MAXIMUM HEART RATE AS A MEANS OF RAPIDLY ESTIMATING OPTIMAL TEMPERATURE FOR AEROBIC SCOPE IN SALMON: ITS POTENTIAL FOR APPLICATION

by

Matthew Thomas Casselman

B.Sc., Simon Fraser University, 2006

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

The Faculty of Graduate Studies

(Zoology)

THE UNIVERSITY OF BRITISH COLUMBIA (Vancouver)

March 2012

© Matthew Thomas Casselman 2012

ABSTRACT

Knowing the optimal temperature (T_{opt}) for aerobic scope of fishes may be useful for predicting responses to warming environmental temperature because T_{opt} is when fish can allocate the most oxygen to activity. However, the broad application of T_{opt} measurements is hampered by the time required to determine T_{opt} of a species. This thesis sought to develop a rapid method of estimating T_{opt} of Pacific salmon species (genus *Oncorhynchus*) based on evidence that suggests the decline in aerobic scope above T_{opt} is triggered by a limitation on maximum heart rate (f_{H}). Thus, maximum f_{H} at elevated temperature is thought to limit oxygen convection through the circulatory system, and hence limits both maximum metabolic rate and aerobic scope.

Measurements of metabolic rate and $f_{\rm H}$ were taken over a range of temperatures at rest and following exhaustive exercise in juvenile coho salmon (*O. kisutch*) to confirm the association between $T_{\rm opt}$ and maximum $f_{\rm H}$. In vivo measurements revealed a maximum $f_{\rm H}$ limitation at the $T_{\rm opt}$ for aerobic scope, supporting the use of $f_{\rm H}$ to estimate $T_{\rm opt}$.

The potential for expediting measurements of maximum $f_{\rm H}$ during acute warming was investigated using anaesthetized coho salmon and pharmacological stimulation of $f_{\rm H}$. In coho salmon sedated with low doses of anaesthetic, pharmacologically stimulated $f_{\rm H}$ was equivalent to the maximum $f_{\rm H}$ measured *in vivo*. Breakpoint analysis of the relationship between maximum $f_{\rm H}$ and temperature demonstrated a limitation on maximum $f_{\rm H}$ that corresponded closely with the $T_{\rm opt}$ for aerobic scope. Further, while $T_{\rm opt}$ measurements took three weeks to complete, maximum $f_{\rm H}$ measurements were completed in three days. Therefore, the novel maximum $f_{\rm H}$ technique considerably reduced the time needed to estimate T_{opt} and may be broadly suited to estimating T_{opt} both within and outside of the *Oncorhynchus* genus.

Potential application of this rapid T_{opt} estimation method is discussed in relation to temperature data collected from two local coho salmon-bearing streams. Temperature data also allowed for the examination of stream warming and cooling dynamics and identification of habitat critical to buffering anthropogenic disturbances to stream temperature. These data highlight the importance of riparian areas for maintaining the thermal integrity of waterways.

PREFACE

A version of Chapter 2 has been published. M.T. Casselman, K. Anntila, and A.P. Farrell. 2012. Using maximum heart rate as a rapid screening tool to determine optimum temperature for aerobic scope in Pacific salmon *Oncorhynchus* spp. Journal of Fish Biology. 80(2): 358-377.

M.T. Casselman carried out the vast majority of experimental design, data collection, and manuscript preparation. A.P. Farrell provided valuable supervision on experimental design, data analysis, and manuscript preparation. K. Anttila provided assistance with data collection and manuscript preparation. Members of the Seymour Salmonid Society and West Vancouver Streamkeepers assisted with data collection for Chapter 3 and Chapter 4.

All procedures were approved by the University of British Columbia Animal Care Committee in accordance with guidelines set out by the Canadian Council on Animal Care (UBC Animal Care protocols A10-0002 and A10-0236).

TABLE OF CONTENTS

ABSTRAC	CT	ii
PREFACE	Ε	iv
TABLE O	F CONTENTS	v
LIST OF T	TABLES	vii
LIST OF I	FIGURES	viii
LIST OF A	ABBREVIATIONS	X
ACKNOW	VLEDGEMENTS	xi
СНАРТЕІ	R 1. INTRODUCTION	1
	The Influence of Temperature	, I 2
1.1	The Importance of $T_{\rm c}$	
1.2	Limits to Maximum Oxygen Delivery	
1.5	1.3.1 Oxygen Delivery and Untake at the Gills	10
	1.3.2 Oxygen Convection by the Circulatory System	
	1.3.3 Oxygen Deliverv at the Tissues	
	1.3.4 T_{opt} and Maximum f_{H}	
1.4	Expediting T_{out} Estimations	
	1.4.1 Estimating T _{opt} for Aerobic Scope	
	1.4.2 The $f_{\rm H}$ Method for Determining $T_{\rm opt}$	
1.5	Study Species	
1.6	Chapter 2 Objectives	
1.7	Applying T _{opt} Measurements	
1.8	Chapter 3 and Chapter 4 Objectives	
CHAPTEI	R 2: USING MAXMIMUM HEART RATE AS A RAPID SCH	REENING
TOOL TO) ESTIMATE OPTIMUM TEMPERATURE FOR AEROBIC	SCOPE IN
SALMON		
2.1	Introduction	
2.2	Materials and Methods	
	2.2.1 Experimental Animals and Care	

	2.2.2	Aerobic Scope and f _H Measurements in Unanaesthetized Fish	28
	2.2.3	MO ₂ Measurements	30
	2.2.4	f _H Measurements	31
	2.2.5	Maximum $f_{\rm H}$ in Anaesthetized Fish	32
	2.2.6	Experimental Apparatus	33
	2.2.7	Data and Statistical Analysis	34
2.3	Resu	lts	35
	2.3.1	Effect of Temperature on \dot{MO}_2 and f_H in Unanaesthetized Fish	35
	2.3.2	Effect of Temperature on $f_{\rm H}$ in Anaesthetized Fish	37
	2.3.3	Comparison of T _{opt} Estimates	39
2.4	Discu	ussion	39
	2.4.1	Accuracy of T_{opt} and f_H Measurements for Unanaesthetized Fish.	40
	2.4.2	Measuring Maximum $f_{\rm H}$ in Anaesthetized Fish	42
CHAPTEI	R 3: TH	EMPERATURE CONDITIONS AND DYNAMICS IN TWO	
COHO SA	LMO	N-BEARING STREAMS	55
3.1	Intro	duction	55
3.2	Mate	rials and Methods	58
	3.2.1	Study Watersheds	58
	3.2.2	Temperature Monitoring and Data Analysis	59
3.3	Resu	lts	60
3.4	Discu	ussion	63
	3.4.1	Resolution of Temperature Monitoring	64
	3.4.2	Stream Temperature Dynamics	67
СНАРТЕІ	R 4: DI	SCUSSION AND CONCLUSIONS	87
4.1	Com	bining Rapid T _{opt} Determinations with Temperature Data	89
4.2	Com	parative Assessments using the $f_{\rm H}$ Protocol	92
4.3	Exter	nding the Applicability of the f _H Protocol	94
4.4	Adap	ting the $f_{\rm H}$ Protocol to Species and Conditions	95
4.5	Conc	lusions and Perspectives	97
REFEREN	NCES		102
	,		

LIST OF TABLES

Table 2.1. Changes in heart rate following pharmacological treatments	47
Table 2.2. Physical and blood characteristics, cardiac collapse, and	
Arrhenius break temperature analysis of heart rate during an	
acute temperature change	
Table 3.1. Temperature logger locations and basic stream temperature	
parameters from the study watersheds	73
Table 3.2. Differences in temperature between the upper and lower loggers	
for each stream reach within the study watersheds	74
Table 4.1. Habitat thermal quality assessment of the Seymour River and	
Brothers Creek watersheds	
Table 4.2. Results from the field application of the $f_{\rm H}$ protocol to wild	
juvenile coho salmon from the Seymour River and Brothers	
Creek watersheds	

LIST OF FIGURES

Figure 1.1. The temperature-dependent response of reaction rates, Arrhenius plot transformation, and Arrhenius break temperature analysis	23
Figure 1.2. Changes in resting metabolic rate, maximum metabolic rate, and aerobic scope with temperature	24
Figure 2.1. Changes in water bath temperature, internal body temperature, and heart rate during acute warming in anaesthetized coho salmon	49
Figure 2.2. Oxygen consumption, heart rate, and aerobic scope over a range of temperatures in resting and post-chase coho salmon	50
Figure 2.3. Arrhenius plot of resting and maximum heart rate	51
Figure 2.4. Heart rate during acute warming of anaesthetized coho salmon	52
Figure 2.5. Arrhenius plots of pharmacologically stimulated maximum heart rate	53
Figure 2.6. Comparison of optimal temperature estimates	54
Figure 3.1. Area map for the Seymour River, Brothers Creek, and Hadden Creek	75
Figure 3.2. Detail map for the Seymour River	76
Figure 3.3. Detail map for Brothers Creek and Hadden Creek	77
Figure 3.4. Summer temperature frequencies on the Seymour River	78
Figure 3.5. Summer temperature frequencies on Brothers Creek	79
Figure 3.6. Summer temperature frequencies on Hadden Creek	80

Figure 3.7. Summer temperature dynamics on the Seymour River	81
Figure 3.8. Temperature dynamics in Brothers Creek reach B1	82
Figure 3.9. Influence of air and input water temperature on warming in Brothers Creek reach B1	83
Figure 3.10. Temperature dynamics in Hadden Creek reach H2	84
Figure 3.11. Influence of air and input water temperature on cooling in Hadden Creek reach H2	85
Figure 3.12. Regression of air <i>versus</i> water temperature for upper and lower loggers in reach H2	86
Figure 4.1. Polynomial quadratic regression of aerobic scope for juvenile coho salmon and Fry curve to define the temperature ranges for stream thermal assessments	.101

LIST OF ABBREVIATIONS

ABT	Arrhenius break temperature
ATP	adenosine triphosphate
Ca ²⁺	calcium
C_{aO2}	oxygen content of arterial blood
C _{a-vO2}	difference in oxygen content of arterial and venous blood
C_{vO2}	oxygen content of venous blood
ECG	electrocardiogram
ETC	electron transport chain
$f_{ m H}$	heart rate
М	mass
$\dot{M}O_2$	rate of oxygen consumption
MS-222	tricaine methanesulfonate
Na ⁺	sodium
NaCl	sodium chloride
NaHCO ₃	sodium bicarbonate
O ₂	oxygen
P_{aO2}	partial pressure of arterial blood
P_{vO2}	partial pressure of venous blood
Ż	cardiac output
Q ₁₀	temperature coefficient
T _{opt}	optimal temperature; maximum aerobic scope available
T _{crit}	critical temperature; zero aerobic scope available
Vs	stroke volume
U _{crit}	critical swimming speed

ACKNOWLEDGEMENTS

I would first like to thank Dr. Tony Farrell for the past two and a half years of supervision, inspiration and endless patience. Tony is an incredible supervisor who motivated and encouraged me throughout my degree. I greatly appreciate all his support, teachings and the frequent computer quandaries that were welcome diversions. I would also like to thank my committee members, Bill Milsom and Rick Taylor, for their valuable input on my thesis.

The people I met during my time at UBC made my Masters degree a truly amazing experience. I would like to thank all members of the Farrell lab for their support. Big thanks to Katja for keeping me on a rigorous experiment schedule and keeping my inbox full; Georgina and Slabado MaGoo for being amazing office-mates (but probably extending my writing by a month); Erika for all her smarts and being the best beer fairy; and Linda for kicking ass (mine included) in the lab.

I would also like to thank those in the Zoology and Forestry departments that have been terrific for both academic and non-academic support. In particular, Alison 'gong show' Collins, Ken Jeffries, Tim Clark, Mike Sackville, Sarah Fortune, and Trisha Atwood.

The Coho Society of the North Shore, the Pacific Salmon Foundation, and the West Vancouver Streamkeepers provided support for my research. Thank you to the members of these organizations for their field assistance and making the community a part of my MSc.

Finally I would like to thank my parents and family for all their love and support.

CHAPTER 1: INTRODUCTION

The rapid pace of climate change has created a pressing need to understand how animals will respond to the temperature regime shifts expected to occur in most ecosystems (IPCC 2007). Warming temperatures are of particular consequence for aquatic ectotherms such as fishes, where minor changes in temperature have implications for energetics, habitat availability, and ultimately distribution and abundance. For instance, increases in sea temperature of less than 2°C in the past three decades have resulted in significant northward shifts of numerous marine fish species (Dulvy et al. 2008; Perry et al. 2005). If meaningful predictions are to be made regarding ecosystem-level responses to warming, a measure of thermal tolerance is required that is not only applicable at the ecosystem level but can also provide such pertinent information in a timely fashion. My thesis aims to develop a methodology that has the potential to act as a rapid screening tool to assist with meaningful predictions of the tolerance of fishes to warming temperature.

Pacific salmon (genus *Oncorhynchus*) are an ecologically, culturally, and economically important group of fishes that have been well studied in all aspects of their physiology and ecology. Using one species of salmon, this thesis investigated whether measurements of thermal tolerance using a proven, ecologically relevant matrix (aerobic scope) could be expedited using alternative measures based on known physiological limitations of salmon (heart rate) at their upper temperature limits. Further, this thesis sought to demonstrate how the thermal tolerance data generated by this investigation could be combined with water temperature measurements from two salmon-bearing streams to assess the thermal suitability of habitat within these watersheds. This information would be useful in assessing how salmon will respond to increasing environmental temperature due to climate change.

1.1 The Influence of Temperature

Temperature affects all levels of biological organization primarily by increasing the rate of biological reactions. Increases in temperature will raise the mean kinetic energy of molecules according to the Maxwell-Boltzmann distribution and exponentially increase the number of molecules with sufficient energy to participate in a reaction (Haynie 2008). In response, reaction rates also increase exponentially (Figure 1.1A). One of the earliest attempts to quantify the temperature dependence of chemical reaction rates was by van't Hoff, who proposed several equations to quantify this relationship that Arrhenius later assimilated into the Arrhenius equation ($k = Ae^{-E_{\alpha}/RT}$) (Logan 1982). The Arrhenius equation and the work underlying it showed that chemical reactions did indeed increase exponentially with temperature. Arrhenius (1915) later used the Arrhenius equation as well as its linear form $(\ln k = -E_a/RT + \ln A)$ in an Arrhenius plot (Figure 1.1B) to assess the temperature dependence of more complex biological reactions, such as the resting heart rate of a tortoise. Arrhenius found that not only was the temperature response of biological reactions also exponential, but the temperature dependence of these reactions was fairly consistent (Arrhenius 1915). Today, the temperature dependence of biological reactions is most commonly described using Q₁₀ values (van't Hoff 1898; as cited in Blackman 1905), the ratio of reaction rates across a 10°C temperature difference. Not surprisingly, and in keeping with the findings of Arrhenius (1915) that biological reactions increase exponentially, most biological reactions have a Q_{10} between 2 and 3, meaning they will double or triple with a 10°C increase in temperature. Thus, the inescapable laws of thermodynamics that drive increases in the

rates of chemical reactions also apply to biological reactions that similarly increase exponentially with temperature. The exponential increase in biological reaction rates has important consequences for the energy requirements of organisms.

Energy produced during cellular respiration is primarily generated via oxidative phosphorylation and the transfer of electrons down the electron transport chain (ETC) to produce biochemical energy in the form of adenosine triphosphate (ATP). The ETC is located in the mitochondria and is an efficient energy-producing pathway that is dependent on oxygen as the terminal electron receptor (Hochachka and Somero 2002). Cells use the ATP generated in the ETC to power biological reactions that support wholeorganism function. Increased rates of ATP-consuming reactions will therefore require increased ATP generation and increase the oxygen required by a cell. While ATP production in the absence of oxygen (anaerobic) is possible, it is far less efficient (Hochachka and Somero 2002). Alternative energy stores to ATP can also be utilized if oxygen is unavailable, but their supplies are finite and can only meet short-term energetic requirements (Pörtner 2001). Therefore, organisms ultimately rely on oxygen for energy production and this allows the use of whole-organism oxygen consumption (\dot{MO}_2) as an indirect measure of metabolic rate – the combined rate of all energy being used by an organism. Measuring $\dot{M}O_2$ can be used to quantify changes in energy utilization by an organism with changing physiological state or environmental conditions.

The temperature-dependence of biological reaction rates is reflected at the wholeorganism level as an exponential increase in resting $\dot{M}O_2$. Ege and Krogh (1914) were the first to characterize this response in an ectotherm using an acute temperature increase in the goldfish (*Carassius auratus*) (as cited in Clarke and Fraser 2004). This metabolic

response has since been characterized in fishes across taxa (Brett 1971; Gollock et al. 2006; Johnston et al. 1991; Lannig et al. 2004), demonstrating resting \dot{MO}_2 increases with temperature according to thermodynamic principles (Gillooly et al. 2001). It is clear that to support such an increase in oxygen demand an organism must increase oxygen supply to the cells carrying out oxidative phosphorylation.

Given that the early experiments of Ege and Krogh (1914) showed that goldfish could increase resting \dot{MO}_2 exponentially, it is apparent in this case at least, that oxygen supply was capable of meeting demand. However, in order to flourish in the environment, animals must increase their metabolic rate above resting in order to forage, grow, reproduce, avoid predators, and perform a host of other activities. Examining how resting \dot{MO}_2 changes with warming will only reveal the limits of thermal tolerance and the temperature at which an organism's capacity to supply oxygen collapses. Resting \dot{MO}_2 alone does not provide sufficient insight as to how temperature effects on oxygen demand will influence whole-organism function.

