double-bagged embryos (0 dpf). Once at UVic, the jar containing TE was placed in a fridge (4.5°C). Hereafter, embryos reared at UVic since 0 dpf will simply be referred to as "UVic E".

2.4 Embryo Treatment Summary

UVic E were incubated at three different temperatures:

1. A low temperature group at 3.0°C
2. A control group at 5.0°C, and
3. A high temperature group at 7.0°C

A control group of 5.0°C was chosen because that is the temperature that sablefish are acclimated to at the hatchery and is also the temperature at which they are found in the natural environment along the BC coast (Mason et al. 1983). A low temperature of 3.0°C was chosen because sablefish embryos could be exposed to temperatures between 2.0° and 3.0°C in the Bering Sea at depths of ~1,000 m if adults were to shift their distribution polewards (Luchin et al. 1999). A high incubation temperature of 7.0°C was chosen on the basis that maximum survival of sablefish embryos has been found to occur up to 6.6°C and high mortalities (>50%) of embryos have been recorded at water temperatures above this (Alderdice et al. 1988; Cook et al. 2018). Embryos were allowed to hatch to yolk-sac larvae (YSL) and remained in the same bucket and incubation temperature as their initial holding setup. In total, the study comprised seven different treatments, two life stages (eggs and YSL) incubated at three temperatures each, in addition to embryos that were transported later in their development and incubated at 5.0°C (Table 1).

Table 1. Incubation temperatures of sablefish (*Anoplopoma fimbria*) embryos (E) and yolk-sac larvae (YSL). TE were sablefish embryos transported later in their development (TE), immediately prior to when a heartbeat could be detected.

Developmental Stage	E	E	E	YSL	YSL	YSL	TE
Incubation Temperature (°C)	3.0	5.0	7.0	3.0	5.0	7.0	5.0

2.5 Preliminary Testing

Prior to performing the acute temperature challenge, the following data were collected:

1. Time required for embryos to adjust to the testing chamber,
2. The lower limit of the acute temperature challenge,
3. Time required to acclimate to 2.0°C from starting point of treatment temperature,
4. Time required to acclimate to a +Δ1.0°C change in temperature,
5. The upper limit of the acute temperature challenge, and
6. Determining whether the O_2 concentration in the water of the testing chamber changed substantially during the acute temperature challenge.

Preliminary data were collected from the first group of embryos ($n = 3$) that had detectable heartbeats, which corresponded to batch 1 (B1) acclimated to 7.0°C. Preliminary testing was conducted in a testing chamber that consisted of a mesh screen submerged in a temperature-controlled water-jacketed glass vessel (250 ml) that was connected to a recirculating water chiller (Figure 7). The mesh screen was divided into multiple sections to keep the embryos separated during testing. The glass vessel was placed on top of a magnetic stir plate and a small magnetic stir bar was placed at the bottom of the glass vessel to ensure uniform mixing of water temperature. When videos of embryos were recorded, stirring of the water was halted to allow the camera to focus precisely on the heart of the embryos and to keep the embryos from moving during recording. Water temperature within the chamber was monitored continuously using a Fisherbrand™ temperature probe.

(1) To determine the amount of time required for embryos to adjust to the testing chamber, three embryos were video recorded at different time intervals, and their heart rates (f_H) at each time interval were compared. The embryos were placed into separate sections of the mesh screen in the chamber and video recorded using a camera- (AmScope™ 5MP USB3.0 CMOS) mounted stereoscope (AmScope™ SM-3T-54S-5M). The testing chamber was filled aerated 7.0°C seawater, the same as the respective acclimation water. Embryos were video recorded for one-minute immediately after they were placed in the chamber, as well as five-minutes after they were placed in the chamber. Embryo heartbeats were counted from the video recordings. No difference in f_H was detected between the two time periods for all three embryos. Therefore, I concluded that pre-experimental adjustment period did not need to extend beyond 5-minutes.

(2) To determine the lower limit of the acute temperature challenge, the goal was to decrease the water of the testing chamber in steps of 1.0°C (at a rate of 1°C 40 min^{-1}) to a temperature that elicited a critical temperature response (in this case, a critical response was expected to be arrhythmia or no heartbeat detection). The same three embryos from preliminary testing (1) were video-recorded for one-minute at each 1.0°C change and their heartbeats were

counted from the videos. Although arrhythmia was not detected at the lower limit for these embryos, no heartbeats could be detected for any of the embryos once the temperature was dropped to 1.0°C. The temperature was then increased to 2.0°C to see if the embryos would recover or if a heartbeat could be detected again. No heartbeats were detected once the temperature had been increased from 1.0 to 2.0°C, even after a 40-minute period. However, heartbeats were detected once the temperature was increased again to 3.0°C. Before the temperature was dropped to 1.0°C, heartbeats were detected at 2.0°C, although in some cases as low as 1 beat-per-minute (bm^{-1}). Based on these results, 2.0°C was selected as the lowest temperature of the acute temperature challenge for this study.

(3) During the experimental phase, each trial began by dropping the temperature of the testing chamber from the initial incubation temperature of the embryos to the lowest temperature of the acute temperature challenge. To determine the time required to adjust from this initial decrease during the challenge, the water of the testing chamber was decreased from 7.0°C to 2.0°C (without stopping) in 1.0°C increments. This cooling process took approximately 40 minutes. Embryos were video recorded at different time intervals, and their heart rates (f_H) at each time interval were compared. This test was done on three new embryos and the water of the testing chamber was replaced with new fully aerated 7.0°C seawater. Embryos were video recorded for a one-minute period once the temperature had stabilized to 2.0°C, and again after a further five-minutes had passed. Embryo f_H was counted from each one-minute video. There was no change in f_H during each time period for any of the embryos. This was most likely because the rate of change in temperature was considerably slow, especially when the temperature was changing from 3.0° to 2.0°C (~30 min), giving the embryos time to adjust. It was assumed that this would also be a sufficient time for embryos in the other two treatments (3.0° and 5.0°C) to

adjust because the overall change in temperature would be less (i.e. compared to a decrease from a starting point of 7.0°C).

(4) To determine the acclimation time to a +1.0°C change in temperature, the temperature of the testing chamber was increased from 2.0° to 3.0°C, and embryos were video recorded for a one-minute period immediately after the water had stabilized to 3.0°C, and then again five minutes later. Heartbeats were counted from the videos for all embryos at each time interval to determine f_H. No difference in f_H was detected for any of the embryos between the two time periods. It is known that biochemical reactions occur at faster rates at warmer temperatures (Ritchie, 2018). Therefore, it is suggested that organisms should take longer to adjust to colder temperatures. Once the temperature had stabilized after a +1°C increase, video recordings of embryos took place after a one-minute period.

(5) To determine the upper limit of the acute temperature challenge, the water temperature of the testing chamber was increased back to the acclimation temperature of 7.0°C and then increased in 1.0°C increments until arrhythmia was detected from video recordings of the three embryos. The temperature was increased in 1.0°C increments to 7.0°C to maintain a constant rate of temperature change. Embryos were video recorded for one-minute at each 1.0°C increment and f_H was determined from the videos. Arrhythmia was not detected until the temperature reached 12.0°C, at which point it was detected in all three embryos. Based on these results, 12.0°C was selected to be the highest temperature of the acute temperature challenge for this study.

(6) To determine the change in oxygen saturation over the course of the acute temperature challenge, O_2 concentration was recorded at the beginning of the challenge during preliminary testing (3) and then again at the end of preliminary testing (5) using a FireSting® O_2

meter. During this time, the O_2 concentration did not change more than 2%. It was therefore deemed unnecessary to continuously aerate the testing chamber during the acute temperature challenges. Providing a continuous stream of air bubbles to the chamber would have also created extra turbulence and increased the difficulty of maintaining proper orientation and position of the embryos on the mesh screen.

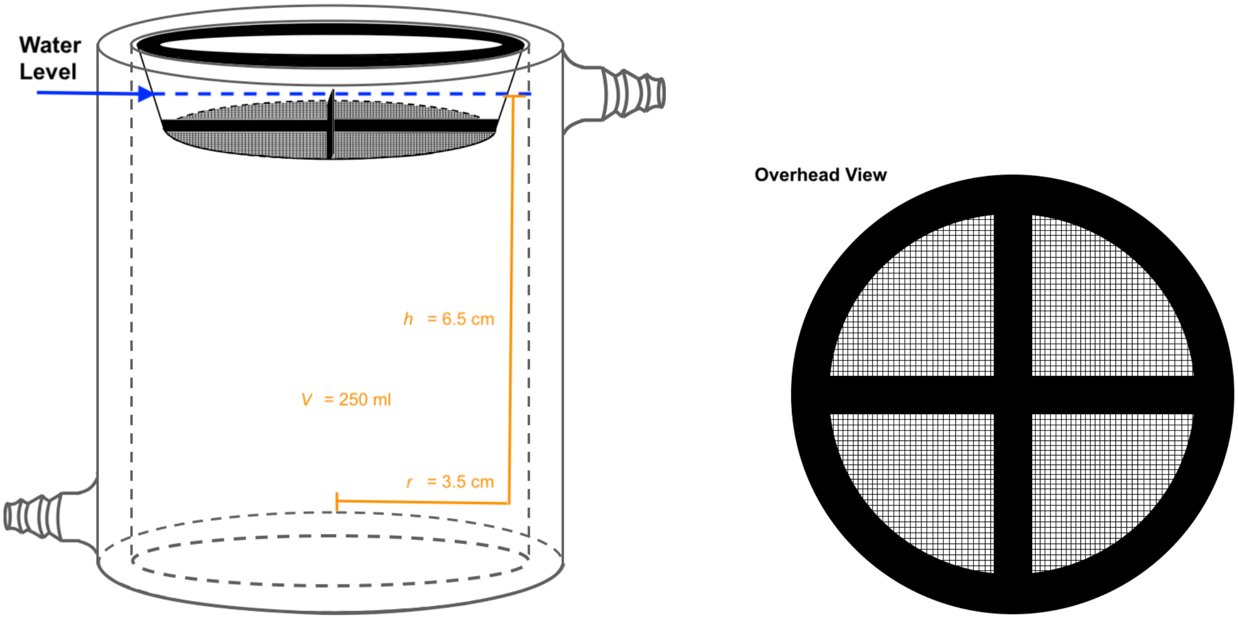

Figure 7. Testing chamber used to assess sablefish (*Anoplopoma fimbria*) embryos and yolk-sac larvae heart rate [f_{H} (b min-1)] during an acute temperature challenge from 2.0° to 12.0°C. The testing chamber consisted of a mesh screen in a temperature-controlled water-jacketed glass vessel (250 ml) that was connected to a recirculating water chiller. Dimensions; V = volume (250 ml), h = height (6.5 cm), r = radius (3.5 cm).

