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Thermal performance covaries with environmental temperature across populations of Atlantic salmon (*Salmo salar*)

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Graduate Program in Biology
A thesis submitted in partial fulfillment of the requirements for the degree in Master of Science
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**THERMAL PERFORMANCE COVARIES WITH ENVIRONMENTAL
TEMPERATURE ACROSS POPULATIONS OF ATLANTIC SALMON (*SALMO
SALAR*)**

(Thesis format: Integrated Article)

by

Kayla J. Gradil

Graduate Program in Biology

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science

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Abstract

Global climate change is projected to have widespread effects that could threaten the viability of natural populations. Physiological processes of aquatic ectotherms critically depend on their thermal environment, such that the optima for performance often correspond to environmental temperatures. Given predicted changes in aquatic thermal environments, it is increasingly important to understand underlying physiological mechanisms utilized by organisms to cope with these changes. Here, I show that three populations of Atlantic salmon (*Salmo salar*) are narrowly adapted to their native summer temperatures, such that thermal tolerance is optimized near average temperatures and collapses near peak temperatures. Further, I found evidence of various physiological mechanisms that drive thermal tolerance at the level of the heart, at both the individual and population level. These results enhance our understanding of thermal adaptation in an ecologically, economically, and culturally important fish.

Keywords

Adaptation, aerobic scope, cardiac output, climate change, heart rate, salmon

Co-Authorship Statement

Chapter 2 has been submitted to *Journal of Evolutionary Biology* with Shawn Garner, Chris Wilson, Anthony Farrell and Bryan Neff as co-authors. **Kayla Gradil** contributed to study design, conducted heart rate trials, analyzed data and drafted the manuscript. **Shawn Garner** contributed to building the experimental setup, provided input on the manuscript and analyzed data. **Chris Wilson** collaborated in rearing fish, **Anthony Farrell** collaborated in experimental setup and provided input on the manuscript and **Bryan Neff** contributed to study design and provided input on the manuscript.

Chapter 3 has been submitted to the *Journal of Experimental Biology* with Chris Wilson, Anthony Farrell and Bryan Neff in addition to Greg Kelly, Ben Dickson and Gurjoth Deol. **Kayla Gradil** contributed to study design, conducted heart rate trials, analyzed data and drafted the manuscript. **Chris Wilson** collaborated in rearing fish, **Anthony Farrell** collaborated in experimental setup and provided input on the manuscript and **Bryan Neff** contributed to study design, data collection and analysis, and provided input on the manuscript. **Greg Kelly** provided equipment and expertise for western blots and **Ben Dickson** and **Gurjoth Deol** conducted western blots and helped analyze data.

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List of Abbreviations

CT_{\max}	critical thermal maximum
ECG	electrocardiogram
IPCC	Intergovernmental Panel on Climate Change
f_H	heart rate
$f_{H\max}$	maximum heart rate
$f_{H\text{peak}}$	highest $f_{H\max}$ reached as a result of temperature increase
T_{opt}	optimum temperature at which aerobic scope is maximal
T_{crit}	upper critical temperature at which aerobic scope is zero
T_{AB}	Arrhenius breakpoint temperature of maximum heart rate
T_{arr}	cardiac arrhythmia temperature
T_{peak}	temperature at which $f_{H\text{peak}}$ occurs
Q_{10}	thermal sensitivity of biological rate processes
OMNRF	Ontario Ministry of Natural Resources and Forestry
OCLTT	oxygen- and capacity-limited thermal tolerance
Hct	hematocrit
HSP90 β	heat shock protein 90 β
Mb	myoglobin
RVM	relative ventricular mass

Chapter 1

1 General Introduction

Temperature highly constrains physiological processes and in turn the form and function of individuals. Indeed, for ectotherms, whose internal physiology is closely linked to the environment, temperature has even been called the “ecological master factor” (Brett 1971), as it is central to an individual’s environmental tolerance and can drive local adaptation and even ecological speciation (Keller and Seehausen 2012). Given that the average global air temperature is predicted to increase by 1.7-4.8°C by the end of the 21st century (IPCC 2013), it is increasingly important for ecophysiologicalists to understand the effects of environmental temperature on ectotherms. Because of their reliance on the environment for heat, ectotherms have relatively narrow thermal windows, or thermal ranges in which aerobic activity can be sustained. Beyond their thermal window, cardiac capacity collapses and whole-animal thermal tolerance is restricted (Pörtner and Knust 2007).

In aquatic animals such as fish, the thermal performance and tolerance is shaped by aerobic scope, which is the difference between routine and maximum oxygen consumption (Pörtner 2001; Pörtner and Farrell 2008). As temperature increases, the kinetic energy of molecules increases, and thereby reaction rates increase exponentially. The inescapable laws of thermodynamics cause the reaction rates that drive metabolism to increase exponentially with temperature, resulting in an exponential increase in resting metabolic rate, and thereby oxygen consumption (Farrell 2009). The main mechanism by which fish mediate this temperature-dependent increase in oxygen demand is by increasing their heart rate (Farrell 2009; Eliason et al. 2011). Therefore, as temperature increases, oxygen delivery increases at a certain rate for both maximum and resting metabolism, but eventually the maximum capacity of the cardiorespiratory system fails to keep pace with increased oxygen demands (Figure 1.1). The temperature at which this occurs is called the thermal optimum (T_{opt}), or the temperature that provides the greatest amount of energy available for aerobic activities. Beyond this temperature, aerobic scope continues to decrease as maximum oxygen delivery begins to plateau and resting oxygen consumption continues to increase exponentially. This loss of scope due to the inability of maximum metabolism to keep pace with oxygen demands as set by resting metabolism reduces the capacity for fitness-related behaviours such as growth, reproduction and aerobic swimming,

and can lead to increased mortality (Pörtner and Knust 2007; Farrell et al. 2008). Beyond T_{opt} , aerobic scope functionally collapses at the upper critical temperature (T_{crit}), at which point an individual cannot even sustain resting metabolism and therefore cannot sustain any aerobic activities, such as avoiding predation, foraging, and tissue growth and repair. The resultant mismatch between oxygen supply and demand forces the individual to progressively switch to anaerobic metabolism (Farrell et al. 2008). Therefore, a thermal niche where resting metabolism is sustained is bounded by T_{crit} . This understanding of temperature-dependent aerobic scope has led to the mechanistic framework of oxygen- and capacity-limited thermal tolerance (OCLTT), which has helped predict the susceptibility of aquatic ectotherms to climate change scenarios (Pörtner and Farrell 2008; Pörtner 2010; Somero 2010; Munday et al. 2012).

Measures of aerobic scope, however, are time consuming, taking 1-2 weeks to assess a single fish. In order to expedite this process, Casselman et al. (2012) developed a high-throughput surrogate method in juvenile coho salmon (*Oncorhynchus kisutch*) for measuring temperature-dependent aerobic scope. Using this method, two fish can be tested in parallel in two hours. As T_{opt} is set by the temperature at which maximum metabolism fails to keep pace with oxygen demands, and heart rate is the primary driver of oxygen delivery in salmonids (Fry 1971; Farrell 2009; Eliason et al. 2011), Casselman et al. (2012) proposed that maximum heart rate (f_{Hmax}) could be used to determine a proxy of T_{opt} called the Arrhenius breakpoint temperature (T_{AB}). Specifically, it was proposed that T_{AB} could be estimated by determining when the Q_{10} (the rate of increase of a biological process in response to a 10°C increase) of f_{Hmax} shifted to a lower exponent. Furthermore, as T_{crit} is set by a collapse of cardiac scope and ultimately aerobic scope (Farrell 2009), the temperature at which heart rate becomes arrhythmic (T_{arr}), or irregular, signals cardiac failure and the approach of T_{crit} . Using this method, T_{opt} and T_{crit} can be approximated by measuring just f_{Hmax} across temperatures, further expediting the process. Using respirometry trials to determine aerobic scope and an acute warming regime to determine cardiac function, Casselman et al. (2012) found that T_{AB} and T_{arr} were within 1-2°C of T_{opt} and T_{crit} , respectively. Therefore, this rapid-screening tool provides a reliable way to indirectly measure T_{opt} and T_{crit} , and has subsequently been used in fish to test differences in thermal tolerance between diploid and triploid rainbow trout (*Oncorhynchus mykiss*) (Verhille et al. 2013), local adaptation and plasticity in cardiac function (Anttila et al. 2014), the upper thermal limits of Arctic cod

(*Boreogadus saida*) (Drost et al. 2014) and the adaptive capacity and plasticity of thermal tolerance in Chinook salmon (Muñoz et al. 2014; Muñoz et al. 2015).

In addition to heart rate, there has been considerable research focused on how physiological mechanisms that facilitate oxygen delivery, in addition to heart rate, shape thermal performance. For example, Eliason et al. (2011) found that Fraser River sockeye salmon (*Oncorhynchus nerka*) populations that encountered warmer temperatures during upstream migration had higher T_{opt} values, mediated in part by higher densities of ventricular β -adrenoceptor (a receptor which mediates cardiac adrenergic stimulation, protecting cardiac function from negative effects of hypoxia at high temperatures) compared to other Fraser River sockeye salmon populations. Accordingly, the authors argued that the temperatures experienced during upriver migration impose selection on heart morphology at the population level. Furthermore, Anttila et al. (2013) found that families of juvenile Atlantic salmon (*Salmo salar*) that had higher critical thermal maxima (CT_{max}), the temperature at which an individual cannot maintain equilibrium, had larger ventricles and higher expression of ventricular myoglobin (which facilitates oxygen storage and transfer from the blood to the ventricle tissue). In sum, physiological mechanisms that facilitate oxygen delivery at both the population and family level have been shown to shape thermal performance and tolerance in salmonids.

Atlantic salmon are an important group to examine thermal tolerance. They are ecologically, culturally and economically important, contributing \$250 million to the Gross Domestic Product in Canada. Atlantic salmon populations are already declining, in part, due to global warming, with decreases in run size in virtually all North American populations (Ford and Myers 2008). Here, I examine the thermal performance and tolerance of juveniles from three Atlantic salmon populations: the LaHave River population from Nova Scotia; the Sebago Lake population from Maine; and the Lac Saint-Jean population from Québec. These populations of Atlantic salmon were chosen as they are being maintained as broodstock in Ontario hatcheries as part of a concerted effort among Ontario Ministry of Natural Resources and Forestry (OMNRF) and local conservation groups to restore the extirpated Lake Ontario Atlantic salmon population. When the restoration program began in the 1990's, the anadromous LaHave population from Nova Scotia was chosen as a source because it was self-sustaining and therefore accessible (Dimond and Smitka 2005). Restoration success with the LaHave population, however, was very low, with

only a few individuals returning to their natal streams in Lake Ontario (Dimond and Smitka 2005). In 2007, the OMNRF began stocking the Sebago Lake population from Maine and the Lac Saint-Jean population from Québec (specifically fish that spawn in Métabetchouane River and Rivière aux Saumons), two landlocked populations, in an effort to increase adult returns. The Sebago population is believed to be an ‘environmental match’ because it was successfully introduced into Lake Champlain, an environment similar to the current Lake Ontario (Dimond and Smitka 2005), while the Saint-Jean population is a ‘genetic match’ based on support for a historical connection between Lake Ontario and Lac Saint-Jean via the St. Lawrence River (Tessier and Bernatchez 2000). As such, these candidate source populations were readily available and ideal for investigating thermal tolerance as they differ in thermal environment (Figure 2.1.), and perhaps thermal performance.

Atlantic salmon have a high juvenile residency time, remaining in streams or rivers for 2-3 years before migration to the ocean (or in the case of landlocked populations, the lake) as smolts. As streams and rivers are small bodies of water and therefore less thermally stable relative to lakes and oceans, they are not able to buffer large changes in air temperature, creating a thermally variable environment that is more prone to sudden spikes in water temperature. As Atlantic salmon juveniles have been shown to suffer from thermal disease (in which the endogenous yolk sac congeals and is not accessible to the fish), with 90% of overall mortality occurring at the juvenile life-stage, temperature is thought to be an important selective pressure. Recently, anomalously high river temperatures in Miramichi River, NB, have caused significant mortality of thousands of juvenile Atlantic salmon (Fisheries and Oceans Canada 2012). Temperature-induced mortality in juveniles, along with adults, has been attributed to the collapse of aerobic scope (Crozier and Zabel 2006; Keefer et al. 2008). Therefore, temperatures experienced as juveniles should exert selective pressure that could shape thermal windows and performance optima.