1.2 The Importance of T_{opt}

Fry (1947) was the first to recognize that temperature also influenced the activity of ectothermic animals as well as resting $\dot{M}O_2$. Like Ege and Krogh (1914), Fry used goldfish to demonstrate this relationship. However, a key distinction between Fry's work and the earlier work of Ege and Krogh (1914) was that Fry incorporated measures of maximum $\dot{M}O_2$ and, quite importantly, realized that activity was intrinsically linked to $\dot{M}O_2$. Building on this, Fry recognized temperature controlled both the resting and the

maximum \dot{MO}_2 and that the capacity for an organism to perform activity lay in the difference between these two measurements, which Fry termed *scope for activity*. By measuring resting and maximum \dot{MO}_2 across a temperature range, Fry observed that resting \dot{MO}_2 increased exponentially across the temperature range, as expected, but maximum \dot{MO}_2 increased exponentially only at lower temperatures (Figure 1.2A). The increase in maximum \dot{MO}_2 then failed to keep pace with resting \dot{MO}_2 and plateaued at a temperature mid-way between the upper and lower temperatures measured for resting \dot{MO}_2 . When Fry plotted the *scope for activity* over the temperature range, the curve was bell-shaped with a distinct maximum (Figure 1.2B). The maximum for this 'Fry curve' (Farrell 2009) defines the optimal temperature (T_{opt}) where the capacity for extraneous activity should be maximal. As a result, Fry postulated that T_{opt} would be a more ecologically relevant measure of thermal tolerance than previous measures that focused on upper and lower thermal limits (Brett 1944; Britton 1924).

Fry's *scope for activity* framework is now known as aerobic scope and further studies of aquatic ectotherms and in particular salmonids, have confirmed the link between T_{opt} for aerobic scope and activity. Gibson and Fry (1954) demonstrated maximal swimming speed coincided with T_{opt} for aerobic scope of lake trout (*Salvelinus namaycush*) and Brett (1964) found equivalent results with juvenile sockeye salmon (*Oncorhynchus nerka*). Recent studies with adult coho salmon (*Oncorhynchus kisutch*) and sockeye salmon have not only reiterated this relationship, but also demonstrated that T_{opt} is species- (Lee et al. 2003) and even population-specific (Eliason et al. 2011). The likely dependence of fitness on swimming ability (Plaut 2001) suggests that temperatures away from T_{opt} that

reduce aerobic scope will have ecological relevancy. Further, growth may also decline at temperatures away from T_{opt} (Brett et al. 1969; Elliott and Hurley 2000).

The ecological importance of T_{opt} has been demonstrated with investigations into the impacts of climate change where warming trends in both freshwater and marine environments are already exposing fishes to adverse temperatures. In the marine environment, Pörtner and Knust (2007) found that temperatures exceeding the T_{opt} of eelpout (*Zoarces viviparus*) in the North and Baltic Seas caused a northern distribution shift and reduced abundance. In freshwater, temperatures in the Fraser River, British Columbia, Canada have on occasions already exceed the T_{opt} of migrating sockeye salmon and have been linked to upstream migratory failure (Farrell et al. 2008). Thus, measures of T_{opt} are potentially useful in predicting when environmental temperature may negatively impact a species.

1.3 Limits to Maximum Oxygen Delivery

It is readily apparent when examining a Fry curve alongside maximum and resting \dot{MO}_2 that T_{opt} for aerobic scope is defined by the plateau of maximum \dot{MO}_2 because resting \dot{MO}_2 continues to increase exponentially above T_{opt} (Figure 1.1A). Fry and Hart (1948) commented that the plateau in maximum \dot{MO}_2 was likely due to a deficiency in oxygen transport. A modern synthesis of this hypothesis is the concept of Oxygen and Capacity-Limited Thermal Tolerance (Pörtner 2001, 2010), whereby thermal tolerance is ultimately set by the maximum capacity of the ventilatory and circulatory systems to deliver oxygen to the tissues. Given the likely fitness consequences of reduced aerobic

scope, there have been investigations (Eliason 2011; Steinhausen et al. 2008) to elucidate the mechanistic basis for the failure of maximum $\dot{M}O_2$ to continue increasing above T_{opt} . Identifying potential mechanisms to explain this maximum $\dot{M}O_2$ limitation requires understanding how oxygen is moved through the cardiorespiratory oxygen cascade, how fish increase oxygen delivery to meet the increased oxygen requirements of activity, and how active fish support the additive oxygen demands of warming temperature.

Oxygenated water flows nearly continuously across the gills of the fish by the combined action of the buccal and opercular pumps. Oxygen diffuses from the water, across the secondary gill lamellae and into the lamellar capillaries where it binds to hemoglobin in red blood cells. Oxygen delivery to and across the gills is governed by ventilation rate and volume, diffusion distance, surface area, gill perfusion and the diffusion gradient for oxygen. Blood is then pumped by the heart from the gills, through the circulatory system, and delivered to the tissue capillaries. Convection of oxygen through the circulation is governed by cardiac output (\dot{Q}) and hemoglobin concentration. At the tissues, the diffusion gradient between the blood and the tissues favors oxygen diffusion from hemoglobin to the cell's mitochondria. Again, oxygen delivery to the tissues is governed by capillary perfusion, diffusion distance, surface area, and the partial pressure gradient. Partially deoxygenated venous blood then returns to the heart, which is the last organ in the circulatory system to receive oxygen before the venous blood is pumped to the gills and re-oxygenated.

During activity, the \dot{MO}_2 of active fish can be 15x that of resting fish (Brett 1964, 1965) with the swimming muscles accounting for nearly all of the increased oxygen consumption (Randall and Daxboeck 1982). To meet this increase in oxygen demand,

fish invoke a suite of physiological mechanisms to increase oxygen transport to the skeletal muscle mitochondria. Fish increase oxygen delivery to the gills by increasing ventilation rate and volume and may even ram ventilate if sufficient swimming velocities are obtained (Steffensen 1985). To increase oxygen uptake across the gills, fish increase lamellae perfusion (Booth 1979; Farrell et al. 1979). Oxygen diffusion from the water to the blood is also aided by a lower venous partial pressure of oxygen (P_{vO2}) from increased tissue oxygen extraction that increases the diffusion gradient (Farrell and Clutterham 2003; Kiceniuk and Jones 1977). Oxygen convection in the circulatory system is increased via \hat{Q} through a combination of increased heart rate ($f_{\rm H}$) and stroke volume (V_s) (Brett 1971; Farrell and Jones 1992; Kiceniuk and Jones 1977). For salmonids, \dot{Q} is primarily increased via $V_{\rm s}$ during activity. Release of additional red blood cells from the spleen will also increase oxygen convection (Gallaugher et al. 1992). Finally, increased capillary perfusion at the tissues will increase the surface area for diffusion while potential Bohr and Root effects that decrease the oxygen-affinity of hemoglobin will increase tissue oxygen removal from the blood at the capillaries. Thus, at a fixed temperature, fish have the capacity to greatly increase oxygen supply when the demands of activity necessitate. For salmon at least, it is thought that the finite capacity for oxygen delivery is expressed during maximal aerobic swimming.

Increased tissue oxygen demand with warming is supported by thermodynamic effects that increase the capacity of the oxygen delivery mechanisms and maximum \dot{MO}_2 . For example, rates of muscle contraction and relaxation increase with temperature (Randall and Brauner 1991) allowing fish to further increase ventilation rate and \dot{Q} (Heath and Hughes 1973; Steinhausen et al. 2008). Elevated temperature will also right-

shift the oxyhemoglobin dissociation curve (Jensen et al. 1998) enabling faster and greater oxygen unloading at the tissues. In addition, oxygen diffusion will occur exponentially faster at higher temperatures, although this effect is small for biologically relevant temperatures (Dejours 1981). As a result, the maximum \dot{MO}_2 that can be obtained increases with temperature. However, the plateau of maximum \dot{MO}_2 past T_{opt} clearly demonstrates that temperature-induced increases in maximal oxygen delivery capacity eventually become limited. The temperature of this limitation is also well below the maximum temperature tolerable by resting fish. Thus, a weak link in the steps of the cardiorespiratory oxygen cascade develops when active fish are warmed beyond T_{opt} .

Data regarding the mechanistic underpinnings for this limitation at T_{opt} are most readily available for salmon. Previous studies (Eliason 2011; Steinhausen et al. 2008) investigated the basis for this limitation at four of the five steps in the cardiorespiratory oxygen cascade: oxygen delivery (i) and uptake (ii) at the gills, oxygen convection by the circulatory system (iii), and oxygen diffusion at the tissues (iv). The role of mitochondrial respiration (v) in limiting maximum $\dot{M}O_2$ above T_{opt} has not been investigated in salmon, although some studies have investigated changes in mitochondrial properties with temperature acclimation (Bouchard and Guderley 2003; St-Pierre et al. 1998). However, Pörtner (2001) suggested the thermal sensitivity of mitochondria is less than that of the more complex ventilatory and circulatory systems. Mitochondrial respiration rates may be maintained above T_{opt} and would not contribute to a maximum $\dot{M}O_2$ limitation, although evidence to support this is lacking. The following sections will therefore outline the current knowledge regarding the limits to oxygen delivery at high temperatures in

three areas (gills, circulation, tissues) covering steps i-iv of oxygen cascade and discuss the contribution of each of these areas to the maximum \dot{MO}_2 limitation at T_{opt} in salmon.

1.3.1 Oxygen Delivery and Uptake at the Gills

Early studies that observed a collapse in fish swimming performance at elevated temperatures speculated that the primary factor in this collapse was the reduced oxygen concentration in water (Brett 1971). This suggestion was based on the fact that the solubility of oxygen in water decreases by ~2% for every 1°C increase in temperature (Dejours 1981). Decreased oxygen availability could then lead to a diffusion limitation for oxygen across the gill lamellae that would reduce arterial partial pressure (P_{aO2}) and blood oxygen content (C_{aO2}). In support of this hypothesis, Heath and Hughes (1973) observed decreased C_{aO2} with temperature in acutely warmed resting rainbow trout (Oncorhynchus mykiss). However, data from active fish obtained in recent studies support neither the speculations of Brett (1971) nor the findings of Heath and Hughes (1973). For example, Steinhausen et al. (2008) found that both P_{aO2} and C_{aO2} were unchanged during an acute temperature increase above T_{opt} in sockeye salmon swimming at ~75% of maximal aerobic capacity. Further, investigation by Eliason (2011) with maximally swimming sockeye salmon also showed no decrease in PaO2 or CaO2 at temperatures above known T_{opt} values. These more recent data suggest that the earlier findings on resting rainbow trout of Heath and Hughes (1973) were likely due to a ventilatory limitation. Thus in the absence of a ventilatory limitation, salmon appear to be able to supply sufficient oxygen delivery to the gills to maintain P_{aO2} and C_{aO2} above T_{opt} .

1.3.2 Oxygen Convection by the Circulatory System

Convection of oxygen through the circulatory system is a product of the CaO2 and the \dot{Q} of the heart, which itself is a product of V_s and f_H . When fish are warmed, increases in \hat{Q} occur entirely through increases in $f_{\rm H}$. This has been demonstrated in resting and swimming salmon (Brodeur et al. 2001; Clark et al. 2008; Gamperl et al. 2011; Sandblom and Axelsson 2007; Steinhausen et al. 2008) and also appears to be a general trend among non-salmonid fishes (Gollock et al. 2006; Mendonça and Gamperl 2010; Stevens et al. 1972). The reliance on $f_{\rm H}$ for increases in Q is likely due to the temperaturedependent frequency of the heart's pacemaker cells (Randall 1970), which will contribute to exponentially increasing both resting and maximum $f_{\rm H}$, at least at lower temperatures. Thermodynamic effects on $f_{\rm H}$ should therefore maintain the scope for increasing $f_{\rm H}$ during warming. However, $f_{\rm H}$ has a finite capacity to increase (Farrell 1991) and would be expected to become limited as it approached this upper threshold, with maximum $f_{\rm H}$ becoming limited at a lower temperature than resting $f_{\rm H}$. Maximum $f_{\rm H}$ may increase slightly above this limitation, but $f_{\rm H}$ scope will decline since resting $f_{\rm H}$ continues to increase exponentially. Accordingly, a limitation on maximum $f_{\rm H}$ would be expected to restrict oxygen convection through the circulatory system and limit maximum \dot{MO}_2 . Indeed, the data of Steinhausen et al. (2008) and Eliason (2011) demonstrate that maximum $f_{\rm H}$ in swimming sockeye salmon became limited and failed to increase exponentially once a temperature that coincided with the plateau in maximum \dot{MO}_2 had been reached. Further, Steinhausen et al. (2008) and Eliason (2011) found $f_{\rm H}$ scope declined past T_{opt} . Thus, there is solid evidence that a limitation on maximum $f_{\rm H}$ plays a

central role in defining T_{opt} by reducing f_H scope, limiting oxygen convection through the circulatory system and causing a decline in aerobic scope.

1.3.3 Oxygen Delivery at the Tissues

Whole-organism tissue oxygen utilization is calculated by the Fick equation $(\dot{MO}_2 = \dot{Q} \times A - V_{O2})$, which is the product of the difference in oxygen content between arterial and venous blood (C_{a-vO2}) and \dot{Q} . As previously discussed, blood is fully saturated with oxygen when leaving the gills and C_{aO2} does not decrease with temperature. Warming increases oxygen consumption at the tissues, decreasing CvO2 and increasing C_{a-vO2}. Thus, P_{vO2} should steadily decrease in the absence of a diffusion limitation. If an oxygen delivery limitation to the tissues did arise at T_{opt} , for instance due to inadequate capillarization, venous oxygen parameters would remain constant and Ca-vO2 would plateau. Steinhausen et al. (2008) observed a general trend towards a steadily increasing C_{a-vO2} and decreasing C_{vO2} with an acute temperature increase in swimming sockeye salmon, although neither changes in Ca-vO2 nor CvO2 reached statistical significance. These results suggest no diffusion limitation was present. However, Steinhausen et al. also found P_{vO2} did not change with warming, supporting the possibility of a diffusion limitation. The findings of Eliason (2011) with maximally swum sockeye salmon did not support a tissue limitation as both PvO2 and CvO2 decreased past T_{opt} . Together, these studies demonstrate there is still a reasonable degree of uncertainty regarding the possibility of a diffusion limitation developing at the tissues of active salmon. However, the gradual onset of the observed changes suggests a diffusion

limitation would likely only develop at temperatures higher than T_{opt} . Further, active salmon may still have some capacity to increase venous oxygen extraction above T_{opt} , apparent as the more rapid decline of f_{H} scope in comparison to aerobic scope.

1.3.4 T_{opt} and Maximum f_{H}

To summarize, the temperature at which maximum \dot{MO}_2 plateaus ultimately defines the T_{opt} for aerobic scope because resting \dot{MO}_2 continues to increase exponentially above T_{opt} . The current evidence from salmon indicates that the plateau of maximum \dot{MO}_2 at T_{opt} is triggered by the failure of maximum f_H to maintain an exponential increase with increasing temperature. Therefore, the temperature at which maximum f_H becomes limited may be a useful predictor of the T_{opt} for aerobic scope. The limitation on maximum f_H has also been proposed to initiate a series of events that lead to the death of exercising salmon at high temperatures (Farrell et al. 2009). The maximum f_H limitation at T_{opt} , which causes a decline in f_H scope and limits \dot{Q} , places a perfusion limitation. Inadequately perfused tissues must switch to anaerobic metabolism, causing metabolic acidosis and an adverse venous environment for the cardiac muscle and the eventual collapse of cardiac function apparent as cardiac arrhythmia (Clark et al. 2008). The death of the salmon follows soon after.

1.4 Expediting *T*_{opt} Estimations

Relatively few studies of salmon or other fishes have incorporated T_{opt} measurements. The lack of T_{opt} application is not so much because the results are uninformative, but rather because it is time consuming to determine T_{opt} for aerobic scope with an exacting methodology. The length of time required to estimate T_{opt} is therefore the most obvious shortcoming of the aerobic scope methodology.

The association of T_{opt} and a maximum $f_{\rm H}$ limitation may provide an analytical method for expedited T_{opt} estimates. Although an exponential increase is typical of biological rates, divergence from this relationship can occur if biological systems are observed over a wide enough temperature range. Crozier (1926) was one of the first to demonstrate a departure from the exponential increase in a biological rate function and interestingly, demonstrated this with the $f_{\rm H}$ of larval fish. Crozier used Arrhenius plots (see Section 1.1) to describe a discontinuity or 'break' in the increase of $f_{\rm H}$ at a certain temperature. Crozier observed that the normally linear increase in $f_{\rm H}$ that occurs on an Arrhenius plot failed to maintain a constant slope throughout the measured temperature range (Figure 1.1C). The temperature at which a break in an Arrhenius plot occurs is known as the Arrhenius break temperature (ABT). These century-old observations highlight an analytical method that may allow a straightforward approach to estimating T_{opt} of salmon. Given that T_{opt} coincides with maximum f_{H} failing to increase exponentially with temperature, the ABT for maximum $f_{\rm H}$ could act as a surrogate measure of $T_{\rm opt}$. Further, this approach may allow T_{opt} estimates to be expedited if the ABT for maximum f_{H} can be

rapidly determined. Understanding why an expedited methodology is desirable, however, requires a familiarity with the current method for estimating T_{opt} for aerobic scope.

1.4.1 Estimating T_{opt} for Aerobic Scope

Estimating T_{opt} for aerobic scope for a fish species requires measurements of resting and maximum $\dot{M}O_2$ across a range of tolerable temperatures in order to generate a Fry curve. Three approaches are used in order to obtain measurements across a suitable temperature range: fish can be acclimated to specific test temperatures over the long-(weeks) (Zeng et al. 2010) or short-term (days) (Eliason et al. 2011); be tested at ambient temperatures (Lee et al. 2003); or receive an acute (hours) temperature increase to the test temperature from a common holding temperature (Clark et al. 2008; Eliason et al. 2011).

Once a fish is at the appropriate temperature, resting \dot{MO}_2 can be obtained using intermittent flow respirometry (Ege and Krogh 1914; Steffensen 1989). Inevitably, handling of the fish is required prior to any resting measurements and fish must be allowed to recover from handling stress and the associated metabolic perturbations (Davis and Schreck 1997). Approximately 12 h has emerged as a standard minimum recovery time, although slightly shorter times are permissible (Clark et al. 2008). Further, if diurnal fluctuations in activity are to be considered, resting \dot{MO}_2 should be continuously monitored over a minimum 24 h period (Steffensen 1989).

