

**THERMAL LIMITS TO THE CARDIORESPIRATORY PERFORMANCE OF ARCTIC
CHAR (SALVELINUS ALPINUS) IN A RAPIDLY WARMING NORTH**

by

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Abstract

The Canadian Arctic is warming at nearly three times the global rate. Consequently, thermal regimes of native cold-adapted species like the Arctic char (*Salvelinus alpinus*) are being rapidly reshaped. The Arctic char is the most northerly-distributed freshwater fish on Earth and is essential to Inuit food security and culture. Anadromous Arctic char migrate between freshwater habitats and the Arctic Ocean many times throughout their lives, which can expose them to an already extreme range of temperatures (<0 to >21°C). My thesis examined the ability of Arctic char to cope with thermal variation, focusing specifically on cardiorespiratory performance.

I used a novel, mobile laboratory in the central Canadian Arctic to assess how acute temperature changes impact cardiac function and aerobic metabolism in migrating Arctic char. Arctic char maintained aerobic performance over an impressive temperature range (4-16°C), but could not recover from exhaustive exercise above 16°C. Furthermore, maximum heart rate, which should increase with acute warming, began to plateau at 16°C, declined at 19°C and became arrhythmic at 21°C. I conducted similar assessments on four other populations to determine how they differed. One population that undertakes physically and thermally challenging migrations had higher maximum aerobic capacity and heat tolerance than another with less harsh migrations. Heart mass was also higher in populations with less challenging migrations.

Next, I acclimated hatchery-reared Arctic char to naturally encountered temperatures (2-18°C) to characterize their thermal plasticity. As before, Arctic char maintained high aerobic performance over a broad temperature range (2-14°C) and warm acclimation improved

swimming performance and dramatically increased (+35-45%) cardiac heat tolerance. However, mortality occurred with chronic exposure to 18°C.

My research indicates that Arctic char have broad thermal performance and acclimation potential that may help mitigate the negative impacts of rapid environmental warming. Nevertheless, I showed that Arctic char already encounter temperatures in the Canadian Arctic that constrain cardiorespiratory performance and impair recovery from exhaustive exercise, which would likely hinder their obligatory return migration. Furthermore, my results suggest that intraspecific diversity in cardiorespiratory physiology, thermal tolerance, and local migration conditions may be important for Arctic char conservation and shape population-specific responses to warming.

Lay Summary

The Arctic char, a type of salmon, is an essential resource in communities of the Canadian Arctic, which is among the most rapidly warming regions on Earth. However, we know alarmingly little about how resident cold-adapted species like Arctic char will respond. Arctic char migrate annually between their spawning grounds in lakes and their feeding habitat in the Arctic Ocean. My thesis shows that water temperatures encountered during this migration can already be warm enough to impair their heart function and their ability to recover from strenuous exercise, which could critically impair upriver return migrations. I also demonstrate that some populations are more susceptible to such impairments, but that Arctic char may be able to adjust their physiology (acclimate) to allow them to better tolerate high temperatures if given sufficient time. As climate change continues, such information will be integral to developing evidence-based management strategies.

Preface:

This research was approved by Fisheries and Oceans Canada (AUP 2016-033, AUP 2017-039, LFSP S-16/17 1028-NU, LFSP S-17/18 1023-NU) and the UBC Animal Care Committee (AUP 2016-033, AUP 2017-039). Informed letters of support were received annually from the Ekaluktutiak Hunters & Trappers Organization for all work conducted in the Kitikmeot Region of Nunavut.

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A version of Chapter 3 has been submitted for publication as ‘Gilbert MJH, Farrell AP. The thermal acclimation potential of maximum heart rate and cardiac heat tolerance in Arctic char (*Salvelinus alpinus*), a northern cold-water specialist.’ M.J.H. Gilbert designed the studies in Chapter 3 and 4, conducted data collection, analysis and presentation, and wrote the chapters with supervision and editorial input from Dr. A.P. Farrell. Y. Zhang assisted in the collection of respirometric data for Chapter 4.

M.J.H. Gilbert designed Chapter 5 based on discussions with Dr. J.S. Moore, Dr. A.P. Farrell and L.N. Harris and it was inspired by findings presented in Eliason et al. (2011) and Moore et al. (2017). M.J.H. Gilbert led data collection, conducted all data analysis and presentation and wrote the chapter with editorial input and supervision by Dr. A.P. Farrell. L.N. Harris and Dr. J.S. Moore assisted in research planning and logistics. K. Kanayok, D. Kanayok, C. Amegainik, R. Ekpakohak, L.N. Harris, B. K. Malley and Dr. J.S. Moore assisted in fieldwork in the Kitikmeot region of Nunavut.

M.J.H. Gilbert designed and wrote Chapter 1 and 6 with editorial input from Dr. A.P. Farrell.

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List of Abbreviations

AAS	absolute aerobic scope
ANOVA	analysis of variance
CT _{max}	critical thermal maximum
FAS	factorial aerobic scope
FWC	Freshwater Creek, Nunavut
f_{Hmax}	maximum heart rate
Hb	haemoglobin
HCT	hematocrit
IQR	interquartile range
K	Fulton's condition factor
MCHC	mean corpuscular haemoglobin content
$\dot{M}O_2$	oxygen uptake rate
$\dot{M}O_{2max}$	maximum oxygen uptake rate
$\dot{M}O_{2mean}$	average oxygen uptake rate between 24-48h of respirometry
$\dot{M}O_{2min}$	minimum oxygen uptake rate
$\dot{M}O_{2standard}$	standard oxygen uptake rate
O ₂ pulse	$(\dot{M}O_{2max}/f_{Hmax})$
OCLTT	oxygen- and capacity-limited thermal tolerance hypothesis
PC	principal component
PCA	principal components analysis
Peak f_{Hmax}	peak maximum heart rate observed during acute warming to arrhythmia
Q ₁₀	Q10 temperature coefficient
RAM	relative atrial mass
RVM	relative ventricular mass
SD	standard deviation
SE	standard error of the mean
SGR	specific growth rate
T _{AB} and T _{warm}	first Arrhenius breakpoint temperature during acute warming
T _{arr}	temperature at which the heart beat first became arrhythmic during acute warming
T _{coldAB}	Arrhenius breakpoint temperature during acute cooling
TPC	thermal performance curve
T _{peak}	temperature at peak maximum heart rate during acute warming
T _{q10<1.5}	temperature at which the heart rate Q10 fell below 1.5
T _{qb}	temperature at which the heart rate Q10 fell below 2

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Chapter 1: Introduction

Temperature is a measure of a system's kinetic energy and thus has a profound influence on the rate at which physiological rates occur. In ectotherms, physiological rates typically increase, often exponentially, with ambient temperature over a biologically relevant range yet become critically limited at extremely high and low temperatures (Fry 1947; Fry 1971; Schulte *et al.* 2011; Dowd *et al.* 2015; Schulte 2015). The shape (amplitude, breadth and skewness) of these temperature-performance relationships, called thermal performance curves (TPCs), can often reflect an ectotherm's life history and their corresponding exploitation of, and exposure to thermal variation (eg. Eliason *et al.* 2011; Healy and Schulte 2012). Furthermore, because the temperature range for adequate function of vital physiological processes sets an organism's fundamental thermal niche, this temperature range is commonly reflected in their biogeographical distribution (Sunday *et al.* 2011; Sinclair *et al.* 2016). In the Anthropocene, the study of these relationships between animal's thermal physiology and their ecology has emerged as an important approach to predicting and understanding the consequences of often dramatic changes in local and global thermal regimes (Sinclair *et al.* 2016).

Along these lines, my dissertation examines the influence of temperature on life-sustaining physiological rates (e.g. heart rate and rates of oxygen uptake) of Arctic char (Salmonidae: *Salvelinus alpinus*) in relation to their thermal ecology. I focus on Arctic char for three main reasons: First, from a conservation perspective, the Canadian Arctic is warming at nearly three times the average global rate (Zhang *et al.* 2019a). Second, the Arctic char is an essential part of northern aquatic ecosystems, food security and culture (Roux *et al.* 2011; Day and Harris 2013; Lysenko and Schott 2019). Third, from a basic science perspective, the Arctic char is the most

northerly distributed freshwater fish on Earth and is thus an intriguing organism in which to study how thermal physiology relates to species life history strategies and biogeographical distributions. The remainder of the introduction provides pertinent background information on thermal biology, cardiorespiratory physiology and Arctic char ecology and concludes with the goal and objectives for my thesis research.

1.1 General Thermal Biology

1.1.1. Thermal performance relative to thermal experience

Within the field of thermal biology, the thermal variability hypothesis (Janzen's hypothesis) is a leading explanation for global trends in the breadth of thermal-performance relationships (Thermal Tolerance Breadth: TTB) (Ghalambor *et al.* 2006; Huey *et al.* 2012). The hypothesis contends that groups of animals that experience greater thermal variation throughout their lives should have greater TTB owing to selection for adequate physiological performance over a wide range of encountered temperatures. The primary support for this hypothesis is that the TTB of ectotherms tends to increase with latitude, which corresponds with increased environmental thermal variation (Janzen 1967; Ghalambor *et al.* 2006; Compton *et al.* 2007; Sunday *et al.* 2011). Accordingly, Janzen hypothesized that the narrow TTB of tropical species restricts their latitudinal and altitudinal ranges (Janzen 1967).

Recently, Payne and Smith (2017) proposed an alternative explanation for limited TTB at in the tropics, and at warm temperatures in general, which I refer to as the "equivalent biological rate hypothesis". Payne and Smith (2017) contend that for a given performance trait (e.g., aerobic metabolism) there is a discrete range of physiological rates that can be supported within an individual and that this range is similar among classes of ectotherms. Consequently, the

absolute temperature range that can be tolerated is necessarily narrower at high temperatures than at low ones given that the rising phase of a thermal performance curve is typically exponential. Thus, under this hypothesis the limited TTB of tropical species has more to do with the warm absolute temperatures they encounter rather than the local thermal variation. This hypothesis is supported by considering TTB as a difference in physiological rates calculated from the Boltzmann–Arrhenius equation, rather than as a difference in absolute temperature, because the positive relationship between TTB and latitude is eliminated (Payne and Smith 2017). Based on the equivalent biological rate hypothesis, polar species, which live at cold temperatures, should exhibit a high TTB relative to their temperate and tropical counterparts. However, while this hypothesis is compelling, there is still a wide range of TTBs found among species within a region, and many at high latitudes have very narrow TTB (e.g. Arctic cod and Antarctic notothenoids)(Somero and DeVries 1967; Drost *et al.* 2016). This variation may be explained by species adaptation to their experienced thermal variation, or it could also be a result of their phylogeny.

Beyond TTB, TPCs for a given trait can also vary dramatically in amplitude and skewness, even among closely related species such as the salmonids (family: Salmonidae) TPCs may be skewed (typically to the left) to varying extents and can have large peaks focused around particular temperatures or be relatively flat (Figure 1.1a)(Dowd *et al.* 2015; Schulte 2015; Farrell 2016). These features are used to characterize species as thermal specialists, whose TPC tend to have narrower TTB (stenothermal) and often large peaks, and generalists whose TPC may be broader (eurythermal) and flatter (Gilchrist 1995; Angilletta Jr *et al.* 2003; Dowd *et al.* 2015; Farrell 2016). While these characterizations are useful for relative comparisons there are no well-defined absolute limits as to what constitutes a thermal specialist or generalist.

In nature, temperature exposures occur over a continuum of time scales, which is critical to consider when assessing thermal performance relationships and interpreting their ecological significance (Fry 1947; Fry 1971; Schulte *et al.* 2011). Many ectotherms routinely experience substantial temperature changes associated with regular spatial and temporal (ontogeny and seasonal and diurnal) changes, but they may also encounter more irregular, acute changes related to weather or when passing through thermal environments. Prolonged temperature exposures may allow some animals to acclimate to the new conditions resulting in a shift in acute temperature-performance relationships, while shorter-term exposure may permit some level of performance at temperatures that would be unsustainable and potentially lethal with prolonged exposure. Thermal polygons, first developed by Fry (Fry *et al.* 1942; Fry 1947), are a useful method to visualize thermal tolerance and performance while incorporating the timescale of exposure and the role of acclimation (Figure 1b)(Baroudy and Elliott 1994; Drost *et al.* 2016; Farrell 2016). Fry thermal polygons are generated by plotting upper and lower acute critical temperatures (y-axis) over all biologically viable acclimation temperatures (x-axis) and are thus bound by upper and lower prolonged critical temperatures (e.g. incipient lethal temperatures, ILT). The area within the polygons can provide an absolute measure of the breadth of an organism's fundamental thermal niche. In my thesis I construct a thermal polygon for Arctic char and generate TPCs that fall within the polygon to characterize the thermal biology of Arctic char relative to their current and predicted thermal regimes (Chapter 6).

1.1.2. Temperature effects on cardiorespiratory performance in fish

The identification of thermally sensitive physiological processes that can limit whole organism performance is naturally a principal goal in the study of thermal physiology (Somero

2010). In many ectotherms, and salmonids in particular, the rate of oxygen delivery to systemic tissues is one such critical process that often becomes constrained at high temperatures and is therefore a likely candidate to influence whole organism temperature tolerance (Pörtner and Farrell 2008; Farrell *et al.* 2009; Eliason *et al.* 2011). This idea was formalized by Fry (1947; Fry and Hart 1948) who assessed the difference between standard and maximum rates of oxygen uptake ($\dot{M}O_{2\text{Standard}}$ and $\dot{M}O_{2\text{Max}}$) over a wide range of temperatures in goldfish (*Carassius auratus*) and then in other fishes (Gibson and Fry 1954). Fry interpreted the difference between $\dot{M}O_{2\text{Standard}}$ and $\dot{M}O_{2\text{Max}}$ as a measure of the capacity of the animal to utilize oxygen to support non-vital functions, which he termed the ‘scope for metabolic activity’. This term has since been refined to ‘aerobic scope’, which importantly recognizes that the measure only relates to oxygen-dependent metabolism. Fry (1947) and many others since (eg. Gibson and Fry 1954; Brett 1964; Brett 1971; Eliason *et al.* 2011; Chen *et al.* 2015) found that while both $\dot{M}O_{2\text{Standard}}$ and $\dot{M}O_{2\text{Max}}$ increase with temperature over low and moderate temperatures, at warm temperatures $\dot{M}O_{2\text{Max}}$ tends to plateau or decrease while $\dot{M}O_{2\text{Standard}}$ does not, resulting in a decline in aerobic scope. The prevalence of this observation resulted in the formation of the oxygen- and capacity-limited thermal tolerance hypothesis (OCLTT), which contends that tissue oxygen supply at extreme temperatures sets whole organism thermal tolerance (Pörtner and Farrell 2008; Pörtner and Peck 2010; Portner *et al.* 2017). However, it is important to note that a pronounced decline in aerobic scope below critical temperatures has not been found in all species examined (Clark *et al.* 2005; Norin *et al.* 2014; Norin and Clark 2016), which may be a result of a combination of biological, physical (kinetic or thermodynamic) or methodological factors. Such contradictory findings have resulted in notable disagreement over the broad applicability of the OCLTT hypothesis among ectotherms (Clark *et al.* 2013; Farrell 2013; Jutfelt *et al.* 2014; Portner 2014; Jutfelt *et al.* 2018).

Nevertheless, thermally limited aerobic scope is very common and seems to be ubiquitous in salmonids (Gibson and Fry 1954; Lee *et al.* 2003; Farrell *et al.* 2008b; Pörtner and Farrell 2008; Farrell 2009; Somero 2010; Clark *et al.* 2011). Such a limitation unarguably constrains the aerobic capacity available to support various activities such as migration and is thus a focal point of my thesis.

When examining the mechanism underlying thermal limitations to aerobic scope and $\dot{M}O_{2\text{Max}}$ it is useful to individually consider the components of the Fick equation for oxygen uptake:

$$\dot{M}O_2 = f_H \cdot SV \cdot (CaO_2 - CvO_2)$$

Where $\dot{M}O_2$ is a function of the amount of oxygen extracted from circulating blood ($CaO_2 - CvO_2$; oxygen content of arterial and venous blood, respectively) and cardiac output (\dot{Q}), which is the product of stroke volume (SV) and heart rate (f_H). The plateau and decline in $\dot{M}O_{2\text{Max}}$ that results in aerobic scope impairments at warm temperatures appears to be, at least in part, the result of a corresponding limitation in \dot{Q} (Farrell 2002; Clark *et al.* 2008b; Steinhausen *et al.* 2008; Farrell *et al.* 2009). Generally maximum heart rate ($f_{H\text{max}}$) increases with temperature, which facilitates an increase in \dot{Q} to match convective oxygen supply with increasing consumption at tissues (Steinhausen *et al.* 2008; Ekström *et al.* 2014). However, at high temperatures f_H plateaus or decreases and ultimately becomes arrhythmic. This f_H limitation constrains \dot{Q} because SV does not markedly change with warming until near critical temperatures (Steinhausen *et al.* 2008; Farrell 2009; Ekström *et al.* 2014; Eliason and Anttila 2017). Furthermore, fish can only partially compensate for limitations in \dot{Q} by increasing blood oxygen extraction because venous blood oxygen levels can only be depleted so far before

adequate oxygen diffusion into vital tissues, including the myocardium, is impaired (Farrell and Clutterham 2003; Lannig *et al.* 2004; Ekström *et al.* 2016a). The occurrence of a cardiac collapse when water temperature becomes too warm has now been documented in a wide variety of species from polar stenotherms such as the Arctic cod (*Boreogadus saida*) (Drost *et al.* 2016) to notable eurytherms such as the goldfish and the Atlantic killifish (*Fundulus heteroclitus*) (Ferreira *et al.* 2014; Safi *et al.* 2019) as well as numerous species in-between (Eliason *et al.* 2011; Casselman *et al.* 2012; Eliason *et al.* 2013; Anttila *et al.* 2014a; Chen *et al.* 2015; Eliason and Anttila 2017). As such, my thesis will largely focus on f_H limitations to $\dot{M}O_2$. It should be noted that oxygen uptake at the gill and reduced haemoglobin- O_2 affinity may lower CaO_2 at high temperatures, which could also contribute to limitations in $\dot{M}O_2$, however, evidence is mixed and such limitations were beyond the scope of my thesis (Heath and Hughes 1973; Steinhausen *et al.* 2008; Eliason *et al.* 2013; Ekström *et al.* 2016a). While there are many studies that demonstrate that a limitation in \dot{Q} mediated by heart rate may underlie aerobic scope limitations at warm temperatures, aerobic scope is also often limited at cold temperatures. Cold limitations to aerobic performance have largely been neglected and while I briefly discuss them in Chapter 2, they are not a focus of my thesis and warrant further investigation.

1.1.3. Mechanisms underlying heart rate collapse at warm temperatures

The response of f_H to warming, and specifically, heat-induced f_H collapse is central to my thesis given its essential role in setting internal oxygen convection and tissue oxygen supply. A number of mechanisms are proposed to underlie temperature limitations to cardiac performance in fish, some of which may not be mutually exclusive. They include: a general

venous oxygen supply limitation, mitochondrial dysfunction, and temperature-dependent depression of electrical excitability (TDEE).

The first of these proposed mechanisms is fairly intuitive; at supra-optimal temperatures the temperature-dependent increase in internal oxygen convection is insufficient to meet an increase in systemic oxygen demand which results in observed declines in venous blood oxygen tension at a time when the heart is beating faster and venous blood has a shorter residence time in the cardiac chambers to deliver essential oxygen to the myocardium (Farrell 2002; Lannig *et al.* 2004; Steinhausen *et al.* 2008; Eliason *et al.* 2013; Ekström *et al.* 2016a). This blood oxygen limitation could disproportionately affect the heart regardless of the presence of a coronary system because, to some extent, the heart relies on luminal, venous blood for oxygen and it is the last organ to receive blood prior to re-oxygenation at the gills (Farrell 2002; Steinhausen *et al.* 2008).

Mitochondrial dysfunction may also contribute to cardiac performance limitations at high temperatures. In some fish species, a temperature-induced reduction in mitochondrial efficiency (decrease in ATP produced per unit O₂ consumed) has been observed under normoxic conditions at temperatures below the onset of cardiac collapse (Iftikar and Hickey 2013; Iftikar *et al.* 2014; Chung *et al.* 2017) A decline in mitochondrial efficiency increases an already elevated tissue oxygen requirement at supra-optimal temperatures and would certainly exacerbate any cellular energy deficiencies that result from an oxygen supply limitation. Succinate, a citric acid cycle intermediate, accumulates in plasma with heat stress (Iftikar and Hickey 2013; Iftikar *et al.* 2014; Gilbert 2015) and may be an indicator of mitochondrial dysfunction at warm temperatures

(Pörtner and Knust 2007). However, this limitation is not apparent in all cases (Penney *et al.* 2014).

The TDEE hypothesis suggests that the electrical excitability of ventricular cardiac myocytes, and potentially other excitable cells, becomes impaired at high temperatures (Vornanen 2016). Many studies report heat-induced cardiac arrhythmias indicative of either an atrioventricular block, or an inability of the ventricle to respond to an upstream depolarization event (Casselmann *et al.* 2012; Ferreira *et al.* 2014; Badr *et al.* 2016). Vornanen (2016) has suggested that the limitation in electrical excitability may result from the differential temperature sensitivity of K^+ and Na^+ channels. Specifically Na^+ currents may be impaired at a lower temperature than K^+ currents resulting in a mismatch in K^+ and Na^+ conductance that can prevent ventricular depolarization (Vornanen *et al.* 2014; Haverinen and Vornanen 2020). However, the generality of this mismatch has yet to be tested.

While any or all of these mechanisms could contribute to temperature induced cardiac impairments, it is important to remember that the heart is not working in isolation. There are potentially many extrinsic regulatory systems working on different timescales to counteract these limitations and maintain cardiac functionality (Farrell *et al.* 2009), among which are the sympathetic and parasympathetic branches of the autonomic nervous system (ANS). The ANS responds rapidly to environmental conditions to regulate numerous parameters of the cardiorespiratory system including, f_H , ventricular contractility and vascular tone in a localized fashion (Sandblom and Axelsson 2011). This regulatory ability can improve cardiac and whole organism heat tolerance in some, but not all instances (Ekström *et al.* 2014; Ekström *et al.* 2019; Gilbert *et al.* 2019). As such, the potential effects of autonomic regulation on thermal

performance and tolerance should be considered, particularly, when interpreting findings generated using methods that eliminate this control system.

1.2 Arctic char

The Arctic provides an intriguing but concerning context for asking both basic and conservation-driven questions related to the thermal biology of fish. Indeed, the field of thermal biology has a long history of studying organisms that thrive in extreme thermal environments to better understand the extent of biological solutions to thermal challenges. Accordingly, Arctic char present themselves as a strong candidate species in which to study the basic science of TTB, thermal plasticity, and physiological and life-history trade-offs. The Arctic char is the most northerly distributed freshwater or diadromous fish in the world, and in the extremely oligotrophic systems at the northern extents of their range, they are often the only fish present (Klemetsen 2010). Despite this impressive northerly distribution, Arctic char can also thrive in more temperate environments and thus overlap and compete with more temperate salmonid relatives such as sockeye salmon (*Oncorhynchus nerka*) in Alaska, lake char (*S. namaycush*) throughout the Canadian north and brown trout (*Salmo trutta*) in northern Europe. There is even a subspecies of Arctic char, *S. alpinus oquassa*, whose range extends into the eastern United States (Oleinik *et al.* 2015; Taylor 2016). Furthermore, Arctic char are often considered to be among the most phenotypically diverse vertebrates on Earth (Sandlund *et al.* 1992; Klemetsen 2010; Swanson *et al.* 2010a; Roux *et al.* 2011; Moore *et al.* 2013; Moore *et al.* 2014; Moore *et al.* 2016) with anadromous, resident (non-migratory), and landlocked forms and numerous distinct ecotypes, often occurring in sympatry. Unsurprisingly, char (*Salvelinus* spp.) biologists have long been enamored by this diversity, a sentiment well expressed by Behnke and Tomelleri (2002) who stated “If any species of fish could be characterized as defying description, it is the

Arctic char”. In my thesis I focus principally on anadromous Arctic char because even within the anadromous form significant variation and complexity exists among migratory strategies and environments, which can make their marine-freshwater migrations decisive events in a population’s thermal and reproductive ecology.

From a conservation perspective there is a clear and dire need for research related to the thermal requirements of native Arctic fish such as the Arctic char as the abiotic effects of global climate change (GCC) are most prevalent at high latitudes (Poesch *et al.* 2016). As a result of this “Arctic amplification” the Canadian Arctic has warmed at close to three times the average global rate (Zhang *et al.* 2019a). Current and anticipated results of global climate change in the Arctic include earlier and more temporally variable snow- and ice-melt periods, changes in precipitation, and warming, more variable water temperatures (Wrona *et al.* 2006a; Wrona *et al.* 2006b; Poesch *et al.* 2016; Zhang *et al.* 2019a). These changes may have a profound effect on the thermal and hydrological regimes encountered by marine and freshwater fishes (Wrona *et al.* 2006b; Poesch *et al.* 2016). However, anadromous fish, such as Arctic char, which rely on access to specific abiotic conditions in marine, riverine and lacustrine environments at specific periods throughout their life-histories may be particularly susceptible as changes in any one of these environments could alter fitness (Reist *et al.* 2006a; Reist *et al.* 2006b; Jonsson and Jonsson 2009). Furthermore, anadromous Arctic char are integral to Inuit economies, food security, and culture making them a vital northern resource (Roux *et al.* 2011; Lysenko and Schott 2019).

1.2.1 The life history and thermal ecology of anadromous Arctic char

Our understanding of the extent of variation in anadromous Arctic char migratory life-history traits (e.g. timing, frequency, fidelity) and associated thermal experience is still

developing; however, there are a few generalizations that can be drawn (Figure 1.2). Young Arctic char rear in freshwater for many years (typically 3-8 but often much longer) before first migrating to sea in the spring to feed. Once at sea, Arctic char will usually spend only a few weeks feeding, because frigid marine overwintering temperatures and possibly osmoregulatory stress necessitates an obligatory annual return to freshwater before winter. Arctic char cannot tolerate super cooling because unlike many cold-adapted fishes, Arctic char do not possess an antifreeze protein (Plasma freezing point = -1.0°C)(Fletcher *et al.* 1988). After this initial migration they return to sea in the spring of most years, but may skip annual migrations putatively due to environmental factors, body condition and reproductive development (Gyselman and Broughton 1991; Gyselman 1994; Swanson *et al.* 2010b). Although data are limited, in terms of thermal experience, this migratory strategy apparently presents many thermal challenges (Figure 1.2). When in fresh water, anadromous Arctic char spend most of their lives (October-June annually) under ice at temperatures between 0 and 4°C (Figure 1.2 and 1.3)(Mulder *et al.* 2018) although young Arctic char may exploit warmer water ($<14^{\circ}\text{C}$) in the summer (July-August) when it is seasonally available (e.g. Figure 1.3)(Dick *et al.* 2009). After moving to sea, they spend most of their time at more moderate temperatures ($\sim 6^{\circ}\text{C}$; Table 1.1) but also frequent areas with temperatures near freezing while diving to feed or to escape predation (Rikardsen *et al.* 2007; Jensen and Rikardsen 2012; Jensen *et al.* 2014; Harris *et al.* 2020b).

Arctic char encounter the least consistent water temperatures during their riverine migrations (Figure 1.2 and 1.3). Many Arctic char bearing rivers are shallow and have limited cover and thus tend to warm faster, and have more variable temperatures than larger rivers, lakes or marine environments. As such, the obligatory return (upriver) migration of Arctic char can be

the point in their lives where they are most likely to encounter their lifetime maximum temperatures (Figure 1.2 and 1.3) (Gilbert *et al.* 2016; Gilbert and Tierney 2018). Indeed, previous research has identified one population of Arctic Char that navigates a shallow river that currently experiences not only large diurnal temperature fluctuations ($>10^{\circ}\text{C}$ per day) but also maximum water temperatures ($>21^{\circ}\text{C}$) warm enough to impair the migration of some Pacific salmon (*Oncorhynchus*) species (Eliason *et al.* 2011; Gilbert *et al.* 2016; Gilbert and Tierney 2018). Therefore, migratory Arctic char require capacity for aerobic performance and growth at temperatures near freezing for much of their lives, but they might also require capacity for critical and physiologically demanding activities (e.g. migration) at much warmer temperatures (Murdoch *et al.* 2014; Gilbert *et al.* 2016; Moore *et al.* 2016; Gilbert and Tierney 2018).

1.2.2 The thermal physiology of Arctic char

Despite many assertions that Arctic char are among the least heat-resistant salmonids, there are significant gaps in our knowledge of their thermal physiology, especially when compared to other commercially and culturally valuable salmonids (Baroudy and Elliott 1994; Elliott and Elliott 2010; Penney *et al.* 2014). The research on Arctic char thermal physiology that has been conducted has largely focused on laboratory-reared juveniles (Table 1.1). Thermal physiology, however, can vary dramatically with thermal history and throughout a fish's life. Indeed, TPCs often left-shift to cooler temperatures as body size increases and fish mature (Björnsson *et al.* 2001; Morita *et al.* 2010; Imsland *et al.* 2019). Furthermore, much of this prior research on Arctic char thermal biology has been conducted using European populations. Arctic char in the central Canadian Arctic have likely been genetically isolated from those in Europe for $>250\,000$ years (Moore *et al.* 2015) and therefore meaningful regional differences in thermal physiology may exist among populations. Even within broad geographical regions, Moore *et al.*

(2017) have found genomic evidence to suggest that, like several other salmonids (Taylor 1991; Eliason *et al.* 2011; Fraser *et al.* 2011), Arctic char may be locally adapted to migratory conditions in their natal systems, a possibility I examine in Chapter 5.

Previous investigations of Arctic char thermal physiology, which provide context for my thesis research, are summarized in Table 1.1. Heat tolerance, namely acutely lethal temperatures such as the temperature at which fish lose equilibrium during acute warming (CT_{Max} ; Table 1.1), has been assessed in many strains throughout their northern distribution. In juvenile Arctic char (<200 g), reported CT_{Max} values range from 26-28°C (Table 1.1) which is remarkably high for a cold-water specialist (Baroudy and Elliott 1994; Anttila *et al.* 2015). In contrast, in larger Canadian Arctic char (Table 1.1) CT_{Max} was only 23°C (Penney *et al.* 2014; Christen *et al.* 2018), which suggests a substantial effect of either body size (life history stage) or genetic background. Accordingly, I also assess CT_{Max} in my research to allow for direct comparisons with this past research. Nevertheless, the ecological relevance of CT_{Max} is widely debated (Terblanche *et al.* 2011) in part because fish often encounter significant, sub-lethal performance impairments at temperatures well below their CT_{Max} . For example, in prolonged thermal tolerance studies with juvenile European Arctic char, feeding ceased and delayed mortality occurred by 21.5°C (Baroudy and Elliott 1994; Thyrel *et al.* 1999), but no equivalent studies exist for larger or western Arctic char. Furthermore, Hansen *et al.* (2016) found that f_{Hmax} in adult Arctic char from Greenland reached a peak at 13.8°C and then the heartbeat became arrhythmic at 15.5°C, both of which typically occur several degrees below CT_{Max} (Fry 1947; Chen *et al.* 2015; Drost *et al.* 2016; Farrell 2016). Arctic char acute cold tolerance is assumed to be their freezing point (-1.0°C)(Fletcher *et al.* 1988) based on observations that they can remain

active and grow at near freezing temperatures (Brännäs and Wiklund 1992; Brännäs and Linnér 2000). However, cold tolerance has received decidedly less attention.

Few studies have developed TPCs for physiological traits in Arctic char, and those that have, have almost entirely been focused on juvenile growth in an aquaculture context. These studies (Table 1.1) have repeatedly shown that optimal temperatures for growth are remarkably high (14-17°C) and similar to other salmonids (Elliott and Elliott 2010). These temperatures are also well above those that Canadian Arctic char would commonly encounter for prolonged periods, if at all (Figure 1.2 and 1.3). Importantly, these studies have all fed char to excess so food availability would not limit growth as it does in the wild. Arctic char growth efficiency increases with decreasing temperature (Larsson and Berglund 2005) and so growth in natural, food-limited environments may be optimized at cooler temperatures as previously suggested (Imsland *et al.* 2019; Harris *et al.* 2020b). Juvenile growth declines rapidly with warming above their optimal growth temperature, approaching zero by ~21°C, but declines more gradually with cooling and can remain positive even at 0°C (Brännäs and Wiklund 1992; Brännäs and Linnér 2000; Larsson *et al.* 2005). As in other salmonids, Arctic char TPCs for growth appear to left-shift to cooler temperatures with increasing body size (Table 1.1; Gunnarsson *et al.* 2011; Imsland *et al.* 2019). The only estimate of the optimal temperature for cardiorespiratory performance in Arctic char was 7.5°C in wild, anadromous Greenlandic char (Larsson *et al.* 2005; Hansen *et al.* 2016). However, this estimate was based on the temperature where the rate at which f_{Hmax} increased with warming began to slow down (the first Arrhenius break point temperature; T_{AB}). T_{AB} generally corresponds well with the optimal temperature for aerobic scope in salmonids (Casselman *et al.* 2012; Chen *et al.* 2015) but this has not been examined in Arctic char.

Interestingly, thermal preference in Arctic char tends to be lower than their optimal temperatures for growth (Table 1.1), which is in contrast to many (but not all) other fish, including other salmonids, in which these temperatures typically align well (Jobling 1981; Jobling 1997). Others have previously suggested that this lower thermal preference is a result of selection for temperatures that optimize growth efficiency over maximum possible growth rate as food is often limited in the wild and foraging comes at a cost (Larsson 2005; Harris *et al.* 2020b). Alternatively, preferred temperatures may reflect prey distribution. As with thermal tolerance and performance, preferred temperatures appear to decrease as body size increases (Table 1.1), however, this has not been thoroughly examined. Preferred temperatures also appear to be higher in summer than in winter and may be lower in wild than hatchery-reared fish (Table 1.1), which suggests that genetic differences or the cooler rearing environments of wild Arctic char may result in lower preferred temperatures.

1.3 Thesis outline and objectives

This introduction presented a case for Arctic char being in need of our research efforts as Earth's most northerly distributed freshwater fish species, and an invaluable cultural, economic, and ecological Arctic resource facing rapid environmental warming. As such, the overarching goal for my thesis was to examine how temperature impacts the cardiorespiratory physiology of Arctic char, and to do so in a manner that advances our fundamental understanding of the thermal biology of fish, and aids Arctic char conservation efforts. My general hypothesis was that Arctic char have a broad thermal performance for cardiorespiratory traits that reflect their variable thermal experience (Figure 1.2 and 1.3).

To address my overarching goal and hypothesis I had three specific objectives, based principally on the fact that an animal facing an adverse environment that they cannot

behaviourally avoid has three main mechanisms that shape their ability to cope over varying timescales (Fuller *et al.* 2010). First, they can simply tolerate the change and attempt to manage the cost for as long as it persists. Second, if given sufficient time (days to weeks) they can acclimate/acclimatize to fully or partially compensate for any loss of function. Third, if the change persists over multiple generations a population can undergo local adaptation. To address these three mechanisms, my three thesis objectives were:

- 1) Provide a general characterization of the acute thermal limits to cardiorespiratory performance in anadromous Arctic char in the central Canadian Arctic relative to their migratory environment (Chapter 2)
- 2) Characterize the thermal acclimation potential of Arctic char over ecologically relevant temperatures with a focus on:
 - a) Acute cardiac heat tolerance (Chapter 3)
 - b) Cardiorespiratory performance over commonly encountered temperatures (Chapter 4)
- 3) Determine how cardiorespiratory performance and thermal tolerance differ among Arctic char populations in the central Canadian Arctic, and whether or not there is evidence of adaptation to local migratory environments (Chapter 5)

In Chapter 6, I integrate my research findings in our broader understanding of the extent of diversity in fish thermal physiology and explore the implications my research has for the conservation of Arctic char in the face of rapid environmental change.

Table 1.1 A summary of prior knowledge regarding the thermal tolerance, performance and preference of Arctic char.

Region	Wild/ Lab.	Mass (g)	Acclimation temp. (°C)	Trait	Temp. (°C)	Notes	Source
Thermal Tolerance							
Finland	Lab.	25.0 ^a , 27.5 ^b	7.7 ^a , 14.9 ^b	CT _{Max}	26.7 ^a 28.0 ^a		Anttila et al., 2015
United Kingdom	Lab.	Parr*	5 ^a , 10 ^b , 15, 20 ^c	CT _{Max} IULT	25.7 ^a - 26.5 ^c 21.5 ^a - 22.7 ^b	*Mass not reported Estimated at >100g	Baroudy and Elliott, 1994
Eastern Canada	Lab.	746.6	9.5-10.5	CT _{Max}	23.0	Saltwater acclimated	Penney et al., 2014
Eastern Canada	Lab.	286.9	10	CT _{Max}	23.0		Christen et al., 2018
Canadian Arctic	Wild/ Lab.	73-98	11.0	Exercise Recovery*	20-21	* Repeated swimming test	Gilbert & Tierney, 2018
Greenland	Wild	851	6-7*	T _{arr}	15.5	*Estimated from SST; Sea-run	Hansen et al., 2016
Thermal Performance							
Europe	Lab.	<50	3.7-22.0*	growth T _{opt}	14.4- 17.2*	*Data from Multiple studies	Larsson et al., 2005
Finland	Lab.	0.7-2.4	5.9-18.0	growth T _{opt}	15.1		Lyytikäinen et al., 1997
Iceland	Lab.	>500	4-15*	growth T _{opt}	8-12	*Data from Multiple studies	Imsland et al., 2019
Greenland	Wild	851	6-7*	f _{Hmax} T _{AB}	7.5	T _{AB} as estimate of AAS T _{opt}	Hansen et al., 2016
Thermal Preference							
Sweden	Lab. (F1)	213-530 ^a 8.2-46.3 ^b	11	Selected T	10.8 ^a 11.8 ^b	Shuttle box	Larsson, 2005
Norway	Lab. (F1)	93-430 ^a	12 Spring-Fall ^a 12 Winter ^b	Selected T	11.6 ^a 8.7 ^b	Shuttle box	Mortensen et al., 2007
Eastern Canada	Lab.	<1	8	Selected T	9.2	Horizontal gradient	Peterson et al., 1979
Canadian Arctic	Wild	1833	Estimated as mean T _b	Mean T _b	5.1- 7.5*	Landlocked; *Depth inferred	Dick et al., 2009
Canadian Arctic	Wild	~1800	Estimated as mean T _b	Mean T _b	9.4*	* Near shore recordings only	Spares et al., 2012
Norway	Wild	768	Estimated as mean T _b	Mean T _b	10.7	Sea-run	Rikardsen et al., 2007
Canadian Arctic	Wild	3848	Estimated as mean T _b	Mean T _b	6.1	Sea-run	Harris et al., 2020

CT_{max} – Critical thermal maximum

IULT – Incipient upper lethal temperature

T_{arr} – Temperature at which cardiac arrhythmia first occurred

T_{opt} – Temperature for optimal performance

f_{Hmax} T_{AB} – Arrhenius breakpoint temperature for maximum heart rate

AAS – Absolute aerobic scope

T_b – Body temperature

Figure 1.1 Conceptual diagrams of thermal performance curves (a) and thermal tolerance polygons (b) for stenothermal and eurythermal fishes. Diagrams for the polar stenotherm, temperate eurytherm and tropical stenotherm are based on data for Arctic cod, Atlantic killifish, and Pacu spp. respectively (Fangue *et al.* 2006; Chu-koo *et al.* 2011; Laurel *et al.* 2015; Drost *et al.* 2016; Di Santo *et al.* 2018).