Indeed, salmon populations have been shown to exhibit local adaptations that reflect their thermal regimes. For example, a landmark study by Eliason et al. (2011) measured aerobic scope in six populations of Fraser River sockeye salmon and found that it was shaped by temperatures experienced during upriver migration. Populations that had more arduous journeys (i.e. swimming through “Hell’s Gates,” a hydraulically challenging river segment), had higher

maximum aerobic scope compared to populations with relatively easy upriver migrations. Similarly, redband trout (*Oncorhynchus mykiss gairdneri*) have been found to adjust their physiology at a local scale, with desert populations being able to tolerate temperatures up to 29°C (Rodnick et al. 2004). Until recently, the assumption has been that such local adaptation was due to genetic differences among populations; such differentiation is facilitated by natal philopatry and thereby low gene flow (Pörtner and Knust 2007; Farrell et al. 2008). This assumption is based on the fact that salmonids are temperature specialists, and as such have a limited capacity for thermal acclimation compared to temperature generalists. For example, CT_{max} increases by only 2°C in response to a 15°C increase in acclimation temperature for sockeye salmon. CT_{max} for goldfish (*Carassius auratus*), a temperature generalists, increases 10°C in response to a 30°C increase in acclimation temperature (Brett 1956). Furthermore, resting f_H does not significantly differ between sockeye salmon that are acclimated to 22°C versus those acclimated to 14°C and acutely warmed to 22°C (Steinhauset et al. 2008). Based on these studies, local adaptation of thermal performance and tolerance in salmon was thought to be mostly due to genetic adaptation rather than developmental acclimation (plasticity).

Recently, however, studies on thermal tolerance in salmonids that have found a surprising amount of plasticity (Anttila et al. 2014; Muñoz et al. 2015). For example, Anttila et al. (2014) measured f_{Hmax} in European Atlantic salmon juveniles from the most northern and southern populations of the species' range. Individuals from these two populations were reared at either 12°C or 20°C for about 3 months as fry and subsequently tested to determine cardiac function. T_{arr} was 21-23°C and peak maximum heart rate (f_{Hpeak}) was ~150 beats per minute (beats min^{-1}) for 12°C-acclimated fish, whereas T_{arr} was 27.5°C and f_{Hpeak} was ~200 beats min^{-1} for 20°C-acclimated fish. Despite the large differences in cardiac function between treatment groups, there were not significant differences in cardiac function between the northern and southern populations, despite a 6.5°C difference in native temperature ranges. Furthermore, Muñoz et al. (2015) assessed cardiac function in Chinook salmon reared in present day (+0°C) and projected future (+4°C) temperature conditions. The T_{AB} values of individuals in the +4°C treatment were 2.1°C higher than the individuals in the +0°C treatment. Similar to Antilla et al. (2013), f_{Hpeak} increased from 153 beats min^{-1} in the +0°C treatment to 180 beats min^{-1} in the +4°C treatment. These findings suggest that plasticity plays a bigger role in shaping thermal tolerance than previously thought. Regardless, adjustments to thermal tolerance via evolutionary adaptation are

likely able to exceed those of plasticity (Anttila et al. 2014), and are therefore more important for long-term viability of populations

The objective of my research was to evaluate the juvenile thermal tolerance of three populations of Atlantic salmon. Using the f_{Hmax} protocol outlined in Casselman et al. (2012), I investigated whether cardiac performance reflected native thermal regimes using a common garden experimental design, such that any population-level differences could be attributed to genetic adaptation. In addition, I investigated the physiological mechanisms underlying cardiac performance at both the individual and population level in these three populations.

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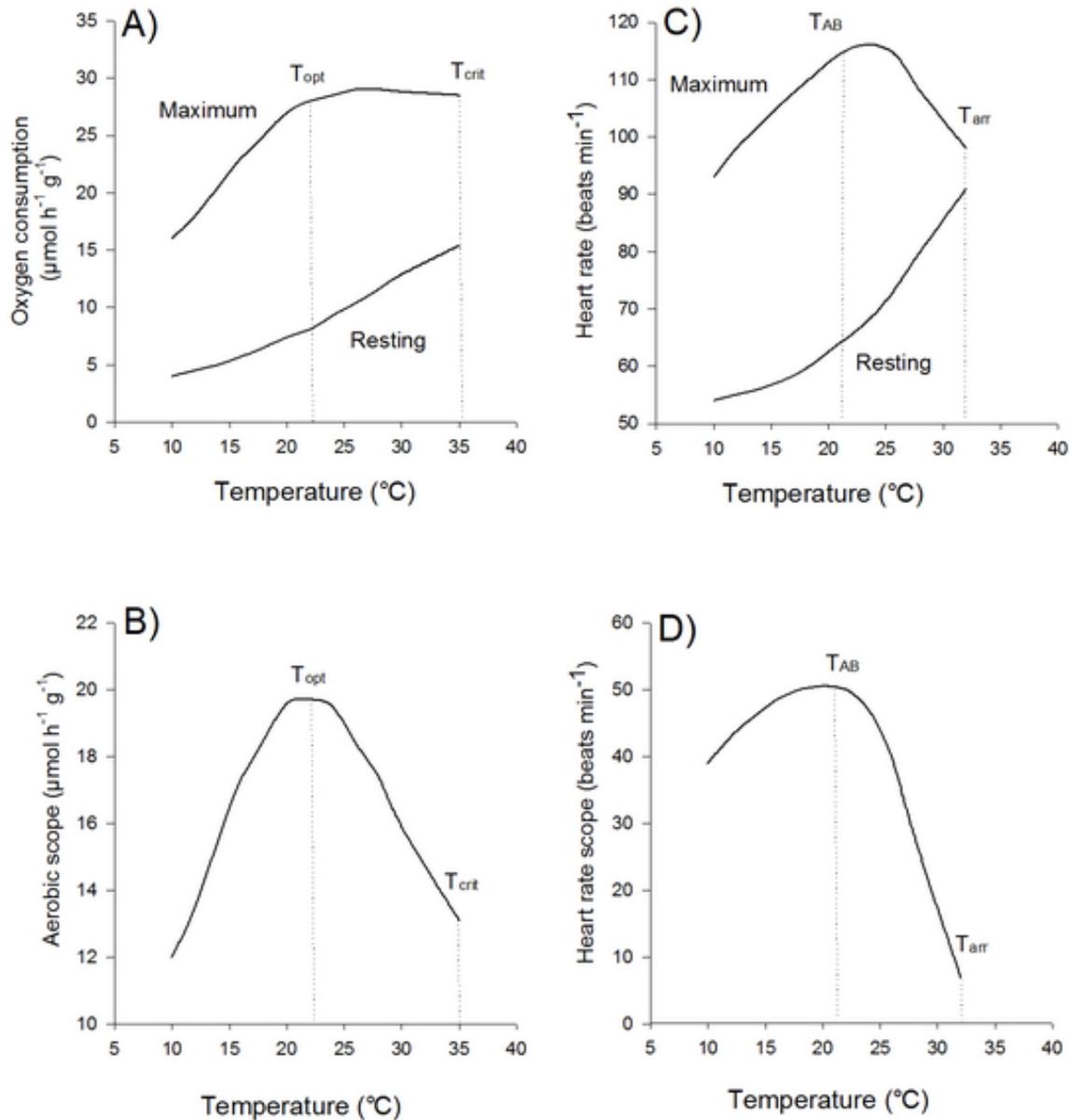


Figure 1.1. Oxygen consumption and heart rate as a function of temperature. (A) Resting and maximum oxygen consumption (VO_2) as a function of temperature. Resting VO_2 increases exponentially with temperature, whereas maximum VO_2 increases exponentially at low temperatures, but then fails to sustain the initial rate of increase, plateauing at high temperatures.

The thermal optimum and critical temperatures are denoted by the dotted lines. (B) The difference between resting and maximum VO_2 , or the aerobic scope, as a function of temperature. Aerobic scope is maximized at T_{opt} and collapses at T_{crit} , denoted by the dotted lines. (C) Resting and maximum heart rate (f_{H}) as a function of temperature. The Arrhenius breakpoint (T_{AB}) and the arrhythmia temperature are denoted by the dotted lines. (D) The difference between resting and maximum f_{H} , or f_{H} scope, as a function of temperature. f_{H} scope is maximized at T_{AB} and collapses at T_{arr} , denoted by the dotted lines.

Chapter 2

2 Thermal capacity covaries with environmental temperature across populations of Atlantic salmon (*Salmo salar*): a common garden experiment implicates local adaptation

Global climate change is projected to have widespread effects that could threaten the viability of natural populations, especially for populations that are adapted to current environmental conditions. In fish, aerobic performance at elevated temperatures is determined largely by cardiac capacity, and the ability of cardiac capacity to respond to temperature can be measured through changes in heart rate. Using a common garden breeding design, we collected heart rate data from three populations of Atlantic salmon (*Salmo salar*) to estimate optimal temperatures and upper critical temperatures for cardiac performance. We found that each population showed evidence of local adaptation to summer temperatures experienced in their native environment, with optimal temperatures (inferred from the Arrhenius breakpoint temperature) falling 2.2-3.8°C below the average native summer temperature for each population. Upper critical temperatures (inferred from the temperature at which heart rate becomes arrhythmic) were nearly identical to peak native summer temperatures (0-0.3°C above the peak), with 43% of individuals across populations having upper critical temperatures that were actually below the peak summer temperatures. Tolerance of current peak summer temperatures is thus limited, and any additional increase in peak temperatures could threaten the persistence of these Atlantic salmon populations.

2.1 Introduction

Understanding the environmental factors that drive local adaptation, and hence drive diversity among populations, has been a focus of evolutionary biology for decades. Previous research has identified many factors that can drive local adaptation including parasites, food supply and habitat quality (e.g. Williams 1966; Taylor 1991; Kawecki and Ebert 2004; Smit and Wandel 2006). More recently, studies have emphasized the role of temperature as a factor driving local adaptation (Eliason et al. 2011; Farrell 2009), in part because of the clear evidence that environments have been warming during the past century (IPCC 2013).

Temperature is particularly important for aquatic ectotherms such as fishes because of its direct impact on an individual's biochemical and physiological processes (Fry 1971). Indeed, a number of well-established metrics can be used to examine a population's thermal performance and tolerance, including the widely used critical thermal maximum (CT_{max}), which measures the temperature at which a fish loses equilibrium (Anttila et al. 2013; Muñoz et al. 2014; Chen et al. 2015). Perhaps more ecologically relevant metrics are the thermal optimum (T_{opt}), which is the temperature that an individual has maximum absolute aerobic scope (i.e. the greatest capacity to do aerobically demanding activity such as swimming), and the upper critical temperature (T_{crit}), which is the temperature at which aerobic scope (the difference between the resting and maximum oxygen uptake rates of the individual) functionally collapses (Farrell 2009). Of particular interest is if these measures reflect native temperatures across populations within a species or closely related species.

There is growing evidence that fish species are adapted to their local environments. For example, CT_{max} and T_{opt} for polar fishes (Drost et al. 2014) are typically much lower than for tropical coral reef fishes (Donelson et al. 2011). Thermal tolerance has been found to covary with local temperatures in subspecies of temperate killifish (*Fundulus spp.*) (Fangue et al. 2006), closely related species of tropical killifish (*Aphyosemion spp.*) (McKenzie et al. 2013), and among *Danio* species (Sidhu et al. 2014). In addition, populations of the same species can be locally adapted to their thermal environments. For example, Eliason et al. (2011) found that in Fraser River sockeye salmon (*Oncorhynchus nerka*), there was strong population-specific local adaptation in thermal tolerance, such that absolute aerobic scope was maximized at temperatures typically experienced during upstream migration. Barrett et al. (2011) found that cold-tolerance in three-

spined sticklebacks (*Gasterosteus aculeatus*) evolved in just three generations when marine sticklebacks were translocated to colder freshwater environments, matching that of the wild freshwater populations. In the other direction, translocation to warmer environments has been associated with increased thermal tolerance in rainbow trout (*Oncorhynchus mykiss*) in hatcheries (Chen et al. 2015). In a non-fish, thermal tolerance in green turtle (*Chelonia mydas*) populations was locally adapted to nesting sites such that turtles that nested on sites with warmer black sand compared to cooler white sand could tolerate a broader range of and higher temperatures (Weber et al. 2015).

Some, but not all, of these studies have used aerobic scope to discriminate thermal performance of individuals and to predict how much of a temperature increase is likely to be tolerated for various species. Such predictions will be particularly important from a conservation perspective given that climate change is predicted to raise average global air temperatures by up to about 5°C by the end of the century (IPCC 2013). The challenge, however, has been to measure absolute aerobic scope both reliably and with a fine temperature resolution (Clark et al. 2014). To solve this difficulty, and recognizing that all fish studied to date increase heart rate with temperature, maximum heart rate (f_{Hmax}) has been proposed as a surrogate for absolute aerobic scope (Casselman et al. 2012). Specifically, if thermal optima and limitations are set by maximum cardiac performance, f_{Hmax} is directly related to aerobic scope. For example, Farrell (2009) proposed that as temperature approaches T_{opt} , increases in maximum cardiac output fail to keep pace with oxygen demand and as temperature approaches T_{crit} , cardiac arrhythmias develop. Therefore, maximum cardiac output sets T_{opt} , while resting cardiac output sets T_{crit} . Since that proposal, f_{Hmax} has been used in a variety of salmonid species to assess the thermal dependence of cardiac performance. For example, this technique was used by Anttila et al. (2014) in European Atlantic salmon to assess cardiac plasticity, by Muñoz et al. (2014) in Chinook salmon (*Oncorhynchus tshawytscha*) to assess plasticity and genetic structure of thermal tolerance, by Chen et al. (2013) to assess differences in thermal tolerance between diploid and triploid rainbow trout, and by Chen et al. (2015) to examine the effect of hatchery selection in rainbow trout.