Generating maximum \dot{MO}_2 requires forcing a fish to actively swim and can be accomplished with exhaustive exercise (Reidy et al. 1995; Scarabello et al. 1991, 1992) or the use of a swim tunnel (Brett 1964; Farrell et al. 2003) and a standardized exercise

protocol, such as the critical swimming speed (U_{crit}) test (Jain et al. 1997). Both of these techniques must also be combined with respirometry. Exhaustive exercise protocols are the most rapid exercise method and can generate maximum \dot{MO}_2 with a brief period (minutes) of manual chasing (Scarabello et al. 1991, 1992).

Minimizing the time required for this protocol by allowing the minimum handling recovery time and using an acute temperature increase combined with exhaustive exercise, would permit resting and maximum $\dot{M}O_2$ values for a single fish to be obtained in ~24 h. However, resting and maximum $\dot{M}O_2$ measurements must be repeated on a sufficient number of fish at a minimum of five temperatures to generate a suitable Fry curve and permit a T_{opt} estimation. Taken together, generating a complete Fry curve necessitates a minimum 3-4 week period of continuous experimentation.

1.4.2 The $f_{\rm H}$ Method for Determining $T_{\rm opt}$

Using maximum $f_{\rm H}$ as an alternative to $\dot{\rm MO}_2$ measurements requires consideration of the methodology, given that the primary goal of any alternative technique should be to reduce experimental duration. For example, simply measuring $f_{\rm H}$ in a maximally swimming fish during an acute temperature increase would provide no advantages in comparison to $\dot{\rm MO}_2$ measurements. Each fish would have to be anaesthetized and undergo surgical procedures to either attach or implant (Clark and Farrell 2011) electrodes. Wireless $f_{\rm H}$ monitoring is possible (Altimiras and Larsen 2000; Rommel 1973), but is highly susceptible to skeletal muscle interference during activity. In addition, a conservative rate of temperature change (2°C h⁻¹; Steinhausen et al. 2008)

would be necessary to allow fish to reach a steady state at each test temperature while a number of temperatures would have to be tested for a wide temperature range. Variations of this protocol are possible, but the necessity of an exercise component to generate maximum $f_{\rm H}$ and the moderate rates of temperature increase required for active fish prevent experimental duration from being significantly reduced.

In lieu of whole-organism activity to stimulate increases in $f_{\rm H}$, the cardiac control mechanisms in salmon present an opportunity to investigate whether pharmacological stimulation of maximum $f_{\rm H}$ is possible. The salmon heart has an intrinsic rhythm set by the pacemaker cells located in the sinoatrial node. This cardiac rhythm is due to 'funny' Na⁺ channels that give pacemaker cells an unstable membrane potential that steadily depolarizes until an action potential threshold is reached. Modulation of the pacemaker rhythm occurs via two mechanisms: cholinergic inhibition and adrenergic excitation. The vagal nerve directly innervates the sinoatrial node and releases acetylcholine that interacts with muscarinic receptors on the pacemaker cells. The binding of acetylcholine triggers a signaling cascade that opens additional ion channels, hyperpolarizing the pacemaker cells, and decreasing action potential frequency and $f_{\rm H}$ (bradycardia). In contrast, adrenaline and noradrenaline released from the chromaffin cells in the head kidney moves through the circulatory system, binds to β -adrenoreceptors on the heart, triggering a cascade that increases the rate and frequency of depolarization and elevates $f_{\rm H}$ (tachycardia). In both cases, pharmacological agents are available that either block or stimulate these pathways. Atropine is a muscarinic antagonist that will block vagal tone and increase $f_{\rm H}$. Isoproterenol is a β -agonist that will stimulate β -receptors and further increase $f_{\rm H}$. Both atropine and isoproterenol have been used extensively to elucidate

cardiac control mechanisms in fishes (Altimiras et al. 1997; Axelsson and Farrell 1993; Randall et al. 1967; Stecyk and Farrell 2006; Wood et al. 1979). However, it is unknown whether pharmacological stimulation alone is sufficient to produce an increase in $f_{\rm H}$ equivalent to that observed in exercising fish.

If maximum $f_{\rm H}$ can be achieved with pharmacological stimulation, ABT analysis could be performed on individual fish. Each fish could be warmed and maximum $f_{\rm H}$ data collected at numerous temperatures across a wide temperature range. This would permit sufficient $f_{\rm H}$ data to be collected from an individual for ABT analysis (that requires a minimum number of temperatures on either side of the 'break'). Pharmacological stimulation would also alleviate concerns regarding the effects of prolonged exercise on cardiac performance (e.g. Steinhausen et al. 2008). The absence of the required exercise might also allow the fish to be restrained or possibly anaesthetized to permit easier $f_{\rm H}$ detection. Increased rates of temperature change may also be possible in anaesthetized fish. Chapter 2 of this thesis explored these possibilities with the overarching goal of expediting $T_{\rm opt}$ measurements in salmon.

1.5 Study Species

Pacific salmon are the focus of this study because of the well-defined mechanistic basis for the collapse of aerobic scope above T_{opt} . Of the five Pacific salmon species, I selected juvenile coho salmon because the fry life stage of coho salmon is characterized by a fluvial residency period of one to two years prior to seaward migration (Quinn 2005) and is one of the longest life history periods within *Oncorhynchus*. This is important, given that thermal tolerance can vary with life stage (Pörtner and Farrell 2008). Using juvenile coho salmon would also allow for the potential application of T_{opt} estimates to assess local coho salmon habitat (see Section 1.7).

An important consideration with the use of juvenile coho salmon is that the evidence supporting maximum $f_{\rm H}$ as the primary factor determining $T_{\rm opt}$ has been derived entirely from studies of adult salmon. However, the adult life stage is not suited to the long-term freshwater holding necessary for this investigation due to the semelparous life cycle of salmon. In contrast, while juvenile coho salmon are amendable to prolonged holding there are no data from juvenile salmon to support the role of $f_{\rm H}$ in setting $T_{\rm opt}$. Thus, the presence of a $T_{\rm opt}$ -maximum $f_{\rm H}$ relationship must be demonstrated prior to using juvenile coho salmon to investigate the possibility of expediting $T_{\rm opt}$ estimates.

1.6 Chapter 2 Objectives

The first objective of Chapter 2 was to demonstrate that a limitation on maximum $f_{\rm H}$ occurs at $T_{\rm opt}$ for aerobic scope in juvenile coho salmon. This objective builds on the evidence from adult salmon that a limitation on maximum $f_{\rm H}$ limits maximum \dot{MO}_2 and aerobic scope. I hypothesized that juvenile coho salmon will exhibit the same signs of cardiac impairment above $T_{\rm opt}$ as adult salmon and that the Arrhenius break temperature for maximum $f_{\rm H}$ will coincide with $T_{\rm opt}$ for aerobic scope.

The second objective in Chapter 2 was to develop a rapid assay to define T_{opt} using pharmacological stimulation of $f_{\rm H}$ in lieu of exercise. This possibility was investigated using anaesthetized juvenile coho salmon. First, I hypothesized that pharmacological

stimulation of maximum $f_{\rm H}$ in anaesthetized coho salmon will produce an equivalent maximum $f_{\rm H}$ to that seen in exercising salmon. Secondly, I hypothesized that during acute warming, the Arrhenius break temperature in maximum $f_{\rm H}$ will occur at $T_{\rm opt}$.

1.7 Applying *T*_{opt} Measurements

Application of T_{opt} requires information on the temperatures encountered by a species. For fishes, this information is most easily obtained by measuring water temperature directly in a species' habitat. Such data can then be combined with T_{opt} estimates to assess, for instance, how current or future temperature regimes may impact fitness and survival.

Examples of T_{opt} application are most abundant in the Fraser River, British Columbia, Canada where over 60 years of historic temperature monitoring, detailed sockeye salmon escapement enumeration, and T_{opt} estimates from several upstream migrating sockeye salmon populations have allowed complex T_{opt} analyses. Farrell et al. (2008) correlated historically encountered river migration temperatures with T_{opt} estimates to suggest population-level T_{opt} adaptation in sockeye salmon based on the respirometry studies of Lee et al. (2003). Further, this association was used to retroactively explain the failed upstream migration of a sockeye salmon population during a year of uncharacteristically warm river temperatures. Eliason et al. (2011) expanded this analysis and confirmed local T_{opt} adaptation and suggested climate warming would have population-specific effects on sockeye salmon. Hague et al. (2011) used climate change scenarios to model future Fraser River temperature and predicted

population-specific climate effects on upstream migratory success. However, outside of the upriver migration of Fraser River sockeye salmon, no other studies have applied T_{opt} estimates to determine how thermal regimes may be affecting other salmon species.

Upriver migration aside, the freshwater riverine environment still remains a priority for application of T_{opt} given most salmon use freshwater habitat to some degree for juvenile rearing (Quinn 2005). The complex interaction of numerous abiotic and biotic factors at the fry life stage does not allow the same straightforward link between $T_{\rm opt}$ and fitness as is possible with adult salmon migration, the dichotomous nature of which results in likely reproduction if migration is successful, or zero lifetime fitness if unsuccessful (Farrell et al. 2008). Regardless, juvenile salmon rearing at temperatures above T_{opt} can be expected to have reduced fitness, given that sub-optimal temperatures limit swimming ability (e.g. Gibson and Fry 1954; see Section 1.3) and in turn affect foraging, growth, competitive ability, and predator avoidance. Consequently, species such as coho salmon, that are dependent on freshwater habitat as a nursery for emergent fry and are limited in their freshwater abundance by habitat availability (Quinn 2005), can see reduced overall abundance if temperatures exceed T_{opt} in the freshwater environment. Therefore, even though a direct link to lifetime fitness cannot be made, there is still significant merit to combining T_{opt} estimates with temperature data for rivers and streams where species such as coho salmon are present.

Recording temperature in waterways has been made dramatically easier by the advent of inexpensive temperature loggers (Dunham et al. 2005). As a result, detailed measures of temperature can be used to record temperature regimes of individual streams and describe temperature dynamics. Combining such temperature information with a

rapid method to estimate T_{opt} may provide species- and population- specific information on salmon habitat thermal quality. Further, temperature dynamics can be used to identify areas important to maintaining temperature conditions in streams. Such information would be useful to managers hoping to conserve salmon populations threatened by warming due to climate change or other anthropogenic disturbances.

1.8 Chapter 3 and Chapter 4 Objectives

The first objective of Chapter 3 was to describe the temperature conditions in two local, coho salmon-bearing waterways. To achieve this, I recorded high-resolution stream temperature data throughout the two study systems. A second objective of Chapter 3 was to examine the temperature dynamics within these streams to determine if particular stream reaches or habitat features acted to maintain or degrade the thermal integrity of waterways. Chapter 4 then discusses the potential for rapid T_{opt} estimations from juvenile coho salmon to be combined with these stream temperature data and summarizes the findings of this thesis.



Figure 1.1. (A). The exponential increase in the rate of chemical and biological reactions with temperature as quantified by the Arrhenius equation ($k = Ae^{-E_{\alpha}/RT}$). (B) Transformation of (A) into an Arrhenius plot using the linear version of the Arrhenius equation ($\ln (k) = -E_{\alpha}/RT + \ln A$). (C) Arrhenius plot showing the Arrhenius break temperature (*****) as a discontinuity in the increase of the reaction rate with temperature.



Figure 1.2. (A) Increases in resting (green line) and maximum (blue line) oxygen consumption of the goldfish (*Carassius auratus*) with temperature. (B) The aerobic scope (maximum *minus* resting oxygen consumption) and optimal temperature for aerobic scope (T_{opt}). Modified from Fry (1947).

CHAPTER 2: USING MAXMIMUM HEART RATE AS A RAPID SCREENING TOOL TO ESTIMATE OPTIMUM TEMPERATURE FOR AEROBIC SCOPE IN SALMON^{*}

2.1 Introduction

It is well established that fishes have optimal temperatures for performance. Important rate functions such as \dot{MO}_2 , growth and swimming ability are maximal at a species-specific T_{opt} (Brett 1971; Elliott and Elliott 2010; Gibson and Fry 1954; Selong et al. 2001). The sharp decline in the capacity of fish to perform above T_{opt} is proposed to be the result of an oxygen limitation brought about by the temperature dependence of aerobic scope (Pörtner 2001, 2010; Pörtner and Farrell 2008). Originally conceived by Fry (1947), aerobic scope is the difference between resting and maximum \dot{MO}_2 and measures the capacity of a fish to perform activity above basic requirements. A Fry curve for aerobic scope (Farrell 2009) describes the relationship between aerobic scope and temperature, with the greatest aerobic scope occurring at T_{opt} . Above T_{opt} , aerobic scope decreases because maximum \dot{MO}_2 fails to keep pace with resting \dot{MO}_2 as temperature increases. With less available oxygen above T_{opt} , reduced growth and abundance and increased mortality are possible (Farrell et al. 2008; Pörtner and Knust 2007).

Measurements of T_{opt} for aerobic scope are already proving to be an effective tool in predicting the affects of seasonal temperature change and future climate change

^{*} A version of this Chapter has been published. M.T. Casselman, K. Anttila, and A.P. Farrell. 2012. Using maximum heart rate as a rapid screening tool to determine the optimal temperature for aerobic scope in Pacific salmon *Oncorhynchus* spp. Journal of Fish Biology. 80(2): 358-377.
scenarios on different fish species. Present applications of T_{opt} estimates include the shifting distribution and abundance of eelpout and Atlantic cod (*Gadus morhua*) in the North Sea (Pörtner et al. 2001; Pörtner and Knust 2007), the impacts of climate change and ocean acidification on coral reef fishes from the Great Barrier Reef, Australia (Munday et al. 2009), the effects of warming on southern catfish (*Silurus meridionalis*) in the Yangtze and Jialing Rivers, China (Zeng et al. 2010), river migration of coho salmon and sockeye salmon in the Fraser River, Canada (Lee et al. 2003), and even local adaptation of sockeye salmon (Eliason et al. 2011).

Despite T_{opt} measurements being broadly applied to fish biology, the more than 25,000 fish species, along with the countless combinations of abiotic and biotic factors that can influence T_{opt} (Pörtner 2010), create a need for a high-throughput method to estimate T_{opt} . Generating a single Fry curve normally takes weeks, as \dot{MO}_2 measurements are required over a range of temperatures in resting and maximally swimming fish while ensuring fish are well recovered from handling (Steffensen 1989). Maximum \dot{MO}_2 can be achieved with a U_{crit} test (Jain et al. 1997). However, somewhat faster T_{opt} estimates are possible if maximum \dot{MO}_2 is measured following exhaustive exercise (Reidy et al. 1995; Zeng et al. 2010).

An alternative and potentially even faster approach is to estimate T_{opt} for maximum \dot{MO}_2 using maximum f_H as a surrogate. The foundation for this idea is the original observation by Fry (1947) that maximum f_H was limited around a purported T_{opt} for brook trout (*Salvelinus fontinalis*) and a later suggestion by Brett (1964) that reduced performance above T_{opt} could be due to a cardiac insufficiency in juvenile sockeye salmon, an idea recently confirmed for adults (Steinhausen et al. 2008). Steinhausen et al.

(2008) found that the upper limit for maximum \dot{MO}_2 and aerobic scope in exercising sockeye salmon was triggered by $f_{\rm H}$ failing to increase significantly above $T_{\rm opt}$, thereby preventing further increases in \dot{Q} with warming. Moreover, it is now clear that temperature-related increases in \dot{Q} in both resting and active fishes are mediated entirely through increased $f_{\rm H}$ (Brodeur et al. 2001; Clark et al. 2008; Cooke et al. 2003; Gamperl et al. 2011; Gollock et al. 2006; Mendonça and Gamperl 2010; Sandblom and Axelsson 2007; Steinhausen et al. 2008). Thus, $f_{\rm H}$ is the primary variable that responds to a need for increased internal oxygen convection during warming.

In view of the central role of $f_{\rm H}$ in determining a fish's response to warming, as particularly well demonstrated in salmon, the present study was carried out to assess the possibility that a limitation on maximum $f_{\rm H}$ in coho salmon can be used to reliably estimate $T_{\rm opt}$ in an expedited manner. This would eliminate the need for direct but more prolonged $\dot{\rm MO}_2$ measurements necessary to generate a Fry curve. As a control, aerobic scope was measured by conventional means over a range of temperatures encompassing $T_{\rm opt}$ for coho salmon (Brett et al. 1958; Edsall et al. 1999; Griffiths and Alderdice 1972) while additionally measuring $f_{\rm H}$ as a confirmation that maximum $f_{\rm H}$ was limited at the $T_{\rm opt}$ for aerobic scope. To expedite $T_{\rm opt}$ estimation and eliminate the problem of activity state altering $f_{\rm H}$, fish were anaesthetized during warming and maximum $f_{\rm H}$ achieved through pharmacological means. Because anaesthetics can directly affect cardiac function (Cotter and Rodnick 2006; Fredricks et al. 1993; Hill et al. 2002; Hill and Forster 2004; Ryan et al. 1993), a comparison was made between tricaine methanesulfonate (MS-222), the most commonly used fish anaesthetic (Carter et al. 2011), and clove oil (Anderson et al. 1997).

2.2 Materials and Methods

2.2.1 Experimental Animals and Care

Juvenile coho salmon were obtained from the Seymour River Hatchery (North Vancouver, BC, Canada) and transported to the University of British Columbia (Vancouver, BC, Canada) in March 2010. Fish were held in a 1,000 l flow-through holding tank supplied with de-chlorinated municipal tap water and kept under a 12 h:12 h light:dark photoperiod while fed to satiation daily (BioClark's Fry, Bio-Oregon Inc.; www.bio-oregon.com). Water temperature increased seasonally from 8°C to 10°C at which point a chilling unit (BHL-1089-3, Frigid Units; www.frigidunits.com) maintained water temperature at 10.0°C (range ± 0.5 °C) for the duration of the experiment. All fish procedures were approved by the University of British Columbia Committee on Animal Care in accordance with the Canadian Council on Animal Care (A10-0236).

Three weeks prior to experimentation fish were anaesthetized (100 mg l⁻¹ MS-222 buffered with 100 mg l⁻¹ NaHCO₃, Sigma-Aldrich; www.sigmaaldrich.com) and individually tagged with a non-absorbable monofilament suture caudal to the dorsal fin. Tags showed no sign of rejection.