2.6 Cardiac Performance During an Acute Temperature Challenge

Cardiac performance of sablefish embryos and YSL was assessed through observations of changes in f_{H} during acute warming. Sablefish embryos and YSL incubated to three temperature treatments (3.0°, 5.0°, and 7.0°C) from five different batches were exposed to an acute temperature challenge from 2.0° to 12.0°C. Heartbeats of individuals were video recorded one at a time at each +1°C increment and reviewed at a later date to determine f_H (b min^{-1}). Each trial took approximately 8 hours to complete and the rate of temperature change was approximately +1°C 40 min^{-1}, which includes the time it took for the temperature to stabilize as well as the time it took to complete all video recordings (Figure 8). The various batches, treatment temperatures, and life stages were exposed to the temperature challenge on separate occasions (Table 2). For a given temperature treatment and life stage, embryos from the different batches were never tested more than 1 day apart in terms of their development (Table 2).

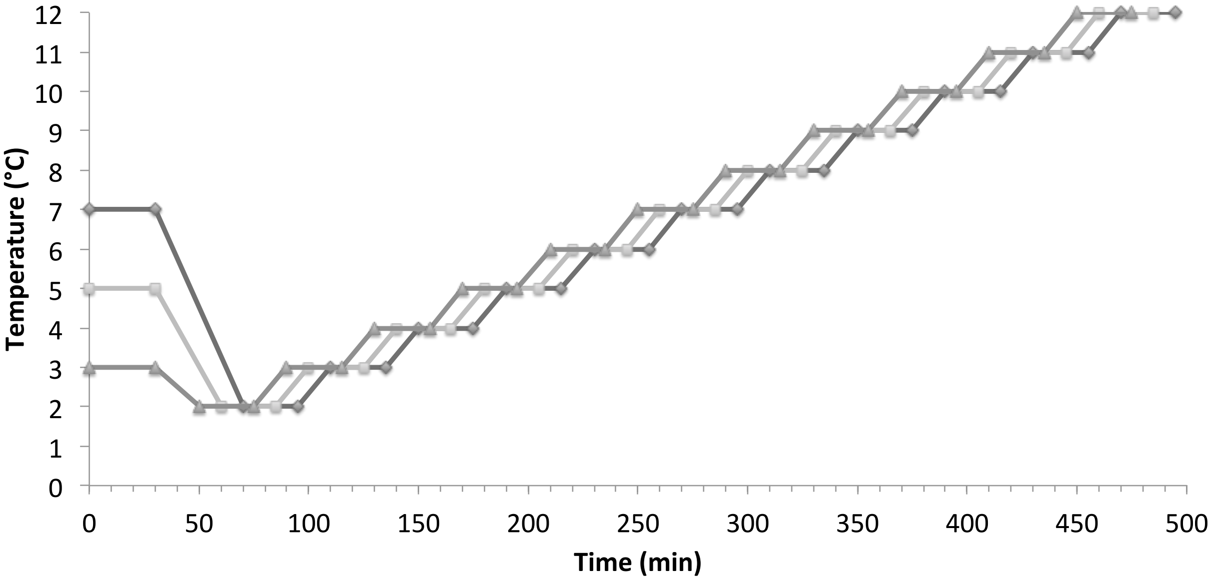

Figure 8. Total Duration (min) and ramping rate (°C/min) of acute temperature challenges that sablefish embryos and yolk-sac-larvae (YSL) incubated at 3°, 5°, and 7°C (triangle, square, and diamond, respectively) were exposed to. Each trial began by decreasing the temperature of the testing chamber from respective incubation temperatures to 2.0°C. The temperature of the chamber was then increased to 12°C in increments of 1.0°C.

Table 2. Developmental stage, in days-post-fertilization (dpf), of sablefish embryos (E) and yolk-sac larvae (YSL) that were subjected to an acute temperature challenge from 2.0° to 12.0°C over approximately an 8 hour time period. Sablefish embryos that were transported to UVic later in development (TE) were acclimated to 5.0°C and exposed to the same challenge. A total of five batches (B#) were tested and all on separate days.

	3.0°C		**5.0°C**		**7.0°C**		**5.0°C**
	E	**YSL**	**E**	**YSL**	**E**	**YSL**	**TE**
B1	20		14		9		14
B2	20		14				
B3	21	23	14	15	9	10	14
B4				15	9	11	
B5		23					

It is known that growth rates of fish often increase linearly with temperature, and this has already been documented in sablefish at various life stages (Alderdice et al. 1988; Cook et al. 2018; Sogard & Olla, 2001). Therefore, it was expected that the heartbeats of sablefish embryos incubated to 7.0°C would be detected first, followed by embryos incubated at 5.0°C, then embryos incubated at 3.0°C. Once embryos were received at UVic, 3 embryos were randomly selected from batch 1 embryos incubated at 7.0°C and placed in the testing chamber in aerated 7.0°C and 30ppt seawater every day and video-recorded to determine when heartbeats could be detected from all individuals. An acute temperature challenge was then initiated on this day. Testing of sablefish YSL was commenced the day that embryos hatched. Upon completion of testing on embryos and YSL incubated at 7.0°C, the same procedure was repeated with embryos and YSL that had been incubated at 5.0° and 3.0°C, as well as the TE which had been incubated at 5.0°C.

The acute temperature challenge was initiated by filling the testing chamber with aerated 30ppt seawater that had been chilled to the same temperature of the treatment water of the individuals being tested at that time. The water in the chamber was decreased to 2.0°C and embryos were individually video-recorded for two-minutes. The chamber water was then increased to 12.0°C in 1.0°C increments (Figure 8). At each increment, embryos were individually video-recorded for two-minutes. Similar to the preliminary testing phase of the experiments, stirring of the water was halted at any point when individuals were being video-recorded. The procedure for testing embryos and YSL were the same, except that the YSL were lightly anaesthetized by the addition of 45 mg l^{-1} tricaine methanesulphonate (MS-222) dissolved in seawater. Slight anesthesia of larvae was necessary because they are motile at this stage. This approach has been used on other species of fish larvae (Drost et al. 2016). In this study, an acute temperature challenge took anywhere from 8-10 hours in total. This study did not test the impacts of atropine or isoproterenol to achieve f_{Hmax}, a method commonly used for adult fishes (Drost et al. 2016).

Video recordings were analyzed at a later date to determine f_H. Videos were viewed at 2-8x the original speed (depending on the temperature) to accurately count heartbeats, since it was not possible to count low f_H values (at colder temperatures) at normal video speed. Heartbeats were counted manually from each two-minute video in one-minute intervals using a hand clicker. The average of each one-minute interval was used to determine mean f_H. In some instances, f_H could only be obtained from a single one-minute interval due to the inherent challenges of this method, highlighted in section 4.7 Limitations of the Study.

2.7 Breakpoint Temperature Indices

To determine T_{AB} for a given individual, an Arrhenius plot was made by plotting mean ln f_H values against 1000 K^{-1} and T_{AB} values were taken as the point at which the two regression lines intersected using piecewise (segmented) regression analysis (Yeager and Ultsch, 1989; Casselman et al. 2012; Gilbert et al. 2020). T_{QB} was determined for an individual when the incremental Q_{10} first decreased and remained below 2.0 (Farrell, 2016). Q_{10} was calculated for all individuals at each 1.0°C change in temperature using the equation $Q_{10} = [(f_{H2})(f_{H1})^{-1}]\hat{}[10(T_2-T_1)^{-1}]$, where f_{H1} and f_{H2} are heart rates at the first (T_1) and second (T_2) temperatures (Anttila et al. 2013; Drost et al. 2014). Calculated Q_{10} values are provided in Appendix B. T_{max} for each individual was taken as being the temperature at which f_H reached its maximum value. T_{AR} was identified for an individual as being the temperature at which the heart first became arrhythmic. Arrhythmia was defined as occurring when an irregularity was observed in the heartbeat displaying either tachycardia or bradycardia, i.e., a shorter or longer pause was observed between two beats compared to the other beats at that temperature, respectively.

2.8 Data Analysis

Mean f_H ± SE was calculated for each individual within a given treatment and then mean f_H ± SE from each treatment were plotted together (Figure 9). For each treatment, individuals were plotted separately and colour-coded by their respective batch (Figures 10, E1, E2). Data were also examined by calculating mean f_H ± SE values for individual batches of each treatment (Figure C3). The percent difference between the lowest and highest f_H value of individuals and batches (mean ± SE) within a given treatment were calculated. The mean percent difference between the lowest and highest f_H value were also compared between life stages within the same temperature treatment, as well as between UVic E and TE incubated at 5.0°C.

Breakpoint indices were calculated and determined for every individual embryo and YSL from all batches and treatment groups. At a given life stage and temperature treatment, data were averaged from all individuals from all batches to calculate mean ± SE. Within a given life stage, breakpoint indices were compared among the three temperature treatments by using a one-way analysis of variance (ANOVA) ($\alpha < 0.05$). Breakpoint indices calculated for TE were compared with the control group of this study (UVic E incubated to 5.0°C) using a Student's t-test ($\alpha < 0.05$).

Once T_{AR} was detected in an individual, f_H values beyond this temperature were not included in the data analysis. Information on sample size and how it changed throughout the acute temperature challenge is provided in Appendix D.

Chapter 3: Results

3.1 Heart Rate vs. Temperature

As expected, mean heart rate (f_H) increased with temperature during the acute temperature challenge for sablefish embryos and YSL incubated at all three temperatures (Figure 9). YSL had consistently higher mean f_H values compared to embryos incubated at the same temperature. With increasing incubation temperature at the YSL stage, mean f_H was always higher at every temperature of the challenge. Interestingly, the mean f_H of embryos incubated at 5.0°C was higher at every temperature when compared to embryos incubated at 7.0°C. Mean f_H of embryos incubated at 3.0°C was the lowest at every temperature, except at 2.0° and 3.0°C where it was the highest among the three treatments at the embryo stage.