The objective of the present study was to assess juvenile thermal tolerance of three populations of Atlantic salmon (the LaHave River, Nova Scotia, Sebago Lake, Maine, and Lac Saint-Jean, Québec). This is the first study of the effects of temperature on cardiac capacity in North

American Atlantic salmon. A recent study on juvenile European Atlantic salmon found no significant population differences in thermal tolerance between northern (cooler) and southern (warmer) populations, with capacity instead being mostly plastic in response to developmental temperature (Anttila et al. 2014). Our study also focuses on juvenile Atlantic salmon because they have a high stream residency time (2-3 years) before migrating as smolts to the ocean (or Lake, in the case of landlocked populations) (Cutts et al. 1999). Considering juvenile survival is negatively correlated with temperatures above optimal levels in salmonids (Crozier and Zabel 2006), temperatures experienced as juveniles should exert selective pressure that shape windows of thermal capacity and performance optima. Among our populations, Sebago Lake is the warmest overall, having the highest average and peak summer temperatures, followed closely by the LaHave River, with Lac Saint-Jean being the coolest (see below). The populations are geographically isolated and show strong natal philopatry, which should facilitate genetic adaptation to their local thermal environments (Dimond and Smitka 2005). We used a multi-generation common garden experiment to assess f_{Hmax} and calculated the Arrhenius breakpoint temperature (T_{AB} ; a proxy for T_{opt}) and the arrhythmia temperature (T_{arr} ; a proxy for T_{crit}). Our common garden approach allowed us to target genetic differences in thermal tolerance, and hence to assess potential local adaptation. We expected the thermal performance to be shaped by summer temperatures (June-September), as this is the warmest and most active, and therefore the most thermally challenging, time of the year for juveniles (Imre and Boisclair 2004). Specifically, we predicted that if local adaptation of aerobic capacity existed for these populations, T_{AB} would be close to average summer temperatures and T_{arr} would be a few degrees higher than maximum summer temperatures (assuming that some scope for heart rate is needed to survive an extreme thermal event).

2.2 Methods

2.2.1 Study populations

Atlantic salmon from three locations were examined in this study. The LaHave River population (NS, Canada; 44.4°N, 64.5°W) has been maintained in hatcheries for 5 generations. The Lac Saint-Jean (QC, Canada; 48.6°N, 72.0°W) and the Sebago Lake (ME, USA; 43.9°N, 70.6°W) populations have been in hatcheries for 2 generations. The LaHave and Sebago populations used in this study were produced at the Ontario Ministry of Natural Resources and Forestry (MNRF) Harwood Fish Culture Station (Harwood, ON) and the Lac Saint-Jean population was produced at the MNRF Codrington Research Facility in November 2012, and were reared at Codrington Research Facility. Eggs were placed in subdivided Heath incubation trays until they hatched (~3 months post-fertilization). The ambient water temperature during incubation mimicked natural conditions because water supplied to the Heath incubation trays was from a spring-fed stream (Marsh Creek). Dead eggs were removed daily to prevent transmission of pathogens such as *Saprolegnia* spp. At 5 months post-fertilization, individuals transitioned from endogenous feeding (yolk sac) to exogenous feeding. Individuals were then fed *ad libitum* using organic fish pellets (EWOS Commercial Feeds, Bergen, Norway). In May 2013, individuals were transferred to a rearing facility at the University of Western Ontario and reared in 650 L tanks. These tanks were supplied with freshwater flow of approximately 1 L min⁻¹ and maintained at 9.5-13°C for the remainder of the experiment.

Of the 25 families originally produced for each population (5 males × 5 females), 5 maternal half-sibling LaHave families died, as well as 3 families from various Sebago parents and 3 families from various Lac Saint-Jean parents. Juvenile families were reared until the fish were sufficiently large for injections and testing. The body mass of test fish at the time of the experimental trials was 12 ± 3 g for LaHave, 14 ± 3 g for Sebago and 12 ± 3 g for Lac Saint-Jean juveniles.

2.2.2 Heart rate measurements

Following Casselman et al. (2012), juvenile cardiac performance was measured as the response of maximum heart rate (f_{Hmax}) to warming using 5-8 offspring from each of the 25 families per population. Test fish were lightly anaesthetized using a 1:1 ratio of MS-222 and sodium

bicarbonate (to prevent activity from influencing f_{Hmax}), and measured for body mass (± 0.1 g) and fork length (± 0.1 cm). One test fish was then placed into each of two holding reservoirs and maintained at the acclimation temperature of 11°C by a recirculating temperature controlled water bath (VWR, Edmonton, AB, Canada). Digital temperature probes were located in each holding reservoir next to the test fish to record the temperature experienced by that fish. The caudal fin of the test fish was loosely fed through a metal cylinder to maintain dorsal-ventral orientation within the holding reservoir. Individuals were ram-ventilated to supply oxygenated water that contained a maintenance dose of anesthetic (75 mg L⁻¹ of MS-222 buffered with 75 mg L⁻¹ sodium bicarbonate). The fish rested on electrodes that were positioned on the bottom of the holding reservoir to allow for non-invasive electrocardiogram (ECG) recordings. Electrodes were connected to a data acquisition system (PowerLab 26T, AD instruments, Dunedin, New Zealand) including a ground, which converted the analog input from the electrodes to digital input. The PowerLab had a built in bio-amplifier, which amplified and filtered the ECG signal to reduce skeletal muscle and ambient electrical interference. The output from the PowerLab system was processed and recorded using LabChart v.7.2.5 (AD instruments, Dunedin, New Zealand).

Each test fish was given 30 min for their heart rate to stabilize, after which resting heart rate (f_{Hrest}) was measured. Then fish were pharmacologically stimulated to reach maximum heart rate using intraperitoneal injections of 2.4 mg kg⁻¹ atropine sulphate (Sigma-Aldrich, St. Louis, MO, USA) to block vagal tone and 8 µg kg⁻¹ isoproterenol (Sigma-Aldrich, St. Louis, MO, USA) to fully stimulate adrenergic β -receptors. Both agents were dissolved in 0.9% NaCl and each injection was followed by a 15 min equilibration period to allow heart rate to stabilize at f_{Hmax} . After the equilibration period, temperature was increased continuously at which point the individual was removed from the apparatus and euthanized by a lethal overdose of MS-222. Heart rates were then calculated by manually identifying peaks of the ECG and determining the number of peaks in a 60 sec continuous heartbeat series.

We conducted trials from October 21, 2013 to August 22, 2014. In total, electrocardiograms were analyzed for 168 LaHave fish, 139 Sebago fish, and 166 Lac Saint-Jean fish. An additional 27 fish (5.4%) were removed from analyses because the electrocardiogram signal was of insufficient quality or the pharmacological injections had incomplete or atypical effects.

2.2.3 Analysis of cardiac performance

After electrocardiogram signals were recorded, they were analyzed to determine cardiac performance measures. Here, we describe how T_{AB} and T_{arr} were calculated. For definitions and calculations of other cardiac performance measures, refer to Table 1. To calculate T_{AB} , the performance curve of f_{Hmax} across temperatures was analyzed using Q_{10} (temperature sensitivity) plots. The Q_{10} of f_{Hmax} (how much f_{Hmax} increases in response to a 10°C increase in temperature) was calculated between each temperature increment as $(f_{Hmax\ n+1} / f_{Hmax\ n})^{(10 / [T_{n+1} - T_n])}$ where $f_{Hmax\ n}$ is the maximum heart rate at temperature step n and T_n is the temperature at step n . The Q_{10} plots were input into SigmaPlot (Systat Software, San Jose, CA, USA) and two regression lines were fit to the biphasic curve by comparing all pair-wise possibilities of residuals of f_{Hmax} at high versus low temperatures, identifying the point at which temperature-induced increases in f_{Hmax} shift to a lower exponent (see Casselman et al. 2012). This point was taken as T_{AB} . The T_{arr} was identified as the first time point when an arrhythmia developed in the continuous ECG. For each cardiac performance measure, a one-way ANOVA was used to test for differences among populations. Individual body mass was initially included in the ANOVA models as a covariate, but its effects were never significant (all $p > 0.05$), so it was removed from the final models.

2.2.4 Analyses of local adaptation

The temperatures experienced by each population while in freshwater were obtained to examine covariation with the measures of cardiac performance determined here. For the LaHave River, temperature data were provided by the Population Ecology Division of Fisheries and Oceans Canada and were collected at the Morgan Falls Fishery on the LaHave River, New Germany, NS, Canada (44.3°N, 64.4°W), between 1997 and 2011 at 11:00 AM AST at an average depth of 1 m. For Sebago Lake, temperature data were provided by the Portland Water District, ME, USA (43.3°N, 70.1°W) and were collected at Crooked-Songo (the most abundant stream of Atlantic salmon for Sebago Lake) between 1990 and 2010 at 10:00 AM EST at a depth of 2 m. For Lac Saint-Jean, temperature data were provided by the Ministère des Ressources naturelles et de la Faune and were collected at the city of Roberval, QC, Canada (area near the shore of Lac-Saint Jean) (48.5°N, 72.2°W) between 1994 and 2012 at 12:00 PM EST at an average depth of 3 m.

For our population comparisons, we calculated the average summer temperatures based on June-

September for all recorded years. For each population we also calculated the average temperature of the 10 warmest days observed across all years as a measure of peak summer temperatures. We compared T_{AB} to average summer temperatures and T_{arr} to peak summer temperatures (average of warmest 10 days in data set) for each population.

2.3 Results

All of the f_{Hmax} performance measures for juvenile salmon showed significant differences among populations (Table 2). The Arrhenius breakpoint temperature (T_{AB}) was significantly different across all populations, with Sebago having the highest T_{AB} followed by LaHave and lastly Lac Saint-Jean. Resting heart rate (f_{Hrest}), the arrhythmia temperature (T_{arr}) and the temperature at which peak heart rate occurred (T_{peak}) were significantly higher in both LaHave and Sebago than in Lac Saint-Jean. Peak heart rate (f_{Hpeak}) itself was significantly higher in LaHave than in either Sebago or Lac Saint-Jean. Individual body mass was not correlated with any of the cardiac performance measures (all $p < 0.05$).

Cardiac performance measures for f_{Hmax} are compared with seasonal water temperatures for each population in Fig 1. In all three populations, when compared with the average summer temperature, the average T_{AB} value was lower by 2.2-3.8°C with rank order of T_{AB} among populations matching that of the average summer temperatures. The average T_{arr} values were essentially the same as the peak summer temperatures for each population (the difference was 0-0.3°C). Similarly, the rank order of T_{arr} among populations matched the rank order of peak summer temperatures. Interestingly, at the individual level, peak summer temperatures were greater than or equal to the T_{arr} of more than 43% of the individuals we examined (LaHave: 46%, Sebago: 37%, Lac Saint-Jean: 46%). Modeling a 1°C rise in temperature, this value rises to 68% of individuals (LaHave: 83%, Sebago: 62%, Lac Saint-Jean: 61%).

Table 2.1. Cardiac performance measures calculated using heart rate data for Atlantic salmon (*Salmo salar*).

Measure	Definition
f_{Hrest}	Resting heart rate, measured 30 minutes after administration of MS-222 using an electrocardiogram machine at acclimation temperature and before pharmacological stimulation.
T_{AB}	Arrhenius breakpoint temperature, calculated as the temperature at which the rate of increase of heart rate due to increases in temperature shifts to a lower exponent. Represents the thermal optimum.
T_{arr}	Arrhythmia temperature, calculated as the temperature at which heart rate becomes arrhythmic. Represents the upper critical temperature.
f_{Hpeak}	Peak heart rate, the highest maximum heart rate observed for an individual across temperatures. Represents the heart being fully exploited. Past this, heart rate cannot increase anymore and other mechanisms to deliver oxygen are progressively exploited.
T_{peak}	Peak heart rate temperature, temperature at which highest maximum heart rate (f_{Hpeak}) is observed. Represents the temperature at which the heart is being fully exploited. Past this, active heart rate can no longer increase to sustain heart rate scope and eventually becomes arrhythmic.

Table 2.2. Cardiac performance measures for three populations of Atlantic salmon (*Salmo salar*). Presented are means \pm 1 SEM for each population, as well as the F-stats, degrees of freedom and p-values from the ANOVA models. Significant differences among population pairs are indicated by different letters.