2.2.2 Aerobic Scope and f_H Measurements in Unanaesthetized Fish

Resting and maximum values for \dot{MO}_2 and f_H for a single group of fish (N = 12) were individually measured at each of five test temperatures (13, 15, 17, 19, and 21°C).

These measurements were completed over a 3-week period, with four fish measured daily as follows. After a 24 h fasting period, fish were individually transferred from the holding tank to one of four separate, custom-made respirometers (~450 ml) in two separate apparatus. Water temperature (10°C) in the respirometers was controlled with in-line, recirculating chillers (F32-MD, Julabo Labortechnik GmbH; www.julabo.de; 3016D, Fisher Scientific; www.fishersci.ca). Fish were left overnight for a minimum of 12 h to adjust to the respirometer. Each experiment began with water temperature being increased at a rate of 2°C h⁻¹ up to the test temperature, which was then held for a period of 1 h before making triplicate measurements of resting \dot{MO}_2 and f_H over a 20 to 30 min period. Afterwards, one fish at a time was removed from its respirometer and placed in a 60 cm diameter circular tank containing aerated water at the test temperature, where it was chased to exhaustion over a 5 min period by combining hand chasing and tail pinches. Depending on the test temperature, fish generally displayed burst swimming for the first 30 s to 1 min, followed by 2 to 3 min of slower swimming with infrequent burst activity, and finally 1 to 2 min of only slow swimming until exhaustion, when the fish became refractory to touch regardless of temperature. The fish was immediately returned to the respirometer and the peak values for \dot{MO}_2 and f_H that were recorded during the 45 to 60 min recovery period were taken as the maximum values for that test temperature. Aerobic scope and scope for $f_{\rm H}$ were calculated as the difference between maximum and resting values. Individual T_{opt} values were assigned to the test temperature at which aerobic scope was the greatest. After the recovery period, swimming activity had partially returned and the fish was placed in a recovery tank where temperature was slowly reduced to the acclimation temperature over a 30 to 80 min period, depending on the test

temperature. The fish was then weighed and returned to the holding tank. The above procedure was then performed on the remaining fish with the experimental start for two fish staggered by 2 to 5 h. Water temperature was matched to 0.1°C between the two test systems using a Fisherbrand[®] Traceable[®] NIST-certified digital thermometer with a Type-K thermocouple (Fisher Scientific). Each fish recovered for a minimum of 3 days in between temperature tests. The order of test temperatures was randomized, as was the order fish were tested on a given day/temperature.

2.2.3 MO₂ Measurements

 \dot{MO}_2 was measured using intermittent-flow respirometry, recording the rate of decrease in water oxygen saturation using a R-type oxygen probe connected to a NeoFox sensor system (Ocean Optics; www.oceanoptics.com) that was calibrated daily at the test temperature. The probe was inserted through the top of the respirometer and monitored changes in oxygen saturation directly in the chamber. Probe output was recorded on a laptop computer that ran the NeoFox Viewer software (Ocean Optics). Water was circulated in the respirometer during \dot{MO}_2 readings using a magnetic stir-bar, which was separated from the fish by a 20 gauge stainless-steel mesh. Each \dot{MO}_2 measurement involved at least a 10% decrease in oxygen saturation, which took 2 to 10 min depending on temperature as well as state of the fish. Water oxygen saturation never decreased below 80% and was quickly (~1 min) restored to \geq 95% by water flow through the respirometer (~250 ml min⁻¹) between recordings, during which time the stir-bar was turned off to reduce interference with the *f*_H recording.

2.2.4 $f_{\rm H}$ Measurements

Bioelectric signals from the heart propagate through water allowing the electrocardiogram (ECG) to be detected wirelessly using submerged electrodes (Altimiras and Larsen 2000). Thus, non-invasive $f_{\rm H}$ recordings were obtained by using the stainless-steel mesh beneath the fish as an electrode grid, with a reference grid above the fish (Johnsson et al. 2001). Both electrodes extended to the interior edges of the respirometer and a small hole was made in the upper grid to allow the insertion of the oxygen probe. The electrodes were connected to a Grass P55 AC amplifier (Astro-Med Inc.; www.astro-med.ca) that amplified (1,000x to 10,000x) and filtered (60 Hz line filter; low-pass: 10 to 30 Hz; high-pass: 0.1 to 0.3 kHz) the ECG signal to reduce skeletal muscle activity and eliminate ambient electrical interference. Output from the amplifier was processed and recorded at a 1 kHz sampling frequency using a Powerlab ML870 data acquisition unit and LabChart software (AD Instruments; www.adinstruments.com). The R-peaks of the QRS complex were manually identified and $f_{\rm H}$ calculated from the mean R-R interval of a continuous beats series (N = 15 heart beats per reading). Proper detection of the ECG required fish to maintain a vertical dorso-ventral orientation between the two electrodes. However, not all fish would necessarily maintain this orientation after exhaustive exercise. Thus, while resting $f_{\rm H}$ values were obtained for all fish at each test temperature, some of the post-exercise $f_{\rm H}$ values were lost at each temperature.

2.2.5 Maximum f_H in Anaesthetized Fish

Anaesthetized juvenile coho salmon from the same population as those used for the aerobic scope trials were tested with pharmacological agents to elicit maximum $f_{\rm H}$. Individual fish were anaesthetized using either 30 ppm clove oil (1:10 clove oil:ethanol) or 75 ppm buffered MS-222 and transferred to the experimental apparatus (see below) where they were maintained in an anaesthetized state at 10°C for 1 h prior to experimentation while monitoring $f_{\rm H}$. An anaesthetized state was maintained using either 30 ppm clove oil (N = 10), 15 ppm clove oil (N = 12), or 50 ppm MS-222 (N = 12). The 15 ppm clove oil and 50 ppm MS-222 represented the minimum concentrations for maintained anaesthesia for the duration of the temperature trial. A control group (N = 8) without pharmacological agents was tested using 30 ppm clove oil.

Pharmacological stimulation of maximal $f_{\rm H}$ was achieved via sequential intraperitoneal injections of 1.2 mg kg⁻¹ atropine sulfate (Sigma-Aldrich) to block vagal tone and 4 µg kg⁻¹ isoproterenol (Sigma-Aldrich) to stimulate cardiac adrenergic βreceptors. Atropine was prepared daily and isoproterenol prepared immediately prior to use. Both agents were dissolved in 0.9% NaCl. Control fish received only saline injections. Each injection was followed by a 15 min equilibration period. Preliminary trials showed that doses used were sufficient to produce a maximal $f_{\rm H}$ response as repeated injections of either atropine or isoproterenol did not further increase $f_{\rm H}$. In addition, these trials revealed the chronotropic and heart rate variability effects of atropine persisted >8 h post-injection, as reported in other studies (Altimiras et al. 1997).

Isoproterenol injections in the absence of atropine were also found to have a chronotropic effect that persisted >1.5 h.

A step-wise temperature increase was applied in 1°C increments at a heating rate of 10°C h⁻¹. A group of size-matched fish was tested to ensure temperature changes in the external bath were near synchronous with the internal body temperature and $f_{\rm H}$ (Figure 2.1A). Preliminary experiments with heating rates of 2°C h⁻¹, 5°C h⁻¹, and 10°C h⁻¹ showed identical $f_{\rm H}$ changes for 5°C h⁻¹ and 10°C h⁻¹ rates, and for 2°C h⁻¹ the only difference was at upper temperatures when fish had been anaesthetised for >6 h. Thus, the 10°C h⁻¹ heating rate was adopted for all experiments. Temperature and $f_{\rm H}$ were allowed to briefly stabilize after each 1°C increment (Figure 2.1A), with the $f_{\rm H}$ measurement being taken at the end of this period (N = 15 heart beats per reading). The experimental endpoint was a shift from rhythmic (Figure 2.1B) to arrhythmic $f_{\rm H}$ (Figure 2.1C) at which point the fish was removed from the apparatus, a blood sample (0.2 to 0.5 ml) was withdrawn from the caudal vein into a heparinized syringe, and length and weight measurements were taken. Fish were then euthanized with a blow to the head, the heart excised to measure wet ventricular mass, and sex was noted. Hematocrit was measured immediately in micro hematocrit capillary tubes spun for 5 min at 5900g.

2.2.6 Experimental Apparatus

The apparatus used to hold anaesthetized fish and measure $f_{\rm H}$ consisted of a short section of PVC pipe cut lengthwise and capped on each end to form a 0.5 l, semicircular holding trough. Fish were orientated with a plastic mesh sling that suspended them in the

centre of the pipe and with weighted-foam pads that maintained the fish in a vertical dorso-ventral orientation. Fish were stationary and fully submerged throughout procedures. Custom-made chromel-A electrodes were positioned beneath the ventral surface of the fish underneath the plastic sling to permit non-invasive recording of the ECG. Duplicate troughs were each supplied with water inflow via a pressure-reduced diversion from the hose of a re-circulating chiller. Inflow was separated between a Y-shaped nozzle inserted in the mouth of the fish and outflowed into the troughs. The flow rate for each trough (~150 ml min⁻¹) was sufficient to provide both gill irrigation and water temperature regulation (range $\pm 0.1^{\circ}$ C). Outflow from the holding troughs returned to the aerated reservoir of the re-circulating chiller. The water total volume of the chiller reservoir and the holding troughs was ~8 l.

2.2.7 Data and Statistical Analysis

Resting and maximum \dot{MO}_2 and f_H values were recorded from all fish after an equal time period at a test temperature. There was no significant difference in \dot{MO}_2 or f_H values between the first and second pair of fish tested daily (P > 0.05; data not shown). Data are presented as mean \pm S.E. For aerobic scope trials, combined resting and post-exercise data were not normally distributed and were separated by treatment (resting *vs*. post-exercise). Data that were normally distributed were tested with a one-way repeated-measures (RM) ANOVA followed by the Holm-Sidak *post hoc* test. Data that were not normally distributed were assessed with a one-way RM ANOVA on ranks with Dunn's *post hoc* test. For the anaesthetized fish, the ABT was calculated for f_H following Yeager

and Ultsch (1989). Arrhenius break temperature analysis of changes in $f_{\rm H}$ with temperature was performed on: 1) the mean $f_{\rm H}$ of all individuals at a test temperature to determine a single ABT for the group (mean analysis) and, 2) each individual separately to determine individual ABTs and calculate a mean group ABT (individual analysis). Regression slopes were compared with a paired t-test. Differences in $f_{\rm H}$ between anaesthetics and pharmacological treatments were assessed with a two-way RM ANOVA and Holm-Sidak *post hoc*. Sampling and temperature response variables (arrhythmias, mean breakpoint) were compared with a one-way ANOVA. Statistical analysis was performed using SigmaPlot 11.0 (Systat Software Inc.; www.sigmaplot.com) with significance assessed as P < 0.05.

2.3 Results

2.3.1 Effect of Temperature on \dot{MO}_2 and $f_{\rm H}$ in Unanaesthetized Fish

All fish fully recovered from multiple temperature and chasing challenges, except for one fish that died while recovering after exhaustive exercise at 21°C (it also had the lowest aerobic scope of all fish tested). Post-exercise, fish resumed feeding soon after being returned to the holding tank and grew during the 3 week experimental period from an initial mass of 16.9 ± 0.2 g to 18.1 ± 0.3 g by the final exercise trial. There was no relationship (P > 0.05; 1-way RM ANOVA) between the number of exercise challenges and resting or post-exercise \dot{MO}_2 . Resting \dot{MO}_2 increased significantly (P < 0.001; 1-Way RM ANOVA; Holm-Sidak *post hoc*) with temperature from 3.7 ± 0.2 µmol h⁻¹ g⁻¹ at 13°C to 7.0 ± 0.3 µmol h⁻¹ g⁻¹ at 21°C, with a Q₁₀ of 2.3 ± 0.1 (Figure 2.2A). Exhaustive exercise increased resting \dot{MO}_2 by 2.5 to 4.3 fold, depending on temperature. Between 13°C and 17°C, maximum post-exercise \dot{MO}_2 increased significantly (P = 0.004; 1-way ANOVA; Holm-Sidak *post hoc*) with a Q₁₀ of 2.2 ± 0.3 and reached 20.3 ± 1.0 µmol h⁻¹ g⁻¹ at 17°C. Post-exercise \dot{MO}_2 did not increase above 17°C and showed signs of declining with increasing temperature since post-exercise \dot{MO}_2 was equivalent for 13°C and 19°C. Thus, based on these mean values, T_{opt} for aerobic scope could be assigned to 17°C (15.5 ± 1.1 µmol h⁻¹ g⁻¹; Figure 2.2C). Using individual T_{opt} values for aerobic scope, T_{opt} was 17.0 ± 0.7°C (17.3 ± 1.0 µmol h⁻¹ g⁻¹).

During the aerobic scope measurements, resting $f_{\rm H}$ at 13°C was 53.5 ± 2.7 beats min⁻¹ and increased significantly (P < 0.05; 1-way RM ANOVA on ranks; Dunn's *post hoc*) with temperature to 110.8 ± 3.9 beats min⁻¹ at 21°C (Figure 2.2B), with a Q₁₀ of 2.6 ± 0.2. Maximum post-exercise $f_{\rm H}$ at 13°C was 96.2 ± 1.3 beats min⁻¹, a 1.8 fold increase over the resting $f_{\rm H}$. However, this factorial scope $f_{\rm H}$ for was not maintained with increasing temperature, decreasing to a 1.1 fold increase at 21°C when maximum $f_{\rm H}$ was 125.8 ± 8.2 beats min⁻¹. Thus, scope for $f_{\rm H}$ was maximal at 17°C (55.1 ± 6.7 beats min⁻¹), and decreased precipitously at higher temperatures to only 10.0 ± 9.7 beats min⁻¹ at 21°C (Figure 2.2C) largely because maximum post-exercise $f_{\rm H}$ increased little above 17°C. Indeed, Q₁₀ values for maximum $f_{\rm H}$ were greatest (1.9) between 13°C and 15°C and lowest (1.1) between 19°C and 21°C. In contrast, the Q₁₀ for resting $f_{\rm H}$ was maintained near 2.6 between all test temperatures. Arrhenius plots reveal discontinuities in temperature effects on rate functions. Such discontinuities were visible for both resting and maximum $f_{\rm H}$ at ~19°C and ~17°C, respectively (Figure 2.3), but proper breakpoint analysis was impossible because of a limited number of test temperatures. However, a two-segment piecewise linear regression revealed a discontinuity in maximum $f_{\rm H}$ values at 16.5°C (P = 0.052) at the point where Q₁₀ equivalents for regression slopes decreased from 1.8 in the lower segment (<16.5°C) to 1.1 in the upper segment (>16.5°C). This discontinuity for maximum $f_{\rm H}$ fell within the previously defined range of $T_{\rm opt}$ for aerobic scope. Matching statistical analysis of resting $f_{\rm H}$ revealed a discontinuity at a higher temperature (18.9°C; P = 0.073) at the point where Q₁₀ values increased from 2.0 in the lower segment (<18.9°C) to 5.2 in the upper segment (>18.9°C).

2.3.2 *Effect of Temperature on* $f_{\rm H}$ *in Anaesthetized Fish*

After 1 h of anaesthesia, initial $f_{\rm H}$ was significantly (P < 0.001) higher for 15 ppm clove oil and MS-222 than either 30 ppm clove oil treatments (Table 2.1). Atropine significantly increased $f_{\rm H}$ by 6.8%, 9.7% and 11.5% in 30 ppm clove oil (P = 0.006), 15 ppm clove oil (P < 0.001) and 50 ppm MS-222 (P < 0.001), respectively. The subsequent isoproterenol injection did not significantly increase $f_{\rm H}$ with any anaesthetic treatment (P > 0.05), suggesting maximal sympathetic stimulation of the heart under these anaesthetic treatments at 10°C. Saline injections had no affect on $f_{\rm H}$.

The significantly higher initial $f_{\rm H}$ with 15 ppm clove oil and MS-222 compared with 30 ppm clove oil treatments was maintained with warming up to 22°C (P <0.05)

(Figure 2.4). Further, $f_{\rm H}$ in the 15 ppm clove oil and MS-222 groups approximated the maximum $f_{\rm H}$ values seen in unanaesthetized fish (Figure 2.4C,D). At temperatures from 20°C to 23°C, cardiac arrhythmias ensued and $f_{\rm H}$ values above 23°C were not different between anaesthetic treatments (P >0.05) likely as a result of reduced statistical power with fewer fish per temperature (Figure 2.4). Neither the temperature nor $f_{\rm H}$ for the onset of cardiac arrhythmias was different among anaesthetic treatments (P >0.05) (Table 2.2).

ABT analysis revealed significant (P < 0.001) discontinuities for both individual (Table 2.2) and mean (Table 2.2; Figure 2.5) responses of $f_{\rm H}$ to warming for each anaesthetic treatment. The range for individual ABTs was greatest (7.5°C) for control fish and least (2.0°C) for the MS-222 anaesthetic. For the individual $f_{\rm H}$ responses, the ABT did not differ among treatments (P > 0.05; Table 2.2) even though the $f_{\rm H}$ corresponding to the ABT in each treatment was significantly higher (P < 0.001) for the 15 ppm clove oil and MS-222 treatments than for either the 30 ppm clove oil treatment or the control fish. Therefore, the ABT was independent of an anaesthetic effect on $f_{\rm H}$. Comparison of regression slopes from individual $f_{\rm H}$ responses revealed the initial Q₁₀ for $f_{\rm H}$ was significantly higher in control (P < 0.001) and MS-222 (P = 0.001) treatments than the pharmacological 30 ppm clove oil treatment. Above the ABT, the Q_{10} for $f_{\rm H}$ was reduced the greatest in the MS-222 treatment (Table 2.2). Thus, although different types and concentrations of anaesthesia affected the maximum $f_{\rm H}$ corresponding to the ABT, the variability of the individual ABTs and the initial Q_{10} for maximum $f_{\rm H}$, none of these effects altered the ABT.

No correlations were found between individual physical characteristics (mass, relative ventricular mass, hematocrit; Table 2.2) and either ABT or the temperature when cardiac arrhythmias started (data not shown).