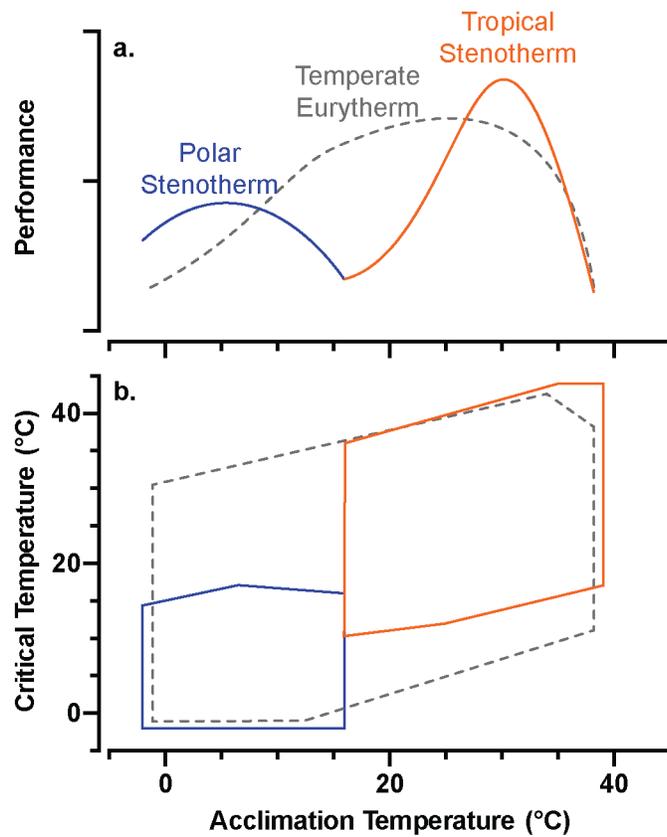


Figure 1.2 The thermal experience of anadromous Arctic char at key stages throughout their life history in the Canadian North. The solid lines show common upper and lower temperatures respectively while the dashed lines show upper and lower extremes. Line colour corresponds with temperature (cold to warm: blue to orange to red). Superscript numbers indicate the source of the temperature estimates: 1. Mulder et al., 2018; 2. Continual lake temperature data, Les N. Harris and Brendan K. Malley, Department of Fisheries and Oceans; 3. Early summer river temperature recordings, Figure 1.3; 4. Gilbert et al., 2016; 5. Harris et al., 2020; 6. Gilbert et al., 2020 (Chapter 2).

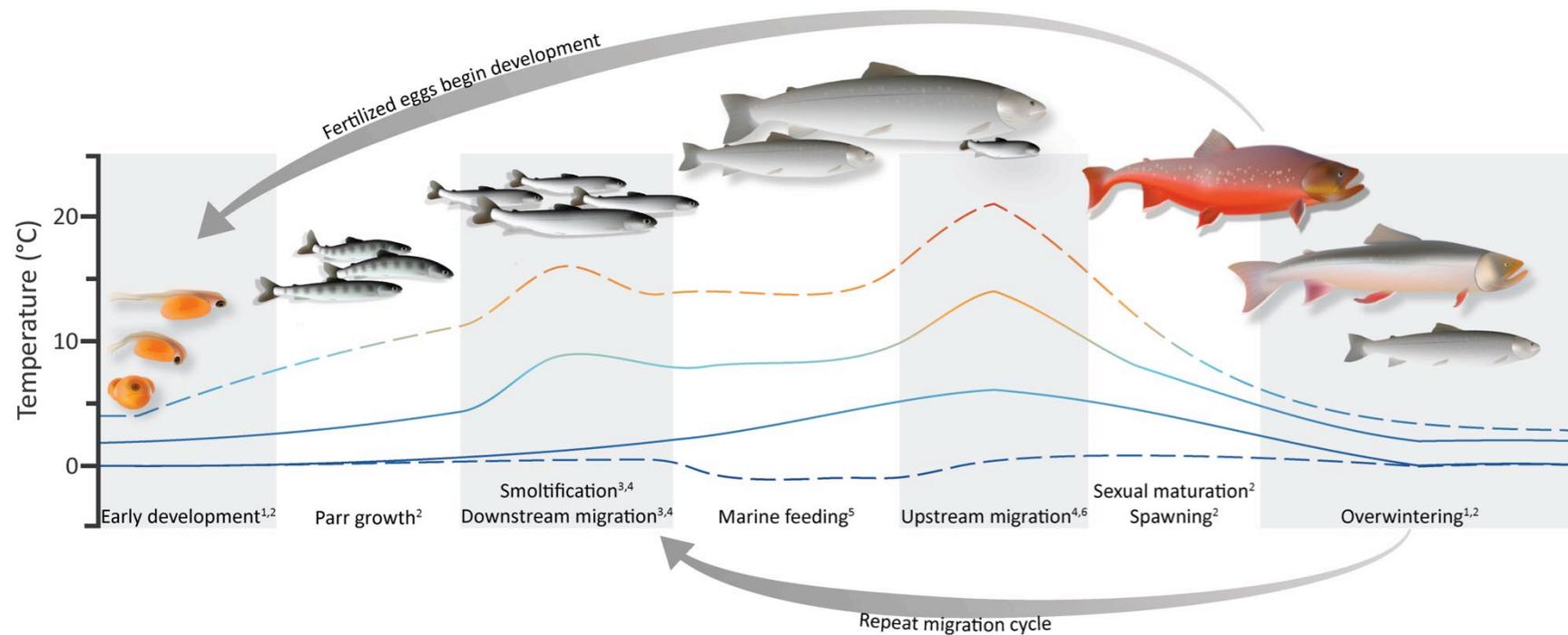
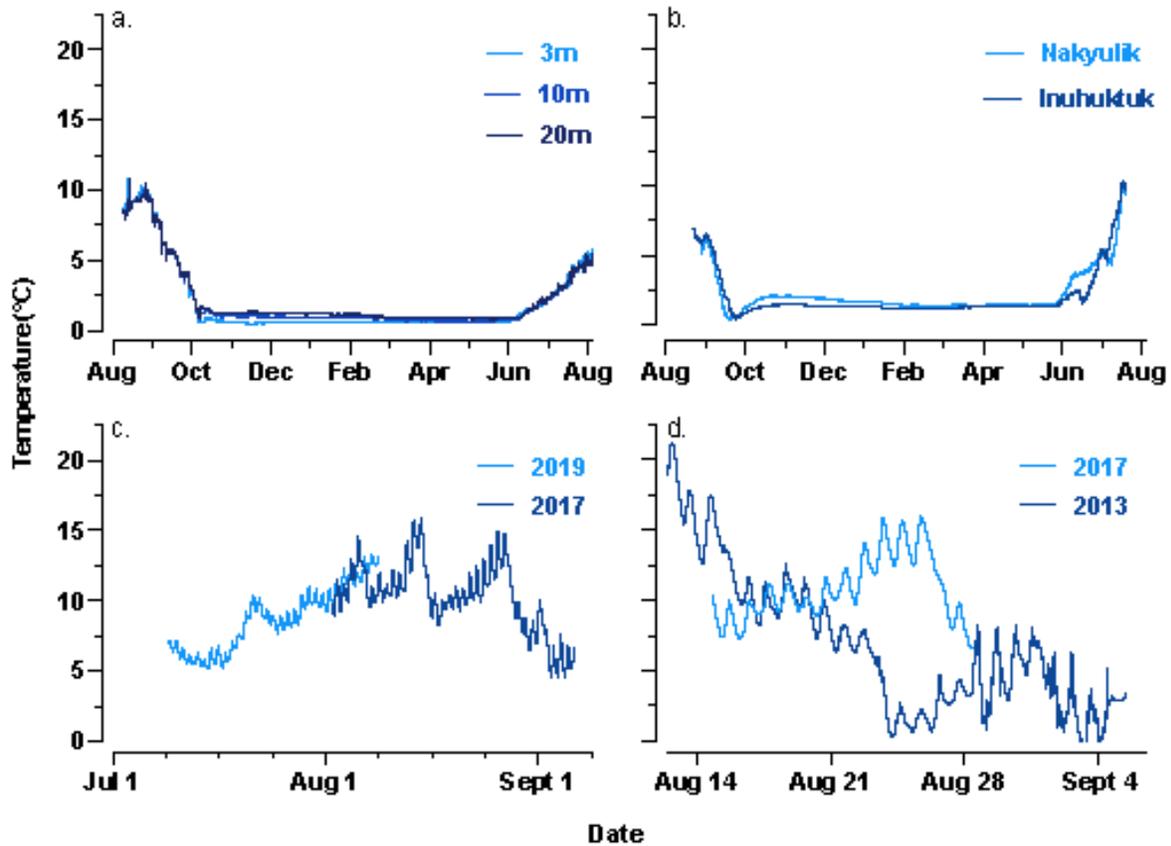


Figure 1.3 Sample lake and river temperatures encountered by anadromous Arctic char in central Canadian Arctic over different time scales. Lake temperatures (a-b) represent the thermal habitat of Arctic char for the vast majority of their lives, year-round during early rearing and September to June when anadromous. Data are shown for Ferguson Lake (a), the largest overwintering lake in Cambridge Bay area of Nunavut, at three depths and Nakyulik and Inuhuktuk (b), two smaller lakes used for early rearing and spawning in the Greiner Lake, Nunavut watershed. Summer water temperatures are shown for Freshwater Creek (c) and Halokvik (30-mile river; d) to demonstrate the thermal variability that can occur during migrations between marine feeding habitats and the lacustrine early rearing, overwintering, and spawning habitat.



Chapter 2: The thermal limits of cardiorespiratory performance in anadromous Arctic char (*Salvelinus alpinus*): A field-based investigation using a remote mobile laboratory¹

2.1 Introduction

The Canadian Arctic has warmed at close to three times the average global rate, raising concern among northerners, scientists, and policy makers alike (Galappaththi *et al.* 2019; Zhang *et al.* 2019a). Despite this concern, we know little about the environmental physiology of many Arctic species, even though such knowledge is fundamental to a mechanistic understanding of the ecological impacts of climate change (Cooke and O'Connor 2010; Cooke *et al.* 2012; Cooke *et al.* 2013; Patterson *et al.* 2016). Indeed, knowledge regarding thermal limitations to metabolism, growth, and exercise performance is increasingly being used to develop evidence-based management strategies for many fishes (Cooke *et al.* 2012; DFO 2012; Patterson *et al.* 2016). For instance, the allowable harvest of Pacific and Atlantic salmon (*Salmo salar*) is now adjusted based on river temperatures, in part, using knowledge that high temperatures by impairing salmon cardiac performance, capacity for aerobic exercise, and recoverability can reduce survival during migration (Farrell *et al.* 2008a; Eliason *et al.* 2011; DFO 2012; Patterson *et al.* 2016). Equivalent knowledge for use in management decisions remains sparse for Arctic fishes, largely due to the exorbitant cost of northern research (Mallory *et al.* 2018), technical limitations of conducting sensitive physiological research in remote field settings (Farrell *et al.* 2003; Mochnacz *et al.* 2017; Gilbert and Tierney 2018), and the absence of field-based infrastructure available to support such research.

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This limited physiological knowledge base is particularly concerning for keystone species such as the Arctic cod (Drost *et al.* 2016) and for my focal species, the Arctic char (*Salvelinus alpinus*), which has immense cultural, subsistence and economic value to northern communities (Roux *et al.* 2011; Day and Harris 2013; Roux *et al.* 2019). What little is known regarding wild Arctic char thermal physiology indicates that, like other anadromous salmonids, their thermal physiology likely varies with life stage, thermal history and population of origin. For instance, juvenile Arctic char in the central Canadian Arctic can maintain their exercise capacity over temperatures from 10-21°C, but nevertheless struggle to recover from exhaustive exercise above 20°C (Gilbert and Tierney 2018). In contrast, the maximum heart rate (f_{Hmax}) of larger sea-run Arctic char in Greenland (presumably acclimated to sea surface temperatures of ~7°C) became thermally limited at just 13°C and the heart failed altogether by 15°C (Hansen *et al.* 2016); the optimal temperature for cardiorespiratory performance was estimated as ~7°C as this was the temperature when the rate of increase in f_{Hmax} with temperature began to significantly decrease. This temperature is well below the maximums seen in Canadian Arctic rivers through which Arctic char migrate in summer (Gilbert *et al.* 2016).

As detailed in Chapter 1 (Figure 1.2 and 1.3), anadromous Arctic char certainly experience dramatic shifts in available and experienced temperatures throughout their life history and broad, circumpolar distribution (Gilbert *et al.* 2016, Harris *et al.* 2020, Mulder *et al.*, 2019). Their up-river return migrations are among the greatest physical and thermal challenges in their lives (Gilbert *et al.* 2016). They can encounter conditions ranging from high-flow rapids to water so shallow that they are only partially submerged, and temperatures that can vary dramatically from ~0 to 21°C (present study; Gilbert *et al.* 2016; Gilbert and Tierney 2018).

This variable thermal history, the rapid warming of the Arctic, and inter-study differences in thermal tolerance provide the impetus for field-based research on the thermal physiology of Arctic char. To this end, I used innovative mobile Arctic research infrastructure and simplified physiological techniques to allow for a field-based assessment of the thermal limits to cardiorespiratory performance for migrating Arctic char in the central Canadian Arctic. Based on their range of encountered migration temperatures, we predicted Arctic char in this region should have a relatively broad cardiorespiratory thermal performance and would tolerate temperatures warmer than those found in the only other comparable study of wild Arctic char (Hansen *et al.* 2016). The overall goal of the study is to provide insight into how current and future thermal regimes may impact Arctic char cardiorespiratory physiology and thus migration success in the face of rapid warming.

2.2 Methods

2.2.1 Study animals and mobile research laboratory

All sampling and animal use was approved by Fisheries and Oceans Canada Freshwater Institute (AUP 2016-033, AUP 2017-039, LFSP S-16/17 1028-NU, LFSP S-17/18 1023-NU). In the summers of 2016 and 2017, anadromous Arctic char (In 2016: $n = 20$ length = 385 ± 13 mm (mean \pm SEM), mass = 605 ± 61 g; In 2017: $n = 12$ length = 407 ± 27 mm, mass = 711 ± 209 mm) were caught by angling with barbless hooks at the mouth or in the lower reach (850 m) of Freshwater Creek, NU, Canada ($69^{\circ}07'N$, $104^{\circ}59'W$; Figure 2.1), where there is an important subsistence fishery for Inuit from the community of Cambridge Bay. Captured fish were transported in an aerated 90 L cooler by all-terrain vehicle (ATV) to a nearby (<7 km) mobile research laboratory (Arctic Research Foundation, Winnipeg, CAN; Figure 2.1) equipped with a 450 L temperature-controlled fish holding system (Aqua Logic Inc., San Diego, USA), where

they were held at commonly encountered river temperatures (~10-12°C). The mobile laboratory was constructed out of a standard 6.1×2.4×2.4 m (20×8×8 feet) shipping container, and equipped with 15×305 W solar panels, 2× 1.1 kW wind turbines, a backup 10 kW diesel generator, 24×2 V batteries and 2×6.8 kW inverter and charging systems (Figure 2.2). The laboratory was also fitted with bench space (~2 m²), equipment storage and a composting toilet.

2.2.2 Respirometry and critical thermal maxima

In 2017, respirometry was conducted at temperatures from 3.7 to 20.0°C (n=12). Arctic char were allowed to recover overnight (>12 h) following capture prior to these experiments. To begin, fish were warmed at 2°C h⁻¹ to the test temperature and held at that temperature for 1 h. They were then chased to fatigue (time to fatigue: 4.4±0.2 min), given 1.3±0.1 min air exposure, during which a ~200 µL caudal blood sample was drawn, and the fish was rapidly sealed in a 30 L respirometer (90 cm long x 20.3 cm diameter). Fatigue was defined as the fish being refractory to a tail and mid-body grab for >5 s. Fish were then allowed to recover for a minimum of 18 h in the respirometer after which they were warmed at rate of 5°C h⁻¹ until they reached their critical thermal maximum (CT_{Max}), the temperature at which they could no longer maintain dorso-ventral equilibrium. This protocol allowed us to reliably estimate maximum ($\dot{M}O_{2Max}$), and minimum ($\dot{M}O_{2Min}$) oxygen consumption rates, calculate absolute and factorial aerobic scope (AAS: $\dot{M}O_{2Max} - \dot{M}O_{2Min}$; FAS: $\dot{M}O_{2Max} / \dot{M}O_{2Min}$), record $\dot{M}O_2$ during acute warming and estimate CT_{Max}. AAS serves as a measure of the net aerobic capacity available above rest while FAS is a measure of the capacity to increase $\dot{M}O_2$ above $\dot{M}O_{2Min}$ in a multiplicative manner. Whether an aerobic function is constrained by AAS or FAS as temperature increases will depend on how its aerobic cost scales with temperature relative to the available scope (see Farrell, 2016; Hasley et al. 2018). Hematocrit (Hct), haemoglobin (Hb) content (HB 201, Hemocue,

Ängelholm, SWE), blood glucose (Accu-Chek Aviva, Roche, Basel, CHE) and blood lactate concentration (Lactate Pro, Arkray KDK, JPN) were measured in duplicate immediately following the chase and at CT_{Max} . Hb values were adjusted as previously described for fish ($Hb_{adjusted}=0.815 \times Hb_{measured}-2.198$) (Clark *et al.* 2008a). Mean corpuscular Hb concentration (MCHC) was also calculated (Hb/Hct). Once the fish was sealed in the respirometer intermittent flow respirometry was performed as previously described (Svendsen *et al.* 2016; Zhang *et al.* 2018a) and the measurement and flush period varied based on the temperature, size of the fish and time post-chase to ensure an adequate $\dot{M}O_2$ signal was obtained ($R^2 > 0.8$) and dissolved oxygen remained above 80% air saturation (Chabot *et al.* 2016). A full water change was conducted between each experiment to limit microbial load and waste accumulation. $\dot{M}O_{2Max}$ was estimated using an iterative algorithm to identify the steepest slope in dissolved oxygen over any measurement period (Zhang *et al.* 2018a). $\dot{M}O_{2Min}$ was taken as the lowest 25% of the data recorded after 12 h of recovery (Chabot *et al.* 2016; Zhang *et al.* 2018a). $\dot{M}O_2$ was recorded once every 12 min during warming from the test temperature at $\sim 1^\circ C$ increments as previously described (Gilbert *et al.* 2019).

2.2.3 Heart rate assessment

Cardiac thermal tolerance was assessed in 2016 on anaesthetized fish fitted with electrocardiogram (ECG) electrodes in a manner similar to previous studies on other salmonids (Casselmann *et al.* 2012; Anttila *et al.* 2014a; Chen *et al.* 2015) and notably with Greenlandic Arctic char (Hansen *et al.* 2016) and Arctic cod (Drost *et al.* 2016). To begin each experiment, two fish were anesthetized in 150 mg L^{-1} buffered tricaine methanesulfonate (TMS; 1:1.5 sodium bicarbonate) at $10^\circ C$ until ventilation slowed, almost stopped and was irregular. Fish were then weighed and transferred to a well aerated anesthetic bath containing 65 mg L^{-1} buffered TMS

where they were placed supine, their gills were irrigated with a pump. Stainless steel electrodes were inserted into the skin over the heart on the right side of the ventral midline and just posterior to the left pectoral fin and an ECG was acquired and processed as previously described (Gilbert *et al.* 2019). The fish were then given an intraperitoneal injection of 1.2 mg/kg atropine sulphate and 4 $\mu\text{g kg}^{-1}$ isoproterenol in a total volume of 1 ml kg^{-1} 0.8% NaCl solution to stimulate their f_{Hmax} . Once the heart rate stabilized (~ 20 min) the anesthetic bath was warmed (n=12) or cooled (n=8) from 5°C and 10°C respectively at a rate of 5°C h^{-1} in 1°C increments every 12 min using a drop-in coil heater-chiller (Isotemp II, Fisher Scientific, Hampton, USA), and a flow through aquarium chiller (Tr15, TECO, Ravenna, ITA). The experiment continued until the heartbeat became arrhythmic or the bath began to freeze at which point the fish was euthanized with an overdose of anesthetic (150 mg/L buffered TMS) followed by spinal pithing. The ECG was analyzed using automated heartbeat detection as in Gilbert *et al.* (2019). The temperature at the first Arrhenius break-point (T_{warmAB}), peak f_{Hmax} , temperature at peak f_{Hmax} (T_{peak}), and temperature at arrhythmia (T_{arr}) were identified for each individual as previously described (Casselmann *et al.* 2012; Chen *et al.* 2015; Hansen *et al.* 2016). I also identified an Arrhenius break-point temperature (T_{coolAB}) for each fish that was acutely cooled from 10°C. Briefly, Arrhenius break-point temperatures were identified using a segmented linear regression to indicate a temperature at which there is a notable transition in the thermal sensitivity of a given rate with further warming or cooling. A deviation from linearity in an Arrhenius plot more generally shows that a rate is not increasing with a constant exponent as predicted based on Arrhenius kinetics. T_{peak} and peak f_{Hmax} are the temperature and f_{H} at which f_{Hmax} ceased to increase or began decreasing with further warming and thus indicate the f_{Hmax} and temperature at

which a cardiac performance limitation occurred. T_{arr} is the temperature at which the heart began skipping beats (became irregular) thus indicating severe cardiac dysfunction.

2.2.4 Analysis

All statistical analyses were conducted in R Studio (R Core Team 2014) except for the segmented regression analysis which, along with all data presentation, was done using Prism v.8.3 (GraphPad Software, San Diego, USA). To account for allometric scaling of $\dot{M}O_2$ with mass, $\dot{M}O_{2Max}$, $\dot{M}O_{2Min}$, and $\dot{M}O_2$ during acute warming were adjusted to a common body mass. To do this, the residuals of linear regressions between $\log(\text{mass})$ and $\log(\dot{M}O_2)$ for each $\dot{M}O_2$ variable were added to the predicted value from the linear regression at the average mass 0.71 kg. The antilog of all $\dot{M}O_2$ values were then divided by the common mass to generate mass-specific values, which follows convention and thus allows for easier comparisons among studies. This adjustment was done prior to all other analyses. Based on relationships identified in previous studies (e.g. Eliason *et al.* 2013; Chen *et al.* 2015), the effect of test temperature on $\dot{M}O_{2Max}$ and $\dot{M}O_{2Min}$ were examined using linear and 2nd order polynomial models and the model with the lowest AICc value was presented. The effect of temperature (fixed effect) on $\dot{M}O_2$ and f_{Hmax} during acute warming was assessed using linear mixed effects models (LMM; (lme4; Bates 2010) with Fish ID included as a random factor to account for the fact that multiple measurements were made on each individual. The f_{Hmax} model was restricted to values recorded below an individual's T_{peak} . Test statistics for LMM were generated using Satterthwaite's degrees of freedom method in the 'lmerTest' package (Kuznetsova *et al.* 2017) and marginal and condition correlation coefficients were calculated using the 'MuMIn' package (Barton and Barton 2015). The normality of model residuals was confirmed using the Shapiro-Wilk test. Arrhenius breakpoint temperatures were determined during cooling and warming as previously

described (Casselman *et al.* 2012; Chen *et al.* 2015). All data are presented as mean±SEM unless otherwise noted and $\alpha = 0.05$.

2.3 Results

2.3.1 Respirometry and CT_{Max}

River temperatures ranged from 8.9 to 14.5°C (mean±SD: 11.0±1.4°C; recorded every 15 min) during the period when fish were captured for the respirometry experiments. Arctic char were acutely brought (2°C h⁻¹) to their test temperature (T) before being chased to fatigue, which lasted 4.4±0.2 min, independent of temperature ($f_{1,10} = 0.49$, $p = 0.50$, $R^2 = 0.05$). Values of $\dot{M}O_{2Max}$ increased linearly with increasing test temperature (Figure 2.3a; eq.1, $f_{1,8} = 8.3$, $p = 0.02$, $R^2 = 0.51$), while $\dot{M}O_{2Min}$ increased exponentially (Figure 2.3a; eq.2, $f_{2,7} = 19.9$, $p = 0.001$, $R^2 = 0.85$). Even so, AAS did not change significantly with increased temperature (Figure 2.3b; eq.3, $f_{1,8} = 0.65$, $p = 0.44$, $R^2 = 0.08$), whereas FAS decreased markedly (Figure 2.3c; eq.4, $f_{1,8} = 23.6$, $p = 0.001$, $R^2 = 0.75$).

$$\text{eq.1: } \dot{M}O_{2Max} = 9.6 \pm 3.0 \cdot T + 231.9 \pm 35.0$$

$$\text{eq.2: } \dot{M}O_{2Min} = 1.2 \pm 0.4 \cdot T^2 - 12.4 \pm 8.1 \cdot T + 80.5 \pm 40.9$$

$$\text{eq.3: } AAS = -3.0 \pm 3.9 \cdot T + 265.9 \pm 45.6$$

$$\text{eq.4: } FAS = -0.34 \pm 0.06 \cdot T + 7.71 \pm 0.65$$

It should be noted that two of the three fish exercised to exhaustion above 15°C died during recovery and had a lower $\dot{M}O_{2Max}$ than would be predicted based on the linear model for the remaining fish (Figure 2.3a). The individual that did recover after being chased at >15°C exhibited the lowest AAS and CT_{Max} . Also, it had the highest blood lactate concentration (15.1

mM) post-CT_{Max}, but whether this elevated lactate was a result of the notably elevated $\dot{M}O_{2Min}$ (Figure 2.3a and 2.7) or a more severe degree of exhaustion is not apparent.

Acute warming following the post-chase recovery period doubled $\dot{M}O_2$ (LMM: $f_{1,8,9}=145.1$, $p<0.001$, marginal $R^2=0.50$, conditional $R^2=0.83$) from 138.8 ± 37.2 mg O₂ h kg⁻¹ at 10°C to 277.7 ± 22.0 mg O₂ h kg⁻¹ at 20°C ($Q_{10}=2.0$). Peak $\dot{M}O_2$ during acute warming was 319.1 ± 33.6 mg O₂ h kg⁻¹ (at 19.8 ± 1.1 °C), a value similar to that measured immediately after exhaustive exercise at warm temperature (Figure 2.3a and 2.4). Likewise, a similar $\dot{M}O_2$ (291.1 ± 42.7 mg O₂ h kg⁻¹) also was seen immediately prior to reaching CT_{Max} (23.0 ± 0.6 °C; range 19.0 to 25.2; Figure 2.4).

2.3.2 f_{Hmax} and cardiac thermal tolerance

River temperatures ranged from 9.7 to 12.4°C (mean: 10.6 ± 0.24) at the site of capture of fish used for the cardiac thermal tolerance experiments. The f_{Hmax} in Arctic char acutely transferred to 5°C was 47.7 ± 1.3 beats min⁻¹. Acute warming of these fish increased their f_{Hmax} (LMM: $f_{1,9,6}=807.5$, $p<0.001$, marginal $R^2=0.92$, conditional $R^2=0.98$), attaining a peak f_{Hmax} of 115.4 ± 4.7 beats min⁻¹ at 19.4 ± 0.5 °C (T_{peak} ; Figure 2.5 and 2.6). Above T_{peak} , f_{Hmax} declined to 99.0 ± 5.9 beats min⁻¹ and became arrhythmic at 21.4 ± 0.5 °C (T_{arr} ; Figure 2.5a,b and 2.6) before the experiment was terminated. The temperature of the first Arrhenius breakpoint in f_{Hmax} during warming (T_{warmAB}) was 12.5 ± 0.3 °C (Figure 2.5a and b), which was similar to common ambient river temperatures (Figure 2.6). The instantaneous Q_{10} for f_{Hmax} decreased progressively with warming (Figure 2.5c), falling below 2.0 at ~11°C; the Q_{10} of 1.0 at ~19°C indicated that, on average, f_{Hmax} had peaked or plateaued (Figure 2.5c). With acute cooling from 10 to 0°C, f_{Hmax} progressively decreased from 76.3 ± 1.3 beats min⁻¹ to 16.1 ± 1.2 beats min⁻¹ (LMM: $f_{1,7,0}=1273.9$, $p<0.001$, marginal $R^2=0.93$ conditional $R^2=0.97$) and exhibited a clear breakpoint at $\sim 3.3\pm 0.5$

°C (Figure 2.5d), below which the thermal dependence of f_{Hmax} increased sharply, as indicated by an instantaneous $Q_{10} > 10$ (Figure 2.5c).

2.3.3 Blood properties

The MCHC decreased (Figure 2.7a; $f_{1,7} = 33.3$, $p < 0.001$, $R^2 = 0.83$) and blood lactate concentration increased (Figure 2.7b; $f_{1,8} = 6.6$, $p = 0.03$, $R^2 = 0.45$) with test temperature when measured immediately after Arctic char were chased to exhaustion. Blood lactate measured immediately after CT_{Max} was negatively correlated with CT_{Max} (Figure 2.7c; $f_{1,8} = 21.2$, $p = 0.001$, $R^2 = 0.76$). Additional blood parameters were correlated with each other, but not with the test temperature or CT_{Max} (Figure 2.8).

2.4 Discussion

Anadromous Arctic char can encounter a broad range of temperatures from ~0 to 21°C during their physically demanding, upriver migrations in the Canadian Arctic (Figure 2.6; Gilbert *et al.* 2016) and even lower temperatures are experienced when at sea (Harris *et al.*, 2020). Here I showed that wild migrating Arctic char can impressively maintain absolute aerobic scope and a high regular heart rate over a large proportion (~4-16°C) of this thermal range. However, factorial aerobic scope (FAS), their ability to recover from exhaustive exercise and their f_{Hmax} became critically limited >16°C, a temperature well within current extremes (Figure 2.6). Moreover, and unexpectedly, f_{Hmax} was greatly depressed with acute cooling to 0°C. As we discuss below, these traits may be intimately linked to the need for Arctic char to repeatedly perform prolonged swimming bouts over a wide range of temperatures in order to complete their migrations and successfully reproduce. My findings were made possible by the use of innovative mobile research infrastructure (Figure 2.1). Thus, the present study demonstrates the critical

importance of such infrastructure and logistical support in any effort to understand pressing conservation and management issues in remote Arctic locations.

2.4.1 Cold performance

Early research on cold-adapted fish species suggested that they may exhibit elevated metabolic rates at cold temperatures to allow elevated growth and activity when compared to related species that were not cold-adapted (Scholander *et al.* 1953; Wohlschlag 1960). This idea, which was termed ‘metabolic cold adaptation’, has largely been disproven and currently has limited support (Holeton 1973; Holeton 1974; Steffensen *et al.* 1994; Steffensen 2002; White *et al.* 2012). Indeed, when Holeton (1973) tested this hypothesis on wild juvenile Arctic char in the Canadian Arctic, he found that their resting $\dot{M}O_2$ was no higher than would be predicted for more temperate salmonids despite the Arctic char being adapted and acclimatized to a cold environment. However, Holeton’s measurements of resting $\dot{M}O_2$ measurements at ambient temperatures (2°C) were technically limited by available equipment, facilities and technology. Although our $\dot{M}O_2$ data do not extend to 0°C, our $\dot{M}O_2$ data for cool temperatures similarly indicate that Arctic char do not exhibit elevated metabolism when compared with other salmonids (e.g. Hvas *et al.* 2017). Instead, f_{Hmax} clearly and sharply declined (high Q_{10}) at <3.3°C, suggesting the potential for Arctic char to undergo metabolic rate suppression rather elevation metabolism at frigid temperatures. Further, I propose this transition in f_{Hmax} may be part of a suite of behavioural and physiological overwintering strategies that conserve energy during a period of limited food availability. Indeed, other species display an active depression of metabolic rate (Guppy and Withers 1999), fasting, and suppression of activity (Speers-Roesch *et al.* 2018). However, as we did not directly characterize acute or prolonged responses of $\dot{M}O_2$ to a

temperature colder than 3°C and Arctic char can continue to feed and grow at 0°C, this possibility remains to be tested.

While the observed limitation in f_{Hmax} at cold temperatures could be part of an adaptive energy conservation strategy, it would likely limit maximum cardiac output and thus aerobic exercise capacity. This cold limitation could, in turn, impact performance during demanding activities such as river migrations or diving down to frigid waters to forage. Indeed, telemetry data suggest that dives to colder, deeper ocean waters are typically very brief (Harris et al., 2019, Spares *et al.* 2012). If this acute cold exposure is an issue, cold acclimation could help mitigate the effects I observed. Fish in the present study were presumably acclimated to near the ambient river temperatures at which they were caught (~11°C), while Arctic char at sea (e.g. ~5-8°C, Harris et al., 2020) or overwintering (e.g. 0.5-2°C; Mulder *et al.* 2018) would be acclimatized to colder water. Indeed, the only other comparable study of f_{Hmax} , which focused on ocean-caught Arctic char, did not find such a pronounced change in f_{Hmax} at cold temperatures (Hansen *et al.* 2016). Furthermore, cold acclimation has previously been shown to increase intrinsic and maximum f_H in many fishes, which helps counteract rate limitations inherently associated with cold exposure (Aho and Vornanen 2001; Drost *et al.* 2016; Eliason and Anttila 2017).

2.4.2 Warm Performance

While debate continues over the direct ecological relevance of CT_{Max} and similar acute lethal thermal tolerance measures such metrics remain useful, relative, comparative indicators of short-term, whole-organism heat tolerance and are unarguably the ceiling for thermal performance (Sandblom *et al.* 2016). Previous measures of CT_{Max} in hatchery-reared (~0.02-0.05 kg) European Arctic char parr (e.g. ~26-28°C; Baroudy and Elliott 1994; Anttila *et al.* 2015) are significantly higher than CT_{Max} for adults in the present study (23°C). In contrast, CT_{max} for adult

Arctic char (0.7 kg) reared in a marine aquaculture in eastern Canada and acclimated to $\sim 10^{\circ}\text{C}$ (23°C ; Penney *et al.* 2014) was identical to the present result. By comparison, adult Arctic cod, a classic polar stenotherm and important food source for Arctic char, have a much lower CT_{Max} ($\sim 15\text{-}17^{\circ}\text{C}$, Drost *et al.* 2016), whereas the CT_{Max} of adult rainbow trout (*O. mykiss*; 0.3-0.7 kg) and Atlantic salmon (0.7 kg), both temperate relatives of Arctic char, is notably higher ($\sim 26\text{-}27^{\circ}\text{C}$; Ekström *et al.* 2014; Penney *et al.* 2014; Gilbert *et al.* 2019). In the present study, elevated blood lactate was associated with a lower CT_{Max} , indicating that anaerobic stress prior to or during acute warming may subsequently decrease heat tolerance, which could be particularly important for exercising (migrating) char.

Sub-lethal thermal limitations to physiological performances, such as a collapse in aerobic scope and heart rate, or cardiac arrhythmia, are arguably of greater ecological relevance than acutely lethal limitations because they occur at lower, more commonly encountered temperatures (Figure 2.6). Penney *et al.* (2014) monitored f_{H} and $\dot{M}\text{O}_2$ in Arctic char during acute warming and found an identical peak f_{H} to that found here ($115 \text{ beats min}^{-1}$). This similarity suggests that acutely warmed adult Arctic char reach their physiological maximum f_{H} prior to CT_{Max} , leaving little to no scope for f_{H} available to support further warming. At 10°C the same study (Penney *et al.* 2014) found that resting f_{H} was $\sim 44 \text{ beats min}^{-1}$ whereas f_{Hmax} at 10°C was $\sim 74 \text{ beats min}^{-1}$ in the present study, highlighting a significant scope to increase f_{H} at this temperature. Despite these similarities, Penney *et al.* (2014) found that the peak $\dot{M}\text{O}_2$ achieved during warming was only $223 \text{ mg O}_2 \text{ h kg}^{-1}$ compared to $\sim 319 \text{ mg O}_2 \text{ h kg}^{-1}$ in the present study, which may indicate a loss of aerobic performance following captive rearing or domestication, as found in Atlantic salmon (Zhang *et al.* 2016).

Hansen et al, (2016) examined cardiac heat tolerance in sea-run char from Greenland and found that T_{warmab} , T_{peak} , T_{arr} , and peak f_{Hmax} were only 7.5°C, 12.8°C, 15.2°C, and 61.8 beats min^{-1} respectively. All of these cardiac thermal performance indicators are all markedly lower (T_{warmab} : -5.0°C T_{peak} : -6.6°C T_{arr} : -6.2°C and peak f_{Hmax} : -53.6 beats min^{-1}) than in the present study, despite similar methodology and fish size. There were, however, key differences between the two studies, including the life history period (marine vs. upstream fall migrating), fish provenance (Greenland vs. central Canadian Arctic) and possibly acclimation temperatures (~7 vs ~11°C). The most parsimonious explanation for these differences is the potential for either thermal acclimation or thermal adaptation among Arctic char populations (as discussed below).

Gilbert and Tierney (2018) found that acutely warmed wild smolts and lab-reared juvenile Arctic char (~0.1 kg) maintained swimming performance up to 21°C, but their recovery from exercise fatigue was impaired above >20°C. This impaired recoverability was associated with highly elevated blood lactate concentrations, which are indicative of increased anaerobic demand and an associated metabolic acidosis. I also observed increased blood lactate levels with temperature and impaired recoverability following exhaustive exercise, albeit at an even lower temperature (>15-16°C) for much larger Arctic char. I measured blood lactate immediately following chasing although lactate release from tissues can continue over a longer time course (Milligan 1996). As such, my results could be a product of increased rate of lactate release into the blood rather than elevated anaerobic metabolism. However, I also observed a decrease in MCHC with increased temperature, which is indicative of red blood cell swelling in response to metabolic acidosis (Nikinmaa *et al.* 1987). Gilbert and Tierney (2018) also found that a handling challenge above 12°C in large migrating adult Arctic char (~4 kg) resulted in an increase in reflex impairment, which was associated with early mortality. Consistent with results for adults

in the present study, these post-handling impairments became particularly severe (>50%) above 15°C and lower than observed for smolts (>20°C). Together these data suggest that access to cold-water refugia in lakes or pools may be critical for recovery if Arctic char encounter and can swiftly pass through warm temperatures during their up-river migration.

Arctic char are clearly more heat tolerant than classically stenothermal polar fishes, likely as a result of the extreme thermal variability they can encounter over the course of their lives (Janzen 1967; Verde *et al.* 2008). Nonetheless, they are among the least heat-tolerant salmonids (Elliott and Elliott 2010; Penney *et al.* 2014; Gilbert and Tierney 2018). As a result, they are already encountering temperatures warm enough to impair vital physiological functions and impact survival. Given that fish only recruit maximum physiological performances (e.g. MO_{2Max} and f_{Hmax}) during demanding activities (e.g. navigating rapids) and operate at a sub-maximal performance level most of the time, further research is needed to examine temperature effects on sub-maximal performances such as routine swimming, growth, feeding and digestion.

2.4.3 Future directions: Sources of variation in Arctic char thermal physiology

The inter-study differences in thermal physiology highlighted above suggest that thermal history, ontogeny, and genetic background likely all contribute to thermal performance in Arctic char in a similar manner to other salmonids. For example, warm-acclimated Atlantic salmon can markedly increase cardiac thermal tolerance (Anttila *et al.* 2014a; Ferreira *et al.* 2014; Safi *et al.* 2019). Ontogeny also has a pronounced effect on thermal physiology (e.g. parr-smolt stages, Elliott and Elliott 2010). Such effects have not been well characterized in Arctic char but likely contribute to incongruences between the present and past studies (see: Harris *et al.* 2019, Larsson 2005; Larsson and Berglund 2005; Mortensen *et al.* 2007; Mulder *et al.* 2019a; Mulder *et al.* 2019b).

