CARDIORESPIRATORY PHYSIOLOGY AND TEMPERATURE TOLERANCE AMONG POPULATIONS OF SOCKEYE SALMON (*ONCORHYNCHUS NERKA*)

by

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ABSTRACT

Elevated summer water temperature has been associated with high mortality in adult sockeye salmon (Oncorhynchus nerka) during their once-in-a-lifetime migration up the Fraser River (British Columbia, Canada) to their spawning grounds. There are over 100 genetically distinct populations of sockeye salmon in the Fraser River watershed, varying in migration distance, elevation gain, river temperature and river flow. This thesis studied the physiological basis for temperature tolerance in sockeye salmon and examined the overall hypothesis that each sockeye salmon population has physiologically adapted to meet their specific upriver migration conditions.

Swimming and cardiorespiratory performance were compared over a range of temperatures across six wild, migrating adult sockeye salmon populations. All populations maintained maximum performance across the entire range of temperatures typically encountered during their upriver migration, with Chilko sockeye salmon emerging as the most high temperature-tolerant. In addition, populations with more challenging migrations had greater aerobic scope, larger hearts and improved coronary supply. These results suggest that sockeye salmon populations have physiologically adapted to cope with their local upriver migration conditions, despite never before having performed the upriver migration.

Temperatures exceeding the population-specific thermal optimum resulted in severely impaired aerobic scope and swimming performance. This study suggests that population-specific thermal limits are set by physiological limitations in aerobic performance. Specifically, fish may be unable to swim at warm temperature due to insufficient oxygen supply to meet demand, triggered via a cardiac limitation due to reduced scope for heart rate.

Given the key role of the heart in limiting thermal tolerance, the role of cardiac adrenergic stimulation was examined as a potential mechanism underlying the observed
differences in thermal tolerance across sockeye salmon populations. Chilko sockeye salmon had a greater density of ventricular β-adrenoceptors, which may provide greater cardiac capacity and protection at temperature extremes, thereby expanding their breadth of thermal tolerance compared to other populations.

This thesis suggests that sockeye salmon populations will be differentially affected by warming river temperatures, raising conservation concerns for biodiversity. This work provides important insight into local adaptation in sockeye salmon and identifies a possible cause for in-river mortality associated with warm temperatures in sockeye salmon.
PREFACE


E. J. Eliason was the primary contributor to the experimental design, data collection, data analysis and manuscript preparation. A. P. Farrell and S. G. Hinch provided supervision, assistance with experimental design and helped with manuscript preparation. T. D. Clark, L. M. Hanson, Z. S. Gallagher, K. M. Jeffries and M. K. Gale provided valuable secondary assistance in the field and during data collection. M. J. Hague and D. A. Patterson provided Fraser River temperature information and modeling expertise.

All procedures were approved by the University of British Columbia’s Animal Care Committee in accordance with the Canadian Council on Animal Care (A06-0328 and A08-0388).
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<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>% compact</td>
<td>percentage compact myocardium</td>
</tr>
<tr>
<td>AIC</td>
<td>Akaike’s Information Criterion</td>
</tr>
<tr>
<td>ATP</td>
<td>adenosine triphosphate</td>
</tr>
<tr>
<td>ATU</td>
<td>accumulated thermal units</td>
</tr>
<tr>
<td>A-V$_{O2}$</td>
<td>tissue oxygen extraction</td>
</tr>
<tr>
<td>bl s$^{-1}$</td>
<td>body lengths per second</td>
</tr>
<tr>
<td>$B_{max}$</td>
<td>$\beta_2$-adrenoceptor density</td>
</tr>
<tr>
<td>CAER</td>
<td>Centre for Aquaculture and Environmental Research</td>
</tr>
<tr>
<td>$C_{aO2}$</td>
<td>arterial oxygen content</td>
</tr>
<tr>
<td>Cl$^-$</td>
<td>chloride</td>
</tr>
<tr>
<td>CLL</td>
<td>Cultus Lake Laboratory</td>
</tr>
<tr>
<td>$C_{O2}$</td>
<td>oxygen content</td>
</tr>
<tr>
<td>COT</td>
<td>cost of transport</td>
</tr>
<tr>
<td>COT$_{net}$</td>
<td>net cost of transport</td>
</tr>
<tr>
<td>COT-\dot{Q}</td>
<td>cardiovascular cost of transport</td>
</tr>
<tr>
<td>COT-\dot{Q}_{net}</td>
<td>net cardiovascular cost of transport</td>
</tr>
<tr>
<td>$C_{vO2}$</td>
<td>venous oxygen content</td>
</tr>
<tr>
<td>DFO</td>
<td>Department of Fisheries and Oceans Canada</td>
</tr>
<tr>
<td>$D_M$</td>
<td>migration distance</td>
</tr>
<tr>
<td>$E_M$</td>
<td>migration elevation</td>
</tr>
<tr>
<td>EPOC</td>
<td>excess post oxygen consumption</td>
</tr>
<tr>
<td>$f_H$</td>
<td>heart rate</td>
</tr>
<tr>
<td>$f_{H_{max}}$</td>
<td>maximum heart rate</td>
</tr>
</tbody>
</table>
\( f_{\text{rest}} \)  
resting heart rate

\( F_M \)  
migration Fraser River flow

\( \text{GSI} \)  
gonadalsomatic index

\( \text{Hb} \)  
haemaglobin

\( \text{Hct} \)  
hematocrit

\( \text{HSI} \)  
hepatosomatic index

\( K^+ \)  
potassium

\( K_d \)  
\( \beta_2 \)-adrenoceptor binding affinity

\( M \)  
body mass

\( \text{MCHC} \)  
mean corpuscular haemaglobin concentration

\( \dot{\text{MO}}_2 \)  
rate of oxygen consumption (measured in mg)

\( \dot{\text{MO}}_{2\text{max}} \)  
maximum oxygen consumption

\( \dot{\text{MO}}_{2\text{rest}} \)  
resting oxygen consumption

\( \text{MS-222} \)  
tricaine methanesulfonate

\( \text{Na}^+ \)  
sodium

\( \text{NaHCO}_3 \)  
sodium bicarbonate

\( \text{OCLTT} \)  
oxxygen- and capacity-limited thermal tolerance

\( P_{\text{aO}}_2 \)  
arterial partial pressure of oxygen

\( P_{\text{O}}_2 \)  
partial pressure of oxygen

\( \text{POF} \)  
post-orbital-fork length

\( \text{POH} \)  
post-orbital-hypural length

\( P_{\text{vO}}_2 \)  
venous partial pressure of oxygen

\( \dot{Q} \)  
cardiac output

\( \dot{Q}_{\text{max}} \)  
maximum cardiac output
$\dot{Q}_{rest}$   resting cardiac output
RDCM   relative dry compact mass
RDVM   relative dry ventricular mass
RR   recovery ratio
RVM   relative wet ventricular mass
SEM   standard error of the mean
SSI   splenosomatic index
T90%   upper temperature experienced by the 90th percentile of fish
$T_aO_2$   arterial oxygen transport
$T_{crit}$   critical temperature
$T_M$   migration Fraser River temperature
$T_{max0-50}$   group of fish swum at temperatures higher than $T_{opt}$ at which 0-50% of maximum aerobic scope was attained
$T_{max50-90}$   group of fish swum at temperatures higher than $T_{opt}$ at which 50-90% of maximum aerobic scope was attained
$T_{min50-90}$   group of fish swum at temperatures lower than $T_{opt}$ at which 50-90% of maximum aerobic scope was attained
$T_{opt}$   optimal temperature
$T_p$   pejus temperature
$T_{vO_2}$   venous oxygen transport
$U_{crit}$   critical swimming velocity
$\dot{V}_{O_2}$   rate of oxygen consumption (measured in ml)
$V_s$   stroke volume
$V_{smax}$   maximum stroke volume
<table>
<thead>
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<th>Symbol</th>
<th>Description</th>
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<tbody>
<tr>
<td>$V_{rest}$</td>
<td>resting stroke volume</td>
</tr>
<tr>
<td>$w$</td>
<td>AIC weight</td>
</tr>
<tr>
<td>β-AR</td>
<td>β-adrenoceptor</td>
</tr>
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ACKNOWLEDGMENTS

First, I want to express my gratitude to my supervisor, Dr. Tony Farrell, for guiding me through both my MSc and PhD over the last 26% of my life. Tony is a first-class scientist who pushed me to reach my potential and gave me every opportunity to succeed. I am grateful for his advice, wisdom and patience.

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DEDICATION

In loving memory to Grandpa Eliason, who taught me the value of gratitude, the power of a smile, to embrace life and most of all, sparked my love and wonder of fish.
CHAPTER 1: INTRODUCTION

Temperature has profound effects on the distribution and physiology of animals. Temperature effects occur over three distinct time scales: acute (direct effects occurring in minutes to hours), acclimation (physiological, morphological and biochemical adjustments occurring over days to weeks) and adaptation (spans generations, due to natural selection acting on individuals). Given that most fish are ectotherms, they are highly susceptible to perturbations in temperature that occur in their aquatic environment. The study of the physiological mechanisms that limit temperature tolerance is a biological problem of fundamental importance.

A central tenet in evolutionary biology is that geographically and reproductively isolated populations are locally adapted to cope with their specific environment (see Schluter, 2000; Taylor, 1991). Heritable traits that enhance survival and reproductive success in a given environment will likely be under strong selection pressure. This thesis examines temperature tolerance and local adaptation using genetically and geographically distinct populations of Fraser River sockeye salmon (Oncorhynchus nerka) as a model.

1.1 The Fry Curve for Aerobic Scope

Fry (1947) established that temperature both controlled and limited metabolic rate in fish, making a direct link between thermal tolerance and oxygen consumption. Fry recognized that temperature tolerance tests (e.g. CT\textsubscript{min} and CT\textsubscript{max}) were restrictive, only differentiating the temperature limits for short-term survival. Instead, Fry realized that it was essential to characterize and understand the temperature limits for a fish to thrive and interact within its environment – e.g. to escape predators, to interact with other animals, to migrate upstream, to
find, digest and assimilate food. As such, Fry examined the effects of temperature on aerobic scope, or the difference between minimum and maximum oxygen consumption. The ‘Fry curve’ for aerobic scope is typically bell-shaped as a function of temperature and represents the maximum oxygen available for activities beyond those considered maintenance, such as swimming, reproduction, feeding and growth. Thus, Fry curves can be used to examine the functional temperature limits for performance.

Oxygen consumption (\(\dot{\text{MO}}_2\)) varies as a function of temperature. Minimum or resting \(\dot{\text{MO}}_2\) (\(\dot{\text{MO}}_{2\text{rest}}\)) represents the metabolic cost to simply exist in a resting, thermally acclimated, non-digesting, non-reproducing fish. \(\dot{\text{MO}}_{2\text{rest}}\) typically increases exponentially with increasing temperature until it approaches lethal temperatures, as expected for temperature effects on rate functions (Fig 1.1). Obviously, in order to feed, reproduce, grow and move, fish must be able to increase \(\dot{\text{MO}}_2\) above minimum levels. As temperatures increases, active or maximum \(\dot{\text{MO}}_2\) (\(\dot{\text{MO}}_{2\text{max}}\)) increases faster than \(\dot{\text{MO}}_{2\text{rest}}\), thus increasing aerobic scope (Fig 1.1). The optimal temperature (\(T_{\text{opt}}\)) coincides with maximal aerobic scope, as do maximal cardiac and swimming performance (Brett, 1971). Beyond \(T_{\text{opt}}\), \(\dot{\text{MO}}_{2\text{max}}\) fails to further increase and rapidly declines, causing a reduction in aerobic scope. The temperatures at which aerobic scope starts to decline are termed the pejus temperatures [\(T_p\), pejus means getting worse (Pörtner, 2001)]. The range of temperatures between the upper and lower \(T_p\) when maximum aerobic scope is maintained is termed the \(T_{\text{opt}}\) window (Fig 1.1). At critical temperatures (\(T_{\text{crit}}\)), aerobic scope approaches zero (Fig 1.1), resulting in a transition to anaerobic metabolism and only passive, short-term survival (Pörtner, 2002; Pörtner, 2001; Pörtner and Farrell, 2008).

\(\dot{\text{MO}}_2\) and aerobic scope varies considerably among species. Likewise, Fry curves take on different shapes. For example, eurythermal species such as goldfish (\textit{Carassius auratus}) and
*Fundulus heteroclitus* have a broader $T_{\text{opt}}$ window compared to more stenothermal fish like sockeye salmon (Fangue et al., 2006; Fry, 1947; Fry, 1957; Lee et al., 2003c). In addition, more athletic fish like wild sockeye salmon have a higher maximum aerobic scope compared to goldfish or hatchery-reared rainbow trout (*Oncorhynchus mykiss*) (Farrell, 2009). Moreover, aerobic scope will vary throughout the life cycle of an individual fish, shifting both the $T_{\text{opt}}$ window and height of aerobic scope (Brett, 1965; Farrell, 2009). Aerobic scope also varies with environmental conditions (Farrell, 2009). For example, hypoxia (low environmental $O_2$) and hypercapnia (high environmental $CO_2$) reduce maximum aerobic scope and constrain the $T_{\text{opt}}$ window (Pörtner and Farrell, 2008). Behaviour has also been demonstrated to alter $T_{\text{opt}}$. For example, competition shifted $T_{\text{opt}}$ and suppressed growth in brook trout (*Salvelinus fontinalis*) (McMahon et al., 2007). Thus, aerobic scope is highly pliable, varying across species, life stages, environmental conditions and with behaviour.

To understand the mechanistic basis of aerobic scope and its dependent relationship with temperature, details of how oxygen is delivered from the water to the mitochondria via the cardiorespiratory system are required. For fish such as salmonids, maximum $\dot{M}O_2$ occurs during maximum aerobic swimming. Therefore, information on cardiorespiratory physiology during swimming is necessary.

### 1.2 Cardiorespiratory Physiology with Swimming

According to the Fick equation for vascular perfusion, whole-animal $\dot{M}O_2$ is determined by the product of cardiac output ($\dot{Q}$) and the difference between arterial and venous oxygen content ($C_{aO_2}$ and $C_{vO_2}$, respectively), which is termed the tissue oxygen extraction ($A-V_{O_2} = \dot{Q}(C_{aO_2} - C_{vO_2})$).
\[ \dot{M}O_2 = Q \times A-V_O2 \]

\( Q = f_H \times V_s \)

A-V\(_O2\) is determined by the partial pressure of oxygen and the capacitance for oxygen in the blood. Accordingly, any change in \( \dot{M}O_2 \) must be due to alterations in some combination of these factors (i.e. \( f_H, V_s, C_{aO2} \) and \( C_{vO2} \)).

In order to support aerobic swimming, oxygen must be transported from the gills to the swimming muscles, which is one of the roles of the cardiovascular system. During maximal aerobic swimming, \( \dot{Q} \) increases 2-3 fold in salmonids (Kiceniuk and Jones, 1977; Stevens and Randall, 1967; Thorarensen et al., 1996). While increases in both \( f_H \) and \( V_s \) contribute to the increase in \( \dot{Q} \) during swimming, \( V_s \) typically increases to a greater extent in salmonids (Kiceniuk and Jones, 1977).

Another factor that can potentially be altered to increase \( \dot{M}O_2 \) is \( C_{aO2} \). Oxygen transport to the tissues (\( T_{aO2} \)) by the circulatory system can be expressed as the product of \( \dot{Q} \) and \( C_{aO2} \). Arterial blood leaves the gills close to fully saturated as a consequence of the counter-current arrangement of blood and water flow at the gills. As a result, \( C_{aO2} \) is near maximal at rest and during swimming (Gallaugher et al., 2001; Kiceniuk and Jones, 1977; Randall and Daxboeck, 1982; Thorarensen et al., 1996). \( C_{aO2} \) could further increase by raising the blood haemoglobin (Hb) concentration either acutely via splenic contraction or chronically via erythropoiesis. However, blood [Hb] appears to be optimized in swimming rainbow trout (Gallaugher et al., 1995; Gallaugher et al., 1998), thus alterations to blood [Hb] may not play a major role during swimming. Therefore, the primary means of increasing \( T_{aO2} \) during swimming is via an increase in \( \dot{Q} \).

The last means of increasing \( \dot{M}O_2 \) during swimming is through increased oxygen extraction from the blood by the tissues, which results in decreased \( C_{vO2} \) and \( P_{vO2} \) and typically a
2-3 fold increase in A-V$_{O2}$ (Farrell and Clutterham, 2003; Kiceniuk and Jones, 1977; Stevens and Randall, 1967). However, evidence has been presented for a minimum P$_{vO2}$ threshold of around 15 torr in cold and 29 torr in warm, normoxic salmonids (Farrell and Clutterham, 2003, Farrell, 2007), which may serve as a mechanism to ensure sufficient oxygen is supplied to the heart (see section 1.3.2 below). Notably, during maximal swimming, tissue oxygen extraction may continue to increase despite a constant P$_{vO2}$ since a decrease in blood pH (due to anaerobic metabolism) may elicit Root and Bohr effects on haemoglobin (a right and downward shift in the oxyhaemoglobin dissociation curve) thus facilitating oxygen unloading (Rummer, 2010).

As fish approach maximal swimming velocity during critical swimming tests, they switch to anaerobic metabolism (Burgetz et al., 1998). As a result, blood becomes acidic (low pH), hypoxemic (low P$_{vO2}$) and hyperkalemic (high [K$^+$]) (Holk and Lykkeboe, 1998; Kiceniuk and Jones, 1977), inhibiting cardiac contractility (Driedzic and Gesser, 1994). Adrenergic stimulation acts to maintain $Q_{max}$ and protect against these noxious venous blood conditions during swimming (Hanson et al., 2006).

During recovery from anaerobic exercise, $\dot{M}O_2$ remains elevated (termed the excess post-exercise oxygen consumption; EPOC) in order to restore oxygen stores and support the metabolic costs associated with restoring high-energy phosphates, biochemical imbalance (e.g. glucose and lactate), ionic and osmotic imbalance and glycogen levels (Scarabello et al., 1992). EPOC represents a cost to the fish and could limit the ability for fish to resume normal activity in a timely manner. Even so, salmonids have been shown to have a remarkable ability to recover rapidly and repeat maximum swim performance after only a brief 30-45 min recovery period (Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003b; MacNutt et al., 2006; MacNutt et al., 2004; Wagner et al., 2006).
Two important concepts emerge from this brief overview of cardiorespiratory physiology during swimming in fish. First, temperature could be acting on any or all of the components linking the transport of oxygen from the environment to the mitochondria. Second, in order to understand the effect of temperature on aerobic scope, I need to measure temperature effects on $\dot{M}O_2$, $f_i$, $V_s$, $P_{aO2}$, $C_{aO2}$, $P_{vO2}$ and $C_{vO2}$, which are considered in section 1.3 below.

1.3 Oxygen and Capacity Limited Thermal Tolerance

Oxygen is the final electron acceptor in the suite of mitochondrial reactions that ultimately create ATP, the energy currency of the cell. The oxygen cascade is composed of several convection and diffusion steps during which oxygen travels down a partial pressure gradient from the environment to the mitochondria (Fig 1.2). First, oxygen-rich water is brought into contact with the respiratory surface. Gill ventilation rate and volume determine this step. Next, oxygen diffuses from the water environment, across the secondary lamellae of the gills, and into the blood where it binds to Hb in red blood cells. The partial pressure gradient between the water and the blood as well as gill anatomy set this step. The circulatory system transports the oxygen-bound Hb by convection to the tissues. Cardiac output and [Hb] govern this step. Finally, oxygen diffuses across the capillary wall and into the cell where it is ultimately used during mitochondrial respiration. This final step is controlled by tissue anatomy and the partial pressure gradient between the blood and mitochondria.

The mechanism of the decline in aerobic scope at temperatures above $T_{opt}$ is poorly understood. Oxygen- and capacity-limited thermal tolerance (OCLTT) suggests that thermal tolerance is set by oxygen limitations due to a mismatch between cellular oxygen supply and
demand (Pörtner, 2001). This oxygen limitation is proposed to occur at the whole organism level, due to capacity limitations in ventilation and circulation (Pörtner, 2002; Pörtner, 2001; Pörtner and Knust, 2007). What is unclear is exactly which step(s) in the oxygen cascade limits oxygen flux, thus setting thermal limits. Specifically, is the mismatch in oxygen supply and demand due to a limitation at the gills, at the heart or at the tissues? Evidence for each of these possibilities has been detailed in Farrell (2009), and is outlined below.

The primary reason for the uncertainty is that few studies have studied the effect of temperature on performance limitations while simultaneously and directly measuring sufficient cardiorespiratory and oxygen status variables in order to identify the limiting factor(s) (Wang and Overgaard, 2007). While a small collection of studies have examined acute temperature effects on some of these variables in resting fish (e.g. Clark et al., 2008b; Gollock et al., 2006; Heath and Hughes, 1973; Sartoris et al., 2003), only Steinhausen et al. (2008) has measured all the necessary variables in fish swimming at close to maximum speed. Therefore, comprehensive cardiorespiratory studies in maximally swimming fish are needed.

1.3.1 Is There a Limitation in Oxygen Uptake at the Gill?

There are two primary reasons why an oxygen limitation at the gill has been proposed. First, it is well known that environmental oxygen availability decreases at high temperatures because water oxygen content decreases by around 2% per °C with increasing temperature (Dejours, 1975). Fish must therefore increase gill ventilation or increase oxygen extraction from the water to compensate. Second, there is decrease in blood oxygen affinity (a right-shift in the oxyhaemoglobin dissociation curve) as temperature increases (Jensen et al., 1998; Perry and
Reid, 1994), which hampers oxygen uptake at the gill, though it facilitates tissue oxygen extraction.

The key piece of evidence required to support the hypothesis that there is an oxygen limitation at the gill (via either insufficient oxygen delivery to the gills or an oxygen diffusion limitation across the gills) is a decrease in $P_{aO2}$ and $C_{aO2}$ at temperatures above $T_{opt}$.

Evidence supporting this hypothesis has been provided by Heath and Hughes (1973) who showed a decrease in $C_{aO2}$ in resting rainbow trout exposed to an acute temperature increase. Notably, hematocrit was not measured to verify whether haemodilution occurred during repeated blood sampling. Similarly, Taylor et al. (1993) found a decrease in $C_{aO2}$ at 18°C in resting and swimming rainbow trout acclimated to seasonal temperatures (4, 11 and 18°C), but hematocrit also decreased by half. A study by Clark et al. (2008b) produced conflicting results. Large resting adult chinook salmon (*Oncorhynchus tshawytscha*) displayed a decrease in $C_{aO2}$ and $P_{aO2}$ during an acute temperature increase while smaller adults did not. However, the holding tubes could have constrained gill movements in the larger chinook salmon, preventing adequate gill ventilation.

In contrast, two studies have found evidence against a limitation in oxygen uptake at the gill. Steinhausen et al. (2008) found that $C_{aO2}$ and hematocrit remained constant in both resting and swimming sockeye salmon exposed to acute warming. Moreover, $P_{aO2}$ actually increased in resting and remained constant in swimming sockeye salmon. Similarly, Sartoris et al. (2003) found that $P_{aO2}$ remained constant during acute temperature increases in resting Atlantic cod (*Gadus morhua*). Therefore, current data are equivocal and further investigation is required.
1.3.2 Is There a Limitation in Oxygen Convection by the Heart?

An oxygen limitation at the level of the heart would be evident by the inability for $\dot{Q}_{\text{max}}$ to increase at temperatures above $T_{\text{opt}}$. Since oxygen demand increases with increasing temperature, $\dot{Q}_{\text{max}}$ must also increase in order to supply sufficient oxygen to keep pace with the tissue oxygen demand. If $\dot{Q}_{\text{max}}$ fails to keep up, insufficient oxygen will reach the muscles and the fish will cease or slow swimming. Indeed, several studies in swimming sockeye salmon and rainbow trout found that both $\dot{\text{MO}}_{2\text{max}}$ and $\dot{Q}_{\text{max}}$ ceased to increase above $T_{\text{opt}}$ (Brett, 1971; Steinhausen et al., 2008; Taylor et al., 1996), providing evidence of a cardiac limitation.

The mechanistic basis of cardiac collapse at high temperature has been considered in detail (Farrell, 1997; Farrell, 2002; Farrell, 2007; Farrell, 2009; Farrell et al., 2009; Pörtner, 2002; Taylor et al., 1997). During warming, the increase in $\dot{Q}$ is almost entirely due to an increase in $f_H$ (Clark et al., 2008b; Sandblom and Axelsson, 2007; Steinhausen et al., 2008). This is true in both resting and swimming fish (Farrell, 2009). Conversely, $V_s$ has been demonstrated to be either insensitive to temperature (Cech Jr. et al., 1976; Clark et al., 2008b; Clark and Seymour, 2006; Gollock et al., 2006; Steinhausen et al., 2008) or to decrease at warm temperatures (Axelsson et al., 1992; Brodeur et al., 2001; Sandblom and Axelsson, 2007). The increase in $f_H$ is likely mediated through a direct temperature effect on the pacemaker rate (Randall, 1970), which reaches a maximum of ~120 beats min$^{-1}$ in many active fish (Davie and Farrell, 1991; Farrell, 1991). The prevailing idea is that maximum $f_H$ is reached at $T_{\text{opt}}$ (Farrell, 2009). Beyond $T_{\text{opt}}$, maximum $f_H$ can no longer increase while resting $f_H$ continues to increase, resulting in a decreased scope for $f_H$. Given that scope for $f_H$ approached zero at high temperature for swimming sockeye salmon (Steinhausen et al., 2008), the inability for $f_H$ to further increase
\( Q_{\text{max}} \) has been identified as a possible initiating factor limiting aerobic performance at high temperature (Farrell et al., 2009).

Another potential factor that could limit aerobic performance at high temperature is oxygen delivery to the heart itself. During exercise, oxygen requirements of the heart increase 3-5 fold, which makes up ~1% of total \( \dot{M}O_2 \) (Farrell and Steffensen, 1987). Salmonid hearts are composed of two types of myocardium; the compact myocardium and spongy myocardium. The outer, compact myocardium receives well-oxygenated arterial blood directly from the gills via the coronary system. As a result, the compact myocardium has a reliable source of oxygen during exercise, just like the skeletal muscles. Certainly, if \( P_{aO_2} \) declines at warm temperatures (see section 1.3.1 above), then oxygen delivery to the compact myocardium could be impaired. In contrast, the inner spongy myocardium of salmonids lacks capillaries and receives oxygen from whatever is leftover in the venous blood by the other tissues. As such, the spongy myocardium has a much less reliable oxygen supply, especially since \( P_{vO_2} \) decreases during swimming.

Though the total amount of oxygen in the blood (\( C_{vO_2} \)) is likely sufficient to meet oxygen demand, a limitation may occur in the rate in which oxygen can be delivered, which depends on the oxygen tension (\( P_{vO_2} \)), contact time of the blood (heart rate) and the arrangement of the spongy myocardium. The spongy myocardium is composed of trabeculae which are arranged in meshwork-like sheets that presumably increase the surface area for oxygen exchange (Pieperhoff et al., 2009). Regardless, if \( P_{vO_2} \) decreases below a threshold level for an adequate rate of oxygen diffusion at high temperature, an oxygen diffusion limitation to the spongy myocardium may occur, resulting in cardiac failure and triggering a limitation in blood oxygen convection to the
swimming muscles. In light of this, individuals possessing a greater percentage of compact myocardium may be able to maintain cardiac performance at higher temperatures.

Cardiac collapse at temperatures above $T_{opt}$ may also relate to the noxious venous blood environment created when exercising at high temperature. Salmonids increase their reliance on anaerobic metabolism when swimming at high temperature (Brett, 1964; Jain and Farrell, 2003; Steinhausen et al., 2008). Anaerobic metabolism leads to the triple threat of acidotic, hypoxemic and hyperkalemic venous blood, which inhibits cardiac contractility (Dridezic and Gesser 1994). Though adrenergic stimulation protected cardiac function and maintained $\dot{Q}_{\text{max}}$ in in situ perfused rainbow trout hearts exposed to the triple threat at optimal temperatures (Hanson et al., 2006), adrenergic protection was diminished at temperatures above $T_{opt}$ (Hanson and Farrell, 2007). The attenuation of the protective and stimulatory effects of adrenaline at high temperature has been attributed to a decline in adrenaline-binding ventricular cell-surface $\beta$-adrenoceptor density ($B_{\text{max}}$) (Keen et al., 1993). As a corollary, individuals possessing an elevated $B_{\text{max}}$ may be able to maintain $\dot{Q}_{\text{max}}$ at higher temperatures.

In summary, a limitation in oxygen convection by the heart could manifest in a number of ways. $\dot{Q}_{\text{max}}$ could fail to increase above $T_{opt}$ due to reduced scope for $f_H$, due to insufficient oxygen delivery to the cardiac myocardium or due to the negative ionotropic and chronotropic effects of acidotic, hypoxic and hyperkalemic venous blood.

1.3.3 Is There a Limitation in Oxygen Delivery to the Tissue Mitochondria?

Muscle oxygen demand increases at high temperature due to temperature effects on rate functions as well as an increase in mitochondria proton leakage which leads to inefficient ATP
production in skeletal muscle (Barron et al., 1987; Pörtner, 2001). Therefore, the muscle must extract more oxygen from the blood in order to meet the increased demand at high temperature. Either a diffusion limitation or a perfusion limitation could lead to insufficient oxygen reaching the mitochondria to meet demand.

A diffusion limitation could develop due to inadequate driving force (low $P_{aO_2}$), insufficient capillary density [white muscle has particularly low capillary density (Egginton et al., 2000)], or ineffective muscle cell morphology (poor mitochondria density or location). A perfusion limitation could result from inadequate $Q$ leading to insufficient muscle capillary perfusion or an issue in blood flow distribution.

Several adjustments can help compensate for the increased oxygen demand at warm temperatures. The right-shift in the oxyhaemoglobin dissociation curve facilitates oxygen extraction (Jensen et al., 1998; Perry and Reid, 1994) as does the similar decrease in oxygen-affinity for myoglobin (Stevens and Carey, 1981). In addition, Krogh’s diffusion coefficient for oxygen increases as biological fluid viscosity decreases with warming temperatures (Taylor et al., 1997).

An oxygen diffusion limitation at the tissues would become apparent if $P_{vO_2}$ was maintained at temperatures above $T_{opt}$, as has been reported in several studies. For example, Steinhausen et al. (2008) found no change in $P_{vO_2}$ with acute temperature increases in swimming sockeye salmon and $P_{vO_2}$ actually increased with temperature in resting fish. Further evidence comes from the observation that when fish quit swimming, venous blood was still partially saturated (Kiceniuk and Jones, 1977), and a venous threshold of $\sim 20$ torr (range = 15 to 29 torr in normoxia) has been proposed (Farrell and Clutterham, 2003; Farrell, 2007).
In contrast, other studies provide evidence against a tissue diffusion limitation. Heath and Hughes (1973) observed a decrease in \( C_vO_2 \) at high temperatures in resting rainbow trout, but as pointed out earlier, hematocrit was not measured. Likewise, Sartoris et al. (2003) reported a decrease in \( P_vO_2 \) in Atlantic cod exposed to an acute temperature increase. Clark et al. (2008b) found a significant decrease in \( P_vO_2 \) and \( C_vO_2 \) at the highest test temperatures in resting adult chinook salmon. Moreover, McKenzie et al. (2004) used optical fibre sensors in red muscle of rainbow trout at 13-15°C to determine that intramuscular \( P_O2 \) never decreased below 45 torr, suggesting that oxygen supply to red muscle was not a limiting factor in exhaustion from swimming. Altogether, evidence is conflicting and further study is required.

1.4 Can Species Comparisons and Acclimation Studies Help Identify Limitations?

An indirect method of assessing potential limitations for exercise performance or thermal tolerance is to compare cardiorespiratory and morphological variables across a) species and b) with acclimation. Identified variables could represent potential locations where evolutionary adaptation has resulted in improved exercise performance or thermal tolerance.

For example, highly athletic fish such as tuna possess a greater \( \dot{M}O_{2\text{max}} \), \( Q_{\text{max}} \), and \( C_{aO2} \), enhanced gill surface area, large, pyramidal-shaped hearts with a higher percent compact myocardium, smaller red muscle fibres with greater capillary and mitochondrial density and a higher \( \beta \)-adrenoceptor density compared to less athletic species (Brill and Bushnell, 1991a; Brill and Bushnell, 1991b; Farrell, 1996; Mathieu-Costello et al., 1992; Mathieu-Costello et al., 1996; Olsson et al., 2000). In addition, aerobic exercise training in salmonids has resulted in higher \( \dot{M}O_{2\text{max}} \), \( Q_{\text{max}} \), Hct, [Hb], \( C_{aO2} \), and A-V \( O_2 \), increased cross-sectional area of red muscle,
increased red muscle capillarity, and cardiac hypertrophy (Davie et al., 1986; Farrell, 1991; Farrell et al., 1990; Farrell et al., 1991; Gallaugher et al., 2001; Kiessling et al., 1994; Thorarensen et al., 1993).

The same principle can be applied for thermal tolerance. For example, acclimation to warm temperature in teleosts resulted in smaller relative ventricular mass with a higher percent compact myocardium, decreased gill epithelial thickness, decreased red muscle capillarization and decreased β-adrenoceptor density (Egginton et al., 2000; Farrell et al., 1988a; Gamperl and Farrell, 2004; Gamperl et al., 1998; Goolish, 1987; Keen et al., 1993; Pelouch and Vornanen, 1996; Taylor et al., 1997) though many of these findings seem counterintuitive. Notably, stenothermic salmonids appear to have a limited capacity to acclimate in comparison with more eurythermal species such as goldfish. For example, a 10°C difference in acclimation temperature changed the upper incipient lethal temperature by only 0-2°C in juvenile chinook salmon (Brett, 1952) compared to ~5°C in goldfish (Fry, 1947).

Rather than applying this principle by comparing across species, I took advantage of the enormous variety in upriver migration environment among genetically isolated populations of sockeye salmon in the Fraser River watershed in order to make intraspecific comparisons in aerobic performance and temperature tolerance.

1.5 Fraser River Sockeye Salmon

Every year, millions of sockeye salmon return to the Fraser River (BC, Canada) to perform the physically demanding upriver migration. During this highly aerobic feat, sockeye salmon must swim continuously against a fast-flowing river for several weeks, swimming 2 to 4
km h\(^{-1}\), which equates to ground speeds of 20 to 40 km day\(^{-1}\) (English et al., 2005; Hinch and Rand, 1998). Sockeye salmon cease feeding in the ocean, prior to entering the river. Therefore, upriver swimming and reproductive maturation (secondary sexual characteristics, gonad growth) are fuelled entirely by endogenous energy stores. Moreover, sockeye salmon are semelparous, meaning that they only spawn once. As a result, individual fish have a single opportunity to complete the journey to their spawning grounds in order to reproduce. Those that don’t make it have zero reproductive success and no lifetime fitness. As a corollary, there is likely strong selection pressure for successful upstream migration.

Fraser River sockeye salmon display a remarkable fidelity to return to their natal stream to spawn (Burgner, 1991). This has resulted in over 100 genetically and geographically distinct populations of sockeye salmon within the Fraser River watershed (Beacham et al., 2005). Populations vary in migration distance (100 to 1100 km), elevation gain (10 to 1200 m), river temperature (9\(^\circ\) to 22\(^\circ\)C), and river flow (2000 to 10,000 m\(^3\) s\(^{-1}\)). Moreover, some populations must traverse major hydrological barriers, such as world-famous Hells Gate, located in the Fraser Canyon ~200 km upstream from the mouth of the Fraser River. Swimming through these difficult stretches requires maximum aerobic scope and anaerobic swimming (Hinch and Bratty, 2000; Rand and Hinch, 1998). As such, some populations have a more difficult upstream migration compared to others.

1.5.1 Environmental Adaptation

Local adaptation has been defined as the process that increases the frequency of traits within a population that augments the reproductive success or survival of individuals possessing
such traits (Taylor 1991). For local adaptation to occur, a given trait must be 1) heritable, 2) differentially expressed across individuals, and 3) be associated with differential survival or fitness. Several correlational studies have provided circumstantial evidence of local adaption in salmonids (for a review, see Taylor, 1991). For example, juvenile Atlantic salmon (*Salmo salar*) and coho salmon (*Oncorhynchus kisutch*) from fast-flowing streams were more stream-lined and possessed longer paired-fins compared to fish residing in lower velocity streams (Riddell and Leggett, 1981; Taylor and McPhail, 1985a). Similarly, adult chum salmon (*Oncorhynchus keta*) and adult pink salmon (*Oncorhynchus gorbuscha*) from larger streams (and were thus exposed to faster flows) possessed larger fins relative to salmon in smaller streams (Beacham, 1984; Beacham, 1985; Beacham and Murray, 1987; Beacham et al., 1988a; Beacham et al., 1988b). Steelhead (*Oncorhynchus mykiss*) and coho populations with longer, more difficult upstream migrations had enhanced prolonged swimming performance compared to more coastal populations (Taylor and McPhail, 1985b; Tsuyuki and Williscroft, 1977). In addition, comparisons among 15 anadromous fish populations across 9 species found that populations with more difficult migrations were more energy efficient compared to those with easier migrations (Bernatchez and Dodson, 1985). Furthermore, a trade-off between egg number and migration distance was reported in chinook salmon (Kinnison et al., 2001).

Intraspecific variability in morphological, physiological and behavioural attributes in Fraser River sockeye salmon may be attributed to population-specific local adaption which facilitates the adult migration and spawning. Indeed, Fraser River sockeye salmon populations with more difficult journeys started their migration with more somatic energy compared to those with shorter, easier migrations (Crossin et al., 2004; Gilhousen, 1980). Moreover, Crossin et al. (2004) demonstrated that Fraser River sockeye salmon populations with more challenging
migrations had fewer eggs and a smaller, more stream-lined body shape. In addition, two Fraser River sockeye salmon populations have been shown to vary in aerobic scope and both possessed a $T_{\text{opt}}$ matching their historical river migration temperature (Farrell et al., 2008; Lee et al., 2003c). Finally, one sockeye salmon population (Chilko, which has a particularly arduous migration to spawn at a high elevation in or adjacent to a glacial lake) had more energetically efficient swimming relative to two other populations (Hinch and Rand, 2000). Collectively, these findings suggest that sockeye salmon arrive at the Fraser River prepared for their specific journey ahead, despite never before having performed the upriver challenge. My thesis builds on this theoretical and empirical support for local adaptation of Fraser River sockeye salmon.