Measure	Sebago	LaHave	Lac Saint-Jean	F	Df	p
f_{Hrest}	69.2 ± 0.6^A	68.6 ± 0.5^A	65.9 ± 0.5^B	10.2	2, 469	<0.001
T_{AB} (°C)	17.0 ± 0.1^A	16.4 ± 0.1^B	14.7 ± 0.1^C	82.0	2, 468	<0.001
T_{arr} (°C)	26.3 ± 0.2^A	26.0 ± 0.1^A	23.5 ± 0.2^B	91.2	2, 469	<0.001
f_{Hpeak} (beats min ⁻¹)	167 ± 2^A	174 ± 1^B	164 ± 2^A	10.1	2, 469	<0.001
T_{peak} (°C)	25.3 ± 0.1^A	25.1 ± 0.1^A	22.4 ± 0.2^B	100.8	2, 469	<0.001

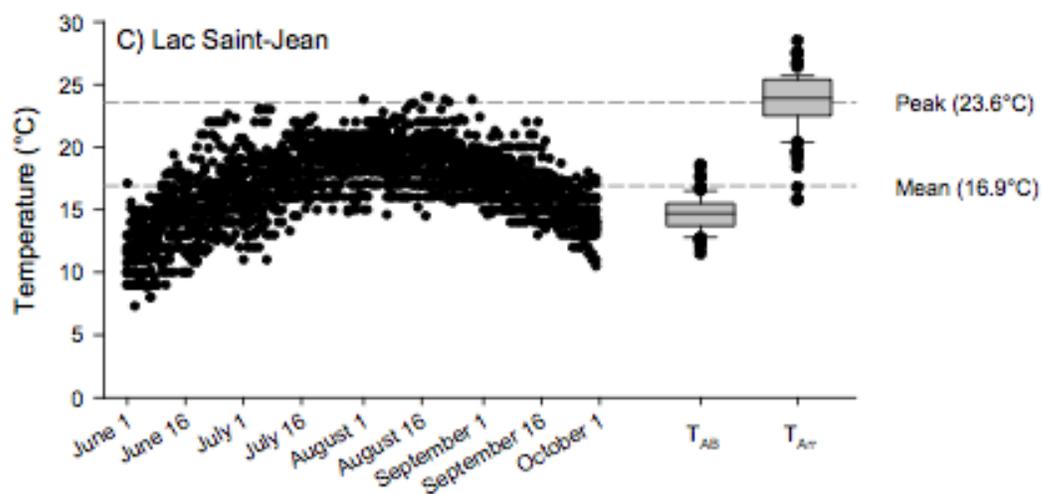
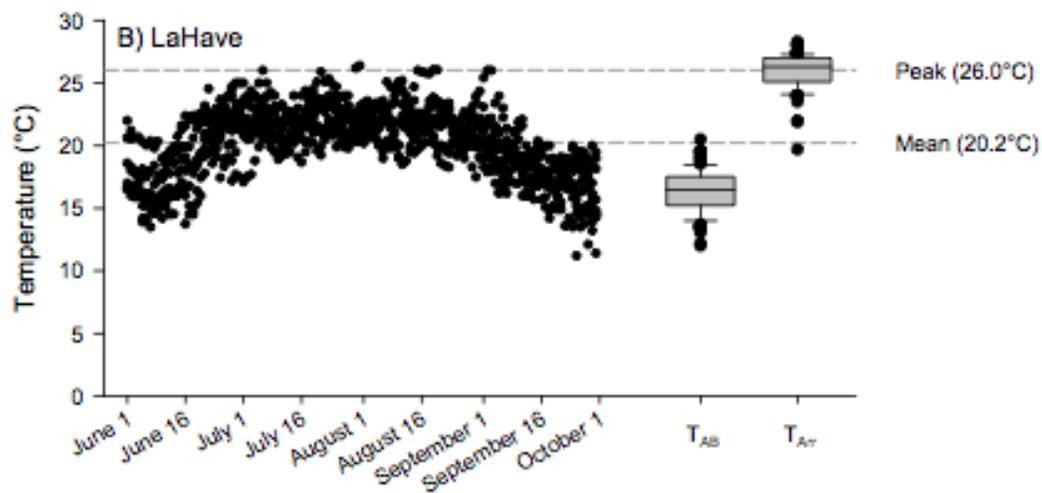
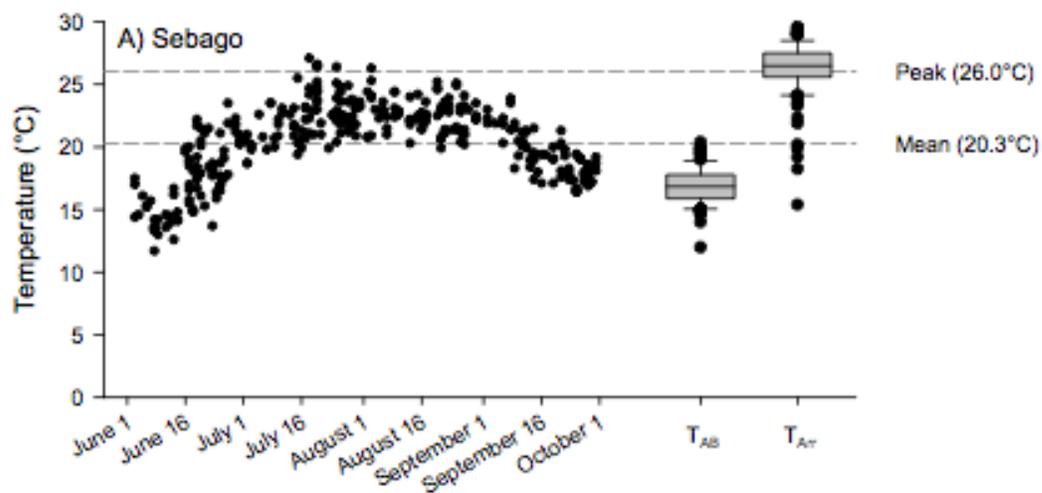


Figure 2.1. Water temperature and cardiac performance measures for three populations of Atlantic salmon (*Salmo salar*). The panels show data for the A) Sebago B) LaHave, and C) Lac Saint-Jean populations. Daily temperatures are shown by date on the left side of each figure with horizontal lines used to display average and peak summer temperatures for each population. Box plots on the right side of each panel show the Arrhenius breakpoint temperature of maximum heart rate $f_{H_{\max}}(T_{AB})$ and the arrhythmia temperature (T_{arr}). The box plots display the median, 25th and 75th percentiles, with whiskers indicating the 10th and 90th percentiles and individual points used to show data outside this interval. For details of temperature measurements at each location, see Methods.

2.4 Discussion

Understanding how cardiac performance is affected by temperature is critical for predicting the fitness of individuals across different thermal environments. In the past, thermal limitations have been assessed using respirometry to measure absolute aerobic scope or using whole-animal thermal tolerance (Eliason et al. 2011; Anttila et al. 2014). An alternative, more rapid, approach developed by Casselman et al. (2012) is to measure maximum heart rate. This approach also allows for a fine-scale temperature resolution, which is helpful in making predictions on population resilience to climate change (e.g., Muñoz et al. 2014). Here, we used maximum heart rate data to calculate the Arrhenius breakpoint temperature (T_{AB}), as a measure of thermal optima (T_{opt}), and the arrhythmia temperature (T_{arr}), as a measure of thermal performance at critical temperatures (T_{crit}), in three populations of Atlantic salmon.

Differences in thermal environments are predicted to drive local adaptation in cardiac performance, with individuals adapted to the temperatures they typically experience. Eliason et al. (2011), for example, found strong thermally specific local adaptation in Fraser River adult sockeye salmon populations, such that the optimum for aerobic scope correlated with the median temperature experienced during upstream spawning migration. Thermal adaptation has also been found in green turtles, such that developing embryos are adapted to the temperature of the nesting beach (Weber et al. 2015). Here, we found that thermal optima, as measured by T_{AB} , were significantly different across all three populations of Atlantic salmon, with Sebago having the highest mean T_{AB} and Lac Saint-Jean having the lowest mean T_{AB} , mirroring average summer temperatures of their native environments. Because we used a common garden experiment over multiple generations, our results implicate genetic differences in T_{AB} . Our data thus add to the growing evidence that temperatures experienced by ectotherms shape thermal performance (Farrell et al. 2008; Eliason et al. 2011; Weber et al. 2015), and provide the first support for local adaptation of thermal performance in populations of Atlantic salmon.

In addition to being adapted to average environmental temperatures, there may also be selection for tolerance to extreme temperatures. For example, Eliason et al. (2011) found that in sockeye salmon from the Fraser River, populations' upper critical temperatures reflected the maximum temperatures they were likely to experience during upriver migration. Here, we found that not only were there differences in the arrhythmia temperatures (T_{arr}) across the three Atlantic salmon

populations, but that these values were almost identical to the peak summer temperatures in the native environments. Specifically, Sebago and LaHave, which experience the same peak temperatures (both 26.0°C), had T_{arr} values that did not differ from each other, whereas T_{arr} values were significantly lower in the cooler Lac Saint-Jean population (peak temperature: 23.6°C). Interestingly, the average T_{arr} values were at most 0.3°C above current peak summer temperatures, with peak summer temperatures falling at or above T_{arr} values for more than 43% of individuals across all populations. Consequently, current peak summer temperatures likely represent an aerobically constrained environment for all three populations. Indeed, additional warming of only 1°C could result in an additional 25% of the fish exceeding their T_{arr} (68% total), which highlights the small thermal buffer against future increases in peak summer temperatures available to these populations. With projected warming, individuals from all three of these populations will have to increasingly exploit behavioural adaptations, such as seeking out thermal refugia during warm days, as has been shown in other populations of Atlantic salmon (Breau et al. 2007). These thermal refugias may become increasingly rare, however, as climate change-induced evaporation has been predicted to cause streams and rivers to become smaller, exposing the refugias to the relatively warmer air. Therefore, such behavior adaptations may not be able to buffer thermal stress in the long-term (Gleick 2004).

In conclusion, we provide evidence supporting local adaptation of thermal tolerance in juvenile Atlantic salmon, adding to the growing evidence of population-specific thermal adaptation across species. Given that many individuals in these populations are currently experiencing temperatures at or above upper thermal limits, it will be important to understand if and how populations of North American Atlantic salmon will be able to adjust these limits as temperatures continue to warm due to climate change. Based on our common garden design over multiple generations, the observed population differences in thermal tolerance are likely due to underlying genetic differences; differences that mirror thermal profiles of the populations' native environments. It remains to be determined, however, if these genetic differences can respond via natural selection, or whether these populations of Atlantic salmon show developmental acclimation to rearing temperature, as has been shown for juveniles of two European Atlantic salmon populations (Anttila et al. 2014). Given our results, assessing the plasticity of upper thermal tolerance (e.g. T_{arr}) is especially important in predicting future viability of North American Atlantic salmon.

2.5 References

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Chapter 3

3 Physiological mechanisms underlying thermal tolerance in Atlantic salmon (*Salmo salar*)

Global climate change is projected to increase air temperature by up to 5°C by the end of the century. Given that temperature directly affects physiological processes in ectotherms, we used three populations of Atlantic salmon (*Salmo salar*) to assess how physiological mechanisms shape upper thermal tolerance. Individual salmon with higher hematocrit (Hct) values, a measure of the oxygen carrying capacity of the blood, and higher peak heart rate (f_{Hpeak}) values preserved cardiac function to a higher temperature during acute warming as indicated by a higher temperature when the heartbeat became arrhythmic (T_{arr}). Also, among the three populations studied, the population that normally occupied the coolest environment had lower mean Hct and f_{Hpeak} . Furthermore, we found that individuals with higher ventricular expression of a constitutive heat shock protein, HSP90, had higher T_{arr} values, perhaps because they were better able to preserve protein conformation and function during acute warming, whereas the size of the ventricle had no effect on T_{arr} values. These findings lend support for the oxygen- and capacity-limited thermal tolerance hypothesis, and highlight several traits that might be selected on in response to climate change.

3.1 Introduction

Climate change models predict an increase of up to about 5°C in average global air temperature by the end of the century (IPCC 2013). As temperature directly affects growth rate, mortality, reproductive success and susceptibility to disease in ectotherms, warming temperatures have the potential to threaten the viability and abundance of these species (Brander 2007). Within fishes, and especially for the cardiorespiratory life-support system, there has been considerable research on how individuals respond to acute changes in the thermal environment and how populations of the same species differ as a result of their differing thermal environments due to phenotypic plasticity and genotype (Farrell 2009). Much of the research on the effects of climate change on fishes has focused on salmon, in part because they are cold-water fish who re-colonized North America following the last glacial refugia (King et al. 2001), and because they are important economically, ecologically and culturally (e.g., Eliason et al. 2011; Muñoz et al. 2015; Antilla et al. 2014; Chen et al. 2015).