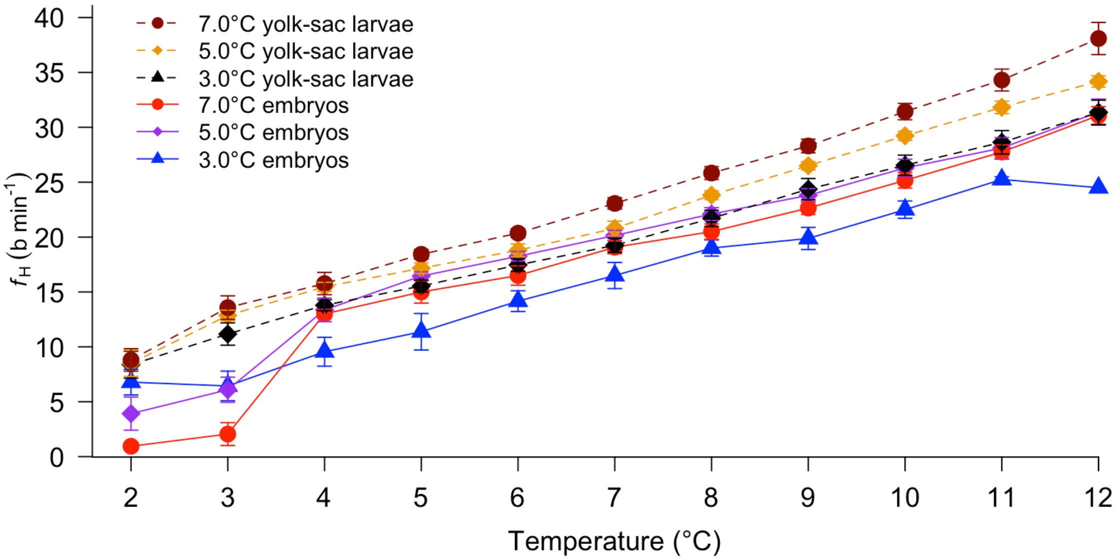

Figure 9. Heart rate [f_H (b min-1)] response of sablefish (*Anoplopoma fimbria*) embryos and yolk-sac larvae acclimated to 3.0°, 5.0°, and 7.0°C during acute warming. All individuals were exposed to an acute temperature challenge from 2.0° to 12.0°C. Data are represented as mean f_H ± SE.

In almost all instances, there appeared to be a sharp increase in mean f_H at lower temperatures of the acute temperature challenge, resulting in higher Q_{10} values. Here, a sharp increase is defined as $Q_{10} > 10$ (Gilbert et al. 2020). Q_{10} values were highest when the temperature increased from 3.0° to 4.0°C for the majority of embryos in all treatments, including TE (Figure 9, Table B1). Embryos acclimated to 3.0°C had the most variability in the temperature at which the highest Q_{10} was recorded (Table B1). The majority of YSL incubated at 3.0°C showed the highest Q_{10} value when the temperature was increased from 3.0° to 4.0°C (Figure 9, Table B1). For YSL incubated at 5.0° and 7.0°C, Q_{10} was highest when the temperature was increased from 2.0° to 3.0°C (Figure 9, Table B1).

When all individuals were considered, the % difference between the lowest and highest f_H values was the greatest among the YSL as compared to embryos incubated at the same temperature (Table 3). Embryos incubated at 3.0°C showed the greatest % difference in f_H among the embryo treatments reared at UVic since 0 dpf. However, the TE had the greatest % difference overall (Table 3). YSL incubated at 3.0°C also showed the greatest mean % difference in f_H among the three YSL treatments when all individuals were considered (Table 3). However, when % difference was compared among batches within a given treatment, the mean % difference was the smallest for YSL (Table 3).

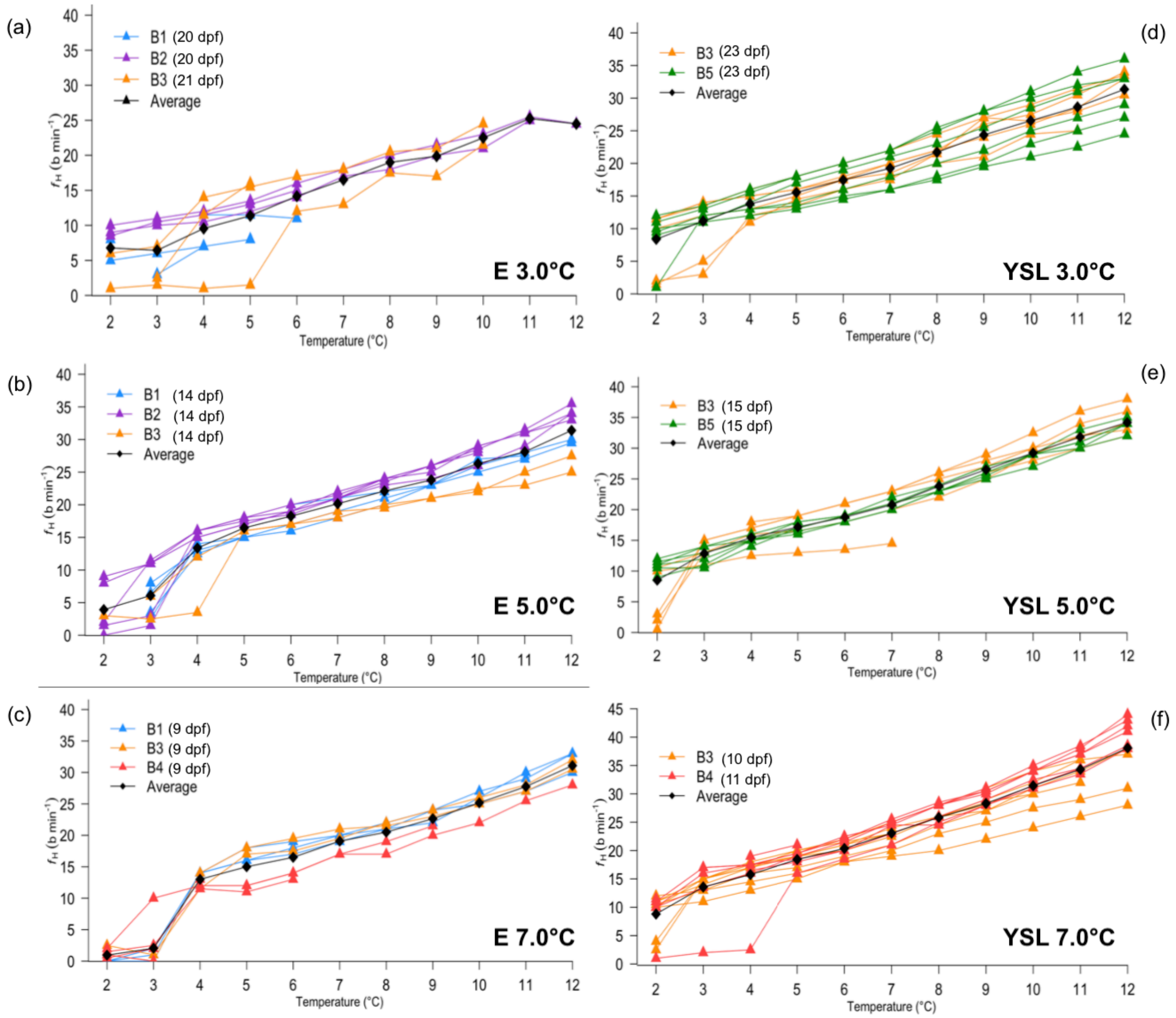

Figure 10. Effect of acute warming on the heart rate [f_H (b min-1)] of sablefish (*Anoplopoma fimbria*) embryos (a), (b), and (c) and yolk-sac larvae (d), (e), and (f) acclimated to 3.0°, 5.0°, and 7.0°C, respectively. All individuals were exposed to an acute temperature challenge from 2.0° to 12.0°C. For each treatment, individual f_H values are plotted and colour-coded by batch, and mean f_H ± SE values are shown by the black line and symbol.

There was consistent overlap in mean f_H ± SE at each temperature of the acute temperature challenge between UVic E and TE incubated at 5.0°C (Figure 11). When mean f_H values were compared, the mean % difference between the two groups was 7.2 ± 1.7 % (Table 4).

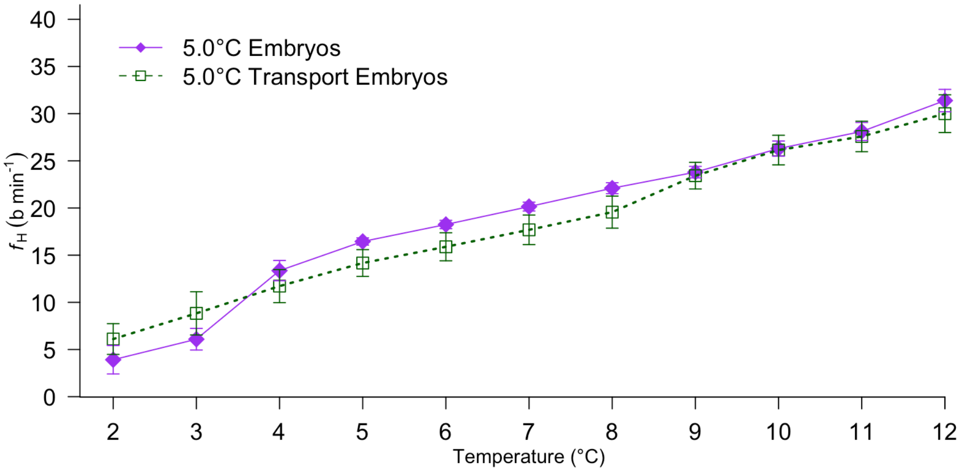

Figure 11. Effect of acute warming on the heart rate [f_H (b min-1)] of sablefish (*Anoplopoma fimbria*) embryos incubated at 5.0°C in the laboratory at UVic since 0 days-post-fertilization and sablefish embryos transported to the UVic laboratory from the hatchery immediately prior to heartbeat detection, also acclimated to 5.0°C. All individuals were subjected to an acute temperature challenge from 2.0° to 12.0°C. Data are represented as mean fH ± SE.

Table 3. The percent (%) difference between the lowest and highest heart rate [f_H (b min^{-1})] value of (a) individuals and (b) mean f_H of batches within a given treatment during an acute temperature challenge of 2.0 to 12.0°C. Data are represented as mean ± SE.