1.5.2 Behavioural and Physiological Responses of Salmon to Temperature

The effect of water temperature on Pacific salmon migration has received substantial attention. Water temperature is known to impact a variety of traits: survival (Crossin et al., 2008; Farrell et al., 2008; Gilhousen, 1990; Macdonald, 2000), behaviour (Berman and Quinn, 1991; Cooke et al., 2004; Crossin et al., 2008; Farrell et al., 2008; Goniea et al., 2006; Hodgson and Quinn, 2002; Keefer et al., 2008a; Newell and Quinn, 2005; Patterson et al., 2007), migration speed (Hanson et al., 2008; Keefer et al., 2008a), swimming performance (Lee et al., 2003c; Farrell et al., 2008; Steinhausen et al., 2008), energetics (Crossin et al., 2004; Hinch and Rand, 1998; Rand et al., 2006), physiology (Crossin et al., 2008; Steinhausen et al., 2008; Young et al., 2006) and disease development (Wagner et al., 2005).

Sockeye salmon are exposed to a wide variety of temperatures during their migration period (Hinch et al., 2006), and Fraser River temperatures have been increasing over the last 60
years (Patterson et al., 2007). An individual fish can encounter temperatures ranging from 11-22°C during the short 3-4 week upriver migration. For example, individual fish routinely experience temperature swings of 3-4°C over 8 days while migrating up the mainstem Fraser (Donaldson et al., 2009). Average peak summer water temperature has increased by ~2°C since the 1950s and 8 of the past 10 summers have been the warmest on record (see Patterson et al., 2007). When faced with unfavourably high temperatures, individual sockeye salmon could make some combination of behavioural and physiological modifications.

Behaviourally, Pacific salmon can slow or cease swimming when exposed to temperatures outside their thermal optimum (Goniea et al., 2006; Keefer et al., 2008b; Salinger and Anderson, 2006). Pacific salmon may also alter the timing of their migration in order to avoid peak temperatures (Hodgson and Quinn, 2002; Quinn and Adams, 1996; Quinn et al., 1997; Robards and Quinn, 2002). Finally, Pacific salmon seeking cold-water refuge have been demonstrated to have improved survival and spawning success (Farrell et al., 2008; Mathes et al., 2010; Roscoe et al., 2010). However, given that sockeye salmon have finite energy reserves, it is not a viable long-term option to excessively slow or cease swimming. Spawning date is highly conserved to ensure egg and juvenile survival (Burgner, 1991), so major alterations in entry timing into the Fraser River are not possible. Upriver migration is energetically expensive, typically depleting more than 50% of stored reserves (Brett, 1995; Crossin et al., 2004) and excessive energy use during migration has been demonstrated to cause premature mortality (Rand and Hinch, 1998). Finally, not all populations have cold refugia available to them. For example, Nechako and Early Stuart sockeye salmon spend weeks migrating up the mainstem Fraser River, which has little-to-no cool water relief (Donaldson et al., 2009).
Phenotypic plasticity can play an important role in temperature tolerance in ectothermic vertebrates. However, the role of physiological plasticity in temperature tolerance is poorly understood in adult salmonids. On one hand, beneficial physiological modifications could enable salmon to cope with warm temperatures. For example, modifications could be made in mitochondrial density, membrane composition and the type and kinetic properties of metabolic enzymes (Guderley and St-Pierre, 2002; Pörtner, 2002). In addition, beneficial changes in muscle capillarization, contractility or muscle fibre type could occur (Egginton and Cordiner, 1997; Egginton and Sidell, 1989; Sidell and Moerland, 1989). Cardiac remodelling to alter the size or composition of the ventricle (Farrell et al., 1988a; Graham and Farrell, 1989), adjustments to the number or binding affinity of adrenaline-binding ventricular β-adrenoceptors (Keen et al., 1993) or alteration in respiratory epithelium thickness (Leino and McCormick, 1993) could enhance oxygen delivery. Therefore, phenotypic plasticity may play an important role in allowing sockeye salmon to adjust to the ever-changing environment during their upstream journey.

On the other hand, acclimatory responses to temperature may play a minor role for salmonids. For example, increasing the acclimation temperature of juvenile sockeye salmon from 10 to 23°C only increased the upper lethal temperature by 0.9°C (Brett, 1952). Moreover, \(f_{\text{Hrest}}\) and \(f_{\text{Hmax}}\) were similar in sockeye salmon acclimated to 22°C [86 and 106 beats min\(^{-1}\), respectively, (Brett, 1971)] compared to sockeye salmon acutely warmed to 22°C [90 and 106 beats min\(^{-1}\), respectively (Steinhausen et al., 2008)]. In addition, swim performance did not vary between cutthroat trout (*Oncorhynchus clarki*) given 48 h or 3 weeks to acclimate to 7, 14, or 18°C (MacNutt et al., 2004). Given that migrating sockeye salmon are simultaneously senescing, not feeding, undergoing massive morphological modifications as they sexually mature and performing an incredible athletic feat as they swim upstream, normal acclimation mechanisms
may be incomplete in adult sockeye salmon. In addition, temperature swings may be too swift during the short 3-4 week migration to allow a full physiological acclimatory response. As a consequence, swimming performance and cardiorespiratory capacity may be set at a level that is sufficient to meet the demand experienced at the highest and lowest temperatures typically encountered by a given population (Pörtner, 2002) and phenotypic plasticity may play a minor role in responding to temperature in migrating salmon. All told, the role of physiological acclimation in migrating sockeye salmon is poorly understood and warrants further investigation.

1.5.3 Conservation Implications

Peak summer river temperature in the Fraser River has warmed by ~2°C over the last 60 years (Fig 1.3). Elevated river temperatures have been repeatedly associated with adult mortality during the upriver migration, raising conservation concerns for this ecologically, economically and culturally important fish species (Hinch et al., 2006; Hinch and Martins, 2011). Current maximum river temperatures exceed $T_{\text{opt}}$ for the two sockeye salmon populations (Gates and Weaver) that have been examined thus far (Farrell et al., 2008; Lee et al., 2003c). En-route mortality for returning adults clearly differs across populations and among years (Hinch and Martins, 2011). For example, in 2004, the Fraser River and its tributaries reached an exceptionally high temperature (>21°C) and an estimated 70-80% of Weaver sockeye salmon died during migration (Farrell et al., 2008). However, the proximate causes of in-river mortality are unknown. Physiological processes are critical in defining temperature-induced mortality (Wang and Overgaard, 2007; Wikelski and Cooke, 2006). Given that the current warming trends are expected to continue (Ferrari et al., 2007; Morrison et al., 2002), it is critical that population-
specific temperature tolerance is defined in order to identify populations most vulnerable to climate change. These discoveries will have important implications for biodiversity and management decisions.

1.6 Thesis Objectives and Hypotheses

The general objective of my thesis was to examine the physiological basis for thermal tolerance in sockeye salmon populations. Specifically, I sought to characterize how cardiorespiratory physiology varies among sockeye salmon populations and determine the mechanism of cardiorespiratory collapse at high temperature. I used an integrative approach, examining several levels of biological organisation, in order to test the overall hypothesis that Fraser River sockeye salmon populations have physiologically adapted to meet their specific upriver migration challenges. I predicted that thermal limits are set at a local level by physiological limitations in aerobic performance due to cardiac collapse. At each level of biological organisation (whole animal, organ and cellular), I made comparisons across populations and temperatures in order to examine this hypothesis.

The form of the thesis is as follows. Chapter 2 provides a detailed description of the Fraser River system, the populations of sockeye salmon I examined and the materials and methods used throughout this thesis. For each experiment, wild, migrating sockeye salmon were collected from the lower Fraser River, very early in the river migration (at the time of capture, the salmon had been in the Fraser River approximately 1-3 days). Therefore, the fish were collected before they encountered the majority of the upriver migration conditions and after they had spent >2 years in a common, cool ocean environment.
The specific research questions are presented in Chapters 3-7. In Chapter 3, I compared cardiorespiratory performance as a function of temperature across four new populations and incorporated data from two previously assessed populations (Lee et al., 2003c). I predicted that populations with more challenging migrations would have greater aerobic, cardiac and heart rate scopes. In addition, I predicted that each population can maintain maximum scope across the entire range of temperatures the adult salmon most frequently encountered during the upriver migration, as has been previously established for two populations (Farrell et al., 2008; Lee et al., 2003c).

Chapter 4 details the swimming physiology for the four upriver populations swum at $T_{opt}$. Specifically, I compared cardiorespiratory performance as well as arterial and venous blood variables among populations during two consecutive swim challenges. I predicted that each population would demonstrate excellent repeat swim performance, as has been demonstrated in the literature (Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003b; MacNutt et al., 2004; MacNutt et al., 2006; Wagner et al., 2006). Moreover, I predicted that these four upriver populations would display similar cardiorespiratory performance since they all encounter similar challenging upriver conditions and demonstrated similar maximum aerobic and cardiac scopes in Chapter 3.

The detailed physiological studies in Chapters 3 and 4 allowed me to examine the mechanism of cardiorespiratory collapse at high temperature in Chapter 5.

In Chapter 5, I pooled the three upriver populations that did not differ in cardiorespiratory performance. I hypothesized that a limitation at the level of the heart, gills or tissue would lead to a mismatch between oxygen supply and demand, and result in impaired aerobic scope and
swim performance. I predicted that aerobic scope is limited at high temperatures due to cardiac collapse.

Since the heart emerged as a key player in temperature tolerance and supporting aerobic swimming in Chapters 3, 4 and 5, I examined cardiac morphology in Chapter 6. I predicted that populations with more challenging migrations would have larger ventricles and a greater proportion of compact myocardium compared to those populations with easier migrations. I also examined how cardiac morphology is affected by temperature exposure.

In Chapter 7, I examined a potential mechanism at the cellular level of the heart for the higher and broader thermal tolerance of one population (Chilko) compared to another co-migrating population (Nechako). I hypothesized that Chilko sockeye salmon would possess an enhanced ability to use adrenaline which would provide greater cardiac capacity and protection at high temperatures, thereby expanding their thermal tolerance.

Chapter 8 concludes my thesis and provides a final synthetic discussion as well as future directions for research.
Figure 1.1. Schematic of resting and maximum oxygen consumption and aerobic scope. See text for details. $T_{\text{opt}}$ = optimum temperature, $T_p$ = pejus temperatures, $T_{\text{crit}}$ = critical temperatures. The $T_{\text{opt}}$ window corresponds to the range of temperatures between the upper and lower $T_p$. 
Figure 1.2. Schematic of the oxygen cascade. Step 1: Oxygen-rich water is brought into contact with the gill. This step is determined by gill ventilation and volume. Step 2: Oxygen diffuses from the environment, across the gills and into the blood where it binds to haemoglobin. This step is determined by the partial pressure gradient of oxygen ($P_{O2}$) between the water ($P_e$) and the blood ($P_v$ to $P_a$) as well as gill anatomy (surface area, diffusion distance and the permeability coefficient of oxygen). Step 3: The circulatory system transports the oxygen-bound haemoglobin by convection to the tissues. This step is governed by cardiac output and the quantity of oxygen per unit arterial blood (which in turn is primarily determined by the haemoglobin concentration). Step 4: Oxygen diffuses across the capillary wall and into the cell, where it is ultimately used during mitochondrial respiration. This final step is determined by the partial pressure gradient between the blood ($P_a$ to $P_v$) and the mitochondria ($P_m$) as well as tissue anatomy (surface area, diffusion distance, the permeability coefficient of oxygen and the quantity of mitochondria). During swimming, more oxygen is extracted by the swimming muscles, resulting in a lower $P_{O2}$ in the venous blood. See Weibel (1984). Note that the heart is composed of two types of myocardium. The outer compact myocardium has a coronary circulation, so it is perfused with oxygen from the arterial system. The inner spongy myocardium is avascular, so it receives oxygen from the venous system.
Figure 1.3. Maximum yearly Fraser River water temperature at Hells Gate from 1950-2009 ($y = 0.0324x - 45.3776$, $p < 0.0001$, $R^2 = 0.25$) (see Patterson et al., 2007).
CHAPTER 2: MATERIALS AND METHODS

2.1 Migration Conditions and Migration Difficulty Indices

Spawning grounds differ in their distance and elevation from the mouth of the Fraser River (Table 2.1; Fig 2.1) and populations initiate up-river migrations at different times of the year. Fisheries managers categorize populations into four major run-timing groups (Early Stuart, Early Summer, Summer and Late) based on the historic timing of Fraser River entry. Mainstem Fraser River discharge decreases from June to November, but temperature typically increases until August and declines thereafter. The Early Stuart run populations enter the Fraser River in early July and experience the highest river flows and moderate temperatures early in their migration and increasing temperatures towards the end of their migration. Early Summer and Summer run populations enter in late July and August and experience the warmest temperatures in the mainstem Fraser River early in their migration and moderate flows. Late run populations enter in the fall and experience the lowest flows and coolest average temperatures compared to the other entry runs (Table 2.1). Therefore, the temperature and water velocity experienced also varies among populations.

Clearly, the relative difficulty of the migration varies considerably and complexly among populations. As a result, the difficulty of population-specific migrations was characterized using several indices. The first was based on whether or not populations pass through Hells Gate, a major hydraulic barrier (Hinch and Bratty, 2000) about 200 km upriver from the mouth of the Fraser River (Fig 2.1) and upstream of the location where fish were sampled for all the
experiments in this thesis. Populations were categorized as “coastal” if they did not pass through Hells Gate and “upriver” if they did (Table 2.1).

Migration difficulty was also characterized for each population based on the river migration distance from the Fraser Delta (Steveston, BC) to the spawning grounds ($D_M$) and the elevation of the main spawning grounds ($E_M$) (Table 2.1). Three additional indices were calculated according to the concepts of physical work and river slope (Crossin et al., 2004; Gilhause, 1980). In physics, “work” is defined as the product of force over a given distance. The amount of work a salmon must do to reach the spawning ground can be estimated using $E_M$ or $F_M$ (elevation or river discharge, as a surrogate for force) and $D_M$ (distance). Migratory work was determined as $k_1 \cdot E_M \cdot D_M$ and migratory effort was determined as $k_2 \cdot D_M \cdot F_M$. In addition, while river distance and elevation do co-vary somewhat, a short migration can be steeper than a long migration. Therefore, river slope ($k_3 (E_M D_M^{-1})$) was included as an additional index. The correction factors $k_1$, $k_2$ and $k_3$ (0.001, 0.0001 and 500, respectively) simplify presentation.

Historic environmental and migratory data were collected for eight sockeye salmon populations. Lower Fraser River discharge ($F_M$) data were obtained from the Water Survey of Canada. Lower Fraser River temperature ($T_M$) data were provided by Fisheries and Oceans Canada (DFO) Environmental Watch Program (see Patterson et al., 2007). For upstream populations, $F_M$ and $T_M$ were measured near Hope (Fig 2.1), centered on the historic date of peak salmon passage through Hells Gate. For coastal populations, $F_M$ and $T_M$ were measured at Mission (Fig 2.1), centered on peak Mission salmon passage. Lower river conditions have been previously used as indices of the total freshwater migratory experience given the generally strong correlation between lower and upper river environmental conditions (Hague et al., 2008). Furthermore, lower river temperature and flow have been correlated to both indirect and direct
estimates of spawning migration mortality (Macdonald et al., 2010; Martins et al., 2011). Median and modal migration temperatures were calculated from the population-level temperature histograms used in the present study (see below, Table 2.1). While river migration speeds vary considerably among individuals and populations, biotelemetry experiments have repeatedly shown that sockeye salmon tend to migrate continuously in freshwater until they reach their natal systems, achieving ground speeds of 15-40 km d⁻¹ depending on river section (English et al., 2005; Hanson et al., 2008). Average ground speeds for each population across the total freshwater migration route were determined using data obtained from radio-biotelemetry studies performed by LGL Environment Ltd from 2002-2007 (Hague et al., 2008; Martins et al., 2011). Migration duration was determined by dividing the migration distance by migration rate (Table 2.1).

The average thermal units accumulated during freshwater migration [i.e. Accumulated Thermal Units (ATU)] were calculated for the “active” part of the migration (i.e. the time when fish were actively migrating upstream, which did not include river or lake holding near the spawning ground). Peak Fraser River entry (average date between 1977-2008 at which 50% of the run-timing group passed Mission minus two days for travel time from Fraser Delta to Mission) and peak Hells Gate passage times (average date between 1977-2008 at which 50% of the run-timing group passed Mission plus 4-5 days travel time, depending on average migration rate for each population) were provided by the Pacific Salmon Commission. Peak spawning date was provided by Fisheries and Oceans Canada Stock Assessment Division (Table 2.1).

Population-level temperature distributions (see Chapter 3) were simulated using an individual-level freshwater migration model which integrated across daily average river and lake temperatures experienced over the “active” period of the spawning migration (i.e. did not include
lake or river holding temperatures prior to spawning) from 1995 to 2008 (modified from Farrell et al., 2008).

Since 1995, several populations of late-run sockeye salmon (e.g. Weaver, Harrison, Lower Adams) have entered the Fraser River up to six weeks earlier than previously observed, a phenomenon that is poorly understood (Cooke et al., 2004; Hinch, 2009). As a result of the early river entry, these salmon encounter considerably warmer temperatures and in-river mortality has exceeded 90% in some years (Cooke et al., 2004; Hinch, 2009). Therefore, the environmental data (Table 2.1) and temperature frequency histograms (Chapter 3) are presented for both historical run timing (before 1995) and the current early entry phenomenon (1995-2008) for comparison.

Each population experiences a broad range of temperatures throughout their brief 1-4 week migration, which varies depending on river entry timing, spawning ground location and year-to-year variation. Some Summer run populations routinely experience temperatures as low as 11°C (e.g. Chilko during the final third of their migration when they enter the Chilcotin River and ascend to their spawning location in or downstream of a glacial lake) and as high as 22°C (e.g. near the mouth of the Fraser River during August when river temperatures tend to peak). Studies using radio tags and thermal loggers have shown that individual fish routinely experience temperature swings of 3-4°C over 8 days during their migration up the mainstem Fraser River (Donaldson et al., 2009). Experimental temperatures were selected to span the entire range of temperatures encountered during migration. In addition, brief exposures to temperatures exceeding those typically encountered in the wild were also used to assess high and low temperature tolerance.
For most populations, the highest temperature experienced during river migration occurs in the lower Fraser River. Since the 1940s, the maximum daily water temperature at Qualark (near Hells Gate in the lower Fraser River) has been 21.5°C, which occurred in 2004. The Early Summer and Summer run groups would experience such peak temperatures, as would any Late run population that entered the river early, as they have been doing since the mid-1990s. In fact, Weaver sockeye (which belong to the Late run group) entered the Fraser River early in 2004, experienced temperatures reaching 21.5°C and over 50% of the population died en route to the spawning area (Mathes et al., 2010). The main exception to this generalization is Early Stuart sockeye salmon, which typically experience cool water in the lower Fraser River but warmer temperatures later in their migration when they are closer to their spawning grounds ~1,000 km upstream (Macdonald et al., 2007). A temperature of 21.5°C is a reasonable maximum temperature experienced by Early Stuart sockeye salmon during the final stage of their river migration. Therefore, 21.5°C is indicated in Chapter 3 as the current temperature maxima experienced by Fraser River sockeye salmon.

2.2 Fish Collection

Wild adult sockeye salmon were collected in the lower Fraser River or Harrison River (a lower Fraser tributary) using a beach seine or gill net while fish were en-route to their spawning grounds and shortly after entry into freshwater (Fig 2.1). Notably, the fish were collected very early in their river migration and before they had experienced most of the upriver migration conditions. The sockeye salmon were transported 25-75 km by land to the DFO Cultus Lake Salmon Research Laboratory (CLL, Cultus Lake, BC, Canada). Following capture, all sockeye
salmon were given a unique cinch tag or PIT tag (Passive Integrated Transponder tag, approximately 8.5 mm x 2 mm size, Biomark Inc., Boise, Idaho) for individual identification, a scale was removed and <0.1 g of the adipose fin was clipped for population identification via DNA analysis (Beacham et al., 2005). The DNA analysis compares one major histocompatibility complex (MHC) loci or five single nucleotide polymorphisms (SNPs) in addition to 14 microsatellite loci and assigns a probability of population identification (Beacham et al., 2005). This method has been demonstrated to correctly assign 94% of individuals to the correct population aggregate (as defined below) using simulations run with the program cBAYES (Beacham et al., 2005; Beacham et al., 2004; Beacham et al., 2010). Due to low sample sizes or an inability to definitively assign population identification between co-migrating, adjacent populations, some populations spawning in adjacent rivers or lakes were grouped as a single population. Chilko is composed of two spawning populations, one that spawns in the lake and one that spawns in the lake outlet (Chilko River). Quesnel comprises two main populations which spawn 47 km apart (Mitchell and Horsefly Rivers; inlet tributaries to Quesnel Lake). Nechako is composed of four populations that spawn within 100 km of each other (Stellako, Nadina, Tachie and Middle River). Early Stuart is made up of 40 small populations that spawn within 100 km of each other (Beacham et al., 2005). Lower Adams, Weaver, Harrison and Gates are all genetically distinct, single populations. All procedures were approved by the University of British Columbia’s Animal Care Committee in accordance with the Canadian Council on Animal Care (A06-0328 and A08-0388).
2.3 Surgical Procedures

In order to measure cardiorespiratory variables, the fish underwent surgery before the swim tests. Individual fish were anaesthetized with buffered tricaine methanesulfonate in freshwater (0.2 g l\(^{-1}\) NaHCO\(_3\) and 0.1 g l\(^{-1}\) MS-222, Sigma, St. Louis, MO), weighed and transferred onto wet foam on a surgical table where their gills were continually irrigated with aerated, chilled freshwater with a lower dose of anaesthetic (0.15 g l\(^{-1}\) NaHCO\(_3\) and 0.075 g l\(^{-1}\) MS-222). Surgical procedures have been detailed elsewhere (Steinhausen et al., 2008). To sample arterial blood, a PE-50 cannula was inserted into the dorsal aorta (Soivio et al., 1973). To measure cardiac output, a 3 mm SB flowprobe (lateral cable exit, Transonic systems, Ithaca, NY, USA) was positioned around the ventral aorta without opening the pericardium (Steffensen and Farrell, 1998). To sample venous blood, a PE-50 cannula was inserted into the ductus of Cuvier and advanced towards the heart into the sinus venosus (Farrell and Clutterham, 2003). Both cannulae were filled and regularly flushed with heparinized saline solution (150 IU ml\(^{-1}\)). The flowprobe lead and cannulae were secured together and sutured to the fish’s body using 2-0 silk. The fish were placed in a Brett-type swim tunnel and allowed to recover overnight at low water velocity of ~0.39 bl s\(^{-1}\) before starting the swim tests.

2.4 Swimming Experiments

The swimming tests were conducted in 2007, 2008 and 2009 at CLL (N = 97). Fish were held at 11-12°C for 1-4 weeks in outdoor 8,000–12,000 l circular aquaria supplied with filtered and UV sterilized freshwater (~40 l min\(^{-1}\); LS-Permabead Filtration System, Integrated Aqua
Systems Inc., Escondido, California) under seasonal photoperiod. The fish were not fed because they had ceased feeding naturally before entering the Fraser River. Three days before the swimming test, fish were placed in 1,400 l circular aquaria and the temperature was progressively increased to the test temperature (13-22°C) by no more than 5°C day\(^{-1}\). The fish were maintained at this temperature for at least one day before the swim tests were conducted.

Following overnight recovery from surgery at their test temperature, resting oxygen consumption (\(\dot{\text{MO}_2}\)), cardiac output (\(\dot{Q}\)), arterial and venous blood were measured at a water velocity of \(~0.39\ \text{bl s}^{-1}\). Then the fish underwent a ramp-\(U_{\text{crit}}\) swim protocol (Jain et al., 1997; Lee et al., 2003c). The velocity of the water was increased every 5 min until approximately 50\% of \(U_{\text{crit}}\) was reached (\(~1.0\ \text{bl s}^{-1}\)). Thereafter, the speed was increased by approximately 0.25 \(\text{bl s}^{-1}\) every 20 min until the fish no longer swam continuously and rested on the back grid for >30 s. The water speed was reduced to the resting velocity and the fish were allowed a 45-min recovery before repeating the same ramp-\(U_{\text{crit}}\) swim protocol. The fish were allowed to recover for 2 h after the second swim test.

\(\dot{Q}\) was measured continuously throughout the swim trial. \(\dot{\text{MO}_2}\) was measured during the second half of every 20-min speed interval. If the dissolved oxygen levels approached 7.0 mg O\(_2\) l\(^{-1}\), \(\dot{\text{MO}_2}\) was deliberately not measured to maintain a normoxic environment in the swim tunnel. Blood samples (~0.7 ml per sample) were collected during the second half of the first 20-min swim interval (mean speed = 1.18 bl s\(^{-1}\), or 56\% of maximum swim speed) during steady swimming. Blood was sampled again when the fish exhibited burst-and-coast swimming near exhaustion (mean speed = 2.1 bl s\(^{-1}\), or 93\% of maximum swim speed). Additional blood samples were occasionally taken at intermediate speeds for some fish. \(\dot{\text{MO}_2}\) and blood were
sampled immediately after the fish quit swimming (fatigue), and again after 45 min of recovery. Final samples were collected after a 2-h recovery period following the second swim.

A subset of fish did not undergo surgery, but were otherwise treated the same as the fish that were instrumented.

I was unable to hold fish at extremely high or low temperatures; therefore, some fish did not undergo the same three-day temperature exposure prior to surgery. Instead, they were allowed to recover overnight from surgery in the swim tunnel at 12°C and in the morning the water temperature was acutely increased or decreased by 4°C h⁻¹ to the test temperature (8-10°C or 22-26°C). After one hour at the test temperature, resting values were recorded as above and then the fish underwent a single ramp-$U_{crit}$ swim protocol, after which the temperature was returned to 12°C over 2 h. Occasionally, some fish at the highest test temperatures displayed cardiac disrhythmias while resting and before the swim test. In the few cases when this occurred, the temperature was immediately decreased and the fish were not used. As such, all fish began their swim test with a regular, rhythmic heart rate.

Upon conclusion of the swim test, the fish was removed from the swim tunnel and sacrificed by a cranial blow. A post-mortem caudal blood sample was collected using a Vacutainer (2-3 ml) and mass (whole body, liver, gonad, spleen, heart), length [standard length, fork length, post-orbital-hypural (POH) length, post-orbital-fork (POF) length], girth and depth were measured for each fish. Gonadosomatic index (GSI), hepatosomatic index (HSI) and splenosomatic index (SSI) were calculated as the mass of the gonad, liver and spleen divided by body mass, respectively (see below for details of heart calculations). In order to estimate the energy status of each fish, proximate constituent analysis was conducted on a ~200 g piece of dorsal muscle, removed from the left side of the fish between the operculum and the dorsal fin.
The concentrations of protein, lipid, moisture and ash were assessed so that gross energy could be estimated (Crossin et al., 2004; Higgs et al., 1979).

### 2.5 Swim Tunnels

Two Brett-type swim tunnels were used to swim individual fish, which have been fully described elsewhere (Lee et al., 2003c; Steinhausen et al., 2008). Both swim tunnels were equipped with a custom-designed heating system which could maintain the set water temperature ± 0.5°C. The velocity of the water was calibrated (± 1 cm s⁻¹) using an anemometer (Valeport Marine Scientific, Dartmouth, UK).

### 2.6 Whole Blood and Plasma Analysis

Whole blood samples were used to measure partial pressure of oxygen (P_{O2}), oxygen content (C_{O2}), haemoglobin concentration (Hb) and hematocrit (Hct). The samples were held at 4°C and analyzed shortly after collection. Blood P_{O2} was measured using a blood gas monitor (PHM 73, Radiometer, Copenhagen, Denmark) which was calibrated and maintained at each temperature using a water jacket. Blood C_{O2} was measured according to the method of Tucker (1967). Hb was measured using either a handheld haemoglobin analyzer (Hemacue 201⁺, Ängelholm, Sweden) calibrated for fish blood (Clark et al., 2008a) or the spectrophotometer method with Drabkin’s solution (Clark et al., 2008a; Drabkin and Austin, 1935). Hct was measured in duplicate using microhematocrit capillary tubes spun at 10,000 g. The remaining
blood was centrifuged at 7,000 g and the plasma was flash frozen in liquid nitrogen and stored at -80°C for subsequent analyses.

Plasma cortisol (ELISA kit, Neogen, Lexington, KY, USA), glucose and lactate (YSI 2300 Stat Plus analyzer), sodium and potassium (Cole-Parmer, model 41- single channel flame photometer) and chloride (Haake Buchler digital chloridometer) were measured on all blood samples (see Farrell et al., 2001a). Plasma testosterone and 17β-estradiol (ELISA kit, Neogen, Lexington, KY, USA) were only determined for the final caudal blood sample from Early Stuart and Chilko sockeye salmon in 2008 and 2009.

2.7 Data Analysis and Calculations for Cardiorespiratory Variables

During an $\dot{M}O_2$ measurement, the inflow and outflow water to the tunnel were turned off and the decrease in oxygen content over time was measured. Oxygen content of the water in the swim tunnel (mg O$_2$ l$^{-1}$) was measured using an Oxyguard probe (Point Four Systems, Richmond, Canada) attached to a Windaq box (Dataq instruments, Akron, ON, USA) interfaced with Labview software (6.0, National Instruments, Austin, TX, USA). The duration of the measurement was sufficient so that the dissolved oxygen decreased by at least 0.3 mg O$_2$ l$^{-1}$, which resulted in a linear regression with $r^2$ values typically $>0.95$. $\dot{M}O_2$ (mg O$_2$ kg$^{-1}$ min$^{-1}$) was calculated as: $\dot{M}O_2 = \Delta[O_2] \cdot \nu \cdot M^{-1} \cdot t^{-1}$ where $\Delta[O_2]$ is the change in water content (mg O$_2$ l$^{-1}$), $\nu$ is the volume of the water minus the volume of the fish (l), $M$ is the mass of the fish (kg) and $t$ is the time (min). Background $\dot{M}O_2$ was measured after each swim trial and determined to be negligible.
$U_{\text{crit}}$ was calculated as in Brett (1965): $U_{\text{crit}} = U_f + (t_f/t_i \cdot U_i)$ where $U_f$ is the water velocity of the last fully completed increment, $t_f$ is the time spent in the final water velocity increment, $t_i$ is the time period for each completed increment, and $U_i$ is the water velocity increment. $U_{\text{crit}}$ was calculated in both body lengths per second (bl s$^{-1}$) and cm per second (cm s$^{-1}$). $U_{\text{crit}}$ was corrected for the solid blocking effect according to Bell and Terhune (1970) using the following equation: $U_F = U_T \cdot (1 + \varepsilon_s)$ where $U_F$ is the corrected flow speed, $U_T$ is the speed in the tunnel without the fish, and $\varepsilon_s$ is the error due to solid blocking. $\varepsilon_s$ is calculated as: $\varepsilon_s = \tau \cdot \lambda \cdot (A_o/A_T)^{1.5}$ where $\tau$ is a dimensionless factor depending on the swim chamber cross section (0.8 in this study), $\lambda$ is the shape factor for the fish (0.5 body length/body thickness), $A_o$ is the cross sectional area of the fish and $A_T$ is the cross sectional area of the swimming chamber. The recovery ratio (RR) was calculated as $RR = U_{\text{crit}2}/U_{\text{crit}1}$ to determine how the first $U_{\text{crit}}$ compared to the second $U_{\text{crit}}$.

To measure $\dot{Q}$, the flowprobe was connected to a flowmeter (Transonic systems, Ithaca, New York, USA) and blood flow was measured at 200 hz using Biopac hardware and Acknowledge software (Biopac systems, Santa Barbara, CA, USA). $\dot{Q}$ was calculated as the mean of at least three 30 s segments. Heart rate ($f_H$) was measured from the flow trace during the 30 s segments using the automated software which was confirmed with manual counting. Stroke volume ($V_s$) was calculated as $\dot{Q} = f_H \cdot V_s$.

Cost of transport (COT) was calculated as: $COT = \dot{M}O_2/U$ where $\dot{M}O_2$ was measured in mg O$_2$ kg$^{-1}$ min$^{-1}$ and $U$ was the swimming speed in m s$^{-1}$, corrected for the solid blocking effect. Net cost of transport (COT$_{net}$) was calculated as: $COT_{net} = (\dot{M}O_2 - \dot{M}O_{2\text{rest}})/U$. Similarly, cost of transport for cardiac output (COT-$\dot{Q}$) and net cost of transport for cardiac output (COT-$\dot{Q}_{net}$) were calculated.
Oxygen extraction (A-V\textsubscript{O}2) was calculated as arterial oxygen content (C\textsubscript{aO}2) - venous oxygen content (C\textsubscript{vO}2) and was only assessed in fish that had both cannulae working simultaneously. Arterial oxygen transport (T\textsubscript{aO}2) to the tissues was calculated as the product of $\dot{Q}$ and C\textsubscript{aO}2. Venous oxygen transport (T\textsubscript{vO}2) to the spongy myocardium and gills was calculated as the product of $\dot{Q}$ and C\textsubscript{vO}2. Mean corpuscular haemoglobin concentration (MCHC) was calculated as $[\text{Hb}] / (\text{Hct}/100)$.

Aerobic scope and cardiac scope were determined as the difference between the resting and maximum values. Scope for heart rate and scope for stroke volume were determined as the difference between the resting values and those measured at maximum cardiac output.

To determine the Fry curves for aerobic scope, a second order polynominal regression was fitted to the aerobic scope data from individual fish of each population swum across a range of temperatures. The same method was used to develop the curves for cardiac scope, scope for heart rate and scope for stroke volume. Optimal temperature (T\textsubscript{opt}) for each population was determined as the temperature corresponding to the peak of the polynomial regression for aerobic scope. The upper and lower pejus temperatures (T\textsubscript{p}) were assigned to 90% of the maximum aerobic scope, with the T\textsubscript{opt} window being defined as the range of temperatures between the upper and lower T\textsubscript{p}. The upper critical temperature (T\textsubscript{crit}) was defined by extrapolating the polynominal regression for aerobic scope to the upper temperature when aerobic scope reached zero. The value of aerobic scope at T\textsubscript{opt} was determined as the average of the individual data points within the T\textsubscript{opt} window for each population. The upper temperature experienced by the 90\textsuperscript{th} percentile of each population (T\textsubscript{90}) was determined from the historic temperature distributions and the percentage of maximum aerobic scope available at T\textsubscript{90} was determined for each population.
There were insufficient data points across a range of temperatures to plot an aerobic scope curve or determine $T_{\text{opt}}$ for Lower Adams sockeye salmon. Similarly, there were insufficient data points at cooler temperatures to define the lower $T_p$ or determine $T_{\text{opt}}$ for Quesnel sockeye salmon. Therefore, aerobic scope at $T_{\text{opt}}$ was based on the plateau of individual data points from fish swum at temperatures corresponding to those typically encountered during upriver migration. For Lower Adams, this temperature range also corresponds with optimal temperatures previously estimated for this population (Steinhausen et al., 2008).

Data from the swim tests conducted in 2007, 2008 and 2009 are presented over three chapters (Chapters 3-5). Chapter 3 presents the overall highest maximum and scope values obtained over the two swims. All fish were included in this chapter. Chapter 4 compares swim 1 and swim 2 in fish that had undergone surgery from four upriver populations (Early Stuart, Nechako, Chilko and Quesnel). Chapter 5 compares cardiorespiratory performance of swim 1 across four different temperature categories (details of the temperature categories are provided in Chapter 5).

2.8 Gross Heart Morphology

2.8.1 Animal Acquisition

Sockeye salmon heart samples were collected from a variety of experiments. In all cases, the fish were collected early in their migration, prior to encountering any of the major upriver migration challenges (as outlined in section 2.2). The three sections below detail the different experimental conditions.
Population Comparisons

Male and female sockeye salmon hearts from seven populations (N = 194, Early Stuart, Chilko, Quensel, Nechako, Lower Adams, Weaver, Harrison) were collected opportunistically from various experiments conducted at CLL in 2007 and 2008 (e.g. the swimming experiment outlined above). However, I restricted population comparisons of cardiac morphology to female sockeye salmon. It is well known that salmonids can rapidly remodel their hearts in response to biological and environmental cues (Gamperl and Farrell, 2004). For example, male salmonids increase RVM up to 2-fold with sexual maturation. In contrast, female salmonid ventricles do not change size with sexual maturation (Bailey et al., 1997; Clark and Rodnick, 1998; Franklin and Davie, 1992). Consistent with this knowledge, cardiac morphology significantly varied with temperature treatment among male but not female sockeye salmon (see Chapter 6). There were insufficient numbers of male fish from a particular temperature treatment and sexual maturation level for all populations in order to make comparisons, so males were excluded from the population analysis.

Temperature Exposure

In 2007, Chilko sockeye salmon (N = 34) were collected from the lower Fraser River and held at CLL in 8,000 – 12,000 l tanks for 4-6 days at 12°C. The fish were then exposed to 14, 16.5 or 19°C (± 0.5°C) for up to 14 days, or until they died. Only fish that were held at their temperature treatment for at least 5 days before dying were included in the analysis.
Swimming Experiment

In 2006, Lower Adams sockeye salmon (N = 16) were collected by purse seine in the Strait of Georgia, held at the DFO & UBC Centre for Aquaculture and Environmental Research (CAER, West Vancouver, BC, Canada) and used in a swimming experiment detailed in Steinhausen et al. (2008). Briefly, the fish were swum at a fixed speed of ~1.35 bl s$^{-1}$, which is approximately 75% of $U_{crit}$, in the Brett-type swim tunnels outlined above. The water temperature was incrementally increased at a rate of 2°C h$^{-1}$ from 15°C to 17, 19, 21, 23 and 24°C, or until the fish quit swimming.

2.8.2 Heart Sampling and Analysis

Regardless of the experiment, the heart tissue was processed by the same method. Following death, fish were weighed (M) and the heart was removed and placed in a vial containing 70% ethanol. The compact and spongy myocardial layers of the preserved ventricles were separated according to established methods (Farrell et al., 2007; Poupa and Carlsten, 1973) to provide an index of the proportion of the ventricle composed of compact relative to spongy myocardium. The two layers were dried to a constant mass (at least 3 days at 60°C) and weighed to the nearest 0.1 mg. Percent ventricular compact mass (% compact) was determined using dry compact ($M_{CD}$) and dry spongy masses ($M_{SD}$): \[ \% \text{ compact} = 100\frac{M_{CD}}{M_{CD} + M_{SD}} \]. Total dry ventricular mass ($M_{VD} = M_{CD} + M_{SD}$) was used to determine relative dry ventricular mass (RDVM): \[ \text{RDVM} = 100\frac{M_{VD}}{M} \]. Since compact myocardium can vary independent of
ventricular mass (i.e. a large ventricle with lower % compact could have the same total compact myocardium as a smaller ventricle with higher % compact), the total compact myocardium was expressed as the relative dry compact mass (RDCM): RDCM = 100M_{CD}M^{-1}.