According to the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis, performance failure at high temperatures is proposed to be due to an oxygen limitation, an idea that builds from pioneer work on fishes (Fry 1947; Brett 1971; Fry 1971). Consequently, aerobic scope (the difference between routine and maximum oxygen uptake) is maximized at an optimal temperature (T_{opt}) and collapses to zero at a higher, critical temperature (T_{crit}) (Pörtner and Knust 2007; Pörtner 2010). At temperatures between T_{opt} and T_{crit} , a fish's capacity for any aerobically powered activity beyond basic needs progressively diminishes. Furthermore, as temperature increases beyond T_{opt} , a mismatch develops between increasing basic oxygen demand and the maximum capacity for oxygen supply to the tissues, heart rate (f_H) reaches a peak (f_{Hpeak}). Once f_{Hpeak} is reached, the capacity of the heart to pump blood, and hence oxygen supply to the tissues, becomes limited (which signals the approach of T_{crit}), as was documented in the early observations of Fry (1947) and Brett (1971), and more recently in a wide variety of fish species, including salmonids (Casselman et al. 2012; Antilla et al. 2013; Chen et al. 2013; Verhille et al. 2013; Chen et al. 2015), Arctic cod (*Boreogadus saida*) (Drost et al. 2014), *Carassius auratus* (Ferreira et al. 2014) and three *Danio* species (Sidhu et al. 2014). Indeed, the upper thermal limits (T_{crit} and critical thermal maximum, CT_{max} , the temperature at which a fish loses equilibrium) have been shown to be associated with the temperature when the heartbeat becomes arrhythmic (T_{arr}) (Ferreira et al. 2014). As such, measurement of maximum heart rate (f_{Hmax}) has

been proposed as a way to estimate individuals' T_{opt} and T_{crit} for aerobic scope (see Casselman et al. 2012; Ferreira et al. 2014).

Within the OCLTT, because thermal tolerance is set by oxygen limitation, various mechanisms that affect oxygen delivery have been studied. At the systemic level, hematocrit (Hct), a measure of the amount of the oxygen binding molecule hemoglobin in the blood and thereby the oxygen carrying capacity of the blood, not only affected swimming capacity in fishes (Farrell and Jones 1992; Gallagher et al. 1995; Brill 1996), but was important in limiting an individual's upper thermal tolerance (Beers and Sidell 2011). Cardiac output has also been shown to affect thermal tolerance. As cardiac output is a product of heart rate and heart size (specifically the size of the ventricle, as it is responsible for pumping blood to the tissues), increased peak heart rate (f_{Hpeak}) and ventricle size should increase the volume of blood pumped each beat, or stroke volume, and thereby affect thermal tolerance (Farrell 2009). Indeed, European Atlantic salmon with higher relative ventricular mass values (RVM; ventricular mass divided by body mass) had significantly higher critical thermal maxima (CT_{max} , the temperature at which a fish loses equilibrium), with RVM explaining 19% of the variation in CT_{max} .

Cardiac protein expression might also contribute to thermal tolerance. For example, expression of heat shock proteins, which function to stabilize protein conformation during heat stress (Somero 2010), have been found to be positively correlated with thermal tolerance in killifish (*Fundulus heteroclitus*) (Fangue et al. 2006). In addition, expression of myoglobin (Mb), an oxygen-binding protein that facilitates oxygen transport within the tissues during functional hypoxia, has been shown to affect thermal tolerance in salmonids; for example, ventricular expression of Mb positively correlated with CT_{max} in European Atlantic salmon (Anttila et al. 2013). Therefore, proteins that help preserve oxygen delivery and thereby aerobic metabolism increase thermal tolerance.

Here, we used a suite of cardiorespiratory traits (Hct, f_{Hpeak} , RVM, ventricular Mb and heat shock protein 90 (HSP90; a constitutively expressed heat shock protein)) to assess the mechanisms associated with upper thermal tolerance in three populations of Atlantic salmon (Sebago Lake from Maine, LaHave River from Nova Scotia and Lac Saint-Jean from Québec) that are known to experience different summer thermal maxima (see Figure 2.1.). We predicted that individuals that are better at delivering oxygen to the tissues would be able to preserve cardiac function for

longer and therefore have higher T_{arr} values. Although such traits have been correlated with whole-animal thermal tolerance (e.g. CT_{max}), their relationships with cardiac capacity have not previously been tested. In addition, we expected some of these traits to vary with environmental temperature. We predicted that the Lac Saint-Jean population, which experiences the coolest peak summer temperature, would have a lower Hct, f_{Hpeak} and RVM than the Sebago and LaHave populations, which experience warmer peak summer temperatures. Understanding these relationships will allow for a more comprehensive mechanistic understanding of thermal tolerance.

3.2 Methods

3.2.1 Study populations

Atlantic salmon used in this study originated from three locations. The Sebago Lake population (43.9° N, 70.6° W) has been maintained in hatcheries for 2 generations, the LaHave River population (44.4° N, 64.5° W) has been maintained in hatcheries for 5 generations and the Lac Saint-Jean population (48.6° N, 72.0° W) has been maintained in hatcheries for 2 generations. Offspring were produced for the three populations at the Ontario Ministry of Natural Resources and Forestry (OMNRF) Harwood Fish Culture Station (Harwood, ON, Canada) in November 2013. Approximately 2,000 eggs were extracted from each of 5 females and fertilized by 5 males in every single-pair mating combination for each population ($n = 25$ families per population). Prior to gamete collection, each spawner was anaesthetized with MS-222. The eggs were then transported and reared at OMNRF Codrington Research Facility (Codrington, ON, Canada).

At Codrington, 400 hundred fertilized eggs per full-sibling family were divided into two replicates of 200 eggs and placed in randomized positions in Heath incubation trays. The ambient water temperature mimicked natural conditions (maintained at 5-13°C), as the Heath incubation trays were spring fed by Marsh Creek. Dead eggs were removed daily to prevent transmission of pathogens such as *Saprolegnia* spp. When the eggs hatched, roughly 4 months post-fertilization, the hatchlings (alevins) were moved to 19 L tanks, also maintained at 5-13°C. Each tank was fitted with a layer of AstroTurf to provide substrate. At 5 months post-fertilization, individuals transitioned from endogenous feeding (yolk sac) to exogenous feeding. Individuals were fed ad libitum using organic fish pellets (EWOS Commercial Feeds, Bergen, Norway).

In May 2013, individuals were transferred to a rearing facility at the University of Western Ontario. Full-sibling families were split and placed in two “fry cups,” until they reached approximately 2 g and could be given population-specific tags using Visible Implant Elastomers (Northwest Marine Technology, Shaw Island, WA, USA). The tanks were supplied with freshwater flow of approximately 1 L min⁻¹ that was maintained at 9.5-13.0°C for the duration of the experiment.

From October 2013 to October 2014, fish from the three populations were tested for cardiac capacity (for full details, see section 2.2.2.). Additionally, peak heart rate (f_{Hpeak}) was calculated as

the maximum heart rate observed during cardiac capacity measurements in beats min^{-1} (bpm). Immediately after cardiac capacity measurements, each fish was euthanized and blood samples and tissues were collected.

3.2.2 Blood and tissue samples

Blood and ventricle tissue samples were taken from a subsample of fish ($N = 33$ for Sebago, $N = 42$ for LaHave and $N = 54$ for Lac Saint-Jean). Blood samples were taken from the caudal peduncle vein with a Fisherbrand heparinized $75 \text{ mm} \pm 0.02 \text{ mm L}^{-1}$ hematocrit capillary tube (Fisher Scientific, Waltham, MA, USA) after the caudal fin was severed. Once the tube was full, it was spun for 5 minutes at 13 g in an IEC microCL 17 centrifuge (Thermo Electron Corporation, Waltham, MA, USA). Hct was then quantified as the ratio of the length of red blood cells to total length of blood in the tube. When there was insufficient blood to fill a tube, the sample was discarded ($n = 18$ fish were discarded for all subsequent analyses).

The heart was then dissected out of the fish and the ventricle was isolated from the rest of the cardiac tissue. Wet mass (mg) of the ventricle was measured and then the ventricle was put in RNA-later (Sigma-Aldrich, St. Louis, MO, USA) and stored -80°C for further analysis. Relative ventricle mass (RVM) was calculated using the formula $\text{RVM} = M_v/M_b \times 1000$, where M_v is ventricle mass (mg) and M_b is body mass (g).

The average mass of the fish used for Hct, RVM, and f_{Hpeak} analyses were $13.5 \pm 2.6 \text{ g}$ for Sebago, $11.7 \pm 2.4 \text{ g}$ for LaHave and $14.7 \pm 1.4 \text{ g}$ for Lac Saint-Jean. The average lengths of these fish were $12.2 \pm 1.3 \text{ cm}$ for Sebago, $8.6 \pm 1.3 \text{ cm}$ for LaHave and $10.4 \pm 1.6 \text{ cm}$ for Lac Saint-Jean.

3.2.3 Western Blots

Ventricle muscle samples were chosen from nine individuals to represent the range of observed T_{arr} values. The sample included individuals from LaHave and Sebago populations, and the relative levels of myoglobin (Mb) and heat shock protein 90 (HSP90) were determined by western blot analysis. Ventricle muscle samples were homogenized by sonication in 0.1 ml of lysis buffer (1:1 v/v of sodium dodecyl sulphate (SDS)) containing protease inhibitor cocktail (Sigma-Aldrich, St. Louis, MO, USA), according to Laemmli (1970). Protein concentration from each aliquot was measured using a Bradford assay (Bradford 1976; Bio-Rad, Hercules, CA, USA). For ventricular Mb, $5 \mu\text{g}$ of protein was loaded into each lane and separated on a 15%

SDS polyacrylamide gel. For HSP90, 60 μg was loaded into each lane and a 7.5% SDS polyacrylamide gel was used. β -actin (Santa Cruz Biotech, Santa Cruz, CA, USA) was used as a loading control. Following electrophoresis for 90 minutes for the 15% gels and 150 minutes for the 7.5% gels, both run at 100 V, proteins were transferred to nitrocellulose membranes (Biotrace, Pall Corp., Penrose, NZ). Blots were first incubated in 5% (w/v) non-fat powdered milk in Tween 20 (TBST) in order to block non-specific protein binding, and then overnight at 4°C in TBST with 5% bovine serum albumin (BSA) and primary antibody (either 1:1000 polyclonal rabbit anti-Mb antibody (M8648, Sigma-Aldrich, St. Louis, MO, USA), 1:1000 mouse monoclonal HSP90 antibody (ab53497, Abcam, Cambridge, MA, USA) or 1:10000 β -actin). Blots were then washed three times for 15 mins in TBST and incubated in TBST with 5% BSA and horseradish peroxidase-conjugated secondary antibody (either 1:2000 goat anti-mouse IgG (ab6789, Abcam, Cambridge, MA, USA) or goat anti-rabbit IgG (ab136636, Abcam)) for 2 h. Signals were detected using the SuperSignal West Pico Chemiluminescent Detection Kit (Fisher Scientific, Waltham, MA, USA) and captured and quantified with a FluorChem 8800 Imager and Quantity 1 software, respectively. Relative protein amounts were indirectly measured as band fluorescence intensity relative to the fluorescence intensity of the actin loading control. Each protein was assayed in duplicate, and the average intensities were calculated to represent protein amount. One outlier from the Mb analysis was removed because the band intensity was much higher (2.8 SD away from the mean) and therefore inconsistent with that of the other samples.

3.2.4 Statistical analysis

Only individuals that had values for T_{arr} , Hct, RVM and f_{Hpeak} were used for analyses. ANOVAs with a Tukey's post-hoc test were used to test for differences in T_{arr} , Hct, RVM, and f_{Hpeak} across the three populations. Next, an ANCOVA was used to examine the relationship between Hct, RVM and f_{Hpeak} (independent covariates) and T_{arr} (dependent variable). Body size was also included as a covariate and population as a fixed factor in this analysis. To visualize these results, we plotted a regression between the residuals of T_{arr} and any significant covariates; the residuals were from an ANCOVA that included all terms in the original ANCOVA except for the significant covariates being plotted. After an outlier was removed from the Mb analysis (2.8 SD away from the mean), non-parametric Spearman's correlations were used to test the relationship between T_{arr} and ventricular HSP90 amount and between T_{arr} and ventricular Mb amount. Lastly,

a non-parametric Spearman's correlation was used to test the relationship between ventricular HSP90 and Mb amounts. All statistics were performed using SPSS v. 22 (Chicago, IL, USA).

3.3 Results

For the Atlantic salmon populations examined in this study, T_{arr} values were significantly different across populations, with Sebago and LaHave having significantly higher T_{arr} values than Lac Saint-Jean ($F = 22.1$, $df = 2$, 108 , $p < 0.001$; Fig. 1A). As predicted, Hct was significantly higher in Sebago than in Lac Saint-Jean, with an intermediate Hct observed in LaHave ($F = 6.4$, $df = 2$, 108 , $p = 0.002$; Fig. 1B). RVM did not reach statistical significance across populations ($F = 1.0$, $df = 2$, 108 , $p = 0.366$; Fig. 1C). Peak heart rate (f_{Hpeak}) in beats min^{-1} was also not

significantly different across populations ($F = 1.8$, $df = 2$, 108 , $p = 0.162$; Fig. 1D).