	E 3°C	E 5°C	E 7°C	YSL 3°C	YSL 5°C	YSL 7°C	TE 5°C
(a) Individuals	36.1 ± 10.7	27.8 ± 2.6	29.6 ± 5.4	39.2 ± 1.8	32.1 ± 4.9	38.7 ± 3.8	100.3 ± 20.4
(b) Mean f_H of batches	22.6 ± 7.8	22.1 ± 2.2	22.1 ± 4.2	3.1 ± 1.2	3.1 ± 0.7	9.3 ± 1.6	58.7 ± 9.3

Table 4. The mean percent (%) difference (± SE) between the lowest and highest mean heart rate [f_H (b min^{-1})] values of embryos (E) and yolk-sac larvae (YSL) incubated at the same temperature, as well as between the control group of this study (E 5.0°C), and embryos transported later in their development (TE 5.0°C). All individuals were exposed to an acute temperature challenge from 2.0° to 12.0°C.

Incubation Temperature	3.0°C	5.0°C	7.0°C	5.0°C
Developmental stage	E vs. YSL	E vs. YSL	E vs. YSL	E vs. TE
Mean % difference	23.2 ± 2.2	7.8 ± 1.2	24.2 ± 0.7	7.2 ± 1.7

3.2 Breakpoint Temperature Indices

It was determined that T_{AB} and T_{QB} were not suitable indices for interpreting the results of this study. T_{AB} was calculated for every single individual and the majority of breakpoint values that were obtained corresponded to inflection points of an increasing slope. In the field of cardiovascular physiology, T_{AB} typically indicates when f_H declines, which is shown by a point of inflection where the slope of the line abruptly *decreases* (Casselman, 2012; Drost et al. 2016; Gilbert et al. 2020). During the acute temperature challenge, the f_H of many embryos increased by 1 beat-per-minute (b m^{-1}) at lower temperatures and then increased by 2 b m^{-1} at higher temperatures, resulting in an increase in slopes. Consequently, reliable breakpoints could not be determined from T_{AB} calculations.

For a given individual, T_{QB} is generally predicted to occur at a lower temperature than T_{max} (Farrell, 2016). However, T_{QB} was only identified in 1 of the 13 individuals that reached T_{max}. In total, T_{QB} was identified for just four individuals. Therefore, it was decided that T_{QB} was not a useful index for this study. Identified T_{QB} values, as well as calculated Q_{10} values, are provided in Appendix B.

Arrhythmia was observed in 22 of the 76 individuals in this study. However, T_{max} was only achieved in 13 of those 22 (Table 5). Among all treatments, the greatest number of individuals to reach T_{max} were among the embryos incubated at 3.0°C. T_{max} was not reached for any UVic E incubated at 5.0°C. Among the TE, only one individual reached T_{max}. There was no significant difference in T_{max} between the embryos incubated at 3.0 and 7.0°C [$t(5) = [1.3]$, $p = 0.3$]. Similarly, there was no significant difference among T_{max} for YSL incubated at the three temperatures [$F(2, 3) = [3.0]$, $p = 0.2$].

Embryos incubated at 3.0°C displayed the highest frequency of arrhythmic behaviour (*n* = 9 individuals) compared to all other treatments (Table 5). Mean T_{AR} was similar among all incubation temperatures for UVIC E and no significant difference was detected [$F(2, 11) = [0.3]$, $p = 0.79$]. There was also no significant difference in mean T_{AR} between the embryos of the control group and TE [$t(2) = 0.3$, $p = 0.8$]. No significant difference in mean T_{AR} was detected among the three YSL temperature groups either [$F(2, 3) = [3.6]$, $p = 0.2$]. No mortalities were observed throughout any acute temperature challenge.

Table 5. The temperature at which heart rate [f_H (b min^{-1})] reaches its maximum value (T_{max}) and the arrhythmic temperatures (T_{AR}) of sablefish (*Anoplopoma fimbria*) embryos (E) and yolk-sac larvae (YSL) that were acclimated to 3.0°, 5.0°, and 7.0°C and were subjected to an acute temperature challenge from 2.0° to 12.0°C. UVic embryos and yolk-sac larvae were incubated at the research facility since 0 days-post-fertilization (dpf), while transported embryos (TE) were incubated at the hatchery and transported to the research facility immediately prior to experimentation. Data are represented as mean ± SE.

Variable	E 3°C	E 5°C	E 7°C	YSL 3°C	YSL 5°C	YSL 7°C	TE 5°C
	n = 9	*n* = 11	*n* = 9	*n* = 12	*n* = 12	*n* = 15	*n* = 8
T_{max} (°C)	9.4 ± 1.1	n/a	6.5 ± 2.5	9.5 ± 1.5	7	10.5 ± 0.5	10.0
n	5	0	2	2	1	2	1
T_{AR} (°C)	8.6 ± 1.0	9.0 ± 3.0	8.5 ± 1.5	10.5 ± 1.5	8	11.7 ± 0.3	10.0 ± 1.0
n	9	2	3	2	1	3	2

3.3 Batches Not Included

Data were not collected from the following batches (B#) and incubation temperatures due to fungal overgrowth resulting in 100% mortality: B4 3.0°C embryos and YSL, B4 5.0°C embryos and YSL, B2 7.0°C embryos and YSL, B5 7.0°C embryos and YSL. Fungal growth is a widely known logistical challenge when working with fish embryos, especially in small volumes without water throughflow (Rahman et al. 2017). Batch B5 was removed from the data analysis for embryos incubated at 3.0° and 5.0°C but included for YSL incubated at these temperatures.

Arrhythmia was detected at almost every temperature of the acute temperature challenge and reliable data could not be collected for B5 embryos incubated at 3.0° and 5.0°C. However, this issue was not present at the YSL stage. This was likely due to the increased probability of choosing embryos that would have not survived to the YSL stage due to the likely decline in overall batch health and increase in natural mortality that occurs with subsequent batches in the reproductive season (Briony Campbell, Golden Eagle Sablefish Hatchery, personal communication, January, 2021). Mortality data could not be collected throughout this experiment to confirm this, due to limitations in the experimental setup and reduced staff working on this project due to COVID-19 safety protocols. TE of B2 were also removed from the data analysis for similar reasons of detecting arrhythmia at almost all temperatures of the acute temperature challenge, an indication of poor embryo health. Embryos from B2 were packaged and transported together in the same cooler as B3 embryos (0 dpf). There were no issues in embryo health with B3, therefore, it is not likely that the issues observed with B2 embryos in the transportation treatment were due to the transportation itself but likely to due poor hatchery conditions for this particular batch.

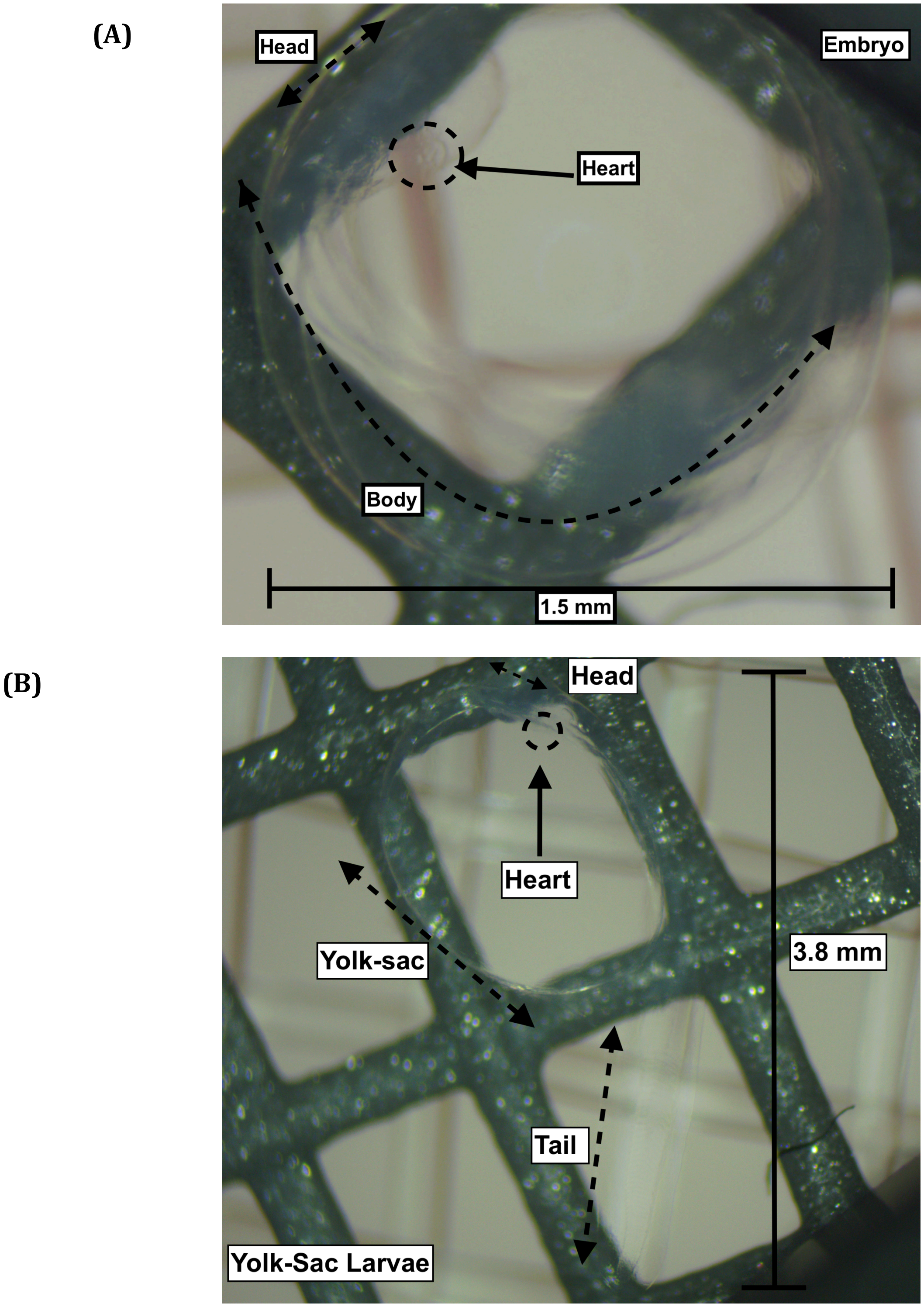

Figure 12. Photo of a sablefish (*Anoplopoma fimbria*) (A) embryos and (B) yolk-sac larvae during an acute temperature challenge. The embryo was from batch 2 and was incubated at 3.0°C. The yolk-sac larvae was from batch 3 and also incubated at 3.0°C. Both photos were taken during separate acute temperature challenges but both at their acclimation temperature of 3.0°C.