To simplify comparisons of our data with the more commonly presented wet ventricular mass (M_{VW}), M_{VW} was measured in a subset of fish (n = 35 from 2 populations, Chilko and Weaver). Immediately after death, the ventricle was blotted dry and weighed to 0.1 g prior to storage in 70% ethanol. Relative wet ventricular mass (RVM) was determined: RVM = 100M_{VW}M^{-1}. Dry ventricular mass (M_{VD}) was determined to be 14.7 ± 0.3% of M_{VW} (no significant differences existed between Weaver and Chilko fish, data not shown), which corresponds to previous studies on salmonids (12-14%, Simonot and Farrell, 2007). Therefore, we extrapolated from M_{VD} to M_{VW} (using a correction factor of 14.7%) for all populations.

2.9 β-Adrenoceptor Experiment

Chilko and Nechako sockeye salmon were collected from the lower Fraser River on August 11 and 12, 2009 and brought to CLL. The fish were placed in 1,400 l circular aquaria at 13°C and the temperature was either maintained at 13°C or increased to 19 or 21°C over 24 h. After four days at the test temperature, the fish were euthanized by a cranial blow and the ventricle was quickly removed, weighed and freeze-clamped in liquid nitrogen. The hearts were stored at -80°C until analysis. Gross body morphology was measured in each fish (body mass, fork length, gonad mass, condition factor). Condition factor = (body mass/length^3) × 100.

Male rainbow trout acclimated to 6°C freshwater at CAER were included as a reference group to validate the assay technique.
Ventricular cell-surface $\beta_2$-adrenoceptor density ($B_{\text{max}}$) and binding affinity ($K_d$) were determined using the tritiated ligand technique [Watson-Wright et al. (1989) as modified for fish hearts (Gamperl et al., 1994; Hanson et al., 2005)]. The frozen ventricles were rinsed in saline to remove any remaining blood and sliced (350 um thickness) using a McIlwain tissue chopper (Brinkman, Rexdale, ON, Canada). Ventricular tissue punches (2 mm diameter) were taken from both the spongy and compact myocardium. Single punches were incubated with various concentrations (0.05 – 3.5 nM) of the hydrophilic $\beta_2$-adrenoceptor ligand [$^3$H] CGP-12177 (Amersham Life Science). Separate punches were incubated at each concentration with the competitive $\beta_2$-adrenoceptor antagonist timolol (10 µM) to determine non-specific binding.

2.10 Statistics

All data are presented as mean ± SEM, unless otherwise indicated. P-values less than 0.05 were considered statistically significant.

2.10.1 Swimming Experiments in Chapters 3, 4 & 5

All data in Chapters 3 and 4 were compared between sexes and among populations. If there were no statistically significant relationships with sex or population, the data were often pooled for subsequent analysis. All data in Chapter 5 were compared among temperature groups (populations were pooled and sex was not considered).

Independent data were compared using a t-test, one-way ANOVA or two-way ANOVA, as appropriate. Dependent data were compared using a paired t-test, one-way repeated measures ANOVA or a two-way repeated measures ANOVA, as appropriate. When the requirement for
normal distribution and equal variance could not be met after transformation, the data were compared using the appropriate nonparametric test (e.g. Mann-Whitney U test, Kolmogorov-Smirnov test, Kruskal-Wallis test). A post-hoc Holm-Sidak or Dunn’s test was used to test for differences among groups.

A Pearson correlation was used to compare aerobic scope with the migration difficulty indices. Three different critical p-values are reported. First, p < 0.05 is indicated, with no correction for multiple comparisons. Second, p < 0.018 is indicated, which is the critical level using the Benjamini and Yekutieli False Discover Rate correction for multiple comparisons (Benjamini and Yekutieli, 2001; Narum, 2006). Finally, p < 0.006 is indicated, which is the critical level using Bonferroni correction for multiple comparisons (Holm, 1979; Rice, 1989).

Linear regression was used to relate maximum aerobic scope with distance to the spawning ground. Linear regression was also used to relate aerobic scope, cardiac scope and scope for heart rate from individual fish.

The goodness of fit of the population-specific aerobic scope curves to the full suite of historic temperature frequency distributions were assessed using AIC (Akaike’s Information Criterion, Burnham and Anderson, 2002). In addition, rigorous sensitivity analyses was conducted to test the robustness of the results from the initial AIC analysis (data not shown). For example, the response and predictor variables were reversed in the regression and fit aerobic scope curves to population-specific temperature distributions. Second, the temperatures used to generate the scope data for the linear regression were restricted to match the minimum and maximum temperatures used to fit the population-specific aerobic scope curves. This reduced the uncertainty introduced from extrapolating scope values beyond the ranges of the observed data. Next, the uncertainty in scope was further reduced by re-fitting the linear regressions using
observed values for each population, removing any assumptions about the true shape of the aerobic scope curve. The regression was also fit using raw temperature frequencies, as opposed to logged values. Finally, alternate modelling approaches were attempted, including a comparison of the scope and temperature distributions using single critical values (e.g. regression between $T_{opt}$ and the median of the temperature distribution across all stocks; regression between upper $T_p$ and the 90$^{th}$ percentile of the temperature distribution). While each approach yielded subtly different results, they all demonstrated that aerobic scope is significantly related to the average thermal migratory experience encountered by each population. Notably, the upriver populations encounter very similar average temperatures, which increases the likelihood that the temperature distribution for a given population matches the aerobic scope curve for another co-migrating population. Indeed, all the upriver populations had a similar temperature median (range 16.4-17.6°C) and mode (range 16.8-17.3°C).

2.10.2 Ventricular Morphology in Chapter 6

To examine whether traveling through hydraulically challenging sections of the river (e.g. Hells Gate) imposes strong selection pressure, the cardiac morphology variables were first compared between upriver and coastal populations using a t-test. Comparisons of cardiac variables in female sockeye salmon among seven populations were analyzed using one-way ANOVA. A Pearson correlation matrix was used to relate the various migration difficulty indices to the three cardiac variables in female sockeye from the seven populations, and three critical p-values are reported, as outline above. Linear regression was used to test for relationships between the cardiac variables and the various measures of migration difficulty and with fail temperature
during the swimming experiment performed by Steinhausen et al. (2008). The effect of temperature on the cardiac variables in Chilko sockeye salmon was assessed using a two-way ANOVA (sex × temperature). When appropriate, a Holm-Sidak post-hoc test was used to distinguish between groups.

2.10.3 β-Adrenoceptor Experiment in Chapter 7

Two-way ANOVA was used to test for differences in gross morphology, $B_{\text{max}}$ and $K_d$ between populations and temperature treatments.
Figure 2.1. Map of the Fraser River, British Columbia, Canada indicating the spawning locations for the eight sockeye salmon populations included in this study.
Table 2.1. Environmental characteristics and migration difficulty indices for eight populations of Fraser River sockeye salmon. Mean ± SEM are presented for $T_M$, $F_M$ and ATU. Minimum and maximum values for migration rate and migration duration are in parentheses. For late run sockeye salmon populations (Lower Adams, Weaver and Harrison), environmental data corresponding to the current early entry phenomenon are shown in parentheses underneath the historical river entry timing information.

<table>
<thead>
<tr>
<th></th>
<th>Early Stuart</th>
<th>Gates</th>
<th>Nechako</th>
<th>Quesnel</th>
<th>Chilko</th>
<th>Lower Adams</th>
<th>Weaver</th>
<th>Harrison</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Spawning region</strong></td>
<td>upriver</td>
<td>upriver</td>
<td>upriver</td>
<td>upriver</td>
<td>upriver</td>
<td>coastal</td>
<td>coastal</td>
<td>coastal</td>
</tr>
<tr>
<td><strong>Run timing group</strong></td>
<td>Early Stuart</td>
<td>Early Summer</td>
<td>Summer</td>
<td>Summer</td>
<td>Summer</td>
<td>Late</td>
<td>Late</td>
<td>Late</td>
</tr>
<tr>
<td><strong>Peak Fraser River entry</strong></td>
<td>Jul-07</td>
<td>Jul-31</td>
<td>Aug-11</td>
<td>Aug-11</td>
<td>Aug-11</td>
<td>Sep-27</td>
<td>Sep-27</td>
<td>Sep-27</td>
</tr>
<tr>
<td><strong>Peak Hells Gate passage</strong></td>
<td>Jul-14</td>
<td>Aug-07</td>
<td>Aug-17</td>
<td>Aug-17</td>
<td>Aug-17</td>
<td>Oct-04</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td><strong>Peak spawning ground arrival</strong></td>
<td>Aug-06</td>
<td>Sep-02</td>
<td>Sep-30</td>
<td>Sep-15</td>
<td>Sep-25</td>
<td>Oct-16</td>
<td>Oct-21</td>
<td>Nov-14</td>
</tr>
<tr>
<td><strong>Lower Fraser temperature ($T_M$) ($^\circ$C)</strong></td>
<td>15.8 ± 1.3</td>
<td>17.7 ± 1.1</td>
<td>17.3 ± 1.0</td>
<td>17.3 ± 1.0</td>
<td>17.3 ± 1.0</td>
<td>11.4 ± 1.4</td>
<td>12.3 ± 1.2</td>
<td>12.3 ± 1.2</td>
</tr>
<tr>
<td><strong>Lower Fraser discharge ($F_M$) (m$^3$ s$^{-1}$)</strong></td>
<td>5686 ± 1331</td>
<td>3860 ± 893</td>
<td>3419 ± 780</td>
<td>3419 ± 780</td>
<td>3419 ± 780</td>
<td>2040 ± 580</td>
<td>2093 ± 577</td>
<td>2093 ± 577</td>
</tr>
<tr>
<td><strong>Migration temperature median (°C)</strong></td>
<td>16.4</td>
<td>17.6</td>
<td>16.2</td>
<td>16.6</td>
<td>16.6</td>
<td>14.2</td>
<td>14.9</td>
<td>14.8</td>
</tr>
<tr>
<td><strong>Migration temperature mode (°C)</strong></td>
<td>17.3</td>
<td>17.3</td>
<td>16.8</td>
<td>16.8</td>
<td>17.3</td>
<td>15.3</td>
<td>15.3</td>
<td>15.3</td>
</tr>
<tr>
<td><strong>Accumulated thermal units (ATU) ($^\circ$C)</strong></td>
<td>502 ± 36</td>
<td>177 ± 11</td>
<td>492 ± 27</td>
<td>341 ± 15</td>
<td>325 ± 16</td>
<td>281 ± 27</td>
<td>87 ± 11</td>
<td>103 ± 11</td>
</tr>
<tr>
<td><strong>Migration distance ($D_M$) (km)</strong></td>
<td>1071</td>
<td>364</td>
<td>958</td>
<td>796</td>
<td>642</td>
<td>480</td>
<td>117</td>
<td>121</td>
</tr>
<tr>
<td><strong>Migration elevation ($E_M$) (m)</strong></td>
<td>690</td>
<td>280</td>
<td>716</td>
<td>728</td>
<td>1174</td>
<td>346</td>
<td>32</td>
<td>10</td>
</tr>
<tr>
<td><strong>Migration duration (d)</strong></td>
<td>30 (23-42)</td>
<td>11 (8-17)</td>
<td>28 (17-43)</td>
<td>21 (15-31)</td>
<td>19 (14-28)</td>
<td>20 (13-47)</td>
<td>5 (3-10)</td>
<td>7 (4-14)</td>
</tr>
<tr>
<td><strong>Migration rate (km d$^{-1}$)</strong></td>
<td>36 (26-46)</td>
<td>35 (21-48)</td>
<td>34 (22-57)</td>
<td>39 (26-51)</td>
<td>34 (23-45)</td>
<td>24 (10-37)</td>
<td>22 (11-44)</td>
<td>18 (9-27)</td>
</tr>
<tr>
<td><strong>Work ($0.001•E_M•D_M$)</strong></td>
<td>739</td>
<td>102</td>
<td>686</td>
<td>579</td>
<td>754</td>
<td>166</td>
<td>4</td>
<td>1</td>
</tr>
<tr>
<td><strong>River slope ($500(E_M•D_M^{-1})$)</strong></td>
<td>322</td>
<td>385</td>
<td>374</td>
<td>457</td>
<td>914</td>
<td>360</td>
<td>137</td>
<td>41</td>
</tr>
<tr>
<td><strong>Migratory effort ($0.0001•F_M•D_M$)</strong></td>
<td>609</td>
<td>141</td>
<td>328</td>
<td>272</td>
<td>219</td>
<td>98</td>
<td>24</td>
<td>25</td>
</tr>
</tbody>
</table>
CHAPTER 3: DIFFERENCES IN THERMAL TOLERANCE AND MAXIMUM CARDIORESPIRATORY PERFORMANCE AMONG SOCKEYE SALMON POPULATIONS

3.1 Introduction

The Fraser River is home to over 100 genetically and geographically distinct populations of sockeye salmon (Beacham et al., 2005), each of which encounters different upriver migration conditions. For example, populations vary in migration distance (100 to 1100 km), elevation gain (10 to 1200 m), river temperature (9° to 22°C), and river flow (2000 to 10,000 m³ s⁻¹) (see Chapter 2, Fig 2.1, Table 2.1). The upriver spawning migration is critical for reproductive success since sockeye salmon are semelparous (only spawn once). Consequently, local migratory conditions are expected to exert strong selection pressure. Indeed, morphological and behavioural characteristics (gross somatic energy, body morphology, egg number and swimming behaviour) have been correlated with river migration distance, elevation and/or work (distance × elevation) in Fraser River sockeye salmon populations (Crossin et al., 2004; Gilhousen, 1980; Hinch and Rand, 2000).

The energetic upriver migration is sustained by the cardiorespiratory system, which provides oxygen to the swimming muscles among other valuable functions. Cardiorespiratory performance can be quantified by measuring aerobic scope, which is defined as the difference between maximum oxygen consumption ($\text{MO}_{2\text{max}}$) and resting oxygen consumption ($\text{MO}_{2\text{rest}}$) (Fry, 1947). Aerobic scope represents the maximum amount of oxygen available for any activity beyond routine maintenance, activities such as swimming, reproduction and growth.
Aerobic scope has a strong temperature dependence (Fry, 1947). $\dot{\text{MO}_2}_{\text{rest}}$ typically increases exponentially with temperature until lethal levels are approached, as expected for a temperature effect on a rate function. $\dot{\text{MO}_2}_{\text{max}}$ similarly increases with increasing temperature but reaches a maximum, which may be a plateau. Then $\dot{\text{MO}_2}_{\text{max}}$ sharply declines as temperature increases toward lethal levels. The temperature at which aerobic scope is maximal is termed the optimal temperature ($T_{\text{opt}}$), which in salmonids corresponds to maximal swimming and cardiac performance (Brett, 1971; Lee et al., 2003c; Taylor et al., 1997). The temperatures at which aerobic scope starts to decline from the maximum are termed the pejus temperatures ($T_p$), which has a lower and upper value. At critical temperatures ($T_{\text{crit}}$), $\dot{\text{MO}_2}_{\text{rest}}$ and $\dot{\text{MO}_2}_{\text{max}}$ intersect and aerobic scope becomes zero. Beyond $T_{\text{crit}}$, there is insufficient oxygen to support the routine needs of the fish and survival becomes passive, time-limited and supported by anaerobic metabolism (Pörtner, 2001; Pörtner and Farrell, 2008).

The central hypothesis of my thesis is that each population has physiologically adapted through natural selection to meet their specific migration challenges. Specifically, I hypothesized that populations with more challenging migrations have greater aerobic, cardiac and heart rate scopes. I predicted that migration distance, elevation gain and work would exert the strongest selection pressure on aerobic scope, given their importance in selecting for morphological traits (Crossin et al., 2004), which has never been tested before. In addition, I hypothesized that each population can maintain maximum scope across the entire range of temperatures most frequently encountered during upriver migration, as has been previously demonstrated for two populations of sockeye salmon (Farrell et al., 2008; Lee et al., 2003c).

Wild, migrating adult sockeye salmon were intercepted in the lower Fraser River, when the fish had only been migrating upstream for 1-3 days and prior to encountering any of the
major selective elements. Individual sockeye salmon were then instrumented to measure cardiovascular variables [cardiac output (\(Q\)), heart rate (\(f_H\)), stroke volume (\(V_s\))] and swum at a single temperature (ranging from 8-26°C) in a Brett-type swim tunnel. Detailed materials and methods are found in Chapter 2 (sections 2.2-2.7 & 2.10).

### 3.2 Results

#### 3.2.1 Gross Morphology and Reproductive Status

Gross body morphology (body mass, fork, standard, POH, and POF lengths, GSI, HSI, SSI) did not differ significantly among the five populations (Table 3.1), although significant differences did exist between sexes. When all populations were pooled, male fish had a significantly greater body mass, fork length, standard length and SSI. Female fish had significantly higher GSI and HSI. None of the swum fish were fully sexually mature (no loose eggs or milt production) but they had begun their sexual maturation process (body colour was starting to turn red, gonads were developing). In addition, plasma cortisol, 17\(\beta\)-estradiol and testosterone did not significantly differ between the two populations tested (Chilko and Early Stuart). Plasma cortisol and 17\(\beta\)-estradiol were significantly higher in females compared to males (cortisol: 619 ± 87 and 380 ± 34 ng ml\(^{-1}\); 17\(\beta\)-estradiol: 0.88 ± 0.18 and 0.07 ± 0.01 ng ml\(^{-1}\), respectively). Plasma testosterone did not significantly differ between sexes (overall mean ± SEM: 2.84 ± 0.52 ng ml\(^{-1}\)).

Gross energy density did not significantly differ among populations or between sexes (mean ± SEM: 8.0 ± 0.2 MJ kg\(^{-1}\), range: 5.6-11.3 MJ kg\(^{-1}\)).
3.2.2 Cardiorespiratory Performance at $T_{\text{opt}}$

For measurements made at $T_{\text{opt}}$, there were no significant differences in $\dot{\text{MO}}_{2\text{rest}}$, $\dot{\text{MO}}_{2\text{max}}$ or aerobic scope between Early Stuart sockeye salmon that had undergone surgery and swam with the added drag of leads and those that had no surgery and had no additional drag during swimming (Table 3.2). Sockeye salmon with leads had a significantly higher $\dot{\text{MO}}_2$ after the 45-min recovery period between the first and second swim, but not after the 45-min or 2-h recovery periods following the second swim. Sockeye salmon swum without leads had an 18-23% significantly higher $U_{\text{crit}}$ compared to those with leads. Notably, both instrumented and uninstrumented fish repeated their swim performance (no significant differences existed between $U_{\text{crit}1}$ and $U_{\text{crit}2}$ within a group). Given these comparable results, fish swum without leads were included in the estimates of $\dot{\text{MO}}_{2\text{rest}}$, $\dot{\text{MO}}_{2\text{max}}$ and aerobic scope presented below. Also, this lack of effect of leads meant that my population-specific $\dot{\text{MO}}_2$ data could reliably be compared with previous literature on fish swum without instrumentation (e.g. Lee et al., 2003c).

No significant differences existed between male and female sockeye salmon for any of the cardiorespiratory variables measured at $T_{\text{opt}}$ (resting, maximum or scope for $\dot{\text{MO}}_2$, $\dot{Q}$, $f_H$ and $V_s$, $p>0.05$); therefore, data for males and females were pooled within a population to increase statistical power (Table 3.3).

Gates sockeye salmon had a significantly higher $\dot{\text{MO}}_{2\text{rest}}$ compared to Early Stuart, Nechako, Quesnel, Chilko and Weaver sockeye salmon at their respective $T_{\text{opt}}$. Weaver sockeye salmon had a significantly lower $\dot{\text{MO}}_{2\text{max}}$ compared to Early Stuart, Nechako, Chilko and Gates sockeye salmon (Table 3.3).
Aerobic scope varied by 69% across populations. Aerobic scope was significantly highest in Early Stuart and Nechako, intermediate in Lower Adams and lowest in Weaver fish. A Pearson correlation matrix revealed that several of the migration difficulty indices correlated significantly with aerobic scope (migration distance, work, duration, rate, ATU; Table 3.4). Among these, aerobic scope had the strongest relationship with migration distance to the spawning ground (Fig 3.1, Table 3.4).

\( \dot{Q} \) and \( f_H \) did not differ significantly among populations (Table 3.3). Nechako had a significantly higher scope for \( V_s \) compared to Early Stuart, Chilko and Lower Adams fish, though \( V_{s_{\text{rest}}} \) and \( V_{s_{\text{max}}} \) did not significantly differ.

3.2.3 Influence of Temperature on Cardiorespiratory Performance

Resting, Maximum and Scope

\( \dot{M}_O_{2_{\text{rest}}} \) increased exponentially with increasing temperature in each population (\( Q_{10} \) ranged from 2.2 to 2.9 across populations, Fig 3.2A). \( \dot{M}_O_{2_{\text{max}}} \) also increased with increasing temperature up to its maximum at \( T_{\text{opt}} \), and then declined thereafter. As a result, aerobic scope displayed a clear peak for each population (Fig 3.2B).

\( \dot{Q} \) measured in resting and exercising fish showed similar patterns to \( \dot{M}_O_{2_{\text{rest}}} \) and \( \dot{M}_O_{2_{\text{max}}} \) with the result that cardiac scope showed a discernible peak (only Chilko data shown, Fig 3.3A, D).

As expected, \( f_{H_{\text{rest}}} \) also increased exponentially with rising temperatures (\( Q_{10} = 2.0 \)) while \( f_{H_{\text{max}}} \) reached a plateau well below \( T_{\text{crit}} \) (Fig 3.3B). \( f_{H_{\text{rest}}} \) and \( f_{H_{\text{max}}} \) intersected at high temperatures
above $T_{opt}$. Remarkably, $f_{H_{\text{max}}}$ decreased below the resting value at the highest temperatures, with the result that scope for $f_H$ became negative at the highest test temperatures (Fig 3.3E). Aerobic scope, cardiac scope and scope for $f_H$ were all positively correlated (Fig 3.4).

In contrast, temperature had no effect on $V_{s\text{rest}}$ (Fig 3.3C). Furthermore, $V_{s\text{max}}$ declined with increasing temperature leading to a decrease in scope for $V_s$ at the highest temperatures (Fig 3.3C, F).

**Associations between Aerobic Scope and Historic River Temperatures**

The coastal Weaver population experiences the coldest temperatures during upriver migration and had the lowest $T_{opt}$ (14.5°C). Upriver populations experience similar river temperatures during migration and accordingly had a similar $T_{opt}$ (range 16.4-17.2°C, Fig 3.5 and Table 3.5). Notably, Weaver sockeye salmon are currently entering the Fraser River much earlier than normal, which exposes them to considerably warmer temperatures compared with their $T_{opt}$ (see the right-shift for the current Weaver temperature histogram, Fig 3.5).

The width of the $T_{opt}$ window (difference between the upper and lower $T_p$ values) ranged from 4-8°C among populations (Table 3.5). Among the upriver populations (Early Stuart, Nechako, Quesnel, Chilko, Gates), the Chilko population displayed the broadest optimal thermal range (Fig 3.5, Table 3.5). For all upriver populations, between 89-98% of maximum aerobic scope consistently fell within the 90th percentile of historic river temperatures encountered by each population (T90%). In contrast, the Weaver population retained only 81% of maximum aerobic scope for their historical T90% and an alarmingly low 45% for the current T90% (Fig 3.5, Table 3.5).
The maximum river temperature (21.5°C) exceeded the upper $T_p$ of every population examined (Fig 3.6). Extrapolation of the aerobic scope curves to $T_{crit}$ resulted in $T_{crit}$ values that varied between 21 and 29°C among populations (Fig 3.6, Table 3.5).

Aerobic scope curves for each population were significantly related to the historic temperature frequencies they typically experience (Fig 3.5, Table 3.6). While all regressions were significant, the AIC weights indicate that there was typically strong support for a single aerobic scope-temperature frequency relationship. In general, the Early Stuart temperature distribution was the best fit for the aerobic scope data for upriver populations; the Gates and current Weaver temperature distributions provided the poorest fit to upriver populations. The historic Weaver temperature distribution was the best fit for aerobic scope of Weaver sockeye salmon.

3.3 Discussion

This study demonstrates that Fraser River sockeye salmon populations differ in their cardiorespiratory performance and suggests that sockeye salmon populations have physiologically adapted to meet the specific challenges of their local upriver migration conditions. This study greatly extends a previous study which suggested that $T_{opt}$ for aerobic scope varied between two sockeye salmon populations (Lee et al., 2003c) by considering aerobic scope of five additional populations. A novel and strong relationship was found between maximum aerobic scope and the river migration distance to the spawning ground. Populations that travel the furthest had the highest aerobic scope, while those traveling short distances had the lowest aerobic scope. In addition, every population examined could maintain maximum
aerobic, cardiac and heart rate scopes across the entire range of temperatures typically encountered during their migration, although it was clear that the current unusual migratory behaviour of Weaver sockeye salmon exposes them to temperatures well beyond optimal.

### 3.3.1 Comparing Instrumented and Un-Instrumented Fish

In order to measure cardiorespiratory performance, fish underwent surgery and dragged leads while swimming. Nevertheless, $\dot{M}O_{2}\text{rest}$, $\dot{M}O_{2}\text{max}$ and aerobic scope were the same for instrumented and uninstrumented Early Stuart sockeye salmon, despite the observation that fish swum without leads achieved higher swim speeds compared to those swum with leads. Indeed, values for $\dot{M}O_{2}\text{rest}$, $\dot{M}O_{2}\text{max}$ and aerobic scope (means: 3.0, 14.6 and 11.8 mg O$_2$ min$^{-1}$ kg$^{-1}$, respectively) are within the previously observed ranges for Early Stuart sockeye salmon (ranges: 2-6, 11-19 and 9-14 mg O$_2$ min$^{-1}$ kg$^{-1}$, respectively, (Lee et al., 2003c; MacNutt et al., 2006). Also, $U_{\text{crit}}$ values for uninstrumented Early Stuart sockeye salmon in the current study (mean maximum $U_{\text{crit}} = 2.44 \pm 0.13$ bl s$^{-1}$) compare favourably with two previous studies examining this same population (2.26 – 2.36 bl s$^{-1}$, Lee et al., 2003c; MacNutt et al., 2006). Both instrumented and un-instrumented groups had excellent repeat swim performance ($U_{\text{crit}} 1 \approx U_{\text{crit}} 2$).

These findings suggest that while the leads did have a substantial drag effect, limiting $U_{\text{crit}}$ by $\sim$20%, oxygen delivery was not significantly effected (i.e. the increased drag resulted in the same swimming effort for a lower $U_{\text{crit}}$). Collectively, these results suggest that the data in the current study are consistent with previously published data. Furthermore, I could pool instrumented and uninstrumented fish for the analysis of aerobic scope and I was confident in
using published aerobic scope data for sockeye salmon swum without instrumentation (e.g. Weaver and Gates populations from Lee et al., 2003c) in my population comparisons.

3.3.2 Baseline Morphology and Reproductive Status

The minimum somatic energy density threshold to sustain life for sockeye salmon has been estimated to be 3.5-4.0 MJ kg\(^{-1}\), and the energy required to reach the spawning grounds is estimated to be between 1.5-2.4 MJ kg\(^{-1}\), depending on the migration distance (Clark et al., 2009; Gilhousen, 1980; Hendry and Berg, 1999; Williams et al., 1986). Given that somatic energy ranged from 6-11 MJ kg\(^{-1}\), every fish in the present study likely had sufficient energy to complete its migration and the energetic challenge of the swim test was small by comparison.

Sex-specific differences in mass, length, GSI, and SSI are all consistent with previously published reports (Clark et al., 2010; Gilhousen, 1980; Idler and Clemens, 1959; Patterson et al., 2004; Sandblom et al., 2009). In contrast to previous findings, body size and morphology did not differ among populations (Crossin et al., 2004). However, morphology was only compared across upriver populations and some populations had low sample sizes within a sex (e.g. Lower Adams) which may have limited statistical power. I would expect to find significant differences in body morphology if coastal Weaver sockeye salmon were included in the analysis (e.g. see Crossin et al., 2004, Lee et al., 2003c).

As expected for fish that were collected early in their river migration and several weeks before spawning, sexual maturation was in progress and incomplete. GSI ranged from 0.7-3.1% in males and 4.0-13.4% in females. Given that GSI reaches ~4 and 17% in fully mature males and females, respectively, (Gilhousen, 1980), the fish in the present study were still maturing. In
fact, both 17β-estradiol and testosterone levels were very low relative to values reported in the literature on migrating adult sockeye salmon (Cooperman et al., 2010; Crossin et al., 2008; Hruska et al., 2007; Sandblom et al., 2009; Young et al., 2006). Sex hormones were significantly depressed in association with increased cortisol levels in Early Stuart sockeye salmon navigating through Hells Gate (Hinch et al., 2006). The stress of sequential swim tests performed here may have had the same effect.

Plasma cortisol levels (range: 79-837 ng ml\(^{-1}\)) were within the range of published values for adult sockeye salmon (e.g. ~50-800 ng ml\(^{-1}\); Cooke et al., 2006; Cooperman et al., 2010; Sandblom et al., 2009; Young et al., 2006). The present values were likely elevated in part because the final blood sample was taken after the swimming experiment and after extensive handling to remove the fish from the swim tunnel. Even so, it has long been established that salmon normally have very high plasma cortisol levels during this final phase of life (e.g. Hane and Robertson, 1959). Chronically high cortisol levels in migrating salmonids have been hypothesized to be either a consequence of stress during the migration, or due to endogenous mechanisms associated with reproductive maturation, or possibly due to enhance home-stream olfactory memory (Carruth et al., 2002; Kubokawa et al., 1999; Sandblom et al., 2009).

Consistent with the literature, females had significantly higher cortisol levels compared to males, which is a phenomenon reported for the entire upriver migration (Carruth et al., 2002; Crossin et al., 2008; Kubokawa et al., 1999; Sandblom et al., 2009; Schmidt and Idler, 1962).
3.3.3 Maximum Cardiorespiratory Performance Among Populations

Aerobic scope at $T_{opt}$ varied considerably (by 69%) across sockeye salmon populations (range: 7.7-13.0 mg O$_2$ kg$^{-1}$ min$^{-1}$) and by 3.6 fold among individuals (range: 4.3-15.4 mg O$_2$ kg$^{-1}$ min$^{-1}$). Given that the cardiorespiratory system sustains swimming during the upriver migration, I hypothesised that aerobic scope would relate to the migratory environment. The substantial intraspecific variability in aerobic scope and 10-fold variation in migration difficulty across the seven populations examined allowed me to test this hypothesis. Coastal populations only travel ~100 km in cooling fall river temperatures, with little change in river elevation to reach their spawning grounds. In contrast, upriver populations must navigate the difficult passages through the Fraser Canyon, including the notorious Hells Gate, often in mid-summer when river temperatures peak. Some upriver populations must travel over 1000 km to reach their spawning grounds while Chilko sockeye salmon ascend ~1200 m in elevation. Because of this high degree of variability, migration difficulty was quantified using various environmental indicies (see Chapter 2): distance, elevation gain, temperature, migration rate, migration duration, work, river slope and migration effort. Elevation does not appear to have exerted a strong selective pressure since neither elevation gain nor river slope had a significant relationship with aerobic scope. However, aerobic scope was significantly related to numerous indices, including work, migration duration, migration rate and accumulated thermal units, with migration distance emerging as the best predictor. These results suggest population level adaptation of maximum aerobic scope to the selection imposed by certain river conditions encountered during migration (see discussion below, Endler, 1986; Schluter, 2000; Taylor, 1991).
This was the first study to compare cardiovascular variables across populations of sockeye salmon. In contrast to the findings for aerobic scope, neither maximum cardiac scope nor maximum scope for $f_{hi}$ varied among the five populations examined. However, I only examined cardiorespiratory performance in upriver populations that travel through Hells Gate. Due to logistical constraints, cardiorespiratory performance was not measured in the population with the lowest aerobic scope (Weaver). Given the findings for aerobic scope and the good correlation between aerobic and cardiac scope, I would expect coastal populations to exhibit lower cardiac performance compared to upriver populations, a subject that should be considered for future studies. Scope for $V_s$ was significantly higher in Nechako sockeye salmon compared to the Early Stuart, Chilko and Lower Adams populations. This demonstrates that the mechanism of achieving the same $\dot{Q}$ differs among populations, a finding that is explored further in Chapter 4.

3.3.4 Cardiorespiratory Performance with Temperature

Aerobic scope, cardiac scope and scope for heart rate were all positively correlated and varied in parallel with temperature, suggesting that the temperature dependence of cardiac performance is linked to that of aerobic capacity at the population level. The optimal water temperature for cardiorespiratory performance matched the typical water temperatures historically encountered by each population. The upriver populations all experience a similar range, mean and mode for river temperature, and accordingly demonstrated a similar $T_{opt}$. In contrast, the coastal Weaver population historically experience colder temperatures and had a corresponding colder $T_{opt}$. All six populations had 81-98% of maximum aerobic scope at the upper 90th percentile of encountered temperatures, clearly demonstrating that each population
could theoretically maintain swimming performance across the majority of river temperatures that they currently encounter. These findings support earlier work demonstrating that aerobic scope matched historic temperatures for two sockeye salmon populations (Gates and Weaver, Farrell et al., 2008; Lee et al., 2003b). Therefore, the present study adds considerably more weight to the idea of intraspecific variability for aerobic scope among Fraser River sockeye populations. This then opens up the possibility that other salmon populations with similar reproductive isolation may also demonstrate local adaptations.

While the overall temperature range may be similar among upriver populations, the timing of river entry and spawning location can create more subtle differences. For example, Early Stuart sockeye salmon, which have a very long river migration, encounter moderate temperatures and the fastest river flow early in their migration, but temperatures escalate (up to ~21.5°C) during the final stages of their migration when they are close to their spawning grounds (Macdonald et al., 2007). Chilko sockeye salmon experience the opposite temperature pattern. They encounter peak summer temperatures (again up to ~21.5°C) early in their migration while traveling through Hells Gate, but the final third of their migration is spent ascending the hydraulically challenging, but up to 10°C cooler, Chilcotin river to reach spawning grounds in or adjacent to a glacier lake. The effect of temporal differences in temperature exposure on salmon physiology and selection pressure is poorly understood. Regardless, the present data suggests that Chilko sockeye salmon possess the broadest and highest thermal tolerance for aerobic scope of all the populations examined due to adaptations to the difficult migration conditions at both warm (Hells Gate) and cold (Chilcotin river) temperatures.

The mechanism of the decline in aerobic scope above \( T_{opt} \) will be examined in detail in Chapter 5. Suffice it to say here that scope for \( f_H \) collapsed at a lower temperature than aerobic
scope in two populations, suggesting that the reduced scope for $f_H$ above $T_{opt}$ may limit $Q_{max}$ and the capacity of the cardiorespiratory system to transport oxygen. This result corroborates earlier work (Steinhausen et al., 2008).

3.3.5 Perspectives and Significance

Collectively, these results suggest that populations have locally adapted to their specific upriver migration environment. Considering that the upriver migration only lasts a few weeks, representing a mere ~2% of a sockeye salmon’s lifespan, this finding is remarkable. However, given the semelparous life history of sockeye salmon, successful upriver migration is essential in order to achieve reproductive success and thus is likely under strong selection pressure. In order for local adaptation to occur, three conditions must be met: 1) the trait must have a genetic basis, 2) variability in trait expression must result in differential survival or reproductive capability, and 3) a functional link between variability in the trait and variability in survival or reproductive success. The correlations presented here provide circumstantial, but promising, evidence for local adaption (Endler, 1986; Schluter, 2000; Taylor, 1991). Conclusive evidence for local adaptation would require breeding studies to generate an F1 and F2 generation, which would demand 4 and 8 years, respectively, a timeframe well beyond the scope of my thesis. Given the present results, such experiments would be worthwhile.

It is highly unlikely that the intraspecific differences observed in the present study were due to a plastic response to encountered river conditions prior to capture and experimentation. The fish were collected only 1-3 days into their upriver migration, after spending more than two years in the much cooler Pacific Ocean and prior to encountering any of the upriver migratory
challenges. In addition, it is highly unlikely that conditions prior to ocean entry (during rearing and downstream smolt migration) caused differential expression of the physiological characteristics that distinguished the adult populations. Foremost, downstream migration occurs at a cooler spring temperature (<12°C), goes with rather than against the current, and reduces in vertical elevation. Therefore, adults have never before experienced nor will they ever experience again the warm river migration conditions that they must overcome to successfully reproduce. As a result, the physiological traits that enabled a successful upriver migration are passed on to the offspring and their genetic basis is conserved by the strong reproductive fidelity of sockeye salmon to their natal spawning area (Burgner, 1991), which are geographically isolated. Thus, I conclude that the population-specific differences observed in the present study were most likely due to genetic adaptation, rather than phenotypic plasticity.

Peak summer temperature in the Fraser River has warmed by ~2°C since the 1950s and is expected to continue along the same trajectory (Ferrari et al., 2007; Morrison et al., 2002). The present study supports the hypothesis that further increases in summer river temperatures will result in population-specific responses in sockeye salmon (Farrell et al., 2008). Populations markedly differ in \( T_{\text{crit}} \) (when aerobic scope is zero), however, the highly aerobic, long upriver migration is clearly impossible at \( T_{\text{crit}} \). Thus, \( T_{\text{crit}} \) is an unreliable management tool, particularly since it also suffers from the inaccuracy of extrapolating from a polynomial curve. It is unknown exactly how much of aerobic scope is required for successful upriver migration. A biotelemetry study with Weaver sockeye salmon suggests that at least 50% of maximum aerobic scope was needed for their short, low elevation upriver migration [<10% of fish reached their spawning area at 18 to 21°C when aerobic scope is 0 to 68% of maximal (Farrell et al., 2008; Mathes et al., 2010)]. However, for upriver populations experiencing greater migration difficulty, perhaps up to
90% of maximum aerobic scope is needed. This suggestion is based on the observation that all
the upriver populations retained 89-97% of maximum aerobic scope at T90%. Future research
should incorporate biotelemetry and biologging techniques in the field with lab-derived
cardiorespiratory data to determine the population-specific functional aerobic scope
requirements.