In temperate salmonids (e.g. *Oncorhynchus* spp.), thermal tolerance tends to be higher in strains from warm habitats (Rodnick *et al.* 2004; Chen *et al.* 2015; Verhille *et al.* 2016; Poletto *et al.* 2017). Based on the current literature European Arctic char may appear more thermally tolerant than Arctic char from Greenland and the Canadian Arctic. European Arctic char are from a different glacial lineage than Greenlandic and northern Canadian Arctic char, and have therefore evolved independently from each other for >250K years (Brunner *et al.* 2001; Moore *et al.* 2015). This isolation may provide a genetic basis for regional differences in thermal physiology. Even within a watershed, the cardiorespiratory thermal performance curves of sockeye salmon appear locally adapted to the conditions they encounter during their upriver migration (Eliason *et al.* 2011). While a recent study has shown that Kitikmeot Arctic char exhibit genetic differences among populations that are consistent with the hypothesis that they may be locally adapted (Moore *et al.* 2017), no study to date has examined this at the phenotypic level.

2.4.4 Conclusions: Thermal barriers to migration

Extreme heat events have repeatedly been shown to impose thermal barriers on the migration of temperate salmonids (Goniaea *et al.* 2006; Keefer *et al.* 2008; Baisez *et al.* 2011; Martins *et al.* 2011; Hinch *et al.* 2012; Martins *et al.* 2012) by critically impairing physiological performances (Cooke *et al.* 2006; Farrell *et al.* 2008a; Farrell 2009; Eliason *et al.* 2011). Here we clearly show that, like these temperate salmonids, migrating Arctic char experience limitations in cardiac performance, factorial aerobic scope, and recoverability at water temperatures that already occur during their migrations in some Arctic rivers (Figure 2.6; Gilbert *et al.* 2016). Such limitations can critically impair migration and they are only going to become more common as the Canadian Arctic is among the most rapidly warming regions on our planet (Zhang *et al.*

2019a). The consequences of migration failure and associated reductions in fitness may be particularly dire in regions like the Kitikmeot, NU where Arctic char are heavily harvested and relied upon as both a subsistence and economic resource (Day and Harris 2013; Roux *et al.* 2019). As such, understanding and mitigating the impacts of extreme temperature events should be an urgent priority for fisheries managers, researchers and conservationists alike.

Figure 2.1 Study area around the hamlet of Cambridge Bay in the Kitikmeot region of Nunavut, Canada. Anadromous Arctic char were captured at the mouth or in the lower 850m of Freshwater Creek, which drains the Greiner watershed into the Arctic Ocean.

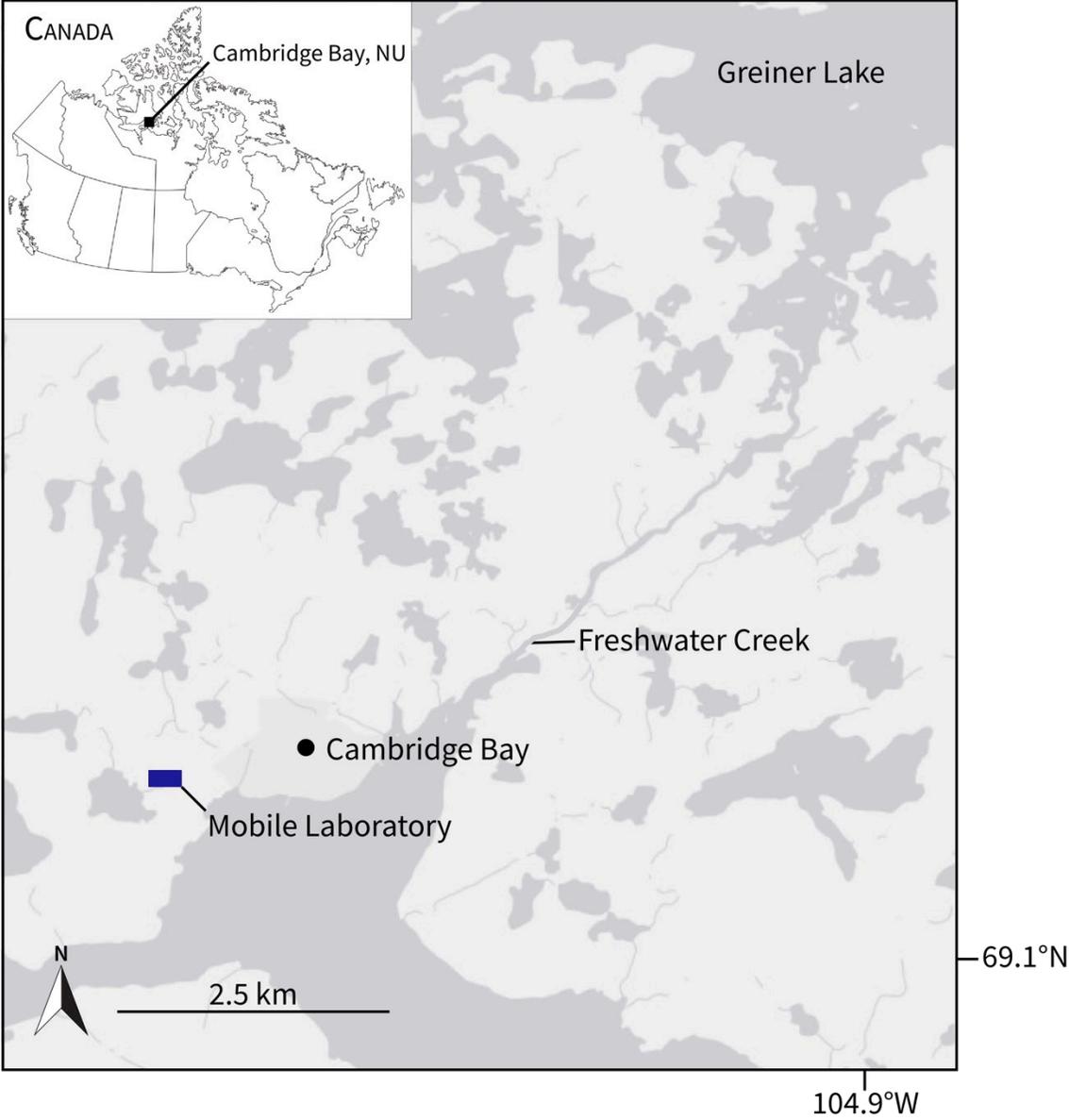


Figure 2.2 The Arctic Research Foundation mobile laboratories staged near Cambridge Bay, Nunavut, Canada. Photos depict (a) the solar arrays and turbines on three laboratories, (b) the turbines, entrance and generator for the marine and aquatic laboratory used in the present study, and (c) cardiac thermal tolerance experiments being conducted inside the laboratory. The shipping containers used to construct the laboratories are 6.1×2.4×2.4m (20×8×8’).



Figure 2.3 The effect of environmentally relevant temperatures on (a) minimum ($\dot{M}O_{2Min}$; open circles; n=10) and maximum oxygen uptake ($\dot{M}O_{2Max}$; closed circles and x; n=12) and aerobic scope of migratory Arctic char following a chase to exhaustion and air exposure. (b) absolute aerobic scope (AAS) and (c) factorial aerobic scope (FAS). Two individuals at warm temperatures died after being exhausted (orange 'x') and were excluded from all regression analyses. A linear and 2nd order polynomial model were compared for each variable. The model with the lowest AICc is presented (blue lines) with its 95% confidence interval (shaded area), R² and p-value.

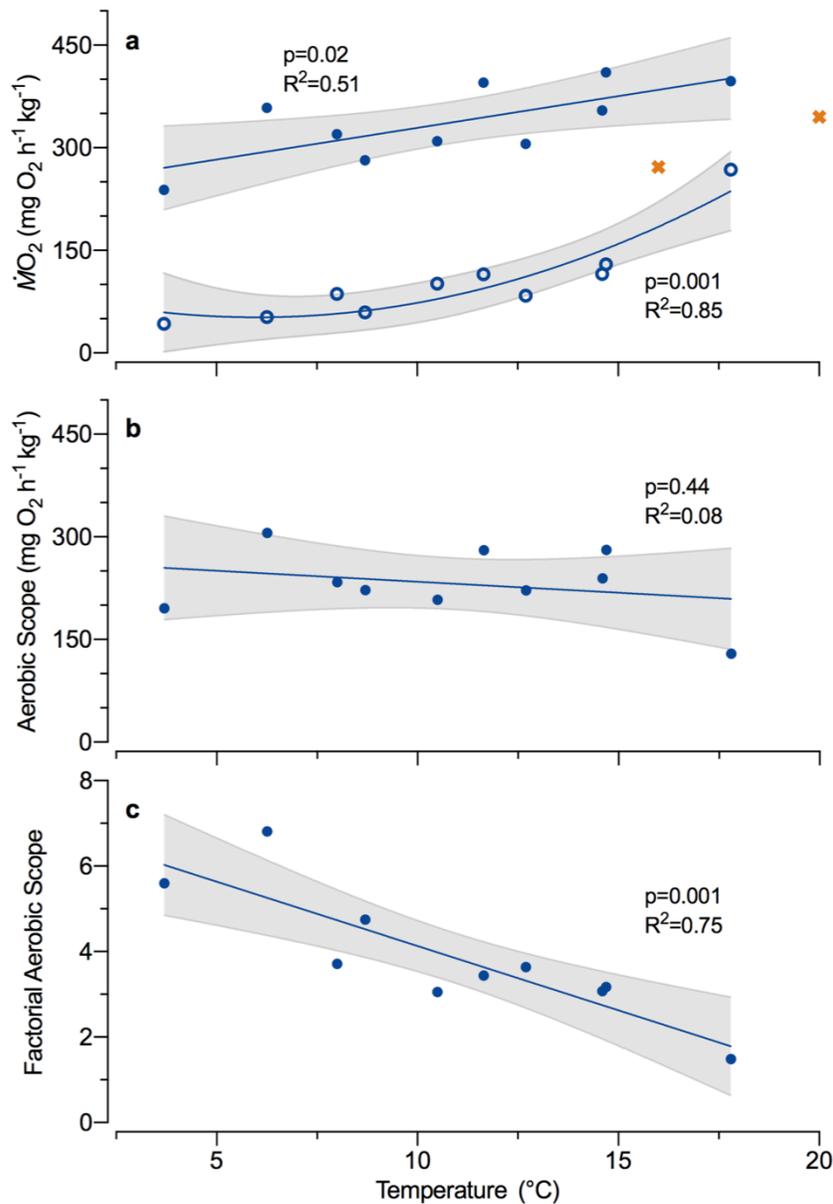


Figure 2.4 Oxygen uptake ($\dot{M}O_2$) of anadromous Arctic char during acute warming to loss of equilibrium (CT_{Max}). Individual $\dot{M}O_2$ values at their measured temperatures (grey circles; n= 8 individuals and 111 data points) and mean \pm SE values in 1°C bins (blue circles and grey shading) are presented. Mean values were calculated at all points where data were available for three or more individuals. The dashed line indicates temperatures at which individuals were removed after reaching CT_{Max} . For reference, the models for maximum $\dot{M}O_2$ and $\dot{M}O_{2Min}$ (Figure 2.3) are presented with their upper or lower 95% confidence intervals, respectively (light blue lines with shaded areas). The cumulative frequency plot (orange line, right axis) indicates the percentage of individuals that reached CT_{Max} .

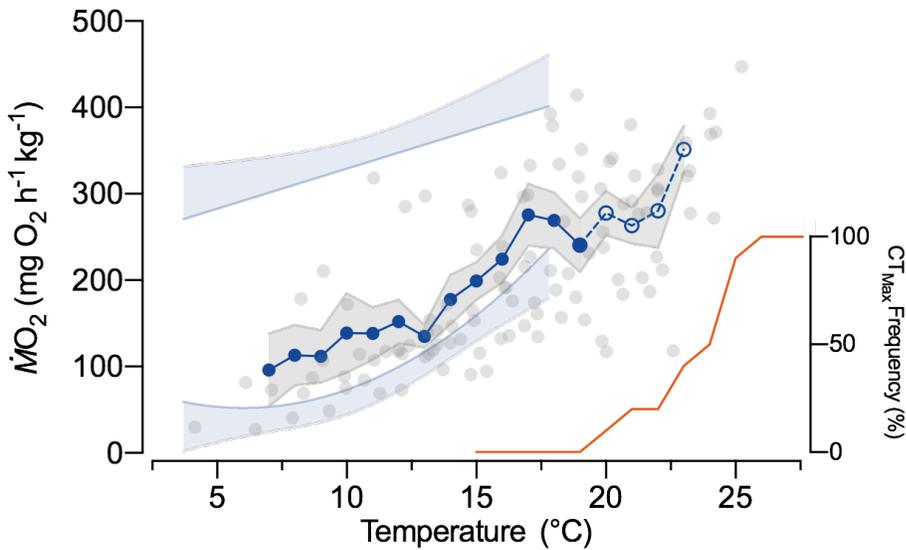


Figure 2.5 Maximum heart rate ($f_{H_{max}}$) of anadromous Arctic char during acute warming from 5°C (orange; n=12) and cooling from 10°C (blue; n=8) as (a) individual and (b) average responses. The cumulative proportion of individuals that reached their temperature at peak $f_{H_{max}}$ (T_{peak}) and arrhythmia (T_{arr}) are inset (b). The thermal sensitivity of $f_{H_{max}}$ is shown using (c) the temperature coefficient (Q_{10}) calculated over 2°C increments, with reference lines indicating rates of change that would correspond to a doubling (2.0), plateau (1.0) or halving (0.5) of $f_{H_{max}}$ over 10°C and (d) an Arrhenius plot of $f_{H_{max}}$ showing the first Arrhenius breakpoints during warming (T_{warmAB}) and cooling (T_{coldAB}). Averaged data (b-d) are presented \pm SE and dashed connecting lines with open circles indicate temperatures where some individuals were removed from the analysis following arrhythmia.

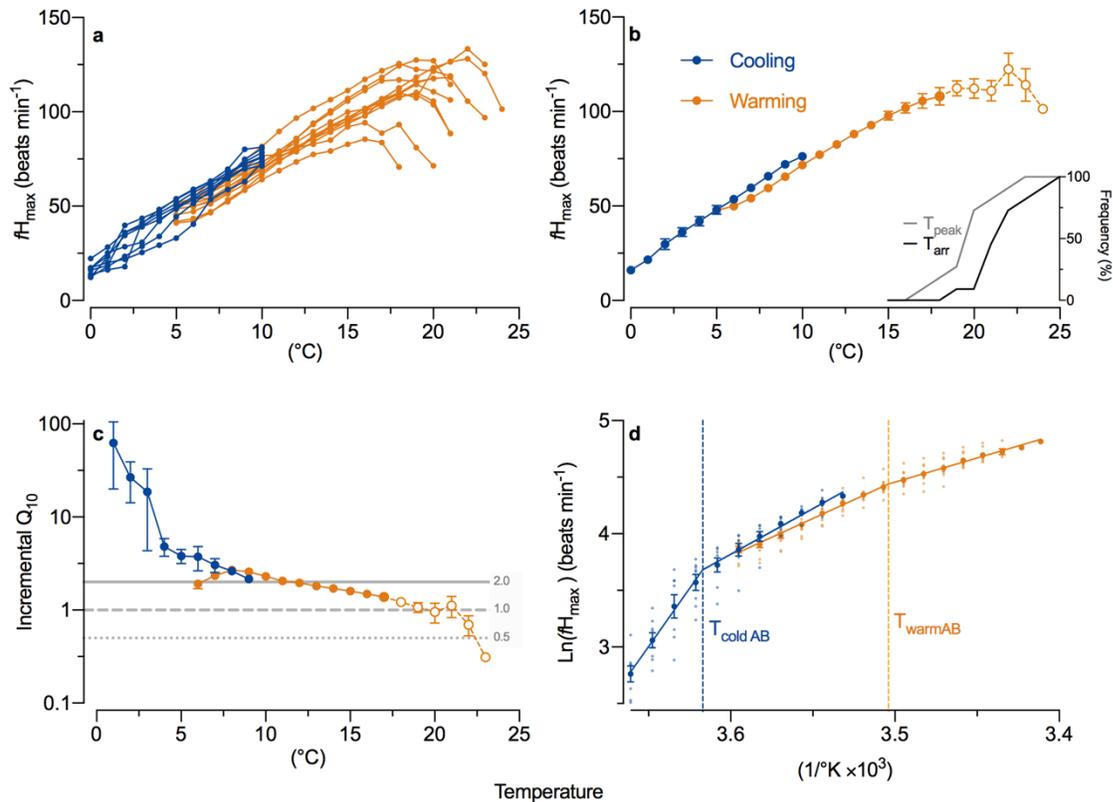


Figure 2.6 Transition temperatures for maximum heart rate (f_{Hmax}) and the critical thermal maximum (CT_{Max}) for anadromous Arctic char relative to frequency distributions for water temperatures during their upriver migration. Arrhenius breakpoint temperatures (cooling: T_{coldAB} warming: T_{warmAB}) and temperatures at peak f_{Hmax} (T_{peak}), onset of arrhythmia (T_{arr}) and loss of equilibrium (CT_{Max}) are shown relative to the average (blue) and warmest (orange; Gilbert et al. 2016) river temperature frequencies recorded during upriver migrations in the Kitikmeot region of Nunavut, Canada. River temperatures were compiled for four rivers from past (Gilbert et al. 2016) and ongoing fisheries research (M.J.H. Gilbert and L.N. Harris unpublished data). Boxes represent the median and interquartile range and whiskers indicate the 5th and 95th percentile of data.

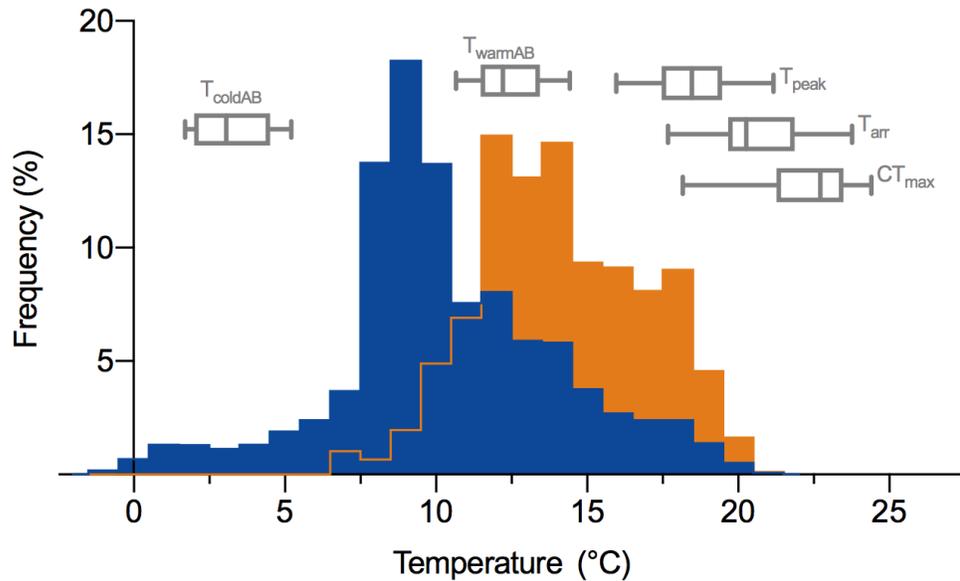


Figure 2.7 Correlations between blood properties and either the test temperature or the critical thermal maximum (CT_{Max}). Blood was drawn immediately following the chase to exhaustion (a and b) or immediately after fish lost equilibrium at their CT_{Max} (c). Significant relationships were identified through a pairwise Spearman's correlation analysis ($\alpha=0.05$; Figure 2.8). Relationships for mean corpuscular haemoglobin concentration (MCHC; a) and blood lactate (b and c) are shown with their corresponding linear model (solid line) and with 95% confidence intervals (shaded).

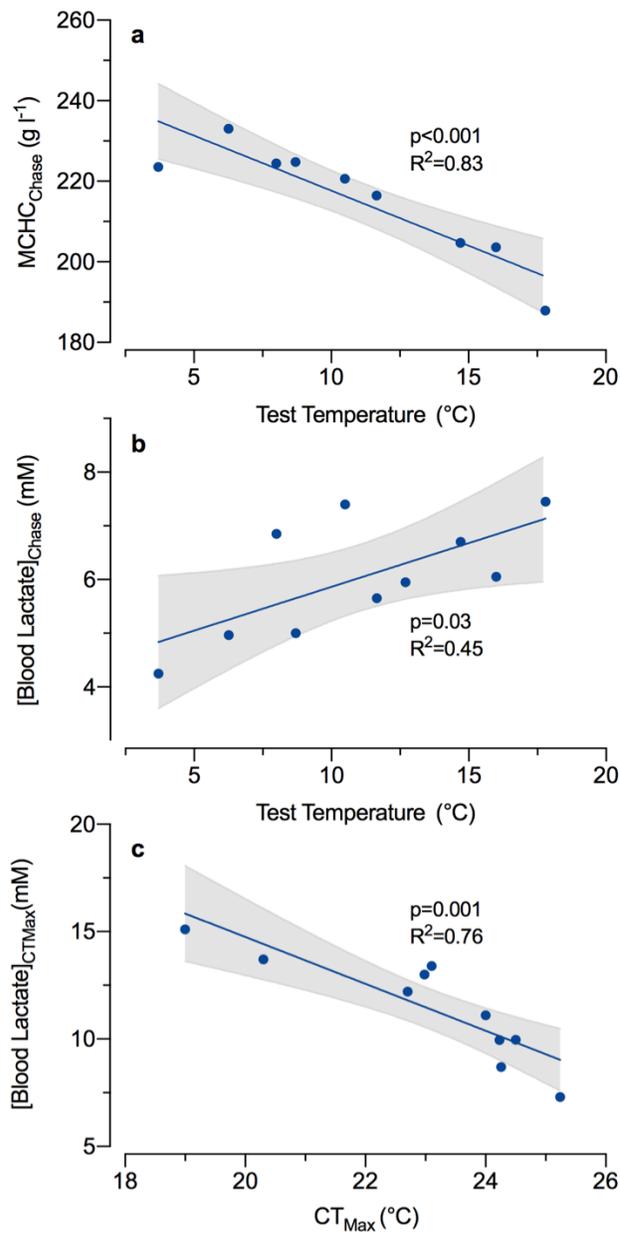
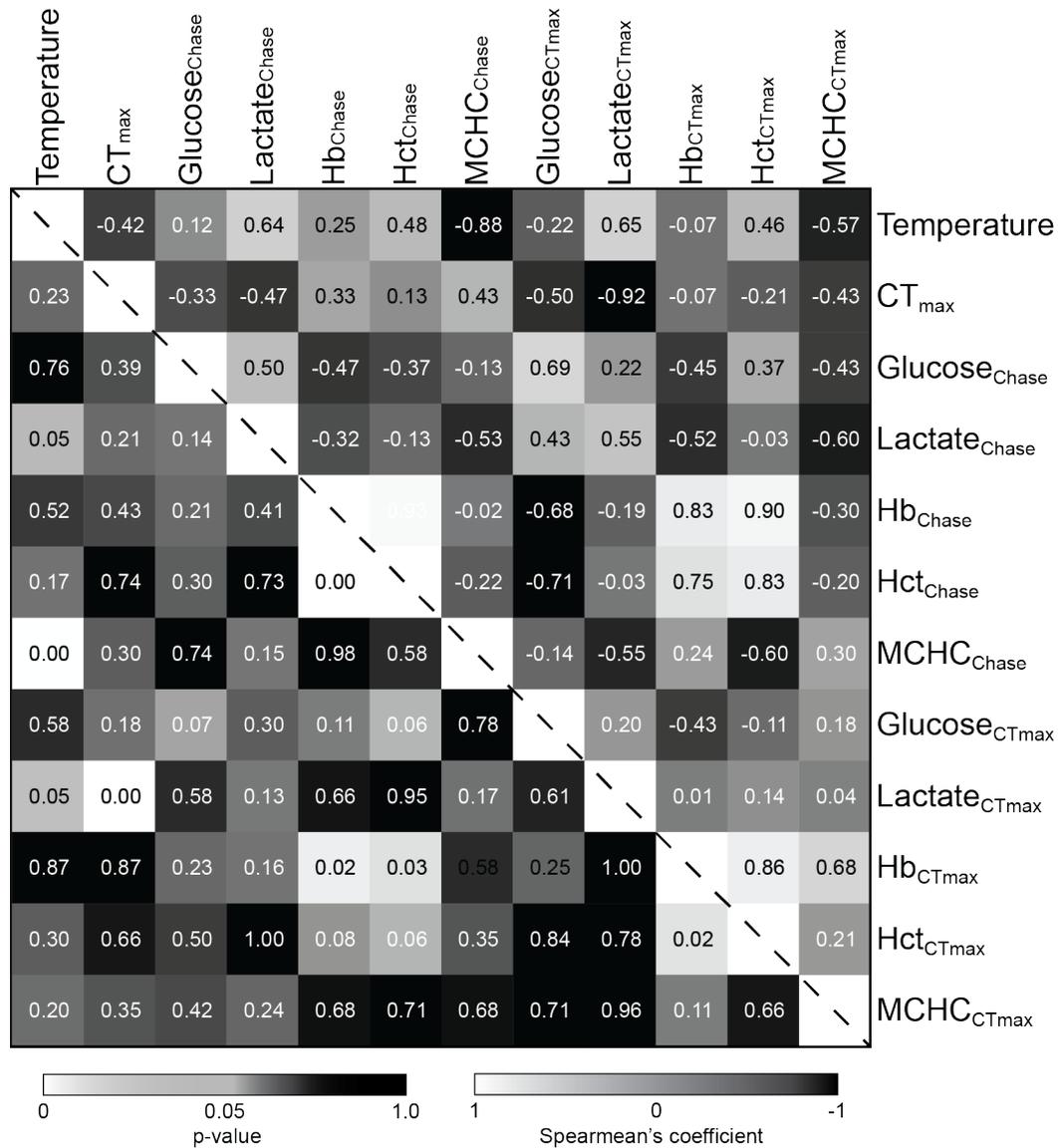


Figure 2.8 Correlation matrix for test temperature, critical thermal maximum (CT_{Max}) and all measured blood parameters. Blood parameters include Glucose, Lactate, haemoglobin (Hb), hematocrit (Hct) and mean corpuscular haemoglobin content (MCHC) immediately following chasing or CT_{Max} . Blood was sampled immediately following the chase to exhaustion (chase) or immediately after fish lost equilibrium at their CT_{Max} (CT_{Max}). Spearman's correlation coefficients are presented above the dashed line and p-values are below.



Chapter 3: The thermal acclimation potential of maximum heart rate and cardiac heat tolerance in the Arctic char (*Salvelinus alpinus*), a northern cold-water specialist²

3.1 Introduction

Temperature is a measure of the kinetic energy within a system, and consequently governs physicochemical and physiological rates. This influence is of particular importance for ectotherms such as fish whose body temperatures tend to match their external environment (Fry and Hart 1948; Farrell *et al.* 2009). As discussed in Chapter 1, one life-supporting physiological rate is heart rate (f_H), which almost universally increases with acute warming in all fishes tested to date (Eliason and Anttila 2017). This increase in f_H drives the increase in cardiac output, to meet concomitant elevations in aerobic metabolic demand. However, f_H has an upper physiological limit and, with acute warming, it reaches a peak or plateau before declining and losing rhythmicity (Hughes and Roberts 1970; Heath and Hughes 1973; Casselman *et al.* 2012; Vornanen 2016). Accordingly, transition temperatures for maximum f_H (f_{Hmax}) during acute warming have been established for a wide variety of fish species and used as indicators of cardiac heat tolerance beyond which circulatory constraints may begin to limit whole animal aerobic performance (Farrell *et al.* 2009; Casselman *et al.* 2012; Chen *et al.* 2015; Drost *et al.* 2016; Farrell 2016; Eliason and Anttila 2017; Skeeles *et al.* 2020).

Over time at a new temperature an ectotherm can alter its physiology to better suit prevailing temperatures (i.e., thermal acclimation), a response that can shape its capacity to cope with seasonal, life-history dependent and stochastic changes in thermal regimes. Given the

2. A version of this chapter has been submitted for publication as: Gilbert MJH, Farrell AP. The thermal acclimation potential of maximum heart rate and cardiac heat tolerance in Arctic char (*Salvelinus alpinus*), a northern cold-water specialist.

importance of f_H in supporting internal oxygen convection, it is not surprising that both the thermal dependence of f_H and cardiac thermal tolerance are plastic traits (Anttila *et al.* 2014a; Badr *et al.* 2016; Eliason and Anttila 2017; Safi *et al.* 2019). Specifically, with warm acclimation fish can reset intrinsic and maximum f_H to a lower rate, increase peak f_{Hmax} , increase the temperature at which peak f_{Hmax} is reached (T_{peak}), and increase the temperature at which arrhythmia first occurs (T_{arr}) (Aho and Vornanen 2001; Anttila *et al.* 2014a; Badr *et al.* 2016; Drost *et al.* 2016; Eliason and Anttila 2017). However, the presence, exact type and extent of these phenotypic changes vary considerably among species (Farrell, 2016).

In light of this species variability, the Arctic char is an intriguing fish in which to study cardiac thermal plasticity as they are the most northerly distributed freshwater fish on the planet, and are among the most cold-adapted salmonids (Reist *et al.* 2006b; Elliott and Elliott 2010). Yet, their life history naturally exposes them to dramatic changes (-1 to 21°C) in temperature (Gilbert *et al.* 2016; Gilbert *et al.* 2020) as discussed in Chapter 1 (Figure 1.2 and 1.3). For example, anadromous Arctic char spawn and overwinter at stable, cold temperatures (0.5-2°C) in freshwater lakes (Mulder *et al.* 2018), spend summer feeding at more moderate temperatures (~6-11°C; Rikardsen *et al.* 2007; Harris *et al.* 2020b) and navigate shallow, physically challenging rivers that undergo extreme and rapid diurnal variations of >10°C with peak temperatures in excess of 21°C (Gilbert *et al.* 2016; Gilbert *et al.* 2020). Given that Arctic char are long-lived and iteroparous, they complete this migration cycle many times (Johnson 1980; Johnson 1989; Klemetsen *et al.* 2003). Such repeated and extreme acute temperature exposures present many opportunities for thermal plasticity to influence organismal performance in a manner that could affect migration success and ultimately fitness. Moreover, with the Arctic warming at over twice the average global rate (Zhang *et al.* 2019a), thermal plasticity may prove

critical in the ability of high-latitude, cold-adapted species, like Arctic char, to persist in their rapidly changing environment.

Given such dramatic thermal variation in anadromous Arctic char, cardiorespiratory thermal plasticity may play an important, but yet to be characterized role in shaping their ability to perform vital activities such as their essential pre-winter up-river migration. To begin addressing this knowledge gap, I acclimated Arctic char to five environmentally relevant temperatures from 2 to 18°C to investigate how thermal acclimation alters their cardiac heat tolerance by measuring maximum heart rate (f_{Hmax}) during acute warming. Given the extent of thermal variation they can encounter throughout their life history, I hypothesized that Arctic char possess a significant capacity for cardiac thermal plasticity, likely greater than most stenothermal polar fishes. However, given their northerly distribution I expected that this capacity would not extend their upper cardiac thermal limits up to or beyond that of their temperate relatives.

3.2 Methods

3.2.1 Study design and animals

All protocols were approved by the University of British Columbia (UBC) Animal Care Committee (AUP 2016-033, AUP 2017-039). Arctic char ($N_{total} = 100$) were obtained from Miracle Springs Hatchery (Mission, BC) where they were reared at ~8°C until they reached ~200 g. At UBC, fish were held in 450 L circular tanks with circular flow at 10°C for two weeks and fed trout pellets (Skretting, Vancouver, BC) in excess once daily before they were measured (mass and length) and were injected with a passive inductive transponder (PIT) tag in the intraperitoneal cavity. Fish were recovered for two days after tagging then divided into five tanks (10-14 fish per tank; 32-451 per fish) where their water temperature was adjusted at a rate of ~2°C per day to their final acclimation temperature (2, 6, 10, 14 and 18°C). Regardless of

temperature, fish were held in 450 L tanks with partial flow through in de-chlorinated municipal tap water on a 12:12 light:dark cycle. During the acclimation period fish were fed in excess twice daily (morning and evening). After 6-9 weeks at their acclimation temperature, maximum aerobic metabolic and exercise capacity were assessed in a separate study (Chapter 4). Fish were then allowed to recover for a minimum of one week during which they were fed once daily to limit growth.

2.2. Assessment of maximum heart rate and cardiac heat tolerance

The acute response of f_{Hmax} was assessed as previously described (Casselman *et al.* 2012; Gilbert *et al.* 2020) with minor modifications. Briefly, Arctic char were anaesthetized (0.15 g L⁻¹ TMS; Syndel, Nanaimo, Canada) at their acclimation temperature and once ventilation nearly ceased they were transferred to the experimental bath (0.07 g L⁻¹ TMS) at 2°C where their gills were continuously irrigated with anaesthetic for the duration of the experiment. Fish were fitted with subdermal ECG electrodes, given sequential injections of atropine (1.2 mg kg⁻¹; Sigma-Aldrich, St. Louis, USA) and isoproterenol (4 µg kg⁻¹; Sigma-Aldrich) to induce f_{Hmax} which was allowed to stabilize for at least 30 min. These drugs have previously been shown to maintain f_{Hmax} for the duration of the experiment (Casselman *et al.* 2012). Acute warming began at 5-6°C h⁻¹ in 1°C stepwise increments until f_{Hmax} became arrhythmic, which presented as clear missing heartbeats and at least a halving of instantaneous f_H . The ECG was acquired, processed and analyzed as previously described (Gilbert *et al.* 2019; Gilbert *et al.* 2020) to identify f_{Hmax} at each 1°C increment, peak f_{Hmax} , T_{peak} and T_{arr} . Incremental Q_{10} for f_{Hmax} was calculated over 2°C increments to characterize the incremental thermal sensitivity of f_{Hmax} during warming and the temperature at which Q_{10} decreased and remained below 2.0 with warming was selected as a transition temperature for Q_{10} (Anttila *et al.* 2014a; Anttila *et al.* 2014b). A Q_{10} of 2.0 is a

common rate of increase for f_H during acute warming (Eliason and Anttila 2017) and was the overall average Q_{10} of f_{Hmax} from 2 to 14°C. Fish were euthanized immediately following the experiment and body mass, length, and ventricular mass were recorded. Six fish were assessed at a time, in a single large insulated tank, and fish from different acclimation temperatures were assessed together to limit the potential influence of order effects.

A minor deviation from this protocol was used for the fish at the 18°C acclimation temperature because they had ceased feeding after 5-7 weeks of acclimation and some mortality had occurred (50%), despite a rapid early growth, high activity and a notable appetite (personal observation). As such, the surviving 18°C-acclimated fish ($n=6$) were only monitored in respirometers for two days in a separate study (maximum aerobic metabolic and exercise capacity were not assessed) and then recovered for three days before their f_{Hmax} response to acute warming was assessed as described above.

3.2.2 Assessment of critical thermal maximum

Additional Arctic char were held at 6°C throughout the f_{Hmax} assessments, after which they were similarly acclimated to 2, 6, 10 and 14°C for 6 weeks ($n = 6, 5, 6 \& 6$, respectively) before assessing their critical thermal maximum (CT_{max}). Fish were not acclimated to 18°C to avoid excessive thermal stress and mortality (see above). During acclimation fish were fed a maintenance diet (once daily on weekdays) to ensure these fish were a similar size to those used in the f_{Hmax} assessments. For the CT_{max} assessment, all acclimation groups were acutely transferred to a single 450 L circular holding tank at 8°C (the mid-point between acclimation temperatures) where they were habituated for 1 h before being warmed at 6°C h⁻¹. Immediately after fish lost equilibrium their PIT-tag was scanned for identification, they were transferred to

cooler water (~14°C) and then brought down to 10°C overnight to recover. There was no mortality for over two weeks following the CT_{max} assessment.

3.2.3 Data Analysis

Differences in body size (mass and length), Fulton's condition factor (K), relative ventricular mass ($m_{\text{ventricle}} \times m_{\text{body}}^{-1} \times 100$; RVM), peak $f_{H_{\text{max}}}$ and critical and transition temperatures (T_{Q10}, T_{peak}, T_{arr}, CT_{max}) between acclimation groups were assessed using analysis of variance (ANOVA) followed by pairwise comparisons with Holm's correction applied to account for multiple testing. The difference between T_{arr} and CT_{max} was assessed using a two-way ANOVA including the upper thermal tolerance metric (T_{arr} or CT_{max}), acclimation temperature and their interaction as fixed effects. Holm-adjusted pairwise comparisons were then made between T_{arr} and CT_{max} at each acclimation temperature.

Differences in $f_{H_{\text{max}}}$ between acclimation groups were assessed in two ways. First, a linear mixed effects model (LME4, Package; Bates *et al.* 2007) was used to examine $f_{H_{\text{max}}}$ as a function of acclimation temperature and acute warming over cold to moderate temperatures (2-14°C; Table 3.2). Body mass was initially included as a covariate in this analysis but it was subsequently excluded as it did not have a significant effect on $f_{H_{\text{max}}}$ ($p=0.64$), did not explain any additional variance ($\Delta R^2 < 0.01$), and importantly did not alter any conclusions. Fish ID, was included in this model as a random factor to account for repeated measures. Second, once this global analysis revealed a pronounced effect of acclimation temperature on $f_{H_{\text{max}}}$, separate ANOVAs were conducted at each acclimation temperature to determine specific temperatures where differences existed between acclimation groups. These ANOVAs were followed by pairwise comparisons between each acclimation group at each test temperature examined (2, 6, 10, 14 and 18°C) with Holm's correction applied at each temperature to account for multiple

comparisons. All data presentation was done using Prism v.8.3 (GraphPad Software, San Diego, USA) and all data analysis was done using R Studio (R Core Team 2014).

3.3 Results

3.3.1 Arctic char body size and heart mass

Body size at the time of the f_{Hmax} assessment following acclimation was not a meaningful covariate of f_{Hmax} (see above; $p=0.63$) and there was substantial overlap between acclimation temperatures (Table 3.1). The RVM numerically increased with acclimation temperature above 6°C ($f_{4,38}=4.2$, $p=0.007$), but was only significantly different between 6 and 18°C acclimation groups (+26%; $p=0.004$; Table 3.1).

3.3.2 Maximum heart rate and cardiac heat tolerance

Regardless of their acclimation temperature, all individual Arctic char increased f_{Hmax} with acute warming (Table 3.2; Figure 3.1). Within an acclimation group, fish exhibited low inter-individual variation in f_{Hmax} over cool temperatures but this variation increased at higher temperatures, particularly as individuals approached their upper thermal limits (Figure 3.1). Nevertheless, all individuals reached a peak f_{Hmax} before f_{Hmax} declined and then became arrhythmic (Figure 3.1). Furthermore, incremental Q_{10} for f_{Hmax} declined with warming in all acclimation groups (Figure 3.2a). The cardiac transition temperatures during acute warming (T_{Q10} , T_{peak} , T_{arr}) all increased progressively with acclimation temperature from 2 to 14°C (Figures 3.2b and 3.3): T_{Q10} by 37% (12.0 ± 0.7 to 16.4 ± 0.4 °C; $f_{4,38}=11.6$, $p<0.001$), T_{peak} by 49% (16.1 ± 0.9 to 24.0 ± 0.4 °C; $f_{4,38}=16.6$, $p<0.001$) and T_{arr} by 35% ($+19.1\pm 0.9$ to 26.2 ± 0.72 °C; $f_{4,38}=10.0$, $p<0.001$). The peak f_{Hmax} reached during acute warming also markedly increased by 33% with acclimation temperature from 2 to 14°C (106 ± 5 to 140 ± 3 beats min^{-1} ; $f_{4,38}=6.3$,

$p < 0.001$; Figure 3.4). Therefore, upper cardiac thermal tolerance of Arctic char increased with warm acclimation up to 14°C.