Chapter 4: Discussion

4.1 Purpose of Study

The ocean is the primary heat reservoir in earth's climate system and has been warming over the last several decades due to anthropogenic-induced climate change (Singh et al. 2022; NOAA, 2022). With ocean warming, temperatures may exceed the upper critical temperatures of many marine species in their current environment (Cheung et al. 2009). Some species may also experience temperatures below their cold tolerance limits during the coldest months of the year if they shift their distribution polewards to seek thermal refuge (Sunday et al. 2012). Therefore, characterizing thermal tolerance limits of marine species is important in order to project where they may redistribute to and to assess their overall likely success of survival in a warming climate.

Of the very few studies that have described thermal tolerance of fish embryos, most have examined developmental rates, survival rates, aerobic scope, aerobic exercise, and CT_{max} (Cook et al. 2018; Flynn & Todgham, 2017; Lee et al. 2016; Martin et al. 2020; Rio et al. 2019). The majority of studies that have quantified fish embryo f_H have focused largely on confirming methodological approaches, rather than using f_H to characterize cardiac plasticity *per se* (Gierten et al. 2020; Kang et al. 2018; Pylatiuk et al. 2014; Sampurna et al. 2018). A few others measured f_H to assess hypoxia tolerance and effects of contaminants (Agathokleous, 2022; Barrionuevo & Burggren, 1999).

The paucity of studies examining fish embryonic f_H, in comparison to adults, is primarily due to logistical constraints – since embryos need to be translucent to visually assess f_H and because f_H cannot be assessed via electrocardiogram as it can be in adults. That said, there appears to be considerable variation among heart rate values of other fish species, even when exposed to similar temperatures as the sablefish embryos in the current study. For example, brook charr embryos at 49 dpf (*n* = 15) had a mean f_H of 43.6 b min^{-1} at 6.0°C (Benfey and Bennett, 2009), while lake whitefish embryos (*n* = 18) tested at 25 dpf had a mean f_H of ~18 b min^{-1} at 5.0°C (Eme et al. 2015).

The purpose of this study was to assess whether sablefish embryos and yolk-sac larvae (YSL) have the potential for cardiac acclimation to temperatures outside of what they currently experience in their natural setting (~5°C) by examining changes in their thermal tolerance limits. Cardiac performance was assessed during an acute temperature challenge from 2.0° to 12.0°C for individuals incubated at 3.0°C, 5.0°C (control), and 7.0°C. This is the first study to provide any heart rate (f_H) measurements of sablefish embryos and YSL under any conditions. It is also the first study that aimed to characterize thermal tolerance of fish embryos and YSL by using temperature breakpoint analysis of cardiac performance during acute warming.

4.2 Sablefish Embryos and YSL Heart Rate Response to Acute Warming

In this study, mean f_H of YSL increased with incubation temperature at every incremental step in the acute temperature challenge (Figure 9). An increase in f_H with incubation temperature reflects changes in the height of the TPCs (Figure 1). In contrast, this was not the case for embryos. Embryos incubated at 7.0°C had consistently lower mean f_H values compared to embryos incubated at 5.0°C (Figure 9). However, there was still a consistent overlap in mean f_H values between these two groups once the challenge extended above 3.0°C. Embryos incubated at 3.0°C had the lowest mean f_H values among the three temperature groups when temperatures in the challenge exceeded 3°C.

To determine whether there were changes to the width of TPCs, both the upper and lower thermal limits would need to be identified (Figure 1). In this study, T_{AR} was used as an indicator that an individual was near its upper thermal tolerance limit. However, T_{AR} was only reached in 29% of all individuals. Although there were no significant differences in T_{AR} among any of the treatments, the number of individuals that reached T_{AR} was noticeably higher for embryos incubated at 3.0°C (Table 5). All embryos (100%) incubated at 3.0°C exhibited arrhythmic behaviour. In contrast, only 18% and 33% of embryos incubated at 5.0° and 7.0°C exhibited arrhythmia, respectively. It is unclear whether the counts of arrhythmia observed by a small number of embryos and YSL in the various treatments represents natural individual variability or, conversely, that these particular embryos were not in good health. As mentioned earlier, high mortalities in these early life stages of fishes are expected and normal and it is therefore possible that not all individuals chosen for testing in this study were at optimal health.

The lower thermal limits were also not well-defined for all individuals in this study. However, during the preliminary trials, temperatures were initially dropped to 1.0°C at which point heartbeats were no longer detected for embryos incubated at 7.0°C. These embryos also had difficulty recovering once having been exposed to 1.0°C. This was evidenced by the fact that embryo f_H at 2.0°C (i.e., prior to a 1.0°C exposure) was higher than embryo f_H at 2.0°C once the temperature was increased again after the exposure to 1.0°C, even after 30 minutes had passed. This suggests that 1.0°C may be (or may be close to) the lower critical temperature of embryos that are incubated at 7.0°C. It was not confirmed whether embryos incubated at 3.0° and 5.0°C also have difficulty recovering f_H after exposure to 1.0°C (nor for YSL incubated at any incubation temperature). However, the available data suggest that embryos incubated at 3.0° and 5.0°C, as well as YSL incubated at all three temperatures, may not have had the same difficulties with cardiac recovery at temperatures as low as 1.0°C. This is primarily inferred from the fact that embryos incubated at 7.0°C had the lowest mean f_H at 2.0° and 3.0°C of the acute temperature challenge and the highest mean Q_{10} value (indicated by the largest increase in f_H) when temperatures increased beyond this point. The reduced thermal sensitivity of embryos incubated at 3.0° and 5.0°C at those lower temperatures suggests they had increased cardiac thermal compensation ability, compared to embryos incubated at 7.0°C. Therefore, it's possible that embryos incubated at 3.0° and 5.0°C may have had a lower thermal tolerance limit than 1.0°C. However, this would need to be verified.

Based on these results, it is unclear whether the thermal tolerance limits of sablefish embryos and YSL incubated at higher and lower temperatures (7.0° and 3.0°C, respectively) have shifted or if they have contracted relative to those incubated at 5.0°C (i.e., which represents the "normal" temperature currently encountered by sablefish embryos off the BC coast). To conclusively determine whether a shift in thermal tolerance occurred for embryos, the following thermal limits would also need to be identified and compared: (1) the lower thermal limits of embryos incubated at 3.0° and 5.0°C, and (2) the upper thermal limits of embryos incubated at 5.0° and 7.0°C. As for YSL, the upper and lower thermal limits of all three temperature treatment groups would need to be identified. Even though the upper and lower thermal limits of YSL were not identified here, there is some evidence of thermal plasticity in cardiac function, since mean f_H increased with incubation temperature.

I was also unable to identify the breakpoint indices T_{AB} and T_{QB}. These indices provide initial indications of change in heart rate during acute warming. Therefore, it remains unknown if there was a difference in the temperature at which the heart began to reduce its thermal compensation ability between incubation temperatures.

4.3 Unexpected Results From Temperature Breakpoint Analysis

The breakpoint analysis of this study revealed several unexpected results. First, T_{max} was not detected for all individuals that experienced arrhythmia (9 of 22). This was surprising because if T_{AR} indicates that the organism is near cardiac collapse, it is expected that its f_H would reach a maximum and decline before reaching T_{AR}. Second, T_{QB} was not detected for all individuals that reached T_{max} (only 1 of 13 individuals). This was also unexpected, because T_{QB} indicates a reduction in thermal sensitivity of f_H to a 10°C rise in temperature, which is expected to occur before f_H plateaus as it reaches a maximum. Finally, T_{AB} was found not to be a suitable index for use in this study due to positive inflection points when mean $\ln(f_H)$ values were plotted against 1000 K^{-1}. Again, this was surprising because the rate at which f_H increases typically decreases before reaching T_{max}.

To date, no other studies have used breakpoint temperature indices to describe the effect of acute warming on f_H of other fish embryos or YSL. However, studies that have identified breakpoint temperature indices in adults or more developed larvae when exposed to an acute temperature challenge report results that oppose all of the unexpected findings listed above (Anttila et al. 2014; Casselman et al. 2012; Drost et al. 2016; Ferreira et al. 2014; Gilbert et al. 2020; Gilbert & Farrell, 2021; Joyce et al. 2018; Skeeles et al. 2020). These findings imply that cardiac performance in embryos and YSL must be different than those of older life stages.

During the embryonic stage, the heart is still maturing and forming new vessels, cardiac output is still growing, and it is hypothesized that the heart begins to beat well before convective blood flow is needed for bulk transport (Burggren, 2004). Under-developed hearts, coupled with a reduced cardiac output, may explain why several individuals in this study exhibited arrhythmia before reaching T_{max}. A greater number of embryos displayed arrhythmic behaviour without reaching T_{max}, compared to YSL. A higher T_{AR} to T_{max} ratio for embryos is likely due to embryo hearts being even more under-developed than YSL. If this is the case, T_{AR} may not necessarily be an indicator of cardiac collapse, *per se*, in fish embryos and YSL if T_{max} has not already been reached. However, if both T_{AR} and T_{max} have been reached, it's more likely that the organism is near cardiac collapse.

The fact that T_{AB} was found not to be a suitable index may be due to a lack of (or underdeveloped) mechanistic controls that help stabilize f_H under changing conditions. Due to cardiac performance of sablefish embryos and YSL not following the "standard TPC" observed in adult fish, the standard characteristics and performance indicators used to describe acclimation potential and thermal tolerance limits may not apply for fish embryos and YSL. It is also possible that the early life stages of sablefish may not have the ability to acclimate their cardiac function because their hearts have not fully developed. Therefore, f_H may not be the best performance metric to use and other indicators such as survival and growth rates may be more useful and reliable when assessing the physiological performance of the early life stages of fishes.