Temperatures exceeding the population-specific upper $T_p$ must at some point limit
upriver swimming due to a functional collapse in aerobic scope. The $T_{opt}$ window is rather
narrow across populations (4-8°C). Thus, only 2-4°C separates $T_{opt}$ from the upper $T_p$, leaving
sockeye salmon with a narrow safety margin for temperature change. In fact, the current
temperature maximum (21.5°C) already exceeds the upper $T_p$ (set at 90% of aerobic scope) for
every population in the current study. As a result, populations are already experiencing
temperatures at their upper limit, and given the individual variability in aerobic scope, some
individuals may be dying en route because they cannot reach the spawning ground due to
insufficient aerobic scope. Given the present data, it is not surprising that no sockeye salmon
population has initiated river migration at temperatures exceeding 21°C (Hyatt et al., 2003), nor
has a historic mean migration temperature been above 19°C (Hodgson and Quinn, 2002).
Nechako and Weaver populations appear especially susceptible to high temperature, which could
prove catastrophic under the continued warming scenario. In particular, Weaver sockeye salmon
could be considered “dead fish swimming” if they continue to enter the Fraser River up to six
weeks earlier than normal, exposing themselves to temperatures higher than their historic norm
and suffering high mortality (Cooke et al., 2004; Farrell et al., 2008; Mathes et al., 2010). In
contrast, Chilko sockeye salmon appear to be “superfish”, and may have greater resilience to
climate change by being able to maintain cardiorespiratory performance at a higher temperature
compared with the other populations studied so far. A potential mechanism for Chilko sockeye salmon’s exceptionally high and broad thermal tolerance relative to the co-migrating Nechako population is explored in Chapter 6.
Figure 3.1. Linear regression between migration distance to the spawning ground and population-specific maximum aerobic scope measured at $T_{opt}$. Means ± SEM are presented.
Figure 3.2. (A) Population-specific estimates of resting (open circles) and maximum (closed circles) oxygen consumption rates in relation to water temperature for sockeye salmon. Each point corresponds to a single fish. (B) Population-specific estimates of aerobic scope, the difference between the maximum and resting oxygen consumption data presented in panel A. An exponential equation was fit to the minimum oxygen consumption rate and a polynomial quadratic equation was fit to the maximum oxygen consumption rate and aerobic scope data sets for each population. Data for Gates and Weaver provided by Lee et al. (2003c).
Figure 3.3. Resting (open circles) and maximum (closed circles) values for (A) cardiac output, (B) heart rate and (C) stroke volume in Chilko sockeye salmon. Each point corresponds to a single fish. Scope, the difference between maximum and resting data presented in A, B and C are shown in (D) cardiac scope, (E) scope for heart rate ($f_H$) and (F) scope for stroke volume ($V_s$). A polynomial quadratic equation was fit to the maximum and scope data, an exponential equation was fit to the resting data for cardiac output and heart rate and no relationship was found with temperature for resting stroke volume.
Figure 3.4. Linear regressions between aerobic scope, cardiac scope and scope for heart rate. Each data point corresponds to an individual fish, the overall $R^2$ and p-value with all populations and temperatures combined is indicated in black.
Figure 3.5. Population-specific estimates of aerobic scope (coloured lines) cardiac scope (black lines) and scope for heart rate (grey lines) in relation to water temperature. The frequency histogram shows simulated distributions of average river temperatures encountered by individual modeled fish from each population during their upriver migration from 1995 to 2008. For Weaver fish, two temperature histograms are presented, one for historical river entry (blue), the other for the current early entry phenomenon (grey). Aerobic scope data for Gates and Weaver were provided by Lee et al. (2003c).
Figure 3.6. Percentage of maximum aerobic scope available for each population in relation to temperature. Dashed line at 21.5°C indicates the maximum Fraser River temperature measured near Hells Gate since the 1940s. Although it is unknown what proportion of aerobic scope is needed to successfully ascend the river, 90% and 50% are indicated as guidelines (dotted lines).
Table 3.1. Gross morphology among populations and between sexes. Post-orbital-hypural (POH) length, post-orbital-fork (POF) length, gonadosomatic index (GSI), hepatosomatic index (HSI), and splenosomatic index (SSI) are indicated.

<table>
<thead>
<tr>
<th></th>
<th>Early Stuart</th>
<th>Nechako</th>
<th>Quesnel</th>
<th>Chilko</th>
<th>Lower Adams</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>male</td>
<td>female</td>
<td>male</td>
<td>female</td>
<td>male</td>
</tr>
<tr>
<td>N</td>
<td>17</td>
<td>9</td>
<td>6</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>mass (kg)</td>
<td>2.41 ± 0.04</td>
<td>2.39 ± 0.08</td>
<td>2.46 ± 0.20</td>
<td>2.11 ± 0.13</td>
<td>2.86 ± 0.20</td>
</tr>
<tr>
<td>fork length (cm)</td>
<td>59.6 ± 0.4</td>
<td>59.9 ± 0.9</td>
<td>60.6 ± 1.2</td>
<td>57.5 ± 1.0</td>
<td>63.5 ± 2.4</td>
</tr>
<tr>
<td>standard length (cm)</td>
<td>54.4 ± 0.4</td>
<td>53.6 ± 1.0</td>
<td>55.1 ± 2.8</td>
<td>51.6 ± 1.2</td>
<td>57.2 ± 2.2</td>
</tr>
<tr>
<td>POH (cm)</td>
<td>49.5 ± 0.5</td>
<td>50.2 ± 0.6</td>
<td>50.5 ± 1.1</td>
<td>48.4 ± 1.0</td>
<td>51.5 ± 2.3</td>
</tr>
<tr>
<td>POF (cm)</td>
<td>54.6 ± 0.4</td>
<td>55.6 ± 0.7</td>
<td>55.8 ± 1.1</td>
<td>54.2 ± 0.8</td>
<td>57.7 ± 2.4</td>
</tr>
<tr>
<td>GSI (%)</td>
<td>2.01 ± 0.09</td>
<td>5.75 ± 0.49</td>
<td>1.49 ± 0.26</td>
<td>5.49 ± 0.31</td>
<td>1.71 ± 0.22</td>
</tr>
<tr>
<td>HSI (%)</td>
<td>1.42 ± 0.05</td>
<td>1.66 ± 0.06</td>
<td>1.32 ± 0.08</td>
<td>1.53 ± 0.14</td>
<td>1.47 ± 0.19</td>
</tr>
<tr>
<td>SSI (%)</td>
<td>0.14 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.14 ± 0.03</td>
<td>0.11 ± 0.02</td>
<td>0.13 ± 0.02</td>
</tr>
<tr>
<td>energy (MJ kg⁻¹)</td>
<td>7.77 ± 0.23</td>
<td>8.08 ± 0.46</td>
<td>8.90 ± 0.25</td>
<td>7.69 ± 0.47</td>
<td>7.02 ± 1.08</td>
</tr>
</tbody>
</table>
Table 3.2. Measurements of oxygen consumption (\(\dot{M}O_2\)), critical swimming velocity (\(U_{\text{crit}}\)) and recovery ratio (RR) in Early Stuart sockeye salmon swum at \(T_{\text{opt}}\) that had (with leads) and had not (no leads) been instrumented with a flowprobe and catheters to measure cardiovascular variables. Mean ± SEM are presented, an asterisk indicates a statistically significant difference between fish with leads and those without (\(p<0.05\)).

<table>
<thead>
<tr>
<th>(\dot{M}O_2) (mg O_2 kg(^{-1}) min(^{-1}))</th>
<th>n</th>
<th>No leads</th>
<th>n</th>
<th>With leads</th>
</tr>
</thead>
<tbody>
<tr>
<td>rest</td>
<td>4</td>
<td>2.6 ± 0.2</td>
<td>8</td>
<td>3.2 ± 0.2</td>
</tr>
<tr>
<td>maximum</td>
<td>4</td>
<td>14.4 ± 1.4</td>
<td>8</td>
<td>14.7 ± 0.4</td>
</tr>
<tr>
<td>scope</td>
<td>4</td>
<td>11.9 ± 1.3</td>
<td>7</td>
<td>11.7 ± 0.3</td>
</tr>
<tr>
<td>fatigue 1</td>
<td>3</td>
<td>8.6 ± 0.2</td>
<td>7</td>
<td>10.0 ± 0.8</td>
</tr>
<tr>
<td>fatigue 2</td>
<td>4</td>
<td>9.0 ± 2.3</td>
<td>7</td>
<td>8.9 ± 0.8</td>
</tr>
<tr>
<td>45-min recovery 1</td>
<td>4</td>
<td>4.2 ± 0.9</td>
<td>7</td>
<td>6.6 ± 0.3*</td>
</tr>
<tr>
<td>45-min recovery 2</td>
<td>4</td>
<td>6.3 ± 1.4</td>
<td>7</td>
<td>5.4 ± 0.8</td>
</tr>
<tr>
<td>2-h recovery 2</td>
<td>4</td>
<td>4.0 ± 0.7</td>
<td>7</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>(U_{\text{crit}}) 1 (bl s(^{-1}))</td>
<td>4</td>
<td>2.41 ± 0.13</td>
<td>9</td>
<td>2.02 ± 0.06*</td>
</tr>
<tr>
<td>(U_{\text{crit}}) 2 (bl s(^{-1}))</td>
<td>4</td>
<td>2.35 ± 0.19</td>
<td>8</td>
<td>1.91 ± 0.06*</td>
</tr>
<tr>
<td>(U_{\text{crit}}) 1 (cm s(^{-1}))</td>
<td>4</td>
<td>144.1 ± 7.6</td>
<td>9</td>
<td>122.1 ± 4.1*</td>
</tr>
<tr>
<td>(U_{\text{crit}}) 2 (cm s(^{-1}))</td>
<td>4</td>
<td>140.6 ± 12.1</td>
<td>8</td>
<td>114.4 ± 3.5*</td>
</tr>
<tr>
<td>RR</td>
<td>4</td>
<td>0.97 ± 0.05</td>
<td>8</td>
<td>0.95 ± 0.02</td>
</tr>
</tbody>
</table>
Table 3.3. Oxygen consumption (\(\dot{M}O_2\)), cardiac output (\(Q\)), heart rate (\(f_H\)) and stroke volume (\(V_s\)) at the optimal temperature (\(T_{opt}\)) (mean ± SEM). \(\dot{M}O_2\) data for Gates and Weaver are taken from Lee et al. (2003c). No cardiac variables were measured in Lee et al. (2003c). Populations with differing letters are significantly different within each variable (p<0.05).

<table>
<thead>
<tr>
<th></th>
<th>Early Stuart</th>
<th>Nechako</th>
<th>Quesnel</th>
<th>Chilko</th>
<th>Lower Adams</th>
<th>Gates</th>
<th>Weaver</th>
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<tbody>
<tr>
<td>n</td>
<td>9-12</td>
<td>4-6</td>
<td>6-7</td>
<td>12-13</td>
<td>4-5</td>
<td>27</td>
<td>24-26</td>
</tr>
<tr>
<td>(\dot{M}O_2)rest (mg O(_2) kg(^{-1}) min(^{-1}))</td>
<td>3.0 ± 0.2(^a)</td>
<td>2.4 ± 0.2(^a)</td>
<td>2.6 ± 0.2(^a)</td>
<td>2.9 ± 0.2(^a)</td>
<td>3.4 ± 0.5(^{ab})</td>
<td>4.0 ± 0.1(^b)</td>
<td>2.8 ± 0.1(^a)</td>
</tr>
<tr>
<td>(\dot{M}O_2)max (mg O(_2) kg(^{-1}) min(^{-1}))</td>
<td>14.6 ± 0.5(^a)</td>
<td>15.3 ± 0.6(^a)</td>
<td>13.7 ± 0.5(^{ab})</td>
<td>13.8 ± 0.6(^a)</td>
<td>12.6 ± 1.4(^{ab})</td>
<td>15.0 ± 0.2(^a)</td>
<td>10.5 ± 0.3(^b)</td>
</tr>
<tr>
<td>(\dot{M}O_2) scope (mg O(_2) kg(^{-1}) min(^{-1}))</td>
<td>11.8 ± 0.5(^a)</td>
<td>13.0 ± 0.6(^a)</td>
<td>11.2 ± 0.6(^{ab})</td>
<td>10.9 ± 0.6(^{ab})</td>
<td>9.0 ± 0.8(^b)</td>
<td>10.9 ± 0.2(^{ab})</td>
<td>7.7 ± 0.2(^c)</td>
</tr>
<tr>
<td>(\dot{Q})rest (ml min(^{-1}) kg(^{-1}))</td>
<td>34.8 ± 2.7</td>
<td>29.9 ± 1.7</td>
<td>34.7 ± 3.9</td>
<td>34.8 ± 2.9</td>
<td>27.8 ± 3.1</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(\dot{Q})max (ml min(^{-1}) kg(^{-1}))</td>
<td>105.5 ± 5.5</td>
<td>110.0 ± 5.6</td>
<td>113.6 ± 10.7</td>
<td>107.1 ± 5.5</td>
<td>85.4 ± 10.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(\dot{Q}) scope (ml min(^{-1}) kg(^{-1}))</td>
<td>70.7 ± 4.7</td>
<td>80.2 ± 6.1</td>
<td>78.9 ± 7.8</td>
<td>72.4 ± 3.9</td>
<td>57.6 ± 7.4</td>
<td>-</td>
<td>-</td>
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<tr>
<td>(f_H)rest (beats min(^{-1}))</td>
<td>70.1 ± 2.3</td>
<td>65.8 ± 2.6</td>
<td>60.9 ± 4.7</td>
<td>67.3 ± 2.7</td>
<td>67.7 ± 6.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(f_H)max (beats min(^{-1}))</td>
<td>95.5 ± 2.8</td>
<td>84.7 ± 4.0</td>
<td>93.1 ± 3.5</td>
<td>94.1 ± 2.1</td>
<td>91.2 ± 7.4</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(f_H) scope (beats min(^{-1}))</td>
<td>25.4 ± 3.8</td>
<td>18.9 ± 3.9</td>
<td>32.2 ± 3.0</td>
<td>26.7 ± 3.7</td>
<td>23.5 ± 9.7</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(V_s)rest (ml beat(^{-1}) kg(^{-1}))</td>
<td>0.49 ± 0.03</td>
<td>0.46 ± 0.02</td>
<td>0.57 ± 0.06</td>
<td>0.53 ± 0.05</td>
<td>0.43 ± 0.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(V_s)max (ml beat(^{-1}) kg(^{-1}))</td>
<td>1.10 ± 0.05</td>
<td>1.30 ± 0.05</td>
<td>1.22 ± 0.11</td>
<td>1.14 ± 0.06</td>
<td>0.93 ± 0.06</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>(V_s) scope (ml beat(^{-1}) kg(^{-1}))</td>
<td>0.60 ± 0.04(^a)</td>
<td>0.85 ± 0.05(^b)</td>
<td>0.65 ± 0.05(^{ab})</td>
<td>0.62 ± 0.05(^a)</td>
<td>0.50 ± 0.02(^a)</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>
Table 3.4. Pearson correlation matrix relating aerobic scope of fish from seven populations and eight migration difficulty variables (see Table 2.1). ATU = accumulated thermal units, $F_M =$ Fraser River discharge. Three critical values are indicated: $p < 0.05$ (no correction for multiple comparisons), $p < 0.018$ (Benjamini and Yekutieli False Discovery Rate) and $p < 0.006$ (Bonferroni). Bold font indicates the migration difficulty variable with the highest correlation coefficient.

<table>
<thead>
<tr>
<th>Variable</th>
<th>Aerobic Scope</th>
</tr>
</thead>
<tbody>
<tr>
<td>migration distance ($D_M$)</td>
<td>0.856†</td>
</tr>
<tr>
<td>migration elevation ($E_M$)</td>
<td>0.653</td>
</tr>
<tr>
<td>work ($0.0001\cdot E_M \cdot D_M$)</td>
<td>0.785*</td>
</tr>
<tr>
<td>river slope ($500(E_M D_M^{-1})$)</td>
<td>0.335</td>
</tr>
<tr>
<td>migration effort ($0.0001\cdot D_M \cdot F_M$)</td>
<td>0.732</td>
</tr>
<tr>
<td>migration duration</td>
<td>0.777*</td>
</tr>
<tr>
<td>migration rate</td>
<td>0.842†</td>
</tr>
<tr>
<td>ATU</td>
<td>0.832*</td>
</tr>
</tbody>
</table>

* $p < 0.05$; † $p < 0.018$, ‡ $p < 0.006$
Table 3.5. Population-specific optimal temperature (T$_{opt}$), upper and lower pejus temperatures (T$_p$) and predicted critical temperatures (T$_{crit}$). T$_p$ range refers to the width of the T$_{opt}$ window (i.e. upper T$_p$ – lower T$_p$). T90% indicates the upper 90$^{th}$ percentile of historic temperatures encountered by each population (1995-2008). % Scope at T90% indicates the percent of maximum aerobic scope available at T90%. Values for current river entry timing for Weaver are shown in parentheses under the historical timing.

<table>
<thead>
<tr>
<th>Population</th>
<th>T$_{opt}$ (°C)</th>
<th>Lower T$_p$ (°C)</th>
<th>Upper T$_p$ (°C)</th>
<th>T$_p$ range (°C)</th>
<th>T90% (°C)</th>
<th>% Scope at T90%</th>
<th>Predicted T$_{crit}$ (°C)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Stuart</td>
<td>17.2</td>
<td>14.4</td>
<td>19.9</td>
<td>5.5</td>
<td>19.0</td>
<td>96</td>
<td>25.8</td>
</tr>
<tr>
<td>Nechako</td>
<td>16.8</td>
<td>14.5</td>
<td>19.0</td>
<td>4.5</td>
<td>18.4</td>
<td>95</td>
<td>24.0</td>
</tr>
<tr>
<td>Quesnel</td>
<td>-</td>
<td>-</td>
<td>18.5</td>
<td>-</td>
<td>18.6</td>
<td>89</td>
<td>25.9</td>
</tr>
<tr>
<td>Chilko</td>
<td>16.8</td>
<td>12.9</td>
<td>20.7</td>
<td>7.8</td>
<td>18.8</td>
<td>98</td>
<td>29.4</td>
</tr>
<tr>
<td>Gates</td>
<td>16.4</td>
<td>13.4</td>
<td>19.5</td>
<td>6.1</td>
<td>19.7</td>
<td>89</td>
<td>26.1</td>
</tr>
<tr>
<td>Weaver</td>
<td>14.5</td>
<td>12.5</td>
<td>16.4</td>
<td>3.9</td>
<td>17.2</td>
<td>81</td>
<td>20.8</td>
</tr>
</tbody>
</table>

(19.1) (45)
Table 3.6. Summary of model selection statistics for regressions between population-specific aerobic scope predictions and population-specific temperature frequency distributions. Best-fit relationships correspond to ΔAIC ≤ 2 (bold font). Both current (WeaverCurrent) and historical (WeaverHistoric) temperature frequency histograms for Weaver are included.

<table>
<thead>
<tr>
<th>Population scopes</th>
<th>Temperature frequencies</th>
<th>AIC</th>
<th>ΔAIC</th>
<th>w</th>
<th>R²</th>
<th>p-value</th>
<th>Bonferroni corrected</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Early Stuart</strong></td>
<td><strong>Early Stuart</strong></td>
<td>182.53</td>
<td>0.00</td>
<td>0.94</td>
<td>0.72</td>
<td>8.35E-12</td>
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<td>Nechako</td>
<td>200.18</td>
<td>17.65</td>
<td>0.00</td>
<td>0.62</td>
<td>1.08E-09</td>
<td>4.54E-08</td>
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<tr>
<td></td>
<td>Quesnel</td>
<td>206.71</td>
<td>24.18</td>
<td>0.00</td>
<td>0.55</td>
<td>2.53E-08</td>
<td>1.06E-06</td>
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<tr>
<td></td>
<td>Chilko</td>
<td>187.98</td>
<td>5.45</td>
<td>0.06</td>
<td>0.72</td>
<td>3.08E-12</td>
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<tr>
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<td>Gates</td>
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<td>0.67</td>
<td>7.68E-11</td>
<td>3.23E-09</td>
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<tr>
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<td>WeaverCurrent</td>
<td>231.99</td>
<td>49.47</td>
<td>0.00</td>
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<td>2.89E-01</td>
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<tr>
<td></td>
<td>WeaverHistoric</td>
<td>226.41</td>
<td>43.88</td>
<td>0.00</td>
<td>0.27</td>
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<td>1.67E-02</td>
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<tr>
<td><strong>Nechako</strong></td>
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<td>179.13</td>
<td>1.99</td>
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<td>0.81</td>
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<tr>
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<td>Nechako</td>
<td>197.43</td>
<td>20.29</td>
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<td>0.73</td>
<td>1.12E-12</td>
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<tr>
<td></td>
<td>Quesnel</td>
<td>205.95</td>
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<td>6.69E-11</td>
<td>2.81E-09</td>
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<tr>
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<td>2.49E-07</td>
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<tr>
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<tr>
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<td>169.31</td>
<td>46.00</td>
<td>0.00</td>
<td>0.64</td>
<td>7.58E-10</td>
<td>3.18E-08</td>
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<td>158.12</td>
<td>34.81</td>
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<td>0.75</td>
<td>2.61E-13</td>
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<td>171.22</td>
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<td>0.90</td>
<td>2.00E-16</td>
<td>8.40E-15</td>
</tr>
</tbody>
</table>
CHAPTER 4: A COMPARISON OF CARDIORESPIRATORY AND SWIMMING
PERFORMANCE AMONG UPRIVER SOCKEYE SALMON POPULATIONS AT $T_{opt}$

4.1 Introduction

The previous chapter compared resting, maximum and scope for $\dot{M}O_2$, $\dot{Q}$, $f_H$ and $V_s$ among sockeye salmon populations and demonstrated that aerobic scope varies according to the difficulty of the upriver spawning migration. However, detailed analyses of how the various components of the cardiorespiratory oxygen convection system change with swimming were not considered. Therefore, this chapter greatly expands on Chapter 3 through a comprehensive assessment of swimming physiology at $T_{opt}$.

The upriver spawning migration is physically demanding for sockeye salmon. During this once-in-a-lifetime migration, Fraser River sockeye salmon swim continuously against a fast flowing river for several weeks at swimming speeds of 2 to 4 km h$^{-1}$ and ground speeds of 20 to 40 km day$^{-1}$ (English et al., 2005, Hinch and Rand, 1998). Moreover, because the fish cease feeding in the ocean, upriver swimming is fuelled entirely by endogenous energy stores. Also, sockeye salmon have a finite amount of time to complete their migration in order to successfully spawn. Upriver populations must negotiate hydraulically challenging river sections through the Fraser Canyon, such as Hells Gate, which requires anaerobic swimming (Hinch and Bratty, 2000; Rand and Hinch, 1998). Consequently, it is critical that sockeye salmon are able to recover rapidly from exhaustive exercise in order to continue their upriver migration. Indeed, previous studies on sockeye salmon, pink salmon, coho salmon, cutthroat trout and rainbow trout showed that salmonids have an excellent ability to repeat their swim performance after a short recovery.
period of 30-60 min (Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003b; MacNutt et al., 2004; MacNutt et al., 2006; Wagner et al., 2006).

This is the first study to compare cardiovascular performance and blood variables across wild sockeye salmon populations. The objective of this study was to examine how the cardiovascular system supports aerobic scope and swim performance and whether the mechanism changes over sequential swim tests or across populations. I compared swimming and cardiorespiratory performance among upriver Fraser River sockeye salmon populations (N = 32, Early Stuart, Chilko, Quesnel and Nechako) performing two sequential Ucrit swim challenges at their Topt. By performing these comparisons at Topt, I removed temperature as a confounding factor in the population comparison. Only fish that had been instrumented were included in the analysis. Detailed materials and methods are found in Chapter 2 (sections 2.2-2.7 and 2.10).

All four populations must navigate through Hells Gate and travel 650 to 1100 km upstream, reaching an elevation of 700 to 1200 m on their spawning grounds. Furthermore, all four populations encounter a similar migration temperature median and mode (Table 2.1, 16-17°C) and have a similar Topt (Table 3.6, ~17°C). I hypothesized that all four populations would be able to repeat their swim performance following a brief 45-min recovery, since there is likely strong selection pressure on the ability to rapidly recover from exhaustive exercise in adult sockeye salmon. Furthermore, since all four populations experience challenging migrations and did not differ in aerobic scope (Chapter 3), I hypothesized that they would have similar cardiorespiratory and swimming performance.
4.2 Results

4.2.1 Swimming Behaviour and Performance

Most of the sockeye salmon ventilated regularly and remained steady and calm during the rest period, with occasional exploratory movements. Many sockeye salmon exhibited unsteady, erratic swimming behaviour or they tended to rest on the bottom during the initial ramping phase of the $U_{\text{crit}}$ swim challenge until they reached swim speeds of $\sim 1 \text{ bl s}^{-1}$. Thereafter, there were typically three clear swimming phases. During the first phase, fish regularly ventilated their gills via opercular pumping while swimming steadily. Throughout phase two, fish continued to swim in a steady manner, but switched to ram ventilation. In the third swim phase, the fish transitioned to burst-and-coast swimming and continued to ram ventilate. Phase three typically started during the penultimate or final swim speed, which corresponded to speeds $\sim 80-90\%$ of maximum or $\sim 2.0 \text{ bl s}^{-1}$. Interestingly, during the first two swim phases, fish occasionally exhibited “side burst” behaviour, where they would slowly fall back in the swim tunnel and then flip onto their sides and burst forward with a few quick tail flicks to regain their position at the front of the swim tunnel. This behaviour differed from the vertical burst-and-coast behaviours exhibited at the fastest swim speeds. Most sockeye salmon spent the duration of the recovery periods ventilating via opercular pumping with occasional light swimming, near the front of the swim tunnel.

$U_{\text{crit}}$ 1, $U_{\text{crit}}$ 2 and RR did not significantly differ among populations or between sexes (Table 4.1). In addition, all populations were able to repeat their swim performance because $U_{\text{crit}}$ 1 did not significantly differ from $U_{\text{crit}}$ 2 (overall mean maximum $U_{\text{crit}} = 2.04 \pm 0.04$).
4.2.2 Cardiorespiratory Performance

There were no significant differences in any of the cardiorespiratory variables between sexes, therefore, the data were pooled.

\( \dot{\text{MO}}_{2\text{max}} \) and aerobic scope were not compared between swim 1 and swim 2 due to missing paired measurements. Likewise, Nechako sockeye salmon were excluded from the \( \dot{\text{MO}}_2 \) analysis because there were insufficient measurements at each swimming speed.

During swimming, \( \dot{\text{MO}}_2 \) significantly increased ~5-fold from resting values (Fig 4.1). \( \dot{\text{MO}}_2 \) did not significantly differ among the three populations at any swimming speed (Fig 4.1, Table 4.2). \( \dot{\text{MO}}_2 \) remained significantly elevated above resting levels at both 45-min recovery periods; however, it had recovered by the 2-h recovery period (Table 4.2). \( \dot{\text{MO}}_2 \) did not significantly differ between swim 1 and 2 at any swimming speed. Accordingly, COT and COT\(_\text{net} \) did not significantly differ between swims (Fig 4.2). Notably, COT did not display the characteristic U-shape, instead, it plateaued between 1.12 and 2.37 bl \( \text{s}^{-1} \).

As expected, \( \dot{\text{Q}} \) significantly increased ~3-fold above resting levels during swimming (Fig 4.3A). \( \dot{\text{Q}} \) did not significantly differ among the four populations at any swimming speed (Fig 4.3A, Table 4.2, 4.3). \( \dot{\text{Q}} \) did not recover back to resting levels during any of the recovery periods, except in Nechako sockeye salmon at 2 h (Table 4.2). In addition, \( \dot{\text{Q}} \) did not significantly differ between swim 1 and swim 2, except at the very first swim speed (0.62 bl \( \text{s}^{-1} \)). Consequently, COT-\( \dot{\text{Q}} \) and COT-\( \dot{\text{Q}}_{\text{net}} \) only significantly differed between swims at 0.62 bl \( \text{s}^{-1} \), although there was a general, non-significant trend for COT-\( \dot{\text{Q}} \) and COT-\( \dot{\text{Q}}_{\text{net}} \) to be higher during swim 2 compared to swim 1 (Fig 4.2).
$V_s$ increased ~2-fold above resting levels during swimming. $V_s$ did not significantly differ among populations at rest, during either swim or during recovery (Fig 4.3B, Table 4.2, 4.3). Even so, scope for $V_s$ during swim 1 was significantly higher in Nechako compared to Quesnel sockeye salmon (Table 4.3). $V_s$ returned back to resting levels by the 45-min recovery time point following both swims (Table 4.2). $V_s$ did not significantly differ between swim 1 and swim 2 at any of the swimming speeds or recovery times, although scope for $V_s$ was significantly higher in swim 2 compared to swim 1 for Quesnel sockeye salmon (Table 4.3).

During the first swim, $f_H$ increased by ~1.5 fold from resting levels. $f_H$ did not significantly differ among populations at any swim speed during swim 1 (Fig 4.3C). Notably, $f_H$ did not recover back to resting levels after swim 1 for Early Stuart, Chilko and Quesnel populations (Table 4.2). Instead, following a brief decrease at fatigue, $f_{H\text{max}}$ was maintained throughout the recovery period and the entire second swim for these three populations (Fig 4.3). In contrast, $f_H$ recovered back to resting levels after swim 1 in Nechako sockeye salmon (Fig 4.3C, Table 4.2). During swim 2, $f_H$ was significantly lower in Nechako compared to the Early Stuart and Quesnel populations until they reached a velocity of 1.37 bl s\textsuperscript{-1} (Fig 4.3C). Moreover, Nechako had a significantly lower $f_H$ relative to Quesnel sockeye salmon during fatigue following the second swim (Table 4.2). $f_H$ remained elevated above resting levels at the 2-h recovery period for Early Stuart, Chilko and Quesnel sockeye salmon (Table 4.2). Despite differences in the $f_H$ response to swimming and recovery, $f_{H\text{max}}$ and scope for $f_H$ did not significantly vary among populations or between swims (Table 4.3).
4.2.3 Oxygen Transport and Removal by Tissues

Since \( \dot{MO}_2, \dot{Q}, V_s \) and \( f_{HI} \) did not significantly differ at rest or during swimming in Early Stuart, Chilko and Quesnel sockeye salmon, I pooled the results for the blood analyses from these three populations. Nechako sockeye salmon were not included in the analysis because \( V_s \) and \( f_{HI} \) differed from the other populations at various time points. Again, there were no significant differences in any of the blood variables between male and female sockeye so the data were pooled.

Blood samples were collected at rest, during steady state swimming when most of the fish were still ventilating by opercular pumping (“steady”, mean speed = 1.18 ± 0.02 bl s\(^{-1}\), or 55.8 ± 0.9% of maximum swim speed), during burst-and-coast swimming with ram ventilation (“burst”, mean speed = 2.05 ± 0.06 bl s\(^{-1}\) or 92.6 ± 1.7% of maximum swim speed), immediately following fatigue, following 45 min of recovery and 2 h after the second swim was terminated.

\( \text{P}_{aO2}, \text{P}_{vO2}, \text{C}_{aO2} \) and \( \text{C}_{vO2} \) all significantly decreased from rest during swimming (Fig 4.4). \( \text{P}_{vO2} \) and \( \text{C}_{vO2} \) reached a plateau of 17.6-24.0 torr and 2.5-3.3 ml dl\(^{-1}\), respectively, during burst swimming and fatigue. \( \text{P}_{aO2}, \text{P}_{vO2}, \text{C}_{aO2} \) and \( \text{C}_{vO2} \) did not differ between swim 1 and swim 2, despite significant decreases in [Hb] and Hct during swim 2. Both \( \text{P}_{aO2} \) and \( \text{P}_{vO2} \) returned to resting levels by the 45-min recovery periods. However, \( \text{C}_{aO2} \) and \( \text{C}_{vO2} \) remained depressed below resting levels during the second 45-min and the 2-h recovery period (Fig 4.4).

Hct only significantly varied between arterial and venous blood samples during fatigue 1; however, both [Hb] and Hct were consistently higher in venous compared to arterial blood throughout both swim tests. Moreover, MCHC was consistently lower in venous compared to arterial blood and significantly differed during several time points (Table 4.4). To verify whether
this was an artefact, I compared paired arterial and venous blood samples from fish that had both cannulae working simultaneously (paired samples, Table 4.5). Paired samples revealed that [Hb] was equivalent between arterial and venous blood samples, except at steady 1 (Table 4.5). However, Hct was significantly higher and MCHC was significantly lower in venous compared to arterial blood, but only during burst swimming and at fatigue (Table 4.5).

Hct and [Hb] were significantly lower during swim 2 compared to swim 1, suggesting that hemodilution may have occurred (Table 4.4). To check for this possibility, comparisons were made between fish that had both cannulae working (~20 blood samples collected total, ≥14 ml of blood) and those with only the venous cannula working (~10 blood samples, ≥7 ml of blood). Both [Hb] and Hct were significantly lower during swim 2 in fish that had two cannulae compared to those that only had one functioning cannula, confirming that [Hb] and Hct were significantly lower during swim 2 due to hemodilution (Fig 4.6). This hemodilution had no effect on P\textsubscript{V\textsubscript{O\textsubscript{2}}}; however, C\textsubscript{v\textsubscript{O\textsubscript{2}}} was significantly lower during the recovery periods in fish with two cannulae relative to those with one cannula (Fig 4.6). There were insufficient fish with only the arterial cannula functioning to perform a similar analysis for arterial blood.

There was a general trend for A-V\textsubscript{O\textsubscript{2}} to increase during swimming. However, A-V\textsubscript{O\textsubscript{2}} did not significantly differ from rest or between swims (Fig 4.6); probably because comparisons were limited to fish with both cannulae working (N = 9). Notably, A-V\textsubscript{O\textsubscript{2}} decreased by ~50% from resting values during the 2-h recovery period, since both C\textsubscript{a\textsubscript{O\textsubscript{2}}} and C\textsubscript{v\textsubscript{O\textsubscript{2}}} remained depressed below resting levels.

Arterial transfer of oxygen to the tissues (T\textsubscript{a\textsubscript{O\textsubscript{2}}}) integrates changes in \dot{Q} and C\textsubscript{a\textsubscript{O\textsubscript{2}}} (T\textsubscript{a\textsubscript{O\textsubscript{2}}} = \dot{Q} \times C\textsubscript{a\textsubscript{O\textsubscript{2}}}). T\textsubscript{a\textsubscript{O\textsubscript{2}}} significantly increased from rest by 2.5-fold during burst swimming and returned back to resting levels by the 45-min recovery period (Fig 4.6). No significant differences were
detected between swim 1 and 2. In contrast, venous transfer of oxygen to the heart and gills \( (T_{\text{vO}_2} = \dot{Q} \times C_{\text{vO}_2}) \) remained constant throughout the entire swimming protocol and did not significantly vary from rest or between swim 1 and 2 (Fig 4.6).

4.2.4 Other Blood Variables

Plasma lactate did not significantly differ between arterial and venous blood samples. Plasma lactate was significantly elevated above resting levels during fatigue and 45 min after swim 1, verifying that the salmon did transition to anaerobic swimming during the swim challenge (Fig 4.4). Plasma lactate levels also remained significantly elevated during swim 2, resulting in a significant difference between swim 1 and swim 2 during steady swimming. Although plasma lactate did tend to recover somewhat by burst 2, it was again significantly elevated above resting levels during fatigue 2 and the second 45-min recovery. However, plasma lactate did not significantly differ from resting levels at the 2-h recovery period (Fig 4.4).

Plasma glucose, chloride and sodium varied minimally from resting levels and no significant differences were detected between arterial and venous blood samples (Table 4.4). In contrast, plasma potassium was highly variable between swim 1 and 2 and between arterial and venous blood samples. In general, plasma potassium was higher in arterial relative to venous blood (Tables 4.4 and 4.5). In addition, plasma potassium tended to be higher during swim 2 compared to swim 1. Remarkably, plasma potassium actually decreased from rest during burst swimming and at fatigue with swim 1 (Table 4.4).
4.2.5 General Trends with Swimming

To summarize the general trends in cardiovascular physiology and oxygen status associate with swimming, fold changes from the initial resting value were examined for $\dot{V}O_2$, $Q$, $A-V_2$, $T_{aO2}$ and $T_{vO2}$ exclusively from fish with both cannulae working from pooled data from the Early Stuart, Chilko and Quesnel populations (Fig 4.7). $\dot{V}O_2$ increased 5-fold during both swims, primarily due to a 3-fold increase in $Q$ (Fig 4.7A). $\dot{V}O_2$ returned to resting levels by the 2-h recovery after swim 2, even though $Q$ remained elevated, because $A-V_2$ decreased by ~50% from resting levels (Fig 4.7A, B & C). The 3-fold increase in $Q$ during both swims was primarily due to a >2-fold increase in $V_s$ (Fig 4.7B). $f_{hi}$ increased ~1.5 fold above resting levels during the first swim and never decreased below maximum levels throughout the entire second swim and both recovery periods. As such, $Q$ remained elevated above rest at both 45-min and the 2-h recovery periods, even though $V_s$ had returned to resting levels. The ~1.5-fold increase in $A-V_2$ was driven by a large decrease in $C_{vO2}$, though the $A-V_2$ response was attenuated since $C_{aO2}$ also decreased (Fig 4.7C). $T_{aO2}$ increased by 2.5-fold during both swims, which was entirely due to the aforementioned increase in $Q$ (Fig 4.7D). $T_{vO2}$ changed very little from rest throughout both swims and the recovery periods because the decrease in $C_{vO2}$ was offset by the increase in $Q$ (Fig 4.7E).

4.3 Discussion

The goal of the present study was to compare cardiorespiratory performance and blood variables across upriver Fraser River sockeye salmon populations swimming two sequential
swim tests. As anticipated, all four populations demonstrated similar increases in $\dot{MO}_2$ and $Q$ with swimming. However, in comparison to the other three populations, Nechako sockeye salmon relied more on $V_s$ than $f_H$ to increase $Q$. This finding suggests that the mechanism of achieving the same $MO_2$ and $Q$ can vary across populations. In addition, despite incomplete metabolic recovery, all populations showed an exceptional ability to repeat their swim performance following a brief 45-min recovery, supporting previous studies on salmonids (Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003b; MacNutt et al., 2004; MacNutt et al., 2006; Wagner et al., 2006). A rapid rate of recovery is clearly beneficial for salmon to ensure a timely migration to reach the spawning grounds.

4.3.1 Swimming Behaviour and Performance

Preliminary, practise swim tests have been demonstrated to improve ramp-$U_{crit}$ swim performance in rainbow trout (Jain et al., 1997), presumably because the fish become habituated to the tunnel and learn how to swim effectively. I was unable to give the sockeye salmon a practise swim due to time and logistical constraints. Although the fish often swam erratically during the initial ramping phase of the $U_{crit}$ swim test, they quickly grew accustomed to the tunnel at faster speeds. Despite swimming with leads, the sockeye salmon demonstrated classic swim behaviours and clearly transitioned to burst-and-coast anaerobic swimming at the highest swim speeds, as confirmed by the appearance of lactate in the plasma at fatigue and during recovery. Advanced sexual maturation has been reported to decrease swim performance in pink salmon (Williams et al., 1986), sockeye salmon (M. Steinhausen, pers. communication) and chinook salmon (E. Eliason, pers. observation). This was not a major concern in the present
study since the sockeye salmon were collected early in their migration, several weeks before their spawning date and none of the fish were fully sexually mature (no loose eggs or milt production).