2.3.3 Comparison of T_{opt} Estimates

The individual T_{opt} estimate for aerobic scope (17.0 ± 0.7°C) was not significantly different (P = 0.27) from the ABT estimated from any of the anaesthetic treatments using individual $f_{\rm H}$ responses. The best agreement was for the individual values of ABT for 15 ppm clove oil treatment (17.1 ± 0.5°C) (Figure 2.6), but the least individual variability of ABT was for 50 ppm MS-222 (16.5 ± 0.2°C) (Table 2.2).

2.4 Discussion

The primary objective of this Chapter was to assess the potential for a highthroughput technique to estimate T_{opt} using maximum f_H as a surrogate measurement for aerobic scope. The aerobic scope protocol used here to generate maximum \dot{MO}_2 at each test temperature required 3 weeks to generate minimal data for a Fry curve (i.e. five temperatures at 2°C increments) using N = 12 fish. In contrast, a finer resolution ABT analysis of maximum f_H in anaesthetised fish took only 3 days. Furthermore, T_{opt} for aerobic scope was statistically indistinguishable from the ABT for maximum f_H when individual comparisons were made to avoid rounding issues (see below). Therefore, my data strongly supports the idea that ABT analysis of maximum f_H can be used a highthroughput method for determining T_{opt} that can act as a surrogate for aerobic scope measurements. Furthermore, using 50 ppm MS-222 as the anaesthetic could potential lead to less variability in the estimate of T_{opt} .

2.4.1 Accuracy of T_{opt} and f_H Measurements for Unanaesthetized Fish

The accuracy of a T_{opt} estimate is dependent upon the quality and quantity of the \dot{MO}_2 measurements used to generate the Fry curve. Resting \dot{MO}_2 measured here is comparable or slightly lower than previously reported values for juvenile coho salmon at similar or lower temperatures (3.7 to 4.4 µmol h⁻¹ g⁻¹; Janz et al. 1991; Davis and Schreck 1997) and far lower than resting values for juvenile rainbow trout (6.3 to 10.5 μ mol h⁻¹ g⁻¹; Scarabello et al. 1991, 1992), indicating that the 12 h recovery period was sufficient. Likewise, post-exercise \dot{MO}_2 values closely match those for juvenile rainbow trout (20.1 to 22.1 μ mol h⁻¹ g⁻¹; Scarabello et al. 1991, 1992). Thus, the factorial scope for $\dot{M}O_2$ (2.5 to 4.3) slightly exceeds that for previous chase studies with rainbow trout (2.1 to 3.2; Scarabello et al. 1991; Scarabello et al. 1992). The T_{opt} for aerobic scope $(17.0 \pm 0.7^{\circ}C)$ is lower than the 19°C to 20°C T_{opt} previously reported for swimming performance in 11°C acclimated under-yearling (Griffiths and Alderdice 1972) and yearling coho salmon (Brett et al. 1958), but is greater than the 15°C T_{opt} for growth in coho salmon smolts (Edsall et al. 1999). The single fish mortality observed here at 21°C likely would not have occurred if T_{opt} of this particular group of fish was as high as that reported previously for under-yearling coho salmon.

Resting and maximum $f_{\rm H}$ in non-anaesthetised juvenile coho salmon have not been previously reported for comparison. Resting $f_{\rm H}$ for juvenile Atlantic salmon (*Salmo salar*) was reported as 25 to 40 beats min⁻¹ at 10°C (Knudsen et al. 1992). The present Q₁₀ for resting $f_{\rm H}$ of 2.6 ± 0.2 is higher than the 1.6 to 2.3 range previously reported for larger resting salmon (Clark et al. 2008; Steinhausen et al. 2008; Taylor et al. 1996), primarily because of a greater temperature sensitivity of resting $f_{\rm H}$ above 19°C. The absolute maximum post-exercise $f_{\rm H}$ of 125.8 beats min⁻¹ at 21°C is near the 120 beats min⁻¹ limit suggested for most fishes (Farrell 1991) and similar to that reported for adult sockeye salmon swimming near $T_{\rm opt}$ (Steinhausen et al. 2008).

Arrhenius break temperature analysis determined that maximum $f_{\rm H}$ ceased to increase to any significant degree above the $T_{\rm opt}$ for aerobic scope. This result supports the hypothesis that $T_{\rm opt}$ coincides with a limitation on maximum $f_{\rm H}$ in salmon. Increases in $f_{\rm H}$ above the ABT were only modest (~6 beats min⁻¹ between 17°C and 21°C) and are unlikely to provide any appreciable additional capacity for oxygen convection. This lack of additional capacity is supported by the decline in maximum $\dot{\rm MO}_2$ above 17°C, which corresponded to the decrease in aerobic scope. Thus, my data are consistent with the suggestion that, as fish are progressively warmed, a maximum $f_{\rm H}$ limitation causes a decline in scope for $f_{\rm H}$ that triggers a decline in scope for $\dot{\rm Q}$, which is followed by a collapse of aerobic scope (Farrell 2009). This supports the use of the ABT for maximum $f_{\rm H}$ as a predictor of $T_{\rm opt}$.

2.4.2 Measuring Maximum f_H in Anaesthetized Fish

Previous investigations have used Arrhenius plots to detect discontinuities in $f_{\rm H}$ across species (Frederich and Pörtner 2000; Lannig et al. 2004; Stenseng et al. 2005). These studies performed analyses that were based on mean values, which does not incorporate individual variability and therefore tends to round the estimate. A better approach demonstrated here and used previously (Iwaya-Inoue et al. 1989) is to use individual rather than mean Arrhenius plots to determine ABTs. In fact, the very earliest work using Arrhenius analysis to detect discontinuities in $f_{\rm H}$ was based on individual data across a wide variety of vertebrates (e.g. Crozier 1926), but these earliest studies preceded the development of analytical methods required for statistical analysis.

My present experiments showed that $f_{\rm H}$ discontinuities are revealed in anaesthetized juvenile coho salmon in a reliable and rapid manner. This result raises the possibility of using this methodology as a tool to screen $T_{\rm opt}$ in fishes. While numerous studies have considered using cardiovascular parameters as a predictor of $\dot{\rm MO}_2$ in salmon (e.g. Brodeur et al. 2001), application has been limited by the high variability of $f_{\rm H}$ and $\dot{\rm MO}_2$ correlations (Priede and Tytler 1977). This is because different activity states and environmental factors alter the relationship of $f_{\rm H}$ and $\dot{\rm MO}_2$ (Thorarensen et al. 1996). By keeping the activity state of the fish constant using anaesthetics and achieving maximum $f_{\rm H}$ by pharmacological means the confounding effects of activity state on $f_{\rm H}$ could be circumvented. Therefore, the main concern then shifts to the reliability of measurements of $f_{\rm H}$ in anaesthetised fish especially given the large amount of literature on the effects of fish anaesthetics on cardiac activity.

Consistent with the present observations, MS-222 anaesthesia brings about tachycardia by reducing parasympathetic tone (Cotter and Rodnick 2006; Ryan et al. 1993) by blocking neuronal Na⁺ channels (Burka et al. 1997; Carter et al. 2011). Clove oil also causes tachycardia (Cotter and Rodnick 2006), likely due to eugenol, the active ingredient in clove oil, blocking parasympathetic nerve action potentials (Moreira-Lobo et al. 2010). Blocking vagal tone in quiescent, unanaesthetized rainbow trout elevates $f_{\rm H}$ by 30 to 50%, depending on temperature (Priede 1974; Wood et al. 1979). In the present study, the competitive muscarinic antagonist atropine increased $f_{\rm H}$ by ~10% across treatments, indicating that vagal tone was reduced by all anaesthetic treatments, but not eliminated. Even so, the cardio-acceleratory effect of 30 ppm clove oil was attenuated in comparison with both 15 ppm clove oil and 50 ppm MS-222, possibly in part because eugenol blocks L-type Ca^{2+} channels (Sensch et al. 2000) and slows pacemaker depolarization. On the other hand, while MS-222 has been shown to negatively affect myocardial function *in vitro* (Hill et al. 2002; Ryan et al. 1993), *in vivo* studies have not reproduced these results (Fredricks et al. 1993; Hill and Forster 2004). Correspondingly, the highest $f_{\rm H}$ values were recorded for the MS-222 trials and these were closest to those seen in unanaesthetized fish. The least amount of variability in ABT also occurred with the MS-222 trials. Therefore, the recommendation is to use 50 ppm MS-222 as the maintenance anaesthetic for ABT analysis of maximum $f_{\rm H}$.

Stimulation of β -adrenergic receptors following atropine treatment has previously been shown to increase $f_{\rm H}$ of rainbow trout by 8 to 15% and in a temperature-dependent manner (Wood et al. 1979). Here, isoproterenol did not significantly increase maximum $f_{\rm H}$, suggesting cardiac β -adrenergic receptors either were fully saturated or were inhibited

by the anaesthetic (Butterworth et al. 1997). Fish were sedated to stage 5 or 6 anaesthesia (Summerfelt and Smith 1990), which can temporarily elevate levels of circulating catecholamines (Iwama et al. 1989). No studies have examined plasma catecholamine levels in salmon under prolonged anaesthesia, so it is unclear if plasma catecholamine levels remain elevated. It seems unlikely that anaesthesia confounded the test results by decreasing maximum $f_{\rm H}$ because maximum $f_{\rm H}$ for fish anaesthetized with MS-222 exceeded that recorded post-exercise, possibly as a result of the membrane destabilizing effects of anaesthetics in general (Butterworth and Strichartz 1990).

The maximum $f_{\rm H}$ of fish sedated with MS-222 greatly exceeded that observed post-exercise at elevated temperatures, although a breakpoint was still observed in anaesthetized fish. A lower maximum $f_{\rm H}$ in unanaesthetized fish could be due to increased vagal tone. Vagal tone was speculated to occur at high temperature in swimming sockeye salmon (Steinhausen et al. 2008) and could act as a cardio-protective mechanism by limiting $f_{\rm H}$ and allowing adequate time for oxygen diffusion to the spongy myocardium as well as maintaining cardiac contractility. In anaesthetized fish, the abovementioned membrane destabilizing affect of MS-222 also likely contributed to $f_{\rm H}$ exceeding the ~120 beats min⁻¹ maximum. However, maximum $f_{\rm H}$ in atropinized, anaesthetized fish still failed to maintain an exponential increase with temperature and displayed a breakpoint. This limitation to increases in maximum $f_{\rm H}$ may be an intrinsic property of the salmon heart resulting from the coincidence of a number of mechanistic limitations (contractility, oxygen delivery, Ca²⁺ exchange) (Lillywhite et al. 1999) at temperatures above $T_{\rm opt}$ as the heart approaches its upper temperature and rate limit. It may be possible to simplify the procedure developed in this thesis by eliminating the protracted recovery period following initial anaesthesia and by removing pharmacological treatments. In my experiment, the 1 h recovery period allowed sufficient time for $f_{\rm H}$ to stabilize in order to demonstrate the possible effects of pharmacological stimulation on $f_{\rm H}$. In future studies aiming to achieve maximum $f_{\rm H}$ of fish the pharmacological stimulation may be given immediately after fish are placed in the apparatus. Arguments can be made against the necessity of atropine and isoproterenol injections, given their relatively minor effects observed in this study. However, there is a distinct possibility of increased vagal tone at higher temperatures without the blocking effect of atropine. Moreover, the lack of response of coho salmon to isoproterenol does not preclude other species from responding differently. I recommend, therefore, that both atropine and isoproterenol injections are retained in future studies in order to further evaluate their effectiveness.

In summary, T_{opt} for aerobic scope in juvenile coho salmon was 17.0 ± 0.7°C and was statistically indistinguishable from the ABT for maximum $f_{\rm H}$ in anaesthetized fish (17.1 ± 0.5°C for 15 ppm clove oil and 16.5 ± 0.2°C for 50 ppm MS-222). Therefore, because the ABT analysis took 3 days *versus* 3 weeks for the Fry aerobic scope curve, I propose that ABT analysis of maximum $f_{\rm H}$ in anaesthetized fish presents itself as a valuable, high-throughput screening tool to assess $T_{\rm opt}$ in salmon. Further, the practicality and simplicity of this methodology lends it to field-based application. Field measurements, for instance, could be used to measure the $T_{\rm opt}$ of rearing coho salmon in their natal streams. Recovery of fish following procedures may also be possible, potentially allowing this methodology to be applied to species of conservation concern.

Broader application of this technique may also be possible given that other fish species have been shown to similarly rely on $f_{\rm H}$ to increase in \dot{Q} during warming (Cooke et al. 2003; Gollock et al. 2006; Mendonça and Gamperl 2010). Interestingly, even though wireless field electrodes should theoretically be unable to detect the ECG in seawater, preliminary studies with Arctic cod (*Arctogadus glacialis*) have shown that an ECG can in fact be detected. This opens the possibility of rapid $T_{\rm opt}$ determinations for fish species in both freshwater and marine environments.

Table 2.1. Changes in heart rate ($f_{\rm H}$) (beats min⁻¹) following sequential pharmacological treatment with atropine and isoproterenol to stimulate maximum $f_{\rm H}$ in juvenile coho salmon (*Oncorhynchus kisutch*) sedated with different anaesthetics at 10°C. Injections were administered intraperitoneally and were given 15 min apart 1 h after fish were introduced to the anaesthetic. The 30 ppm sham-injected control group received saline injections only.

Druge Administered	30 ppm Clove Oil	30 ppm Clove Oil	15 ppm Clove Oil	50 ppm MS-222		
Drugs Administered	N = 8	Stimulated $N = 10$	N = 12	N = 12		
1. Pre-injection	$61.0 \pm 1.3a$	$57.4 \pm 1.1a$	$65.9 \pm 1.5b$	$68.6 \pm 0.9b$		
2. Atropine (1.2 mg kg^{-1})	59.6 ± 1.3a	$61.3 \pm 1.2a^*$	$72.3 \pm 1.2b^*$	$76.5 \pm 1.0b*$		
3. Isoproterenol (4 μ g kg ⁻¹)	$58.9 \pm 1.4a$	$62.4 \pm 1.1a$	$72.4 \pm 1.1b$	$76.8 \pm 1.3b$		
All values are mean \pm S.E. Different letters indicate significant differences (<i>P</i> < 0.05) between anaesthetic						
treatments. A * denotes a significant difference from the previous $f_{\rm H}$.						

	30 ppm Clove Oil	30 ppm Clove Oil	15 ppm Clove Oil	50 ppm MS-222		
	Unstimulated	Stimulated	Stimulated	Stimulated		
	$N = \delta$	N = I0	N = IZ	N = IZ		
Sampling						
Mass (M) (g)	$22.7 \pm 2.5a$	$18.6 \pm 1.4a$	$15.8 \pm 3.4a$	$18.4 \pm 0.8a$		
Relative ventricular mass (% M)	-	$0.102 \pm 0.001a$	$0.099 \pm 0.001a$	$0.099 \pm 0.003a$		
Hematocrit (%)	-	$27.7 \pm 2.1a$	$31.0 \pm 0.4ab$	$34.6 \pm 1.6b$		
Cardiac Collapse						
Pre-arrhythmia $f_{\rm H}$ (beats min ⁻¹)	$129.7 \pm 5.0a$	$127.6 \pm 4.5a$	$135.5 \pm 7.2a$	$143.2 \pm 5.7a$		
Arrhythmia temperature (°C)	$22.6 \pm 0.6a$	$22.5 \pm 0.6a$	$23.3 \pm 0.4a$	$22.9 \pm 0.6a$		
Mean Breakpoint Analysis						
Arrhenius break temperature (°C)	17.4	19.4	16.8	16.8		
Breakpoint $f_{\rm H}$ (beats min ⁻¹)	103.1	116.7	118.7	128.4		
Q ₁₀ -equivalent slope (Low/High)	2.1/1.7	1.9/1.4	2.0/1.5	2.1/1.4		
Individual Breakpoint Analysis						
Arrhenius break temperature (°C)	$17.6 \pm 0.7a$	$18.2 \pm 0.6a$	$17.1 \pm 0.5a$	$16.5 \pm 0.2a$		
Range (Min-Max)	12.4-19.9	14.5-21.3	15.2-20.4	15.5-17.5		
Breakpoint $f_{\rm H}$ (beats min ⁻¹)	$105.1 \pm 4.8a$	$106.0 \pm 3.0a$	$121.1 \pm 3.0b$	$125.5 \pm 2.5b$		
Q ₁₀ -equivalent slope (Low)	$2.1 \pm 0.05a$	$1.9 \pm 0.02b$	$2.0 \pm 0.02ab$	$2.1 \pm 0.02a$		
Q ₁₀ -equivalent slope (High)	$1.6 \pm 0.06a^*$	$1.6 \pm 0.06a^*$	$1.5 \pm 0.04a^*$	$1.5 \pm 0.04a^*$		

Table 2.2. Physical and blood characteristics, cardiac collapse, and breakpoint analysis of heart rate ($f_{\rm H}$) during an acute temperature change (10°C h⁻¹) in sedated juvenile coho salmon (*Oncorhynchus kisutch*) under different anaesthetics with or without pharmacological stimulation of $f_{\rm H}$.

All values are mean \pm S.E. Different letters indicate significant differences between anaesthetic treatments. A * indicates a significant difference (*P* <0.05) from previous Q₁₀ value. Low and High refer to breakpoint slopes at temperatures less than/greater than the Arrhenius break temperature.



Figure 2.1. (A) Simultaneous recording of water bath temperature (——), internal body temperature (— —), and heart rate ($f_{\rm H}$) (——) in an anaesthetized juvenile coho salmon (*Oncorhynchus kisutch*) during a step-wise acute temperature increase (10°C h⁻¹). Insert in the main panel displays plateau of $f_{\rm H}$ for ~1 min prior to subsequent temperature changes. Letters indicate where the electrocardiogram trace was sampled for lower panels. (B) Electrocardiogram trace prior to warming at 10°C. (C) Electrocardiogram trace of arrhythmias and the collapse of cardiac function at ~23°C.



Figure 2.2. (A) Resting ($-\Delta$) and maximum post-exercise ($-\Delta$) oxygen consumption (\dot{MO}_2) and (B) resting ($-\Delta$) and maximum ($-\Delta$) heart rate (f_H) of juvenile coho salmon (*Oncorhynchus kisutch*) over a range of temperatures (13°C to 21°C). (C) Mean individual differences between resting and maximum \dot{MO}_2 ($-\Delta$) and f_H ($-\Box$) at a given temperature are presented as aerobic scope and f_H scope. Dissimilar letters indicate a significant difference (P < 0.05) between temperatures within a group. For all samples N = 12 unless noted in brackets. Values are mean \pm S.E.