An ANCOVA was conducted with T_{arr} as the dependent variable, population as the fixed factor and Hct, RVM, f_{Hpeak} and body mass as covariates. The results of the ANCOVA are shown in Table 1. Hct and f_{Hpeak} both significantly and positively varied with T_{arr} , while RVM and body mass did not. Residuals of T_{arr} were calculated from an ANCOVA with only RVM, f_{Hpeak} and body mass as covariates and plotted against Hct (Fig. 2A). Residuals of T_{arr} were then calculated again from an ANCOVA with only RVM, Hct and body mass as covariates and plotted against f_{Hpeak} (Fig. 2B).

Ventricular HSP90 and Mb amounts were assayed separately using western blots, and quantified relative to a control band (α -actin). The difference in ventricular HSP90 amount between populations did not reach significance ($t = -0.7$, $n = 9$, $p = 0.522$). Similarly, there was no significant difference in ventricular Mb amount between populations ($t = 1.0$, $n = 8$, $p = 0.376$). A non-parametric correlation revealed a significant positive relationship between individual values for T_{arr} and ventricular HSP90 ($r = 0.783$, $n = 9$, $p = 0.013$; Fig. 3), but not for T_{arr} and ventricular Mb ($r = -0.50$, $n = 8$, $p = 0.21$; Fig. 3). There was also no significant relationship between ventricular Mb and HSP90 amounts ($r = -0.24$, $n = 8$, $p = 0.570$).

Table 3.1. Summary of ANCOVA results for the effects of hematocrit (Hct), relative ventricular mass (RVM), peak heart rate (f_{Hpeak}) and body mass (covariates) on arrhythmia temperatures (T_{arr}) across three populations of Atlantic salmon (*Salmo salar*).

Dependent variable	Factors	F-stat	Degrees of freedom	P-value
T_{arr}	Population	20.9	2, 108	<0.001
	Hct	5.0	1, 108	0.027
	RVM	0.2	1, 108	0.642
	f_{Hpeak}	20.1	1, 108	<0.001
	Body mass	0.4	1, 108	0.535

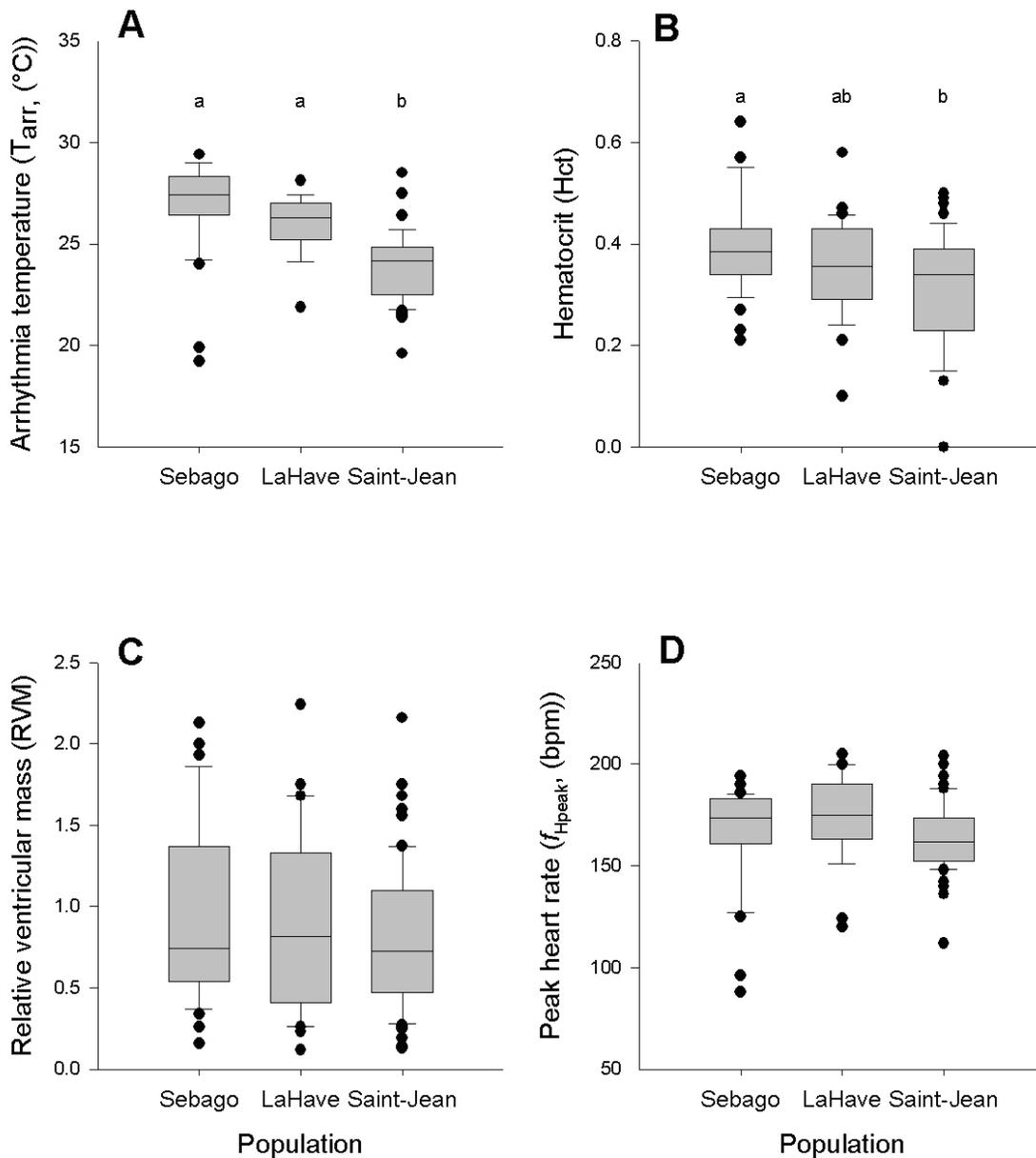


Figure 3.1. Cardiac capacity across three populations of Atlantic salmon (*Salmo salar*). Shown are the (A) arrhythmia temperatures (T_{arr}), (B) hematocrit (Hct), (C) relative ventricular mass (RVM), and (D) peak heart rate (f_{Hpeak}) in beats min^{-1} (bpm). Letters above box plots denote significant differences among populations (see text for statistics). Box plots show median with 25 and 75 percentiles, whiskers show 10 and 90 percentiles and outliers are indicated by dots. Hct was calculated as the volume of red blood cells divided by total blood volume. RVM was calculated as $RVM = M_v/M_b \times 1000$, where M_v is ventricle mass (mg) and M_b is body mass (g). Sample sizes were $N = 30$ for Sebago, $N = 26$ for LaHave and $N = 56$ for Lac Saint-Jean.

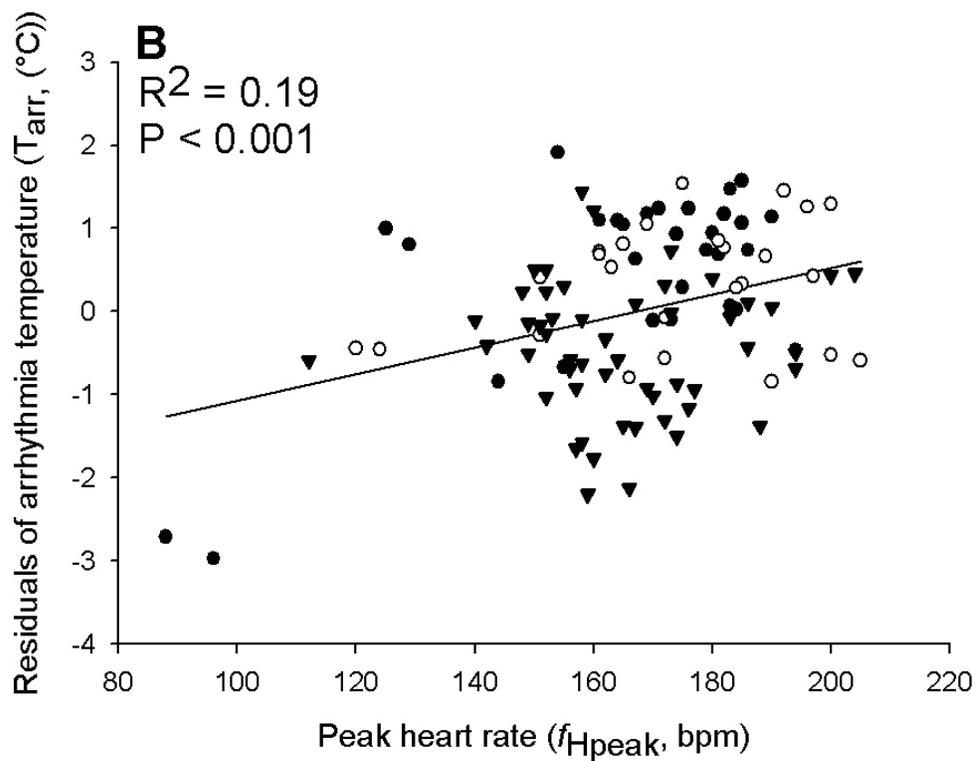
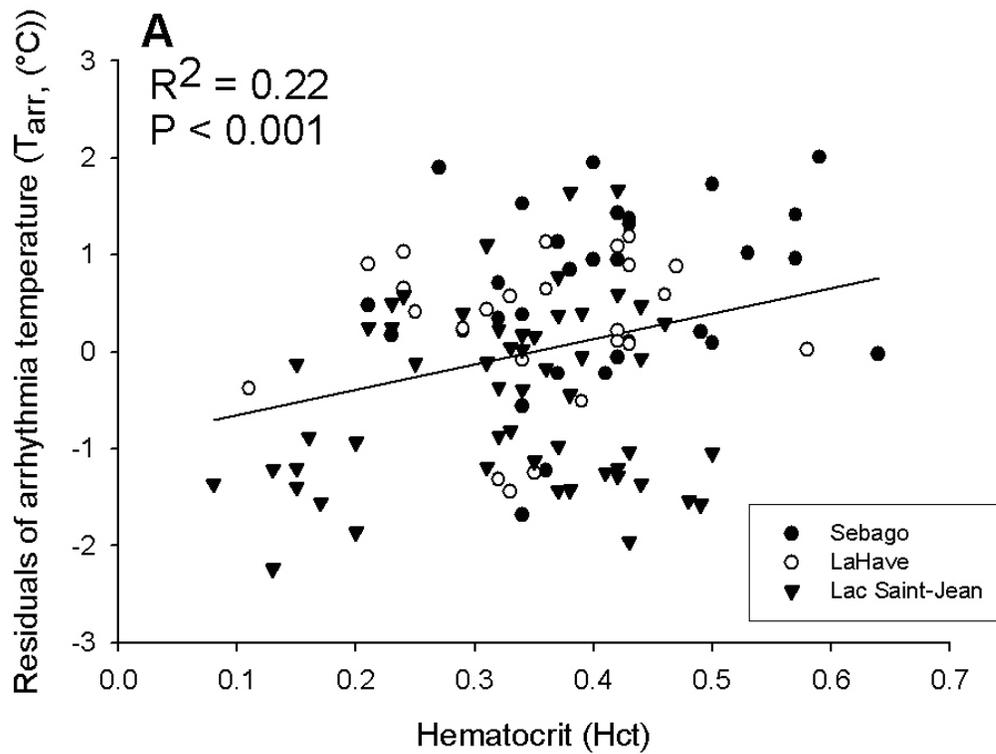


Figure 3.2. Correlation between physiological measures and thermal tolerance in three populations of Atlantic salmon (*Salmo salar*). Shown are (A) hematocrit (Hct) versus the

residuals of arrhythmia temperature (T_{arr}), and (B) peak heart rate (f_{Hpeak}) in beats min^{-1} (bpm) versus the residuals of arrhythmia temperature (T_{arr}). The lines represent significant linear regressions. Residual T_{arr} values are from an ANCOVA analysis with T_{arr} as the dependent variable, population as the fixed factor and RVM, f_{Hpeak} and body mass as covariates for (A) and RVM, Hct and body mass as covariates for (B). Hct was calculated as the volume of red blood cells divided by total blood volume. Sample sizes were $N = 30$ for Sebago, $N = 26$ for LaHave and $N = 56$ for Lac Saint-Jean.