The $U_{\text{crit}}$ values obtained in the present study for adult sockeye salmon swum with leads (mean overall maximum $U_{\text{crit}} = 2.0 \text{ bl s}^{-1}$) were within the reported range for un-instrumented adult salmonids: sockeye salmon (1.4-2.4 bl s$^{-1}$, Brett and Glass, 1973; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003c; MacNutt et al., 2006), chinook salmon (2.1 bl s$^{-1}$, Geist et al., 2003), pink salmon (1.6-3.2 bl s$^{-1}$, Farrell et al., 2003; MacNutt et al., 2006; Williams et al., 1986), coho salmon (1.4-1.9 bl s$^{-1}$, Farrell et al., 2003; Lee et al., 2003a; Lee et al., 2003c), Arctic charr Salvelinus alpinus (L.) (2.8 bl s$^{-1}$, Jones et al., 1974), mountain whitefish Prosopium williamsoni (1.4 bl s$^{-1}$, Jones et al., 1974), Arctic cisco Coregonus autumnalis (1.9 bl s$^{-1}$, Jones et al., 1974), least cisco Coregonus sardinella Valenciennes (2.0 bl s$^{-1}$, Jones et al., 1974), wild-caught rainbow trout (2.2 bl s$^{-1}$, Jones et al., 1974), and hatchery-reared rainbow trout (2.1-2.8 bl s$^{-1}$, Jain et al., 1997; Jones et al., 1974).

$U_{\text{crit}}$ did not differ between swim 1 and 2 or among populations, supporting the observation that migrating, adult Pacific salmon have excellent repeat swim performance (Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003b; MacNutt et al., 2004; MacNutt et al., 2006; Wagner et al., 2006). Collectively, these comparisons suggest that the fish in the present study had recovered well from surgery since the repeatability of swim test decreases when sockeye salmon or rainbow trout are diseased or exposed to toxicants (Jain et al., 1998; Tierney and Farrell, 2004; Wagner et al., 2005).
4.3.2 Cardiorespiratory Performance with Comparisons across Populations

\( \dot{M}O_2 \) measured at \( T_{opt} \) (15-20°C) did not differ among the four upriver sockeye salmon populations and ranged between 2.4 and 3.2 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\) (also see Chapter 3). This range is comparable to resting values in other adult salmonids: sockeye salmon at 11-21°C (1.6-4.4 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\), Farrell et al., 1998; Farrell et al., 2003; Jain et al., 1998; Lee et al., 2003c; Steinhausen et al., 2008; Wagner et al., 2005; Wagner et al., 2006), chinook salmon at 8-17°C (2.0-3.4 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\), Clark et al., 2008b; Geist et al., 2003), pink salmon at 9-22°C (1.1-4.3 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\), Farrell et al., 2003; MacNutt et al., 2006; Williams et al., 1986), coho salmon at 8-10°C (2.2-2.9 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\), Farrell et al., 2003; Lee et al., 2003a; Lee et al., 2003c).

Studies on sexually immature rainbow trout report considerably lower \( \dot{M}O_2 \) values ranging from 0.8-1.3 mg O\(_2\) kg min\(^{-1}\) (Claireaux et al., 2005; Eliason et al., 2008; Kiceniuk and Jones, 1977; Taylor et al., 1996; Thorarensen et al., 1996). The finding that adult Pacific salmon have a comparatively higher \( \dot{M}O_2 \) relative to immature rainbow trout is not surprising since adult salmon undergo considerable morphological changes (developing gonads and secondary sexual characteristics) which undoubtedly has an oxygen cost. They may have also been more restless in the swim tunnel due to the migratory life stage (Lee et al., 2003c; Wagner et al., 2006). In addition, the fish in the present study were only allowed an overnight recovery due to logistical issues and to time constraints. Farrell et al. (2003) demonstrated that \( \dot{M}O_2 \) significantly declined for fish given a 48-h habituation period to the swim tunnel compared to those only given an overnight recovery. Moreover, some of the studies with rainbow trout attempted to measure standard metabolic rate and thus measurements were made over several days under dark conditions (e.g. Eliason et al., 2008). These comparisons emphasize the
importance of considering experimental apparatus and design when comparing across studies, particularly with \( \text{MO}_2 \). To what degree aerobic scope was underestimated in these population comparisons will require further study, though the oxygen cost of sexual development and restlessness are likely unavoidable when measuring \( \text{MO}_{2\text{rest}} \) in adult salmonids.

All the populations increased \( \text{MO}_2 \) by ~5-fold during swimming, attaining \( \text{MO}_{2\text{max}} \) values ranging between 13.7-15.3 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\) and aerobic scope values of 10.9-13.0 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\) (see Chapter 3). This increase is at the high end of the 3- to 5-fold increase reported for other adult salmonids (Farrell et al., 2003; Geist et al., 2003; Lee et al., 2003c; MacNutt et al., 2006; Williams et al., 1986). Similarly, the present study’s \( \text{MO}_{2\text{max}} \) values are at the high end relative to previous studies on adult salmonids: sockeye salmon (5.8-15.1 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\), Brett and Glass, 1973; Farrell et al., 2003; Hinch et al., 1996; Jain et al., 1998; Lee et al., 2003, MacNutt et al., 2006, Wagner et al., 2005, Wagner et al., 2006), chinook salmon (11.2 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\), Geist et al., 2003), pink salmon (12.6-16 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\), Farrell et al., 2003; MacNutt et al., 2006; Williams et al., 1986) and coho salmon (8.7-9.8 mg O\(_2\) kg\(^{-1}\) min\(^{-1}\), Farrell et al., 2003; Lee et al., 2003a; Lee et al 2003c).

\( \dot{Q} \) at rest and during swimming were indistinguishable across populations. Only two previous studies have examined \( \dot{Q} \) in sockeye salmon [Davis, 1968 (as reported in Brett, 1971); Steinhausen et al., 2008]. \( \dot{Q}_{\text{rest}} \) reported here (30-35 ml min\(^{-1}\) kg\(^{-1}\)) is slightly higher than \( \dot{Q}_{\text{rest}} \) at 15°C for Lower Adams sockeye salmon (25 ml min\(^{-1}\) kg\(^{-1}\), Steinhausen et al., 2008) but comparable with \( \dot{Q}_{\text{rest}} \) in adult chinook salmon at 13°C (29 ml min\(^{-1}\) kg\(^{-1}\), Clark et al., 2008b). \( \dot{Q} \) steadily increased with increasing swimming velocity until the fish fatigued. Both \( \dot{Q}_{\text{max}} \) (100-118 ml min\(^{-1}\) kg\(^{-1}\)) and \( \dot{Q} \) measured at ~75% of \( U_{\text{crit}} \) (80 ml min\(^{-1}\) kg\(^{-1}\)) in the present study exceeded \( \dot{Q} \) for Lower Adams sockeye salmon swimming at ~75% of \( U_{\text{crit}} \) (~68 ml min\(^{-1}\) kg\(^{-1}\)) and Steinhausen
et al., 2008). Davis (1968) only reported $Q$ in ml min$^{-1}$ ($Q_{\text{max}}$ at 20°C = ~165 ml min$^{-1}$) and did not report body mass for the adult sockeye salmon of unknown origin. However, if we assume the sockeye salmon were ~2.3 kg, $Q_{\text{max}}$ is estimated to be ~72 ml min$^{-1}$ kg$^{-1}$, which is substantially lower than the present study. Moreover, $Q_{\text{max}}$ greatly exceeded $Q_{\text{max}}$ values reported for other salmonids from hatchery sources: rainbow trout at 10-18°C (42-69 ml min$^{-1}$ kg$^{-1}$, Brodeur et al., 2001; Claireaux et al., 2005; Kiceniuk and Jones, 1977; Taylor et al., 1996; Thoraresnsen et al., 1996) and immature chinook salmon at 8-10°C (66 ml min$^{-1}$ kg$^{-1}$, Gallaugher et al., 2001). Therefore, the wild, upriver sockeye salmon used here have a greater cardiac capacity compared with the limited dataset available for other salmonid species.

$V_{\text{rest}}$ was similar across populations (0.46-0.57 ml beat$^{-1}$ kg$^{-1}$) and within the range reported for other salmonids (e.g. 0.38-0.63 ml beat$^{-1}$ kg$^{-1}$, in sockeye salmon, chinook salmon and rainbow trout, Claireaux et al., 2006; Clark et al., 2008b; Gallaugher et al., 2001; Kiceniuk and Jones, 1977; Steinhausen et al., 2008). Similar to $Q$, $V_{s}$ steadily increased with increasing swim speed until the fish fatigued. $V_{\text{max}}$ (1.08-1.29 ml beat$^{-1}$ kg$^{-1}$) also exceeded the range reported for hatchery-reared salmonids (0.66-1.04 ml beat$^{-1}$ kg$^{-1}$ in rainbow trout and immature chinook salmon, Claireaux et al., 2006; Gallaugher et al., 2001; Kiceniuk and Jones, 1977).

$f_{\text{Hrest}}$ ranged between 61-70 beats min$^{-1}$ across populations, which is similar to $f_{\text{Hrest}}$ reported for Lower Adams adult sockeye salmon at 15°C (65 beats min$^{-1}$, Steinhausen et al., 2008) but slightly higher than $f_{\text{Hrest}}$ reported in adult chinook salmon at a slightly cooler temperature of 13°C (58 beats min$^{-1}$, Clark et al., 2008b), Stamp River adult sockeye salmon at 13-16°C (49 beats min$^{-1}$, Smith et al., 1967) and Weaver and Harrison sockeye salmon at 11-13°C (43-52 beats min$^{-1}$, Sandblom et al., 2009). Free-swimming adult sockeye salmon equipped with biologgers exhibited a lower $f_{\text{Hroutine}}$ (35-44 beats min$^{-1}$ at 13°C, Clark et al., 2010) and (50-
59 beats min\(^{-1}\) at 10°C, Clark et al., 2009). Differences in \(f_H\) among studies may partially be attributed to the \(Q_{10}\) effect. Also, free-swimming fish were able to swim throughout their environment and measurements were made over several days which would likely result in lower values since \(f_H\) appears to lag behind other cardiorespiratory variables during recovery (see Fig 4.3 and 4.7).

\(f_{H_{\text{max}}}\) ranged between 81-95 beats min\(^{-1}\) across populations, which is similar to \(f_{H_{\text{max}}}\) in tethered Lower Adams sockeye salmon swimming at \(~75\%\) of \(U_{\text{crit}}\) at 15°C (81 beats min\(^{-1}\), Steinhausen et al., 2008) and tethered Stamp River sockeye salmon at 13-16°C (83 beats min\(^{-1}\), Smith et al., 1967). In free-swimming sockeye salmon on the spawning ground or in a raceway at 10-13°C, \(f_{H_{\text{max}}}\) reached \(~75-79\) beats min\(^{-1}\) (Clark et al., 2009, Clark et al., 2010).

Although \(\dot{Q}\) did not differ among populations, \(f_H\) was lower and \(V_s\) was higher at some of the time points in Nechako sockeye salmon relative to the other populations. During the first swim, \(f_H\) steadily increased with swimming speed in all populations. However, \(f_H\) remained elevated at maximal levels throughout the first 45-min recovery, the entire second swim and the duration of second recovery period in Early Stuart, Chilko and Quesnel sockeye salmon. In contrast, \(f_H\) recovered back to resting levels in Nechako sockeye salmon. Notably, this recovery may be partially attributed to the low scope for \(f_H\) in Nechako sockeye salmon since \(f_H\) at the first 45-min recovery period did not differ from \(f_{H_{\text{max}}}\) or \(f_{H_{\text{rest}}}\). As such, Nechako sockeye salmon rely more on \(V_s\) and less on \(f_H\) in order to achieve the same \(\dot{Q}\) as the other three populations. A similar phenomenon was reported by Nelson et al. (1994), who found that despite differences in exercise physiology between two populations of Atlantic cod, swimming performance and aerobic scope were identical.
No significant differences were detected in $U_{\text{crit}}$, $\dot{MO}_2$, $Q$, $V_s$ or $f_H$ between male and female sockeye salmon. Despite significant differences in relative ventricular mass (RVM) between males and females (male RVM was 2-25% higher than female RVM depending on the temperature, see Chapter 6), this did not translate to significant differences in $\dot{Q}_{\text{max}}$ or $V_{\text{smax}}$. Similarly, Gallaugher et al. (2001) found that a 13% increase in RVM in exercise-trained immature chinook salmon did not result in differences in $\dot{Q}$. In contrast, sexually mature male rainbow trout with larger ventricles were demonstrated to have higher $\dot{Q}_{\text{max}}$ and $V_{\text{smax}}$ compared to sexually mature females in an in situ perfused heart preparation (Franklin and Davie, 1992). However, the male trout had ~2-fold larger RVM compared to females, which is a much more dramatic difference than the present study. In addition, Sandblom et al. (2009) reported significantly higher $f_{H\text{rest}}$ in female compared to male sockeye salmon confined in holding tubes, while biologging and telemetry studies on free-swimming fish report that male salmonids spent a greater proportion of time with a high $f_H$ (Altimiras et al., 1996; Clark et al., 2009; Lucas et al., 1993), likely due to increased activity and aggressive behaviour on the spawning grounds. Regardless, no significant differences in $f_H$ were detected between sexes in the present study.

4.3.3 Oxygen Transport and Removal by Tissues

Resting Hct, [Hb] and MCHC were within expected levels (Clark et al., 2009; Sandblom et al., 2009). However, Hct and [Hb] decreased throughout the experiment until Hct reached ~22-24% during the final sample at the 2-h recovery. This was clearly due to haemodilution since fish with both cannulae operational (and thus had twice the amount of blood samples removed) had a significantly lower Hct and [Hb] during the second swim relative to fish with
only one cannula operational. Approximately 20 blood samples or ~14 ml of blood was collected from fish with paired cannulae. Overall mean body mass was 2.3 kg, so assuming a blood volume of 3.5 ml 100 g\(^{-1}\) body mass (Olson, 1992), each sockeye salmon had on average ~80.5 ml of blood. Thus, around 17% of the blood volume was removed and replaced with saline throughout the experiment. Notably, reduced [Hb] due to hemodilution during the second swim also decreased \(C_vO_2\), without affecting \(P_vO_2\). I could not confirm that that \(C_aO_2\) was also reduced due to insufficient numbers of fish with only the arterial cannula functioning. However, since \(T_aO_2\) was identical between swim1 and swim 2, the decreased Hct and [Hb] did not result in a differential perfusion limitation to the tissues between swims and accordingly, \(U_{crit}\) did not differ between swims. Similarly, Gallaugher et al. (1995) previously found that \(U_{crit}\) was not impaired in rainbow trout until Hct declined below 22%.

No significant differences in Hct, [Hb] or MCHC were detected between males and females, which is consistent with a previous study on sockeye salmon (Sandblom et al., 2009). In contrast, Clark et al. (2009) reported significantly higher [Hb] in female compared to male sockeye salmon on the spawning ground.

Resting \(P_aO_2\) and \(C_aO_2\) were within the expected range for salmonids (Clark et al., 2008b; Gallaugher et al., 1992; Gallaugher et al., 2001; Farrell et al., 1998; McKenzie et al., 2004; Steinhausen et al., 2008; Thorarensen et al., 1993). Both \(P_aO_2\) and \(C_aO_2\) declined during swimming by 34 and 23%, respectively. Several studies on salmonids report a similar decrease in \(P_aO_2\) with swimming (Farrell et al., 1998; Gallaugher et al., 1992; Gallaugher et al., 2001; McKenzie et al., 2004; Steinhausen et al., 2008; Thorarensen et al., 1993) but \(C_aO_2\) remained constant during swimming in several studies (Kiceniuk and Jones, 1977; Gallaugher et al., 2001; McKenzie et al., 2004; Thorarensen et al., 1993). Conversely, another report found a 21%
decrease in $C_{aO_2}$ when sockeye salmon swam ~75% of $U_{crit}$ (Steinhausen et al., 2008). Two possibilities may account for the decrease in $C_{aO_2}$ during swimming. A normal problem encountered by salmon migrating upriver is the accumulation of fungus on the gills and body. In addition, the surgery to implant the flowprobe and sinus venosus cannula may have caused some gill damage. As such, the gill surface area for diffusion may have been limited and/or the diffusion distance may have increased, resulting in impaired $C_{aO_2}$. Regardless, $T_{aO_2}$ was exceptionally high in the present study, primarily due to the extremely high $Q$.

As expected, $P_{vO_2}$ and $C_{vO_2}$ also significantly declined during swimming by 57 and 70%, respectively, due to increased oxygen uptake to support the increased oxygen demand at the tissues. A threshold value for $P_{vO_2}$ during swimming has been proposed, which would ensure adequate oxygen supply to the spongy myocardium (Davie and Farrell, 1991; Farrell, 2002; Farrell, 2007; Farrell and Clutterham, 2003). Notably, the minimum $P_{vO_2}$ values measured here were 18-24 torr, which compare well with previous studies which suggest a $P_{vO_2}$ threshold of 15-16 and 29 torr in normoxic rainbow trout at 6-10°C and 13-15°C, respectively (Farrell and Clutterham, 2003).

4.3.4 Repeat Swim Performance

$U_{crit}$ swim tests involve aerobic swimming at the lower swim speeds, followed by a transition to anaerobic metabolism as the fish near $U_{crit}$ (Jones, 1982), as is evident by the accumulation of lactate in the blood (Black, 1955). Furthermore, during exhaustive exercise, the blood becomes acidic (low pH due to $CO_2$ and lactate accumulation), hypoxemic (low $P_{vO_2}$ and $C_{vO_2}$ due to oxygen extraction by the tissues) and hyperkalemic (high [K$^+$] due to K$^+$ loss from
working muscles) (Holk and Lykkeboe, 1998; Kiceniuk and Jones, 1977). As expected, plasma lactate levels increased and $P_{\text{vO}_2}$ and $C_{\text{vO}_2}$ decreased during swimming. However, plasma $K^+$ was highly variable, decreasing during swim 1, and increasing during swim 2. Moreover, plasma $K^+$ was significantly higher in the arterial relative to the venous blood. The difference in $[K+]$ between the arterial and venous blood may be attributed to the effect of pH and haemoglobin-oxygen saturation on $K^+$ movement across red blood cells. Specifically, red blood cells take up $K^+$ when blood pH and haemoglobin-oxygen saturation is low and lose $K^+$ when pH and haemoglobin-oxygen saturation is high (Nielsen and Lykkeboe, 1992b).

The elevated $\dot{\text{MO}_2}$ following an anaerobic swim challenge is termed the excess post oxygen consumption (EPOC). EPOC represents the $\dot{\text{MO}_2}$ cost to restore oxygen stores, high energy phosphates and glycogen and reverse biochemical, ionic and osmotic imbalances (Gaesser and Brooks, 1984; Scarabello et al., 1992). An extended EPOC and prolonged rate of recovery could be detrimental to migrating sockeye salmon since they must migrate upstream in a timely manner. However, as described above, sockeye salmon from all four upriver populations were able to repeat their swim performance after a 45-min recovery and $\dot{\text{MO}_2}$ had returned to resting levels by 2 h after the second swim challenge, which is a comparable timeframe to an earlier study for sockeye salmon (Lee et al., 2003b).

Complete recovery of cardiovascular and metabolic indicators was not required in order for the fish to repeat their $U_{\text{crit}}$ swim performance. The same $Q_{\text{max}}$, $V_{\text{max}}$, $f_{\text{Hmax}}$, and $T_aO_2$ were obtained during swim 2 as compared with swim 1, even though $\dot{\text{MO}_2}$, $\dot{Q}$, $f_H$, and plasma lactate remained significantly elevated and $C_{\text{vO}_2}$ remained significantly depressed at the outset of swim 2.

There are several potential explanations for this. Naïve fish may swim inefficiently or prematurely quit swimming during the first swim, but improve during the second attempt. The
increased scope for $Q$ and $V_s$ during the second swim in Quesnel sockeye salmon may be attributed to these types of behavioural differences between swims.

The consistent repeat swim performance was not due to a larger anaerobic contribution during swim 2 relative to swim 1 since lactate did not accumulate in the plasma. In fact, plasma lactate was highest during the 45-min recovery period following swim 1. A similar finding was reported for sockeye salmon swum twice and even three times (Farrell et al., 1998, Jain et al., 1998). In the present study, plasma lactate levels were always less than 10-13 mmol l$^{-1}$, which is the proposed threshold above which sockeye salmon and rainbow trout cannot repeat their swim performance (Farrell et al., 1998; Jain and Farrell, 2003; Stevens and Black, 1966).

Training effects may physiologically allow fish to improve or maintain swim performance during the second swim, despite incomplete metabolic recovery. For example, faster recovery rates for lactate, creatine phosphate and respiratory gases during a second exhaustive burst swim test were suggested to be training effects (Scarabello et al., 1992). Notably, plasma lactate decreased between steady and burst swimming during swim 2, which suggests that lactate may have been used as a fuel or metabolically cleared during the lower speeds of the second swim. Indeed, light swimming has been demonstrated to accelerate recovery ability in rainbow trout (Milligan et al., 2000).

More efficient swimming during the second swim (lower COT) could allow for repeat swim performance (Farrell et al., 1998). However, this was not the case in the current study since COT did not differ between swims. In fact, COT-$\dot{Q}$ was consistently, though not significantly, higher during the second swim, which may have assisted metabolic recovery. Notably, neither COT nor COT-$\dot{Q}$ displayed the classic U-shaped curve with speed (e.g. Hoyt and Taylor, 1981; Lee et al., 2003c; Prange, 1976; Prange and Schmidt-Nielsen, 1970; Wakeman and Wohlschlag,
1982). Instead, both plateaued at speeds higher than ~1 bl s\(^{-1}\). Thus, upriver sockeye salmon maintained their swimming efficiency across the entire range of swim speeds. While \(\text{COT}_{\text{net}}\) and \(\text{COT-}\dot{Q}_{\text{net}}\) steadily increased with higher swim speeds, both plateaued once the fish transitioned to burst swimming, providing further evidence that high velocity swimming was fuelled by anaerobic metabolism.

An important consideration in this study system is that adult sockeye salmon must ‘multi-task’ during their upriver migration. Namely, while swimming almost continually upstream to their spawning grounds, sockeye salmon must also undergo sexual maturation (grow their gonads and develop secondary sexual characteristics). Thus, sockeye salmon may divert blood away from the gonads and to the muscle when swimming at high speeds, and blood flow distribution may vary across sequential swims. This idea is supported by the observation that gut blood flow in digesting salmon decreases with increased swimming speeds (Thorarensen et al., 1993). These ideas should be tested experimentally.

4.3.5 Summary and General Trends in Oxygen Convection with Swimming

In summary, comprehensive studies that have directly measured all the cardiovascular and oxygen transport variables in the Fick equation for vascular perfusion (\(\dot{V}O_2 = Q \times A-V_{O2}\)) are rare in swimming fish (e.g. Steinhausen et al., 2008). Most studies have estimated \(\dot{Q}\) from the Fick equation (e.g. Kiceniuk and Jones, 1977). All the variables were measured here for four populations of sockeye salmon swimming at \(T_{\text{opt}}\) to test two hypotheses. As hypothesized, all four populations had similar cardiorespiratory and swimming performance, though Nechako sockeye salmon relied more on \(V_s\) than \(f_H\) to achieve the same \(\dot{Q}\). I similarly found support for the
hypothesis that all four populations can repeat their swim performance following a brief 45-min recovery and it appears that sockeye salmon are able to recover while swimming aerobically at intermediate speeds.

In addition, a clear picture emerged for the quantitative changes in the various cardiorespiratory components during swimming. The 5-fold increase in \( \dot{V}O_2 \) came about primarily though a 3-fold increase in \( Q \), which was driven by a 2-fold increase in \( V_s \), \( f_H \) and \( A-V_O2 \) both increased \(~1.5\)-fold. Though \( V_s \) returned to rest by the final 2-h recovery, \( f_H \) remained elevated at maximal levels and as a result, \( Q \) remained elevated by \(~1.5\)-fold. However, \( VO_2 \) did return back to resting levels since \( A-V_O2 \) actually decreased by \(~50\%\) at the 2-h recovery. During swimming, \( T_{aO2} \) met the tissue oxygen demand entirely through an increase in \( Q \). \( T_{vO2} \) maintained a constant oxygen delivery to the spongy myocardium and gills throughout the swim tests and recovery since increases in \( Q \) offset decreases in \( C_{vO2} \).
Figure 4.1. Oxygen consumption (ṀO₂) with swimming speed over two consecutive swim challenges in three populations of sockeye salmon. There were no significant differences among populations or between sexes. Shaded areas indicate the recovery periods, starting with the fatigue value collected immediately following the U_{crit} test.
Figure 4.2. (A) Cost of transport (COT), (B) net cost of transport (COT$_{\text{net}}$), (C) cardiovascular cost of transport (COT-$\dot{Q}$) and (D) net cardiovascular cost of transport (COT-$\dot{Q}_{\text{net}}$) over two consecutive swim challenges. Since there were no significant differences in $\dot{M}$O$_2$ or $\dot{Q}$ among populations, all populations were combined. Dashed line indicates typical swim speed at which the fish transitioned from steady swimming to burst swimming. Mean ± SEM are presented. There were no significant differences between swim 1 and swim 2 in COT or COT$_{\text{net}}$. Significant differences between swims in COT-$\dot{Q}$ and COT-$\dot{Q}_{\text{net}}$ are indicated by an asterisk.
Figure 4.3. (A) Cardiac output, (B) stroke volume and (C) heart rate with swimming speed over two consecutive swim challenges in four populations of sockeye salmon. There were no significant differences in cardiac output or stroke volume among populations or between sexes. Heart rate was significantly lower in Nechako sockeye salmon compared to some of the other populations during the first three speeds of the second swim, see text for details.
Figure 4.4. Arterial and venous (A) partial pressure of oxygen ($P_{O2}$), (B) oxygen content ($C_{O2}$) and (C) plasma lactate levels in Early Stuart, Chilko and Quesnel populations combined, over two consecutive swim challenges. Mean ± SEM are presented, there were no significant differences between sexes. Significant differences from rest are indicated by an asterisk (*), significant differences between swims are indicated by the symbol ($\psi$). Lactate did not significantly differ between arterial and venous blood samples. $P_{O2}$ and $C_{O2}$ significantly differed between arterial and venous blood samples at every time point, except $C_{O2}$ did not differ at 2 h recovery.
Figure 4.5. Venous (A) haemoglobin, (B) hematocrit, (C) partial pressure of oxygen and (D) oxygen content in fish with both the arterial and venous cannulae operational (2 cannulae, n = 9) and fish with only the venous cannula functioning (1 cannula, n = 11), over two consecutive swim challenges. Mean ± SEM are presented, significant differences between fish with 2 cannulae and those with 1 cannula working are indicated by an asterisk (*). Note that there were insufficient blood samples during burst swimming in the second swim to compare between groups.
Figure 4.6. (A) Arterial oxygen transport ($T_{aO2}$), (B) venous oxygen transport ($T_{vO2}$) and (C) tissue oxygen extraction ($A-V_{O2}$) in Early Stuart, Chilko and Quesnel populations combined, over two consecutive swim challenges. Measurements were made at rest, during steady swimming (steady), during burst swimming (burst) immediately after the fish quit swimming (fatigue), 45 min after the fatigue (45 min) and 2 h after the conclusion of the second swim test (2 h). Mean ± SEM are presented, significant differences from rest are indicated by an asterisk (*), there were no significant differences between swim 1 and swim 2 or between sexes.
Figure 4.7. Fold changes from rest for (A) oxygen consumption ($\text{VO}_2 = \dot{Q} \times (A-V\text{O}_2)$), (B) cardiac output ($\dot{Q} = f_H \times V_s$), (C) tissue oxygen extraction ($A-V\text{O}_2 = \text{CaO}_2 - \text{CvO}_2$), (D) arterial oxygen delivery ($T_{aO2} = \dot{Q} \times \text{CaO}_2$) and (E) venous oxygen transport ($T_{vO2} = \dot{Q} \times \text{CvO}_2$). $f_H$ = heart rate, $V_s$ = stroke volume, $\text{CaO}_2$ = arterial oxygen content, $\text{CvO}_2$ = venous oxygen content. Only fish from Early Stuart, Chilko and Quesnel with both cannulae working were included in this analysis.
Table 4.1. Measurements of critical swimming speed ($U_{crit}$) and the recovery ratio (RR) in four populations of sockeye salmon at their $T_{opt}$. Mean ± SEM are presented, there were no significant differences in $U_{crit}$ between sexes, among populations or between swim 1 and swim 2 ($p > 0.05$).

<table>
<thead>
<tr>
<th>Population</th>
<th>n</th>
<th>$U_{crit}$ (m s$^{-1}$)</th>
<th>$U_{crit}$ (cm s$^{-1}$)</th>
<th>RR</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>$U_{crit}$ 1</td>
<td>$U_{crit}$ 2</td>
<td>$U_{crit}$ 1</td>
</tr>
<tr>
<td>Early Stuart</td>
<td>8-9</td>
<td>2.02 ± 0.06</td>
<td>1.91 ± 0.06</td>
<td>122.1 ± 4.1</td>
</tr>
<tr>
<td>Chilko</td>
<td>9-13</td>
<td>1.99 ± 0.08</td>
<td>1.95 ± 0.09</td>
<td>117.8 ± 3.8</td>
</tr>
<tr>
<td>Quesnel</td>
<td>6</td>
<td>2.02 ± 0.11</td>
<td>1.98 ± 0.04</td>
<td>121.1 ± 6.3</td>
</tr>
<tr>
<td>Nechako</td>
<td>3-4</td>
<td>1.94 ± 0.11</td>
<td>1.96 ± 0.06</td>
<td>111.0 ± 5.6</td>
</tr>
</tbody>
</table>
Table 4.2. Recovery measurements for oxygen consumption ($\dot{M}O_2$), cardiac output ($Q$), heart rate ($f_H$) and stroke volume ($V_s$) in four sockeye salmon populations after two consecutive $U_{crit}$ swim challenges. Measurements were made at rest, immediately after the fish quit swimming (fatigue), 45 min after fatigue (45-min recovery) and 2 h after the conclusion of the second swim test (2-h recovery). Mean ± SEM are presented. There were no significant differences between sexes. Significant differences from rest are indicated by an asterisk (*), significant differences between swim 1 and swim 2 are indicated by a dagger (‡) and significant differences among populations are indicated by differing letters.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Rest</th>
<th>Fatigue 1</th>
<th>Fatigue 2</th>
<th>45-min recovery 1</th>
<th>45-min recovery 2</th>
<th>2-h recovery 2</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\dot{M}O_2$ (mg O$_2$ kg$^{-1}$ min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early Stuart</td>
<td>5-8</td>
<td>3.2 ± 0.2</td>
<td>10.0 ± 0.8</td>
<td>8.9 ± 0.8</td>
<td>6.6 ± 0.3*</td>
<td>5.4 ± 0.8*</td>
<td>3.9 ± 0.4</td>
</tr>
<tr>
<td>Chilko</td>
<td>8-13</td>
<td>2.9 ± 0.2</td>
<td>10.3 ± 0.9</td>
<td>9.6 ± 1.1</td>
<td>5.7 ± 0.7*</td>
<td>5.9 ± 0.6*</td>
<td>3.3 ± 0.4</td>
</tr>
<tr>
<td>Quesnel</td>
<td>5-6</td>
<td>2.7 ± 0.2</td>
<td>8.7 ± 1.0</td>
<td>9.3 ± 1.2</td>
<td>5.4 ± 1.1*</td>
<td>3.6 ± 0.2*</td>
<td>3.1 ± 0.2</td>
</tr>
<tr>
<td>Nechako</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$\dot{Q}$ (ml min$^{-1}$ kg$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early Stuart</td>
<td>7-9</td>
<td>34.8 ± 2.7</td>
<td>72.0 ± 4.3</td>
<td>79.9 ± 5.8</td>
<td>45.3 ± 2.5*</td>
<td>49.7 ± 3.7*</td>
<td>47.9 ± 2.7*</td>
</tr>
<tr>
<td>Chilko</td>
<td>9-13</td>
<td>34.8 ± 2.9</td>
<td>77.0 ± 6.4</td>
<td>80.0 ± 8.2</td>
<td>49.8 ± 4.9*</td>
<td>56.2 ± 5.9*</td>
<td>53.6 ± 6.2*</td>
</tr>
<tr>
<td>Quesnel</td>
<td>5-6</td>
<td>34.7 ± 3.9</td>
<td>67.6 ± 8.1</td>
<td>89.2 ± 9.0</td>
<td>49.6 ± 6.6*</td>
<td>55.4 ± 4.4*</td>
<td>51.4 ± 6.5*</td>
</tr>
<tr>
<td>Nechako</td>
<td>3-4</td>
<td>29.9 ± 1.7</td>
<td>77.4 ± 8.5</td>
<td>75.5 ± 10.2</td>
<td>44.9 ± 1.6*</td>
<td>51.8 ± 1.9*</td>
<td>40.5 ± 3.8</td>
</tr>
<tr>
<td>$V_s$ (ml beat$^{-1}$ kg$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early Stuart</td>
<td>7-9</td>
<td>0.49 ± 0.03</td>
<td>0.83 ± 0.05*</td>
<td>0.92 ± 0.07*</td>
<td>0.46 ± 0.02</td>
<td>0.55 ± 0.04</td>
<td>0.59 ± 0.05</td>
</tr>
<tr>
<td>Chilko</td>
<td>9-13</td>
<td>0.53 ± 0.05</td>
<td>0.91 ± 0.06*</td>
<td>0.93 ± 0.08*</td>
<td>0.56 ± 0.05</td>
<td>0.60 ± 0.06</td>
<td>0.62 ± 0.07</td>
</tr>
<tr>
<td>Quesnel</td>
<td>5-6</td>
<td>0.57 ± 0.06</td>
<td>0.76 ± 0.08</td>
<td>0.96 ± 0.04*</td>
<td>0.53 ± 0.05</td>
<td>0.57 ± 0.03</td>
<td>0.54 ± 0.04</td>
</tr>
<tr>
<td>Nechako</td>
<td>3-4</td>
<td>0.46 ± 0.02</td>
<td>1.09 ± 0.12*</td>
<td>1.13 ± 0.12*</td>
<td>0.57 ± 0.03</td>
<td>0.64 ± 0.02</td>
<td>0.54 ± 0.02</td>
</tr>
<tr>
<td>$f_H$ (beats min$^{-1}$)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early Stuart</td>
<td>7-9</td>
<td>70.1 ± 2.3</td>
<td>87.6 ± 3.5*</td>
<td>88.0 ± 5.0$^{ab}$</td>
<td>99.6 ± 3.6*</td>
<td>90.3 ± 3.7*</td>
<td>82.8 ± 2.8*</td>
</tr>
<tr>
<td>Chilko</td>
<td>9-13</td>
<td>67.3 ± 2.7</td>
<td>84.0 ± 2.7*</td>
<td>85.7 ± 2.0$^{ab}$</td>
<td>89.2 ± 2.3*</td>
<td>94.0 ± 3.2*</td>
<td>86.3 ± 3.1*</td>
</tr>
<tr>
<td>Quesnel</td>
<td>5-6</td>
<td>60.9 ± 4.7</td>
<td>88.5 ± 7.4*</td>
<td>92.6 ± 6.2$^{ab}$</td>
<td>93.1 ± 8.5*</td>
<td>97.7 ± 7.2*</td>
<td>95.1 ± 7.7*</td>
</tr>
<tr>
<td>Nechako</td>
<td>3-4</td>
<td>65.8 ± 2.6</td>
<td>71.2 ± 0.7</td>
<td>66.3 ± 3.8$^b$</td>
<td>78.7 ± 2.3</td>
<td>80.4 ± 1.7</td>
<td>74.8 ± 4.2</td>
</tr>
</tbody>
</table>
Table 4.3. Maximum measurements for cardiac output (Q), heart rate (fH) and stroke volume (Vs) in four sockeye salmon populations taken over two Ucrit swim challenges. Scope is the difference between maximum and resting values for each individual fish. Mean ± SEM are presented. There were no significant differences between sexes. Significant differences between swim 1 and swim 2 are indicated by a dagger (‡) and significant differences among populations are indicated by differing letters.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Max 1</th>
<th>Max 2</th>
<th>Scope 1</th>
<th>Scope 2</th>
</tr>
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<tr>
<td>Q (ml min⁻¹ kg⁻¹)</td>
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<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early Stuart</td>
<td>8-9</td>
<td>100.3 ± 5.2</td>
<td>104.1 ± 6.4</td>
<td>65.5 ± 4.7</td>
<td>68.2 ± 5.3</td>
</tr>
<tr>
<td>Chilko</td>
<td>9-13</td>
<td>105.0 ± 4.9</td>
<td>103.2 ± 9.3</td>
<td>70.2 ± 3.2</td>
<td>68.0 ± 6.5</td>
</tr>
<tr>
<td>Quesnel</td>
<td>5-6</td>
<td>101.9 ± 9.2</td>
<td>117.7 ± 12.1‡</td>
<td>67.2 ± 6.6</td>
<td>83.6 ± 7.6‡</td>
</tr>
<tr>
<td>Nechako</td>
<td>3-4</td>
<td>107.3 ± 6.7</td>
<td>104.5 ± 1.9</td>
<td>77.4 ± 7.0</td>
<td>74.1 ± 1.2</td>
</tr>
<tr>
<td>Vs (ml beat⁻¹ kg⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early Stuart</td>
<td>8-9</td>
<td>1.08 ± 0.05</td>
<td>1.10 ± 0.06</td>
<td>0.58 ± 0.04ab</td>
<td>0.60 ± 0.04</td>
</tr>
<tr>
<td>Chilko</td>
<td>9-13</td>
<td>1.11 ± 0.05</td>
<td>1.12 ± 0.09</td>
<td>0.59 ± 0.04ab</td>
<td>0.57 ± 0.07</td>
</tr>
<tr>
<td>Quesnel</td>
<td>5-6</td>
<td>1.09 ± 0.10</td>
<td>1.28 ± 0.11</td>
<td>0.52 ± 0.07a</td>
<td>0.69 ± 0.04‡</td>
</tr>
<tr>
<td>Nechako</td>
<td>3-4</td>
<td>1.25 ± 0.07</td>
<td>1.29 ± 0.07</td>
<td>0.80 ± 0.07b</td>
<td>0.82 ± 0.06</td>
</tr>
<tr>
<td>fH (beats min⁻¹)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Early Stuart</td>
<td>8-9</td>
<td>93.1 ± 2.2</td>
<td>94.6 ± 2.8</td>
<td>23.0 ± 3.7</td>
<td>23.5 ± 3.3</td>
</tr>
<tr>
<td>Chilko</td>
<td>9-13</td>
<td>94.4 ± 1.9</td>
<td>92.0 ± 3.2</td>
<td>27.1 ± 3.5</td>
<td>27.4 ± 5.4</td>
</tr>
<tr>
<td>Quesnel</td>
<td>5-6</td>
<td>94.0 ± 3.6</td>
<td>91.3 ± 3.6</td>
<td>33.1 ± 3.6</td>
<td>34.6 ± 2.2</td>
</tr>
<tr>
<td>Nechako</td>
<td>3-4</td>
<td>85.9 ± 3.9</td>
<td>81.3 ± 3.0</td>
<td>20.1 ± 4.3</td>
<td>17.5 ± 5.0</td>
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</table>
Table 4.4. Haematological variables from Early Stuart, Chilko and Quesnel populations combined over two consecutive swim challenges. Arterial and venous blood samples were taken at rest, during steady state swimming (steady) and burst-and-coast swimming (burst), immediately after the fish quit swimming (fatigue), 45 min after the fatigue (45 min) and 2 h after the conclusion of the second swim test (2 h rec). Haemoglobin concentration (Hb), hematocrit (Hct) and mean cell haemoglobin concentration (MCHC) are indicated. Mean ± SEM are presented. There were no significant differences between sexes. Significant differences from rest are indicated by an asterisk (*), significant differences between swim 1 and swim 2 are indicated by a dagger (‡) and significant differences between arterial and venous blood are indicated by differing letters.