In contrast, peak f_{Hmax} , T_{peak} , and T_{arr} all appeared to decrease with acclimation from 14 to 18°C (Figures 3.3 and 3.4). Also, the numerical increase in T_{Q10} with acclimation from 14 to 18°C did not reach statistical significance ($p = 0.348$).

While f_{Hmax} increased with acute warming in all fish, the initial f_{Hmax} and the rate of increase with warming differed among acclimation temperatures (Table 3.2; Figures 3.2 and 3.4). Values of f_{Hmax} at the start of warming (2°C) decreased progressively with increasing acclimation temperature from 2°C to 14°C, but did not decrease further with acclimation to 18°C (Table 3.2; Figures 3.1f and 3.4). These differences in f_{Hmax} at a common temperature among acclimation groups persisted up to ~10°C, indicating a resetting of f_{Hmax} with thermal acclimation. Beyond 10°C, however, f_{Hmax} converged among acclimation groups such that no significant differences for f_{Hmax} existed by ~18°C among the acclimation groups (Figure 3.1f and 3.4). This convergence in f_{Hmax} above 10°C occurred because the rate at which f_{Hmax} increased during warming at lower temperatures slowed at lower temperatures in cold-acclimated char relative to warm-acclimated char. This result is further illustrated by the pronounced leftward shift in the incremental Q_{10} relationship for cold-acclimated char (Figure 3.2). Consequently, acclimation to temperatures up to 14°C provided a greater f_{Hmax} scope to support acute warming (Figure 3.4).

3.3.3 Critical thermal maximum

The value of CT_{max} increased by only 11% with acclimation temperature from 2 to 14°C (from 24.5 ± 0.2 to 27.2 ± 0.1 °C; $f_{3,19} = 59.8$, $p < 0.001$), a relative change lower than the cardiac

transition temperatures (see above). Also, CT_{max} was greater than T_{arr} in all acclimation groups but the difference between T_{arr} and CT_{max} decreased with increasing acclimation temperature (Two-way ANOVA T_{arr} vs. CT_{max} : $f_{1,52}=53.6$, $p<0.001$, Acclimation temperature: $f_{3,52}=24.0$, $p<0.001$, Interaction: $f_{3,52}=4.5$, $p=0.007$) and was not significant at $14^{\circ}C$ ($p=0.619$; Figure 3.3). Therefore, Arctic char acute cardiac heat tolerance approached acute whole organism heat tolerance with increasing acclimation temperature.

3.4 Discussion

Anadromous Arctic char must perform critical physiological functions over temperatures ranging from <0 to $>21^{\circ}C$ because they encounter distinct thermal regimes during reproduction, growth, and migration (Gilbert *et al.* 2016; Mulder *et al.* 2018; Mulder *et al.* 2019b; Gilbert *et al.* 2020; Harris *et al.* 2020b). When we progressively increased acclimation temperature of Arctic char from a typical winter temperature ($2^{\circ}C$) to a warm summer temperature ($14^{\circ}C$) cardiac transition temperatures (T_{Q10} , T_{peak} , T_{arr}) for f_{Hmax} during acute warming all increased (a total of $+35-45\%$). Moreover, the heartbeat remained rhythmic during acute warming up to temperatures above the maximum currently experienced by wild, migrating Arctic char ($\sim 21^{\circ}C$). Additionally, Arctic char compensated for an elevated f_{Hmax} with warm acclimation by lowering f_{Hmax} over moderate temperatures and increasing peak f_{Hmax} , thereby maintaining or increasing the scope to increase f_{Hmax} with acute warming. Thus, the present study demonstrates that, despite being cold adapted and having an extreme northerly distribution, Arctic char possess a substantial ability to alter their cardiac physiology through acclimation to better suit warm temperatures that periodically occur throughout their thermally variable life history. However, Arctic char could not adequately offset thermal stress through acclimation at $18^{\circ}C$ because acute cardiac thermal tolerance became impaired and fish mortality occurred.

3.4.1 Cardiac and whole organism heat tolerance

The ability to increase heat tolerance through warm acclimation is a well-established mechanism through which fish, including many salmonids, compensate for seasonal and life-history dependent shifts in their thermal regimes. For example, Atlantic salmon a temperate salmonid relative of Arctic char, markedly increased peak f_{Hmax} (~30%) and T_{arr} (~22 to 27°C) when acclimation temperature was increased from 12 to 20°C. Similar improvements were seen in the present study with acclimation between 6 and 14°C (peak f_{Hmax} : +26%; T_{arr} : ~22 to 26°C). Contrary to our expectations, acute cardiac thermal tolerance of Arctic char was similar to temperate salmonids including rainbow trout and Atlantic, Chinook (*O. tshawytscha*) and coho salmon (*O. kisutch*), when compared at similar acclimation temperatures (Casselman *et al.* 2012; Anttila *et al.* 2014a; Anttila *et al.* 2014b; Muñoz *et al.* 2014; Chen *et al.* 2015). For instance, similar-sized Atlantic salmon (~300 g) acclimated to 12°C had a peak f_{Hmax} , T_{peak} , and T_{arr} of ~135 beats min^{-1} , 21.5°C, and 25.3°C respectively, which were all intermediate to Arctic char acclimated to 10 and 14°C here (peak f_{Hmax} : 120 to 140 beats min^{-1} ; T_{peak} : 20-24°C, T_{arr} : 23-26°C). However, while most temperate salmonids can acclimate well to 18°C, and often even warmer temperatures (Myrick and Cech 2000), Arctic char in the present study did not.

Arctic cod, an important food resource for Arctic char, are a polar marine fish that do not experience the same variation in temperature throughout their lives. Correspondingly, over comparable acclimation temperatures (0.5-3.5°C) Arctic cod have a much lower cardiac (T_{arr} : 12.4-13.5°C vs 19.3°C) and whole organism acute heat tolerance (14.9-15.5°C vs. 24.5°C) (Drost *et al.* 2016) compared to Arctic char in the present study. Furthermore, T_{arr} , T_{peak} and peak f_{Hmax} did not increase in Arctic cod when their acclimation temperature was increased from 0.5 to 6.5°C (Drost *et al.* 2016). While their CT_{max} did increase by 15% to 17°C (Drost *et al.* 2016),

this is still over 10°C colder than warm-acclimated char in the present study. Thus, Arctic char are much more eurythermal than Arctic cod, a stereotypical Arctic marine stenotherm.

The present study highlights the importance of considering sub-lethal measures of acute thermal tolerance when assessing thermal acclimation capacity. Anttila *et al.* (2015) acclimated Arctic char to 7.7 and 14.9°C and found only a 1.3°C increase in CT_{max} , leading the authors to conclude that Arctic char have limited potential for the thermal acclimation. Here I also showed a limited increase in CT_{max} (2.0°C; +8%) over a similar acclimation temperature range (6 to 14°C), and by only 11% from 2 to 14°C. However, T_{Q10} , peak f_{Hmax} , T_{peak} and T_{arr} all increased by a much greater extent from 6 to 14°C (+22%, +26%, +33% and +18% respectively). The fact that cardiac thermal tolerance was much more thermally plastic than CT_{max} meant that the difference between the cardiac transition temperatures (especially T_{arr}) and CT_{max} decreased as acclimation temperature increased. Consequently, CT_{max} for cold-acclimated Arctic char is unlikely to be an ecologically relevant indicator of acute heat tolerance because char would encounter cardiac arrhythmias and associated circulatory constraints that would limit whole organism performance at much lower temperatures. These findings for Arctic char support the more general hypothesis proposed by Sandblom *et al.* (2016) that absolute ceilings to thermal tolerance more fixed or ‘concrete’ than sub-lethal performances, which are generally more plastic.

3.4.2 Resetting maximum heart rate

Many fishes compensate for prolonged changes in temperature using multiple mechanisms (Hazel and Prosser 1974; Keen *et al.* 2017), including modifying sinoatrial (SA) currents to reset cardiac pacemaker rates (Haverinen and Vornanen 2007; Eliason and Anttila 2017). Cold exposure depresses f_{Hmax} so fish tend to increase sino-atrial pacing rate during cold

acclimation as a compensatory process (Aho and Vornanen 2001; Haverinen and Vornanen 2007). Conversely, warming increases heart rate and so fishes often lower the SA pacemaker rate during warm acclimation (Aho and Vornanen 2001; Haverinen and Vornanen 2007). For example, Anttila *et al.* (2014a) found that warm acclimation of Atlantic salmon from 12 to 20°C reduced f_{Hmax} at 12°C by 26%. Similarly, Arctic cod progressively decreased f_{Hmax} with increasing acclimation temperature from 0.5 to 3.5 to 6.5°C (Drost *et al.* 2016). The present study with Arctic char similarly found that f_{Hmax} decreased progressively with acclimation between 2 and 14°C, such that 14°C-acclimated char had a 21-29% lower f_{Hmax} over test temperatures between 2 and 10°C than 2°C-acclimated char.

Resetting intrinsic f_H is not a universal acclimation mechanism among teleosts and, therefore, may differ among other Arctic species. For instance, f_{Hmax} did not change with acclimation temperature in Arctic shorthorn sculpin (*Myoxocephalus scorpius*), and intrinsic f_H actually decreased with cold acclimation (Farrell *et al.* 2013). Atlantic killifish, a temperate eurytherm that naturally experiences temperatures from -1°C to at least 33°C, elicited an unusual compensatory response by markedly decreased f_{Hmax} with both cold (5°C) and warm (33°C) acclimation relative to an intermediate acclimation temperature (15°C) (Safi *et al.* 2019). The speed with which intrinsic f_H resetting occurs and even whether it always occurs within a species also requires further study. While Ekström *et al.* (2016b) suggested that intrinsic f_H resetting in rainbow trout may take greater than 6 weeks, which was not the case in the present study for Arctic char, Sutcliffe *et al.* (2020) found that one group of rainbow trout reset intrinsic f_H within 24 h, but another group did not reset intrinsic f_H after 10 weeks of temperature acclimation.

3.4.3 Ecological relevance and conservation implications

In the Canadian Arctic, anadromous Arctic char hatch and spend most of their lives at cold temperatures ($<4^{\circ}\text{C}$), but spend summers at more moderate temperatures and even encounter much warmer temperatures during their annual migration. Indeed, recent recordings of river temperatures during Arctic char migrations in the central Canadian Arctic reached a peak temperature $>21^{\circ}\text{C}$, with 1- and 7-day averages as high as 18.7°C and 14.9°C , respectively (Gilbert *et al.* 2016; Gilbert *et al.* 2020). As such, wild Arctic char may already have, not only the opportunity, but also the need for warm acclimation. A recent field study in the central Canadian Arctic that examined the cardiac heat tolerance of wild Arctic char migrating upriver at temperatures of $\sim 11^{\circ}\text{C}$ reported a peak f_{Hmax} , T_{peak} and T_{arr} of $115 \text{ beats min}^{-1}$, 19.4°C and 21.4°C , respectively (Gilbert *et al.* 2020), values similar to those obtained here for Arctic char acclimated to either 6°C or 10°C . Furthermore, f_{Hmax} at 10°C for these wild Arctic char ($\sim 72 \text{ beats min}^{-1}$) was again similar to that for Arctic char acclimated to 6°C here ($71 \text{ beats min}^{-1}$). Thus, the present data likely apply well to wild cohorts as these wild char were returning from the marine environment where body temperatures in the region are typically $\sim 6^{\circ}\text{C}$ (Harris *et al.* 2020b) to river temperatures of $\sim 11^{\circ}\text{C}$. Gilbert *et al.* (2020)(Chapter 2) also found that, as I showed here, the incremental Q_{10} for f_{Hmax} fell dramatically over commonly encountered temperatures, falling below 2.0 at $\sim 13^{\circ}\text{C}$, which is similar to 6°C acclimated Arctic char here.

Hansen *et al.* (2016), studied anadromous Arctic char in Greenland using similar methods, but reported a much lower upper acute cardiac thermal tolerance than what we found for even 2°C -acclimated char, with peak f_{Hmax} , T_{peak} and T_{arr} being only $61.8 \text{ beats min}^{-1}$, 12.8°C and 15.2°C , respectively. Such large quantitative differences between Arctic char from different

areas are likely the result of genetic or prolonged (i.e., developmental or ontogenetic) differences in thermal acclimation, as previously suggested (Gilbert *et al.* 2020; Harris *et al.* 2020b).

When considering the ecological relevance of thermal plasticity, it is important to note wild fish often experience greater thermal variation than captive fish used in laboratory acclimation studies and the resulting phenotype is not necessarily what would be expected at the mean experienced temperature (Morash *et al.* 2018). The Arctic char is no exception in this regard; body temperature can vary little over winter ($\sim 0.5\text{-}2^{\circ}\text{C}$; Mulder *et al.* 2018), but at sea varies between -1 and 14°C with an average of $\sim 6^{\circ}\text{C}$ (Harris *et al.* 2020b). Body temperature during river migration can be even more volatile (Gilbert *et al.* 2016), perhaps testing their upper thermal limits in certain watersheds.

3.4.4 Conclusion

Climate change is progressing rapidly at northern latitudes and is predicted to increase the frequency and amplitude of extreme heat events (Zhang *et al.* 2019a). In some Arctic rivers these events already cause extraordinarily warm water temperatures ($>21^{\circ}\text{C}$) and large acute temperature changes ($>10^{\circ}\text{C}$ in a day, $>20^{\circ}\text{C}$ over two weeks) (Gilbert *et al.* 2016; Gilbert *et al.* 2020). As shown here and in previous studies, such conditions are sufficient to impair heart function and recovery from exhaustive exercise, which has been associated with elevated mortality (Gilbert and Tierney 2018; Gilbert *et al.* 2020). Here, I showed that warm acclimation ($10\text{-}14^{\circ}\text{C}$) has the potential to partially mitigate such impairments. However, further research is needed to determine the time required for such plasticity to occur and whether wild fish behaviorally exploit natural thermal variation in a manner that would permit warm acclimation prior to extreme heat exposure.

Table 3.1 Sample size (N), fork length (mm), body mass (g), Fulton’s condition factor (K) and relative ventricular mass (RVM; %) in Arctic char following acclimation to 2, 6, 10, 14 and 18°C. Dissimilar superscript letters indicate significant differences between acclimation treatments ($\alpha=0.05$).

	2°C	6°C	10°C	14°C	18°C
Sample size (n)	9	10	9	9	6
Fork Length (mm)	336±7	344±6	356±6 ^a	353±3 ^a	324±6 ^b
Mass (g)	440±24	498±24	516±33	468±20	398±32
Condition (K)	1.16±0.03	1.22±0.05	1.13±0.04	1.06±0.04	1.17±0.07
RVM (%)	0.12±0.01	0.11±0.00 ^a	0.12±0.00	0.13±0.01	0.14±0.00 ^b

Table 3.2 Linear mixed effects model characterizing the response of maximum heart rate (f_{Hmax}) to acute warming from 2-14°C in Arctic char acclimated to 2, 6, 10, 14 and 18°C. The lowest acclimation temperature (2°C) was used as the reference term and fish ID was included as a random effect.

Fixed effect	Coefficient	SE	df	<i>t</i>	<i>p</i>
Intercept	3.515	0.031	35	114.49	<0.001
Acute Temperature (°C)	0.078	0.002	34.9	38.14	<0.001
Acclimation: 6°C	-0.126	0.042	35	-2.99	0.005
Acclimation: 10°C	-0.232	0.042	35	-5.49	<0.001
Acclimation: 14°C	-0.409	0.043	35	-9.43	<0.001
Acclimation: 18°C	-0.36	0.047	35	-7.68	<0.001
Interaction: 6°C	0.007	0.003	34.9	2.35	0.025
Interaction: 10°C	0.012	0.003	35	4.33	<0.001
Interaction: 14°C	0.019	0.003	34.9	6.48	<0.001
Interaction: 18°C	0.007	0.003	34.9	2.3	0.027

Model: $\text{Ln}(f_{Hmax}) \sim \text{Acute Temperature} \times \text{Acclimation temperature} + (\text{Fish ID}|1)$

marginal R^2 : 0.95

conditional R^2 : 0.99

Figure 3.1 Maximum heart rate (f_{Hmax}) during acute warming in Arctic char acclimated to five temperatures (2, 6, 10, 14 and 18°C). Warming occurred from 2°C and continued until the onset of cardiac arrhythmia. Data are presented for all individuals (a-e; 2-18°C respectively) with mean (\pm SE) values overlaid and compared (f). Individual traces stop at the arrhythmia temperature below which f_{Hmax} had always plateaued or began declining. Mean values are only presented at temperatures below which the first individual within an acclimation group exhibited arrhythmia. In some instances error bars are encompassed within their associated symbol.

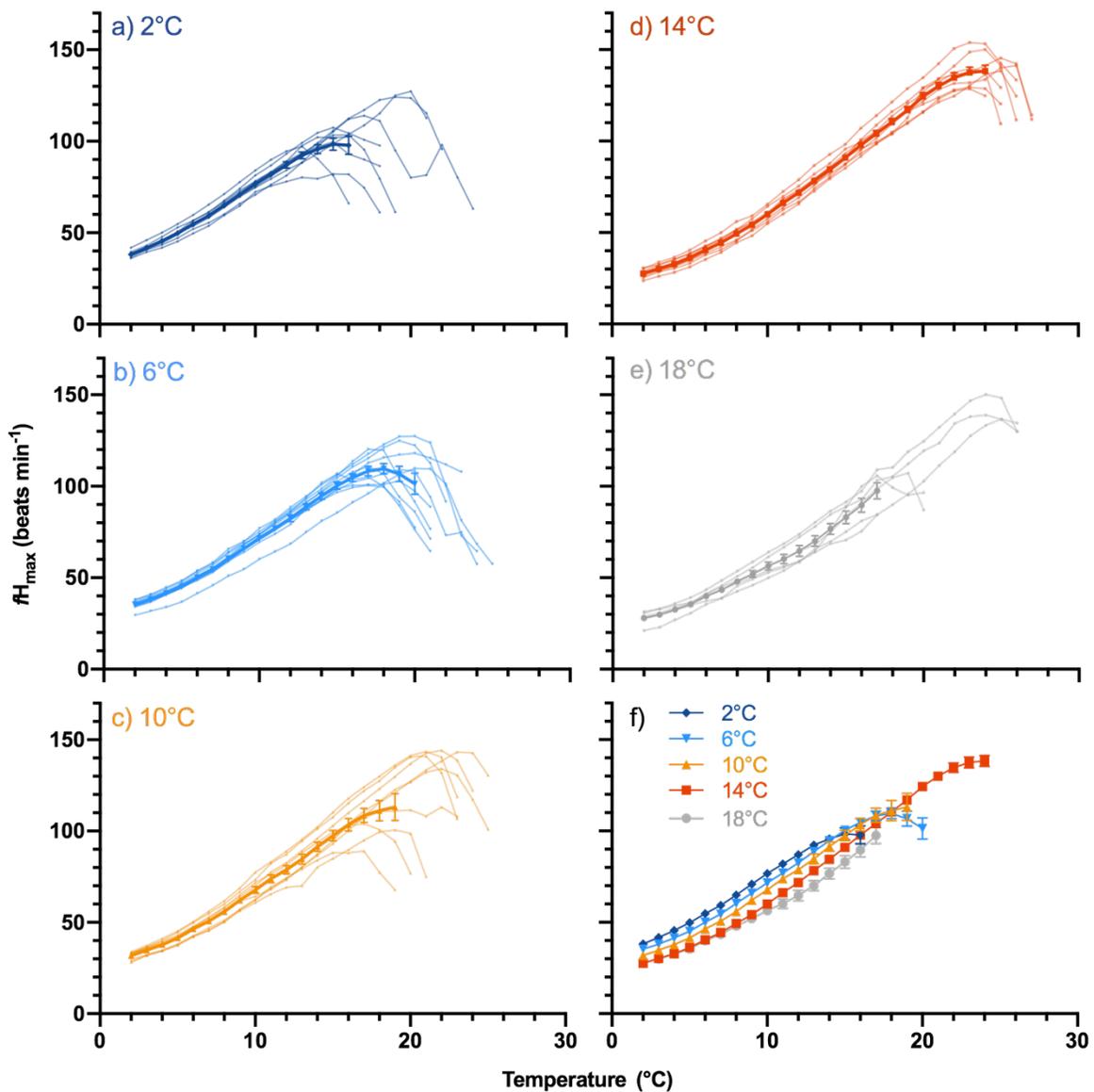


Figure 3.2 The change in thermal sensitivity of maximum heart rate (f_{Hmax}) during acute warming in Arctic char acclimated to five temperatures (2, 6, 10, 14 and 18°C). Thermal sensitivity is presented as the mean (\pm SE) Q_{10} temperature coefficient calculated over 2°C increments (a). Grey horizontal lines highlight a Q_{10} of 2.0, a typical rate of increase for f_{Hmax} with acute warming, and a Q_{10} of 1.0 indicating no change in f_{Hmax} with further warming, while values below 1.0 indicate a decrease. The percentage of fish from each acclimation temperature who's incremental Q_{10} fell below and then did not exceed 2.0 with further warming is shown (b) as an indicator of the decline in rate of increase in f_{Hmax} with acute warming.

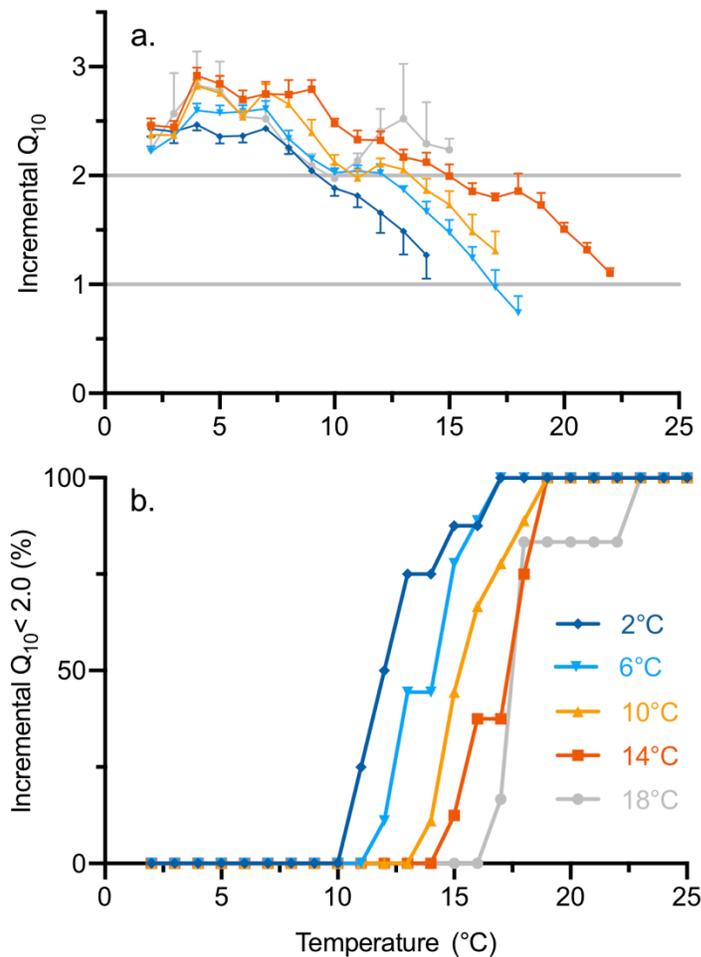


Figure 3.3 The effect of thermal acclimation (2, 6, 10, 14 and 18°C) on transition and critical temperatures during acute warming in Arctic char. Mean (\pm SE) temperatures at loss of equilibrium (CT_{max}), the onset of cardiac arrhythmic (T_{arr}), the occurrence of peak maximum heart rate (T_{peak}), and at which Q_{10} did not exceed 2.0 with further warming are shown. Dissimilar letters indicate significant differences between acclimation temperatures ($\alpha=0.05$). Broken lines with open symbols for 18°C are used to indicate that the fish may have been thermally stressed rather than acclimated since there was mortality during the acclimation period.

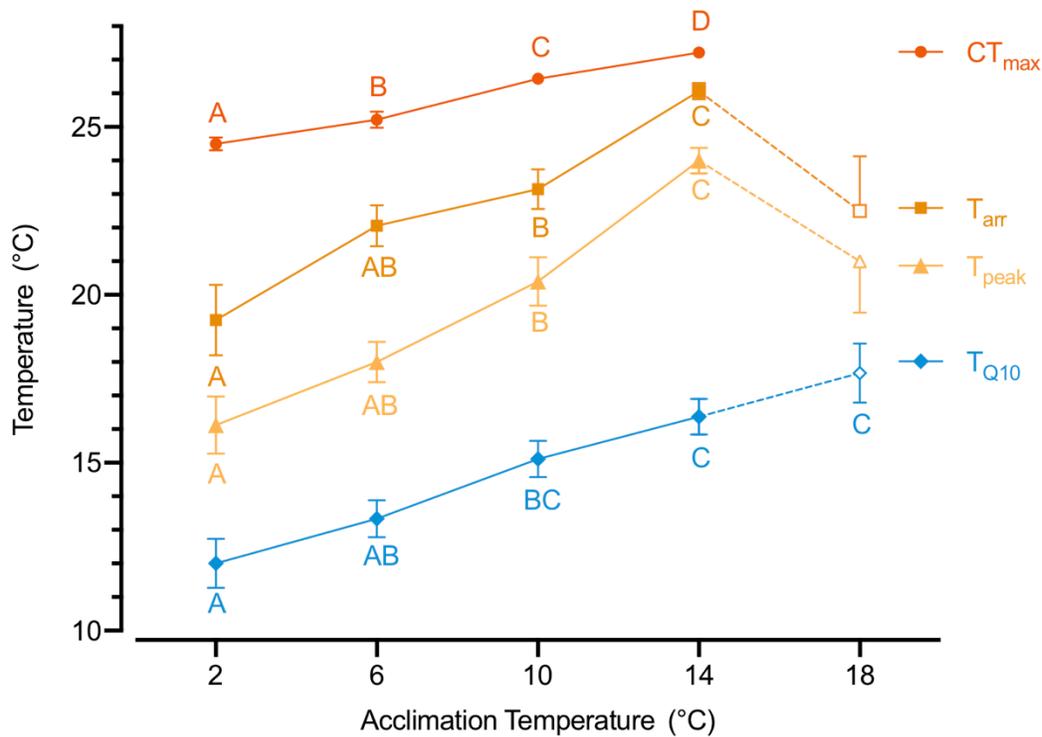
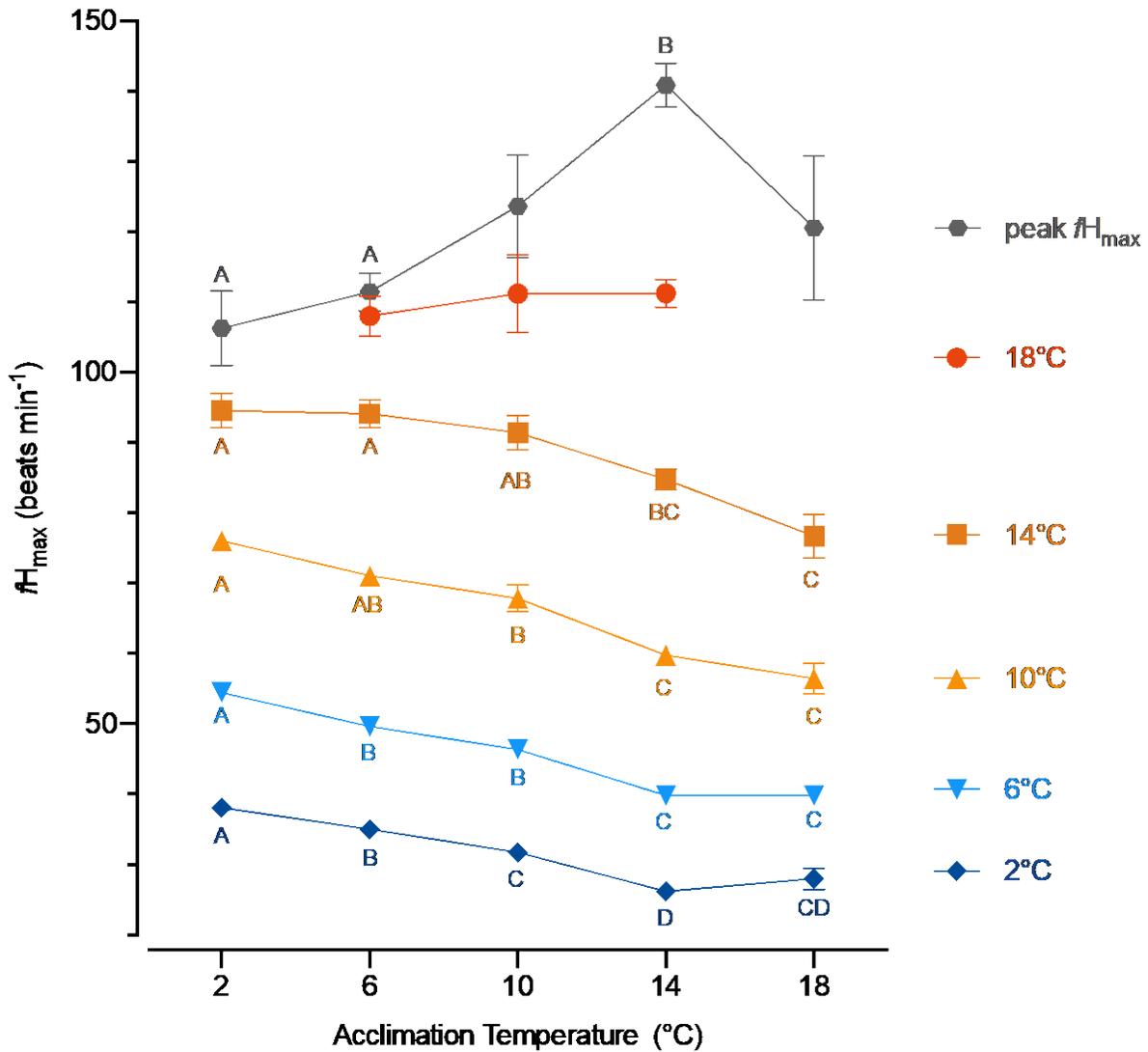


Figure 3.4 The effect of temperature acclimation (2,6,10,14 and 18°C) on maximum heart rate (f_{Hmax}) and peak f_{Hmax} during acute warming in Arctic char. Warming began at 2°C and continued until the onset of cardiac arrhythmia. Mean (\pm SE) f_{Hmax} during acute warming is shown at each acclimation temperature and at T_{peak} . Dissimilar letters indicate significant differences between acclimation groups at a given test temperature ($\alpha=0.05$).



Chapter 4: The thermal acclimation potential of Arctic char (*Salvelinus alpinus*): Growth, swimming performance and aerobic capacity at ecologically relevant temperatures

4.1 Introduction

Fish encounter thermal variation on times scales ranging from minutes (acute) to months (chronic) throughout their lifetime (Fry 1947; Fry 1971; Schulte *et al.* 2011; Sandblom *et al.* 2014). As described previously in Chapters 1 to 3, Arctic char can experience extreme acute temperature changes both when diving and ascending through thermal gradients and when migrating through the dynamic thermal regimes of shallow rivers (e.g., Figure 1.3). Such acute exposures are likely rare events in their life history, but are nevertheless ecologically important because of their potential to rapidly impair physiology, which if severe enough could result in mortality or otherwise reduce fitness (Chapter 2,3 and 5) (Eliason *et al.* 2011; Gilbert and Tierney 2018). Nevertheless, most fish, including Arctic char, more commonly encounter less extreme but more prolonged temperature changes when seasons change (Figure 1.2 and 1.3) and when they move between habitats (e.g., to and from marine feeding and freshwater overwinter habitats; Figure 4.1). These prolonged thermal exposures may provide Arctic char the opportunity, and need, to adjust their physiology (acclimatize) to improve the performance of vital functions under the prevailing thermal conditions.

Arctic char in the central Canadian Arctic spend the vast majority of their life (~75%) overwintering in freshwater, under ice, at temperatures below 4°C (0.5-2°C, Figure 1.2, 1.3, and 4.1) (Mulder *et al.* 2018). In contrast, summer (~2 months) is spent either in their marine feeding habitat or in freshwater at more moderate temperatures (~6-10°C; Table 1.1, Figure 1.2 and

4.1)(Dick *et al.* 2009; Harris *et al.* 2020b). The rivers used by anadromous Arctic char to travel between these two habitats are more thermally variable, but are commonly warmer, between 8-12°C, with 7 day averages already as high as ~15°C, and this migration can last for well over a week (e.g. 7.5 day average at Halokvik, NU). Furthermore, rivers in which these warm temperatures occur are typically shallow and so thermal refugia may not be available. Thus, the life history of Arctic char in the central Canadian Arctic may present sufficient time and the need to acclimatize to and perform critical functions over temperatures ranging from 0 to 15°C for prolonged periods throughout their lives. This thermal range is likely even greater near the southern regions of their distribution and will only increase with climate change (Wrona *et al.* 2006b; Poesch *et al.* 2016; Zhang *et al.* 2019a). Indeed, 24 h average river temperatures in the central Canadian Arctic can already exceed 18°C in extreme cases (e.g. Figure 1.3).

In Chapter 2 and in past research (Gilbert *et al.* 2020) I showed that, during acute warming from ~8-18°C, Arctic char maintain, but do not markedly improve, either swimming performance or the maximum capacity to supply tissues with oxygen - absolute aerobic scope (AAS). However, factorial aerobic scope (FAS) and post-exercise recovery became severely reduced (only ~twice $\dot{M}O_{2min}$) at warm temperatures (16-21°C) (Chapter 2; Gilbert and Tierney 2018). A similar comparison of respiratory performance, however, has not been conducted after thermal acclimation. In Chapter 3 I showed that Arctic char can increase cardiac heat tolerance during warm acclimation and adjust their maximum heart rate (f_{Hmax}) to better suit their acclimation temperature. All the same, Arctic char, as with all fish, have an upper limit for thermal acclimation. For example, their maximum growth rate increases with temperature from 0 to ~10-14°C but then dramatically decreases, approaching zero by ~21°C (Table 1.1.) (Larsson *et al.* 2005; Imsland *et al.* 2019). Similarly, prolonged exposure to 18°C impaired cardiac heat

tolerance when compared to 14°C-acclimated Arctic char (Chapter 3). As such it stands to reason that Arctic char may also be able to improve swimming performance and aerobic scope with warm acclimation, up to a point, beyond which limitations occur. Indeed, temperate salmonids and Arctic cod, an important prey species for Arctic char, do just that (Brett 1964; Griffiths and Alderdice 1972; Drost *et al.* 2016; Hvas *et al.* 2017).

Based on the thermal ecology of Arctic char outlined above, I acclimated Arctic char to ecologically relevant temperatures ranging from 2-18°C to determine how prolonged thermal exposure impacts growth, swimming performance and respiratory physiology. I examined these three traits at five temperatures (2,6,10,14, and 18°C) in Arctic char acclimated to each temperature and 6°C acclimated Arctic char acutely exposed to each temperature. I selected 6°C as my starting point for the acute transfer to simulate known thermal transitions experienced by Arctic char acclimated to their summer marine feeding temperatures (6.0°C), when they dive to deeper cold water (Harris *et al.* 2020b) and when they migrate through thermally variable rivers (Figure 4.1)(Gilbert *et al.* 2016). Based on previous findings for growth (Table 1.1) and maximum heart rate (Chapter 3), I predicted growth, swimming performance, and cardiorespiratory performance would all increase with acclimation temperature up to 14°C but would decrease at 18°C. Furthermore, based on my previous research, I expected the swimming and cardiorespiratory performance to be greater following thermal acclimation compared to an acute thermal challenge (Chapters 2 and 3).

4.2 Methods

4.2.1 Acclimation, growth and mortality

Arctic char (Nauyuk strain, $N_{\text{total}}=104$, mass: 298 ± 48 g, fork length: 306 ± 21 g, mean \pm SD) were obtained from Miracle Springs Hatchery (Mission, BC) and then acclimated at UBC

to 2, 6, 10, 14, and 18°C (Table 4.1) as described in Chapter 3. The remaining fish were held separately at 6°C for use in the acute temperature exposure experiments (see below). Arctic char were fed *ad libitum*, twice daily, and grew for 6-9 weeks at their acclimation temperature before the respirometry measurements. Immediately prior to these measurements, Arctic char were fasted for either 48 h (10, 14, and 18°C) to 72 h (2 and 6°C), their PIT-tag was scanned and they were weighed. The number of days at their acclimation temperature was used to calculate the specific growth rate (SGR; % body mass day⁻¹) at each acclimation temperature:

$$SGR = \frac{\ln(m_f) - \ln(m_i)}{t} \cdot 100$$

Where m_f is the final mass (g), m_i is the initial mass and t is duration of growth in days. Arctic char were weighed only once during the growth trial to eliminate unnecessary handling stress and were checked at least twice daily for any mortality and moribund fish (e.g. exhibiting loss of equilibrium), which was only observed at 18°C (see Results). A moribund fish was immediately euthanized to limit suffering and was considered a mortality for the purpose of the study.

4.2.2 Aerobic swimming performance

Aerobic swimming performance was assessed after a minimum one-week recovery from the respirometry experiments (see below) using a 30-litre swim-tunnel respirometer (Loligo systems, Tjele, Denmark) and a ramp-velocity swim test, as commonly used to establish critical swimming performance (U_{crit}) (Jain *et al.* 1997; Tierney 2011; Zhang *et al.* 2019b). Arctic char were tested either at their acclimation temperature or after an acute transfer from 6°C (4°C h⁻¹) to 2, 10 or 14°C. Arctic char were weighed, transferred into the swim tunnel with little (<10 s) or no air exposure and then habituated to the swim tunnel at a water velocity (U) of 16 cm s⁻¹ (~0.5 body lengths per second; bl s⁻¹). Arctic char $\dot{M}O_2$ was monitored before, during and after the

swimming performance assessment. Habituation lasted at least 2 h, or as long as it took for oxygen uptake ($\dot{M}O_2$) to fall below standard $\dot{M}O_2$ ($\dot{M}O_{2\text{standard}}$) as estimated in the respirometry tests described below, after which U was incrementally increased by 5 cm s^{-1} every 90 s until U reached $\sim 50\%$ of an estimated U_{crit} from preliminary swim trials. Most Arctic char swam steadily at this U , but if they did not (resting at the rear of the tunnel), which was more common at the cold temperatures, they were left in the swim tunnel overnight at a U of $\sim 1 \text{ bl s}^{-1}$ and the test was restarted the following morning. The overnight habituation to the swimming tunnel resolved the lack of motivation to swim with only one exception, which was excluded from the analysis. This lack of motivation to swim was more common at the cold temperatures. The U was increased every 15 min after the initial rapid ramp using increments of either 12 cm s^{-1} at 6, 10, and 14°C or 6 cm s^{-1} at 2°C . These increments ensured at least two completed U steps of steady swimming prior to the gait transition speed (U_{gt}). The U_{gt} was defined as the speed at which Arctic char transitioned from a primarily steady (aerobic) swimming mode to an unsteady, burst-and-coast swimming mode (Peake and Farrell 2004; Peake 2008). This transition was identified using a recognized criterion (MacNutt *et al.* 2004): the time when a fish first swims from rear to the front of the swim tunnel test section at least three times within 1 min. This time was converted to U_{gt} using the equation used to determine U_{crit} Brett (1964a). The swimming test was terminated at U_{gt} so that only aerobic swimming performance was assessed; burst-and-coast swimming can heavily influence traditional U_{crit} estimates, which makes U_{crit} more dependent on the length of the swim tunnel test section than U_{gt} (Tudorache *et al.* 2007; Tudorache *et al.* 2010). As such a longer swim tunnel test section is desirable to assess U_{crit} ; however, in the present study I wanted to optimize $\dot{M}O_2$ measurements by reducing the swim-tunnel volume (short test section) and thus U_{gt} was a more appropriate metric of swimming performance.