Figure 2.3. Arrhenius plot of resting (-D-) and maximum post-exercise (-D) heart rate $(f_{\rm H})$ of juvenile coho salmon (*Oncorhynchus kisutch*) repeatedly tested over a range of temperatures (13°C to 21°C). Discontinuities were approximated using a two segment piecewise linear regression (Region 1: y= b₀ - b₁ (x - T₁), x < T₁; Region 2: y = b₀ + b₂ (x - T₁), x > T₁) that was fitted to resting $f_{\rm H}$ (b₀ = 4.374, b₁ = -13.524, b₂ = -5.778, T₁ = 3.424, P = 0.073, R² = 0.997) and maximum $f_{\rm H}$ (b₀ = 4.784, b₁ = -1.029, b₂ = -5.148, T₁ = 3.452, P = 0.052, R² = 0.998). Resting and maximum $f_{\rm H}$ showed discontinuities at 18.9°C and 16.5°C, respectively. Q₁₀-equivalents for slopes are given for each segment of the regression. A * denotes the discontinuity.



Figure 2.4. Changes in heart rate (f_H) of juvenile coho salmon (*Oncorhynchus kisutch*) during acute warming (10 °C h⁻¹) in each of four anaesthetic treatments. (A) 30 ppm clove oil with no pharmacological treatment (\checkmark). (B) 30 ppm clove oil ($-\Box$), (C) 15 ppm clove oil ($-\Box$), and (D) 50 ppm MS-222 ($-\Delta$) following sequential atropine and isoproterenol injections. Resting and maximum f_H ($-\Box$) from aerobic scope trials are shown for reference. Symbols connected by solid lines represent the range of f_H values used for Arrhenius break temperature analysis. n is in brackets for temperatures where cardiac arrhythmias reduced the number of individuals remaining at each temperature. * denotes a significant difference (P < 0.05) from control and pharmacologically stimulated 30 ppm clove oil treatments. ** denotes a significant difference from all other treatments.



Figure 2.5. Arrhenius break temperature (ABT) analysis of mean heart rate ($f_{\rm H}$) during an acute temperature change for juvenile coho salmon (*Oncorhynchus kisutch*) under 30 ppm clove oil without pharmacological stimulation (\diamondsuit) and 30 ppm clove oil (\blacksquare), 15 ppm clove oil (\bigcirc), and 50 ppm MS-222 (\triangle) with pharmacological stimulation of $f_{\rm H}$. Solid lines associated with symbols for each treatment show ABT regressions and * denotes the break point. Resting and maximum $f_{\rm H}$ from aerobic scope trials (\frown) are shown for reference.



Figure 2.6. Comparison of optimal temperature values for juvenile coho salmon (*Oncorhynchus kisutch*) estimated using measurements of aerobic scope or Arrhenius break temperature analysis of heart rate in acutely warmed, pharmacologically stimulated fish in two different anaesthetics (15 ppm clove oil or 50 ppm MS-222).

CHAPTER 3: TEMPERATURE CONDITIONS AND DYNAMICS IN TWO COHO SALMON-BEARING STREAMS

3.1 Introduction

The ecological relevance of T_{opt} for aerobic scope in the life history of fishes has been suggested by linking changes in distribution and survival with environmental temperature (Farrell et al. 2008; Pörtner and Knust 2007). These previous studies examined this relationship in thermally homogeneous study systems and found associations between single-point environmental monitoring, T_{opt} , and the observed ecosystem effects. For example, Farrell et al. (2008) combined sockeye salmon enumeration records with point source temperature data from the Fraser River to suggest temperatures in excess of T_{opt} for sockeye salmon led to mortality during upstream migration. This particular correlation was facilitated by two factors. First, homogenous temperature conditions in the Fraser River are approached as a result of thorough mixing, limited tributary input, and a low surface area to volume ratio (Patterson et al. 2007). Thus, migrating sockeye salmon cannot behaviorally thermoregulate to any significant degree (Donaldson et al. 2009). Second, migration occurs over a narrow time frame with successful migration largely determined by swimming ability, which is directly influenced by the temperature dependence of aerobic scope. Failure of salmon to complete migration results in no lifetime fitness for this semelparous species (Farrell 2009) Thus, the temporally limited influence of temperature and the spatially invariable

conditions of the Fraser River required only straightforward temperature monitoring to extrapolate ecological consequences of temperatures exceeding T_{opt} .

A proposed use of the rapid T_{opt} method presented in Chapter 2 is the investigation of the thermal tolerance of coho salmon living in low-order streams. Successful residence in freshwater is influenced by growth (Quinn and Peterson 1996), which is determined in part by the cumulative thermal history of a fish over several months during the summer (Holtby 1988). Temporal and spatial temperature variation is also high in low-order streams due to both natural and anthropogenic factors (Caissie 2006; Poole and Berman 2001). Small streams naturally have a high degree of diurnal temperature fluctuation due to their low thermal capacity (Caissie 2006). Anthropogenic disturbances such as deforestation (Holtby 1988), flow alterations (Sinokrot and Gulliver 2000) and reduced groundwater exchange (Story et al. 2003) will also increase diurnal temperature fluctuation. Temperature monitoring would therefore need to be of sufficient temporal resolution to capture the full range of temperatures occurring in a small stream. Spatially, groundwater input (Ebersole et al. 2003a), tributaries (Nielsen et al. 1994) and channel geomorphology (Matthews et al. 1994) create variability in stream temperature that would not be captured by monitoring at a single location (e.g. Farrell et al. 2008). This natural and anthropogenic variability will create a heterogeneous environment in which salmonids can behaviorally thermoregulate to avoid adverse temperatures (Nielsen et al. 1994). Accurately reconstructing the thermal history of fish in streams would be best performed with thermal biotelemetry tags (e.g. Matthews et al. 1994). A less complex method of estimating thermal history that does not require tagging fish is to use an array of water temperature loggers.

The objective of this chapter was to use water temperature monitoring to describe the temperature conditions of two local, coho salmon-bearing waterways. Temperature during the summer (1 June to 15 Sept) was the primary focus due to the importance of this period for coho salmon growth. Temperature was recorded in the streams for a portion of the summer of 2010 and all of summer 2011. Both study watersheds have significant human development (damming or urbanization), which is expected to create high stream temperature variation. To capture the spatial and temporal variation associated with these disturbances, temperature loggers were strategically deployed throughout both streams. Adequate characterization of the temperature variation occurring at each logger would allow the thermal history of fish within different stream reaches to be approximated. The potential for these temperature data to be incorporated with coho salmon T_{opt} estimates is subsequently discussed in Chapter 4. The array of loggers also allowed for the examination of temperature dynamics across each watershed and within specific stream reaches. Temperature dynamics can provide important information on how human disturbances may have altered temperature conditions and the importance of habitat features in maintaining thermal conditions. Therefore, a second goal of this chapter was to assess stream temperature dynamics to determine the factors driving changes in temperature across each system and within specific stream reaches. Understanding the factors contributing to overall stream temperature may be important to mitigating thermal degradation due to habitat alternations or the impacts of climate change.

3.2 Materials and Methods

3.2.1 Study Watersheds

Water temperature was monitored in two coho salmon-bearing waterways: the Seymour River and Brothers Creek (Figure 3.1).

The Seymour River watershed (Figure 3.2) covers 176 km² in North Vancouver, BC, Canada. The Seymour River has naturally spawning populations of all Pacific salmon species with the exception of sockeye salmon. Coho salmon used in Chapter 2 were sourced from the Seymour River. From its headwaters the Seymour River flows south into Burrard Inlet. This flow is interrupted ~19 km from the mouth of the river by the Seymour Falls Dam that prevents upstream salmon migration and restricts adult and juvenile salmon to the lower portion of the watershed. The Seymour Falls Dam is a drinking water reservoir and regulated water release during summer months may create flow conditions that make the Seymour River susceptible to warming. Despite the dam construction, downstream reaches of the Seymour River support juvenile coho salmon. Habitat areas have been further enhanced with off-channel pools and in-stream structures. The lowest ~ 2 km of the Seymour River are characterized by urbanization and channelization. Juvenile coho salmon are present throughout the Seymour River (M. Casselman, personal observation) but are primarily found above the Spur 4 (Reaches S1-4; Figure 3.2).

Brothers Creek watershed (Figure 3.3) covers 6 km² in West Vancouver, BC, Canada. Brothers Creek is a tributary of the Capilano River that drains into Burrard Inlet. Hadden Creek is a tributary of Brothers Creek. Although the headwaters of Brothers Creek are intact, significant urban development has occurred throughout the lower reaches. Development in the headwaters of Hadden Creek has resulted in the complete loss of riparian vegetation in some areas. Urbanization and the construction of impassable fish barriers have limited juvenile coho salmon rearing to just upstream of the Mathers Avenue logger on Brothers Creek (Reaches B2,B3; Figure 3.3). On Hadden Creek, however, hatchery-raised juvenile coho salmon are released above fish barriers into upper Hadden Creek (Reaches H1-H3; Figure 3.3).

3.2.2 Temperature Monitoring and Data Analysis

A total of 16 HOBO® Pro temperature loggers (± 0.2°C accuracy; Onset Computer Corporation; www.onsetcomp.com) were strategically deployed on the Seymour River (ten loggers; Figure 3.2; Table 3.1) and Brothers Creek (seven loggers; Figure 3.3; Table 3.1) in July 2010. Temperature loggers were calibrated and synchronized prior to deployment. Loggers were secured to in-stream structures, encased in protective sleeves to prevent direct solar input, and recorded water temperature every 10 min. Streams were divided into reaches according to logger placement with each reach defined by an upper and lower logger. Loggers recorded temperature year round. The July 2010 installation of loggers only allowed for a portion of 2010 summer temperatures to be recorded. Temperature data from the Upper Seymour logger is only available for 2011 as the first logger placement was lost.

For the summer (1 June to 15 Sept), the frequency of temperatures recorded at each logger was calculated in 0.5°C increments. The frequency of temperatures was only calculated during the summer as this represents the time for maximum growth of coho salmon and when deleterious temperatures are most likely to be encountered. Daily maximum, mean, and minimum temperatures at each logger were used to calculate seasonal maximum temperature and diel variation. Summer temperature dynamics within stream reaches were evaluated using differences in daily maximum and mean temperatures between the upper and lower loggers. To determine what processes drive stream temperature, year-round stream temperature data was correlated with mean daily air temperature and rainfall data from weather stations located within or nearby the study watersheds (Environment Canada 2011; Figure 3.1). Water temperature correlations with environmental data were fit with linear regressions using Sigmaplot 11 (Systat Software Inc.; www.sigmaplot.com).

3.3 Results

Temperature frequencies recorded from the Seymour River, Brothers Creek and Hadden Creek indicated that there was a reduction in the frequency of high temperatures for 2011 *versus* 2010 in both the Seymour River and Brothers Creek watersheds (Figures 3.4-3.6). Reductions in the frequency of high temperatures were most apparent in the upper Seymour River (Figure 3.4B,C,D) and were least apparent in lower Hadden Creek

(Figure 3.6B,C). Summer maximum temperatures in 2011 were up to 4.2°C lower in the Seymour River and 2.1°C lower in Brothers Creek compared to 2010 (Table 3.1).

Temperature dynamics in Seymour River were characterized by a gradual, downstream increase in temperature. In 2010, a progressive increase in seasonal maximum temperature occurred at each downstream logger (Table 3.1), with the exception of the Pool 88 logger where the seasonal maximum was 0.6°C lower than the logger immediately upstream. This downstream warming trend was also apparent from the increased frequency of warmer temperatures (Figure 3.4) and overall temperature increase within reaches (Table 3.2) in both study years. Downstream warming in the Seymour River was less evident in 2011, likely due to colder overall conditions, although maximum temperatures in the lower Seymour remained warmer than in the upper Seymour (above Spur 4).

The Seymour River did not display any significant warming or cooling trends within stream reaches, but unseasonable and abrupt temperature fluctuations were apparent in upper reaches of the river. The fluctuations can be attributed to flow alterations at Seymour Falls Dam. For example, in 2011, the seasonal warming beginning in early July was reversed in August when temperature in the upper river decreased from ~14°C to ~10°C, after which seasonal warming resumed (Figure 3.7A). In addition, a marked increase in temperature unassociated with a rain event occurred in the early summer, probably as dam flow switched from predominately bottom to surface water release. Substantial late-summer warming that coincided with high rainfall events (>90 mm) likely occurred during times of minimum dam discharge. Abrupt temperature
changes that occurred in upper Seymour River were generally not reflected in the lower river, although seasonal warming was curtailed in early August (Figure 3.7B).

Water temperature within Brothers Creek and Hadden Creek displayed variable downstream dynamics. In Brothers Creek, both increases and decreases in seasonal maximum temperature occurred in the downstream direction, whereas only a decreasing maximum temperature occurred on Hadden Creek (Table 3.1). Changes in temperature frequency generally matched the downstream trends in seasonal maximum on both creeks, with an upward shift in the temperature frequency for Brothers Creek (Figure 3.5) and a downward shift for Hadden Creek (Figure 3.6).

Warming of Brothers Creek occurred primarily within reach B1. Temperature at the lower logger in reach B1 was consistently higher than the upper logger (Figure 3.8A) resulting in stable summer warming in this reach (Figure 3.8B). The increase in mean daily temperature within reach B1 was the same in each study year (Table 3.2). Rain events had minimal effects on stream temperature in reach B1 and only temporarily reduced warming (Figure 3.8). The degree of warming was also independent of both air temperature (Figure 3.9A) and input water temperature (Figure 3.9B) during the summer, as well as the remainder of the year. Warming in reach B1 was partially reversed by downstream cooling in reach B2, although this trend was only apparent in 2010 (Table 3.2).

Cooling on Hadden Creek was observed within reach H2. The mean difference in mean daily temperature between loggers was greater in 2010 than 2011 (Table 3.2), possibly due to the absence of data for early summer temperature differences in 2010. During 2011, the complete summer temperature record showed variable degrees of temperature reductions of input water temperature in reach H2. Minimal cooling occurred

during the first half of the summer (June to mid-July), as temperature at the upper and lower logger was approximately equal (Figure 3.10A). Temperatures diverged in the second half (mid-July onwards) (Figure 3.10B) and increasing air and input water temperature was associated with a progressively greater degree of cooling within reach H2 (Figure 3.11A,B). Closer examination of diel temperature fluctuations showed temperature concurrently increased at both loggers (Figure 3.10C) but diel variation was greater at the upper logger (Table 3.1). Maximum temperature differences thus occurred at peak daily water temperatures (Figure 3.10D) and this differential temperature gain possibly accounted for the gradual divergence in temperature during the summer. Differences in daily warming between the upper and lower logger may be partially explained by the reduced influence of air temperature on water temperature at the lower logger (slope = 0.71, $R^2 = 0.91$, P < 0.0001) versus the upper logger (slope = 0.83, $R^2 = 0.89$, P < 0.0001) (Figure 3.12A,B).

Rain events dramatically reduced upstream temperature in reach H2 and this corresponded with a loss of the temperature differential between loggers (Figure 3.10). Cooling steadily returned during periods of no rainfall (Figure 3.10A).

3.4 Discussion

The temperature data I collected were largely able to describe the spatial and temporal water temperature variation within the Seymour River and Brothers Creek systems. As a result, the temperature data are of sufficient resolution to be incorporated with T_{opt} estimates for resident coho salmon, although application would be limited to a

generalized thermal history of fish within different stream reaches. Analysis of temperature dynamics revealed different temperature trends in each study system, both largely influenced by human disturbances. Temperatures across the Seymour River demonstrated that the otherwise natural temperature regime of this river could be significantly perturbed by flow alterations at the upstream Seymour Falls Dam. In Brothers Creek and Hadden Creek, increased headwater temperatures due to riparian habitat loss in upper stream reaches could be partially buffered by cooling processes within intact stream reaches. One shortcoming of the present two-year data set is that both summer seasons were notably cooler and summer water temperatures greatly reduced from preceding years (B. Smith, Seymour River Hatchery, personal communication). Therefore, the possibility of higher temperatures in these watersheds remains to be fully assessed. Ongoing data collection will be important to assess how future temperature changes may impact thermal conditions in these streams. Overall, my study provides an assessment of both temperature conditions and the factors contributing to the temperature dynamics in each study system.

3.4.1 Resolution of Temperature Monitoring

The temporal resolution of temperature monitoring was able to capture the full extent of diel temperature fluctuation in each system. Infrequent temperature recording can lead to underestimation of both diel variation as well as daily maximum temperature (Dunham et al. 2005). Accurate measurement of diel variation is important if cumulative stream temperature data are used to calculate the frequency of temperatures encountered,

as summarized for the two study streams (Figures 3.4-3.6). Further, thermal habitat assessments for fishes that include a temporal component based on such data (e.g. Scruton et al. 1998) could underestimate the extent of adverse conditions if monitoring frequency is too low. Similar concerns would exist if stream temperature conditions were assessed with criteria based on daily maximum temperature (US EPA 2003). These issues were avoided by the high recording frequency of temperature loggers and aided by the relatively moderate diel temperature variation in these streams (Caissie 2006). For the purposes of T_{opt} application, the temporal resolution of the present data is sufficient to estimate the thermal history of fishes, although spatial variability in streams must also be taken into account when determining how well temperature data from stationary loggers represents the thermal history of fish.

Microhabitats in streams will increase spatial temperature variability. Cold-water patches can originate from emergent hyporheic (intragravel) flow (Ebersole et al. 2003a), groundwater or riparian shading (Clark et al. 1999), pool thermal stratification (Matthews et al. 1994) or tributary input (Nielsen et al. 1994) and have an area as small as a few cm. Such localized cold patches would not be captured in mean channel temperature monitoring, although the cumulative effect of such processes might be incorporated in overall cooling trends. As a result, the temperature monitoring on Brothers Creek and the Seymour River was not of sufficient spatial resolution to identify these areas. Microhabitat is an important consideration when applying T_{opt} estimates in small streams because fish can behaviorally thermoregulate to exploit cooler habitats (Berman and Quinn 1991; Nielsen et al. 1994; Stevens and DuPont 2011; Tiffan et al. 2009; Torgersen et al. 1999), thus having a different thermal history from that estimated using water

temperature data. Application of T_{opt} in small streams therefore will be dependent upon the spatial resolution of temperature data.