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>Hb (g l⁻¹)</th>
<th>Hct (%)</th>
<th>MCHC (g l⁻¹)</th>
<th>Glucose (mmol l⁻¹)</th>
<th>Chloride (mmol l⁻¹)</th>
<th>Sodium (mmol l⁻¹)</th>
<th>Potassium (mmol l⁻¹)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>arterial</td>
<td>12</td>
<td>92.4 ± 4.6</td>
<td>31.0 ± 1.7</td>
<td>300.5 ± 6.6</td>
<td>5.6 ± 0.5</td>
<td>127.5 ± 0.9</td>
<td>140.1 ± 1.7</td>
</tr>
<tr>
<td></td>
<td>venous</td>
<td>19-20</td>
<td>95.9 ± 3.5</td>
<td>33.0 ± 1.5</td>
<td>293.7 ± 6.0</td>
<td>5.3 ± 0.4</td>
<td>128.2 ± 1.5</td>
<td>142.4 ± 1.7</td>
</tr>
<tr>
<td>steady 1</td>
<td>arterial</td>
<td>13-14</td>
<td>90.3 ± 4.2</td>
<td>30.4 ± 1.6</td>
<td>299.4 ± 6.8</td>
<td>5.1 ± 0.5</td>
<td>129.9 ± 1.4</td>
<td>143.8 ± 1.6</td>
</tr>
<tr>
<td></td>
<td>venous</td>
<td>18-20</td>
<td>89.4 ± 2.9</td>
<td>30.7 ± 1.0</td>
<td>292.2 ± 5.9</td>
<td>5.1 ± 0.4</td>
<td>130.3 ± 1.3</td>
<td>146.7 ± 1.7</td>
</tr>
<tr>
<td>steady 2</td>
<td>arterial</td>
<td>12</td>
<td>81.3 ± 4.7</td>
<td>26.1 ± 1.9</td>
<td>315.7 ± 9.4a</td>
<td>5.5 ± 0.5</td>
<td>127.3 ± 1.8</td>
<td>140.5 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>venous</td>
<td>15-16</td>
<td>86.0 ± 3.7</td>
<td>30.1 ± 0.8</td>
<td>291.8 ± 7.7b</td>
<td>5.6 ± 0.4</td>
<td>127.4 ± 1.5</td>
<td>144.9 ± 1.9</td>
</tr>
<tr>
<td>burst 1</td>
<td>arterial</td>
<td>6</td>
<td>91.8 ± 5.4</td>
<td>33.9 ± 2.5†</td>
<td>273.1 ± 8.1†a</td>
<td>6.6 ± 0.5</td>
<td>130.2 ± 1.3</td>
<td>145.9 ± 2.1</td>
</tr>
<tr>
<td></td>
<td>venous</td>
<td>7</td>
<td>90.4 ± 3.3</td>
<td>37.9 ± 1.7†</td>
<td>239.4 ± 3.2†b</td>
<td>6.0 ± 0.7</td>
<td>126.8 ± 1.8</td>
<td>142.1 ± 2.4</td>
</tr>
<tr>
<td>burst 2</td>
<td>arterial</td>
<td>3</td>
<td>86.5 ± 12.9</td>
<td>26.5 ± 4.7†</td>
<td>329.1 ± 9.7†a</td>
<td>6.5 ± 0.9</td>
<td>129.9 ± 4.9</td>
<td>139.8 ± 4.2</td>
</tr>
<tr>
<td></td>
<td>venous</td>
<td>8</td>
<td>84.8 ± 4.4*</td>
<td>29.4 ± 1.8†</td>
<td>290.7 ± 6.0‡b</td>
<td>5.1 ± 0.4</td>
<td>126.1 ± 2.4</td>
<td>141.3 ± 2.5</td>
</tr>
<tr>
<td>fatigue 1</td>
<td>arterial</td>
<td>11</td>
<td>87.3 ± 3.0†</td>
<td>31.1 ± 1.4‡a</td>
<td>282.3 ± 4.9b</td>
<td>6.2 ± 0.6</td>
<td>133.3 ± 1.2a</td>
<td>152.9 ± 2.0†</td>
</tr>
<tr>
<td></td>
<td>venous</td>
<td>15-16</td>
<td>93.3 ± 2.7</td>
<td>37.0 ± 1.3b</td>
<td>253.8 ± 5.5ab</td>
<td>6.3 ± 0.4*</td>
<td>131.7 ± 1.3</td>
<td>152.1 ± 2.1*</td>
</tr>
<tr>
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<td>arterial</td>
<td>11</td>
<td>79.3 ± 5.0†</td>
<td>26.8 ± 2.1†</td>
<td>301.0 ± 7.7a</td>
<td>6.4 ± 0.7</td>
<td>130.0 ± 2.0</td>
<td>146.1 ± 2.2</td>
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<td>89.7 ± 5.5†</td>
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<td>126.5 ± 1.2</td>
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Table 4.5. Haematological variables from Early Stuart, Chilko and Quesnel populations combined over two consecutive swim challenges. Only fish with paired arterial and venous cannulae were included. Haemoglobin concentration (Hb), hematocrit (Hct) and mean cell haemoglobin concentration (MCHC) are indicated. Mean ± SEM are presented. Significant differences between arterial and venous blood are indicated by differing letters.

<table>
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<th></th>
<th>n</th>
<th>Hb (g l⁻¹)</th>
<th>Hct (%)</th>
<th>MCHC (g l⁻¹)</th>
<th>Potassium (mmol l⁻¹)</th>
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<td>297.7 ± 8.1</td>
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<td>281.3 ± 12.7&lt;sup&gt;b&lt;/sup&gt;</td>
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CHAPTER 5: CARDIORESPIRATORY COLLAPSE AT HIGH TEMPERATURE IN SOCKEYE SALMON

5.1 Introduction

Chapter 3 provided convincing evidence for a decline in aerobic scope and swim performance outside $T_{\text{opt}}$ in every sockeye salmon population examined and confirmed the previously held notion that aerobic and cardiac scopes are closely linked (e.g. Brett, 1971; Farrell et al., 2009). The purpose of this chapter is to examine the mechanism of the decline in aerobic scope above $T_{\text{opt}}$ in sockeye salmon.

Temperature has been coined the “ecological master factor” because of its role in biochemistry, physiology, behaviour and ecology (Fry, 1971). As previously discussed, all fish have an optimum temperature ($T_{\text{opt}}$) for performance, outside of which whole animal performance declines until eventually death occurs. The oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis attributes the decline in aerobic scope above an animal’s optimum temperature to capacity limitations of the organ systems that deliver oxygen to the tissues (Pörtner, 2001; Pörtner and Knust, 2007). Any one of the steps in the oxygen cascade (from the environment to the mitochondria) could become problematic outside the $T_{\text{opt}}$ window. Among these steps include the capacity for oxygen delivery to the gills, oxygen diffusion across the gills, oxygen transport via the blood, oxygen extraction by the tissues, and oxygen use by the mitochondria (Weibel, 1984). Thus, a limitation could occur at the organ level of the heart, the gills, or the muscle (Brett, 1971; Farrell, 1997; Farrell, 2002; Farrell, 2007; Farrell, 2009; Farrell
et al., 2009; Heath and Hughes, 1973; Pörtner, 2002; Steinhausen et al., 2008; Taylor et al., 1997), though evidence supporting a limitation at any single site is incomplete.

I used the OCLTT hypothesis as a framework to examine cardiorespiratory collapse at high temperature in sockeye salmon. I hypothesized that the limitation on aerobic performance above \( T_{\text{opt}} \) is initiated by an oxygen limitation at the level of the heart.

It is possible to examine most of the critical steps in the oxygen cascade by simultaneously measuring \( \dot{M}O_2 \), \( Q \), and oxygen status [partial pressure (\( P_{O2} \)) and content (\( C_{O2} \))] in arterial and venous blood as a function of increasing temperature. For example, if there was a limitation in delivering oxygen to the gill or oxygen diffusion at the gill, I would expect \( P_{aO2} \) and \( C_{aO2} \) to decrease above \( T_{\text{opt}} \). Alternatively, a limitation at the level of the heart would be apparent by a plateau or decrease in \( Q_{\text{max}} \) at temperatures above \( T_{\text{opt}} \). Finally, if sufficient blood was delivered to the working muscles, but a limitation in oxygen diffusion to mitochondria was present, \( P_{vO2} \) would remain constant above \( T_{\text{opt}} \).

Most of the studies to date examining temperature effects on aerobic scope in fish have not directly measured all the required variables outlined above (e.g. Brett, 1971; Fry, 1947; Taylor et al., 1993). Some variables have been measured in resting fish acutely exposed to increasing temperatures (e.g. Clark et al., 2008b; Gollock et al., 2006; Heath and Hughes, 1973; Sartoris et al., 2003), however, only Steinhausen et al. (2008) has directly measured all these variables in fish swimming near \( \dot{M}O_2_{\text{max}} \). Therefore, this is the first comprehensive study to simultaneously and directly measure all the necessary variables in fish swimming at \( U_{\text{crit}} \) in order to address these mechanistic questions.

To maximize statistical power, my approach was to pool data for Early Stuart, Chilko and Quesnel sockeye salmon since they did not differ in maximum cardiorespiratory performance.
(Chapter 4) and had a similar $T_{\text{opt}}$ (~17°C, Chapter 3). I pooled the data into four temperature groups based on aerobic scope (Fig 5.1). The $T_{\text{opt}}$ grouping ($n = 33$) combined data for sockeye salmon that attained 90-100% of population-specific maximum aerobic scope. The temperature range for the $T_{\text{opt}}$ grouping was 15-20°C for all three populations. The $T_{\text{min}50-90}$ grouping ($n = 8$) included sockeye salmon at temperatures below $T_{\text{opt}}$ attaining only 50-90% of maximum aerobic scope. The $T_{\text{min}50-90}$ grouping corresponded to 12°C and 9-10°C, respectively, for Early Stuart and Chilko populations and no Quesnel sockeye salmon were included. The $T_{\text{max}50-90}$ grouping ($n = 11$), included fish swum at temperatures above $T_{\text{opt}}$, and attaining 50-90% of maximum aerobic scope. This grouping typically corresponded to 22-23°C for Early Stuart and Quesnel sockeye salmon and 24-25°C for Chilko sockeye salmon. The final $T_{\text{max}0-50}$ grouping ($n = 8$) included fish above $T_{\text{opt}}$ whose aerobic scope was only 0-50% of maximum. This corresponded to 23-26°C for Early Stuart and Quesnel sockeye salmon and 25-26°C for Chilko sockeye salmon. While many fish were swum twice (e.g. all fish in the $T_{\text{opt}}$ grouping and several fish in the $T_{\text{min}50-90}$ and $T_{\text{max}50-90}$ groupings), only the first swim was compared across the four groupings. This approach avoided the potentially confounding effect of incomplete recovery from the first swim. Detailed materials and methods are found in Chapter 2 (sections 2.2-2.7 and 2.10).
5.2 Results

5.2.1 Cardiorespiratory and Swimming Performance

Resting, Swimming, Maximum & Scope

The cardiorespiratory response of the four temperature groupings with swimming are shown in Fig 5.2. The corresponding rest, maximum and scope values for each temperature grouping are shown in Fig 5.3.

As defined, aerobic scope was highest at $T_{opt}$. Also, aerobic scope did not significantly differ between $T_{min50-90}$ and $T_{max50-90}$, and was lowest at $T_{max0-50}$ (Fig 5.3). Therefore, the four temperature groupings created the equivalent of a Fry aerobic scope curve.

The response for aerobic scope reflected the different responses of $\dot{M}O_2^{rest}$ and $\dot{M}O_2^{max}$ to temperature. As expected, $\dot{M}O_2^{rest}$ increased significantly from the lowest to the highest temperature grouping (Fig 5.3). While swimming significantly increased $\dot{M}O_2$ for the $T_{min50-90}$, $T_{opt}$ and $T_{max50-90}$ groupings, it did not for the $T_{max0-50}$ grouping (Fig 5.2). Furthermore, $\dot{M}O_2^{max}$ increased significantly between $T_{min50-90}$ and $T_{opt}$, did not differ between $T_{opt}$ and $T_{max50-90}$, and decreased significantly at $T_{max0-50}$ (Fig 5.3).

$\dot{Q}_{rest}$ and $\dot{Q}_{max}$ varied with temperature with similar patterns as $\dot{M}O_2^{rest}$ and $\dot{M}O_2^{max}$, respectively (Fig 5.3). $\dot{Q}$ significantly increased with swimming for the $T_{min50-90}$, $T_{opt}$ and $T_{max50-90}$ groupings, but not the $T_{max0-50}$ group (Fig 5.2). Cardiac scope at $T_{max0-50}$ was significantly lower compared to the other three temperature groupings (Fig 5.3).
The relative contribution of $f_H$ and $V_s$ to $Q$ varied with temperature and with swimming. $f_{Hrest}$ significantly increased with each temperature grouping (Fig 5.3). In contrast, $V_{srest}$ did not change substantially among temperature groups, but was significantly lower at $T_{max0-50}$ compared with $T_{opt}$ (Fig 5.3).

At $T_{opt}$, swimming increased both $V_s$ and $f_H$ (Fig 5.2). Both $T_{min50-90}$ and $T_{max50-90}$ groupings only increased $V_s$ with swimming, $f_H$ did not change significantly from rest at any swimming speed (Fig 5.2). At $T_{max0-50}$, $f_H$ actually decreased during swimming and $V_s$ did not change from rest (Fig 5.2). Notably, at the lowest swim speeds, $V_s$ was similar across temperature groupings. However, differences became apparent at higher swim velocities: $V_s$ was highest in the $T_{min50-90}$ grouping and decreased with increasing temperature groups (Fig 5.2).

$f_{Hmax}$ significantly increased with temperature until $T_{max50-90}$ and plateaued between the two warmest groups (Fig 5.3). Scope for $f_H$ was highest at $T_{opt}$, approached zero for $T_{max50-90}$ and was actually negative for $T_{max0-50}$. $V_{smax}$ significantly decreased above $T_{min50-90}$. As a result, scope for $V_s$ was maintained between $T_{min50-90}$ and $T_{max50-90}$ but decreased significantly at $T_{max0-50}$.

For comparison among these cardiorespiratory variables with temperature, scope is presented as a percentage of its highest value (Fig 5.4). Scope was highest at $T_{opt}$ for $\dot{MO}_2$, $Q$ and $f_H$ and highest at $T_{min50-90}$ for $V_s$ (Fig 5.4). While scope for $\dot{MO}_2$, $Q$ and $V_s$ decreased by 13-25% of maximum at $T_{max50-90}$, scope for $f_H$ plummeted by 80% of maximum at $T_{max50-90}$ and became almost -40% of maximum for $f_H$ at $T_{max0-50}$. At $T_{max0-50}$, scope for $\dot{MO}_2$, $Q$ and $V_s$ declined to 16-20% of maximum.

No cardiac disrhythmis or deaths accompanied swimming at either $T_{min50-90}$ or $T_{opt}$. In contrast, every fish swum at $T_{max0-50}$ exhibited an irregular heart rate immediately after failing the swim test. In addition, 57% of these fish exhibited cardiac disrhythmis during swimming,
shortly before fatigue (Fig 5.5). Despite decreasing the temperature immediately following fatigue, 29% of the $T_{\text{max0-50}}$ fish died, even though they had swum at 1.1-1.5 bl s$^{-1}$ before reaching fatigue. While 27% of $T_{\text{max50-90}}$ fish exhibited cardiac disrhythmias during swimming or fatigue, none died.

**Swimming Performance**

$T_{\text{max0-50}}$ fish attained a lower maximum swim speed compared to the other three groupings (Fig 5.2, 5.6), which is consistent with $\dot{M}O_2$ and $\dot{Q}$ not changing significantly during the swim test of this group.

Cost of transport (COT) at the slowest swim speed (0.4 bl s$^{-1}$ = “rest”) was ~ 2-fold higher in the $T_{\text{max50-90}}$ and $T_{\text{max0-50}}$ groups relative to the $T_{\text{opt}}$ and $T_{\text{min50-90}}$ groups (Fig 5.6). COT decreased with increasing swimming speed and was maintained ~0.12-0.26 mg O$_2$ kg$^{-1}$ m$^{-1}$ across all groups. The $T_{\text{max50-90}}$ and $T_{\text{max0-50}}$ groups tended to have a higher COT compared to the $T_{\text{opt}}$ and $T_{\text{min50-90}}$ groups across all speeds.

Similarly, COT-$\dot{Q}$ was significantly lower at $T_{\text{min50-90}}$ compared to the $T_{\text{max50-90}}$ and $T_{\text{max0-50}}$ groups at the slowest speed (Fig 5.6). COT-$\dot{Q}$ decreased with increasing swimming speeds until ~0.87 bl s$^{-1}$, after which it was maintained at around 0.7-1.9 ml kg$^{-1}$ m$^{-1}$ in $T_{\text{min50-90}}$, $T_{\text{opt}}$ and $T_{\text{max50-90}}$. In contrast, COT-$\dot{Q}$ declined steadily in $T_{\text{max0-50}}$ until the fish quit swimming. Again, warmer temperature groups tended to have a higher COT-$\dot{Q}$ relative to the cooler groups across all speeds. The opposite temperature pattern was observed in COT$_{\text{net}}$ and COT-$\dot{Q}_{\text{net}}$, both of which tended to be higher in colder groups.
5.2.2 Oxygen Transport and Removal by Tissues

Resting $P_aO_2$ and $C_aO_2$ were not significantly affected by an acute increase in temperature from 12°C (the starting temperature) to the test temperature (22-26°C) in either the $T_{max50-90}$ or $T_{max0-50}$ groupings (Table 5.1). The tendency for $P_aO_2$ to increase with temperature can likely be attributed to a decreased affinity for haemoglobin (right-shift in the oxyhaemoglobin dissociation curve).

$P_aO_2$ and $C_aO_2$ did not significantly differ across temperature groups at any of the swimming speeds (Table 5.2). There was an overall trend for $P_aO_2$ and $C_aO_2$ to decrease with swimming relative to rest (Table 5.2, Fig 5.7).

There were no significant differences in [Hb], Hct, or MCHC across temperature groups or with swimming, except that MCHC was significantly lower at fatigue relative to rest in the $T_{max50-90}$ grouping (Table 5.3). As a result, any changes in $C_aO_2$ or $C_vO_2$ reflected changes in Hb saturation. In fact, when $C_aO_2$ was divided by [Hb], there were no significant differences across temperatures or with swim speed (data not shown).

$T_aO_2$ was significantly lower during burst swimming and fatigue for the $T_{max0-50}$ grouping when compared with $T_{opt}$ (Fig 5.8, Table 5.2). While the $T_{min50-90}$, $T_{opt}$ and $T_{max50-90}$ groupings increased $T_aO_2$ by 294%, 153% and 80%, respectively, during burst swimming compared with rest, at $T_{max0-50}$ fish were unable to significantly increase $T_aO_2$ from resting values.

Unlike arterial blood, both $P_vO_2$ and $C_vO_2$ varied significantly among temperature groups (Table 5.2). Resting $P_vO_2$ declined above $T_{opt}$. Resting $P_vO_2$ was only 10 torr at $T_{max0-50}$, or 25% of the $T_{opt}$ value. As a result, resting $C_vO_2$ at $T_{max0-50}$ was only 16-20% of the $C_vO_2$ for the other three temperature groupings.
At fatigue, $C_{\text{VO2}}$ and $P_{\text{VO2}}$ were 114% and 77%, respectively, lower in the $T_{\text{max50-90}}$ group compared to $T_{\text{opt}}$. Limited blood samples at $T_{\text{max0-50}}$ prevented analysis for swimming and fatigue (Table 5.2), but these limited numbers showed a decreasing trend for both resting and fatigue $P_{\text{VO2}}$ and $C_{\text{VO2}}$ at temperatures above $T_{\text{opt}}$ (Fig 5.7).

The increase in oxygen uptake by swimming muscles was reflected in the significant decline of both $P_{\text{VO2}}$ and $C_{\text{VO2}}$ with swimming (Table 5.2). Analysis of A-V$_{\text{O2}}$ was restricted to paired arterial and venous samples (Table 5.2). With this caveat, the trend of increasing A-V$_{\text{O2}}$ with swimming and at warmer temperatures did not reach statistical significance, likely due to low statistical power (Table 5.2).

At rest, $T_{\text{VO2}}$ was over 4-fold lower at $T_{\text{max0-50}}$ compared to $T_{\text{opt}}$ (Table 5.2). In addition, $T_{\text{VO2}}$ was maintained at resting levels throughout the swim test at $T_{\text{opt}}$, while it significantly declined at burst and fatigue in $T_{\text{max50-90}}$ fish (Fig 5.8).

5.2.3 Other Blood Variables

Plasma glucose did not vary significantly with temperature or swimming except for the $T_{\text{max0-50}}$ grouping where plasma glucose significantly declined with swimming (Table 5.3). In contrast, plasma lactate varied significantly with both temperature and swimming. Resting plasma lactate was more than 3-fold significantly higher in $T_{\text{max0-50}}$ compared to $T_{\text{opt}}$ fish. At fatigue, the $T_{\text{opt}}$, $T_{\text{max50-90}}$ and $T_{\text{max0-50}}$ groupings all displayed significant elevations in plasma lactate, increasing by 2 to 4-fold relative to resting levels. Moreover, plasma lactate was significantly higher in $T_{\text{max50-90}}$ fish at fatigue compared to the other groups (Table 5.3).
Plasma sodium varied significantly with both temperature and swim speed (Table 5.3). It tended to be highest at $T_{opt}$ and increased with swimming speed for $T_{opt}$ and $T_{max50-90}$ fish. Plasma potassium did not vary significantly with temperature (Table 5.3). Plasma potassium tended to decrease with swimming speed and was lowest at fatigue. Plasma chloride varied significantly with temperature, but not with swimming (Table 5.3). In general, plasma chloride was significantly higher at $T_{opt}$ relative to $T_{max0-50}$.

5.3 Discussion

This study is the most comprehensive assessment of the oxygen cascade in fish swimming at temperatures bracketing their $T_{opt}$. It greatly extends upon the study by Steinhausen et al. (2008), which swam fish at a constant speed (~1.35 bl s$^{-1}$ or ~70% of maximum) while acutely increasing the water temperature, by swimming individual fish to $U_{crit}$ and at discrete temperatures.

Aerobic scope and swim performance collapsed at temperatures above $T_{opt}$, which is consistent with previous assertions that a limitation in maximum cardiorespiratory performance inhibits exercise at high temperatures in salmonids (Brett, 1971; Farrell, 1997; Farrell, 2002; Farrell, 2009; Farrell et al., 2009; Lannig et al., 2004; Mark et al., 2002; Pörtner et al., 2004; Pörtner and Knust, 2007; Sartoris et al., 2003; Steinhausen et al., 2008; Taylor et al., 1996). In addition, novel findings with respect to venous oxygen status, heart rate and stroke volume were revealed.

The present study provides clear evidence for a cardiac limitation in fish swimming at warm temperatures. At temperatures above $T_{opt}$, $Q_{max}$ failed to increase because $f_{Hrest}$ reached its
maximum and could not further increase during swimming. Moreover, cardiac disrhythmias developed at the highest temperatures grouping. As discussed further below, changes in both \( P_{aO2} \) and \( C_{aO2} \) were minor at warm temperature, so neither oxygen delivery to or across the gills presented themselves as a major problem in terms of the oxygen cascade, despite a decrease in oxygen content in the water. Instead, a perfusion limitation developed because \( T_{aO2} \) failed to increase above \( T_{opt} \) given that \( \dot{Q} \) did not increase and \( C_{aO2} \) was unchanged. A diffusion limitation at the swimming muscles likely followed the cardiac limitation since \( P_{vO2} \) and \( C_{vO2} \) did decrease significantly at warm temperatures.

5.3.1 Fish Performance during Swim Challenge

Swimming a sufficient number of fish to resolve any subtle changes that occur in the oxygen cascade as a function of warming had many inherent challenges. Arterial and venous cannulae had to remain functional for multiple samples, yet blood sampling was selective to minimize hemodilution. In addition, adult Pacific salmon often acquire a fungal infection on the gills during migration. My fish were no exception, which may have contributed to the individual variability for \( P_{aO2} \) (see below). To compensate and increase statistical power, I pooled three populations and created four temperature groupings relative to \( T_{opt} \). Notably, a similar analysis of the temperature responses in Chilko sockeye salmon for \( \dot{M}O_2, \dot{Q}, f_H \) and \( V_s \) (see Chapter 3) mimicked the responses observed here for the pooled populations. In addition, the variance was small for \( \dot{M}O_{2rest}, \dot{M}O_{2max} \) and aerobic scope in each of the pooled temperature categories (see Fig 5.3). Furthermore, aerobic, cardiac and heart rate scopes measured over the full range of
temperatures and populations were all positively correlated (see Chapter 3). Therefore, I have confidence that pooling was valid.

Sex-specific differences in cardiorespiratory physiology and blood oxygen status were not apparent at $T_{opt}$ (see Chapters 3 and 4). Sex-specific differences were not considered in the analysis presented here due to low n-values. However, each temperature grouping contained approximately equal numbers of males and females, which offsets concerns regarding sex-differences. Nevertheless, the possibility that temperature tolerance as well as the physiological response to temperature varies between males and females has not been excluded and therefore should be considered in future studies.

As expected, fish swum above $T_{opt}$ and attaining less than 50% of maximum aerobic scope had a much lower maximum swim velocity compared to the other groups. This result provides evidence of the link between aerobic scope and swim performance.

Also, lactate was elevated at temperatures above $T_{opt}$ and at fatigue. It is well known that as fish approach $U_{crit}$, swimming gait transitions to burst-and-coast behaviours, which activates the white glycolytic muscles relying on anaerobic metabolism, producing lactate and lowering blood pH (Brauner et al., 2000). Moreover, fish are known to increase their reliance on anaerobic swimming at high temperatures (Brett, 1964; Jain and Farrell, 2003; Steinhausen et al., 2008), which was clearly evident here.

There was a clear trend for increasing COT and COT-Q with increasing temperature, though the characteristic “U-shaped” pattern of COT with speed (e.g. Hoyt and Taylor, 1981; Lee et al., 2003c; Prange, 1976; Prange and Schmidt-Nielsen, 1970; Wakeman and Wohlschlag, 1982) was not observed in any of the temperature groups. The remaining cardiovascular and oxygen status results are discussed in the sections below.
Arterial [Hb] and Hct did not significantly vary with temperature or swimming, supporting previous findings (Clark et al., 2008b; Steinhausen et al., 2008). A minor decrease in MCHC was observed at warm temperatures during swimming, which was primarily due to a general non-significant trend for increased Hct. Similar observations have previously been made in resting chinook (Clark et al., 2008b). Previous studies have shown a variable response of Hct with temperature. Hct has been shown to increase by up to 27% due to splenic contraction in acutely warmed resting rainbow trout (Sandblom and Axelsson, 2007), to decrease by 50% in warm-acclimated rainbow trout (Taylor et al., 1993) and to have minimal effects with temperature (see Farrell, 1997). Thus, though splenic contraction can be a short-term solution to increase [Hb] during swimming or acute temperature changes, this was not observed in the present study.

Plasma ions were differentially affected by temperature and swimming. Both plasma chloride and sodium were reduced above $T_{\text{opt}}$, in contrast to resting chinook salmon (Clark et al., 2008b). Plasma potassium was insensitive to temperature. The decrease in plasma potassium with swimming sharply contrasts previous results (Holk and Lykkeboe, 1998; Nielsen et al., 1994; Nielsen and Lykkeboe, 1992a; Steinhausen et al., 2008). As discussed in Chapter 4, increased plasma potassium during swimming has been attributed to potassium loss from working muscles and associated with reduced excitability of muscle cells, which is suggested to contribute to muscle fatigue (both cardiac and skeletal) (Bangsbo et al., 1996; Sjøgaard, 1996; Holk and Lykkeboe, 1998; Nielsen and Lykkeboe, 1992a). The decreased potassium levels observed here are interesting and warrant further study.
5.3.2 The Possibility of a Limitation in Gill Oxygen Uptake

Water oxygen content decreases by around 2% °C⁻¹ with increasing water temperature (Dejours, 1975), limiting environmental oxygen availability at high temperatures. In addition, haemoglobin oxygen affinity decreases (the oxyhaemoglobin dissociation curve shifts to the right) with high temperature exposure (Jensen et al., 1998; Perry and Reid, 1994), which hampers oxygen uptake at the gill although it facilitates tissue oxygen extraction. Accordingly, a limitation in oxygen uptake at the gills (either water delivery to the gills or diffusion of oxygen across the gills) has been proposed as a mechanism causing decreased cardiorespiratory and swimming performance at elevated temperatures in fish (Brett, 1971; Heath and Hughes, 1973; Taylor et al., 1997). In support of this hypothesis, Heath and Hughes (1973) showed that $CaO_2$ decreased with acute increases in water temperature in resting rainbow trout though hematocrit was not measured concurrently to check for haemodilution. Similarly, Taylor et al. (1993) found a decrease in $CaO_2$ at 18°C in resting and swimming rainbow trout seasonally acclimated to 4, 11 and 18°C, but hematocrit was halved. Clark et al. (2008b) found that large, but not smaller, resting adult chinook salmon decreased $CaO_2$ and $PaO_2$ during acute warming, but narrow holding tubes may have constrained gill movements in the larger fish.

In contrast, the present study found that $CaO_2$ and $PaO_2$ did not significantly differ across the four temperature categories. Similarly, Steinhausen et al. (2008) found that $CaO_2$ and hematocrit remained constant in both resting and swimming sockeye salmon exposed to acute increases in temperature. Moreover, $PaO_2$ increased in resting and remained constant in swimming sockeye salmon exposed to increasing temperatures. Likewise, Sartoris et al. (2003) found that $PaO_2$ remained constant during acute warming in Atlantic cod. Thus, there is
accumulating evidence that neither water delivery to the gills nor oxygen diffusion across the gills become limited at temperatures warmer than $T_{opt}$.

$P_{aO2}$ and $C_{aO2}$ did tend to decrease with swimming. A similar phenomenon was observed in swimming sockeye salmon exposed to acute temperature increases (Steinhausen et al., 2008). If instead fish had maintained or increased $C_{aO2}$ during swimming, $T_{aO2}$ would have been higher, which would have been particularly beneficial for warm fish.

Notably, there were a couple experimental concerns, which relate to the variation in $P_{aO2}$ among individual fish. $P_{aO2}$ and $C_{aO2}$ varied considerably, ranging from 49-128 torr and 6.7-14.8 ml dl$^{-1}$, respectively, at rest, and from 42-104 torr and 6.1-16.1 ml dl$^{-1}$, respectively, at fatigue. As described in Chapter 4, this may be attributed to the progressive accumulation of fungus on the body and gills. Gill fungal infection could increase the diffusion distance and decrease the maximum surface area for oxygen, creating variable $P_{aO2}$. In addition, gill damage during surgery (implantation of the flowprobe and venous catheter occurs in the opercular cavity adjacent to the gills) may have created similar gill diffusion problems that might account for the tendency for $C_{aO2}$ to decrease with swimming. Even so, arterial oxygen saturation was not substantially hampered during swimming.

5.3.3 The Possibility of a Limitation in Cardiac Performance

The idea that the temperature dependence of aerobic scope is closely linked with that of cardiac scope is clearly supported by the similarity of the temperature-induced changes for $Q$ and $\dot{M}O_2$ in resting and swimming fish (also see Chapter 3). A striking observation was that maximum $\dot{M}O_2$, $Q$ and $T_{aO2}$ all failed to increase above $T_{opt}$, and all three decreased at $T_{max0.50}$. 
This finding provides evidence of a cardiac limitation at high temperatures, supporting earlier studies (Brett, 1971; Steinhausen et al., 2008; Taylor et al., 1996).

Warming increased $\dot{Q}$ in resting and swimming fish entirely via an increase in $f_H$, corroborating previous work (Clark et al., 2008b; Sandblom and Axelsson, 2007; Steinhausen et al., 2008). Such increases in $f_H$ are probably mediated through a direct temperature effect on the pacemaker rate (Randall, 1970). The highest $f_H$ was achieved in resting fish in the highest temperature group (mean: 123.9 beats min$^{-1}$; range: 117-135 beats min$^{-1}$), and thus sometimes exceeded the proposed maximum of 120 beat min$^{-1}$ in active fish (Davie and Farrell, 1991; Farrell, 1991). However, at temperatures above $T_{opt}$, $f_H$ was unable to increase above resting levels during swimming and even decreased below resting levels for the $T_{max0-50}$ grouping. The negative scope for $f_H$ is a novel finding for fish. Because scope for $f_H$ became limiting at a lower temperature compared to scope for $V_s$ or $Q$, the present study provides support for the previous proposal (Farrell, 2009; Steinhausen et al., 2008) that reduced scope for $f_H$ may be the mechanism that limits $Q_{max}$ above $T_{opt}$.

Cardiac disrhythmias at the highest test temperatures provide further evidence of a cardiac limitation. Disrhythmias were never present in resting fish at any temperature, nor in swimming or fatigued fish at $T_{opt}$ or $T_{min50-90}$. Yet, every fish in the $T_{max0-50}$ group exhibited cardiac disrhythmias during fatigue and 50% displayed disrhythmias during swimming. What is truly remarkable is that the fish at the highest test temperatures continued to swim, albeit to a lower swim speed, despite severe disrhythmias and dramatic declines in $P_{vO_2}$, $C_{vO_2}$, $T_{aO_2}$ and $T_{xO_2}$, demonstrating an impressive tenacity that is obviously fuelled by anaerobic swimming given the high plasma lactate levels. This tenacity, however, could result in delayed mortality. Fish that displayed the most severe disrhythmias (29% of the $T_{max0-50}$ fish) died, despite quickly
decreasing the water temperature following fatigue. In every case, the cardiac disrhythmia was preceded by bradycardia. While irregular heart rates have been reported in resting rainbow trout, Atlantic cod and chinook salmon acutely exposed to high temperatures (Clark et al., 2008b; Gollock et al., 2006; Heath and Hughes, 1973), this is the first known study to report bradycardia followed by disrhythmia in a swimming fish at high temperature.

The mechanism of the bradycardia and subsequent cardiac disrhythmia is unclear. During anaerobic swimming, venous blood becomes acidotic (low pH), hypoxemic (low $P_{vO_2}$) and hyperkalemic (high $K^+$) (Holk and Lykkeboe, 1998; Kiceniuk and Jones, 1977), all of which can inhibit cardiac contractility (Dridezic and Gesser, 1994). High temperatures can exacerbate these conditions as salmonids rely more on anaerobic metabolism (Brett, 1964; Jain and Farrell, 2003; Steinhausen et al., 2008), though hyperkalemia was absent here. Simulated exercise conditions at high temperature with in situ rainbow trout hearts severely impaired maximum cardiac performance (Hanson and Farrell, 2007), thus it is possible that the deleterious venous blood environment caused the bradycardia and disrhythmias. However, bradycardia could also be under central nervous system control via the vagus nerve. A reduction in $f_H$ would increase the residence time of blood in the lumen of the heart which may enhance oxygen delivery to the spongy myocardium (see below). The critical experiments to examine these questions require the use of atropine or vagotomy to determine if heart rate increases when the vagal tone is blocked.

Since scope for $f_H$ declined above $T_{opt}$, $\dot{Q}$ could only increase by an increase in $V_s$. This was not evident. Resting $V_s$ failed to increase with increasing temperatures, corroborating previous studies (Brodeur et al., 2001; Clark et al., 2008b; Gamperl et al., 2011; Gollock et al., 2006; Mendonça and Gamperl, 2010; Sandblom and Axelsson, 2007; Steinhausen et al., 2008). In addition, a novel negative relationship between temperature and $V_s$ was observed here in
swimming fish. $V_{\text{max}}$ decreased at the highest temperatures, resulting in a decreased scope for $V_s$. This result contrasts a previous report, which suggested that $V_s$ was insensitive to temperature in salmonids exercising at ~75% of $U_{\text{crit}}$ (Steinhausen et al., 2008). However, the present study pushed fish to swim at higher temperatures and higher speeds, resulting in substantially reduced scope relative to previous studies, which may have resulted in the observed decrease in $V_{\text{max}}$.

Several potential reasons have been suggested for why $V_s$ does not increase in conjunction with $f_H$ at high temperature, especially since it can triple with swimming (Brett, 1971; Kiceniuk and Jones, 1977; Stevens et al., 1967). First, venous return and end-diastolic volume must first increase in order for cardiac contractility to increase $V_s$ (Sandblom and Axelsson, 2007) because cardiac end-systolic volume is essentially zero in salmonids (Franklin and Davie, 1992). In addition, cardiac contractility could be inhibited by the deleterious blood environment at high temperatures (e.g. low pH, high K$^+$, low $P_{\text{Vo2}}$) (Hanson et al., 2006). Finally, the negative force-frequency relationship for fish cardiac muscle dictates that cardiac contractility decreases with increasing contraction frequency (Hove-Madsen, 1992; Shiels et al., 2002). Accordingly, the high $f_H$ at high temperatures decreases both filling time and ventricular contractions which may increase end-systolic volume and reduce $V_s$. In support of this concept, a recent study by Gamperl et al. (2011) demonstrated that elevated temperature, per se, does not limit the ability of rainbow trout to increase $V_s$ since zatebradine treatment halved $f_H$ at the highest test temperature but $V_s$ doubled to maintain $Q$. This result suggests that perhaps the increase in $f_H$ associated with high temperature is an inescapable direct effect of temperature on the pacemaker, which prevents any beneficial changes in $V_s$.