4.4 Ecological Relevance

It is apparent from the TPCs generated in this study that there is some degree of thermal compensation of cardiac function with temperature in sablefish embryos and YSL, but this study did not describe their full cardiac acclimation potential. Nevertheless, it is important to keep in mind the natural oceanic conditions that are ecologically relevant for sablefish. Sablefish embryos and YSL are currently exposed to temperatures around 5°C at depths of around 1,000 m off the coast of British Columbia (Mason et al. 1983). Typically, the embryos and larvae of most marine fish species are found in near-surface waters (Conway et al. 1997; Liu et al. 1993; Parker et al. 2021; Sabatés et al. 2007; Somarakis et al. 2004). Near-surface waters have been warming at a much faster rate than at depths near 1,000 m (Altieri & Gedan, 2014; Voss & Mikolajewicz, 2001). By the end of the 21st century, it is projected that waters at depths of 600-1,000 m will warm by ~0.8°C (Manuel et al. 2021). Therefore, even if sablefish embryos incubate at temperatures as low as 3°C, it seems likely that they will not be exposed to their upper critical temperature of (8.6 ± 1.0°C) by the end of the 21st century. Sablefish embryos and YSL that incubate at higher temperatures (i.e., 5.0° and 7.0°C) are not likely to be threatened while developing at depth, as the majority of individuals incubated at these temperatures appear to have an upper thermal tolerance limit that exceeds 12.0°C. Depending on the region, water temperatures in the Bering Sea (*i.e.,* the region where adult sablefish could expand northward in response to a warming ocean) range approximately between 2.0° and 3.0°C at depths near 1,000 m, and temporal variations at those depths are less than 0.3°C (Luchin et al. 1999).

Although this study examined the embryonic and YSL stages of sablefish, the thermal limits of exogenously feeding (i.e., post-yolksac) larvae have not yet been tested and it is possible that they could be vulnerable to increasing temperatures as they migrate from depths od 1,000 m to the surface layer. Larvae at this stage are largely neustonic and are therefore exposed to the widest range of water temperatures in comparison to other sablefish life history stages. Being planktonic, they are also at the whim of surface currents (Lobel & Robinson, 1986). It is therefore possible that post-yolksac sablefish larvae may be most threatened by ocean warming, as they are exposed to the most rapidly changing temperatures in the ocean. Assessing the thermal tolerance limits of post-yolksac sablefish larvae should therefore be a priority for understanding how the species will respond to climate change.

Finally, the lower thermal limit of sablefish embryos and YSL incubated at 3.0°C was not reached in this study. This suggests that sablefish embryos may be able to tolerate temperatures at more northern latitudes if the species shifts its distribution. However, it would be of interest to increase the duration of exposure at these low temperatures to investigate whether sablefish embryos and YSL can survive for extended periods at northern latitudes and overwinter successfully. Therefore, sablefish may have the capacity to expand their distribution to northern latitudes and overwinter successfully, as exposure to temperatures below the cold critical limits of the embryos is not currently of concern. However, keeping in mind that this study did not identify the T_{AB} and T_{QB} breakpoint indices, it is unclear at which temperatures cardiac performance of sablefish embryos and YSL will begin to decline.

4.5 Effects of Transportation on Sablefish Embryo Cardiac Performance

In addition to examining acclimation potential, this study is also the first to investigate whether the timing of transportation of sablefish embryos at different stages in development had an effect on their cardiac performance. Determining whether transportation has effects on cardiac performance may be of interest for other researchers who need to transport fish embryos from the field to the laboratory. Sablefish embryos incubated at 5.0°C were transported from the sablefish hatchery later in their development (TE) before being exposed to the same acute temperature challenge as the other embryo treatments. Cardiac performance of the TE was compared with the control group of this study (UVic E incubated at 5.0°C). T_{max} and T_{AR} were characterized for a similar number of individuals in the two groups. There was consistent overlap in mean f_H between these two groups at each temperature of the acute temperature challenge. Therefore, there appears to be no indication that transportation affected the heart rate response of sablefish embryos when incubated at the same temperature. However, these results remain somewhat inconclusive due to small sample size and the fact that T_{max} and T_{AR} were not reached for all individuals in each treatment. Future studies should also compare other breakpoint temperature indices that characterize physiological performance. Although no other studies appear to have assessed the impacts of embryo transportation, a few have examined cortisol response to embryo handling. For example, Ghaedi et al. (2014) found no difference in whole-body cortisol from acute handling stress when spent 5-minutes out-of-water.

4.6 Limitations of the Study

Although collecting heart rate measurements has been considered one of the quicker and more field-friendly approaches for defining thermal tolerance limits (Anttila et al. 2014; Casselman et al. 2012), this study was not without its challenges. Microscopic measurement of heart rates required very fine and precise tuning to observe heartbeats properly, due to the small size of the sablefish embryos and YSL. Individuals also needed to be meticulously oriented for the heart to be observed under the microscope. It was also difficult to keep embryos and YSL from moving in order to get reliable two-minute video recordings. In addition, one of the biggest challenges was that heartbeats could not be detected in real time due to their relatively low f_H values, especially at colder temperatures. Therefore, at a given temperature of the acute temperature challenge, individuals were video recorded and then videos were then replayed at higher speeds to ensure that individuals were correctly oriented and that the microscope was properly focused on the heart before continuing the trial and increasing the temperature. There were several occasions where I decided to forego recording certain individuals if too many unsuccessful attempts were made and too much time had passed at any given temperature.

There are also several limitations to using this method alone. Measuring both f_H and AS during acute warming confirms the precision of the results obtained from breakpoint analysis of each performance measure. Undertaking AS measurements might have helped clarify some of the unexpected results achieved in this study (see section 4.4). In addition, Leeuwis et al. (2021) pointed out the importance of considering the multi-stressor effects of hypoxia and high temperatures on the cardiorespiratory response of sablefish. In their study, adult sablefish f_H and cardiac output did not increase with temperature and had reduced thermal tolerance and maximum O_2 consumption while hypoxic. Therefore, assessing multiple performance measures in a multi-stressor context is likely to provide a more accurate prediction of how sablefish at all life stages will respond to climate change.

Chapter 5: Overall Conclusions and Future Work

A summary of the major results:

1. It appears that the upper thermal limit for sablefish embryos was only identified in embryos incubated at 3.0°C, and the lower thermal limit was only likely identified for embryos incubated at 7.0°C.
2. Neither the upper or lower limits were identified for YSL.
3. There was an increase in mean f_H with incubation temperature for YSL throughout the entire acute temperature challenge.
4. Embryos incubated at 5.0°C had higher mean f_H values throughout the challenge, compared to embryos incubated at 7.0°C. However, there was consistent overlap in mean f_H once the challenge extended beyond 3.0°C.

Heart rate measurements during an acute temperature challenge of sablefish embryos and yolk-sac larvae (YSL) acclimated to various temperatures provided initial insight to their overall success in a warming climate. The results of this study suggest that the early life stages of sablefish may not be exposed to lethal temperatures in the near future, but future impacts on overall physiological decline remain unknown. This study also suggests that the transportation of embryos may not have physiological consequences for sablefish embryos. Again, other performance indicators would need to be identified to confirm if this is generally the case.

Future studies should extend the acute temperature challenge for sablefish to include temperatures below 2.0°C and above 12.0°C to identify the missing upper and lower thermal tolerance limits in this study. Future studies should also increase sample size to gain more precise confidence intervals. This study also recognizes the limitations that come with drawing confident conclusions solely from heart rate measurements. In addition, temperature is not the only environmental variable that may pose as a threat to marine organisms. Therefore, generating multiple thermal performance curves for various physiological parameters (e.g., AS + f_H) and under various environmental conditions (e.g., temperature + hypoxia) will be necessary to create confident predictions in how species will respond to climate change.

This study also highlights the logistical challenges in assessing physiological performance and tolerance during the early life stages of fish as compared to juveniles and adults. However, given that the early life stages of most fish are widely believed to be the most sensitive to environmental conditions (Brett, 1970; Burggren & Reyna, 2011), coupled with their inability to actively move to more favourable areas, and the fact that recruitment is tied to larval survival (Norcross & Shaw, 1984), further research is clearly warranted. The novel data presented here lay the groundwork for future researchers to continue to characterize the thermal tolerances of the early life stages of sablefish, and the likely response of this important species to ocean warming.

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Appendix A: Preliminary Heart Rate Results of Arctic cod

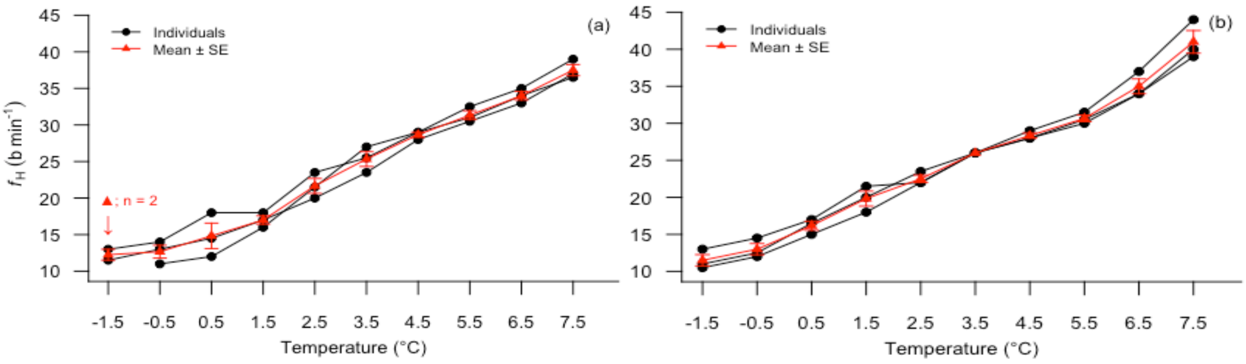

Figure A 1. Heart rate [f_H (b min^{-1})] response of Arctic cod (*Boreogadus saida*) embryos held at 3.5°C and acclimated to **(a)** ambient (*n* = 3) and **(b)** 7.5 pH (*n* = 3) to acute warming. Embryos were exposed to an acute temperature challenge from -1.5° to 7.5°C. Heart rate values for individual embryos are reported in black and the mean ± SE for each treatment is reported in red.

Table A 1. Q_{10} values of Arctic cod (*Boreogadus saida*) embryos acclimated to 8.0 pH (*n* = 3) and 7.5 pH (*n* = 3) at 3.5°C. Embryos were exposed to an acute temperature challenge from -1.5° to 7.5°C.