Identifying microhabitat areas could increase the spatial resolution of temperature monitoring. Handheld water temperature monitoring has been used to detect and determine the extent of microhabitat in streams (Ebersole et al. 2003b). Likewise, attempts have also been made to associate channel morphology with microhabitat abundance (Ebersole et al. 2003a) although definite linkages have yet to emerge. Alterations to streambed morphology have been suggested to reduce hyporheic exchange and microhabitat abundance (Poole and Berman 2001) while channelization has been shown to decrease microhabitat interconnectivity (Ebersole et al. 2003b). Channelization and other human disturbances in the Seymour River and Brothers Creek may have already reduced or eliminated microhabitats in these systems. Therefore, temperature data as recorded could be an accurate representation of temperature conditions, making T_{opt} application more straightforward. Although small scale temperature variation was not a part of this work, future temperature monitoring on these systems could include identification of microhabitat and if present, the extent of cooling in these areas. Information on the availability and quality of cold-water refuge could be then incorporated with application of T_{opt} estimates, under the assumption that fish can find and will use these cooler areas to behaviorally thermoregulate. A natural continuation of this temperature monitoring would be determining the extent of microhabitat use by fish.

3.4.2 Stream Temperature Dynamics

Temperature dynamics in the Seymour River and Brothers Creek were also examined. Stream temperature is determined by a suite of atmospheric and streambed heat exchange processes (Caissie 2006). For the most part, atmospheric processes make up the greatest proportion of heat flux in streams with solar radiation accounting for the majority of atmospheric heat gain whereas net long-wave radiation and evaporative cooling account for most of the heat loss (Johnson 2004; Webb and Zhang 1997). Streambed heat flux also contributes to the heat exchange in streams, although overall less so than atmospheric processes. Groundwater input can warm or cool streams depending on season (Shepherd et al. 1986) and conductive heat loss to the streambed can also play a role in streambed heat flux (Johnson 2004). Processes promoting heat loss, therefore, are important in moderating heat gain and can even lead to cooling in stream reaches with limited solar input (Scruton et al. 1998). The overall temperature regime of a stream is a product of the total heat flux by these factors and is dependent upon input water temperature and total stream discharge (Poole and Berman 2001). Typically, these atmospheric and streambed processes act to increase stream temperature in the downstream direction (Caissie 2006). Pronounced temperature dynamics can arise if the balance of these heat fluxes is altered by human disturbances to riparian habitat or the stream flow regime. As a result, normally gradual downstream warming can become much more acute.

Progressive downstream warming in the Seymour River is characteristic of streams with sufficient balance between natural warming and cooling processes that

buffer against significant heat gains (Caissie 2006). A lack of obvious temperature dynamics within the stream segments and the large scale of the Seymour River make it difficult to determine the processes driving stream temperature in this system. It is readily apparent from summer temperature dynamics, however, that releases from the reservoir on the Seymour River can profoundly alter water temperature throughout the system. The reversal of the seasonal warming trend in August likely resulted from a loss of warm, surface spillage and a change to primarily bottom reservoir release that reduced input water temperature by ~4°C in the upper river. Sub-optimal temperatures will have negative consequences for coho salmon growth (Edsall et al. 1999), ultimately having the same effect as temperatures above T_{opt} but without the possibility of approaching lethality. Application of T_{opt} would therefore have to account for such cold temperature when determining the suitability of temperature conditions. Rapid increases in water temperature were also observed, likely due to rain events suddenly increasing reservoir levels and causing surface dam spillage, although the rate of increase (~1°C h⁻¹) would likely not cause undo stress in fish.

Future temperature regimes of the Seymour River can be expected to be warmer than that observed in either study year. Trends towards earlier summer snowmelt (Stewart et al. 2005) will result in lower mid-summer flows and extended periods where reservoir discharge is minimal and at a higher temperature. Low flows not only reduce the thermal buffering capacity of streams, but also reduce the extent of hyporheic cooling (Poole and Berman 2001). Given the lack of significant cooling in any reach of the Seymour River, future years may be marked by high temperatures that would be of particular consequence for coho salmon residing in the lower reaches of the watershed.

Minimum summer discharge from the reservoir should therefore be of sufficient volume to allow natural heat exchange processes to occur and reduce the frequency of high temperatures (Sinokrot and Gulliver 2000).

The temperature dynamics in Brothers Creek suggest streambed heat exchange processes may provide heat flux into some stream reaches, although the possibility of warming via surface water inputs cannot be eliminated. Reach B1 had the greatest absolute temperature gain of any stream reach within the studied watersheds. Such warming could not be attributed to atmospheric processes as riparian vegetation is generally intact and low solar input would be anticipated, although the extent of riparian shading in this reach was not quantified. Convective heat gain from surrounding air is also a relatively minor component of heat gain (Johnson 2004). Accordingly, warming was not influenced by air temperature during the summer or the remainder of the year (Figure 3.9A). Warming in reach B1 may therefore occur either through streambed heat exchange or tributary input. Streambed processes such as hyporheic exchange (Shepherd et al. 1986) and conductive heat exchange with the streambed (Caissie 2006; Johnson 2004) can dominate heat flux in the absence of solar input. Typically, these processes result in heat loss during the summer as warm surface water infiltrates streambed gravel, exchanges heat with the cooler stream substratum and hyporheic water, and reemerges at a lower temperature (Poole and Berman 2001). However, the snowmelt in 2011 that maintained low surface water temperatures could result in warming if subsurface water was warmer than in-stream water temperature. The possibility of streambed heat gain could be investigated by measuring intra-gravel temperature in this reach (Shepherd et al. 1986). Alternatively, warming could be due to surface water input from tributaries not

identified in this study. Further investigation is needed to determine the source of warming in reach B1, but the pronounced warming in this short length of stream suggest it is caused by human habitat alteration. Regardless of the source of heat gain, headwaters are an important area for maintaining source water temperatures (Scruton et al. 1998). If warming in Brothers Creek reach B1 persists at high temperatures, downstream thermal conditions could negatively affect coho salmon.

Streambed heat exchange processes may also be responsible for moderating high input temperatures in Hadden Creek, although atmospheric processes likely play an important role. In contrast to the low input temperatures in Brothers Creek, input temperatures in Hadden Creek were the highest observed in this study. Loss of riparian vegetation in reach H1 likely caused the high input temperatures, as removal of canopy cover is well known to cause significant increases in solar input and stream temperature (Johnson and Jones 2000; Lynch et al. 1984; Rishel et al. 1982). In reach H2, the high input water temperatures from reach H1 were buffered by cooling processes that reduced daily maximum temperature up to 4.9°C. Riparian vegetation present in reach H2 would significantly limit solar input as well as reduce wind near the water surface, thereby insulating against convective heat transfer (Poole and Berman 2001). Blocking of solar input can also allow for a net heat loss via long wave radiation and evaporation (Caissie 2006; Johnson 2004). Heat loss via atmospheric processes, however, may only confer part of the cooling in reach H2.

Conductive heat loss to the streambed and hyporheic flow probably account for a portion of cooling in reach H2. Burton and Likens (1973) suggested conduction was responsible for the observed cooling when streams transitioned from clear-cut areas to

intact, forested reaches. Likewise, Story et al. (2003) found cooling via streambed conduction accounted for ~35% of an observed 2.3°C cooling in a small stream, with a further ~25% of cooling attributed to hyporheic flow. The influence of streambed processes in reach H2 is apparent from the different intercept and slope for the regressions between mean daily air and water temperature at the upper and lower loggers (Figure 3.12). Linear regressions of these variables will have slopes close to 1 and intercepts closer 0°C if air temperature influence dominates (Erickson and Stefan 2000). In reach H2, the lower logger has both a higher intercept and lower slope than the upper logger, suggesting a moderating streambed component. Interestingly, cooling in reach H2 was only apparent at stream temperatures above 15°C and increased in magnitude with higher input water temperatures and presumably, lower flow (Figure 3.11). Further, rain events and assumed high flow eliminating this cooling. Together, these observations suggest that the streambed processes present in reach H2 are only of sufficient magnitude to cool the stream during low flow periods. Given that high flows and warm input water temperatures are unlikely to occur at the same time, the effects of future climate warming on summer stream temperatures may be effectively moderated in this reach of the stream. This is critical given that snowmelt and rainfall dominated streams like Hadden Creek will be most affected by climate change (Mantua et al. 2010).

Together, the temperature data collected in this study demonstrate that anthropogenic disturbances have significantly altered the temperature regimes in these mountainous, high-gradient waterways. Managing for these disturbances and the additive affects of climate warming may be possible through flow compensation and the preservation of riparian areas and channel morphology. Such measures would maintain

water temperature-moderating processes and thermal heterogeneity in streams, both important for resident fishes. Salmon-bearing streams with contrasting channel morphology, such as low-gradient floodplain type streams, will also benefit from similar preservation strategies. Maintenance of channel complexity should be of particular focus on such streams given that hyporheic exchange has greater importance as channel width increases and the extent of riparian cover decreases (Poole and Berman 2001).

Logger	Watershed	Distance Upstream (m)	Year	Seasonal Maximum (°C)	Mean Diel Variation (°C)	Mean Diel Varation Range (°C)
Upper Seymour	Seymour	18500	2010* 2011	16.7	- 1.4	0.3 - 5.2
Hatchery Pool	Seymour	17810	2010** 2011	16.6 16.4	2.1 1.7	0.4 - 3.9 0.4 - 3.3
Spur 7	Seymour	16230	2010** 2011	16.7 16.4	2.7 2.1	0.6 - 4.4 0.4 - 4.5
Pat's Pool	Seymour	15340	2010** 2011	17.1 16.5	2.3 2.0	0.7 - 4.2 0.4 - 4.4
Spur 4	Seymour	11650	2010** 2011	19.5 16.1	3.6 2.6	0.7 - 5.3 0.5 - 4.8
Cribbing	Seymour	6970	2010** 2011	20.7 17.3	4.1 3.2	1.0 - 6.2 0.6 - 5.8
Twin Bridges	Seymour	4860	2010** 2011	20.7 17.5	3.5 3.2	1.1 - 5.6 0.7 - 5.3
Pool 88	Seymour	3230	2010** 2011	20.1 17.4	2.9 3.0	0.9 - 5.0 0.7 - 5.1
Parkway	Seymour	730	2010** 2011	21.5 17.3	2.5 2.7	0.5 - 3.8 0.6 - 4.9
Upper Brothers	Brothers	4430	2010** 2011	15.3 13.7	0.7 0.9	0.3 - 1.9 0.3 - 2.3
Mathers Avenue	Brothers	2020	2010** 2011	19.2 17.1	1.6 1.7	0.2 - 3.0 0.4 - 4.1
Newdale Avenue	Brothers	1590	2010** 2011	16.7 16.8	1.2 1.5	0.4 - 1.9 0.3 - 3.4
Lower Brothers	Brothers	130	2010** 2011	17.9 16.9	1.6 1.6	0.4 - 2.9 0.3 - 3.4
Upper Hadden	Brothers	3030	2010** 2011	22.3 20.5	1.7 2.3	0.2 - 3.6 0.5 - 5.0
Stevens Drive	Brothers	2270	2010** 2011	18.8 18.2	1.4 1.4	0.4 - 2.6 0.3 - 3.3
Upper Levels	Brothers	1770	2010** 2011	18.7 17.9	1.6 1.4	0.4 - 3.2 0.3 - 3.1

Table 3.1. Temperature logger locations in the Seymour River and Brother Creek watersheds and basic temperature parameters recorded from each logger during the summer (1 June to 15 Sept).

Reach	Reach Length	Year	Mean Difference Between Loggers	Difference in Daily Maximum Between Loggers	
	(m)		(°C)	Mean	Range
S1	690	2010* 2011	0.1	0.2	-2.5 - 1.2
S2	1580	2010** 2011	0.0 0.1	0.3 0.3	-0.5 - 1.1 -0.7 - 1.2
S3	890	2010** 2011	0.3	0.0	-0.5 - 1.8 -1.5 - 1.0
S4	3690	2010** 2011	0.5	1.2	-1.0 - 3.2 -0.8 - 3.1
S5	4680	2010**	0.8	0.9	-0.8 - 2.4
S6	2110	2011 2010** 2011	0.3	0.0	-0.5 - 1.0
S7	1630	2011	0.1	-0.1	-0.7 - 0.9
S8	2500	2011 2010** 2011	0.3 0.4 0.2	0.1 0.3 0.0	-0.3 - 1.0 -1.0 - 1.6 -0.6 - 0.7
B1	2410	2010** 2011	3.2 3.0	3.6 3.5	2.4 - 4.4 1.5 - 4.9
B2	430	2010** 2011	-1.1 0.1	-1.3 0.1	-2.5 - 0.2 -2.1 - 2.3
B3	1460	2010** 2011	0.5 0.6	0.7 0.6	-0.1 - 1.3 -0.6 - 1.4
H2	760	2010**	-2.1	-2.3	-4.9 - 0.3
H3	500	2011 2010** 2011	-1.1 -0.1 -0.1	-1.6 0.0 -0.1	-3.4 - 0.1 -0.4 - 0.2 -0.6 - 0.2

Table 3.2. Mean difference and difference in daily maximum temperatures between the upper and lower loggers in reaches of the Seymour River, Brothers Creek, and Hadden Creek during the summer (1 June to 15 Sept).

Reaches are abbreviated; (S) Seymour River. (B) Brothers Creek. (H) Hadden Creek. * 2010 data are unavailable due to the upper logger being lost. ** 2010 data are from 24 July to 15 Sept.



Figure 3.1. Area map for the Seymour River, Brothers Creek, and Hadden Creek. The (★) indicates the location of Environment Canada weather stations that were the source for air temperature and rainfall data for the associated waterway.



Figure 3.2. Detail map of the Seymour River showing logger locations (\bullet) and the numbered stream reaches (S) between upper and lower loggers.



Figure 3.3. Detail map of Brothers Creek and Hadden Creek showing logger locations (\bullet) and the numbered stream reaches for Brothers Creek (B) and Hadden Creek (H) between upper and lower loggers.



Figure 3.4. Frequency distribution of temperatures for all logger locations on Seymour River for a portion of the summer in 2010 (24 July to 15 Sept) and all of the summer in 2011 (1 June to 15 Sept).



Figure 3.5. Frequency distribution of temperatures for all logger locations on Brothers Creek for a portion of the summer in 2010 (24 July to 15 Sept) and all of the summer in 2011 (1 June to 15 Sept).



Figure 3.6. Frequency distribution of temperatures for all logger locations on Hadden Creek for a portion of the summer in 2010 (24 July to 15 Sept) and all of the summer in 2011 (1 June to 15 Sept).



Figure 3.7. Summer temperature dynamics on the Seymour River. (A) Water temperature (thick line) at the Upper Seymour logger, mean air temperature (thin line), rain events >10 mm (\bigstar), and rain events >20 mm (stacked \bigstar) at the Upper Seymour logger from summer 2011. Gaps in air temperature indicate when data were unavailable. (B) Water temperature (dark line) at the Parkway temperature logger from summer 2011.



Figure 3.8. Temperature dynamics in Brothers Creek reach B1 (A) Water temperature at the Upper Brothers logger (thick black line), Mathers Avenue logger (thick grey line), mean air temperature (thin black line), rain events >10 mm (\bigstar), and rain events >20 mm (stacked \bigstar) from summer 2011. (B) Temperature difference between the Upper Brothers and Mathers Avenue loggers from summer 2011.



Figure 3.9. (A) Daily mean air temperature and (B) mean daily input water temperature *versus* the difference in temperature between the upper and lower loggers in reach B1 during the summer and the remainder of the year from 24 July 2011 to 15 Sept 2011. Days when mean daily air or water temperature was $\leq 0^{\circ}$ C are not shown.



Figure 3.10. Temperature dynamics in Hadden Creek reach H2. (A) Water temperature at the Upper Hadden logger (thick black line), Stevens Drive logger (thick grey line), mean air temperature (thin black line), rain events >10 mm (\bigstar), and rain events >20 mm (stacked \bigstar) from 1 June 2011 to 15 Sept 15 2011. (B) Temperature difference between the Upper Hadden and Stevens Drive loggers from 1 June 2011 to 15 Sept 2011. (C) Detailed view of daily temperature dynamics. (D) Detailed view of the difference in temperature between loggers.



Figure 3.11. (A) Mean daily air temperature and (B) mean daily input water temperature *versus* the difference in temperature between the upper and lower loggers in reach H2 during the summer and the remainder of the year from 24 July 2010 to 15 Sept 2011. Days when mean daily air or water temperature was recorded as $\leq 0^{\circ}$ C are not shown.



Figure 3.12. Regression (solid line) of mean daily air temperature *versus* mean daily water temperature on Hadden Creek at (A) the Upper Hadden logger and (B) the Stevens Drive logger from 24 July 2010 to 15 Sept 2011. Days when air or water temperature recorded as $\leq 0^{\circ}$ C were not included for analysis.

CHAPTER 4: DISCUSSION AND CONCLUSIONS

The primary objective of this thesis was to develop a rapid method for estimating T_{opt} for aerobic scope that could be readily applied to fishes and thus provide an ecologically relevant measure of thermal tolerance in a rapid manner.

Salmon were studied because the physiological basis for the decline in aerobic scope above T_{opt} has been particularly well studied in this group of fishes. Moreover, recent evidence for adult salmon suggested the decline in aerobic scope above T_{opt} is triggered by a limitation on maximum f_{H} , thus providing a mechanism for the development of a rapid T_{opt} estimation method. Chapter 2 presented a methodology using juvenile coho salmon where, after defining T_{opt} for aerobic scope for this life stage and demonstrating T_{opt} coincided with a limitation on maximum f_{H} , this f_{H} limitation was reproduced using pharmacological agents to stimulate maximum f_{H} in anaesthetized fish while acutely warming them. The temperature of this limitation on maximum f_{H} was demonstrated to occur at the same temperature as T_{opt} for aerobic scope. Furthermore, the experimental time needed to estimate T_{opt} with this novel protocol proved to be considerably less than with an abbreviated aerobic scope methodology (3 days *versus* 3 weeks). Therefore, the maximum f_{H} protocol developed here provided an equivalent but much faster estimate of T_{opt} than \dot{MO}_2 measurements.