It is important to note that the heart is a muscle requiring oxygen, and its requirements increase 3 to 5-fold during exercise, equating approximately to 1% of total $\overline{MO}_2$ (Farrell and
Steffensen, 1987). Salmonid hearts have two oxygen supply routes to their two distinct types of myocardium (see Chapter 6). The outer, compact myocardium is perfused with oxygenated blood from the arterial coronary circulation. Given that \( P_{aO2} \) and \( C_{aO2} \) were maintained with increasing temperature, and that coronary blood flow increases concomitantly with cardiac output during swimming (Axelsson and Farrell, 1993; Gamperl et al., 1995), \( T_{aO2} \) to the compact myocardium likely wasn’t limited, except perhaps at the highest temperatures when \( T_{aO2} \) declined. The inner, spongy myocardium is avascular and relies on the leftover oxygen in venous blood returning to the heart. Since both \( P_{vO2} \) and \( C_{vO2} \) decrease during exercise due to increased oxygen extraction by the muscles, a diffusion limitation could develop. The temperature-induced increase in \( f_H \) could then exacerbate the situation by decreasing the residence time of the blood in the lumen and thereby decreasing diffusion time between heart beats. The bradycardia observed here during swimming at high temperatures could serve to alleviate a diffusion limitation.

Some have suggested that a threshold value for \( P_{vO2} \) exists in order to guarantee sufficient oxygen supply to the spongy myocardium (Davie and Farrell, 1991; Farrell, 2002; Farrell, 2007; Farrell and Clutterham, 2003). However, in the present study, \( P_{vO2}, C_{vO2}, \) and \( T_{vO2} \) declined in salmon swimming above \( T_{opt} \). Thus, a state of cardiac hypoxia could have developed, possibly contributing to the disrhythmias discussed above, cardiac collapse and a concomitant decrease in oxygen delivery to the tissues, ultimately leading to decreased swimming performance.

5.3.4 The Possibility of a Limitation in Tissue Oxygen Extraction

As oxygen demand increases with warming, several situations could limit oxygen delivery to the mitochondria of the locomotory muscles. A diffusion limitation could occur due
to inadequate capillary density, ineffective muscle morphology (e.g. poor mitochondria density or location) or insufficient driving force (low $P_aO_2$). A perfusion limitation could result from inadequate $Q$ or insufficient capillary perfusion at the muscle. Evidence exists for both possibilities in fish swimming at high temperatures.

Steinhausen et al. (2008) found evidence of a diffusion limitation since $P_vO_2$ was maintained during acute temperature increases in swimming sockeye salmon. In addition, when fish quit swimming, venous blood is still partially saturated (Farrell and Clutterham, 2003; Farrell, 2007; Kiceniuk and Jones, 1977).

In contrast, $P_vO_2$ and $C_vO_2$ decreased above $T_{opt}$ in resting sockeye salmon in the present study. In fact, resting $P_vO_2$ decreased to 10 torr and resting plasma lactate became elevated in the highest temperature group, signifying insufficient oxygen delivery to tissue mitochondria. Similarly, warming decreased $C_vO_2$, $P_vO_2$ or both in resting rainbow trout, Atlantic cod and adult chinook salmon (Clark et al., 2008b; Heath and Hughes 1973; Sartoris et al., 2003). Thus, a tissue diffusion limitation was likely not manifest at $T_{opt}$.

Given the dramatic decline in $T_aO_2$ and the obvious increase in plasma lactate during swimming and at fatigue in the highest temperature group relative to $T_{opt}$, there was clearly a mismatch between oxygen supply and demand at the tissues, suggesting a perfusion limitation. There were insufficient venous blood samples in $T_{max0.50}$ during swimming and at fatigue to include in the analysis across groups; however, the scatterplot in Fig 5.7 shows a steady decline in $C_vO_2$ and $P_vO_2$ at fatigue above $T_{opt}$. Therefore, I suggest that there may not have been an immediate diffusion limitation at the muscle in swimming fish at high temperature. However, a decrease in $C_vO_2$ and $P_vO_2$ does not definitively preclude a diffusion limitation since the decrease may not have been proportional to the oxygen demand (Wagner, 1996). Therefore, these data do
not definitely exclude the possibility that both a diffusion and perfusion limitation may have been occurring.

The role of muscle morphology in limiting oxygen diffusion at high temperature and with exercise is ripe for future research. A diffusion limitation for oxygen uptake due to low capillarity in white muscle (Egginton and Sidell, 1989; Mosse, 1978) has been suggested to be an important mechanism “governing” systemic tissue utilization and thus ensuring an adequate $P_{\text{vo2}}$ threshold to supply the spongy myocardium with oxygen (Farrell et al., 2009). It would be particularly interesting to compare cardiac and skeletal muscle morphology (e.g. capillary, mitochondria and lipid density and location) in sockeye salmon populations differing in cardiorespiratory capacity and temperature tolerance.

5.3.5 A Possible Death Spiral for Salmon Swimming above $T_{\text{opt}}$

A “death spiral” for salmon swimming at temperatures above $T_{\text{opt}}$ was proposed by Farrell et al. (2009). Here, I provide further evidence for and expand upon the death spiral progression. My results are entirely consistent with the death spiral starting with a plateau in maximum heart rate above $T_{\text{opt}}$, which prevents $Q_{\text{max}}$ from further increasing to satisfy the increased tissue oxygen demand. With no compensatory increase in $C_{\text{aO2}}$, a perfusion limitation to swimming muscles creates a mismatch between oxygen supply and demand, as evidenced by elevated lactate levels. Low $P_{\text{vo2}}$ levels coupled with low pH due to anaerobic swimming likely impair cardiac contraction, further exacerbating the perfusion limitation and causing a positive feedback loop. At sufficiently low $P_{\text{vo2}}$ levels, a diffusion limitation to the swimming muscles likely develops as well and eventually swimming ceases. At temperatures well above $T_{\text{opt}}$. 
corresponding to precipitous declines in aerobic scope that would certainly prevent successful upstream migration, the situation is dire. Even in resting fish, $T_{aO2}$ levels are insufficient to meet the increased oxygen demand, as shown by high resting lactate levels. Swimming actually decreases $f_H$ below resting levels and maximum $V_s$ plummets, leading to a massive collapse of $Q$.

A perfusion limitation, which is likely followed by a diffusion limitation, develops at the swimming muscles. Dramatic declines in $P_{vO2}$, $C_{vO2}$ and $T_{vO2}$, coupled with low pH, create a deleterious venous environment for the spongy myocardium, which weakens cardiac contraction and may be the cause of the bradycardia and cardiac disrhythmias. Eventually, fish quit swimming and at excessively warm temperatures, cardiac function cannot recover and the fish perish.
Figure 5.1. Schematic of the four categories of cardiorespiratory performance with temperature. 
\( T_{\text{opt}} \) included fish swum at the optimal temperature range, at which 90-100\% of maximum aerobic scope was attained. \( T_{\text{min50-90}} \) included fish that were swum at temperatures lower than \( T_{\text{opt}} \) at which only 50-90\% of maximum aerobic scope was measured. \( T_{\text{max50-90}} \) included fish swum at temperatures above \( T_{\text{opt}} \), when 50-90\% of maximum aerobic scope was measured. \( T_{\text{max0-50}} \) included fish whose aerobic scope was only 0-50\% of maximum.
Figure 5.2. (A) Oxygen consumption (\(\dot{\text{MO}_2}\)), (B) cardiac output, (C) stroke volume and (D) heart rate with swimming speed across the four temperature groups. Mean ± SEM are shown.
Figure 5.3. Resting and maximum (A) oxygen consumption ($\dot{V}O_2$), (B) cardiac output ($\dot{Q}$), (C) stroke volume ($V_s$), (D) heart rate ($f_H$) at the four temperature categories. Scope for $\dot{V}O_2$ (E), $\dot{Q}$ (F), $V_s$ (G) and $f_H$ (H) are shown. All values are presented as mean ± SEM. Significant differences among temperature categories are indicated by differing letters (p<0.05).
Figure 5.4. Percent of maximum aerobic scope, cardiac scope, scope for heart rate ($f_H$) and scope for stroke volume ($V_s$) for each temperature category.
Figure 5.5. Individual blood flow traces for two Chilko sockeye salmon at 17°C ($T_{\text{opt}}$) and 26°C ($T_{\text{max0-50}}$) at rest (A, C) and during swimming (B, D, E). Swimming traces were recorded during the final swim speed before each fish fatigued (measured at 2.3 bl s$^{-1}$ and 1.5 bl s$^{-1}$ for the 17 and 26°C fish, respectively). Trace D was recorded 5 min before trace E, at the same swimming speed.
Figure 5.6. (A) Cost of transport (COT), (B) net cost of transport (COT$_{net}$), (C) cardiovascular cost of transport (COT-\(\dot{Q}\)) and (D) net cardiovascular cost of transport (COT-\(\dot{Q}_{net}\)) with swimming speed across the four temperature groups. Mean ± SEM are shown.
Figure 5.7. Arterial and venous (A, B) oxygen content ($C_{O2}$) and (C, D) partial pressure of oxygen ($P_{O2}$) at rest (open symbols) and fatigue (filled symbols) in four temperature groups ($\Delta = T_{min50-90}; \bigcirc = T_{opt}; \square = T_{max50-90}; \bigtriangleup = T_{max0-50}$). Each data point corresponds to an individual fish. A quadratic equation was fit through the venous data. Resting $C_{vO2}$: $R^2 = 0.37$, $p = 0.0007$; Fatigue $C_{vO2}$: $R^2 = 0.39$, $p = 0.002$; Resting $P_{vO2}$: $R^2 = 0.51$, $p < 0.0001$; Fatigue $P_{vO2}$: $R^2 = 0.41$ $p = 0.001$. 
Figure 5.8. (A) Arterial oxygen transport ($T_{aO2}$), (B) venous oxygen transport ($T_{vO2}$) and (C) arterial plasma lactate across the four temperature groups and with swimming. Refer to Tables 5.2 and 5.3 for statistical information.
Table 5.1. Arterial partial pressure of oxygen (P$_{aO2}$) and oxygen content (C$_{aO2}$) in resting fish, measured at 12°C and at the test temperature. Mean ± SEM are presented, there were no significant differences within a temperature group (p>0.05).

<table>
<thead>
<tr>
<th></th>
<th>P$_{aO2}$</th>
<th></th>
<th>C$_{aO2}$</th>
<th></th>
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</thead>
<tbody>
<tr>
<td></td>
<td>n</td>
<td>12°C</td>
<td>test temp</td>
<td>12°C</td>
</tr>
<tr>
<td>$T_{\text{max}50-90}$</td>
<td>5</td>
<td>67.5 ± 3.3</td>
<td>81.0 ± 5.6</td>
<td>14.2 ± 0.6</td>
</tr>
<tr>
<td>$T_{\text{max}0-50}$</td>
<td>7</td>
<td>63.5 ± 6.4</td>
<td>72.8 ± 4.8</td>
<td>12.0 ± 0.4</td>
</tr>
</tbody>
</table>
Table 5.2. Oxygen status variables across the four temperature categories and with swimming. Arterial and venous partial pressure of oxygen ($P_{aO2}$ and $P_{vO2}$), oxygen content ($C_{aO2}$ and $C_{vO2}$), oxygen extraction ($AVO2$), arterial oxygen transport ($T_{aO2}$) and venous oxygen transport ($T_{vO2}$) are indicated. Mean ± SEM, temperatures groups with differing letters within a swim speed are statistically different, an asterisk indicates a statistically significant difference from rest within a temperature group (p<0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>rest</th>
<th>steady</th>
<th>burst</th>
<th>fatigue</th>
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<tr>
<td>$P_{aO2}$ (torr)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{min50-90}$</td>
<td>-</td>
<td>60.7 ± 9.3</td>
<td>62.9 ± 3.5</td>
<td>49.8 ± 2.4</td>
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<tr>
<td>$T_{opt}$</td>
<td>98.4 ± 7.4</td>
<td>96.5 ± 5.8</td>
<td>64.7 ± 9.6*</td>
<td>69.7 ± 4.9*</td>
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<td>58.1 ± 4.3</td>
<td>60.7 ± 5.7</td>
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<td>$T_{max50-50}$</td>
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<td>70.8 ± 3.7</td>
<td>71.7 ± 7.4</td>
<td>75.4 ± 7.9</td>
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<tr>
<td>$P_{vO2}$ (torr)</td>
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<td></td>
</tr>
<tr>
<td>$T_{min50-90}$</td>
<td>28.0 ± 1.3^{ab}</td>
<td>24.0 ± 1.1</td>
<td>-</td>
<td>17.5 ± 2.5</td>
</tr>
<tr>
<td>$T_{opt}$</td>
<td>41.6 ± 2.4^{a}</td>
<td>32.6 ± 1.9*</td>
<td>17.6 ± 2.8*</td>
<td>23.4 ± 2.0*</td>
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<tr>
<td>$T_{max50-90}$</td>
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<td>21.1 ± 3.3</td>
<td>11.6 ± 3.7*</td>
<td>13.2 ± 2.18*</td>
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<td>8.5 ± 0.5</td>
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<td>$C_{vO2}$ (ml dl$^{-1}$)</td>
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<td>4.9 ± 1.2</td>
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<tr>
<td>$T_{opt}$</td>
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<td>5.7 ± 0.4*</td>
<td>2.5 ± 0.3*</td>
<td>3.0 ± 0.4^{a}*</td>
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<tr>
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<td>4.3 ± 0.8</td>
<td>1.0 ± 0.4*</td>
<td>1.4 ± 0.4^{b}*</td>
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<tr>
<td>$AVO2$ (ml dl$^{-1}$)</td>
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<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>$T_{min50-90}$</td>
<td>1.4 ± 0.6^{b}</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>$T_{opt}$</td>
<td>1.6 ± 1.6</td>
<td>-</td>
<td>-</td>
<td>6.8 ± 1.7*</td>
</tr>
<tr>
<td>$T_{max50-90}$</td>
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<td>$T_{aO2}$ (ml O$_2$ min$^{-1}$ kg$^{-1}$)</td>
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<tr>
<td>$T_{min50-90}$</td>
<td>3.4 ± 0.5</td>
<td>8.6 ± 1.0</td>
<td>13.4 ± 0.1^{ab}*</td>
<td>11.1 ± 1.1^{a}*</td>
</tr>
<tr>
<td>$T_{opt}$</td>
<td>5.8 ± 0.4</td>
<td>9.4 ± 0.4*</td>
<td>14.7 ± 1.2^{a}*</td>
<td>10.7 ± 0.8^{a}*</td>
</tr>
<tr>
<td>$T_{max50-90}$</td>
<td>7.6 ± 0.3</td>
<td>9.8 ± 0.4</td>
<td>13.7 ± 2.0^{a}*</td>
<td>8.9 ± 1.7^{ab}</td>
</tr>
<tr>
<td>$T_{max50-50}$</td>
<td>7.2 ± 0.5</td>
<td>8.7 ± 1.2</td>
<td>7.4 ± 0.7^{b}</td>
<td>4.1 ± 1.4^{b}</td>
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<tr>
<td>$T_{vO2}$ (ml O$_2$ min$^{-1}$ kg$^{-1}$)</td>
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<tr>
<td>$T_{min50-90}$</td>
<td>2.8 ± 0.4^{b}</td>
<td>3.8 ± 0.7</td>
<td>3.6 ± 0.4</td>
<td>-</td>
</tr>
<tr>
<td>$T_{opt}$</td>
<td>4.1 ± 0.3^{a}</td>
<td>5.1 ± 0.4</td>
<td>3.7 ± 0.4</td>
<td>3.0 ± 0.4</td>
</tr>
<tr>
<td>$T_{max50-90}$</td>
<td>4.2 ± 0.4^{ab}</td>
<td>3.8 ± 0.7</td>
<td>1.4 ± 0.5*</td>
<td>1.6 ± 0.5*</td>
</tr>
<tr>
<td>$T_{max50-50}$</td>
<td>0.8 ± 0.6^{b}</td>
<td>-</td>
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Table 5.3. Arterial haematological variables across the four temperature categories and with swimming. Haemoglobin concentration (Hb), hematocrit (Hct), mean cell haemoglobin concentration (MCHC), plasma sodium (Na⁺), plasma potassium (K⁺) and plasma chloride (Cl⁻) are indicated. Mean ± SEM, temperatures groups with differing letters within a swim speed are statistically different, an asterisk indicates a statically significant difference from rest within a temperature group (p<0.05).

<table>
<thead>
<tr>
<th>Variable</th>
<th>rest</th>
<th>steady</th>
<th>burst</th>
<th>fatigue</th>
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<tr>
<td>Hb (g l⁻¹)</td>
<td>T_{min50-90} 92.3 ± 9.0 89.9 ± 10.6 93.5 ± 11.8 81.5 ± 12.6</td>
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<tr>
<td></td>
<td>T_{opt} 92.4 ± 4.6 90.3 ± 4.2 91.8 ± 5.4 87.3 ± 3.0</td>
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<tr>
<td></td>
<td>T_{max50-90} 100.3 ± 10.9 95.8 ± 2.7 89.3 ± 6.3 89.1 ± 6.3</td>
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<tr>
<td></td>
<td>T_{max0-50} 89.0 ± 5.0 98.3 ± 6.0 91.6 ± 7.5 91.8 ± 2.3</td>
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<tr>
<td>Hct (%)</td>
<td>T_{min50-90} 30.7 ± 3.4 29.2 ± 1.6 30.6 ± 3.5 29.6 ± 5.2</td>
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<tr>
<td></td>
<td>T_{opt} 31.0 ± 1.7 30.4 ± 1.6 33.9 ± 2.5 31.1 ± 1.4</td>
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<tr>
<td></td>
<td>T_{max50-90} 32.9 ± 1.8 31.4 ± 0.8 34.2 ± 2.2 36.1 ± 1.4</td>
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<tr>
<td></td>
<td>T_{max0-50} 32.6 ± 1.5 32.1 ± 0.9 35.8 ± 3.4 37.5 ± 3.3</td>
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<tr>
<td>MCHC (g l⁻¹)</td>
<td>T_{min50-90} 302.2 ± 9.1 306.4 ± 19.7 305.1 ± 6.3 278.3 ± 10.9</td>
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<tr>
<td></td>
<td>T_{opt} 300.5 ± 6.6 299.4 ± 6.8 273.1 ± 8.1 282.3 ± 4.9</td>
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<tr>
<td></td>
<td>T_{max50-90} 308.4 ± 32.7 306.7 ± 13.5 267.7 ± 25.1 249.0 ± 22.0*</td>
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<tr>
<td></td>
<td>T_{max0-50} 274.5 ± 15.8 305.7 ± 11.7 259.1 ± 19.4 250.4 ± 16.4</td>
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<tr>
<td>Glucose (mmol l⁻¹)</td>
<td>T_{min50-90} - 9.6 ± 0.7 9.0 ± 0.3 9.8 ± 0.7</td>
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<tr>
<td></td>
<td>T_{opt} 5.6 ± 0.5 5.1 ± 0.5 6.6 ± 0.5 6.2 ± 0.6</td>
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<tr>
<td></td>
<td>T_{max50-90} 7.6 ± 1.9 8.3 ± 1.2 6.9 ± 1.0 7.8 ± 1.1</td>
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<tr>
<td></td>
<td>T_{max0-50} 10.2 ± 2.0 - 5.4 ± 1.9* 7.3 ± 1.6*</td>
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<tr>
<td>Lactate (mmol l⁻¹)</td>
<td>T_{min50-90} 0.8 ± 0.4a 1.3 ± 0.3 2.3 ± 0.5 4.0 ± 0.9a</td>
<td></td>
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<tr>
<td></td>
<td>T_{opt} 1.3 ± 0.2a 1.4 ± 0.2 3.5 ± 0.5 5.3 ± 0.6abx</td>
<td></td>
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</tr>
<tr>
<td></td>
<td>T_{max50-90} 2.3 ± 0.5ab 2.5 ± 0.4 5.0 ± 0.6 9.5 ± 0.9c</td>
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<tr>
<td></td>
<td>T_{max0-50} 4.5 ± 0.7b - 5.0 ± 1.4 8.0 ± 0.7b</td>
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<tr>
<td>Na⁺ (mmol l⁻¹)</td>
<td>T_{min50-90} 142.3 ± 2.9a 138.1 ± 1.1 133.1 ± 6.0 -</td>
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<tr>
<td></td>
<td>T_{opt} 140.1 ± 1.7a 143.8 ± 1.6 145.9 ± 2.1 152.9 ± 2.0a</td>
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</tr>
<tr>
<td></td>
<td>T_{max50-90} 120.7 ± 6.8b 140.4 ± 5.2* 138.9 ± 3.2* 139.6 ± 4.7abx</td>
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<tr>
<td></td>
<td>T_{max0-50} 137.7 ± 4.1ab - 135.9 ± 2.6 137.6 ± 2.6b</td>
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<tr>
<td>K⁺ (mmol l⁻¹)</td>
<td>T_{min50-90} 3.3 ± 0.1 3.9 ± 0.5 4.3 ± 0.8 4.0 ± 1.8</td>
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<tr>
<td></td>
<td>T_{opt} 4.9 ± 0.4 4.6 ± 0.4 2.7 ± 0.7 2.6 ± 0.3*</td>
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</tr>
<tr>
<td></td>
<td>T_{max50-90} 6.2 ± 1.0 4.5 ± 0.7 2.8 ± 0.6* 1.7 ± 0.3*</td>
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<tr>
<td></td>
<td>T_{max0-50} 3.8 ± 0.3 - 3.9 ± 0.9 1.7 ± 0.6</td>
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</tr>
<tr>
<td>Cl⁻ (mmol l⁻¹)</td>
<td>T_{min50-90} 121.0 ± 2.0ab 122.0 ± 5.2 117.4 ± 2.3ab 123.6 ± 3.8ab</td>
<td></td>
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<tr>
<td></td>
<td>T_{opt} 127.5 ± 0.9ab 129.9 ± 1.4 130.2 ± 1.3a 133.3 ± 1.2a</td>
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<tr>
<td></td>
<td>T_{max50-90} 119.3 ± 6.0ab 119.8 ± 2.1 118.3 ± 4.5ab 119.9 ± 5.2b</td>
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<td></td>
<td>T_{max0-50} 112.0 ± 3.1b - 113.4 ± 3.9b 111.9 ± 2.7b</td>
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CHAPTER 6: DIFFERENCES IN GROSS CARDIAC MORPHOLOGY AMONG
SOCKEYE SALMON POPULATIONS AND IN RELATION TO TEMPERATURE
TREATMENT

6.1 Introduction

The preceding chapters demonstrated that aerobic scope is correlated with migration
difficulty among Fraser River sockeye salmon populations (Chapter 3) and that cardiac and
aerobic scope are tightly related (Chapters 3, 4 and 5). Furthermore, I proposed that cardiac
collapse precipitates a decrease in aerobic swimming performance at temperatures above \( T_{opt} \)
(Chapter 5). Given the key role of the heart in temperature tolerance and supporting aerobic
swimming, I sought to determine whether there were differences in cardiac morphology across
sockeye salmon populations related to migration difficulty and whether cardiac morphology was
affected by temperature exposure.

Relative ventricular mass (RVM) varies considerably across fish species [ranging over
10-fold from 0.03 to 0.4% of body mass; (Santer, 1985)]. Some of these interspecific differences
can be attributed to diversity of habitat, life history and activity levels. Like all other muscles,
cardiac mass is a primary determinant of force development and a larger heart can presumably
generate higher cardiac outputs (\( Q \)) and greater arterial blood pressures. Correspondingly,
athletic fish tend to have larger, more powerful hearts that generate higher \( Q \) and arterial blood
pressure compared with sedentary species, though Antarctic icefishes are an important exception
(Gamperl and Farrell, 2004). Such species distinctions also extend to ventricular composition.
Salmonid ventricles are composed of two distinct layers of myocardium. The outer compact
myocardium is perfused with well-oxygenated arterial blood via a coronary circulation. The inner spongy myocardium is avascular, so it relies on a more variable and lower oxygen tension from the venous blood returning to the heart. Some athletic species (e.g. salmonids and tunas) have 30-50% compact myocardium (Farrell et al., 1988a; Poupa and Lindström, 1983), while most sluggish fish (e.g. hagfishes, Atlantic cod) only have spongy myocardium. While the influence of athleticism on interspecific ventricular design is clear across fish species, its influence among populations within a fish species is unknown.

I predicted that cardiac morphology would vary among sockeye salmon populations according to migration difficulty, mimicking the patterns observed in aerobic scope (Chapter 3). Specifically, I hypothesized that sockeye salmon populations with more challenging migrations would have a larger relative ventricular mass (to allow the heart to generate more power output) and a greater percent compact myocardium (to have a more secure supply of oxygen while swimming). Furthermore, since the total amount of compact myocardium depends on both the size of the ventricle (RVM) and proportion of compact myocardium (% compact) (e.g. a large ventricle with a low proportion of compact myocardium can have the same total amount of compact myocardium as a smaller ventricle with a higher percent compact), I also assessed relative dry compact mass (RDCM). Again, I predicted that populations facing more difficult migrations would display a higher RDCM.

In making comparisons among sockeye salmon populations, I first broadly categorized migration difficulty by dividing the populations into those that pass through Hells Gate, a hydraulically challenging river segment (upriver populations) and those that do not [coastal populations (Table 2.1)]. I predicted that Hells Gate may impose a major selection pressure on the cardiovascular system, especially since Chapter 3 revealed that the coastal Weaver
population possessed the lowest $\text{MO}_{2}\text{max}$ and aerobic scope. I also considered that the river environment may impose selection at a finer scale since Chapter 3 showed that migration distance correlated significantly with aerobic scope across sockeye salmon populations. In addition, migration distance, elevation gain and work (distance $\times$ elevation) were the best predictors for various energetic, morphological and reproductive attributes among Fraser River sockeye salmon populations (Crossin et al., 2004). Therefore, my primary hypothesis was that migration distance, elevation gain and work correlate with the heart morphology indices. However, I also took into account the possibility that heart morphology may interact with the environment in a more complex manner. For example, warm temperatures may necessitate a greater percent compact myocardium because of the requirement for a more reliable, stable oxygen supply when $P_v^{O_2}$ is reduced (see Chapter 5). In addition, swimming at a greater rate or against a stronger river current may require a larger heart to supply a greater $Q$. Therefore, I included ATU and various new indices that had not been previously considered (e.g. migration rate, migration duration, migration effort) in the analysis.

It is well known that individual fish show remarkable cardiac plasticity and variability. Indeed, salmonids in particular can rapidly remodel their ventricle in response to various environmental and biological cues (Gamperl and Farrell, 2004). For example, ventricle mass and composition are known to change with temperature acclimation, exercise-training, anemia and sexual maturation in fishes (Bailey et al., 1997; Clark and Rodnick, 1998; Franklin and Davie, 1992; Gamperl and Farrell, 2004; Goolish, 1987; Graham and Farrell, 1989; Pelouch and Vornanen, 1996; Simonot and Farrell, 2007; West and Driedzic, 1999). Specifically, RVM increased during sexual maturation in male, but not female salmonids (Clark and Rodnick, 1998; Franklin and Davie, 1992; Graham and Farrell, 1992; West and Driedzic, 1999). In addition, cold
acclimation significantly increased RVM, but decreased % compact in rainbow trout (Farrell et al., 1988a, Gamperl and Farrell, 2004; Graham and Farrell, 1989). Therefore, I also examined cardiac morphology in male and female sockeye exposed to different holding temperatures. I hypothesized that male sockeye salmon would have a greater RVM relative to females and that only males would demonstrate cardiac remodelling. I additionally predicted that RVM would decrease and % compact would increase at warmer temperatures in males, supporting previous observations in rainbow trout (Farrell et al., 1988a, Graham and Farrell, 1989). Finally, to further examine the effect of temperature, I compared cardiac morphology in sockeye salmon exposed to an acute temperature increase while swimming at a constant velocity (Steinhausen et al., 2008). I hypothesized that a greater % compact would translate to a higher temperature tolerance.

Detailed materials and methods are provided in Chapter 2 (sections 2.1, 2.2, 2.8 and 2.10.2).

6.2 Results

6.2.1 Population Comparisons with Migration Difficulty

Population comparisons were restricted to females because male cardiac morphology was shown to significantly differ with temperature treatment (see below). Notably, there were no significant relationships between GSI and any of the cardiac variables within a population (data not shown).

Upriver sockeye salmon had significantly higher RVM, % compact and RDCM compared to coastal sockeye salmon (p<0.01). In addition, RVM, % compact and RDCM varied
across populations by 40, 27 and 60%, respectively (Table 6.1). Chilko and Quesnel fish had significantly higher RVM than Weaver fish and all populations had significantly higher RVM compared to Harrison fish. Early Stuart and Nechako had significantly higher % compact (~44%) compared to Quesnel, Lower Adams, Weaver and Harrison (~36%). RDCM exhibited more of a gradient across populations. Early Stuart, Nechako, Chilko and Quesnel had the highest RDCM (~0.0090%), Lower Adams and Weaver displayed an intermediate RDCM (~0.0075%) and Harrison exhibited the lowest RDCM of all (0.0060%).

Each cardiac morphology variable was significantly correlated with migration difficulty (Table 6.2). Linear regressions between the migration difficulty indices with the strongest Pearson correlation coefficient and each cardiac variable are shown in Figure 6.1. RVM, % compact and RDCM had the strongest correlation coefficients with migration rate, migration effort (distance × Fraser River discharge) and migration distance, respectively. In addition, RDCM significantly correlated with aerobic scope.

6.2.2 Temperature Effects and Sex Differences

Cardiac morphology varied significantly with holding temperature (>5 days of thermal acclimation to 14, 16.5 and 19°C) for males, but not for females (Figure 6.2). Males had a 17% higher RVM at 19°C compared with 14°C. Males also had a significantly higher RVM compared with females at 16.5°C and 19°C. Male fish held at 16.5°C and 19°C had a RDCM that was 19-21% significantly higher compared with male fish held at 14°C. Again, males had a significantly higher RDCM compared to females at 16.5°C and 19°C. Percent compact myocardium did not vary significantly between sexes, or within males as a function of holding temperature. Notably,
GSI did not significantly differ among temperature treatments within sex (p>0.05), thereby reducing the possibility that the temperature effects on ventricular composition were related to differences in the state of maturity.

6.2.3 High Temperature Swimming Experiment

There were no statistically significant differences in any of the cardiac variables between male and female Lower Adams sockeye salmon used in the high temperature swimming experiment performed by Steinhausen et al. (2008). Therefore, male and female fish were pooled in order to assess the relationship between the temperature at which the fish failed to continue swimming at approximately 75% of \(U_{\text{crit}}\) (fail temperature) and the various cardiac variables (Figure 6.3). No relationship was found between any of the cardiac variables and fail temperature (p>0.05).

6.3 Discussion

The present study clearly demonstrates for the first time that cardiac morphology can vary among wild populations of the same fish species. As predicted, the differences in ventricular morphology among Fraser River sockeye salmon populations were related to the difficulty of the upriver migration. These findings add to similar discoveries for population-specific variation in aerobic scope (Chapter 3) and gross morphology [e.g. body mass, egg number and energy content (Crossin et al., 2004; Gilhousen, 1980)] according to migration difficulty. The population differences in ventricular morphology likely represent adaptations to
the upriver environment since all fish were sampled early in the migration, prior to encountering the major selective elements. Individuals from a given sockeye salmon population are therefore prepared for the athletic task that lies ahead during the upriver migration, even though they themselves have never experienced the upstream migration conditions. Consistent with previous studies, ventricular morphology was shown to be sexually dimorphic in sockeye salmon in some respects and plastic with response to environmental temperature in male fish.

6.3.1 Population Differences in Ventricular Morphology

The range for RVM (0.09-0.19%) among individual female sockeye salmon corresponds well with values reported for other sexually mature salmonids: sockeye salmon (0.11-0.13%, Clark et al., 2009; Sandblom et al., 2009), rainbow trout (0.07-0.24%, Bailey et al., 1997; Clark and Rodnick, 1998; Franklin and Davie, 1992; Graham and Farrell, 1992) but was lower than in sexually mature male chinook salmon near the spawning ground (0.24%, Clark et al., 2008b).

Substantial individual variation was also observed for % compact, ranging between 25 and 50%. This range corresponds with previously reported values for mature sockeye salmon (43%, Sandblom et al., 2009) and chinook salmon (53%, Clark et al., 2008b).

RDCM is a new metric that integrates the two cardiac measures, RVM and % compact, to illustrate how much total myocardium relative to body mass is independent of the venous circulation and instead has a secure oxygen supply via the coronary circulation. RDCM can increase either by maintaining RVM while increasing % compact, or by maintaining % compact while increasing RVM or by increasing both % compact and RVM. As such, a large ventricle with a smaller percentage compact myocardium could have the same total amount of compact
myocardium as a small ventricle with a large percentage myocardium. In both cases, the same total amount of heart is perfused with stable, oxygenated blood via the coronary circulation. RDCM was observed to vary substantially across individual sockeye, between 0.005% and 0.011%.

Beyond individual variation, cardiac morphology also varied considerably across populations. Indeed, RVM, % compact and RDCM varied by 40, 27 and 60% across the seven Fraser River sockeye salmon populations investigated here. Clear differences in cardiac morphology existed between coastal and upriver populations, suggesting that the difficult journey through the Fraser Canyon and Hells Gate likely imposes strong selection pressure for a greater RVM, % compact and RDCM. In addition to this broad classification, other aspects of the upriver migration also appear to influence cardiac morphology. RVM only correlated with migration rate (p < 0.05, no correction for multiple comparisons), suggesting that fast swimming requires large ventricles. Alternatively, the primary selection force for RVM may simply have been Hells Gate. Percent compact myocardium correlated with several of the indices (i.e. migration distance, migration duration and ATU) but migration effort had the strongest correlation (p < 0.006, Bonferroni level). As such, long river distances with a strong current may necessitate a higher percentage compact myocardium. Finally, RDCM correlated with every difficulty index examined except river slope, and migration distance had the strongest relationship (p < 0.006, Bonferroni level). Additionally, RDCM correlated with aerobic scope. Therefore, RDCM appears to be under strong selection pressure across many levels of migration difficulty. None of the ventricular morphology variables significantly correlated with river slope, supporting an earlier finding by Crossin et al. (2004) which suggested that river slope was not a major selective element for gross morphology among Fraser River sockeye salmon populations.
Collectively, these correlational analyses suggest that migratory difficulty is likely a strong selective force responsible for the population-specific differences in cardiac morphology. As discussed in Chapter 3, correlations only provide circumstantial, though promising, evidence of local adaptation (Endler, 1986; Schluter, 2000; Taylor, 1991) and conclusive evidence would require breeding studies. I cannot reject the possibility that differences may be due to developmental plasticity. However, given that the fish were collected early in their migration, had never before encountered the upstream migration conditions and spent the last >2 years in a common ocean environment, I conclude that the observed differences among populations were most likely due to genetic adaptation rather than environmental acclimation (refer to Chapter 3 for further discussion).

6.3.2 Temperature and Sex Differences

Male sockeye salmon had up to 25% more RVM compared to females, depending on the temperature. These results correspond well to previously reported values for sexually maturing sockeye salmon (males had 11-13% greater RVM compared to females, Clark et al., 2009; Sandblom et al., 2009). In contrast, studies performed on sexually mature rainbow trout found a more dramatic, up to 2-fold difference between male and female fish (Bailey et al., 1997; Clark and Rodnick, 1998; Franklin and Davie, 1992).

The much larger RVM in mature male rainbow trout has been demonstrated to increase $V_s$ and cardiac power output, which has been hypothesized to support increased functional demands placed on the hearts of spawning male salmon (Franklin and Davie, 1992, Gamperl and Farrell 2004). However, clear evidence supporting this hypothesis from wild migrating fish is
lacking. In the present study, the smaller difference in RVM resulted in no differences in $Q$ or $V_s$ between male and female sockeye salmon (Chapter 4). Recent studies suggest that $f_H$ was similar between sexes in sockeye salmon on the spawning ground, though males spent more time with elevated heart rates and had a higher routine $\dot{\text{MO}_2}$, both of which can be attributed to increased activity (Clark et al., 2009). In addition, no differences in arterial blood pressure were observed between mature male and female sockeye salmon (Sandblom et al., 2009). Perhaps, compared to wild conditions, the hatchery environment exacerbates the sexual diochotomy because hatchery-reared rainbow trout had significantly smaller RVM compared to wild, migrating trout (Graham and Farrell, 1989), which could potentially allow for a greater scope for cardiac growth with sex development. Collectively, these observations suggest that the remarkable ventricular growth observed in mature male rainbow trout may not be a general characteristic shared with all salmonids. Clearly, more research is needed to address these ideas.

Temperature clearly remodelled the heart in male, but not female, sockeye salmon. RVM and RDCM were 17-21% significantly higher in warm compared to cool temperature-treated male sockeye salmon, which was not due to differences in sexual maturation since GSI did not differ. Percent compact did not significantly differ with temperature treatment. These findings sharply contrast with my hypotheses and previous reports in the literature for rainbow trout. Rainbow trout acclimated to 5°C had a 20-40% higher RVM but a 15-30% decrease in % compact compared to fish held at 15°C (Farrell et al., 1988a, Graham and Farrell, 1989). A larger RVM at cold temperatures has been postulated to compensate for the decrease in contractility associated with cold, helping to maintain stroke volume, cardiac output and power output (Gamperl and Farrell, 2004). Why such a difference in the cardiac remodelling response to temperature exists within the genus *Oncorhynchus* is unclear. It could possibly be attributed to
the different temperatures chosen for the studies, although this remains to be tested. Regardless, the higher RVM with no change in % compact in warm-temperature treated sockeye resulted in a concomitant higher RDCM, meaning that a larger total amount of myocardium was perfused with blood from the coronary rather than the venous circulation. This could enhance oxygen delivery to the ventricle at warm temperature, matching the increased oxygen demand.