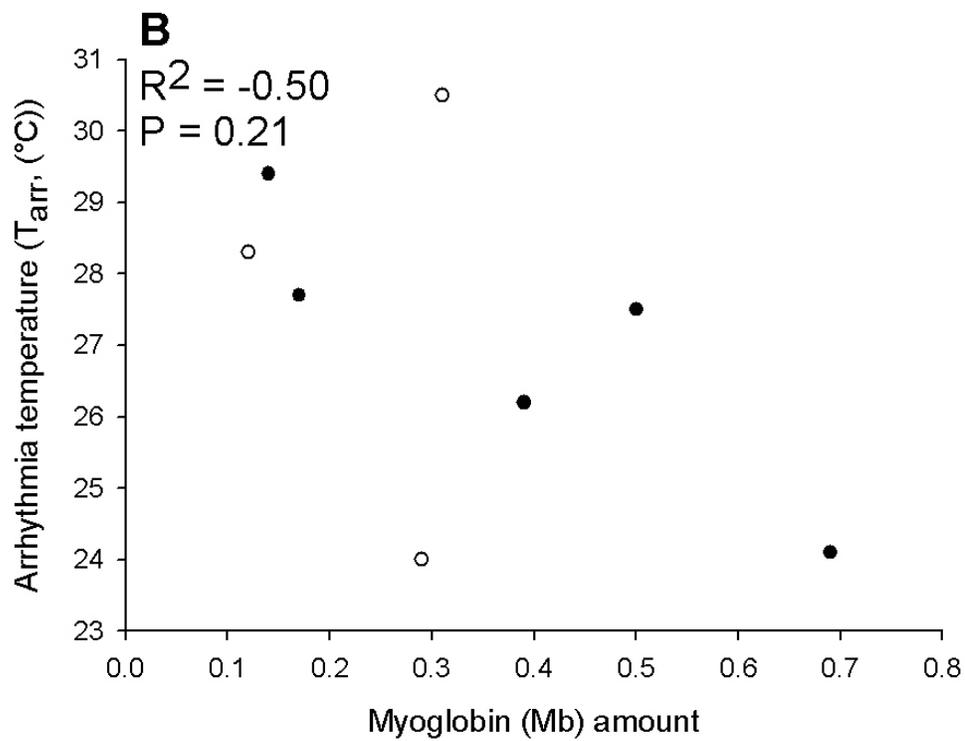
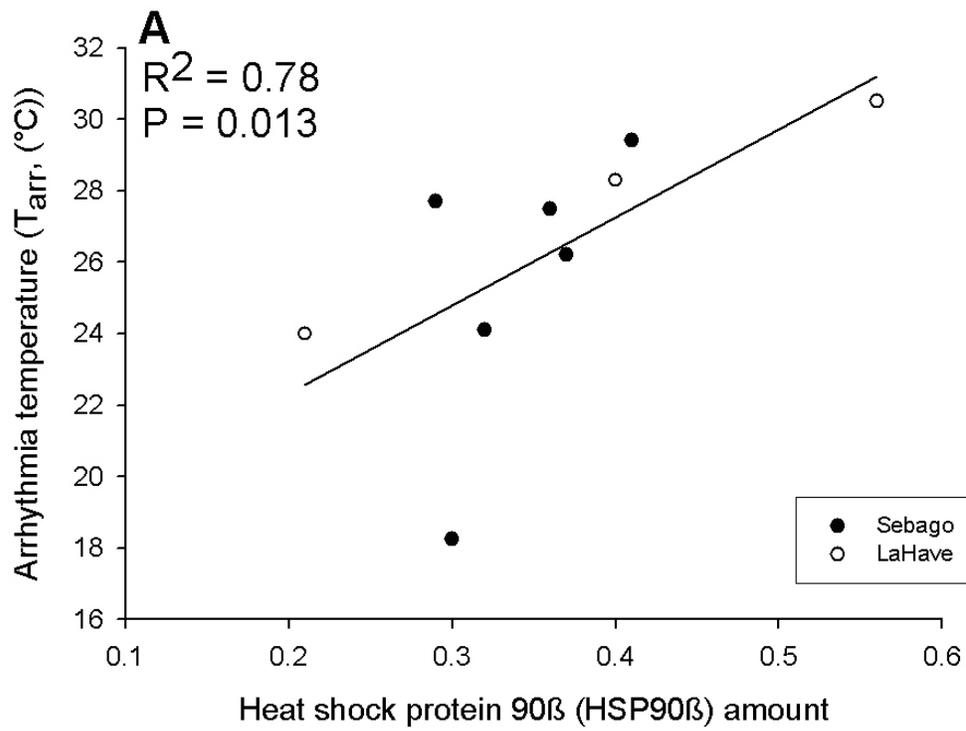


Figure 3.3. Correlation between ventricular protein amount and arrhythmia temperature (T_{arr}) in Atlantic salmon (*Salmo salar*). Shown is (A) the Spearman's correlation between the amount of ventricular heat shock protein 90 β (HSP90 β) and T_{arr} , and (B) the Spearman's correlation between the amount of ventricular myoglobin (Mb) and T_{arr} . There was a significant positive correlation between ventricular HSP90 β amount and T_{arr} , but not between ventricular Mb amount and T_{arr} . The line in (A) represents the line of best fit. Protein amount was quantified as fluorescence intensity relative to that of control band (β -actin) using western blots. Samples sizes were $N = 9$ for HSP90 β and $N = 8$ for Mb analysis.

3.4 Discussion

Temperature increases associated with climate change will threaten the viability and abundance of ectotherms, such as the three populations of Atlantic salmon examined here, because temperature directly affects the cardiorespiratory physiology of fish in critical ways (Farrell 2009). Predicting the resilience of such populations to climate change requires a mechanistic understanding of physiological traits that affect thermal tolerance. Here, we used the oxygen- and capacity-limited thermal tolerance hypothesis (Fry 1971; Pörtner and Farrell 2008) as a framework to explore organ and cellular level mechanisms that are associated with thermal tolerance at the population and individual level.

Environmental oxygen demands have been shown to shape physiological traits that facilitate oxygen delivery such as hematocrit (Hct) and cardiac output (a product of heart size (RVM) and peak heart rate (f_{Hpeak})). Oxygen demands are lower in cooler environments, as reaction rates that drive metabolism are not being increased by warm temperature; therefore we predicted that Hct, RVM and f_{Hpeak} would be lower in the cooler Lac Saint-Jean population compared to the warmer populations. For example, polar fishes have lower Hct than fishes from warmer regions (Scholander and Van Dam 1957; Wells 1990). Indeed, we found that the Lac Saint-Jean Atlantic salmon population had a lower Hct compared to the two other populations. Moreover, across all populations, individual Hct was positively correlated with the cardiac arrhythmia temperature (T_{arr}), explaining 22% of the variation in T_{arr} . We did not find, however, a significant difference in f_{Hpeak} or RVM across populations. Our findings suggest that Hct, but not cardiac output, are shaped through evolutionary processes by environmental oxygen demands at the population level.

Cardiac output has been shown to be important in facilitating oxygen delivery during temperature stress (e.g., Anttila et al. 2013). As cardiac output is a product of heart size and heart rate, we predicted that individuals with higher RVM and f_{Hpeak} would be able to maintain cardiac function for longer at extreme temperatures and therefore have a higher T_{arr} . We found that f_{Hpeak} positively correlated with T_{arr} , indicating that individuals who sustained a higher heart rate could maintain oxygen delivery during acute temperature stress, with f_{Hpeak} explaining 19% of the variance in T_{arr} . On the other hand, we did not find that RVM affected T_{arr} , which was inconsistent with a previous study on Atlantic salmon (Anttila et al. 2013). This discrepancy between our findings and those of Anttila et al. (2013) may be due to the fact that they correlated

RVM with the critical thermal maximum (CT_{max}), which is the temperature at which a fish loses equilibrium. CT_{max} occurs at temperatures above T_{arr} , so it is possible that a larger stroke volume is important to maintain cardiac output only after the heart becomes arrhythmic at T_{arr} . Regardless, our data show that cardiac output mediated by f_{Hpeak} contributes to higher T_{arr} values in multiple populations of Atlantic salmon.

Cardiac protein expression of myoglobin (Mb) and heat shock protein 90 (HSP90) has also been shown to affect thermal tolerance. Anttila et al. (2013) found that the amount of Mb in the ventricle, which binds oxygen from the blood and transports it to the site of respiration in the muscle tissue, had a significant positive correlation with CT_{max} in European Atlantic salmon. We found no relationship, however, between the amount of ventricular Mb and T_{arr} in three North American Atlantic salmon populations. Our sample size was smaller than Anttila et al. (2013), and the amount of variation in CT_{max} explained by Mb was relatively small in their study, so it is possible that we did not have sufficient statistical power to detect the relationship. On the other hand, Anttila et al. (2013) found no correlation between the amount of HSP90 and CT_{max} , yet we found a significant positive relationship between the amount of HSP90 in the ventricle and T_{arr} . Other studies have found that heat shock proteins allow for higher upper thermal tolerance (Fangue et al. 2006), similar to our results. As with the RVM results, it is possible that different mechanisms mediate T_{arr} and CT_{max} . In fact, Muñoz et al. (2015) found that there was a negative correlation between T_{arr} and CT_{max} and suggested that this relationship may be due to a tradeoff between aerobic (as measured by T_{arr}) and anaerobic (as measured by CT_{max}) capacity. Such a tradeoff is consistent with findings of Reidy et al. (2000) that there was a tradeoff between aerobic and anaerobic swimming performance in Atlantic cod (*Gadus morhua*). More research is warranted to better elicit the roles of Mb and heat shock proteins in thermal tolerance under both aerobic and anaerobic conditions.

In conclusion, our data from multiple levels of organization lend support for the oxygen- and capacity-limited thermal tolerance. Specifically, individuals with higher Hct, f_{Hpeak} , and ventricular HSP90 expression had higher T_{arr} values. These traits could be targets for selection in response to climate change depending on the underlying additive genetic variance. RVM and the amount of ventricular Mb, on the other hand, were not related to T_{arr} values, and these results differed from a previous study on European Atlantic salmon that examined CT_{max} (Anttila et al. 2014). Taken together, these data from Atlantic salmon suggest that different physiological

mechanisms are exploited to extend upper thermal tolerance from the aerobic through the anaerobic stages.

3.5 References

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Chapter 4

4 General discussion

Knowledge of thermal performance is critical for understanding how species have adapted to their current environments and predicting how species will respond to climate change (Somero 2010). Here, I present the first evidence supporting local adaptation of thermal performance in Atlantic salmon, an economically and ecologically important species for fisheries in North America. Across three populations of North American Atlantic salmon, I showed that juvenile thermal performance reflected native summer temperatures, such that cardiac capacity was maximized near average summer temperatures and collapsed near peak summer temperatures. In addition, I describe physiological mechanisms underlying cardiac capacity, which I argue ultimately determine thermal performance in Atlantic salmon and are in turn set by oxygen limitation, according to the oxygen- and capacity- limited thermal tolerance (OCLTT) hypothesis.

4.1 Oxygen- and capacity- limited thermal tolerance

The OCLTT is the primary hypothesis describing optima and critical limits of temperature. The theoretical basis for this hypothesis was first proposed by Pörtner and Knust (2007), and stems from the early observations of Fry (1947) that although basic metabolic rate increases exponentially with temperature, maximum metabolic rate increases exponentially only at low temperatures, and declines at high temperatures. Under the OCLTT, the thermal optimum (T_{opt}) is the temperature at which oxygen supply, and therefore potential for aerobic activity, is the greatest, whereas the upper critical temperature (T_{crit}) is the temperature at which aerobic scope functionally collapses. Indeed, fitness-related traits have been shown to be directly affected by temperature. Gibson and Fry (1965) and Brett (1964) found that swimming speed was maximized at T_{opt} in lake trout (*Salvelinus namaycush*) and sockeye salmon. Further, Brett et al. (1969) and Elliot and Hurley (2000) found that growth rate is maximized at T_{opt} in brown trout (*Salmo trutta*). The OCLTT has also been used to explain ecology of fishes. For instance, Pörtner and Knust (2007) found that eelpout (*Zoarces viviparus*) expanded their northern range and had reduced abundance when temperatures in their historical range exceed T_{opt} . Farrell et al. (2008) found that a massive mortality event involving Fraser River sockeye salmon was linked to

anomalously high seasonal temperatures that exceeded T_{opt} . Therefore, since its conception about a decade ago, the OCLTT has been supported by many taxa in both lab and wild settings.

Although the OCLTT is widely accepted, two recent studies have been critical of the hypothesis. In one such study, Clark et al. (2013) questioned the OCLTT based on data from juvenile barramundi (*Lates calcarifer*), a tropical ectotherm. In that species, Norin et al. (2011) acclimated fish to 38°C, and subsequently measured their aerobic scope as well as their preferred thermal regime. Fish whose aerobic scope was maximized at 38°C preferred a median temperature of ~32°C, spending less than 10% of their time in temperatures above 36°C, suggesting that individuals preferred temperatures well below their T_{opt} . Clark and colleagues (2013) argued that according to the OCLTT, individuals should prefer to stay at their T_{opt} . One critical misunderstanding of Clark et al. (2013), however, is that fish will adjust their thermal window to reflect environmental temperatures. The OCLTT does not assume that optimum and preferred temperatures will match. Indeed, mismatches between physiological optimal and preferred temperatures are documented for various fish species (Angiletta 2009). For example, landlocked juvenile sockeye salmon use diurnal vertical migration such that they migrate at dawn to warm surface waters to forage and migrate back down to deep cooler waters at dusk to lower their routine metabolic rate to maximize food conversion and thereby growth rate (Brett 1971). At a biogeographic level, juvenile and adult sockeye salmon inhabit lakes, rivers and oceans below (=colder) their T_{opt} (Lee et al. 2003, Eliason et al. 2011; Chen et al. 2013) to avoid predatory tuna that inhabit more southern, warmer waters (Block et al. 1997). As such, there are many ecological reasons that fish may prefer suboptimal temperatures.