	8.0 pH			7.5 pH		
Temp [°C]	Embryo 1	Embryo 2	Embryo 3	Embryo 1	Embryo 2	Embryo 3
-1.5						
-0.5	2.1		3.4	3.0	3.8	3.6
0.5	12.3	2.4	3.0	4.9	9.3	16.1
1.5	1.0	17.8	4.9	10.5	6.2	6.8
2.5	14.4	19.2	5.1	1.3	7.4	5.0
3.5	2.3	9.8	5.0	5.3	5.3	2.7
4.5	3.6	2.0	5.8	2.1	2.1	3.0
5.5	1.9	3.1	2.4	2.4	2.0	2.3
6.5	2.5	2.1	2.2	3.0	3.5	5.0
7.5	2.0	3.0	3.1	5.1	3.9	5.7

Appendix B: Temperature Sensitivity (Q_{10}) and Q_{10} Breakpoint Temperatures (T_{QB})

Table B 1. Number of sablefish embryos (E) and yolk-sac larvae (YSL) that experienced a sharp increase (Q10 > 10) during an acute temperature challenge from 2.0° to 12.0°C. Embryos and YSL incubated at the research facility since 0 days-post-fertilization (dpf) were acclimated to 3.0°, 5.0°, and 7.0°C. Embryos transported later in their development (TE) were acclimated to 5.0°C at the hatchery. For instances where an individual experienced a sharp increase more than once, the point of the sharpest increase is indicated here.

Temp Increase	**E 3.0°C**	**E 5.0°**	**E 7.0°C**	**YSL 3.0°C**	**YSL 5.0°C**	**YSL 7.0°C**	**TE 5.0°C**
2-3		1	1	1	3	8	
3-4	4	9	4	3	1		2
4-5	3	1	2			1	1
5-6	1						
6-7	1						

Table B 2. Temperature sensitivity (Q10) of sablefish (Anoplopoma fimbria) embryos (E) and yolk-sac larvae (YSL) subjected to an acute temperature challenge from 2.0° to 12.0°C. Embryos and YSL incubated at the research facility since 0 days-post-fertilization (dpf) were acclimated to 3.0°, 5.0°, and 7.0°C. Embryos transported later in their development (TE) were acclimated to 5.0°C at the hatchery. Data are represented as mean ± SE. Data do not include instances where there was a sharp increase in heart rate, corresponding to a Q10 > 10.

Temp (°C)	**E 3°C**	**E 5°C**	**E 7°C**	**YSL 3°C**	**YSL 5°C**	**YSL 7°C**	**TE 5°C**
2-3	4.9 ± 1.1	1.5 ± 1.5		5.3 ± 0.6	4.3 ± 1.0	4.4 ± 2.0	
3-4	2.2 ± 0.8			3.3 ± 0.5	4.7 ± 0.8	4.7 ± 0.7	5.5 ± 0.3
4-5	3.1 ± 0.4	3.5 ± 0.5	2.1 ± 0.7	3.2 ± 0.4	3.2 ± 0.5	3.1 ± 0.3	3.4 ± 0.9
5-6	3.3 ± 0.9	2.3 ± 0.2	3.1 ± 0.6	3.4 ± 0.2	2.5 ± 0.2	3.2 ± 0.3	3.2 ± 0.4
6-7	3.1 ± 1.0	2.8 ± 0.3	3.7 ± 0.7	2.8 ± 0.2	2.8 ± 0.2	3.5 ± 0.4	2.3 ± 0.4
7-8	2.8 ± 0.6	2.6 ± 0.2	2.2 ± 0.4	3.5 ± 0.4	3.0 ± 0.2	3.3 ± 0.3	2.9 ± 0.3
8-9	1.7 ± 0.5	2.2 ± 0.3	3.0 ± 0.5	3.2 ± 0.7	3.0 ± 0.2	2.6 ± 0.2	3.2 ± 0.4
9-10	2.8 ± 1.0	2.9 ± 0.4	2.6 ± 0.6	2.7 ± 0.3	2.8 ± 0.2	2.9 ± 0.2	3.1 ± 0.3
10-11	2.1 ± 1.4	2.2 ± 0.3	2.8 ± 0.5	2.2 ± 0.1	2.4 ± 0.2	2.2 ± 0.2	2.2 ± 0.3
11-12	0.7 ± 0.0	2.6 ± 0.4	3.1 ± 0.2	2.1 ± 0.3	2.1 ± 0.2	2.7 ± 0.4	2.1 ± 0.2
Average	**2.7 ± 0.1**	**2.5 ± 0.1**	**2.9 ± 0.1**	**3.2 ± 0.1**	**3.1 ± 0.1**	**3.2 ± 0.2**	**3.2 ± 0.1**

Table B 3. Identified Q10 breakpoint temperatures (TQB) of sablefish (Anoplopoma fimbria) embryos (E) and yolk-sac larvae (YSL) subjected to an acute temperature challenge from 2.0° to 12.0°C. Embryos and YSL incubated at the research facility since 0 days-post-fertilization (dpf) were acclimated to 3.0°, 5.0°, and 7.0°C. Embryos transported later in their development (TE) were acclimated to 5.0°C at the hatchery. Data are represented as a single data point or mean ± SE where applicable.

Variable	E 3°C	E 5°C	E 7°C	YSL 3°C	YSL 5°C	YSL 7°C	TE 5°C
T_{QB} (°C)	n/a	n/a	n/a	11.0 ± 0	11.0	11.0	n/a
n	0	0	0	2	1	1	0

Appendix C: Individual f_H, Treatment Mean f_H, and Batch Mean f_H

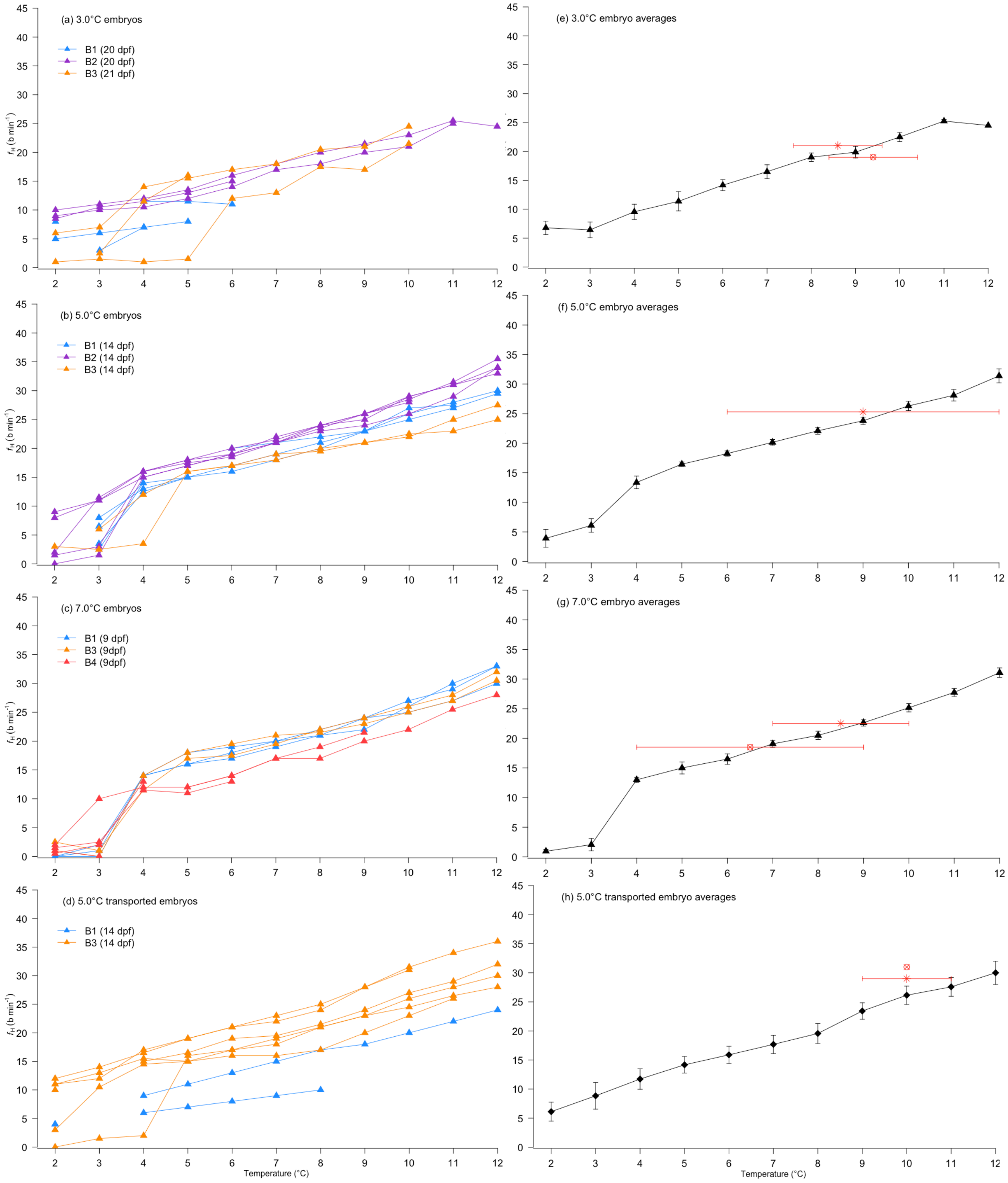

Figure C 1. Effect of acute warming on the heart rate [f_H (b min^{-1})] of sablefish (*Anoplopoma fimbria*) embryos acclimated to 3.0°, 5.0°, and 7.0°C. Embryos (a), (b), and (c) were incubated at the research facility since 0 days-post-fertilization (dpf), while transported embryos (d) were incubated at the hatchery and transported to the research facility later in their development. Graphs on the left represent individual f_H values during an acute temperature challenge from 2.0 to 12.0°C. Data on the right are represented as mean ± SE. Mean T_{max} (⊗) and mean T_{AR} (✳) ± SE are indicated for all treatments.