The individual cardiac plasticity in male migrating, adult sockeye salmon is quite remarkable given that when the fish enter the river, they are 4-6 weeks from death, have ceased feeding, are in the midst of tremendous physiological flux as secondary sexual characteristics develop and the gonads grow all while performing the enormous athletic task of returning upstream to their spawning ground. This finding demonstrates that male sockeye salmon retain the ability to adjust morphological features when faced with changing environmental variables, even once they have commenced their upstream migration.

Individual variability in cardiac anatomy has been linked to differences in $Q_{\text{max}}$, $\dot{MO}_{2\text{max}}$ and swim performance in rainbow trout (Claireaux et al., 2005). Specifically, poor swimmers had more rounded hearts compared to good swimmers. I similarly sought to examine the possible role of individual variation in cardiac composition on high temperature tolerance. However, no relationship was found between ventricular morphology and high temperature swim performance in fish subjected to a high temperature challenge while swimming near maximally (Steinhausen et al., 2008). Unfortunately, only three fish were truly classified as “poor” swimmers, resulting in very low statistical power. Therefore, these results do not preclude the possibility that population differences in cardiac composition are related to temperature tolerance, or that the cardiac responses to holding temperature could improve temperature tolerance. Rather, more experimental work is needed to test these ideas.
Figure 6.1. Linear regressions between migration difficulty indices and (A) relative ventricular mass (B) percent compact myocardium and (C) relative dry compact mass. Population means ± SEM are presented. The migration difficulty indices with the strongest Pearson correlation coefficient are presented (Table 6.2). $F_M$, Fraser River Discharge, $D_M$, distance to spawning grounds. Only female sockeye salmon were compared.
Figure 6.2. (A) Relative ventricular mass (RVM), (B) percentage compact myocardium (% compact), and (C) relative dry compact mass (RDCM) are shown for male and female Chilko sockeye salmon acclimated to 14, 16.5 and 19°C for at least 5 days before death. N values are indicated in parentheses. An asterisk indicates a statistically significant difference between male and female fish. Significant differences with temperature treatment among male sockeye are indicated by differing letters. There were no significant differences with temperature treatment among female sockeye (p>0.05).
Figure 6.3. (A) Relative ventricular mass (RVM), (B) percentage compact myocardium (% compact), and (C) relative dry compact mass (RDCM) as a function of the temperature at which Lower Adams sockeye salmon failed to continue swimming at approximately 75% of $U_{\text{crit}}$ (fail temperature). Each point corresponds to an individual fish. Males and females are indicated (open triangles and closed circles, respectively); however, none of the cardiac variables differed significantly with sex. Therefore, statistical analysis was performed on the entire group. No relationship was found between any of the cardiac variables and fail temperature ($p>0.05$).
Table 6.1. Relative ventricular mass (RVM), percentage compact myocardium (% compact) and relative dry compact mass (RDCM) for each sockeye salmon population (mean ± SEM). Only female sockeye salmon from each population were compared. Populations with differing letters are significantly different within each variable.

<table>
<thead>
<tr>
<th>Population</th>
<th>Spawning location</th>
<th>n</th>
<th>RVM (%)</th>
<th>% compact</th>
<th>RDCM (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early Stuart</td>
<td>upriver</td>
<td>7</td>
<td>0.141 ± 0.004&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>45.2 ± 0.8&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.0096 ± 0.0004&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Nechako</td>
<td>upriver</td>
<td>9</td>
<td>0.141 ± 0.007&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>42.5 ± 0.8&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>0.0090 ± 0.0004&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Quesnel</td>
<td>upriver</td>
<td>11</td>
<td>0.154 ± 0.003&lt;sup&gt;a&lt;/sup&gt;</td>
<td>36.3 ± 0.8&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0084 ± 0.0002&lt;sup&gt;ab&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chilko</td>
<td>upriver</td>
<td>35</td>
<td>0.150 ± 0.002&lt;sup&gt;a&lt;/sup&gt;</td>
<td>38.9 ± 0.7&lt;sup&gt;bc&lt;/sup&gt;</td>
<td>0.0088 ± 0.0001&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>Lower Adams</td>
<td>upriver</td>
<td>21</td>
<td>0.145 ± 0.003&lt;sup&gt;ab&lt;/sup&gt;</td>
<td>36.0 ± 0.7&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0079 ± 0.0003&lt;sup&gt;bc&lt;/sup&gt;</td>
</tr>
<tr>
<td>Weaver</td>
<td>coastal</td>
<td>11</td>
<td>0.134 ± 0.004&lt;sup&gt;b&lt;/sup&gt;</td>
<td>35.7 ± 1.5&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0072 ± 0.0003&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Harrison</td>
<td>coastal</td>
<td>13</td>
<td>0.110 ± 0.003&lt;sup&gt;c&lt;/sup&gt;</td>
<td>36.3 ± 0.6&lt;sup&gt;c&lt;/sup&gt;</td>
<td>0.0060 ± 0.0001&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
</tbody>
</table>
Table 6.2. Pearson correlation matrix relating relative ventricular mass (RVM), percentage compact myocardium (% compact), relative dry compact mass (RDCM) of female fish from seven sockeye salmon populations and eight migration difficulty variables (see Table 2.1). ATU = accumulated thermal units, $F_M$ = Fraser River discharge. Bold font indicates the migration difficulty variable with the highest correlation coefficient for a given physiological variable. Three critical values are indicated: $p < 0.05$ (no correction for multiple comparisons), $p < 0.018$ (Benjamini and Yekutieli False Discovery Rate) and $p < 0.006$ (Bonferroni).

<table>
<thead>
<tr>
<th>Variable</th>
<th>RVM</th>
<th>% compact</th>
<th>RDCM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Migration distance ($D_M$)</td>
<td>0.626</td>
<td>0.801*</td>
<td>0.933‡</td>
</tr>
<tr>
<td>Migration elevation ($E_M$)</td>
<td>0.744</td>
<td>0.482</td>
<td>0.812*</td>
</tr>
<tr>
<td>Work ($0.0001 \cdot E_M \cdot D_M$)</td>
<td>0.662</td>
<td>0.725</td>
<td>0.913‡</td>
</tr>
<tr>
<td>River slope ($500(E_M \cdot D_M^{-1})$)</td>
<td>0.736</td>
<td>0.170</td>
<td>0.607</td>
</tr>
<tr>
<td>Migration effort ($0.0001 \cdot D_M \cdot F_M$)</td>
<td>0.426</td>
<td><strong>0.899‡</strong></td>
<td>0.866†</td>
</tr>
<tr>
<td>Migration duration</td>
<td>0.598</td>
<td>0.785*</td>
<td>0.906‡</td>
</tr>
<tr>
<td>Migration rate</td>
<td><strong>0.787</strong>*</td>
<td>0.558</td>
<td>0.880†</td>
</tr>
<tr>
<td>ATU</td>
<td>0.580</td>
<td>0.833*</td>
<td>0.925‡</td>
</tr>
<tr>
<td>Aerobic scope</td>
<td>0.354</td>
<td>0.748</td>
<td>0.893†</td>
</tr>
<tr>
<td>RVM</td>
<td>-</td>
<td>0.164</td>
<td>0.764*</td>
</tr>
<tr>
<td>% compact</td>
<td>-</td>
<td>-</td>
<td>0.761*</td>
</tr>
<tr>
<td>RDCM</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
</tbody>
</table>

* $p < 0.05$; † $p < 0.018$, ‡ $p < 0.006$
CHAPTER 7: THE EFFECT OF TEMPERATURE ACCLIMATION ON MYOCARDIAL β-ADRENOCEPTOR DENSITY AND LIGAND BINDING AFFINITY IN TWO POPULATIONS OF SOCKEYE SALMON

7.1 Introduction

The preceding chapters clearly demonstrated a link between maximum aerobic scope and maximum cardiac performance (Chapters 3, 4 and 5) and provided evidence that the decline in aerobic scope above $T_{opt}$ is driven by cardiac collapse (Chapter 5). Similar to aerobic scope, ventricular composition also differed among populations and is likely under strong selection pressure by the upriver migration conditions (Chapters 3 and 6). Therefore, I again turned to the heart in order to examine whether adrenergic cellular stimulation was an important mechanism for high thermal tolerance in sockeye salmon.

Adrenergic stimulation is critical for increasing cardiac performance during exercise and maintaining cardiac performance at temperature extremes in salmonids (Hanson et al., 2006; Hanson and Farrell, 2007; Keen et al., 1993; Shiels et al., 2002). Adrenergic stimulation has both chronotropic (rate) and ionotropic (force) effects on the teleost heart (Ask, 1983; Axelsson et al., 1987; Cobb and Santer, 1973; Farrell et al., 1986; Farrell et al., 1982; Laurent et al., 1983; Temma et al., 1986; Vornanen, 1989). These effects are mediated through the β-adrenoceptor (β-AR) signalling pathway, which primarily involves a β$_2$ subtype in salmonids (Ask et al., 1980; Ask et al., 1981; Gamperl et al., 1994; Keen et al., 1993). Cardiac adrenergic stimulation is possible through both sympathetic and humoral (catecholamines are released from chromaffin tissue within the head kidney) routes in salmonids, though many other fish lack sympathetic
cardiac innervation (Donald and Campbell, 1982; Farrell and Jones, 1992; Laurent et al., 1983). Indeed, circulating catecholamines increased 10-fold above resting levels in rainbow trout swum to 2 bl s\textsuperscript{-1} and as much as 92-fold in rainbow trout chased to exhaustion (Butler et al., 1986; Perry et al., 1996).

Cardiac sensitivity to adrenaline changes with temperature acclimation in rainbow trout (Ask et al., 1981; Farrell et al., 1996), which has partly been attributed to changes in cell surface β-AR density (Gamperl et al., 1998; Keen et al., 1993). Specifically, cardiac adrenergic stimulation protects rainbow trout cardiac function at low temperatures (Hanson and Farrell, 2007; Graham and Farrell, 1989; Keen et al., 1993), and β\textsubscript{2}-AR density ($B_{\text{max}}$) increased almost 3-fold in cold-acclimated rainbow trout (Keen et al., 1993). However, adrenergic stimulation and protection diminishes at high temperatures in rainbow trout (Farrell et al., 1997; Hanson and Farrell, 2007). In light of these findings, an elevated $B_{\text{max}}$ at warm temperatures could improve cardiac performance and protection, leading to enhanced thermal tolerance.

I sought a mechanistic explanation for the observed differences in thermal tolerance among populations of Fraser River sockeye salmon. Chilko and Nechako sockeye salmon are co-migrating populations that enter the river at the same time and encounter similar warm water temperatures and velocity conditions in the lower Fraser River and Hells Gate (Table 2.1, 3.5). Both the Chilko and Nechako populations have difficult migrations, traveling 642 and 958 km upstream and reaching 1174 and 716 m in elevation, respectively. However, Chilko sockeye salmon spend the final third of their migration ascending the steep, cool Chilcotin River and spawn in or near a glacial lake. Chilko sockeye salmon correspondingly displayed an exceptionally high and broad thermal tolerance compared with Nechako sockeye salmon (Chapter 3). I hypothesized that Chilko sockeye salmon would have a greater β\textsubscript{2}-AR density in
ventricular tissues compared to Nechako sockeye salmon. To test this hypothesis, I compared cell surface $B_{\text{max}}$ and $\beta_2$-AR binding affinity ($K_d$) from Chilko and Nechako sockeye salmon exposed to 13, 19 and 21°C for 4 days. Detailed materials and methods are provided in Chapter 2 (sections 2.2, 2.9 and 2.10.3).

7.2 Results

All Chilko sockeye salmon survived the 4-day treatments at 13, 19 and 21°C, as did all the Nechako sockeye salmon at 13 and 19°C. In contrast, only 4 out of 21 Nechako sockeye salmon (19%) survived the 4-day treatment at 21°C.

There were no significant differences in gross body morphology among temperature treatments or between sexes within a population, except for the significantly higher gonadosomatic index (GSI) of females compared to males in both populations (Table 7.1). Body mass, fork length and condition factor did not significantly differ between the two populations. However, Chilko sockeye salmon had a significantly higher relative ventricular mass (RVM) and a higher GSI compared to Nechako sockeye salmon (Table 7.1).

Independent of the temperature treatment, Chilko sockeye salmon had a 2-fold higher $B_{\text{max}}$ compared to Nechako sockeye salmon (Fig 7.1). Furthermore, $B_{\text{max}}$ significantly increased when Chilko sockeye salmon were warmed to 19° and 21°C from 13°C (Fig 7.1). In contrast, temperature exposure had no effect on $B_{\text{max}}$ in Nechako sockeye salmon. Thus, not only did Chilko sockeye salmon have a greater $B_{\text{max}}$ compared to Nechako, they actually increased $B_{\text{max}}$ in response to warming.
In contrast, $K_d$ did not significantly differ between populations or with temperature treatment (Fig 7.2).

7.3 Discussion

Nechako and Chilko sockeye salmon populations clearly differ in both ventricular $B_{\text{max}}$ and the ability to alter cell surface $B_{\text{max}}$ within a short (4-day) thermal acclimation period. The response to warming in Chilko sockeye salmon sharply contrasts with previous studies that showed a decreased in $B_{\text{max}}$ with warm acclimation in rainbow trout (Gamperl et al., 1998; Keen et al., 1993). Chilko sockeye salmon clearly have a higher thermal tolerance compared to Nechako sockeye salmon, as is evident from the respective aerobic scope Fry curves (Chapter 3), and the observation that only 19% of Nechako sockeye salmon survived the 4-day temperature treatment at 21°C, while all the Chilko sockeye salmon survived. I propose that the elevated $B_{\text{max}}$ for Chilko sockeye salmon may provide greater cardiac performance and protection at temperature extremes and thus may be one of the mechanisms leading to their broader and higher thermal tolerance relative to the Nechako population.

7.3.1 Differences in $B_{\text{max}}$ between Populations

Rainbow trout acclimated to 6°C were included as a reference group to confirm the quality of the assay. The present study’s results ($B_{\text{max}} = 36.3$ fmol mg protein$^{-1}$ and $K_d = 0.23$ nM) fall within expected values. Previous studies reported a $B_{\text{max}}$ of 23-26 fmol mg protein$^{-1}$ and $K_d$ of 0.13-0.19 nM for rainbow trout acclimated to 12-14°C (Gamperl et al., 1998; Hanson et al.,
Gamperl et al. (1994) reported a higher $B_{\text{max}}$ and comparable $K_d$ (40 fmol mg protein$^{-1}$ and 0.25 nM) for rainbow trout acclimated to a colder temperature ($8^\circ$C) in seawater. These results for rainbow trout provide confidence in the assay technique.

$B_{\text{max}}$ for Chilko sockeye salmon was at least twice as high as any other salmonid (Gamperl et al., 1994; Gamperl et al., 1998; Hanson et al., 2005; Olsson et al., 2000). Similarly, their $B_{\text{max}}$ was also more than twice that of non-salmonid athletic fish species: mahimahi ($Coryphaena hippurus$) (46.9 fmol mg$^{-1}$ protein$^{-1}$), skipjack tuna ($Katsuwonus pelamis$) (41.3 fmol mg$^{-1}$ protein$^{-1}$), yellowfin tuna ($Thunnus albacares$) (25.7 fmol mg$^{-1}$ protein$^{-1}$), and Pacific mackerel ($Scomber japonicus$) (27.2 fmol mg$^{-1}$ protein$^{-1}$) (Olsson et al., 2000). Only the winter flounder ($Pleuronectes americanus$) ($B_{\text{max}} = 252$ fmol mg$^{-1}$ protein$^{-1}$) has a higher $B_{\text{max}}$, however, the binding affinity was extremely low, leading the investigators to propose that flounder hearts may also be populated by $\beta_3$-adrenoreceptors (Mendonca and Gamperl, 2009). In contrast, Nechako sockeye salmon displayed $B_{\text{max}}$ values that were similar to mahi-mahi, skipjack tuna and previous estimates of sockeye and chinook salmon (Gamperl et al., 1998; Olsson et al., 2000). Both sockeye salmon populations displayed $B_{\text{max}}$ values well above those determined for African catfish ($Claris gariepinus$) (14.3-17.8 fmol mg$^{-1}$ protein$^{-1}$), warm acclimated-rainbow trout (12-14$^\circ$C, 23-26 fmol mg$^{-1}$ protein$^{-1}$) and an Antarctic nototheniid ($Trematomus bernacchii$) (10.5 fmol mg$^{-1}$ protein$^{-1}$) (Hanson et al., 2005; Olsson et al., 2000).

This experiment on wild fish was conducted under a very controlled setting and many of the potential confounding factors for this population comparison were removed or minimized. For example, all the salmon were collected at the same time and over a period of two days. The fish were collected very early, ~1-3 days into the upriver migration; therefore, they had yet to experience the majority of the upriver conditions and had experienced a common ocean and river
migration environment prior to capture. As a result, population differences were unlikely due to a plastic response to differential environmental conditions encountered prior to capture. Furthermore, all fish were held for the same amount of time and in the same tanks according to temperature treatment, thus eliminating the possibility for differential plastic responses after capture. Also, the differences in $B_{\text{max}}$ were unlikely due to variation in the level of sexual maturation since previous studies suggest that gonadal steroid hormones do not modulate $B_{\text{max}}$ in mature rainbow trout or chinook salmon (Gamperl et al., 1994; Gamperl et al., 1998). Finally, $B_{\text{max}}$ was expressed per mg protein; therefore, the significant difference in RVM was not a factor. However, it is interesting to note that the larger RVM in Chilko sockeye salmon amplifies the difference in the total number of receptors on the ventricle.

7.3.2 Temperature Effects on $B_{\text{max}}$

The increase in $B_{\text{max}}$ with warming in Chilko sockeye salmon was a novel response for fish and was not seen in Nechako sockeye salmon. Two previous studies with rainbow trout showed the opposite pattern, namely, an 11% decrease in $B_{\text{max}}$ per °C increase in temperature (Gamperl et al., 1998; Keen et al., 1993).

Notably, the acclimation duration in the present study (4 days) was much shorter than previous studies (typically >3 weeks, Gamperl et al., 1998; Hanson et al., 2005; Keen et al., 1993). The assay used in the present study cannot determine whether the additional $\beta$-adrenoceptors were synthesized de novo or whether they were simply released from vesicles contained within the cell. Moreover, the time interval required to alter $\beta_2$-AR expression is unknown for fish and is an area of research should be pursued in future studies.
A case has been made for the importance of β2-AR to stimulate maximum cardiac performance during exercise and at high temperature in salmon (Butler et al., 1986; Hanson et al., 2006; Hanson and Farrell, 2007; Randall and Perry, 1992) and to protect maximum cardiac function against a harmful acidotic and hypoxic venous environment, especially at high temperatures (Hanson and Farrell, 2007; Holk and Lykkeboe, 1998; Kiceniuk and Jones, 1977). In vitro and in situ perfused heart studies have consistently shown that acidic and hypoxic conditions lead to impaired cardiac contraction (Driedzic and Gesser 1994; Farrell et al., 1986; (Farrell et al., 1988b; Farrell and Milligan, 1986; Gesser et al., 1982; Gesser and Jorgensen, 1982; Kalinin and Gesser, 2002) and adrenergic stimulation plays a key role in maintaining or enhancing cardiac function under these conditions (Driedzic and Gesser, 1994; Hanson et al., 2006; Hanson and Farrell, 2007; Nielsen and Gesser, 2001).

When the ventricular cell-surface β2-AR is activated, the signalling pathway ultimately increases intracellular calcium delivery to the cardiomyocytes, which enhances both the force and rate of cardiac contraction. Thus, calcium handling in the cardiomyocytes may play a critical role in temperature tolerance in sockeye salmon. Calcium cycling and sarcoplasmic reticulum function has been demonstrated to be critical for the broad temperature tolerance in bluefin tuna (Castilho et al., 2007; Landeira-Fernandez et al., 2004; Shiels et al., 2011). Specifically, bluefin tuna have more sarcoplasmic reticulum Ca\(^{2+}\) ATPase (SERCA2) in their cardiomyocytes, which regulates Ca\(^{2+}\) uptake into the sarcoplasmic reticulum, compared to warmer species that do not have to cope with a wide range of temperatures (Castilho et al., 2007; Landeira-Fernandez et al., 2004). Future studies should examine whether Chilko sockeye salmon similarly have more SERCA2 compared to populations with a more narrow optimal thermal range.
Given that the reduction in aerobic scope at high temperatures can likely be attributed to a limitation in maximum cardiovascular performance (Chapter 5), increased $B_{\text{max}}$ could lead to superior thermal tolerance. Consequently, the exceptionally high and broad thermal tolerance of Chilko sockeye salmon may be due, at least in part, to elevated $B_{\text{max}}$. This hypothesis should be further investigated using perfused heart studies with Chilko and Nechako sockeye salmon.
Figure 7.1. Ventricular β-adrenoceptor density ($B_{\text{max}}$). Significant differences between populations are indicated by * ($p < 0.001$). Significant differences between temperature treatments existed only for Chilko sockeye salmon and are indicated by differing letters. Rainbow trout were included as a reference group to confirm the assay technique.
Figure 7.2. Ventricular β-adrenoceptor [3H]CGP-12177 dissociation constant ($K_d$). No significant differences were detected among sockeye salmon groups. Rainbow trout acclimated to 6°C in freshwater were included as a reference group to confirm the assay technique.
Table 7.1. Gross morphology for fish used in β-AR experiment. Relative ventricular mass (RVM) and gonadosomatic index (GSI) are indicated. Mean ± SEM are presented. Significant differences within and between populations are indicated by differing letters and an asterisk, respectively. N-values for GSI are indicated in parentheses.

<table>
<thead>
<tr>
<th></th>
<th>Chilko</th>
<th>Nechako</th>
<th>Rainbow trout</th>
</tr>
</thead>
<tbody>
<tr>
<td>n</td>
<td>16</td>
<td>18</td>
<td>7</td>
</tr>
<tr>
<td>body mass (kg)</td>
<td>2.14 ± 0.10</td>
<td>2.31 ± 0.10</td>
<td>1.88 ± 0.08</td>
</tr>
<tr>
<td>fork length (cm)</td>
<td>58.0 ± 0.7</td>
<td>58.5 ± 0.6</td>
<td>47.1 ± 1.2</td>
</tr>
<tr>
<td>condition factor</td>
<td>1.09 ± 0.02</td>
<td>1.14 ± 0.03</td>
<td>1.81 ± 0.08</td>
</tr>
<tr>
<td>RVM %</td>
<td>0.156 ± 0.003</td>
<td>0.145 ± 0.004*</td>
<td>0.118 ± 0.009</td>
</tr>
<tr>
<td>GSI (males) %</td>
<td>2.3 ± 0.2 (9)a</td>
<td>1.4 ± 0.1 (10)y*</td>
<td>1.8 ± 0.5</td>
</tr>
<tr>
<td>GSI (females) %</td>
<td>5.1 ± 0.3 (7)b</td>
<td>3.8 ± 0.2 (8)*</td>
<td></td>
</tr>
</tbody>
</table>
CHAPTER 8: CONCLUSIONS

The general objective of this thesis was to examine the physiological basis for thermal tolerance among sockeye salmon populations. I hypothesized that thermal limits are set at a local level by physiological limitations in aerobic performance due to cardiac collapse.

In support of this hypothesis, my research suggests that sockeye salmon populations in the Fraser River watershed have physiologically adapted to meet the specific challenges of their local upriver migration conditions. Thermal optima for each population coincided with the river temperatures typically encountered during upstream migration. Temperatures exceeding the population-specific thermal optimum resulted in severely impaired aerobic scope and swimming performance. My research further suggests that fish are unable to swim at warm temperatures due to insufficient oxygen supply to meet the swimming muscles’ demand, triggered via a cardiac limitation. Finally, I identified that thermal tolerance differs across sockeye salmon populations and suggest a potential mechanism for enhanced thermal tolerance in Chilko sockeye salmon. All told, important management and conservation implications may emerge from my research. I identified a possible cause for in-river mortality associated with warm temperatures in sockeye salmon and I identified certain populations most vulnerable to climate change.

8.1 Local Adaptation in Fraser River Sockeye Salmon Populations

The lifetime fitness of millions of sockeye salmon that annually return to the Fraser River depends on a physically demanding upriver migration. During this once-in-a-lifetime event, fish swim continuously against a fast flowing river for several weeks at ground speeds of 20 to 40 km
day$^{-1}$ (English et al., 2005). Feeding ceases in the ocean and upriver swimming is fuelled entirely by endogenous energy stores. Because sockeye salmon return to natal spawning grounds with remarkable fidelity, the Fraser River is home to more than 100 genetically and geographically distinct populations (Beacham et al., 2005), each of which experiences variable upriver migration conditions, depending on when they enter the river and where they spawn. Thus, populations vary in migration distance (100 to 1100 km), elevation gain (10 to 1200 m), river temperature ($9^\circ$ to $22^\circ$C), and river flow (2000 to 10,000 m$^3$ s$^{-1}$). Reproductively isolated populations can potentially adapt to the environmental conditions that induce maximal aerobic challenges, which for sockeye salmon likely occur during the upriver spawning migration. Indeed, local migratory conditions apparently exert strong selection pressure for adaptation because morphological and behavioural characteristics (gross somatic energy, body morphology, egg number and swimming behaviour) correlate with river migration distance, elevation gain and/or work (distance x elevation gain) in sockeye salmon (Crossin et al., 2004; Hinch and Rand, 2000). Therefore, I hypothesized that physiological adaptation in sockeye salmon occurs locally at the population level, reflecting the specific river migration conditions.

I applied an established conceptual and mechanistic framework for understanding temperature effects on aquatic ectotherms, the oxygen- and capacity-limited thermal tolerance (OCLTT) hypothesis (Pörtner 2002, Pörtner and Knust, 2007, Pörtner and Farrell, 2008). OCLTT attributes the decline in aerobic scope (the difference between resting and maximal oxygen consumption rates) above an animal’s optimal temperature ($T_{\text{opt}}$) to capacity limitations of the organs systems that deliver oxygen to the tissues. The expectation is that local adaptations should extend to multiple levels of the cardiorespiratory system, explaining intraspecific variation in thermal tolerance and aerobic scope.
Eight sockeye salmon populations spanning a range of river migration difficulties were used to varying degrees in my study. Migration difficulty was quantified using various environmental river characteristics: distance, elevation gain, temperature, migration rate, duration, work, river slope and migration effort. I predicted that migration distance, elevation gain and work would exert the strongest selection pressure on aerobic scope given their importance in selecting for morphological traits (Crossin et al., 2004). I measured individual cardiorespiratory performance (N = 97) as a function of temperature in four populations. Aerobic scope curves for each population were significantly related to the historic range in river temperature they experienced, a finding consistent with two additional Fraser River sockeye salmon populations (Farrell et al., 2008; Lee et al., 2003c). The coastal Weaver sockeye salmon experience the coldest temperatures and had the lowest $T_{\text{opt}}$ (14.5°C) whereas the upriver populations experience similar river temperatures and accordingly had a similar $T_{\text{opt}}$ (range 16.4-17.2°C). The upriver Chilko population displayed an unusually broad optimal thermal range that corresponded with the lower temperatures encountered during their difficult migration in the Chilcotin watershed and the high temperatures encountered while migrating through Hells Gate. In addition, significant differences in maximum aerobic scope among the populations were positively correlated with the distance to the spawning ground. These results suggest population level adaptation of maximum aerobic scope to selection imposed by river conditions encountered during migration.

Given that cardiac capacity and aerobic scope are tightly related (Farrell, 2009), I expected populations with the greatest migratory demands to display similar adaptations in cardiac morphology and performance. Relative ventricular mass (RVM), percentage compact myocardium (% compact; the proportion of the ventricle supplied with coronary blood flow) and
relative dry compact mass (RDCM) significantly differed among populations. All three morphological variables were significantly greater for upriver compared with coastal populations, suggesting that the hydraulically challenging sections of the river may impose selection on heart morphology. In addition, correlations between cardiac morphology and migration difficulty, and maximum aerobic scope with RDCM, provide promising evidence for local adaptation to river conditions on an even finer scale (Endler, 1986; Schluter, 2000; Taylor, 1991). Furthermore, aerobic scope, cardiac scope and scope for heart rate were all positively correlated, and varied in parallel with river temperature, suggesting that the temperature dependence of cardiac performance is linked to that of aerobic capacity at the population level. In contrast, maximum cardiovascular performance did not significantly differ among upriver populations, though notably, coastal populations were not included in the analysis. I predict that cardiovascular performance in coastal populations would be reduced compared to upriver populations, mimicking the trend observed with aerobic scope.

Altogether, this is the first ever large-scale study to demonstrate how wild fish within a single watershed are physiologically fine-tuned to their migration environment. I found a strong relationship between the difficulty of river migration and the cardiorespiratory physiology and cardiac morphology of the populations examined. Furthermore, optimal water temperature for aerobic swimming matched the typical water temperatures historically encountered by each population.

The failed attempt to transplant coastal sockeye salmon to upriver spawning grounds in order to help re-establish populations decimated by the 1913 Hells Gate rockslide (Ricker, 1972) provides a cautionary tale to managers. It is becoming clear that coastal populations are not adapted for the more arduous upriver migration and are ill-equipped to complete the more
difficult migration (Taylor, 1991). Combining my results with those found in the literature (Crossin et al., 2004; Gilhousen, 1980; Lee et al., 2003c), the upriver, more athletic populations (Early Stuart, Nechako, Chilko and Quesnel) can be characterized as having more somatic energy at the start of their migration, fewer eggs, a smaller, more fusiform body shape, higher aerobic scope, more energetically efficient swimming behaviour and larger hearts with more compact myocardium compared to coastal populations (Weaver and Harrison). Lower Adams sockeye salmon fall somewhere in-between these extremes.

8.2 Mechanism of Cardiorespiratory Collapse at High Temperature

Sockeye salmon exposed to temperatures above their population-specific $T_{\text{opt}}$ had severely impaired aerobic swimming performance. However, the mechanism of this decline in aerobic scope is poorly understood. Using the OCLTT hypothesis as a framework, I predicted that an oxygen limitation could occur at the level of the gills, the heart or the muscle. By simultaneously measuring oxygen consumption, cardiac output and arterial and venous oxygen status in fish swimming to $U_{\text{crit}}$, I comprehensively examined these possibilities for the first time.

Corroborating earlier work for sockeye salmon swimming at ~75% of $U_{\text{crit}}$ (Steinhausen et al., 2008), my data showed that scope for $f_H$ collapsed at a lower temperature than either aerobic scope, cardiac scope or scope for $V_s$. Thus, my data give weight to the idea that reduced scope for $f_H$ above $T_{\text{opt}}$ is the triggering factor that limits maximum $\dot{Q}$ and the capacity of the cardiorespiratory system to transport oxygen. There was no evidence of a gill limitation since $P_{aO_2}$ and $C_{aO_2}$ remained constant at temperatures above $T_{\text{opt}}$. Furthermore, there did not appear to be an immediate diffusion limitation at the muscle since $P_{vO_2}$ and $C_{vO_2}$ did decline with further
warming above \( T_{opt} \), though a diffusion limitation may have developed at the tissues at the warmer temperatures (Wagner, 1996). All told, the initiating step leading to a mismatch between oxygen supply and demand at the swimming muscle appears to be a cardiac limitation due to reduced scope for heart rate.

8.3 Potential Mechanism for Enhanced Thermal Tolerance

I sought a mechanistic explanation for the observed intraspecific variation in thermal tolerance. Cardiac adrenergic stimulation protects salmonid cardiac function at low temperatures (Keen et al., 1993; Shiels et al., 2002) and against the negative effects of acidosis and hypoxia during exercise (Hanson and Farrell, 2007), but protection diminishes at high temperatures associated with declining aerobic scope (Hanson and Farrell, 2007; Keen et al., 1993). Therefore, I hypothesized that the unusually broad and high thermal tolerance of the Chilko population would reflect a greater density of adrenaline-binding ventricular \( \beta \)-adrenoceptors compared with the co-migrating Nechako population that has a narrower and lower thermal tolerance. I determined ventricular \( \beta \)-adrenoceptor density \( (B_{max}) \) and binding affinity \( (K_d) \) in fish that had been held for 4 d at 13, 19 or 21°C. At all three temperatures, Chilko had a significantly higher \( B_{max} \) compared with Nechako sockeye salmon \( (K_d \) did not differ) and over twice that previously measured for salmonids. In contrast to rainbow trout (Gamperl et al., 1998; Keen et al., 1993), \( B_{max} \) increased significantly when Chilko sockeye salmon were warmed to 19 and 21°C from 13°C. Thus, not only did Chilko sockeye salmon have a greater \( B_{max} \) compared to Nechako, they actually increased \( B_{max} \) in response to warming. Consequently, elevated ventricular \( \beta \)-adrenoceptor expression for Chilko sockeye salmon may provide greater cardiac capacity and
protection at temperature extremes, expanding their thermal tolerance compared with the Nechako population.

8.4 Conservation and Management Implications

Warm river temperatures have been repeatedly associated with high in-river mortality in ecologically, economically and culturally important Fraser River sockeye salmon. Mortality clearly differs across populations and among years in sockeye salmon (Hinch and Martins, 2011). My results support the hypothesis that continued increases in summer river temperatures will result in population-specific responses of sockeye salmon (Farrell et al., 2008).

The sockeye salmon populations included in my study clearly differ in $T_{crit}$ (when aerobic scope is zero and fish survival is passive, time-limited and supported by anaerobic metabolism). While upstream migration is obviously impossible at $T_{crit}$, exactly how much aerobic scope is required for successful river migration is unknown. A biotelemetry study with Weaver sockeye salmon suggests that at least 50% of aerobic scope is needed [<10% of fish reached their spawning area at 18-21°C when aerobic scope is 0-68% of maximal (Farrell et al., 2008; Mathes et al., 2010)]. However, given that all the upriver populations studied here have 89-97% of maximum aerobic scope at the upper 90th percentile of historic temperatures encountered, perhaps ~90% of aerobic scope is necessary over a broader time scale for upriver populations experiencing greater migration difficulty. Accordingly, temperatures exceeding the population-specific upper $T_p$ (temperature corresponding to 90% of maximum aerobic scope, which includes current temperature maxima of 21.5°C) could limit successful migrations due to a functional collapse in aerobic scope. Empirically, no sockeye salmon population has initiated river
migration at a temperature exceeding 21°C (Hyatt et al., 2003), nor has a historic mean migration temperature been above 19°C (Hodgson and Quinn., 2002). However, Chilko sockeye salmon may emerge as “superfish” with greater resilience to climate change by being able to maintain cardiorespiratory performance at higher temperatures. Conversely, Weaver and Nechako populations appear especially susceptible to high temperature. If Weaver sockeye salmon continue to enter the Fraser River up to six weeks earlier than normal (Cooke et al., 2004), exposing themselves to such high temperatures, high en-route mortality will continue (Cooke et al., 2004; Farrell et al., 2008; Mathes et al., 2010).

In summary, while warming water temperatures are undoubtedly a global issue for fishes at the species level, I propose a concern at the population level for Fraser River sockeye salmon. Since current warming trends in the Fraser River (1.9°C during the last 60 years) are expected to continue (Ferrari et al., 2007; Morrison et al., 2002), survival of sockeye salmon populations will require some combination of behavioural adaptations (to avoid high temperatures by entering the river when it is cooler) and physiological adaptations (a higher $T_p$ to increase high temperature tolerance). Substantial shifts in entry timing are unlikely due to energy and time constraints to achieve highly conserved spawning dates. On the other hand, warming river temperatures could exert strong selective pressure for physiological adaptation. Physiological adaptation requires trait heritability, trait variability and differential fitness. Evidence of all three have been presented here: local adaptation of cardiorespiratory traits, individual variability in these traits and zero lifetime fitness for fish failing to complete their upriver migration. The salmonid genome clearly has the capacity for higher thermal tolerance [current thermal extremes are documented for redband trout ($\textit{Oncorhynchus mykiss}$) which experienced 15-27°C diurnally, acutely tolerated 29°C and demonstrated a plateau in aerobic scope at 26°C (Rodnick et al.,
suggesting that there is potential for future physiological adaptation in Fraser River sockeye salmon. I suggest that adaptations at the level of the heart that sustain cardiac performance at high temperatures, such as the increased ventricular $\beta$-adrenoceptor density displayed in Chilko sockeye salmon, could be beneficial in this regard. The current challenge is determining whether the rates and extents of physiological adaptation for Fraser River sockeye salmon will allow them to adapt quickly enough to cope with the current warming trend.

**8.5 Future Directions**

This thesis has identified numerous future directions for further research into questions surrounding the physiological basis of thermal tolerance and local adaptation in fishes.

First, the correlational evidence relating physiological variability to migration difficulty presented here only provides promising, but not definitive, evidence of local adaptation. Therefore, breeding studies should be conducted to look for more conclusive evidence of adaptation. In addition, this thesis only examined a single, brief stage of the life cycle of a sockeye salmon. Indeed, the upriver migration represents only ~2% of a sockeye salmon’s entire life. It would be interesting to examine thermal tolerance and physiological variability across populations in other life stages. In addition, cardiovascular physiology should be examined in adult sockeye salmon from coastal populations to determine if the trends for aerobic scope and cardiac morphology extend to cardiac performance.

This thesis also opens up a myriad of questions regarding cardiorespiratory collapse at high temperature. Comparisons of ultrastructure in heart and skeletal muscle (e.g. capillary and mitochondria density) across populations varying in thermal tolerance and athletic ability would
provide insight into the role of muscle morphology in limiting oxygen diffusion at high temperature and with exercise. Furthermore, the cause of the bradycardia and cardiac disrhythmia during high temperature swimming is unknown. Studies using atropine to block vagal tone should be conducted to determine if the bradycardia and disrhythmia are under cholinergic control. Notably, I was unable to investigate the possibility that thermal tolerance differs between male and female sockeye salmon, which is an area of research that should be pursued. Studies measuring blood flow distribution would also be beneficial, though there are problems with microsphere technique in fish (Farrell et al., 2001b). Direct measurement of gonadal blood flow during swimming and high temperature exposure would provide insight into important trade-offs between swimming and gonad development since all tissues cannot be simultaneously perfused. Finally, the capacity for migrating adult sockeye salmon to acclimate and the role of phenotypic plasticity in temperature tolerance is still poorly understood. Temperature studies should be used to examine the possibility and timecourse for acclimation from the gene to whole animal level.

A major concern emerging from my thesis is that populations are already experiencing temperatures at their upper thermal limit. Since Fraser River temperatures are expected to continue to warm along the same trajectory (~2°C over 60 years, Ferrari et al., 2007; Morrison et al., 2002), populations will have to adapt in order to cope. However, we don’t know which or if any populations will be able to adapt quickly enough to keep pace with the warming temperatures. Therefore, studies examining the rate and extent of physiological adaptation are necessary.
REFERENCES


migration timing and high en route mortality of sockeye salmon in the Fraser River, British Columbia. *Fisheries* 29, 22-33.