Other studies that seemingly contradict the OCLTT are those that find that aerobic scope is maintained at upper critical temperatures. For example, in a study of the semi-terrestrial air-breathing toad (*Rhinella marina*), Overgaard et al. (2012) found that there was no significant decrease in aerobic scope as temperature increased, even immediately below their lethal limit of 41-42°C, suggesting that oxygen delivery does not significantly affect thermal tolerance in this species. These authors concluded that this finding directly contradicted the OCLTT. This conclusion was later rebutted by Portner and Giomi (2013) and Farrell (2013) who suggested that breathing air, which is more oxygenated than water, may alleviate constraints associated with oxygen limitation. As such, the contradicting evidence put forward by Overgaard et al. (2012) somewhat extends beyond the framework defined by the OCLTT; however, more work is

certainly necessary to resolve how thermal tolerance is shaped by oxygen limitation in air breathers. Ern et al. (2014) later found a consistent pattern in the tropical giant freshwater shrimp (*Macrobranchium rosenbergii*), such that individuals maintained oxygen transport capacity up until the critical maximum temperature (CT_{max}) of 41°C, and that anaerobic metabolism was not being exploited. This finding suggests that mechanisms other than those involved in oxygen delivery, such as protein dysfunction, limit aerobic performance at high temperatures in this species and perhaps other crustaceans. Therefore, more work is needed to integrate possible other mechanisms that limit performance at high temperatures into the OCLTT. Further, this finding is less contradictory than if the opposite were found, i.e. if individuals were surviving long-term after aerobic scope collapse. In sum, the OCLTT remains the unifying framework to explain thermal tolerance in ectotherms and has important utility in predicting responses to climate change, but requires more research to resolve the fine-scale mechanisms underlying thermal tolerance.

In order to measure oxygen limitations that shape thermal tolerance, aerobic scope must be measured across temperatures. However, aerobic scope measures are time-consuming, and so Casselman et al. (2012) developed a method to expedite measurements of transition temperature associated with aerobic scope (i.e. T_{opt} and T_{crit}). As heart rate (f_H) is central in determining a fish's response to warming (Farrell et al. 2008), this method measures maximum f_H (f_{Hmax}) to determine these temperatures of interest. This method, used in both Chapter 2 and 3, involves anaesthetizing fish to standardize activity state and pharmacologically stimulating f_{Hmax} . Although Casselman et al. (2012) found that resting f_H in unanaesthetized fish was not significantly different from resting f_H in anaesthetized fish, other studies have found that anesthesia directly affects cardiac function (Hill et al. 2002; Cotter and Rodnick 2006). Here, I anaesthetized the experimental fish and found that resting f_H was not in the normal range for unanaesthetized resting juvenile Atlantic salmon (mean of 67 beats min^{-1} at acclimation temperature of 11°C vs. normal range of 25-40 beats min^{-1} at acclimation temperature of 10°C; Knudsen et al. 1992). This is likely because anaesthesia reduces vagal tone, increasing f_H . Therefore, I did not make any conclusions based on our resting f_H values, as they are likely confounded by the anesthetic. I advise that future resting f_H values in salmonid species should be collected from unanaesthetized fish to avoid confounding drug interactions. Such a protocol can follow Casselman et al. (2012), whereby fish are chased to exhaustion at incrementally warmer test temperatures and

subsequently placed in a small chamber with electrodes on the bottom. f_{Hmax} (as stimulated pharmacologically once the fish are anaesthetized), on the other hand, was not significantly different from that of an exercising salmon (Casselman et al. 2012) and can therefore reliably be obtained by the high-throughput method of Casselman et al. (2012). Obtaining accurate resting f_H and f_{Hmax} , and, in doing so, scope for f_H , would be a powerful extension of Casselman's method that could be used to quantify the effects of warming temperature on aerobic capacity, and by extension, the viability of populations.

4.2 Thermal adaptation

In order to understand how species will adapt to future temperature changes, it is first important to understand how species are adapted to their current temperatures. After all, if populations are not currently adapted to their thermal regime, or if there is no variation in thermal tolerance within a population on which selection can act, it is unlikely that a population could respond to climate change. Studies of many taxa have found that populations are narrowly adapted to their current thermal environments, including fishes, turtles, snakes, and lizards (Nilsson et al. 2009; Franklin et al. 2007; Drost et al. 2014; Weber et al. 2015; Wei-Guo et al. 2010). Local adaptation in salmonids is of particular interest, as these fishes are economically, ecologically, and culturally important, and have life histories that may facilitate adaptation to local temperatures. However, few studies have examined thermal adaptation in salmon. Farrell et al. (2008) and Eliason et al. (2011) found that the thermal performance of Fraser River sockeye salmon adults reflected temperatures experienced during upstream migration. Furthermore, Chen et al. (2015) found that rainbow trout translocated from their native habitat of North America to Western Australia had higher thermal tolerance than their native counterparts after 19 generations. However, these studies did not use a common garden or reciprocal transplant design, so developmental acclimation, rather than local adaptation, could explain these population differences. Anttila et al. (2014) attempted to tease apart developmental acclimation and genetic adaptation by rearing juveniles from two populations of European Atlantic salmon at the same temperatures (12°C and 20°C), but found no difference in thermal tolerance between populations. Although critical for predicting the vulnerability of species to climate change, genetic differences in thermal performance among populations have been poorly characterized in salmon.

In my thesis, I used a common garden breeding design to examine genetic differences in thermal tolerance among three populations of Atlantic salmon, while excluding the potential effects of developmental acclimation. In chapter 2, I found that differences in thermal performance reflected native thermal regimes, such that thermal performance was maximized near average summer temperatures and collapsed near peak summer temperatures, as predicted. These findings suggest that environmental temperatures encountered as juveniles shape thermal performance at the population level. In chapter 3, I investigated the fundamental mechanisms that might be underlying differences in thermal tolerance among populations. I predicted that because cold environments require less oxygen, the cooler Lac Saint-Jean population would have less capacity for traits that facilitate oxygen delivery, such as cardiac output and hematocrit (which govern convection of oxygen through the cardiorespiratory system). As predicted, I found that hematocrit varied across populations, with the cooler, Lac Saint-Jean population having the lowest hematocrit. Contrary to my predictions, I did not find that cardiac output (measured as heart size (RVM) and peak heart rate ($f_{H_{peak}}$)) was lower in the Lac Saint-Jean population. Environmental factors other than temperature are also important in shaping cardiac output, potentially explaining why I did not find differences in RVM or $f_{H_{peak}}$ in populations with different temperature regimes. For example, in Fraser River sockeye salmon, Eliason et al. (2011) found that cardiac output was determined largely by the difficulty of the populations' upriver migrations. In my populations, cardiac demands may have differed based on flow rate. LaHave is an anadromous population with juveniles inhabiting the hydraulically challenging LaHave River, whereas Sebago and Lac Saint-Jean are landlocked populations with juveniles inhabiting relatively slow-flowing streams. Therefore, LaHave may have higher cardiac demands based on flow rate alone. Regardless of the specific physiological mechanism underlying differences in thermal tolerance, I provide the first evidence of local adaptation in a salmonid that can be attributed to genetic differences rather than developmental acclimation.

Existing phenotypic plasticity may allow species to respond to small increases in temperature; however, large-scale increases in temperature, such as those predicted as a result of climate change, will likely require genetic adaptation if species are able to persist in their current environments. Recently, there has been evidence that both phenotypic plasticity and additive (heritable) genetic variation contribute to thermal performance in salmon. For example, Muñoz et al. (2015), found additive genetic variation and plasticity underlying the Arrhenius breakpoint

(T_{AB} ; a proxy for T_{opt}) but not the arrhythmia temperature (T_{arr} ; a proxy for T_{crit}), in a population of Chinook salmon. In Atlantic salmon, Anttila et al. (2013) suggested that there was genetic variation among families of European Atlantic salmon in CT_{max} , albeit the conclusions were limited by weak statistical power. Such issues of statistical power are common in quantitative breeding design; in my study, some families suffered such high mortality that I also had low statistical power to detect genetic variation within populations. Anttila et al. (2014) later found that there was plasticity underlying both T_{AB} and T_{arr} , suggesting that Atlantic salmon already have a higher chance of short-term survival than Pacific salmon in the face of increasing temperatures. However, it is unknown whether this plasticity would keep pace with climate change, as plasticity led to only a partial recovery of thermal performance and the genetic architecture underlying cardiac performance remains unresolved in Atlantic salmon. It is important to investigate the genetic mechanisms underlying thermal tolerance, as genetic adaptation is likely necessary if this species is going to persist in the face of climate change.

4.3 Concluding remarks

As global warming increasingly threatens natural ecosystems, research that integrates ecological, genetic, and physiological data will provide crucial information to predict biological resilience. In my thesis, I found variation among populations in cardiac performance over a thermal range, and the potential for adaptation to temperature change in three populations of Atlantic salmon. However, the ability of Atlantic salmon to persist in the wild will likely rely on limiting the rate of temperature change so that populations have the time to adapt. Although my thesis presents a hopeful future for Atlantic salmon, I suggest that mitigating climate change is essential to measuring the persistence of salmon and the ecosystem they support.

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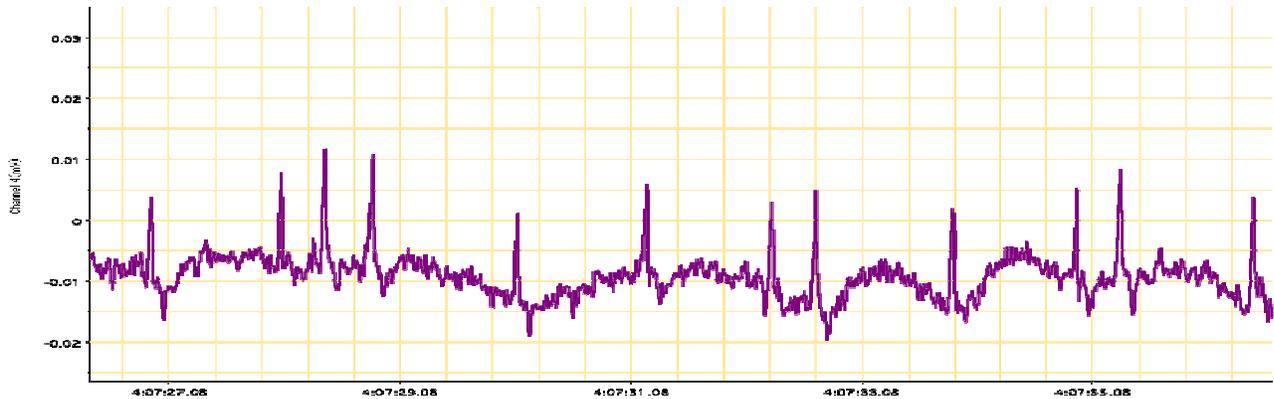
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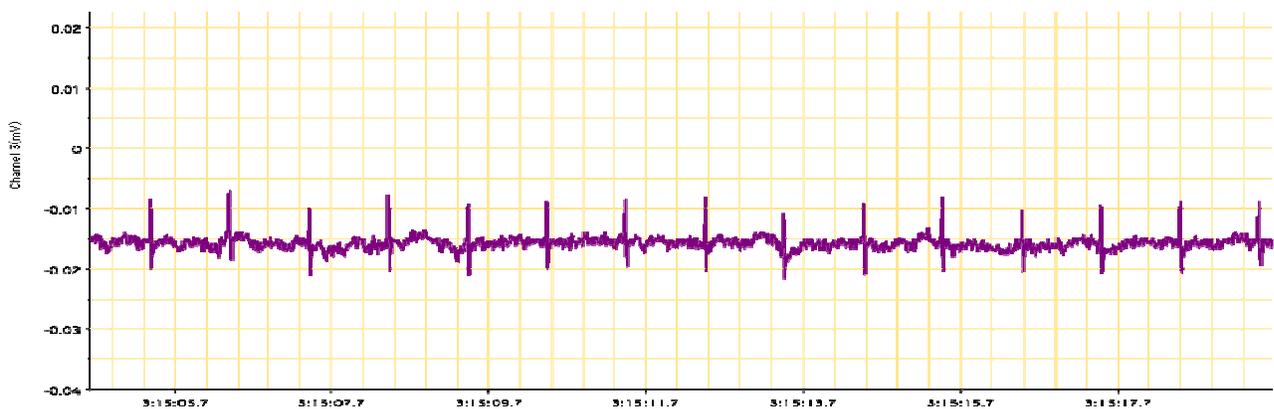
Appendix

Appendix A. Electrocardiogram (ECG) traces in millivolts (mV) from an individual Atlantic salmon (*Salmo salar*) during acute temperature increase. Each inflection of the ECG trace is the QRS wave, or the depolarization and repolarization of the ventricle. (A) A rhythmic ECG trace at 11°C, the acclimation temperature. (B) An ECG trace at the arrhythmia temperature (T_{arr}) at 25.6°C. For details of heart rate analysis, see section 2.2.3.

(A)



(B)



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