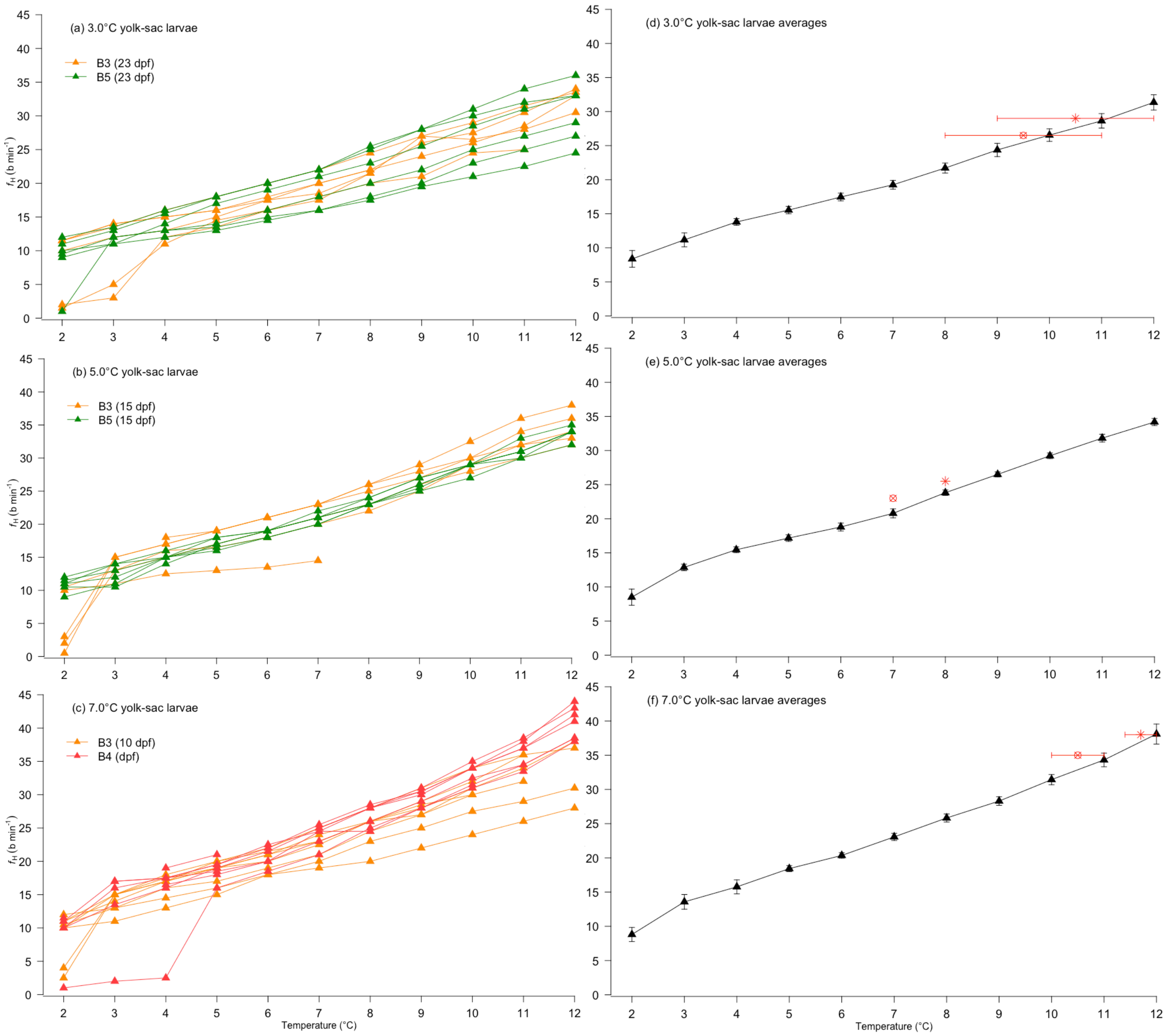

Figure C 2. Effect of acute warming on the heart rate [f_H (b min^{-1})] of sablefish (*Anoplopoma fimbria*) yolk-sac larvae (YSL) acclimated to 3.0°, 5.0°, and 7.0°C. All YSL were incubated at the research facility since 0 days-post-fertilization (dpf). Figures on the left represent individual f_H values during an acute temperature challenge from 2.0° to 12.0°C. Data on the right are represented as mean ± SE. Mean T_{max} (⊗) and mean T_{AR} (✳) ± SE are indicated for all treatments.

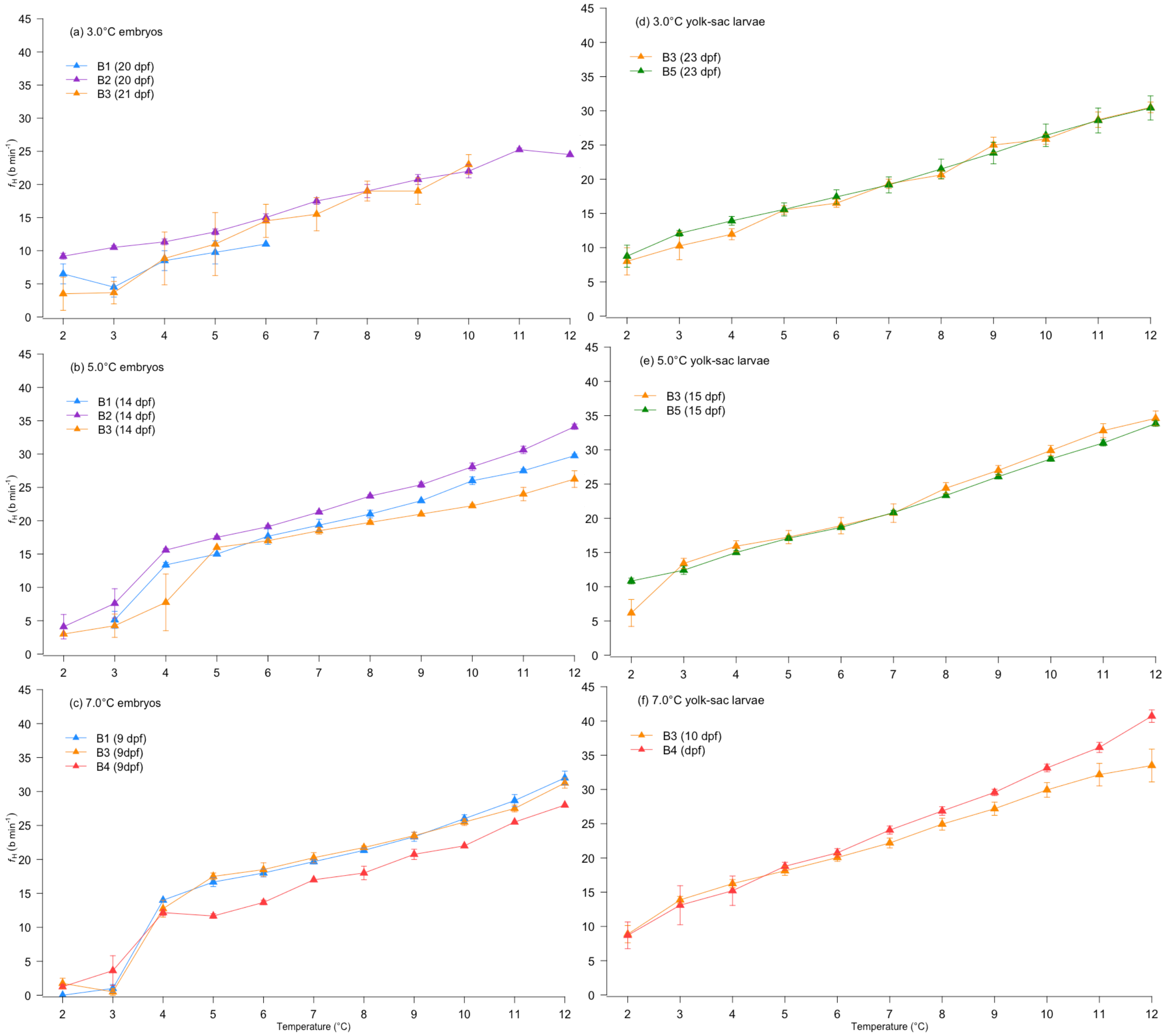

Figure C 3. Effect of acute warming on the heart rate [f_H (b min^{-1})] of sablefish (*Anoplopoma fimbria*) embryos **(a), (b),** and **(c)** and yolk-sac larvae **(d), (e),** and **(f)** acclimated to 3.0°, 5.0°, and 7.0°C. All individuals were subjected to an acute temperature challenge from 2.0° to 12.0°C. Each line represents the mean f_H ± SE response of individuals from a single batch.

Appendix D: Sample Size Change Throughout the Acute Temperature Challenge

Table D 1. Sample size of sablefish (Anoplopoma fimbria) embryos (E) included in the data analysis. Embryos incubated to 3.0°, 5.0°, and 7.0°C were exposed to an acute temperature challenge from 2.0° to 12.0°C. Sample size is reflected as a total value (Tot) for each incubation temperature, as well as for each batch (B#) within a given incubation temperature.

°C	E 3°C				E 5°C				E 7°C				TE 5°C		
	B1	B2	B3	**Tot**	B1	B2	B3	**Tot**	B1	B3	B4	**Tot**	B1	B3	**Tot**
2	3	3	3	**9**	4	5	2	**11**	3	2	4	**9**	2	6	**8**
3	3	3	3	**9**	4	5	2	**11**	3	2	4	**9**	2	6	**8**
4	2	3	3	**8**	4	5	2	**11**	3	2	4	**9**	2	6	**8**
5	1	3	3	**7**	4	5	2	**11**	3	2	3	**8**	2	6	**8**
6	1	3	2	**6**	3	5	2	**10**	3	2	3	**8**	2	6	**8**
7	0	2	2	**4**	3	5	2	**10**	3	2	2	**7**	2	6	**8**
8	0	2	2	**4**	3	5	2	**10**	3	2	2	**7**	2	6	**8**
9	0	2	2	**4**	3	5	2	**10**	3	2	2	**7**	1	6	**7**
10	0	2	2	**4**	3	5	2	**10**	3	2	1	**6**	1	6	**7**
11	0	2	0	**2**	3	5	2	**10**	3	2	1	**6**	1	5	**6**
12	0	2	0	**2**	2	5	2	**9**	3	2	1	**6**	1	4	**5**

Table D 2. Sample size of sablefish (Anoplopoma fimbria) yolk-sac larvae (YSL) included in the data analysis. YSL incubated to 3.0°, 5.0°, and 7.0°C were exposed to an acute temperature challenge from 2.0° to 12.0°C. Sample size is reflected as a total value (Tot) for each incubation temperature, as well as for each batch (B#) within a given incubation temperature.

°C	YSL 3.0°C			YSL 5.0°C			YSL 7.0°C		
	B3	B5	**Tot**	B3	B5	**Tot**	B3	B4	**Tot**
2	6	6	**12**	6	6	**12**	8	6	**14**
3	6	6	**12**	6	6	**12**	8	6	**14**
4	6	6	**12**	6	6	**12**	8	6	**14**
5	6	6	**12**	6	6	**12**	8	6	**14**
6	6	6	**12**	6	6	**12**	8	6	**14**
7	6	6	**12**	6	6	**12**	8	6	**14**
8	6	6	**12**	5	6	**11**	8	6	**14**
9	5	6	**11**	5	6	**11**	8	6	**14**
10	5	6	**11**	5	6	**11**	8	6	**14**
11	5	6	**11**	5	6	**11**	6	6	**12**
12	4	6	**10**	5	6	**11**	4	6	**10**