4.2.3 Respirometry

Intermittent-flow respirometry was conducted on 4-6 fish simultaneously with each Arctic char individually housed in 9.9 litre respirometer (Loligo systems) $\dot{M}O_2$ was as assessed either at an Arctic char's acclimation temperature, or following an acute change from 6°C as described above. Automated intermittent flush cycles (Aquaresp v.3, Denmark, Aquaresp.com), controlled as previously described (Chapter 2; Svendsen *et al.* 2016; Zhang *et al.* 2020), lasted 360-500 s and consisted of a flush duration (50-210 s), wait period (60-100 s) and an $\dot{M}O_2$ measurement window (120-340 s). The durations varied with temperature and body mass to ensure that the dissolved oxygen concentration (DO) remained above 80% air saturation during any given $\dot{M}O_2$ measurement cycle. The decline in DO during a measurement cycle ($R^2 > 0.8$) was used to continuously monitor $\dot{M}O_2$ for 48 h and ensure $\dot{M}O_{2\text{standard}}$ (the minimum $\dot{M}O_2$ required to support maintenance metabolism) could be accurately assessed (Chabot *et al.* 2016). $\dot{M}O_{2\text{standard}}$ was calculated as the 20th percentile ($q_{0.2}$; Chabot *et al.*, 2016) of all $\dot{M}O_2$ measurements between 12 h to 48 h. I assumed the initial stress of introducing fish into the respirometer had abated after 12 h (Chabot *et al.* 2016; Zhang *et al.* 2018a). The $\dot{M}O_{2\text{mean}}$ (used as a proxy for mean daily energy expenditure) was calculated as the average of all $\dot{M}O_2$ measurements between 24 h and 48 h. As an indicator of activity level or agitation, the percent of $\dot{M}O_2$ measurements that were greater than twice $\dot{M}O_{2\text{standard}}$ was also calculated. After 48 h fish were agitated to induce maximum $\dot{M}O_2$ ($\dot{M}O_{2\text{max}}$) by rotating a nylon strap inside the respirometer, as previously described (Adams 2020; Zhang *et al.* 2020). This forced exercise period had two agitation phases. First, Arctic char were agitated briefly (10 s) to induce a short transient peak in $\dot{M}O_2$. Second, Arctic char were exercised to exhaustion (5 min) to induce a sustained peak. This two-stage protocol was designed based on personal observations that brief

bouts of intense activity in Arctic char can induce elevations in $\dot{M}O_2$ up to or near $\dot{M}O_{2max}$ and based on concerns that physiological disturbances associated with exhaustion (e.g. hyperkalemia and acidoses) may impair rather than increase $\dot{M}O_2$ as intended. The latter was of particular concern for Arctic char because they often appear to cease ventilation (low $\dot{M}O_2$ with little to no opercular movement) during sustained forced exercise (personal observation). This forced exercise protocol was not performed on fish acclimated to 18°C to avoid post-exercise mortality. The flush pump was manually controlled during and shortly after the forced exercise periods to maximize the likelihood of capturing the true peak in $\dot{M}O_2$ while still maintaining DO >75% air saturation (Zhang *et al.* 2019b; Zhang *et al.* 2020). Fish were then returned to their previous intermittent flow cycle and allowed to recover for at least 12 h. An off-line analysis using a 90-s iterative algorithm was applied to all DO measurements during and for at least 5 min following forced exercise to properly identify the peak in $\dot{M}O_2$, which was assigned as $\dot{M}O_{2max}$. All $\dot{M}O_2$ values recorded outside of the period around exercise were then inspected Arctic char if spontaneously achieved a higher value it was assigned as $\dot{M}O_{2max}$ instead. Absolute aerobic scope (AAS = $\dot{M}O_{2max} - \dot{M}O_{2standard}$) and factorial aerobic scope (FAS = $\dot{M}O_{2max} / \dot{M}O_{2standard}$) were then calculated as metrics of the aerobic capacity available to support aerobic functions above $\dot{M}O_{2standard}$ (Fry 1947). For 18°C acclimated Arctic char, $\dot{M}O_{2max}$, AAS and FAS were calculated using $\dot{M}O_{2max}$ estimates derived from spontaneous activity, and were not included in statistical comparisons.

4.2.4 Analysis

Data analysis and presentation were conducted using Prism v.8.3 (GraphPad Software, San Diego, USA) and R Studio (R Core Team 2014) software ($\alpha=0.05$). Data are presented as mean \pm se unless otherwise stated. The effect of temperature on SGR was assessed in two ways.

First, differences in growth among acclimation temperatures were identified using a Kruskal-Wallis test followed by Dunn's test for pairwise comparisons, as the data did not meet the assumptions for parametric analyses. Second, the temperature-growth relationship was modeled using second-order polynomial, Gaussian, and Lorentzian equations. The Lorentzian model had the lowest Akaike information criterion (AIC) and highest R^2 (Δ AIC and R^2 for Gaussian: -1.8 and +0.03, Polynomial: -3.1,+0.05) and was thus selected as the final model. Both analyses were conducted a second time following the removal of three outliers, which were identified as growth rates that were outside 1.5 times the interquartile range (IQR), and the Lorentzian was again the final model (Δ AIC and R^2 for Gaussian: -4.1 and +0.05, Polynomial: -7.2,+0.09).

The effects of test temperature and acclimation status (acutely exposed vs. acclimated) on U_{gt} and all $\dot{M}O_2$ related variables were assessed using two-way analyses of variance (ANOVAs). The effects of allometric scaling of $\dot{M}O_{2standard}$, $\dot{M}O_{2mean}$, $\dot{M}O_{2max}$, and AAS with body mass were accounted for by adjusting all values to the overall average body mass (0.480 kg) prior to all analyses as described in chapter 2. This analysis is equivalent to conducting the analyses on the residuals of a $\log(\dot{M}O_2)$ vs. $\log(\text{mass})$ regression. All $\dot{M}O_2$ variables were then expressed in mass-specific units ($\text{mgO}_2 \text{ h}^{-1} \text{ kg}^{-1}$) to allow for appropriate comparisons with literature values including those in chapter 6 (Figure 6.5).

All ANOVAs were followed by Holm-Sidak adjusted pairwise comparisons among all temperatures in thermally acclimated Arctic char, and separately in acutely exposed 6°C-acclimated Arctic char. Pairwise comparisons were also made between thermally acclimated Arctic char and acutely exposed 6°C-acclimated Arctic char at each study temperature. The effects of temperature on $\dot{M}O_{2standard}$, $\dot{M}O_{2mean}$, $\dot{M}O_{2max}$, and AAS were also modeled as second-order polynomial equations as previously described in Chapter 2. These models were created for

Arctic char tested at their acclimation temperature and for 6°C acclimated Arctic char acutely transferred to each temperature.

The oxygen pulse at maximum aerobic workload was calculated by dividing $\dot{M}O_{2\max}$ by the mean $f_{H\max}$ at each temperature calculated in Chapter 2. This analysis assumes that fish are at $f_{H\max}$ whenever they are at $\dot{M}O_{2\max}$. Based on the Fick equation for $\dot{M}O_2$, changes in oxygen pulse ($\dot{M}O_2 \cdot f_H^{-1}$) are indicative in changes in cardiac stroke volume (SV) and/or arteriovenous oxygen extraction measured as the difference between arterial and venous oxygen content ($CaO_2 - CvO_2$):

$$\frac{\dot{M}O_2}{f_H} = SV \cdot (CaO_2 - CvO_2)$$

The effect of temperature on oxygen pulse was assessed by two-way ANOVA and Holm-Sidak pairwise comparisons as described above for other $\dot{M}O_2$ metrics.

4.3 Results

4.3.1 Growth and mortality

Arctic char SGR increased with acclimation temperature from 2 to 14°C (+108% overall, $Q_{10} = 1.8$; $p < 0.001$), but decreased between 14 and 18°C (-48%; $Q_{10} = 0.2$; $p = 0.026$) (Table 4.1, 4.2 and 4.3, Figure 4.2a). When three outliers (outside 1.5•IQR at 14 and 18°C) were excluded from the analysis the same pattern remained (Table 4.3, Figure 4.2a). The temperature-growth model generated a temperature for maximal growth of 12.2°C (12.7°C without the outliers) (Table 4.2). During the initial phase of acclimation to 18°C, Arctic char had a notable appetite, but feeding ceased over time and mortality started after 5 weeks of acclimation (Figure 4.2b); ultimately, 4 of the 13 Arctic char died during acclimation to 18°C and two more became

moribund shortly (<1 h) after minimal handling (netting) at the start of the respirometry experiments.

4.3.2 Aerobic swimming performance

Thermally acclimated Arctic char significantly increased U_{gt} by 40% from 2 to 10°C (Table 4.3, Figure 4.3). However, U_{gt} was unchanged between 10 and 14°C acclimation temperatures. When 6°C-acclimated char were acutely exposed to 2°C, U_{gt} significantly decreased by 16%, but U_{gt} of 6°C-acclimated char remained unchanged when tested following acute warming to 10°C or 14°C. While U_{gt} for 6°C-acclimated Arctic char acutely exposed to 2°C was similar to that for 2°C-acclimated char when, their U_{gt} when acutely exposed to 10°C and 14°C was significantly lower (-16%) compared with Arctic char acclimated to those two temperatures (Table 4.3, Figure 4.3).

4.3.3 Respirometry

Thermally acclimated Arctic char exponentially increased $\dot{M}O_{2standard}$ between 2 and 18°C (overall $Q_{10} = 2.1$; Table 4.3). When 6°C-acclimated Arctic char were acutely exposed to the same temperature range the overall increase in $\dot{M}O_{2standard}$ was even greater (overall $Q_{10} = 3.1$) (Table 4.2 and 4.3, Figure 4.4 and 4.5). When 6°C-acclimated Arctic char were acutely exposed to 2°C $\dot{M}O_{2standard}$ was significantly lower than that of 2°C acclimated Arctic char (-30%; Table 4.3). Furthermore, when acutely exposed, $\dot{M}O_{2standard}$ at 10°C was similar to that of 10°C acclimated char but was significantly greater at 14 and 18°C than in Arctic char acclimated to those two temperatures (+22 and 32% respectively; Figure 4.4).

The $\dot{M}O_{2mean}$ was 12-37% higher than $\dot{M}O_{2standard}$ and similarly increased exponentially with acclimation temperature from 2 to 18°C with a similar exponent (overall $Q_{10} = 2.2$; Table 4.3). When 6°C-acclimated Arctic char were acutely exposed to the same temperature range, the

overall Q_{10} (3.5) was again greater than that with thermal acclimation (Table 4.2 and 4.3, Figure 4.4 and 4.5). When 6°C-acclimated Arctic char were acutely exposed to 2°C, $\dot{M}O_{2\text{mean}}$ was significantly lower than 2°C acclimated char (-32%). In contrast, when acutely exposed, $\dot{M}O_{2\text{mean}}$ at 10°C was similar to 10°C acclimated Arctic char but was much greater at 14 and 18°C than in Arctic char acclimated to those temperatures (+31 and 41% respectively; Table 4.3, Figure 4.4).

The percentage of time Arctic char spent with an $\dot{M}O_2$ greater than twice $\dot{M}O_{2\text{standard}}$ was generally higher at warmer temperatures (Table 4.3, Figure 4.4). This difference was more pronounced in 6°C acclimated Arctic char acutely exposed to warmer temperatures (10-18°C) than in Arctic char acclimated to those temperatures. Thus, the slight increase in thermal sensitivity of $\dot{M}O_{2\text{mean}}$ compared with $\dot{M}O_{2\text{standard}}$ was in part a result of more spontaneous activity at 14 and 18°C.

Arctic char $\dot{M}O_{2\text{max}}$ increased with acclimation temperature between 2 and 6°C (overall +27%, $Q_{10} = 1.8$) and was similar from 6 to 14°C (2 to 14°C $Q_{10}:1.4$) (Table 4.2 and 4.3, Figure 4.4 and 4.5). When 6°C-acclimated Arctic char were acutely exposed, $\dot{M}O_{2\text{max}}$ decreased with cooling to 2°C ($Q_{10}:1.9$), did not change with warming to 10°C, increased at 14°C and remained elevated at 18°C (2-18°C $Q_{10}:1.4$). The $\dot{M}O_{2\text{max}}$ of 6°C acclimated Arctic char and those acclimated to the test temperature did not significantly differ at any temperature.

Arctic char AAS increased with acclimation temperature from 2 to 6°C (+27%) and was similar from 6 to 14°C (2 to 14°C: +34%). The AAS exhibited during spontaneous activity at 18°C was ~20% lower than that achieved through forced exercise at 14°C. When 6°C-acclimated Arctic char were acutely exposed, AAS decreased with cooling to 2°C ($Q_{10}:1.9$), did not change with warming to 10°C, increased slightly at 14°C and remained elevated at 18°C (2-18°C $Q_{10}:1.4$). The AAS of 6°C acclimated Arctic char acutely exposed to 2°C was lower than at all

other test temperatures although the difference was only significant at 14°C (+44%), and 18°C (+34%)(Figure 4.4). There were no significant differences in AAS at any point between 6°C acclimated Arctic char and those acclimated to the test temperature (Table 4.3).

The FAS was similar with acclimation at 2 and 6°C, decreased at 10°C (-33%) and was similar at 10 and 14°C (Figure 4.4). In 6°C-acclimated char, FAS decreased progressively with acute increases in test temperature and was 70% lower at 18°C than at 2°C (Table 4.3). When 6°C-acclimated char were acutely exposed to 2°C, FAS was significantly higher than 2°C-acclimated char as a result of the depressed $\dot{M}O_{2\text{standard}}$ in 6°C-acclimated char at 2°C.

4.3.4 Oxygen pulse

The $\dot{M}O_{2\text{max}}$ and $f_{H\text{max}}$ (Chapter 3) were positively correlated for acclimated Arctic char (Figure 4.6a), but $f_{H\text{max}}$ increased with acclimation temperature to a proportionately greater extent than $\dot{M}O_{2\text{max}}$, except between 2°C and 6°C. Thus, the oxygen pulse was 35% lower at 14°C than 2°C (Table 4.3, Figure 4.6b). In 6°C-acclimated Arctic char acutely tested at 2°C to 18°C, the oxygen pulse again progressively decreased (-45% total) with temperature.

4.4 Discussion

With the Canadian Arctic being among the most rapidly warming regions on our planet (Zhang *et al.* 2019a), wild Arctic char already experience prolonged temperature exposures from 0 to 15°C and thus require aerobic capacity, growth and swimming performance over this range. Additionally, they must be able to acutely tolerate a temperature change between -1°C and 21°C (Figures 1.2 and 1.3) (Dick *et al.* 2009; Gilbert *et al.* 2016; Harris *et al.* 2020b). Beyond confirming these expectations, I needed to determine if Arctic char will be able to cope with the ongoing warming. Therefore, I acclimated hatchery-reared Arctic char of a central Canadian Arctic background (Nauyuk Lake, NU) to temperatures ranging from 2-18°C and studied them

not only at their acclimation temperature but also following an acute temperature exposure from a 6°C acclimation temperature. While growth rate, aerobic swimming performance, and AAS either increased or were maintained with acclimation temperatures from 2-14°C, at 18°C feeding ceased over time, SGR declined and nearly 50% mortality accrued. Furthermore, FAS declined markedly with both increasing acclimation and acute test temperature. The poor whole animal performance of 18°C-acclimated Arctic char compares well with the impaired cardiac heat tolerance I observed in Chapter 3. Collectively, my results suggest that this strain (and size class) of Arctic char can only tolerate temperature up to 18°C for brief (a few weeks) but not prolonged periods without mortality or reduced performance.

4.4.1 Growth and delayed mortality

A previous study of small (<50 g) European Arctic char fed *ad libitum* found that growth rate increased steadily with warming from 0°C, reaching a peak between 14 and 17°C, but then growth declined dramatically at higher temperature, and ceased at 20-21°C (Table 1.1)(Larsson *et al.* 2005). In larger (>500 g) European Arctic char fed *ad libitum* the optimum temperature for growth rate was 10-12°C (Imsland *et al.* 2019). Consistent with these earlier works, I found an optimum temperature for growth rate around 12°C. Wild Arctic char, however, commonly select much cooler temperatures during their peak growth period in the summer (5-10°C; Table 1.1; Figure 4.1) even though there is periodically access to warmer temperatures. One possible explanation for this discrepancy between optimal growth and preferred temperatures is that growth efficiency increases with decreasing temperature (Larsson and Berglund 2005; Imsland *et al.* 2019) and wild Arctic char do not have *ad libitum* access to high quality food (foraging comes at a cost). As such, growth in larger Arctic char in natural, food-limited environments would likely be optimized at cooler temperatures than in a laboratory or aquaculture setting.

Previous studies have also characterized prolonged heat tolerance of Arctic char and found that the upper temperature at which feeding ceased was 21.5°C (Thyrel *et al.* 1999) and the incipient upper lethal temperature (that which produces 50% mortality during prolonged exposure) was 21.5-22.7°C (Table 1.1) (Baroudy and Elliott 1994). I found that all fish eventually stopped feeding after 5 weeks at 18°C. While the previous estimates are notably above 18°C, these studies were conducted on smaller (~15-100 g), European Arctic char over a shorter time scale, any or all of which could account for the discrepancies.

Delayed mortality during acclimation was recently observed in adult Atlantic salmon at 22-23°C (Hvas *et al.* 2017; Gamperl *et al.* 2020) and in juvenile, warm-adapted rainbow trout at 25°C (Adams 2020). In the present study, similar delayed mortality was observed in Arctic char at 18°C. In all three species, heat-induced mortality, loss of appetite and growth impairments occurred despite fish having a substantial AAS, although all exhibit a collapse of FAS to between 2-4 fold $\dot{M}O_{2\text{standard}}$ at high temperatures. As such, whether or not reduced aerobic scope contributed to these adverse outcomes depends on how aerobic scope is budgeted among competing demands (Farrell 2016), a possibility that warrants an in-depth discussion here.

To support growth and maintain long term homeostasis (i.e. prevent loss of function and mortality) the available aerobic scope must support all functions a fish undertakes including (but not limited to) routine activity, digestion, growth, and recovery following any anaerobic metabolism. If the aerobic cost of these processes is fixed over a range of temperatures then AAS is a suitable measure of capacity (Figure 4.7a and b). However, if aerobic costs increase exponentially with temperature (Figure 4.7c and d), then FAS is more indicative of available aerobic capacity (Figure 4.7c and d). Previous studies have shown that the peak $\dot{M}O_2$ required for digestion (peak specific dynamic action; SDA) increases exponentially with temperature but

remains about twice $\dot{M}O_{2\text{standard}}$. In the present study I showed that the cost of routine activity ($\dot{M}O_{2\text{mean}} - \dot{M}O_{2\text{standard}}$; Figures 4.4 and 4.5) also increases with temperature. If other aerobic costs such as growth (anabolism above maintenance needs) and exercise recovery also increase then, a limitation in FAS could critically constrain such functions. To demonstrate this, I have produced a conceptual aerobic budget with fixed and exponentially rising aerobic costs over temperature (Figure 4.7). I based this model on data collected here ($\dot{M}O_{2\text{standard}}$, $\dot{M}O_{2\text{mean}}$, $\dot{M}O_{2\text{max}}$), an estimated SDA of twice $\dot{M}O_{2\text{standard}}$ (Farrell 2016; Adams 2020), and a $\dot{M}O_2$ during recovery from anaerobic exercise of 2.4 fold $\dot{M}O_{2\text{standard}}$ (Zhang *et al.* 2018a). If prolonged, these aerobic limitations could result in the need to suppress appetite (and selection for such a behavior) and could also disrupt cellular homeostasis leading to stress and delayed mortality (Hvas *et al.* 2017; Adams 2020).

4.4.2 Swimming performance

In many (but not all) temperate salmonids (e.g. cutthroat trout (*O. clarkii*), and sockeye and Atlantic salmon) U_{crit} has an optimum acclimation temperature at which it is maximized (Brett 1964; Taylor *et al.* 1996; MacNutt *et al.* 2004; Hvas *et al.* 2017). For instance, Hvas *et al.* (2017) found that U_{crit} of Atlantic salmon increased with acclimation temperature from 3 to 13°C, but then remained unchanged up to 18°C before decreasing at 23°C, the only temperature at which delayed mortality was observed. In the present study, a similar pattern existed for U_{gt} versus acclimation temperature, but it only increased up to 10°C and plateaued between 10°C and 14°C; I did not assess U_{gt} in 18°C-acclimated char, but observed mortality during the growth study. In the only other temperature acclimation study with Arctic char (similar size: 312 mm; same origin: Nauyuk Lake, Nunavut), U_{crit} increased by ~8% from 5-10°C (Beamish 1980), whereas I saw a ~17% increase in U_{gt} from 6-10°C. That study found that U_{crit} at 10°C was 2.1bl

s^{-1} , which is identical to the estimate at 10°C for the present study generated by converting to U_{crit} using $U_{crit} = 1.09 \times U_{gt}$ as previously shown for Arctic char at 11°C (Gilbert and Tierney 2018).

Multiple studies have examined acute temperature effects on the swimming performance of Arctic char acclimated to between 10 and 13°C. For example, acute exposure to temperatures ranging from ~ 10 to 21°C did not change U_{crit} in wild Arctic char smolts (199 mm), or U_{crit} and U_{gt} in laboratory-reared Arctic char (201 mm) (Gilbert and Tierney 2018), although exercise recovery and repeat swimming performance was impaired above 20°C. Pettersson *et al.* (2010) found that U_{crit} decreased 24% in 10°C-acclimated Arctic char acutely cooled to 4°C but acute warming to 17°C had no effect on U_{crit} . My results for 6°C-acclimated Arctic char are consistent with these findings as I found that U_{gt} decreased at 2°C but was unchanged when tested at 10 and 14°C. Collectively, strong evidence exists that acute cooling of Arctic char from a common summer acclimation temperature tends to impair sustained swimming performance but acute warming does not generally improve it. Thus, one benefit of thermal acclimation is that it clearly increases the range over which swimming performance can increase with warming (Figure 4.3). This pattern, of course, may vary somewhat depending on the initial acclimation temperature, especially below 6°C, which has yet to be examined.

The relatively low swimming performance of 6°C-acclimated Arctic char tested at 10°C and 14°C likely reflects the benefits of multiple thermal acclimation processes. During cold acclimation (e.g. 0-6°C), swimming musculature is markedly remodeled in many salmonids because lower temperatures constrain physiological rates (Rome *et al.* 1985; Guderley 2004). This remodeling includes changes in membrane composition to increase fluidity, isoform switching in structural and regulatory proteins (e.g. troponin 1), and general elevations in

metabolic enzyme activities (e.g. citrate synthase)(Guderley and Gawlicka 1992; St-Pierre *et al.* 1998; Kraffe *et al.* 2007; Alderman *et al.* 2012; Liu *et al.* 2019). As, I have previously demonstrated for heart function (Chapter 3), such changes can improve performance at cold temperatures (Rome *et al.* 1985) they also can limit performance at warm temperatures (Guderley 2004). While such physiological differences in swimming musculature likely played some role in the differences observed in U_{gt} here, swimming behaviour may have also contributed. When Arctic char were acutely warmed from 6°C they appeared more active or agitated, increasing the time spent above twice $\dot{M}O_{2standard}$, which could reduce the available aerobic scope apportioned to swimming. A future analysis of swimming $\dot{M}O_2$ from the present study will provide insight into how Arctic char are using their available aerobic scope to support swimming, and if indeed, the apparent cost of transport is elevated (i.e. decreased swimming efficiency) in 6°C-acclimated fish when tested at 10 and 14°C.

4.4.3 Oxygen uptake and aerobic scope

In salmonids, and teleost fishes in general, resting $\dot{M}O_2$ increases with acclimation temperature with a Q_{10} around 2.4 (Brett 1964; Clarke and Johnston 1999; Hvas *et al.* 2017) which agrees well with the increase that I observed in $\dot{M}O_{2standard}$ and $\dot{M}O_{2mean}$ with acclimation from 2 to 18°C (Q_{10} : 2.1 and 2.2 respectively). In wild Arctic char captured through the ice at the end of winter, Holeton (1973) found that $\dot{M}O_{2standard}$ at 2°C was approximately half that measured in 2°C-acclimated Arctic char in the present study. These differences may be explained by the fact that food availability and activity is notably limited overwinter in wild Arctic char and indeed many fast through much if not all of the winter (Dutil 1986; Boivin and Power 1990; Jørgensen *et al.* 1997; Hawley *et al.* 2017). Prolonged food deprivation, declining condition factor and activity suppression could drive a suppression of $\dot{M}O_2$, which would conserve energy,

a possibility that certainly warrants further investigation. It should be noted that while the $\dot{M}O_{2\text{standard}}$ at 2°C here was higher than in Holeton (1973), it was similar or below that estimated in other less cold-adapted salmonids (Brett 1964; Clarke and Johnston 1999; Hvas *et al.* 2017) and thus does not provide support for the theory of “metabolic cold adaptation” (Holeton 1973; Holeton 1974).

As discussed with regard to heart rate (Chapter 3), thermal compensation of physiological rates is a common product of thermal acclimation (Gamperl and Farrell 2004; Guderley 2004; Sandblom *et al.* 2014; Keen *et al.* 2017). As a simple example, Evans (1990) acclimated rainbow trout to 10 and 20°C and then acutely warmed or cooled them to the other study temperature. $\dot{M}O_{2\text{standard}}$ was initially depressed with acute cooling but increased during cold acclimation. Conversely, $\dot{M}O_{2\text{standard}}$ was excessively high following acute warming but decreased during warm acclimation. This thermal compensation is thus apparent as a reduction in Q_{10} temperature coefficients following acclimation, i.e., partial thermal compensation (Sandblom *et al.* 2014). I observed a similar partial thermal compensation for $\dot{M}O_{2\text{standard}}$ and $\dot{M}O_{2\text{mean}}$ (Acute vs. Acclimated Q_{10} 3.1-3.5 vs. 2.1-2.2) and possibly for $\dot{M}O_{2\text{max}}$ and fish activity/agitation (time spent above twice $\dot{M}O_{2\text{standard}}$) at warm temperatures, although the effect of acclimation was not statistically significant for the latter two (Figure 4.4). Despite this thermal compensation AAS and FAS were not notably different between Arctic char at their acclimation temperature and those acclimated to 6°C and acutely exposed to each temperature, except at 2°C where 6°C-acclimated char had a higher FAS because of their low $\dot{M}O_{2\text{standard}}$.

The relationship between temperature and AAS varies among, and even within salmonid species (see Figure 6.5 for synthesis)(Brett 1964; Farrell 2009; Clark *et al.* 2011; Verhille *et al.* 2016; Eliason and Anttila 2017; Hvas *et al.* 2017; Abe *et al.* 2019). For instance, thermal

specialists like sockeye salmon exhibit a pronounced peak in AAS, indicating a clear optimal temperature (T_{opt}) (Eliason *et al.* 2011; Eliason *et al.* 2013), while others including some strains of rainbow trout and Atlantic salmon have flatter curves, a lower peak AAS and a less apparent T_{opt} (see Figure 6.5; Verhille *et al.* 2016; Hvas *et al.* 2017). The present study suggests that Arctic char are more of a thermal generalist in this regard, albeit with left-shifted thermal performance curves (Figure 6.5); $\dot{M}O_{2max}$ and AAS both increased by ~27% with acclimation temperature from 2 to 6°C and then plateaued between 6 to 14°C. Furthermore, while it is evident that some fish could not tolerate prolonged exposure to 18°C and died ($\dot{M}O_{2max}$ and AAS=0), the surviving fish still exhibited a notable spontaneous $\dot{M}O_{2max}$ and AAS (spontaneous AAS at 18°C was ~80% of AAS at 14°C). In fact, based on the model for AAS (Table 4.2), with temperature acclimation Arctic char can maintain 90% of their peak AAS between 5.2 and 17.8°C ($\Delta 12.6^\circ\text{C}$). In contrast, sockeye salmon AAS typically falls to below 90% peak AAS within ~3°C of the population's T_{opt} (T_{opt} : 14.5 to 17.2°C) (Eliason *et al.* 2011).

Eliason *et al.* (2013) found that stroke volume (SV) during maximal exercise in sockeye salmon decreased with increasing temperature while arteriovenous oxygen extraction remained constant or increased. Similarly, Farrell *et al.* (1996) found that the maximum SV of *in situ* perfused trout hearts declined by 2.5% per °C with warming from 14°C to 22°C. In the present study, oxygen pulse (oxygen uptake per heart beat) at $\dot{M}O_{2max}$ was calculated to provide insight into how, beyond f_H (Chapter 3), the other components of the Fick equation for $\dot{M}O_2$ (i.e. SV and arteriovenous oxygen extraction) influence $\dot{M}O_{2max}$ with rising temperatures. This analysis revealed that during acute and prolonged warming the relative increase in f_{Hmax} was greater than the corresponding elevation in $\dot{M}O_{2max}$. As such, the oxygen pulse declined by ~2.8% per °C, which indicates that SV and/or arteriovenous oxygen extraction declined. Given the similarity

with past research in trout and salmon, I would expect that the observed decline in oxygen pulse was also the result of a decline in SV (Farrell *et al.* 1996; Eliason *et al.* 2013).

4.4.4 Conclusions

Growth, swimming performance and AAS all increased or were maintained over the entire range of temperatures (~0-14°C) that Arctic char currently encounter for prolonged periods in the central Canadian Arctic (Table 1.1; Figure 1.2, 1.3, 4.1). As such these prolonged thermal performance relationships seem well suited to the current thermal ecology of Arctic char in this region. However, given how dramatically Arctic char health can decline from 14 to 18°C, I would expect that their prolonged thermal limits may already be tested at the southern limits of their distribution, and in the near future at higher latitudes as climate change progresses.

Together with my findings from Chapter 3, my results here regarding swimming performance and partial thermal compensation of $\dot{M}O_2$ suggest that thermal acclimation may meaningfully influence physiological performances in wild Arctic char as they naturally encounter distinct thermal regimes throughout their lives (Figure 4.1). For instance, together with my findings regarding cardiac heat tolerance (Chapter 3), the fact that aerobic swimming performance markedly increased with warm acclimation (vs. acute exposure) suggests that acclimation prior to upstream migration through warm rivers may influence migration duration and success. Further research is needed to determine the time course for such acclimatory responses to occur and whether or not Arctic char exploit available thermal variation in a manner that would facilitate acclimation.

Table 4.1 Arctic char body size, condition factor, and specific growth rate (SGR) following acclimation to environmentally relevant temperatures. Data are presented as mean±SE and dissimilar superscript letters for SGR indicate significant difference between acclimation temperatures.

	2°C	6°C	10°C	14°C	18°C
Sample size (n)	10	10	10	10	12
Fork Length (mm)	336±6	342±6	361±7	356±7	324±4
Mass (g)	438±30	471±25	529±36	564±46	393±26
Condition (K)	1.16±0.03	1.16±0.04	1.11±0.03	1.23±0.06	1.17±0.00
SGR (% day⁻¹)	0.61±0.03 ^a	0.71±0.03 ^a	0.91±0.08 ^{ab}	1.27±0.2 ^b	0.65±0.1 ^a

Table 4.2 Parameters for models describing the effect of temperature on Arctic char specific growth rate (SGR), oxygen uptake rates ($\dot{M}O_2$) and absolute aerobic scope (AAS). $\dot{M}O_2$ models were constructed separately for Arctic char tested at their acclimation temperature or 6°C-acclimated char acutely tested at each experimental temperature.

Growth	Amplitude (% day⁻¹)	T_{opt} (°C)	Width (°C)	R²	df		
SGR (Full dataset)	1.2±0.10	12.2±0.8	8.6±1.6	0.23	49		
SGR (Outliers excluded)	1.12±0.07	12.7±0.5	9.2±1.2	0.42	46		
<i>SGR = Amplitude / (1 + ((T - T_{opt}) / Width)²)</i>							
<i>SGR: Specific growth rate (% day⁻¹)</i>							
Acclimated $\dot{M}O_2$	a₀	a₁	a₂	R²	df	F	p
$\dot{M}O_{2\text{standard}}$	28.1±5.37	2.0±1.3	0.10±0.07	0.79	43	82.9	<0.001
$\dot{M}O_{2\text{mean}}$	31.8±7.68	2.3±1.9	0.19±0.09	0.80	43	87.0	<0.001
$\dot{M}O_{2\text{max}}$	221.0±31.6	24.4±9.4	-0.94±0.58	0.39	37	11.7	<0.001
AAS	200.2±33.0	19.3±9.9	-0.83±0.6	0.21	37	4.9	0.013
Acute $\dot{M}O_2$							
$\dot{M}O_{2\text{standard}}$	22.7±6.4	0.7±1.5	0.30±0.07	0.90	38	188.5	<0.001
$\dot{M}O_{2\text{mean}}$	21.6±13.7	1.7±3.2	0.43±0.16	0.84	39	102.3	<0.001
$\dot{M}O_{2\text{max}}$	235.1±25.0	16.4±5.9	-0.23±0.28	0.66	39	38.5	<0.001
AAS	214.5±23.0	14.8±5.4	-0.47±0.26	0.36	39	11.0	<0.001
<i>$\dot{M}O_2 = a_0 + a_1T + a_2T^2$</i>							
<i>$\dot{M}O_2$: oxygen uptake (mgO₂ kg⁻¹ h⁻¹)</i>							
<i>T: Temperature (°C)</i>							

Table 4.3 Outputs from two-way analyses of variance (ANOVAs) characterizing the effect of temperature (2, 6, 10, 14 and 18°C), acclimation status (acclimated vs. acutely exposed), and their interaction with specific growth rate (SGR), gait transition speed (U_{gt}), and variables related to oxygen uptake ($\dot{M}O_2$). A p-value in bold font indicates significant effects ($\alpha = 0.05$).

	Temperature			Acclimation status			Interaction		
	F	df	p	F	df	p	F	df	p
SGR (% day ⁻¹)	6.1	4,47	<0.001						
U_{gt}	41.6	3,41	<0.001	22.4	1,41	<0.001	10.7	3,41	<0.001
$\dot{M}O_{2standard}$	193.9	4,77	<0.001	0.1	1,77	0.701	10.4	4,77	<0.001
$\dot{M}O_{2mean}$	135.1	4,77	<0.001	3	1,77	0.086	6.4	4,77	<0.001
$\dot{M}O_{2max}$	20.9	4,77	<0.001	4.4	1,77	0.039	2.2	4,77	0.076
AAS	7.1	4,77	<0.001	3.4	1,77	0.069	1.3	4,77	0.280
FAS	47.8	4,77	<0.001	4.9	1,77	0.030	6.3	4,77	<0.001
%>2x $\dot{M}O_{2standard}$	15.1	4,77	<0.001	2.9	1,77	0.095	1.2	4,77	0.320
O_2 pulse	37.8	4,77	<0.001	0.6	1,77	0.459	1	4,77	0.427

Figure 4.1 Temperature frequency distributions for Arctic char lake, marine, and river habitats in the central Canadian Arctic. Lake temperatures were recorded year round in three lakes in the Cambridge Bay Area used by Arctic char for early rearing, spawning and overwintering. Arctic char marine body temperatures were obtained via acoustic telemetry while fish were feeding in the Kitikmeot Sea (Harris *et al.* 2020b). Mean river temperatures during migratory periods were compiled from available data between 2012 and 2019 and the single warmest river temperature distribution (Nulahugyuk, 2012) is also presented.

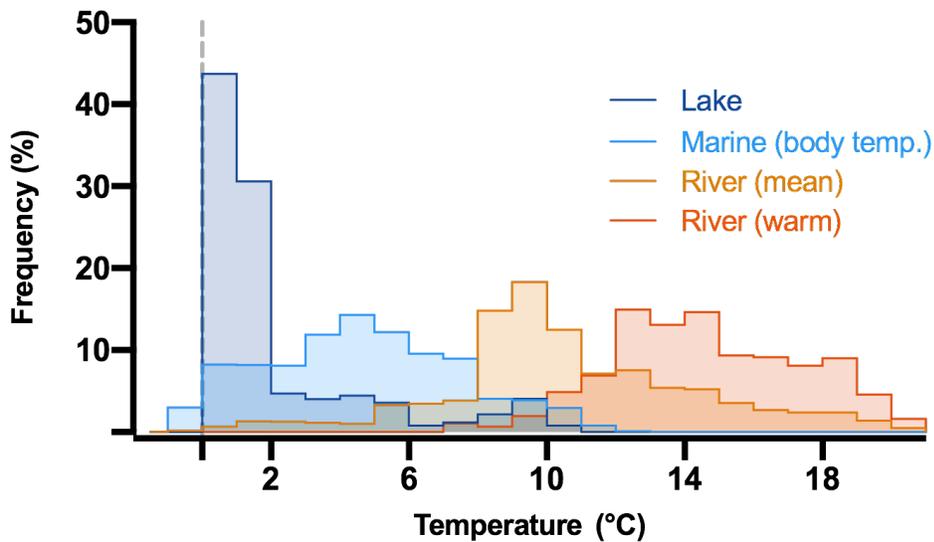


Figure 4.2 The effect of acclimation temperature on growth rate (a) and mortality (b) in captive-reared Arctic char fed ad libitum. Median (\pm interquartile range; $n=10$ per temperature except at 18°C , $n=12$) and individual (light grey points) specific growth rate over a 6-8 week temperature acclimation period are shown along with the thermal performance model for growth (black line) and its 95% confidence interval (grey bounds). The model (details found in Table 5.2) estimated optimal growth temperature as 12.7°C . Dissimilar letters or numbers indicate significant differences between acclimation temperatures with (letters) or without (numbers) outliers (orange circles) included in the analysis. Mortality data are only included for 18°C (b) because no mortality occurred at any of the other acclimation temperatures.

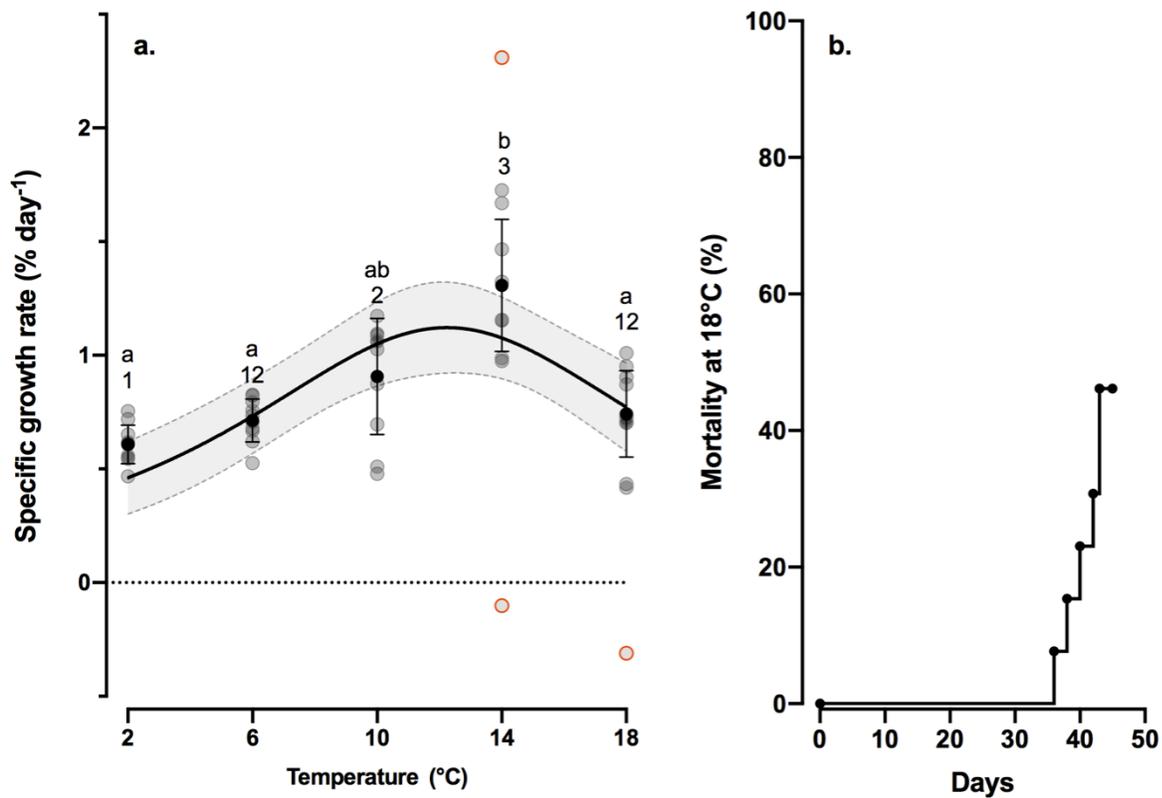


Figure 4.3 The effect of thermal acclimation and acute transfer from 6°C on maximum aerobic swimming performance (gait transition speed, U_{gt}) of Arctic char over ecologically relevant test temperatures (2, 6, 10 and 14°C). Dissimilar letters or numbers indicate significant differences between temperatures within acclimated or acutely exposed Arctic char, respectively. An asterisk indicates a significant difference between thermally acclimated and acutely exposed Arctic char at a common temperature at which U_{gt} was assessed.

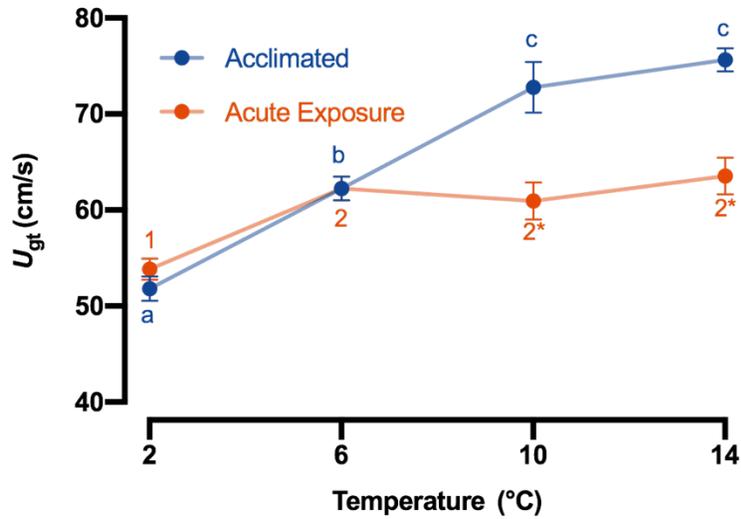


Figure 4.4 The effect of thermal acclimation on oxygen uptake ($\dot{M}O_2$) and aerobic scope in Arctic char at ecologically relevant temperatures. Data are shown at five temperatures (2, 6, 10, 14, and 18°C) following acclimation or acute transfer from 6°C. Standard $\dot{M}O_2$ ($\dot{M}O_{2\text{standard}}$), the 24 to 48 h mean $\dot{M}O_2$ ($\dot{M}O_{2\text{mean}}$) and maximum $\dot{M}O_2$ ($\dot{M}O_{2\text{max}}$) are presented with the percentage of time spent above $2 \times \dot{M}O_{2\text{standard}}$ shown as a measure of activity or agitation. Absolute aerobic scope (AAS: $\dot{M}O_{2\text{max}} - \dot{M}O_{2\text{standard}}$) and factorial aerobic scope (FAS: $\dot{M}O_{2\text{max}} / \dot{M}O_{2\text{standard}}$) are presented as measures of aerobic capacity. Open, unconnected symbols indicate that acclimated fish were not chased to exhaustion at 18°C. Dissimilar letters or numbers indicate significant differences between temperatures in acclimated or acutely exposed char respectively. An asterisk indicates a significant difference between acclimated and acutely exposed char at a common test temperature.

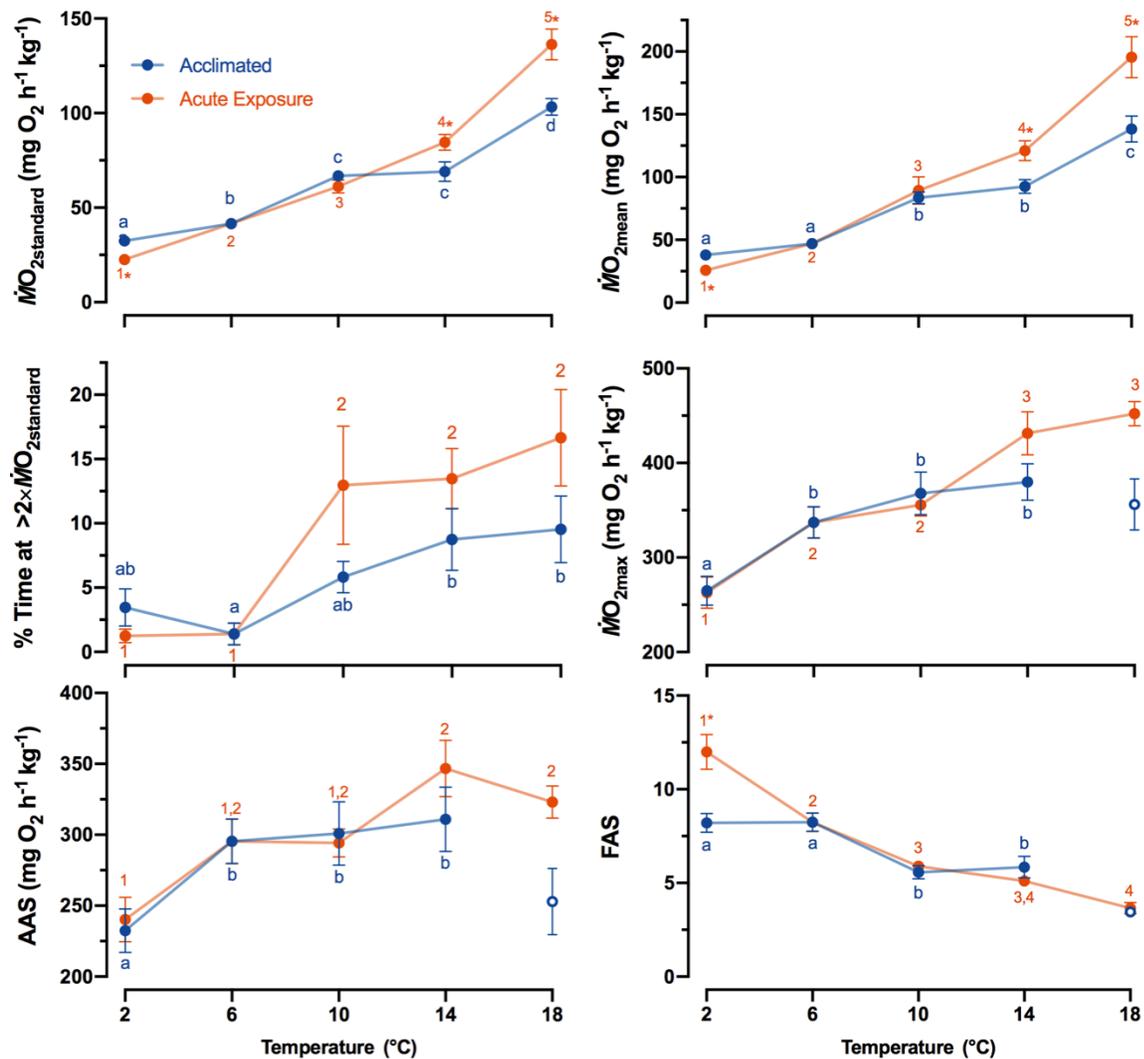


Figure 4.5 Thermal performance curves (TPCs) for oxygen uptake ($\dot{M}O_2$) and absolute aerobic scope in Arctic char acclimated or acutely exposed (from 6°C) to ecologically relevant temperatures. Second order polynomial TPCs are presented for standard $\dot{M}O_2$ ($\dot{M}O_{2\text{standard}}$), the 24 to 48 hr mean $\dot{M}O_2$ ($\dot{M}O_{2\text{mean}}$), maximum $\dot{M}O_2$ ($\dot{M}O_{2\text{max}}$), and absolute aerobic scope ($\dot{M}O_{2\text{max}}$ - $\dot{M}O_{2\text{standard}}$). Model parameters are presented in Table 5.2. Open, unconnected symbols indicate that acclimated fish were not chased to exhaustion at 18°C and were not included in the TPC analyses. Dissimilar letters or numbers indicate significant differences between temperatures in acclimated or acutely exposed char respectively.

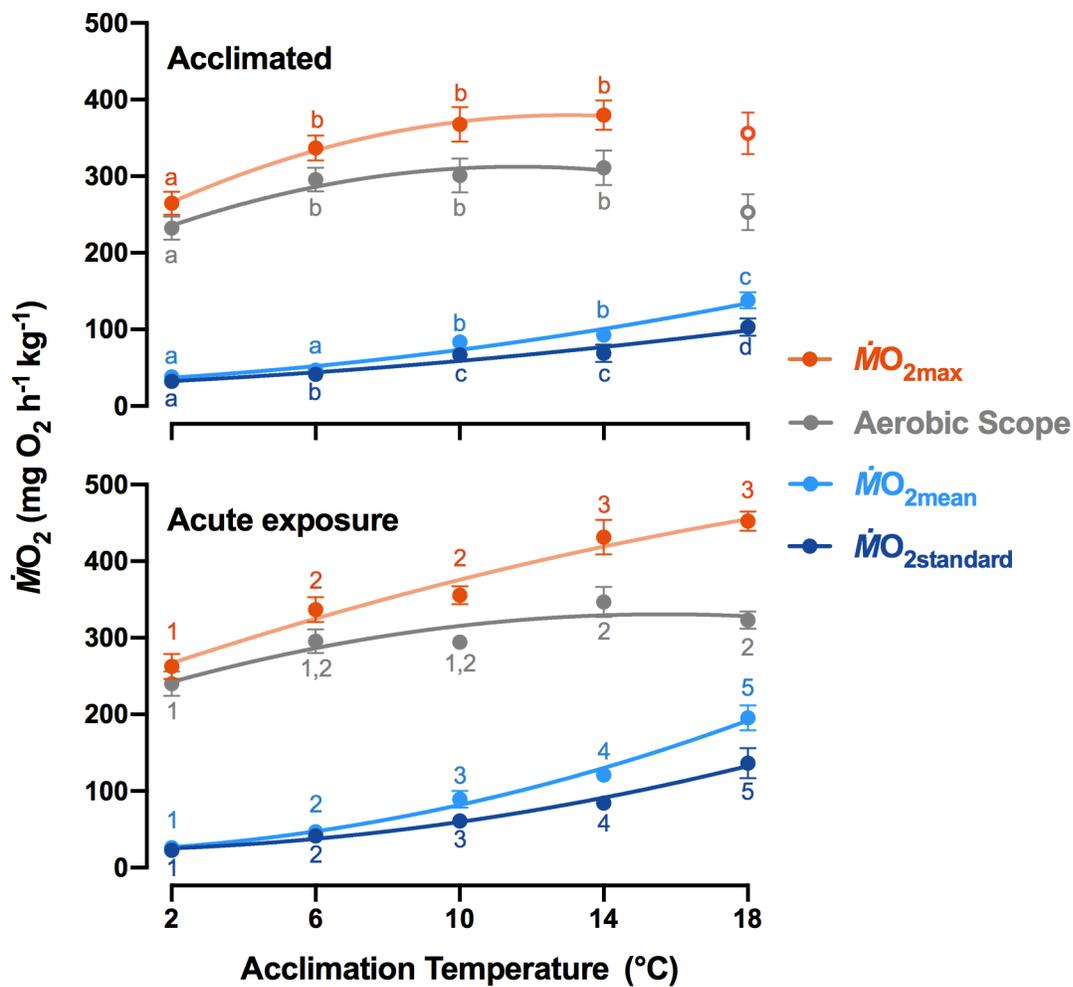


Figure 4.6 The relationship between maximum heart rate ($f_{H_{max}}$) and maximum oxygen uptake ($\dot{M}O_{2max}$) in Arctic char acclimated or acutely exposed (from 6°C) to ecologically relevant temperatures. (a) Maximum $\dot{M}O_2$ ($\dot{M}O_{2max}$) are compared to estimates of $f_{H_{max}}$ at each temperature (2, 6, 10, 14, and 18°C). $f_{H_{max}}$ was measured in separate experiments on the same cohorts of fish (Chapter 3). (b) Oxygen pulse (O_2 pulse; $\dot{M}O_{2max}/f_{H_{max}}$) was calculated as a measure of stroke volume and arteriovenous oxygen extraction based on the Fick equation for $\dot{M}O_2$. Dissimilar letters or numbers indicate significant differences between temperatures in acclimated or acutely exposed char respectively. Open, unconnected symbols at 18°C indicate that fish were not exercised to exhaustion.

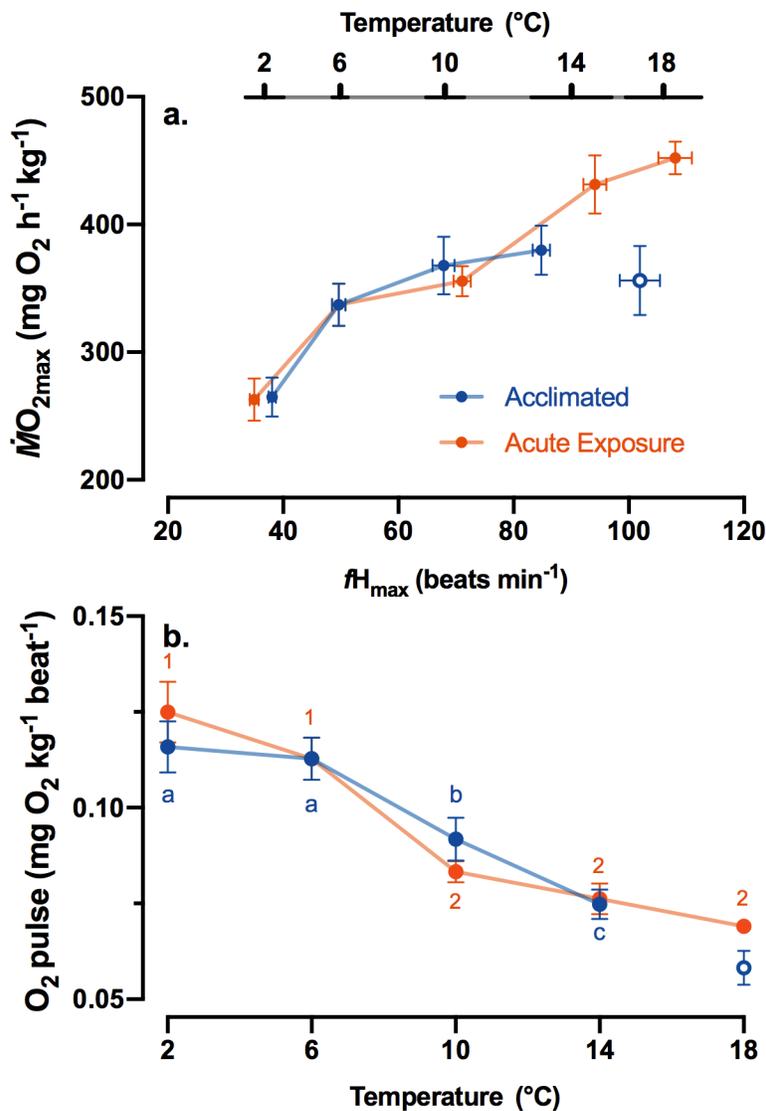
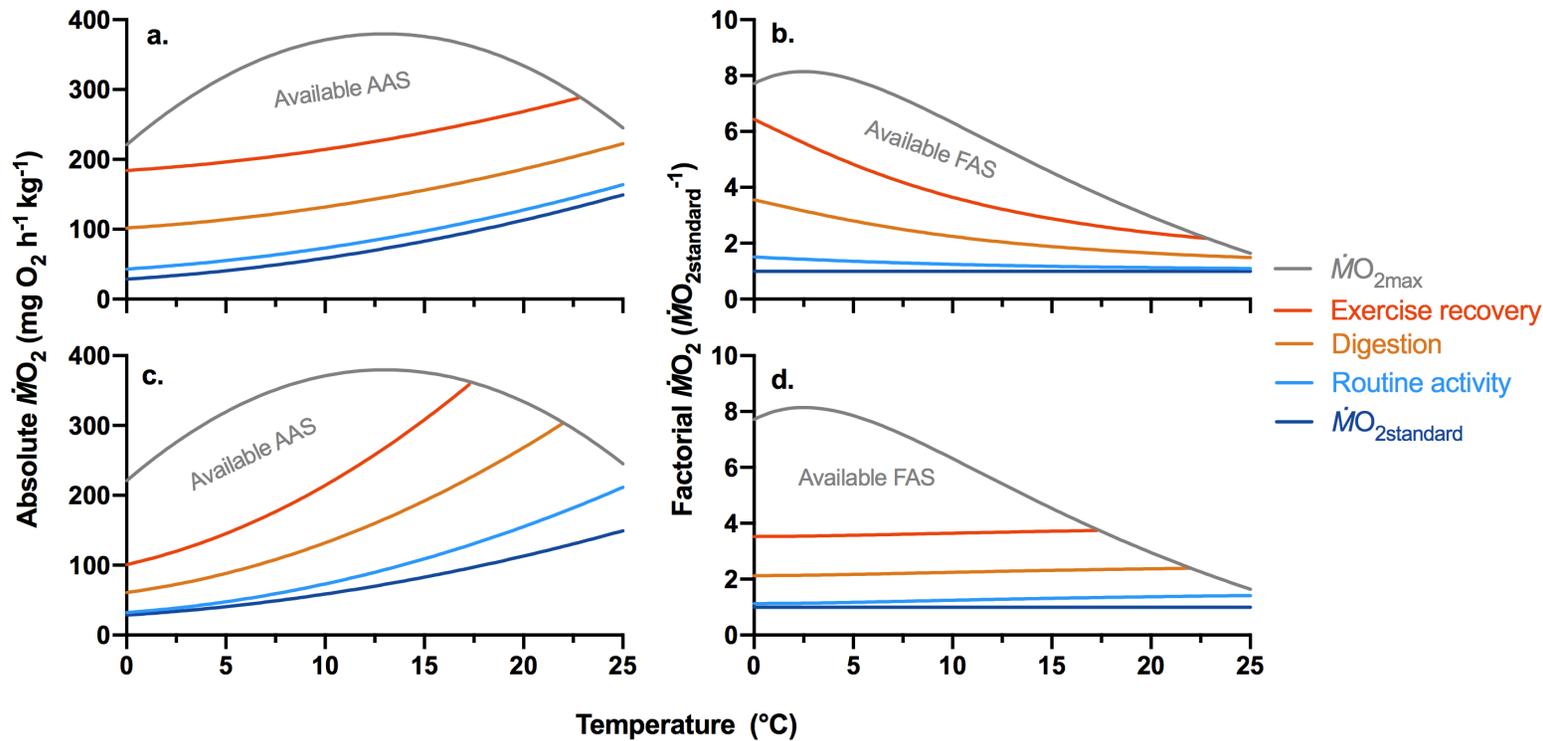


Figure 4.7 Conceptual aerobic budget for common processes during prolonged exposure to environmentally relevant temperatures. $\dot{M}O_2$ is expressed in absolute units (a and c) and relative to $\dot{M}O_{2\text{standard}}$ (b and d). Models for $\dot{M}O_{2\text{max}}$ and $\dot{M}O_{2\text{standard}}$ are from Arctic char acclimated from 2 to 18°C in the present study (Table 5.2). $\dot{M}O_{2\text{mean}}$ from the same fish was used as an estimate for $\dot{M}O_2$ during routine activity. $\dot{M}O_2$ during digestion and exercise recovery were estimated as twice and 2.4-times $\dot{M}O_{2\text{standard}}$, respectively, based on Adams (2020) and Zhang *et al.* (2018a). To simulate aerobic costs that are fixed over temperature (a and b), a single estimate of the cost above $\dot{M}O_{2\text{standard}}$ for each process was generated at 10°C and added sequentially to the temperature- $\dot{M}O_{2\text{standard}}$ model. To simulate aerobic costs that increase with temperature (c-d), the cost above $\dot{M}O_{2\text{standard}}$ were modeled over all temperatures and added sequentially to the temperature- $\dot{M}O_{2\text{standard}}$ model.



Chapter 5: Population-specific cardiorespiratory and thermal physiology of migratory Arctic char in a rapidly warming north

5.1 Introduction

Within salmonid species (family: Salmonidae), population differences in behaviour, morphology and physiology are common (Taylor 1991; Carlson and Seamons 2008; Fraser *et al.* 2011). Such differences often confer a fitness advantage under local environmental conditions and can arise through local adaptation (heritable changes arise over multiple generations) and phenotypic plasticity (either acclimation/acclimatization over days to weeks or developmental changes). For instance, through local adaptation, sockeye salmon from populations that undertake long, physically and thermally challenging upriver migrations tend to have larger hearts, a greater proportion of compact myocardium, higher aerobic scope and more fusiform bodies than those from coastal populations, which undertake shorter migrations (Crossin *et al.* 2004; Eliason *et al.* 2011). Similarly, inland coho salmon that have longer migrations have greater swimming performance than coastal coho salmon (Taylor and McPhail 1985). Local migratory thermal regimes can also drive adaptive changes in thermal physiology. For example, adult sockeye and Atlantic salmon from populations that typically experience higher temperatures during their spawning migrations have higher upper thermal limits for aerobic scope and cardiac performance (Eliason *et al.* 2011; Gradil *et al.* 2016). The prevalence of local adaptation to migratory environments in anadromous Pacific salmonids is thought to stem from the substantial variation in migratory conditions among populations and the fact that their

lifetime reproductive success is entirely contingent on the completion of their spawning migration (Farrell *et al.* 2008b).

While local adaptation in salmonids is common, environmentally driven phenotypic plasticity can also give rise to similar beneficial phenotypic variation. In theory, sufficiently rapid phenotypic plasticity can lessen or remove local selective pressures by permitting adequate behaviour and physiological performance over a broader range of environmental conditions. For example, cardiac thermal tolerance did not differ between a northern and southern strain of Atlantic salmon despite the fact that they likely encounter dramatically different thermal environments. Instead, both strains were able to markedly improve cardiac heat tolerance with warm acclimation (Anttila *et al.* 2014a). Evidence for adaptation to local migratory conditions does exist in Atlantic salmon (Taylor 1991; Fraser *et al.* 2011; Gradil *et al.* 2016), however, the underlying selective pressures may be relaxed for Atlantic salmon and other iteroparous (repeat spawning) species such as Arctic char, as they may have multiple opportunities to attempt to spawn on their natal spawning grounds.

As in other salmonids, upstream migratory distances, elevation gains and thermal regimes differ drastically among Arctic char populations (Figure 1.3, 2.6 and 5.1; Table 5.1; Moore *et al.* 2017). Furthermore, in the central Canadian Arctic, discharge in coastal rivers is typically dominated by snowmelt (i.e., nival flow regimes) and commonly declines exponentially as summer progresses (Church 1974; Déry *et al.* 2016). In extreme cases, this decline in water flow renders some migratory routes impassible at the time when Arctic char would normally return to freshwater following their summer marine feeding period; returning Arctic char can then become stranded (Gilbert *et al.* 2016). Arctic char appear to have evolved two behavioural adaptations

that help them avoid such challenging and sometimes impassable migratory conditions. First, in non-spawning years Arctic char, reduce their natal fidelity and instead overwinter in more accessible non-natal freshwater systems rather than face challenging migrations in their natal watershed. This behaviour creates a less energetically and physically demanding migration that maximizes their marine feeding window and thus likely improves over-winter condition and survival (Moore *et al.* 2017). Second, when they do return home to spawn, Arctic char with long migrations or those prone to low water levels may do so far earlier than other populations that have a less difficult river migration (Johnson 1989; Gyselman 1994; Gilbert *et al.* 2016; Moore *et al.* 2017). This strategy reduces their risk of stranding in excessively shallow water later in the summer while still affording them the benefits of homing (e.g., access to a proven spawning area).

These putative behavioral adaptations to challenging migratory environments may be paralleled by adaptations of their cardiorespiratory and thermal physiology that would facilitate migration success under challenging conditions (e.g., high temperature and low flows), as previously found in other salmonids. Indeed, numerous (~90) single nucleotide polymorphisms (SNPs) were recently identified among Arctic char populations and were associated with characteristics of their local migratory environments (Moore *et al.* 2017). Further, some of these SNPs fell near or within genes involved in glucose and glycogen metabolism (e.g. *glycogen phosphorylase muscle form* and *fructose-1,6-bisphosphatase 1-like*) and in cardiac development and function (e.g. *Myocyte-specific enhancer factor 2C* and *nebulette*). These genetic-environment associations point toward the possibility of local adaptation of metabolic and cardiac function.

Despite this known genetic and behavioural variation associated with migratory environments no study has previously examined the prevalence of corresponding functional differences in physiology among populations. To this end, I investigated the cardiorespiratory and thermal physiology of four populations Arctic char selected because they undertake migrations of varying difficulty. My general hypothesis was that cardiorespiratory and thermal physiology would vary among populations in manner that reflects differences in their migratory environments. As such, I predicted that Arctic char from populations that undergo strenuous migrations through long, likely thermally variable rivers (Palik and Halokvik Rivers) may have larger hearts, improved aerobic performance and greater heat tolerance relative to those that undertake less challenging migrations through more thermally stable rivers (Ekalluktok and Jayko Rivers).

5.2 Methods

5.2.1 Study area and fish capture

All sampling and animal use was approved by Fisheries and Oceans Canada Freshwater Institute (AUP 2018-046, AUP 2017-039, LFSP S-18/19 1030-NU, LFSP S-17/18 1023-NU). The cardiorespiratory and thermal physiology of Arctic char was characterized *in situ* at four river sites on the south coast of Victoria Island, NU in the central Canadian Arctic (Figure 5.1). The site locations were selected based on differences in local migratory conditions (Table 5.1) and associated genetic differences among local populations (Moore et al., 2017). Specifically, rivers at Palik and Halokvik were chosen because navigation upstream requires strenuous swimming over a long distance, while the Ekalluktok and Jayko rivers involve much shorter migrations (see Table 5.1). In addition to being the shortest river, Jayko is also more

geographically isolated from the others (Figure 5.1.). The “migratory harshness” of each river was characterized, as previously described (Eliason *et al.* 2011; Moore *et al.* 2017), by using the minimum migration distance, elevation gain, and the product of the two, which was considered a measure of total migration work. River temperatures were recorded continuously for the duration of experimentation at each site (Table 5.1). August 2018 was unseasonably cold and thus 2018 river temperatures may not be representative of other years.

For the purpose of this chapter, Arctic char captured at each site are assumed to be native to the freshwater system nearest their site of capture. Arctic char were caught by angling and with 139 mm mesh gill nets set along the shoreline at the mouth of Palik (in 2018), and Ekalluktok (in 2017) and the outflow of Jayko (in 2018), while angling and a commercial weir were used at the mouth of Halokvik in 2017 as previously described (Moore *et al.* 2017). Following capture, Arctic char were held for up to 96 h in mesh pens submerged near the capture site until experimentation began.

5.2.2 Respirometry, critical thermal maximum and maximum heart rate assessment

Respirometry and maximum heart rate experiments were conducted as described in Chapter 2 with only a few notable differences. The mobile laboratory used in Chapter 2 was only available at the Palik site, and so experiments at the other sites were conducted in a canvas wall-tent (2.4 x 3.0 or 3.0 x 3.7 m) with a small generator (2000W, Honda, Tokyo, JP) and a custom, mobile solar power system (Newo Global Energy, Camrose, AB). Given these infrastructure limitations, equipment to precisely control temperature over prolonged periods was only available at Palik. As such respirometry experiments were conducted at temperatures as narrow

as possible (range: 8.1-12.1°C; mean±SD = 10.2±1.1°C) and that were readily achieved through a combination of cooling with ambient water and periodic heating with submersible 800W titanium aquarium heaters (Finnex, Countryside, IL). The assessment of CT_{\max} following respirometry was conducted inside the respirometers exactly as described in Chapter 2.

As temperature control was limited, the assessment of maximum heart rate ($f_{H\max}$) during acute warming began at ~7-11°C rather than ~5°C as in Chapter 2 except at Palik, where warming began at 1°C (n=9) and a subset of individuals were started at 10°C (n=4) to verify that the starting temperature did not alter the outcome. By beginning the warming at a higher temperature, the first Arrhenius break-point temperature could not reliably be identified for every individual as in Chapter 2. Instead, the temperature at which the 2°C incremental Q_{10} permanently fell below 1.5 ($T_{q10<1.5}$) was used as the marker of the decline in slope between $f_{H\max}$ and temperature during acute warming. An incremental Q_{10} of 1.5 was chosen because it was intermediate to the Q_{10} at the start of warming (~2) and that at peak $f_{H\max}$ ($Q_{10} = 1$; Figure 5.3).

5.2.3 Analysis

Statistical analyses were conducted in R Studio (R Core Team 2014) and data presentation was done using Prism v.8.3 (GraphPad Software, San Diego, USA). Overall, body mass varied from 1031 g to 6776 g. Therefore, to account for allometric scaling, ventricular mass, atrial mass, $\dot{M}O_{2\min}$ and $\dot{M}O_{2\max}$ were all adjusted to a common body mass, as described in chapter 2 and 4, by summing the residual values from the respective log-log(body mass) linear regression with the predicated value at the average mass of 3.5 kg. This adjustment was done

prior to the calculation of all mass-specific $\dot{M}O_2$ values and relative ventricular (RVM) and atrial masses (RAM) as a percentage of the common body mass (3.5 kg). Mass-specific $\dot{M}O_2$ values were presented to allow for comparisons among studies where appropriate (e.g. similar methods and fish sizes).

Differences in body mass, fork length, Fulton's condition factor (K ; $\text{mass} \times \text{length}^{-3} \times 10^5$), RVM and RAM among populations were assessed by analysis of variance (ANOVA) followed by Holm-adjusted pairwise comparisons. Differences in oxygen uptake variables ($\dot{M}O_{2\text{Min}}$, $\dot{M}O_{2\text{Max}}$, AAS and FAS), CT_{max} , cardiac transition temperatures ($T_{q10 < 1.5}$, T_{peak} , and T_{arr}), and peak $f_{H\text{max}}$ among populations were assessed by ANOVA with body mass included as a covariate. Because there was a much greater size range than in previous chapters, mass was retained as a covariate for these analyses in the present Chapter to show the effect of body mass on each variable (Table 5.2). If population was significant as an explanatory variable then Holm-adjusted pairwise contrasts of least-squares means were performed to account for the effect of mass. Potential effects of migratory harshness (distance, elevation, or work) were not assessed statistically as there was insufficient replication along this axis (i.e., $n=4$) to draw impactful conclusions. Instead, patterns of differences among populations are interpreted relative to aspects of their migratory environment.

Principal components analysis (PCA) was conducted on the correlation matrix of traits related to cardiac physiology including RVM, RAM, peak $f_{H\text{max}}$, $T_{q10 < 1.5}$, T_{peak} , and T_{arr} . The first and second principal components (PC) were plotted against each other and 68% confidence limit ellipses were plotted for Arctic char from each population to show an estimate of one standard

deviation around the centre of the data. Differences in PC1 and PC2 among populations were also assessed by ANOVA and holm-adjusted pairwise comparisons.

5.3 Results

5.3.1 Gross morphology, condition and heart size

Body mass and fork length differed among all four Arctic char populations, being greatest in Halokvik followed by Ekalluktok, Jayko and then Palik (Table 5.1 and 5.2). The greatest K was in Arctic char from Ekalluktok followed by Halokvik, and was similar for Jayko and Palik Arctic char. The RVM for Arctic char was significantly greater in Halokvik and Palik (+14% and +17%) than that for Ekalluktok (Table 5.1 and 5.2), while RAM was significantly greater in Palik Arctic char compared with all other populations (+25 to +33%; Table 5.1 and 5.2).

5.3.2 Respirometry and CT_{max}

The overall mean (\pm SE) $\dot{M}O_{2min}$ was 81.3 ± 2.8 mg kg⁻¹ h⁻¹. While the ANOVA indicated that $\dot{M}O_{2min}$ differed among populations, post-hoc analysis revealed no significant pairwise differences (Table 5.2, Figure 5.2). The $\dot{M}O_{2max}$ was greatest for Palik and Halokvik Arctic char, intermediate in Ekalluktok, and lowest for those from Jayko (-20% vs. Palik; Figure 5.2). The AAS was numerically similar for Palik, Halokvik and Ekalluktok Arctic char, and numerically lowest for those from Jayko (-17-20%); a statistically significant difference existed only between Halokvik and Jayko. Mean (\pm SE) FAS was 4.1 ± 0.2 and did not differ significantly among Arctic char populations (Table 5.2).

The overall mean (\pm SE) CT_{\max} for Arctic char was 20.3 ± 0.2 and CT_{\max} was highest for those from Halokvik, intermediate for Palik and Ekalluktok, and lowest for Jayko Arctic char; the maximum difference of 2.1°C (10%) was between Jayko and Halokvik (Figure 5.4d and e).

5.3.3 $f_{H\max}$ and cardiac thermal tolerance

Mean $f_{H\max}$ for Arctic char at 12°C , a common migration temperature, was 69.0 ± 0.7 beats min^{-1} and did not differ among populations (Table 5.2). As expected, acute warming increased $f_{H\max}$ and all but one individual (39 of 40) displayed a clear peak or plateau in $f_{H\max}$ (Figure 5.3a-e), as indicated by Q_{10} decreasing below 1.0 in all populations (Figure 5.3f), before the heartbeat became arrhythmic. The $T_{q_{10}<1.5}$ was significantly greater for those from Halokvik than from Palik and Jayko, and was intermediate for Ekalluktok Arctic char (Table 5.2, Figure 5.4a and e). Peak $f_{H\max}$ was greatest in Arctic char from Halokvik and Ekalluktok, intermediate for Palik and lowest for those from Jayko; the maximum difference of 18 beats min^{-1} (17%) was between Jayko and Halokvik (Table 5.2, Figure 5.4f). The T_{peak} followed a similar pattern, but was significantly higher for Halokvik and Ekalluktok than for Palik Arctic char, and T_{peak} for Jayko Arctic char was significantly lower than those from all other populations; the maximum difference of 3.9°C (18%) was again between Jayko and Halokvik (Table 5.2, Figure 5.4c and e). The T_{arr} was similar for Palik, Halokvik, and Ekalluktok Arctic char and was 2.3 to 3.7°C (10-15%) lower for Jayko Arctic char (Figure 5.4b and e).

Population specific differences in cardiac physiology are apparent by the separation of each population in the principle components analysis and by significant differences in PC1 and PC2 among populations (Figure 5.5). Most notably, Arctic char in Halokvik had a markedly different cardiac thermal tolerance phenotype than those in Jayko. Variation in PC1 was largely

driven by differences in cardiac heat tolerance while variation in PC2 was largely due to heart mass, which is apparent in the component loadings (Table 5.3). Note that only PC1 and PC2 had Eigenvalues >1.

5.4 Discussion

The estimated work (distance x elevation) required for Arctic char to complete their annual upstream migration in the central Canadian Arctic can differ by more than two orders of magnitude among populations (Table 5.1; Moore *et al.* 2017). Furthermore, migrating Arctic char from some populations have already encountered extreme heat and thermal variation (Gilbert *et al.* 2016; Gilbert *et al.* 2020). The present study demonstrates that, like other salmonids (Taylor 1991; Crossin *et al.* 2004; Eliason *et al.* 2011; Fraser *et al.* 2011; Abe *et al.* 2019), Arctic char from different populations possess a population-specific cardiorespiratory and thermal physiology that appears to reflect differences in their local migratory environments (Table 5.4 and Figure 5.5). The most compelling evidence for this association is that Arctic char from Halokvik had a markedly higher $\dot{M}O_{2\max}$ (23%), AAS (26%), peak $f_{H\max}$ (20%), $T_{q10<1.5}$ (20%), T_{peak} (22%), T_{arr} (16%), and CT_{\max} (11%) than Jayko Arctic char, with the other two populations being intermediate. Halokvik River is among the longest (>50km), most energetically demanding (greatest work) migratory routes in the Kitikmeot region, with large summer temperature fluctuations and peak temperatures that can exceed 21°C in August, when Arctic char perform their obligatory upriver migration (Figure 1.2; McGowan 1990). Conversely, Jayko is among the shortest, least imposing rivers in the region and the upriver migration typically occurs in early September when ambient temperatures are falling. Jayko is also more geographically isolated from the other three populations that I studied. As in sockeye salmon

(Eliason *et al.* 2011), I discovered that heart size (RVM and RAM) also tended to be larger in Arctic char from populations with more challenging migratory environments which may confer greater stroke volume and pumping capacity, thereby contributing to the differences in aerobic performance that I observed. Below I discuss how local adaptation, phenotypic plasticity, and variation in migratory behavior could contribute to the observed physiological differences and address the potential conservation and management implications.

5.4.1 Population-specific cardiorespiratory and thermal physiology

Anadromous Arctic char that undertake more challenging spawning migrations must exhibit appropriate migratory behaviours and physiological capabilities to reach their intended spawning grounds. Those that do not cannot reproduce in that particular freshwater system and either die trying or divert their course to a more accessible system (i.e., migration failure). The other alternative, over-wintering at sea, is not a possibility due to prolonged sub-zero temperatures. Indeed, Nulahugyuk Arctic char encountered peak temperatures $>21^{\circ}\text{C}$ during their upstream migration and repeatedly exercise to exhaustion while navigating shallow water through much of the river (Gilbert *et al.* 2016). Furthermore, their migration must be complete before the end of July before the river becomes too shallow to navigate. Upstream migration for most populations in the region does not even begin until mid- to late-August (Johnson 1980; McGowan 1990). Thus, Nulahugyuk Arctic char that do not migrate early enough (and cannot cope with the local thermal and hydrological regimes) will not be able to reach their intended spawning lake (Gilbert *et al.* 2016; Gilbert and Tierney 2018). If these traits (e.g., migration timing and repeat exercise performance) are heritable and vary within a population they would likely evolve through natural selection. In the present study, Halokvik and possibly Palik Arctic

char would likely experience such selective pressures to a greater extent than those at Ekalluktok and Jayko, where migratory conditions are less challenging.

Along these lines Moore *et al.* (2017) recently showed that the frequency of specific SNPs within Arctic char populations in the Cambridge Bay area was correlated with migratory work. Some of these SNPs were in or near genes that relate directly to cardiac function and development including *MEF 2C* and *Nebulette*. The MEF 2C protein helps regulate cardiac myogenesis (Akazawa and Komuro 2003; Yogev *et al.* 2013) and is thought to contribute to exercise-induced cardiac hypertrophy in Atlantic salmon (Castro *et al.* 2013). *Nebulette* is involved in the development and organization of cardiac actin filaments and can influence contractile properties (Moncman and Wang 2002; Bang and Chen 2015). Changes in either or both of these genes could directly account for Arctic char from Palik and Halokvik having high RVM and RAM, which correspond with their strenuous migrations (Table 5.2 and 5.4). Intuitively, such genetic differences could also impact cardiac responses to exercise and acute warming and thus contribute to populations with more difficult migrations having high aerobic performance ($\dot{M}O_{2\max}$ and AAS, Table 5.4) and cardiac thermal tolerance (e.g. T_{arr} , Table 5.4) especially when compared to populations, such as that from Jayko, with less demanding migrations.

The same environmental variation that drives local adaptation can also elicit compensatory changes in behaviors and physiology, which in turn can relax selective pressure. For instance, exercise training can increase heart size, $\dot{M}O_{2\max}$, AAS, swimming efficiency and performance, and growth in some fishes (Farrell *et al.* 1990; Farrell *et al.* 1991; Davison 1997; Castro *et al.* 2011; Castro *et al.* 2013; Gilbert *et al.* 2014; Zhang *et al.* 2016). As such,

differences in activity and migratory difficulty among Arctic char from populations could elicit a training effect that would contribute to those with more strenuous migrations having greater RVM, RAM, $\dot{M}O_{2\max}$ and AAS. Conversely a lack of exercise could limit these traits in Jayko Arctic char. Training effects may be particularly relevant for Arctic char as they are long-lived (commonly 20 years but up to 40 years) and repeat an upstream migration annually (they rarely skip seaward migrations) after they first migrate to sea as smolts.

Similarly, thermal history can have a profound impact on thermal tolerance. For instance, Anttila *et al.* (2014a) found that Atlantic salmon acclimated to 20°C versus 12°C increased peak $f_{H\max}$, T_{peak} and T_{arr} by 30%, 23% and 24%, respectively. While the timescales for some acclimatory responses can be on the order of weeks, others such as resetting intrinsic heart rate can be very rapid (rainbow trout; Sutcliffe *et al.* 2020), and meaningful changes ($>2^{\circ}\text{C}$) in CT_{\max} can occur within a day (sheepshead minnow; Fanguie *et al.* 2014). Given that August 2018 was unseasonably cold (mean August temperature was 5.3°C in 2018 versus 9.5°C in 2017) Arctic char studied at Palik and Jayko in 2018 may have experienced cooler temperatures over the course of the summer than those examined at Halokvik and Ekalluktok in 2017. Indeed, Arctic char from Palik and Jayko had a significantly lower peak $f_{H\max}$ and T_{peak} than those from Halokvik and Ekalluktok. Nevertheless, Arctic char from Palik differed by maintaining a rhythmic heartbeat $\sim 5^{\circ}\text{C}$ above their T_{peak} and thus had the highest T_{arr} . As such, there are likely factors beyond their recent thermal history that contributed to the observed differences in cardiac thermal tolerance among populations. The thermal plasticity of cardiac heat tolerance and aerobic performance is thoroughly examined in Chapters 3 and 4. However, it should be noted that thermal plasticity and thus thermal reaction norms may also differ among Arctic char

populations as it does in brook char (*Salveinus fontinalis*) (Stitt *et al.* 2014), a possibility that warrants further investigation.

Beyond local adaptation and acclimatization, life history differences among the study populations may have contributed to differences in aerobic and thermal physiology. As discussed above, Arctic char from systems with challenging migratory conditions may avoid returning home in non-spawning years (Gilbert *et al.* 2016; Moore *et al.* 2017) and will instead overwinter in a more accessible freshwater system. As such, it is possible that only Arctic char in good physiological condition and with the potential to spawn in the next year will even attempt the migration at Halokvik, while systems like Jayko and Ekalluktok may serve as local repositories for non-spawning fish with a lower condition. On-going genetic population assignment analysis will determine the extent of population mixing within my samples to clarify the extent to which such behavioral sorting contributes to physiological differences at each location. Regardless of the proximate cause (i.e., genetics, plasticity, behavioural sorting) of the observed differences, the present study suggests that differences in migratory environments may promote functional differences in Arctic char cardiorespiratory and thermal physiology among rivers, which may improve local migration success.

Each sampling location in the present study is fished as part of the commercial and subsistence Arctic char harvests for the community of Ikaluktutiak (Cambridge Bay, NU). Consequently, body mass, length and condition factor of Arctic char have been monitored regularly at these sites for over 40 years. In general, Arctic char from Halokvik are the largest in the region, followed by Ekalluktok, Palik and Jayko (Day and Harris 2013). This pattern is similar to what was found here, although I did select smaller Arctic char at Palik for some

respirometry experiments to optimize the fish mass to respirometer volume ratio to more easily maintain DO levels in the respirometer. I did, however, ensure that there was substantial overlap in size across all four populations to allow me to more effectively account for size differences and allometric scaling.

5.4.2 Conservation Implications

In anadromous salmonids, life-history and physiological variation within and among populations can help a species buffer against environmental variation by creating a diversified “portfolio” of migratory phenotypes (Schindler *et al.* 2010). Consequently, the preservation of genetic and phenotypic diversity can be critical to effective fisheries management (Hilborn *et al.* 2003; Hutchinson 2008). As such, managers must identify levels of diversity (e.g. subspecies, meta-populations, populations or ecotypes) that warrant specific conservation action (e.g. COSEWIC’s designatable units; COSEWIC 2015). To this end, Harris *et al.* (2016) determined that some Arctic char stocks in the Cambridge Bay area are genetically distinct from each other, with Jayko Arctic char being particularly distinct from other commercially harvested stocks. These findings were confirmed by subsequent genomic analysis by Moore *et al.* (2017). If such genetic diversity is linked to functionally significant biological variation it may warrant targeted management action such as adjusting river-specific quotas, and may grant specific populations or groups special legal status (Green 2005; COSEWIC 2015). In this regard, previous studies have demonstrated substantial differences in migration timing among Cambridge Bay area Arctic char populations (McGowan 1990), and in other salmonids run timing can be heritable (Carlson and Seamons 2008). The present study builds on these genetic, genomic and ecological data by

providing evidence that these populations also differ significantly in their cardiorespiratory and thermal physiology and in a manner that may improve their local migratory performance.

The Canadian Arctic is warming at nearly three times the average global rate (Zhang *et al.* 2019a) and earlier, more rapid snowmelt is likely causing shifts in the timing and magnitude of peak and low flow events (Bonsal 2019). Given that populations have unique migratory environments, life histories (e.g., annual fidelity and migration timing) and physiology the impacts of this environmental change will be determined by how these factors interact. For late-run populations using short river systems (e.g., Ekalluktok and Jayko) the declining flows and rising temperatures may have little to no impact on migration success in the near term. However, for populations that undertake strenuous migrations, extreme heat events and shallow water have already been encountered (e.g., Halokvik), and such harsh environmental conditions will likely test an Arctic char's upper thermal tolerance and aerobic capacity. However, in order to make more specific predictions about population level responses to environmental change detailed and consistent environment data (e.g., river temperature and discharge) during the migratory window are needed. Indeed, in temperate rivers such as the Fraser River long-term continuous temperature and hydrological monitoring have been instrumental in allowing researchers to identify relationships between river conditions and salmon physiology, phenology and migration success, information that is now used in the formulation of management strategies (Cooke *et al.* 2006; Crossin *et al.* 2008; Farrell *et al.* 2008b; Eliason *et al.* 2011; Martins *et al.* 2011; Martins *et al.* 2012; Eliason and Farrell 2016; Patterson *et al.* 2016).

5.4.3 Conclusions and future directions

The present study provides evidence that Arctic char from populations with more strenuous migrations tend to have greater cardiorespiratory performance and heat tolerance (Table 5.4 and Figure 5.5). These differences were most pronounced between Halokvik and Jayko Arctic char, which have among the most and least strenuous migrations, respectively, in the Cambridge Bay area. Together with previously described variation in their migration timing and genetics (McGowan 1990; Day and Harris 2013; Harris *et al.* 2016; Moore *et al.* 2017), these findings suggest that Halokvik and Jayko Arctic char may be important conservation units and may be differentially impacted by on-going climate change.

The current experimental design, time and financial limitations, and more generally the extreme remote nature of the central Canadian Arctic, precluded data collection over multiple years at each site, over more locations, or in a common garden environment. As such, despite the marked and compelling differences I identified among populations, I cannot firmly attribute those differences to adaptive processes. As such, similar studies with a common-garden design, additional populations, and captive F1 and F2 offspring, would all be highly revealing as to the heritable basis of the observed differences. Furthermore, given the extent of variation I found in the Cambridge Bay area alone, it is reasonable to expect that substantial physiological differences exist among regions, and particularly those with Arctic char of different post-glacial origins (Moore *et al.* 2015), a possibility that warrants further investigation.

Table 5.1 Migration characteristics, gross morphology, relative ventricular and atrial mass (RVM and RAM), and sample sizes for Arctic char from each study population. Migratory work is the product of distance and elevation gain. River temperatures are from a single sampling period at each location (6-10 days) and may not represent common migration temperatures. Data are presented as mean±SD. Dissimilar letters indicate significant differences within a trait among populations.

	Palik	Halokvik	Ekalluktok	Jayko
Coordinates	68.945N, 108.532W	69.175N, 107.104W	69.406N, 106.314W	69.736N 103.255W
Distance (km)	28.5	50.4	3.8	5.4
Elevation (m)	64	118	15	3
Migratory work	1824	5947	57	16
Temperature (°C)	7.1±1.0	10.8±2.3	9.4±0.7	4.6±0.6
Body mass (g)	2522±922 ^a	4932±930 ^c	4053±1309 ^b	3055±701 ^d
Fork length (mm)	617±75 ^a	739±50 ^b	671±67 ^c	658±53 ^d
Condition factor (K)	1±0.1 ^a	1.2±0.1 ^b	1.3±0.1 ^c	1.1±0.1 ^a
RVM (% body mass)	0.12±0.01 ^a	0.12±0.02 ^a	0.10±0.01 ^b	0.11±0.01 ^{ab}
RAM (% body mass)	0.029±0.005 ^a	0.024±0.006 ^b	0.022±0.004 ^b	0.023±0.004 ^b
n _{gross morphology}	32	20	20	20
n _{respirometry}	12	8	8	10
n _{Hmax}	13	7	10	10

Table 5.2 Analysis of variance (ANOVA) of gross morphology, heart size, oxygen uptake cardiac transition temperatures and peak f_{Hmax} among populations. Body mass was included as a covariate where shown and bold p-values indicate significant effects ($\alpha = 0.05$).

	Main effect: Population			Main effect: Body mass		
	F	df	p	F	df	p
Body mass	28.1	3,88	<0.001			
Fork length	15.2	3,88	<0.001			
Condition (k)	29.5	3,88	<0.001			
RVM	6.5	3,84	<0.001			
RAM	9.7	3,80	<0.001			
$\dot{M}O_{2min}$	25.3	3,34	<0.001	52.7	1,34	<0.001
$\dot{M}O_{2max}$	99.9	3,35	<0.001	105.7	1,35	<0.001
AAS	52.8	3,33	<0.001	39.2	1,33	<0.001
FAS	1.0	3,33	0.402	1.2	1,33	0.274
CT_{max}	6.0	3,27	0.003	7.1	1,27	0.013
T_{arr}	9.1	3,35	<0.001	0.0	1,35	0.915
T_{peak}	13.2	3,35	<0.001	0.2	1,35	0.637
$T_{q10<1.5}$	5.4	3,35	0.004	0.1	1,35	0.725
peak f_{Hmax}	6.1	3,35	0.002	6.2	1,35	0.018
f_{Hmax} at 12°C	1.5	3,35	0.237	5.6	1,35	0.024

Table 5.3 Principal component (PC) coefficients (loadings) for variables related to cardiac thermal tolerance and heart size. The percentage of variance explained by each PC is shown in parentheses. Note that only PC1 and PC2 (bolded) had Eigenvalues greater than 1.

	PC1 (45.2%)	PC2 (24.4%)	PC3 (11.4%)	PC4 (8.7%)	PC5 (5.9%)	PC6 (4.4%)
T _{arr}	0.47	0.08	-0.57	0.37	-0.44	0.34
T _{peak}	0.52	-0.05	0.3	-0.16	0.49	0.61
peak f_{Hmax}	0.48	0.08	0.54	-0.25	-0.59	-0.26
T _{q10<1.5}	0.5	-0.15	-0.4	-0.22	0.38	-0.61
RVM	-0.1	0.69	-0.29	-0.64	-0.04	0.14
RAM	0.13	0.7	0.22	0.57	0.27	-0.23

Table 5.4 Summary population rankings for traits related to heart size, aerobic metabolism and thermal tolerance. Dissimilar letters indicate significant differences within a trait among populations.

	Halokvik	Palik	Ekalluktok	Jayko
Migratory work	1	2	3	4
RVM	2 ^b	1 ^b	4 ^a	3 ^{ab}
RAM	2 ^a	1 ^b	4 ^a	3 ^a
$\dot{M}O_{2min}$	2	1	3	4
$\dot{M}O_{2max}$	2 ^b	1 ^b	3 ^{ab}	4 ^a
AAS	1 ^b	3 ^{ab}	2 ^{ab}	4 ^a
CT _{max}	1 ^c	3 ^{ab}	2 ^{bc}	4 ^a
T _{arr}	2 ^b	1 ^b	3 ^b	4 ^a
T _{peak}	1 ^b	3 ^c	2 ^b	4 ^a
T _{q10<1.5}	1 ^b	3 ^{ab}	2 ^{ab}	4 ^a
peak f_{Hmax}	1 ^b	3 ^c	2 ^{bc}	4 ^a

Figure 5.1 Study area near Cambridge Bay in the Kitikmeot region of Nunavut, Canada. Anadromous Arctic char were studied at four commercially harvested rivers (Palik, Halokvik, Ekalluktok, and Jayko River) that serve as migratory routes between natal lakes and the Arctic Ocean. The sampling year (parentheses), minimum migration distance (Dist.), elevation gain (Elev.) and work (dist. x elev.) for are shown for each river.

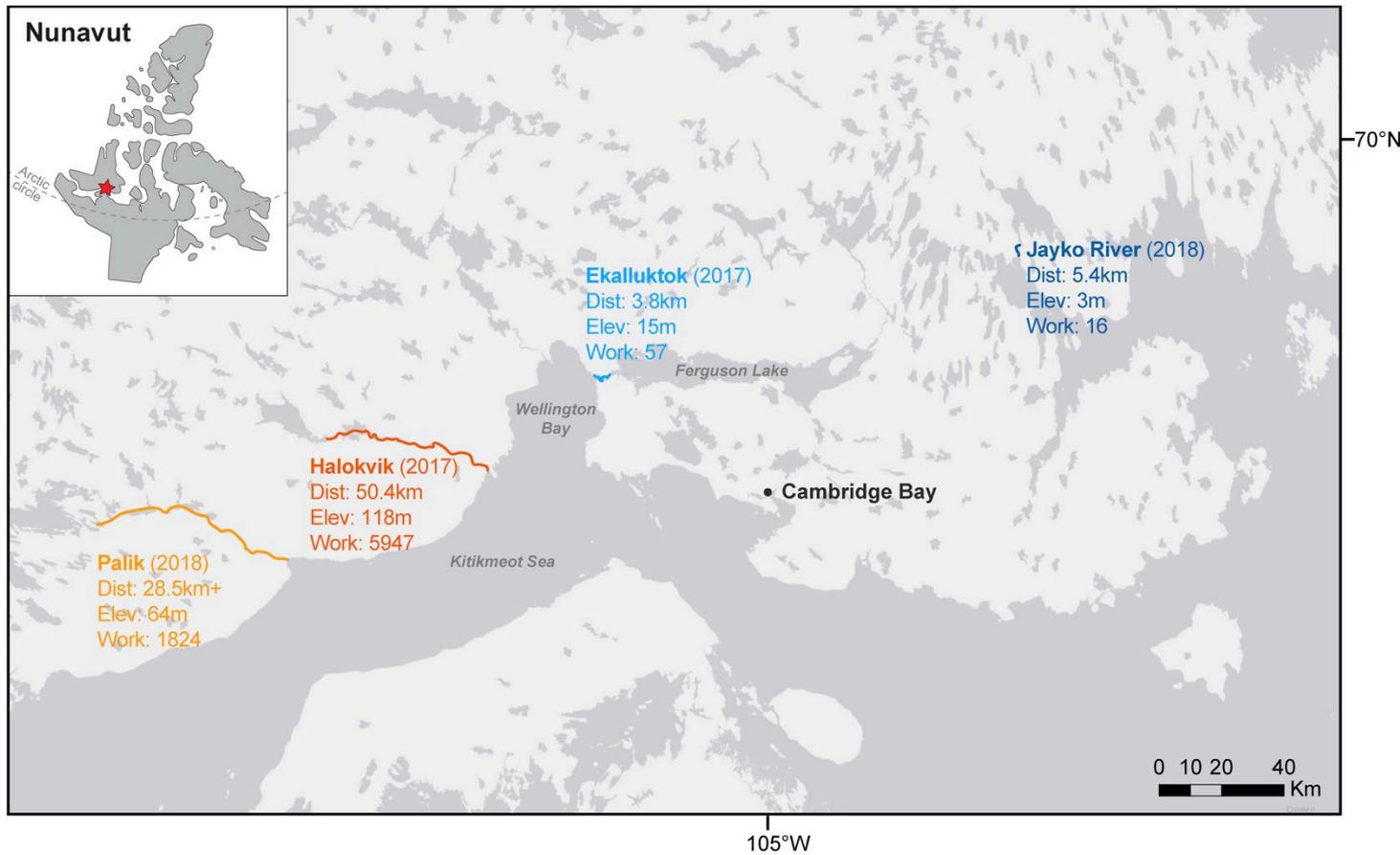


Figure 5.2 Minimum ($\dot{M}O_{2min}$) and maximum oxygen uptake ($\dot{M}O_{2max}$) and absolute aerobic scope (AAS) of migratory Arctic char from four populations at $\sim 10^{\circ}\text{C}$ following a chase to exhaustion and air exposure. Populations are ordered by migratory work (Table 5.1) with migrations up Jayko and Ekalluktok Rivers requiring much less work than those at Palik and Halokvik. Dissimilar letters indicate significant differences within a trait between populations. In some instances error bars are encompassed by their associated symbol

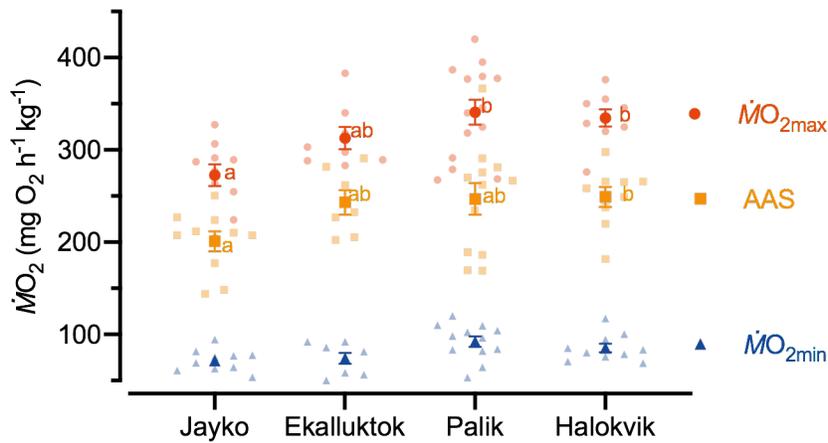


Figure 5.3 Maximum heart rate (f_{Hmax}) and corresponding incremental Q_{10} values during acute warming in anadromous Arctic char from four populations. f_{Hmax} was measured continuously during acute warming (6°C h^{-1}) of anaesthetized Arctic char and experiments were terminated at the onset of cardiac arrhythmia. Data are presented for all individuals from each population with mean (\pm SE) values overlaid (a-d) and compiled below (e). Individual traces stop at the arrhythmia and mean traces stop once three individuals were removed from the data set following the onset of arrhythmia. Mean (\pm SE) Q_{10} values are shown for changes in f_{Hmax} over 2°C increments (f). In some instances error bars are encompassed by their associated symbol.

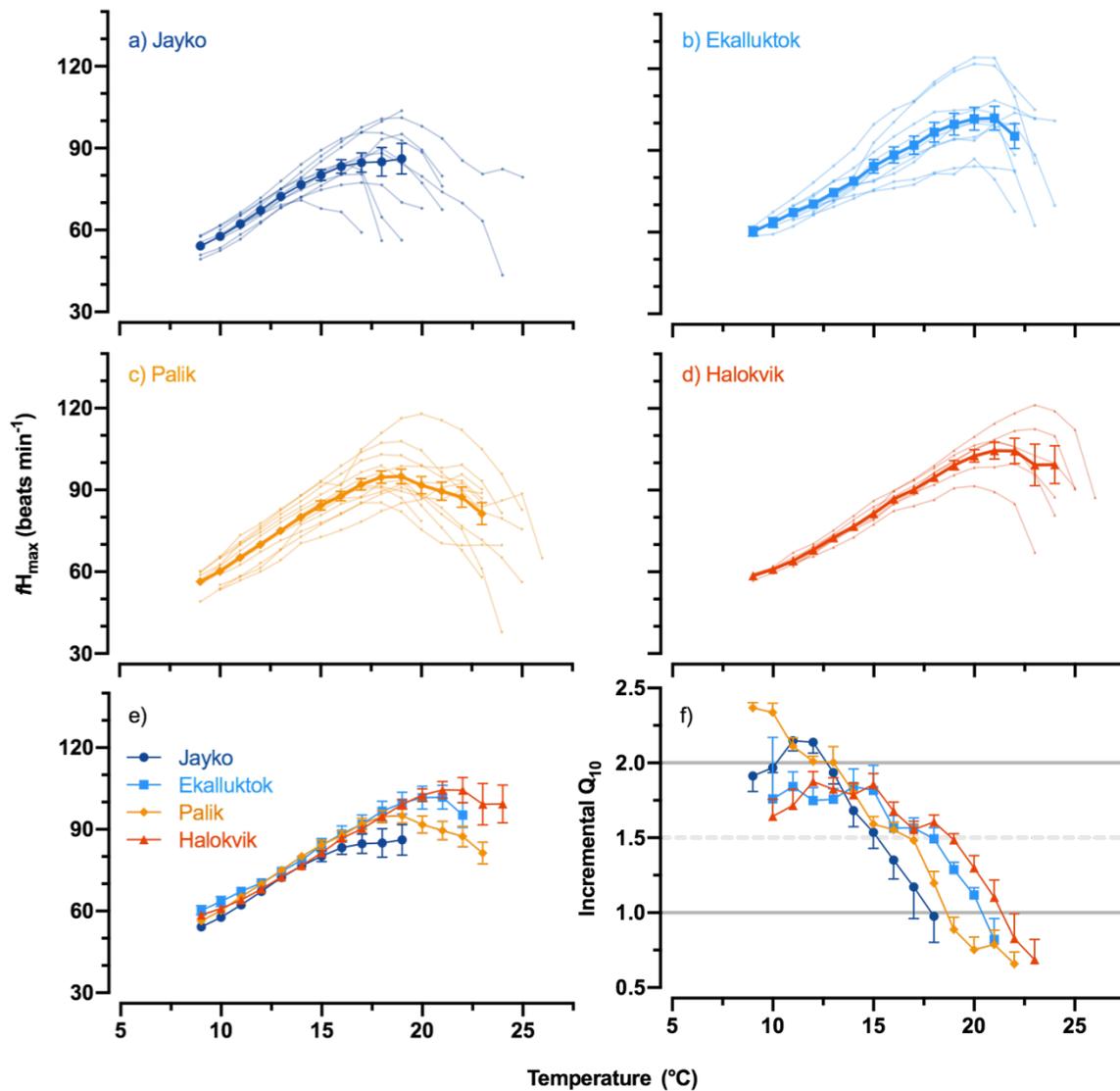


Figure 5.4 Population specific transition temperatures and peak maximum heart rate (peak f_{Hmax}) during acute warming in Kitikmeot Arctic char. Transition temperatures ($T_{q10<1.5}$, T_{peak} , T_{arr} , CT_{max}) for Arctic char from each population are presented as cumulative frequency distributions (a-d) to show intraspecific variation and as mean \pm SE (e). Peak f_{Hmax} is presented as mean \pm SE with individual values overlaid (f). Dissimilar letters indicate significant differences within a trait among populations. In some instances error bars are encompassed by their associated symbol

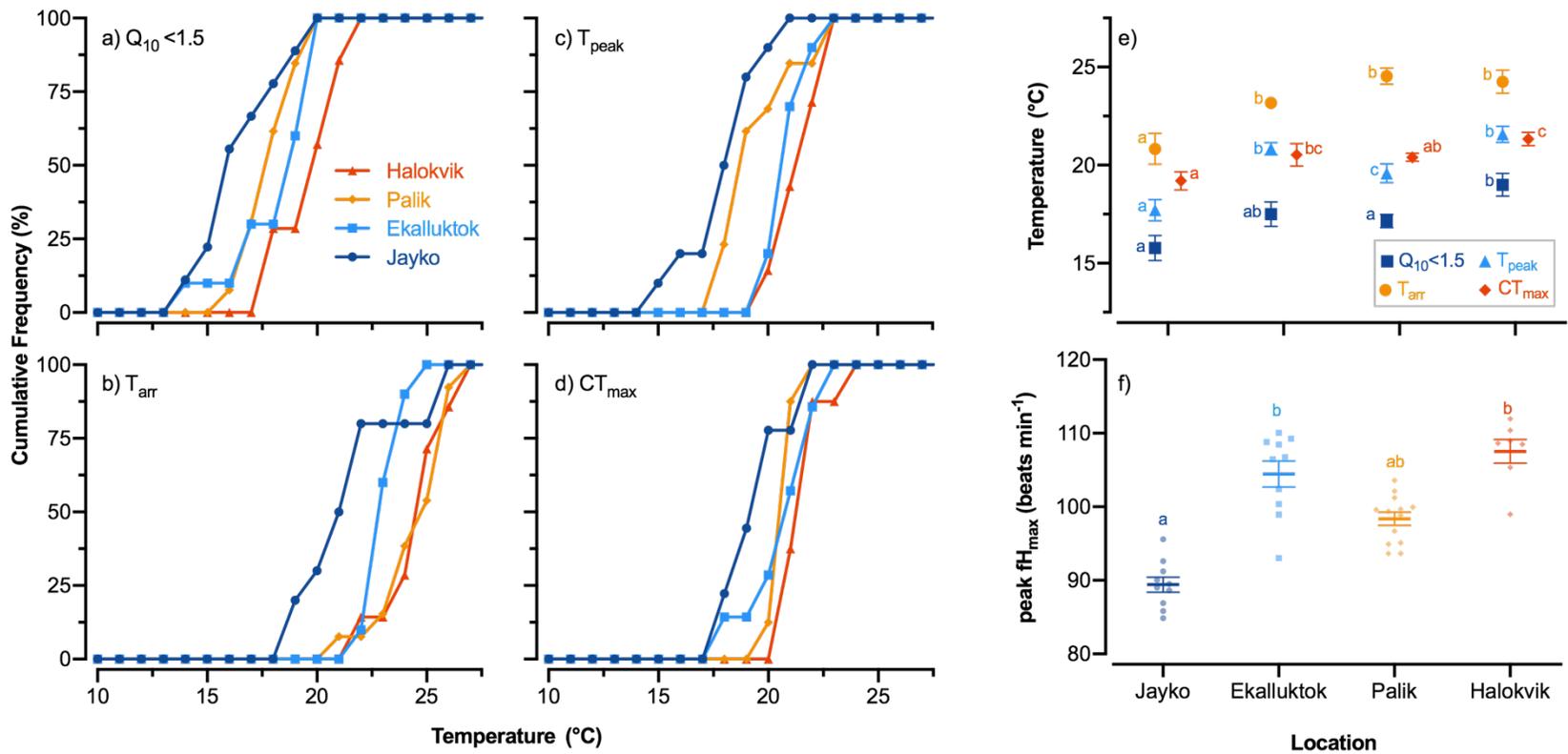
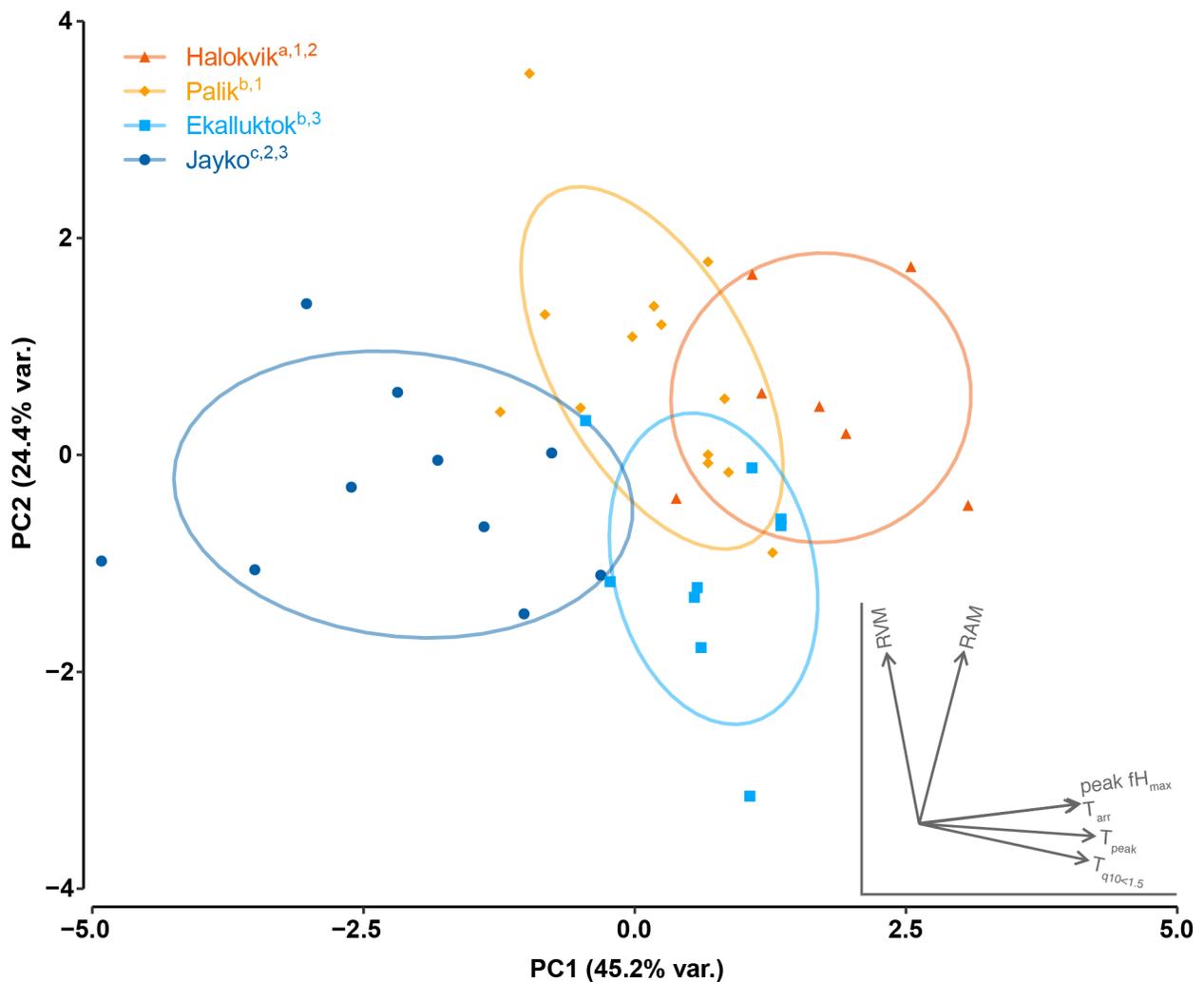


Figure 5.5 Principal components analysis (PCA) of heart size and cardiac transition temperatures among Arctic char from four populations in the Kitikmeot region of Nunavut. The PCA included relative ventricular mass (RVM), relative atrial mass (RAM), peak maximum heart rate (peak f_{Hmax}), and cardiac transition temperatures ($T_{q10<1.5}$, T_{peak} , T_{arr}). The PC1 and PC2 explained 45.2 and 24.4% of the variation respectively. Each population is identified by its 68% data ellipse, indicative of ~ 1 standard deviation around the mean. Dissimilar letters or numbers among populations indicate significant differences in PC1 or PC2 respectively. The inset shows directional vectors for each variable included in the analysis (Table 5.3).



Chapter 6: General discussion

6.1.1 Overview and principle findings

The overall goal for my thesis was to characterize the thermal limits to cardiorespiratory performance of Arctic char in the central Canadian Arctic. Moreover, I sought to conduct my research in a manner that would improve our understanding of the extent of biological solutions to thermal challenges in fish and inform Arctic char conservation efforts. To address my goal, I had three specific objectives structured around complementary physiological mechanisms that fish use to cope with environmental challenges: acute thermal tolerance, acclimation/acclimatization and local adaptation. Based on these themes, my results can be distilled into three core findings:

- 1) In the Canadian Arctic, migrating Arctic char encounter temperatures that can exceed their acute tolerance limits and, indeed, constrain cardiac performance and exercise recovery, while at the same time raising basic energetic demand (Chapters 2-5). Together these challenges may decrease migration success and ultimately reproductive success.
- 2) Arctic char possess a significant capacity for thermal acclimation that reflects the thermal range normally encounter throughout their life history (Chapter 3-4). This acclimation potential will likely improve their ability to cope with environmental warming.
- 3) Arctic char cardiorespiratory and thermal physiology differs among populations in manner suggestive of local adaptation to migratory environments (Chapter 5). Such physiological variation across heterogenous environments will likely influence population-specific responses to climate change.

This final chapter discusses the successes and challenges that I faced while conducting physiological research in an Arctic context. I examine how my findings relate to other salmonids and Arctic fishes and I consider the conservation implications of my work. Lastly, I highlight a few of the numerous avenues for future research that arose over the course of my investigations.

6.1.2 Environmental physiology of fish in the Canadian Arctic

The Canadian Arctic is an extreme, remote and demanding environment, making it an often prohibitively expensive (Mallory *et al.* 2018) and logistically challenging region in which to carry out any research, not to mention environmental physiology, which often requires a large, stable power supply and a controlled environment (i.e., a laboratory). Consequently, field-based research on the environmental physiology of Arctic fishes has been limited. In fact, in the studies that do exist, several authors have remarked that their study design was meaningfully restricted by logistical challenges (Holeton 1973; Farrell *et al.* 2013; Drost *et al.* 2014; Gilbert and Tierney 2018). A major take away of my thesis is that innovation and investment in Arctic research infrastructure is needed to remove barriers to conducting high quality physiological research in the Canadian north (Figure 6.1, 6.2, 6.4).

Through my collaboration with the Arctic Research Foundation (Winnipeg, MB), I had access to a mobile research laboratory (Figure 2.2) at both Freshwater Creek (2016-2017) and Palik (2018-2019). The substantial onboard power supply, the fish holding system and the precise temperature control equipment allowed me to conduct assessments of a quality that I could perform at UBC but without having to either transport fish from the north (Drost *et al.* 2016) or use fish from a hatchery (Chapter 3 and 4). Indeed, using the mobile laboratory allowed me to measure f_{Hmax} at frigid temperatures (Figure 6.1) and develop thermal performance curves for

$\dot{M}O_{2min}$, $\dot{M}O_{2max}$ and aerobic scope over a broad range of ecologically relevant temperatures (Figure 6.4 and 6.5). In contrast, at the other research sites (Halokvik, Ekalluktok and Jayko; Chapter 5) I had to work out of a temporary research camp consisting of a wall-tent lab, small generator and mobile solar power system because transport of the mobile laboratory was too costly and needed to be done over sea-ice in winter, making relocation from other research sites more than once per year impractical. Consequently, precise, multi-day, temperature control was unfeasible at these three sites; aerobic scope was assessed only at a single, common, intermediate temperature ($\sim 10^{\circ}\text{C}$) and acute cardiac heat tolerance assessments were started at a higher, ambient water temperature.

Unfortunately, mobile research infrastructure will remain a luxury in the Canadian Arctic for the near future because of its cost and logical constraints for transport. Thus, regardless of recent developments in infrastructure many important experiments are currently impractical in the Canadian Arctic, but are feasible at better-equipped field stations further south. For instance, Eliason *et al.* (2011) generated full thermal performance curves for numerous cardiorespiratory traits on multiple populations of wild Sockeye salmon under common conditions by using mobile swimming tunnels and large fish holding systems at the Cultus Lake Salmon Research Laboratory in southern BC. Numerous hatchery facilities are also suitable and commonly used for such research (Chen *et al.* 2015; Zhang *et al.* 2018b; Adams 2020). The new Canadian High Arctic Research Station in Cambridge Bay, NU operated by Polar Knowledge Canada offers enormous potential in this regard if it is equipped with facilities needed to support aquatic and marine research. Given the rapid rate of climate change in the Canadian Arctic there is certainly an urgent need for such investment.

In addition to infrastructure development, my research highlights the need to tailor research methodologies specifically for field applications in remote settings. For example, the assessment of $f_{H_{max}}$ during acute warming is a rapid, high-throughput method that generates high quality data regarding cardiac heat tolerance and, critically, the technique is amenable for use at minimally equipped field laboratories (Chapter 2)(Drost *et al.* 2014) and even more rudimentary field settings (Chapter 5). Similarly, my research shows that forced exercise, in the form of a chase-to-exhaustion protocol, coupled with automated, intermittent-flow respirometry, was an effective field-based method for assessing aerobic demand and capacity in large salmonids (Figures 6.4 and 6.5). Indeed, Little *et al.* (2020) recently showed that exercising adult coho salmon in a swim-tunnel or through chasing produced similar estimates of $\dot{M}O_{2max}$ and AAS, provided that the correct analytical methods were applied to the data. In the field, chasing fish obviates the need for traditional swim-tunnel respirometry (Brett 1964; Farrell *et al.* 2003; Eliason *et al.* 2011). While mobile swim-tunnel respirometers have been used effectively in temperate regions (Farrell *et al.* 2003; Lee *et al.* 2003; Eliason *et al.* 2011), transporting such equipment to and throughout the Arctic is another matter altogether (Jones *et al.* 1974). Furthermore, Chapter 2 shows that strategically timed blood samples for metabolite and haematological analysis can help reveal mechanisms or consequences associated with higher order findings, reinforcing why such sampling has become so common place in conservation physiology (Clark *et al.* 2008a; Cooke *et al.* 2012; Stoot *et al.* 2014).

Along the same lines, current best practices in environmental physiology are constantly evolving as technological and analytical tools improve (Chabot *et al.* 2016; Norin and Clark 2016; Harianto *et al.* 2019; Zhang *et al.* 2019b; Little *et al.* 2020; Zhang *et al.* 2020). Taking

advantage of such advances is particularly important in remote field settings where sample sizes and infrastructure are limited. For instance, the application of an iterative (i.e. sliding window, or rolling) regression in the analysis of $\dot{M}O_{2\max}$ allowed me to produce field estimates that had similar variance to those generated in laboratory studies. Such improvements to the signal-to-noise ratio undoubtedly helped me to detect differences in $\dot{M}O_{2\max}$ and AAS among acclimation temperatures in the lab (Chapter 4) and populations in the field (Chapter 5).

Lastly, my thesis highlights how laboratory-based and field-based research can be used together to elevate the impact of both, as previously discussed (Payne *et al.* 2016; Speers-Roesch and Norin 2016; Gilbert and Tierney 2018). For example, I designed my laboratory-based acclimation studies (Chapter 3 and 4) by first compiling the available field-based data on the thermal tolerance (Gilbert and Tierney 2018; Gilbert *et al.* 2020) and thermal habit use (Figure 1.2 and 1.3; Gilbert *et al.* 2016; Mulder *et al.* 2018; Harris *et al.* 2020b) for anadromous Arctic char in the Canadian North. I then selected acclimation and acute test temperatures based on actual measurements of specific environmental exposures that occur at key life stages in wild Arctic char. As such, my experimental designs greatly improved the direct ecological relevance of my laboratory-based findings. The principal findings should still be tested in wild fish, but with a smaller, more pragmatic study (e.g., sampling at the end of winter and the beginning of the upstream migration) that is much more achievable and more efficiently uses limited resources compared to a full-scale, exploratory field-based acclimation study.

6.1.3 Acute heat tolerance

The assessment of $f_{H\max}$ during acute warming in anaesthetized fish is increasingly becoming adopted as an important tool for ecologically relevant characterizations of heat tolerance

in fish (Eliason and Anttila 2017). The technique was first developed by Casselman *et al.* (2012) with coho salmon, using two pieces of physiological knowledge. Foremost, the need to increase Q with warming is primarily achieved through increasing f_H (Steinhausen *et al.* 2008; Farrell 2009; Ekström *et al.* 2014; Penney *et al.* 2014; Farrell 2016). Second, during warming f_H becomes limited at ecologically relevant temperatures below CT_{max} a limitation that can generally constrain whole-animal oxygen supply and therefore aerobic functions (Steinhausen *et al.* 2008; Farrell 2009; Eliason *et al.* 2011; Eliason *et al.* 2013; Farrell 2016). Since then, the f_{Hmax} technique has been applied around the globe on Arctic fishes (Drost *et al.* 2014; Hansen *et al.* 2016; Gilbert *et al.* 2020), numerous salmonids (Anttila *et al.* 2014a; Anttila *et al.* 2014b; Muñoz *et al.* 2014; Chen *et al.* 2015; Adams 2020), and some impressively warm-tolerant species such as goldfish, killifish and zebrafish (Ferreira *et al.* 2014; Marchant and Farrell 2019; Safi *et al.* 2019). In my thesis, I show that increasing the acclimation temperature allowed Arctic char to markedly increase their cardiac heat tolerance (Figure 6.2) and reset their f_{Hmax} (Figure 6.1), as previously documented in other salmonids (Anttila *et al.* 2014a; Adams 2020) and more distantly related fishes (Ferreira *et al.* 2014; Safi *et al.* 2019). However, the acclimation potential of the Arctic char heart was clearly exceeded at 18°C and this was associated with numerous other adverse, whole-animal effects beyond a decrease in cardiac heat tolerance (Chapters 3 and 4). Importantly, I found that f_{Hmax} (Figure 6.1a) and T_{peak} (Figure 6.2) in wild migrating Arctic char were comparable to those in similarly sized laboratory-acclimated Arctic char at 6-10°C, a temperature range encountered in their natural marine and riverine habitats (Harris *et al.* 2020b). This comparison also provides further support for the benefits of parallel laboratory and field studies. Also of note, as previously shown in Atlantic salmon (Anttila *et al.* 2014b), f_{Hmax}

decreased with increasing body size in Arctic char. Specifically, Freshwater Creek Arctic char (Chapter 2) with an average mass of ~0.6 kg and had a ~20% higher f_{Hmax} at most temperatures than ~1-7 kg adult Arctic char from the other populations I studied (Chapter 5; Figure 6.1b). Such mass- f_H relationships are indeed common within and among vertebrate species (Lillywhite *et al.* 1999), however they do not exist in all cases (Clark and Farrell 2011). While f_{Hmax} was lower in larger fish there was no apparent relationship between body size and cardiac transition temperatures (Figure 6.2; Chapter 2, 3 and 5).

In general, my estimates of cardiac transition temperatures for Arctic char were markedly higher (~4-10°C) than the stenothermal Arctic cod (Figures 6.1 and 6.2) (Drost *et al.* 2014; Drost *et al.* 2016). Arctic cod are an important prey item of Arctic char, but as a permanent marine resident, they do not encounter the same level of thermal variation (maximum temperature in the Central Canadian Arctic ~12°C). Adult Atlantic salmon, a temperate salmonid relative of Arctic char, tend to have modestly higher cardiac transition temperatures than my data for Arctic char when the two species are compared at similar sizes (Figure 6.2). In contrast, Hansen *et al.* (2016) produced estimates for T_{peak} (12.8°C) and T_{arr} (15.2°C) in sea-run Greenlandic Arctic char, presumably for similar acclimation temperatures, that were ~6-8°C below mine (Figure 6.2; Chapter 2,3 and 5). In fact, the estimates for upper critical temperatures produced by Hansen *et al.* (2016) overlap with numerous estimates of optimal temperatures for Arctic char growth including mine (Chapter 4; Table 1.1)(Larsson *et al.* 2005; Imsland *et al.* 2019) and are temperatures commonly encountered by migrating Arctic char in the Canadian Arctic (Figure 1.2 and 1.3). Moreover, the cardiac transition temperatures reported by Hansen *et al.* (2017) align better with Arctic cod than with other estimates in Arctic char (Figure 6.2).

Provided methodological issues are not generating the difference between the two studies, the possibility exists that Greenlandic Arctic char may be cold-adapted relative to the Canadian Arctic char populations that I studied, one that warrants further investigation.

Among the key benefits of the $f_{H_{max}}$ -based thermal tolerance assessment is that multiple transition temperatures are generated which serve as various sub-lethal (early) indicators of the onset of heat-induced deterioration of cardiac function. In contrast, CT_{max} , while easier to measure, arguably holds less direct ecological relevance because important performance limitations commonly occur at lower temperatures thereby rendering fish unable to carry out ecologically vital functions, such as feeding and migration. Nevertheless, as discussed in Chapter 2 and 3, CT_{max} remains a useful comparative indicator of whole-organism thermal tolerance among species and is unarguably the ceiling for thermal performance within a species or a population. Indeed, I found that just like cardiac thermal tolerance, CT_{max} in Arctic char was considerably higher than Arctic cod ($\sim 6-10^{\circ}\text{C}$) (Drost *et al.* 2016), but only moderately lower than more commonly studied temperate salmonids such as Atlantic salmon, brook char, and rainbow trout ($\sim 2-6^{\circ}\text{C}$; Figure 6.2; Chapters 2,3, and 5) (Ekström *et al.* 2014; Penney *et al.* 2014; Gilbert *et al.* 2019; O'Donnell *et al.* 2020).

That CT_{max} is generally several degrees ($\sim 3-4^{\circ}\text{C}$) above T_{arr} within a species is now well documented (Chen *et al.* 2015; Drost *et al.* 2016; Eliason *et al.* 2017), and is indeed what I found in Chapters 2 and 3. Even so, in large adult Arctic char, CT_{max} was below T_{arr} and more similar to T_{peak} (Figure 5.4). Therefore, I examined whether CT_{max} declines with increasing body mass in Arctic char by compiling my CT_{max} data (Chapter 2, 3 and 5) with previous data available for Arctic char (Figure 6.3); CT_{max} had a strong negative relationship with body mass ($r^2 = 0.75$,

$p < 0.001$; $CT_{max} = -3.21 \times \log_{10}(\text{mass}) + 32.3$; Figure 6.3). Multiple factors may have contributed to such a difference and as previously discussed (Gilbert *et al.* 2019), there is no *a priori* reason to predict that CT_{max} is oxygen-limited given that the task of simply remaining upright (the measurement end point) is not necessarily aerobically demanding. Indeed, under optimal conditions or in hypoxia tolerant species CT_{max} may be a result of the direct effects of temperature on nervous function (Friedlander *et al.* 1976). With that said, acute warming often agitates fish, increasing oxygen demand above maintenance needs, as demonstrated in Chapters 2 and 4 (Figures 2.4, 4.4 and 4.5). Given that larger salmonids often have a greater abundance of muscle mass per unit body mass and a higher-mass specific anaerobic capacity (Goolish 1989; Goolish 1991), they may generate a more substantial or rapid oxygen deficit and associated metabolic disequilibrium (e.g., respiratory and metabolic acidoses) when agitated at high temperature. Furthermore, a potentially influential factor in the methodology of assessing CT_{max} is whether or not fish are physically confined. CT_{max} is often conducted on free-swimming fish in large tanks, as in Chapter 3. However, in order to obtain $\dot{M}O_2$ data during acute warming, researchers commonly conduct CT_{max} assessments within a respirometer (Chapter 2 and 5) (Rodnick *et al.* 2004; Ekström *et al.* 2014; Penney *et al.* 2014; Gilbert *et al.* 2019). Such confinement may cause fish to struggle more than they would otherwise and may also restrict ventilation. Either or both of these factors could logically be exacerbated in larger fish and thus limit oxygen availability and thereby confound and decrease the CT_{max} measurement. Beyond CT_{max} , both my past and present research suggests that recovery from exhaustive exercise is impaired more by acute warming in larger Arctic char as discussed in Chapter 2 (Chapter 2 and 4; Gilbert and Tierney 2018) and previously shown in coho salmon (Clark *et al.* 2012). Indeed

numerous studies (Clark *et al.* 2008b; Clark *et al.* 2012; Messmer *et al.* 2017; Portner *et al.* 2017; Leiva *et al.* 2019) have suggested that larger fish are more sensitive to warming (but see: Rodnick *et al.* 2004; Leiva *et al.* 2019).

6.1.4 The effects of temperature on cardiorespiratory physiology

Arctic char $\dot{M}O_{2\min}$ and $\dot{M}O_{2\text{mean}}$ both increased considerably with acute warming over commonly encountered temperatures, more than tripling between $\sim 2^{\circ}\text{C}$ and 18°C ($Q_{10}= 3.2\text{-}3.5$; Chapters 2 and 4; Figure 6.4). Thermal acclimation decreased this thermal dependence, but did not fully compensate for warming ($Q_{10}=2.5$; Figure 4.4). My estimates of $\dot{M}O_{2\min}$ for wild Arctic char (Chapters 2 and 5) correspond very well with $\dot{M}O_{2\text{mean}}$ in laboratory-reared Arctic char (Figure 6.4), which is almost certainly an artifact of the necessarily limited duration for $\dot{M}O_2$ recording (>20 h) in field experiments (Chabot *et al.* 2016). This similarity indicates that estimates for $\dot{M}O_{2\min}$ for wild char in my experiments likely represent a routine $\dot{M}O_2$ and not standard or basal $\dot{M}O_2$.

Over environmentally relevant temperatures, $\dot{M}O_{2\max}$ of Arctic char is less dependent on temperature than $\dot{M}O_{2\min}$ and $\dot{M}O_{2\text{mean}}$, increasing with warming up to a plateau (Chapter 2 and 4; 6°C acclimated, 14°C acute). As such, AAS only increases with warming over their cold thermal range ($2\text{-}6^{\circ}\text{C}$) and plateaus thereafter (Figure 6.5), while FAS declines with rising temperature, possibly reaching an activity-limiting low level at $16\text{-}18^{\circ}\text{C}$ (Chapter 2 and 4; Figure 4.6). Field-based estimates of mass-specific $\dot{M}O_{2\max}$ in Arctic char were $\sim 10\%$ lower than laboratory-based estimates at the same temperatures (Figure 6.4). This difference is likely a result of both negative allometric scaling and important methodological differences between field- and laboratory-based studies. Specifically, in the field, Arctic char were chased to exhaustion outside of the

respirometers then briefly exposed to air before being rapidly sealed inside a respirometer. In contrast, based on a recent methodological advancement (Zhang *et al.* 2020), I exercised an Arctic char inside of a respirometer in the laboratory to maximize the likelihood of capturing the true peak $\dot{M}O_2$. Indeed, we found that differences between these two methods resulted in an ~20% difference in $\dot{M}O_{2\max}$ estimates for the same rainbow trout (Figure 6.4) (Zhang *et al.* 2020).

It should be noted that Jayko Arctic char which undertake the shortest, seasonally latest and arguably least difficult migration in the Kitikmeot region of Nunavut are also clear low outliers in the comparison of $\dot{M}O_{2\max}$ values throughout my thesis (Figure 6.4). As discussed in Chapter 5, similar differences have been found between sockeye salmon populations that undertake short migrations to near coastal spawning habitat (e.g., Weaver Creek) compared to those that undertake much longer migrations (e.g., Nechako), which results in similar differences in AAS (Figure 6.5).

Arctic char apparently have a much higher and broader TPC for AAS than Arctic cod, which may be expected based on the more thermally variable and aerobically challenging life history of Arctic char. Interestingly, Atlantic salmon, like Arctic char, are iteroparous and have a thermally variable life history, have a similarly flat TPC for AAS (Figure 6.5) (Hvas *et al.* 2017). However, while adult Arctic char experience significant delayed mortality at 18°C (Chapter 4), the more temperate adult Atlantic salmon do not do so until ~22-23°C (Hvas *et al.* 2017; Gamperl *et al.* 2020). In contrast, many semelparous Pacific salmon appear to have narrow TPC for AAS, one that likely reflect thermal specialization to common and historically dependable temperatures during their once-in-a-lifetime upriver migration (Clark *et al.* 2011; Eliason *et al.* 2011; Abe *et al.* 2019). Furthermore, adult Pacific salmon tend to have a much higher peak AAS

(Figure 6.5), which may in part be a matter of either their thermal optima occurring at a warmer temperatures (i.e. hotter is better; Angilletta *et al.* 2010), or their greater energetic needs for upstream migration. Thus, just as $\dot{M}O_{2\max}$ and AAS appear adapted to local migratory challenges within Arctic char (Chapter 5) and sockeye salmon (Eliason *et al.*, 2011) they also appear adapted across salmonid species (Figure 6.5), as Pacific salmonids often undertake much more aerobically challenging migrations than Arctic char at much warmer temperatures.

Such species comparisons are highly relevant from a conservation perspective as the ranges of both Pacific (primarily pink and chum salmon) and Atlantic salmon are rapidly expanding northward into the Canadian Arctic (Dunmall *et al.* 2012; Dunmall *et al.* 2016). Chum salmon have even recently been caught in the central Canadian Arctic near Cambridge Bay NU, (A. Kanayok, personal communication, March 10, 2020). Given the impressive swimming and aerobic capacity of temperate salmonid species (Pacific salmon in particular) and the current thermal regimes of Arctic rivers, it is unlikely that upriver migration success will restrict their Arctic range expansion (Gilbert and Tierney 2018). Rather, because these salmon lack antifreeze proteins and Arctic-adapted life-histories invasions will more likely be constrained by cold performance, namely the ability of temperate salmonids to overwinter and complete their reproductive cycle in northern rivers. As such, studies of the overwintering requirements and cold performance of temperate salmon are likely going to be important to understanding their potential to establish viable populations in the Arctic.

6.1.5 Conservation and management implications

When considering conservation implications, it is critical to acknowledge what is at stake. The Cambridge Bay area alone supports an annual commercial Arctic char harvest of

~50,000 kg, worth over \$1.4 million to the local community's annual economy, employing dozens of Nunavummiut (Moore *et al.* 2017). More importantly, Arctic char are fundamental to food security, culture, and aquatic ecosystems in the Kitikmeot (central) region of Nunavut and throughout the Canadian Arctic (Lysenko and Schott 2019). Inuit have heavily relied on Arctic char as a subsistence resource for millennia and continue to do so given the often prohibitively high price of other healthy foods in Arctic communities (Lysenko and Schott 2019). In fact, the Arctic char is the single most harvested species in Nunavut. Thus, Arctic char remain an inextricable part of the Inuit way of life. Unfortunately, the rapid rate of climate change in the North (Zhang *et al.* 2019a) makes the future uncertain for many valuable northern species including Arctic char. As such, managers trying to preserve the species must not only consider the impacts of harvest but also the effects of climate change.

In conservation risk assessment the vulnerability of a given population to some adverse outcome (e.g., overharvesting or predation) can be defined as the product of their susceptibility (e.g., population size and reproductive rate) to the driving stressor and the likelihood of harmful exposure (e.g., fishing pressure or predator abundance) (Turner *et al.* 2003). As it relates to my research on Arctic char upriver migrations, vulnerability can be considered as the probability of migration failure. Thus, a population's vulnerability increases as a product of their physiological susceptibility to challenging migratory conditions (e.g., long distances, high temperatures and low flows) and the likelihood of encountering those conditions (Figure 6.6). Among other traits, physiological susceptibility is likely influenced by heat tolerance, repeat exercise capacity, and aerobic scope. Physiological susceptibility can be decreased through acclimation or local adaptation, as demonstrated in Chapters 3 to 5, while migration harshness is likely exacerbated

by climate change and either improved or worsened by changes in migration timing. For Arctic char earlier migrations may improve flow conditions (higher water levels), but increase the risk of encountering high temperatures and reduce their marine feeding window. Conversely, while delayed migrations may reduce heat exposure and maximize marine feeding, they increase the risk of encountering low, possibly impassable water levels and frigid temperatures. As discussed in Chapter 5, among the populations studied, Halokvik Arctic char appear to be the least physiologically susceptible to challenging migratory conditions. However, because they already encounter migratory conditions that may test their physiological limits, they may still be the most vulnerable to migration failure as climate change progresses among the populations I studied (Figure 6.6). This is of particular concern because, if Halokvik Arctic char are indeed adapted to challenging local migratory conditions, they may be or become an important source of genetic diversity in the region.

While this framework provides a useful way to examine potential interactions between physiological, ecological and environmental factors, for it to be applied in a more predictive manner we need better data on migration conditions. While collaborators and I have periodically documented extreme conditions that likely impair Arctic char migrations (e.g., low flows and high temperatures), we do not know how prevalent such conditions are throughout even the Cambridge Bay area, not to mention the entire Canadian or global Arctic. Once such data is available, more quantitative predictions can be made and incorporated into fisheries and climate change risk assessments along with other vulnerability analyses (e.g. fisheries productivity and susceptibility analysis; Tallman *et al.* 2019). Indeed, a similar logic has been applied in the management of Pacific and Atlantic salmon (Cooke *et al.* 2012; DFO 2012; Patterson *et al.*

2016). For instance, such information has been used as support for temperature-dependent closures of salmon fisheries (recreational and commercial). Based on my findings, such action may be justified at Halokvik, which already experiences extreme temperatures ($>21^{\circ}\text{C}$) and is fished with a weir that temporarily confines migrating Arctic char to shallow, warm water.

6.1.6 Future directions

Throughout my thesis I have highlighted many areas that require further investigation. Here I will expand upon a few that warrant further explanation. First, as previously stated, meaningful evidence exists to suggest that Arctic char susceptibility to high temperatures varies among populations (Chapter 5) and likely among regions (Table 1.1: Figure 6.2; Hansen *et al.* 2016). As such, an international collaborative research effort is needed to determine how Arctic char thermal physiology varies throughout its Holarctic range to establish how Arctic char from each region will differentially respond to warming.

Second, selective pressures (or lack thereof) do not necessarily influence physiological traits in a uniform manner over the range of experienced conditions, but rather can reshape environmental reaction norms and plasticity. As such, differences in physiological traits such as thermal tolerance and AAS among populations may only be apparent in specific environmental contexts where plasticity is needed. For example, compared to landlocked Arctic char, anadromous Arctic char experience a broad range of salinities throughout their lives (0 to 30 ppt) and thus can exhibit greater plasticity in their hypoosmoregulatory ability (e.g., Na^+/K^+ -ATPase isoform switching and up regulation at the gill) (Bystriansky *et al.* 2007; Ojima *et al.* 2009). If compared in freshwater both life history types appear to have similar osmoregulatory phenotypes; however, in seawater there is a clear difference between the two. Along these lines, I

would hypothesize that populations of anadromous Arctic char that migrate through thermally variable rivers not only have high aerobic performance and thermal tolerance (Chapter 5) but may also have greater thermal plasticity than those from more stable environments. To this end, acclimated thermal performance curves should be compared among anadromous Arctic char populations that encounter different degrees of thermal variation throughout their lives.

Furthermore, such studies could investigate landlocked Arctic char that spend their entire lives in lakes with access to cooler, generally more stable thermal regimes and may thus have lower thermal plasticity and tolerance.

Finally, while I focused exclusively on Arctic char, other anadromous species in the Canadian Arctic warrant equal attention. For instance, Dolly Varden (*S. malma*) are closely related to Arctic char, also have a complex life-history, and are an important subsistence resource (Sandstrom *et al.* 2009). Lake char (i.e., lake trout; *S. namaycush*), which are freshwater residents at temperate latitudes, remarkably display anadromy at high latitudes (Swanson *et al.* 2010a; Harris *et al.* 2014; Kissinger *et al.* 2016; Kissinger *et al.* 2017; Kissinger *et al.* 2018; Harris *et al.* 2020a). Furthermore, the aerobic scope of freshwater lake char can become limited at temperatures that are relatively low (~15°C) for a temperate salmonid (Kelly *et al.* 2014). Both anadromous lake char and Dolly Varden presumably face similar challenges to Arctic char, however, very little is known about their environmental physiology and how it will influence their ability to cope with environmental change.

Figure 6.1 Maximum heart rate ($f_{H_{max}}$) during acute warming in wild adult Arctic char relative to (a) hatchery Arctic char at multiple acclimation temperatures and (b) adult Atlantic salmon and Arctic cod. Freshwater Creek (FWC) and Palik Arctic char were presumed to be acclimated to between ~ 6 and 10°C based on marine temperature preferences and prevailing river temperatures. Hatchery reared Arctic char ($\sim 0.35\text{kg}$) were acclimated to $2, 6, 10,$ and 14°C , Adult Arctic cod to 3.5°C ($n=9$) and Atlantic salmon to 12°C ($n=6$). Data are presented as mean ($\pm\text{SE}$) and only at temperatures below which the first individual within a group exhibited arrhythmia. In some instances, error bars are encompassed by their associated symbol. Data are from: 1. Chapter 2; 2. Chapter 3; 3. Chapter 5; 4. Drost et al., 2016; 5. Unpublished data, M.Gilbert, 2016.

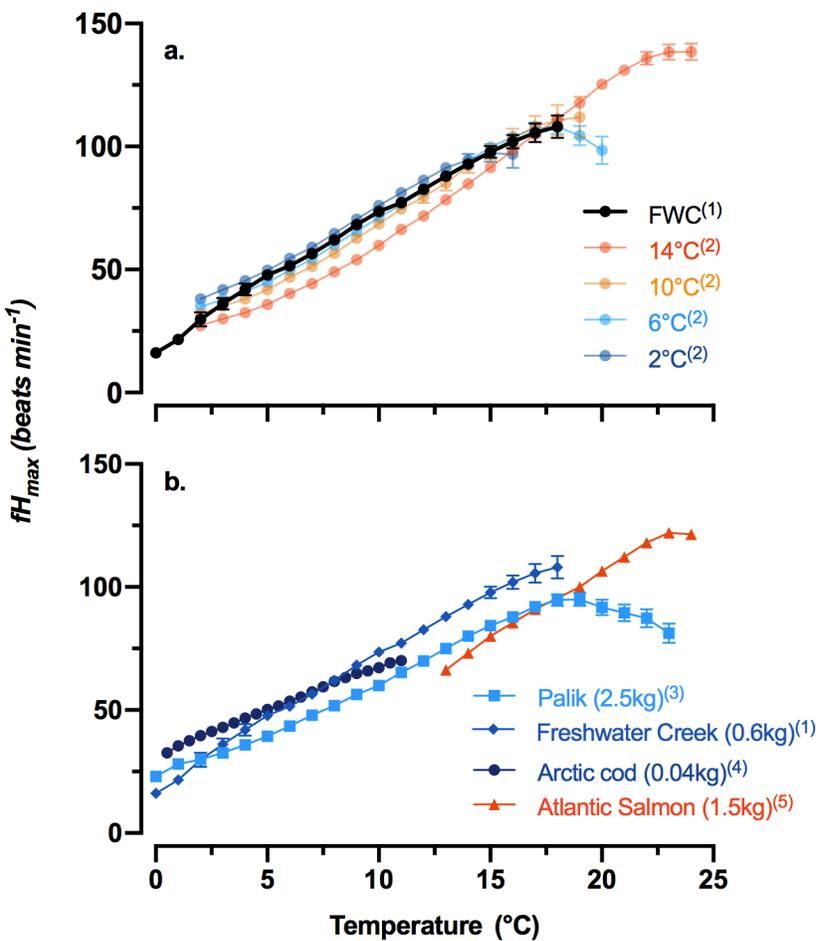


Figure 6.2 Fry thermal tolerance polygon for hatchery Arctic char in relation to the acute heat tolerance of adult Arctic cod, Atlantic salmon and five populations of wild anadromous Arctic char. The polygon is based on data from Chapter 4. 18°C was used as the upper limit for the polygon because close to 50% mortality was observed during acclimation. 0°C was used as the lower acute and acclimated limit because while Arctic char may be able to tolerate slightly lower temperatures (plasma freeze point = 1.0°C)(Fletcher *et al.* 1988), they overwinter in freshwater where 0°C is the coldest temperature they are forced to cope with. CT_{max} for 18°C-acclimated Arctic char was estimated to have fallen to levels similar to Arctic char acclimated to 6 and 10°C based on the declines observed in other heat tolerance metrics (T_{peak} and T_{arr}). Data at 0°C was taken as the y-intercept for regression models over 2-14°C. Data from Chapter 2 and 3 are distributed evenly around an assumed acclimation temperature of ~8°C. Data for Arctic cod (0.03-0.12 kg), Greenlandic Arctic char (0.85 kg), and Atlantic salmon (1.3-1.4 kg) are from: 1. Drost *et al.*, 2016; 2. Hansen *et al.*, 2016; 3 Unpublished data, M.Gilbert, 2016; 4. Hines *et al.* 2019.

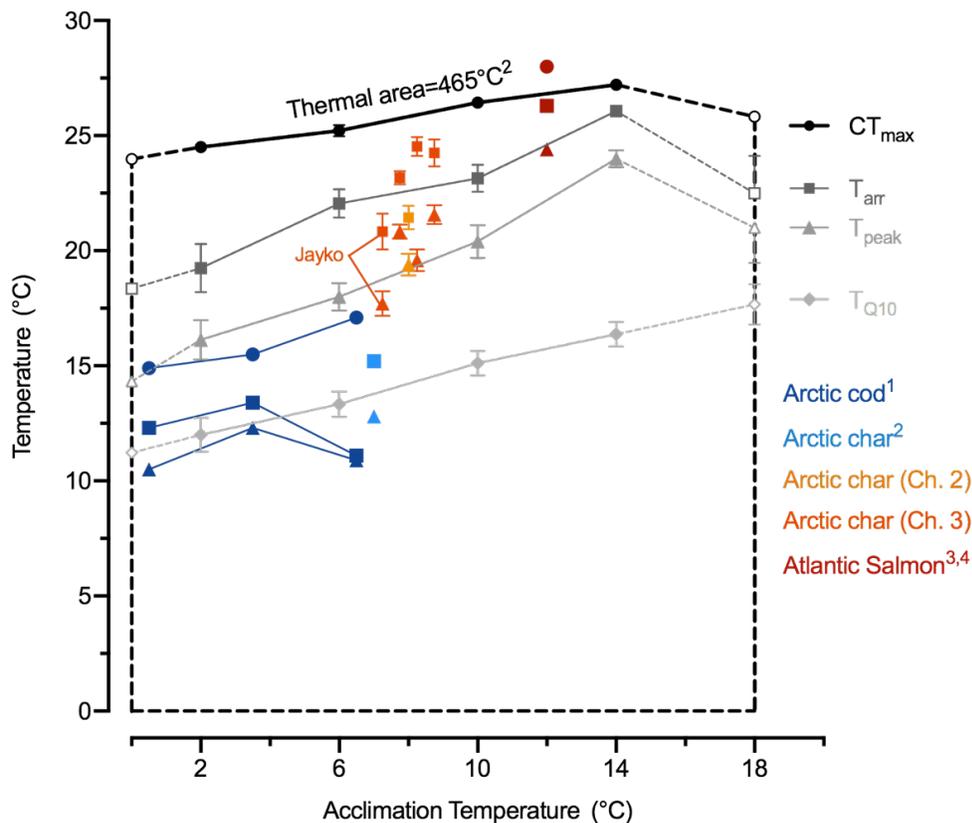


Figure 6.3 The relationship between body mass and critical thermal maxima (CT_{max}) in Arctic char. Each point is the mean of a population or treatment group (15 data points over 7 studies). Measured or inferred acclimation temperatures were restricted to 5-15°C. Data from the present thesis are shown in grey and data from previous studies (see Table 1.1) are in black.

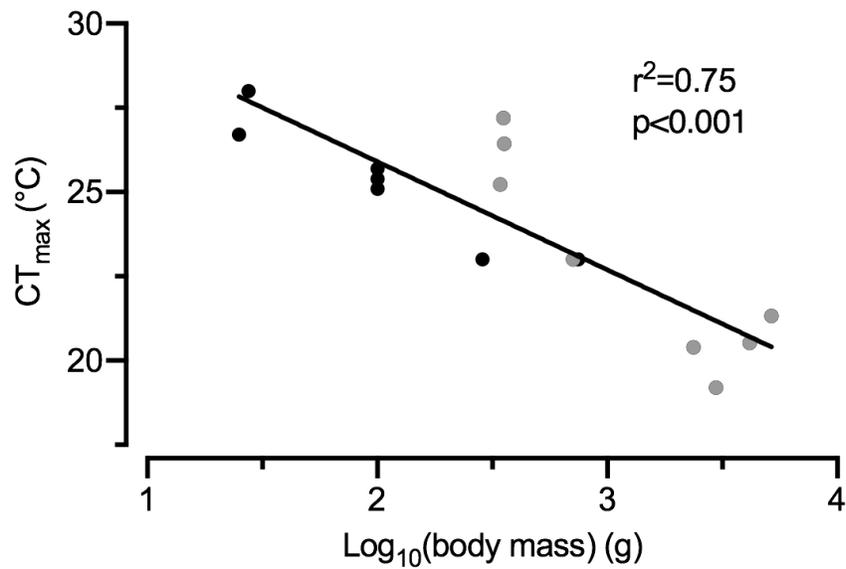


Figure 6.4 Maximum and minimum (or mean) oxygen uptake ($\dot{M}O_2$) of wild and hatchery reared Arctic char acutely exposed to environmentally relevant temperatures in field and laboratory settings. Hatchery Arctic char (Chapter 4) were acclimated to 6°C, which is similar to the assumed acclimation temperature of wild Arctic char (Chapter 2 and 5). Individual values are shown for data from Chapter 2, while data for populations and each test temperature are presented as mean (\pm SE) for Chapter 4 and 5. The Jayko population is highlighted as a notable outlier for the $\dot{M}O_{2max}$ data set.

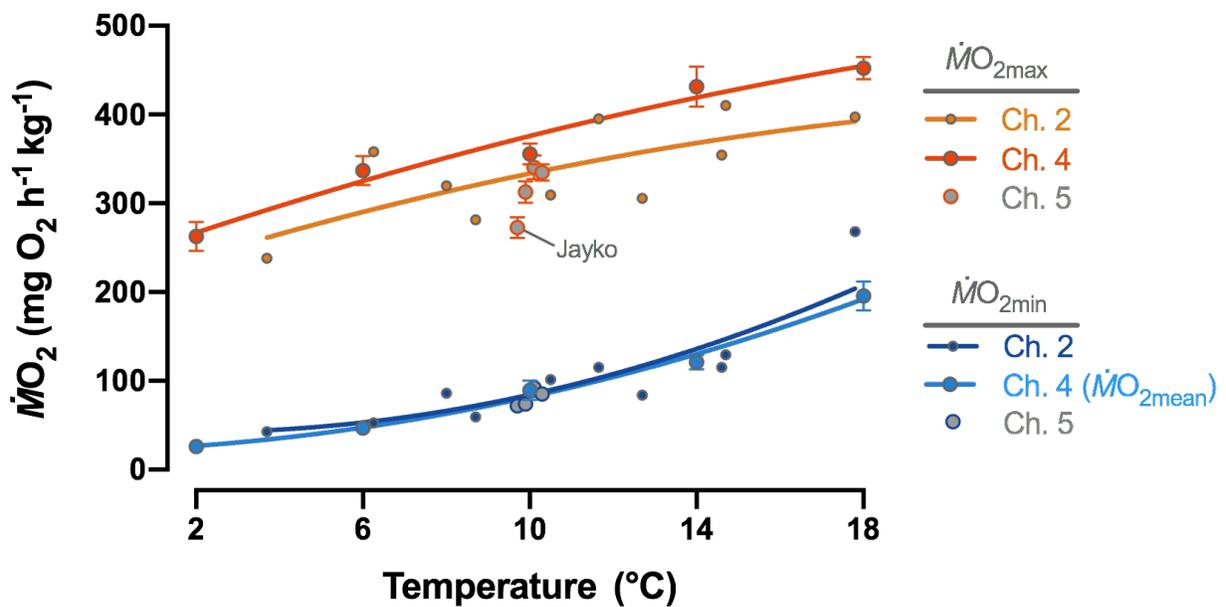


Figure 6.5 Thermal performance curves (TPC) for absolute aerobic scope (AAS) in wild and hatchery Arctic char relative to Pacific salmon species, Atlantic salmon, and Arctic cod. TPCs are shown relative to the average (light grey) and warmest (dark grey; Gilbert et al. 2016) river temperature frequency distributions recorded during upriver migrations in the Kitikmeot region of Nunavut, Canada. River temperatures were compiled for five rivers from past (Gilbert et al. 2016) and ongoing fisheries research (M.J.H. Gilbert and L.N. Harris unpublished data). Data sources are indicated by superscript numbers: 1. Clark et al., 2011; 2. Abe et al., 2019; 3. Eliason et al., 2011; 4. Lee et al., 2003; 5. Chapter 4; 6. Chapter 2; 7. Hvas et al. 2017; 8. Drost et al., 2016.

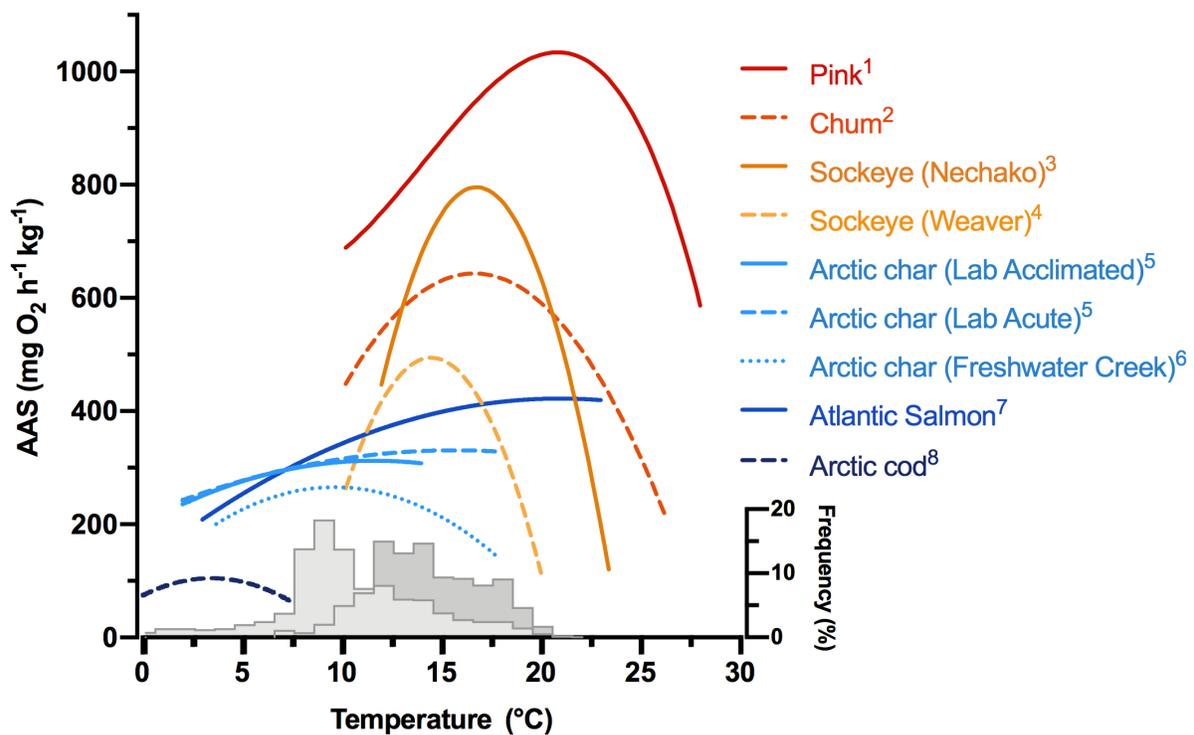
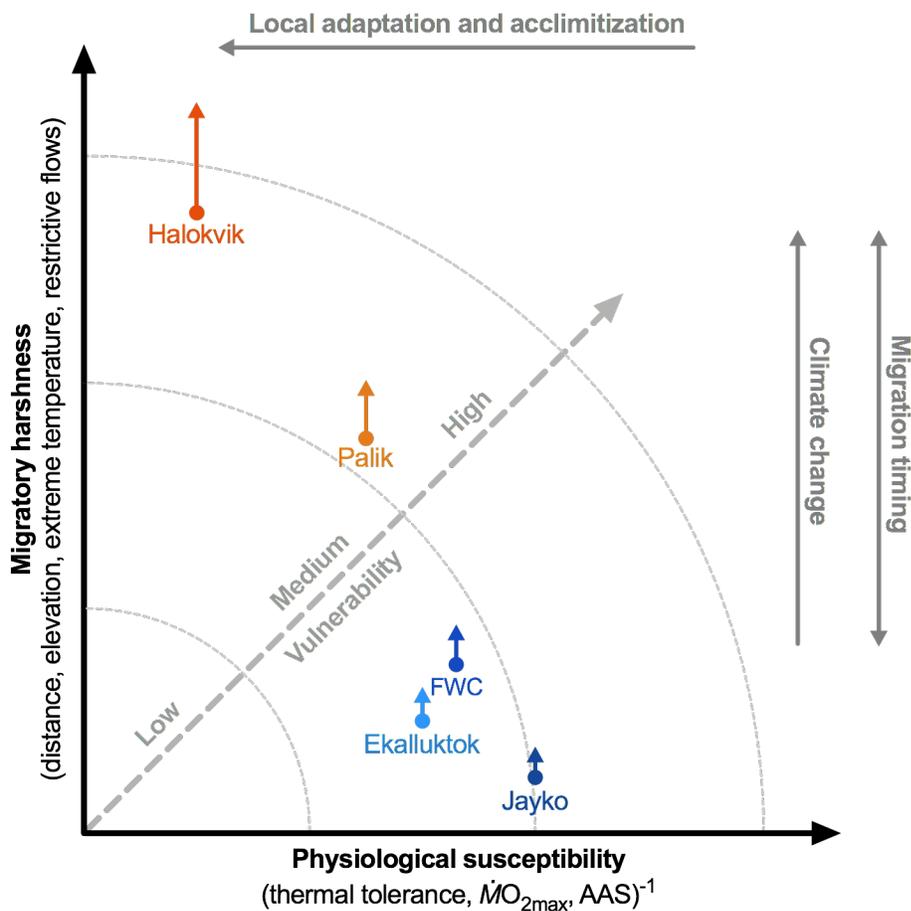


Figure 6.6 A conceptual framework of anadromous salmonids' vulnerability to migration failure as a product of their physiological susceptibility and prevailing migratory conditions.

Susceptibility can be characterized by heat tolerance, maximum aerobic performance, absolute aerobic scope and repeat exercise performance, among numerous other traits. Migration harshness can be characterized by distance, elevation gain and the likelihood of encountering thermal and hydrological barriers to migration. Hypothetical estimates are plotted for the five populations of wild Arctic char studied in this thesis (FWC: Freshwater Creek) based on the relative differences in traits among populations. Plausible effects of climate change (coloured arrows) on migratory harshness are presented based on the assumption that long shallow rivers will be more prone to warming and earlier snow melt. Acclimatization and local adaptation can decrease salmonids' susceptibility to harsh migratory conditions while migration timing can markedly alter the conditions they encounter.



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