While this novel T_{opt} estimation method has potential for broad application, there are important tradeoffs with this method that must be recognized. Specifically, the f_{H} protocol and corresponding Arrhenius break temperature analysis only generate a T_{opt} estimate. In comparison, a Fry curve defines T_{opt} and quantifies how aerobic scope

declines as temperatures increase above T_{opt} and approach T_{crit} , the temperature at which aerobic scope collapses (Pörtner 2001). Quantifying the rate of decline of aerobic scope for a species can be an important component in determining the extent to which variable environmental temperatures affect performance. Species that are eurythermal and maintain a high aerobic scope over a broad temperature range will not be as affected by elevated temperature as species that are stenothermal and can maintain aerobic scope only over a narrow temperature range. Thus, a T_{opt} estimate generated with the $f_{\rm H}$ protocol would indicate a temperature at which conditions in a fish's habitat are beyond optimal, but more comprehensive measurement of T_{opt} via $\dot{\rm MO}_2$ may be necessary to fully understand the implications of elevated temperature. Section 4.1 discusses the incorporation of the $f_{\rm H}$ protocol with temperature data from Chapter 3. Subsequent sections discuss the potential for further application of the $f_{\rm H}$ protocol, how $T_{\rm crit}$ data might be derived from the $f_{\rm H}$ data, and how the protocol can be adapted to the various situations that might be encountered during application.

A second objective of this thesis was to comprehensively describe the temperature conditions and dynamics in coho salmon-bearing streams. Two local waterways were selected for water temperature monitoring. The temperature data revealed important but variable temperature dynamics within each stream that showed the potential for certain stream reaches to either help maintain or degrade habitat thermal quality. What emerged from this monitoring was that minimum flows and riparian areas on these streams are critical for moderating water temperature. Because the monitoring was of short duration, multi-year temperature trends could not be described. Local volunteers, however, were trained in the collection of temperature data and the expectation is that ongoing stream

temperature monitoring will be able to assess whether or not the factors that moderate water temperature will continue to do so as climate change progresses and stream temperature increases.

4.1 Combining Rapid *T*_{opt} Determinations with Temperature Data

Numerous approaches have been used to assess the suitability of environmental temperatures for salmon and other species within the family Salmonidae (Flebbe et al. 2006; Isaak et al. 2010; Rieman et al. 2007; Scruton et al. 1998; Williams et al. 2009). Studies that have incorporated T_{opt} estimates focused on temperatures during the narrow migration window of adult salmon on the Fraser River (Farrell et al. 2008; Hague et al. 2011). A more comprehensive approach may be necessary when applying T_{opt} estimates to juvenile coho salmon since rearing success in streams is influenced by the cumulative thermal history of fish over several months (Holtby 1988; Quinn and Peterson 1996).

One approach that may be appropriate for applying T_{opt} estimates to juvenile coho salmon is that of Scruton et al. (1998), who used stream temperature data to calculate the frequency of suitable summer rearing temperatures. Originally, this method categorized temperatures using previous thermal tolerance data. Alternatively, T_{opt} estimates from the $f_{\rm H}$ protocol and Fry curve data can be used to define temperature categories. What follows is an example that illustrates how T_{opt} and temperature data could be combined to assess temperature conditions, and how the results can be interpreted to determine the implications for coho salmon in these streams. Two sets of categorical temperature criteria were generated based on the coho salmon T_{opt} estimates in Chapter 2 and then used to assess the temperature data from Chapter 3. One set of criteria was based the f_{H} protocol and defined temperature as either above or below T_{opt} . The second set of criteria, based on \dot{MO}_2 measurements and the calculated Fry curve (Figure 4.1A), defined various temperature ranges for coho salmon, including an *optimal* range where aerobic scope is >80% of maximum (Figure 4.1B). This percentage was arbitrarily set, as it is unknown what level of aerobic scope is required by coho salmon in this environment.

Temperature analysis using data collected in Chapter 3 and the $f_{\rm H}$ protocol criteria shows these data can indicate the stream reaches where coho salmon would have potentially encountered adverse temperature conditions and where fish could seek refuge from high temperatures (Table 4.1). In 2010, the lower reaches of the Seymour River (S5-S8) and the upper reaches of Hadden Creek (H1) had a high frequency of temperatures exceeding T_{opt} , suggesting sub-optimal conditions for performance and growth. Impacts of such temperatures to fish cannot be determined with this data, but speculation can be made regarding behavioral responses. Fish frequently encountering high temperatures would be expected to either seek out local thermal refuge (Ebersole et al. 2003b) or relocate to more thermally favorable habitat (Berman and Quinn 1991). Loss of microhabitat refugia in the Seymour River and Brothers Creek due to urbanization (Poole and Berman 2001) would likely force fish to thermoregulate via relocation. The $f_{\rm H}$ protocol criteria results from 2010 also suggest coho salmon could seek thermal refuge in upstream reaches of the Seymour River (S1-S4) and downstream Brothers Creek reaches (B2-B3), assuming relocation is not impeded by thermal or

physical barriers. Thus, coho salmon could avoid high temperatures during the warmest of the two study years, although thermal refugia availability could also be limited by inter- and intra-specific competition (Hartman 1965; Mason and Chapman 1965). Together, the T_{opt} estimate from the f_{H} protocol and temperature monitoring provide a straightforward and useful overview of stream conditions as well as the potential for coho salmon to behaviorally thermoregulate and minimize exposure to temperatures exceeding T_{opt} during the summer months.

Stream temperature analysis with Fry curve data and the added designation of an optimal range allows for a broader assessment than that provided by the $f_{\rm H}$ protocol, yielding an indication of the growth capacity for coho salmon in these streams. Growth is also maximal at an optimal temperature (Brett et al. 1969; Elliott and Hurley 2000) and the similarity of growth curves and Fry curves has led to juxtaposition of the two curves (Farrell 2009), suggesting interdependence. Coincidentally, growth of coho salmon with excess ration is maximal at 17°C (Everson 1973; as cited in Sullivan et al. 2000), matching the $T_{\rm opt}$ for aerobic scope as determined in Chapter 2. Coho salmon growth at this life stage is a determinate of survival (Quinn and Peterson 1996) and therefore has direct implications for fitness. Results of the temperature assessment therefore suggest the capacity for coho salmon growth would have been near maximal in 2010 in all stream reaches but restricted in 2011 in the Seymour River due to a high proportion of colder conditions.

An important caveat in the application of aerobic scope as a surrogate for growth and fitness is that T_{opt} for growth can decrease with reduced food availability (Brett et al. 1969; Elliott 1975; Elliott and Hurley 2000; Everson 1973). The daily energy intake of

naturally foraging coho salmon may be ~60% of maximum, reducing T_{opt} for growth by 2-3°C (Sullivan et al. 2000). Competition can also reduce the T_{opt} for growth (McMahon et al. 2007). A lower threshold than the upper end of the optimal range temperature for aerobic scope, therefore, may be more appropriate for estimating growth potential. Further study is needed on the relationship between aerobic scope and growth, but a T_{opt} estimate (and therefore the $f_{\rm H}$ protocol) may be an index suitable for both a general assessment of conditions and an approximation of upper thermal thresholds for lost growth and fitness.

4.2 Comparative Assessments using the $f_{\rm H}$ Protocol

The $f_{\rm H}$ protocol may be best suited for screening for $T_{\rm opt}$ differences among species or populations. Eliason et al. (2011) found population-level differences in $T_{\rm opt}$ for aerobic scope amongst sockeye salmon that correlated with differences in the $T_{\rm opt}$ for $f_{\rm H}$ scope. Therefore, it would be expected that such population-level differences in cardiac performance would be reflected as an equivalent difference using the $f_{\rm H}$ protocol, although this has yet to be tested. Such comparative assessments of populations would be particularly useful in prioritizing conservation efforts, as those populations with the lowest $T_{\rm opt}$ and highest susceptibility to increasing temperature could be quickly identified and then perhaps more thoroughly tested. The Weaver Creek population of sockeye salmon had the lowest $T_{\rm opt}$ of the six sockeye salmon populations considered by Eliason et al. (2011) and was therefore identified as the most vulnerable to climate change. It is unclear, however, whether or not the $f_{\rm H}$ protocol can resolve between $T_{\rm opt}$

values for populations that are separated by only a few degrees. The Weaver Creek population of sockeye salmon with a T_{opt} of 14.5°C and the Chilko River population with a T_{opt} that is 2.3°C higher might be a good test of the methodology.

Comparison of species within the family Salmonidae that have markedly different thermal tolerances would further test the resolving power of the $f_{\rm H}$ protocol. For instance, redband rainbow trout Oncorhynchus mykiss gairdneri have one of the highest thermal tolerances of any trout species (Rodnick et al. 2004). On the other hand, bull trout Salvelinus confluentus are amongst the least thermally tolerant salmonids and show an optimal temperature below that of Weaver Creek sockeye salmon (~13°C; Selong et al. 2001). Performing the $f_{\rm H}$ protocol on each of these species would demonstrate the results for the extremes of thermal sensitivity and possibly provide enhanced analytical capacity to differentiate temperature tolerant and intolerant species. For instance, the ABT would obviously expected to be at a lower temperature for bull trout, but the reduction in the slope of the upper break regression might be more pronounced above the T_{opt} for this species, implying a higher degree of cardiac impairment than in redband rainbow trout. Subsequent studies could use species with more similar thermal tolerances to determine the minimum resolution of the protocol. Initially, these studies would likely have to be paired with measures of aerobic scope (as in Chapter 2) in order to confirm that any differences observed with the $f_{\rm H}$ protocol did indeed correspond to differences in aerobic scope. Population-level comparisons would then be a natural continuation of this investigation if the $f_{\rm H}$ protocol did have sufficient resolution.

4.3 Extending the Applicability of the $f_{\rm H}$ Protocol

It may be possible to use the arrhythmias that are triggered at extreme temperatures during the $f_{\rm H}$ protocol to extend the applicability of the results from the $f_{\rm H}$ protocol. Clark et al. (2008) observed arrhythmias near $T_{\rm crit}$ and use of anaerobic metabolism (e.g. collapse of aerobic scope) for resting Chinook salmon (*Oncorhynchus tshawytscha*). In addition, Farrell et al. (2009) suggested that as a component of the 'death spiral' for maximally swimming salmon, cardiac arrhythmias would occur as result of the increasing reliance on anaerobic metabolism at high temperature, therefore marking the failure of fish to maintain adequate aerobic scope. In Chapter 2, arrhythmias were observed in $f_{\rm H}$ trials at 22.9 ± 0.6°C (MS-222) and this temperature roughly corresponds with the estimated $T_{\rm crit}$ in Figure 4.1. However, since unanaesthetized fish were only tested up to 21°C, the connection between the collapse of aerobic scope and arrhythmias in the $f_{\rm H}$ protocol remains only a possibility.

If future studies were able to draw a definitive link between arrhythmias in the $f_{\rm H}$ protocol and $T_{\rm crit}$, the $f_{\rm H}$ protocol could provide an indication of the breadth of a fish's thermal tolerance. Although the rate of decline of aerobic scope away from $T_{\rm opt}$ would still be unknown, the 'optimal range' for a fish could be estimated based on the temperature difference between the ABT and cardiac arrhythmias. Such information may be useful in the temperature assessment presented in Section 4.1. Interestingly, salmon are estimated to have a $T_{\rm opt} \sim 7^{\circ}$ C below the collapse of aerobic scope (Farrell 2009) and the temperature difference between the ABT and the cardiac arrhythmias in the MS-222 trials was also $\sim 7^{\circ}$ C.

Further confirmation of the relationship between the temperature difference of T_{opt} , cardiac arrhythmias and the breadth of a fish's thermal tolerance could be achieved by performing the $f_{\rm H}$ protocol on markedly eurythermal and stenothermal species. Goldfish are a classic example of a highly eurythermal species that maintains high aerobic scope over a 15°C range (Fry and Hart 1948). In contrast, coral reef fishes of the family Pomacentridae only maintain a high aerobic scope over 2-3°C and display a rapid collapse of aerobic capacity (Johansen and Jones 2011; Nilsson et al. 2009). A corresponding temperature difference between T_{opt} and arrhythmias and the breadth of the Fry curve in these species would lend further support to this difference defining the thermal tolerance of a fish as well as arrhythmias defining the collapse of aerobic scope.

4.4 Adapting the $f_{\rm H}$ Protocol to Species and Conditions

This thesis restricted the investigation of an expedited T_{opt} estimation method to one species within *Oncorhynchus*. However, the temperature dependence of $f_{\rm H}$ (Randall 1970) and strong evidence that many fish species rely almost entirely on $f_{\rm H}$ for increasing oxygen convection through the circulatory system when temperature is elevated (Brodeur et al. 2001; Clark et al. 2008; Gamperl et al. 2011; Gollock et al. 2006; Mendonça and Gamperl 2010; Sandblom and Axelsson 2007; Steinhausen et al. 2008; Stevens et al. 1972) suggest broad potential for this technique. Application of this technique to include other species and even potential field application will likely require modifications to certain aspects of the methodology. Changing the method of $f_{\rm H}$ detection, increasing the resolution of temperature intervals, and need to decrease as well as increase temperature are all potential modifications to the technique that may be required with other species and conditions. Simplification of $f_{\rm H}$ detection to monitoring the ECG without directly wired electrodes is an appealing aspect of the anaesthetized methodology, but morphological limitations in some species may require alternate detection methods.

From an analytical perspective, applying this technique to fishes where T_{opt} and the collapse of aerobic scope are separated only by a few degrees (e.g. coral reef fishes) may not provide a sufficient number of temperature points to calculate the ABT. Therefore, it may be necessary to reduce the 1°C temperature interval between f_{H} measurements used in this study to 0.5°C increments or smaller in order record sufficient temperatures below the ABT for an accurate ABT calculation. Further, a similar situation could arise if field application of this technique is undertaken to estimate T_{opt} of wild fish in their native environment. Ambient environmental temperature could already be within a few degrees or even exceed the T_{opt} for the species being studied. In this case, the temperature of the anaesthetized fish could be reduced to a common starting temperature prior to acute warming.

Further methodological considerations not considered here would likely be necessary given the diversity in fish species and environmental conditions likely to be encountered during application. The methodology outlined in this thesis therefore should be considered a proof-of-content blueprint that other researchers applying this technique should verify and modify to suit their specific applications.

4.5 Conclusions and Perspectives

F.E.J. Fry originally conceived the *scope for activity* concept to provide an ecologically relevant measure of thermal tolerance. More than 50 years later, after both application and renaming, the significance of aerobic scope has finally been demonstrated for aquatic ectotherms with the work of Pörtner and Knust (2007) and Farrell et al. (2008). Both of these studies presented their findings in the context of climate change, a modern dilemma that is already strongly influencing and will continue to impact all types of biological phenomena (Rosenzweig et al. 2008). Conservation measures to preserve and protect species threatened by climate change are typified both by a sense of urgency and limited resources. Prioritizing conservation efforts requires quickly identifying the species that are most vulnerable to climate change while assessing if it is even possible to safeguard species given future climate scenarios. In this regard, this thesis outlined a method that has the potential to greatly assist in the identification of aquatic fish species susceptible to changing environmental temperature.

Contributions were made in this thesis to both the understanding of thermal tolerance in salmon and adaptation of the aerobic scope methodology to meet the challenges of climate change. While comprehensive aerobic scope measures from juvenile salmon were measured over 50 years ago, no previous studies had measured how $f_{\rm H}$ in juvenile salmon responded to temperature. This study was not only the first to describe how both resting and maximum $f_{\rm H}$ change with temperature in juvenile coho salmon, but also the first to relate the changes to aerobic scope and demonstrate that similar cardiorespiratory limits occur at high temperature in juvenile and adult salmon.
Building on this relationship, this thesis then developed a relatively simple methodology that accurately and rapidly predicts T_{opt} for aerobic scope in Pacific salmon, making this method an important tool for screening different salmon species and populations, and determining those most vulnerable to warming environmental temperatures. Such a method will not only be important locally, but also has potential application to a much broader range of species. Finally, the simplicity and applicability of this technique may be best demonstrated by the ease with which field measurements were obtained from coho salmon in local waterways (Table 4.2) and proficiency with which local volunteers acquired the technique.

Reach	Year	Total Time (h)	Fry Curve			$f_{\rm H}$ Protocol	
			Low (<13°C)	Optimal (13-20°C)	High (>20°C)	<16.5°C	>16.5°C
S1	2010*	1296	24%	76%	0%	100%	0%
	2011	2568	90%	10%	0%	100%	0%
S2	2010*	1296	36%	64%	0%	99%	1%
	2011	2568	89%	11%	0%	100%	0%
S3	2010*	1296	17%	83%	0%	99%	1%
	2011	2568	88%	12%	0%	100%	0%
S4	2010*	1296	20%	80%	0%	88%	12%
	2011	2568	86%	14%	0%	100%	0%
S5	2010*	1296	13%	86%	1%	74%	26%
	2011	2568	73%	27%	0%	99%	1%
S6	2010*	1296	10%	89%	1%	71%	29%
	2011	2568	69%	31%	0%	98%	2%
S7	2010*	1296	6%	94%	0%	68%	32%
	2011	2568	65%	35%	0%	98%	2%
S8	2010*	1296	3%	96%	1%	59%	41%
	2011	2568	57%	43%	0%	98%	2%
B1	2010*	1296	7%	93%	0%	74%	26%
	2011	2568	54%	46%	0%	99%	1%
B2	2010*	1296	13%	87%	0%	99%	1%
	2011	2568	55%	45%	0%	100%	0%
B3	2010*	1296	11%	89%	0%	91%	9%
	2011	2568	51%	49%	0%	100%	0%
H1	2010*	1296	1%	85%	14%	46%	54%
	2011	2568	12%	88%	0%	59%	41%
H2	2010*	1296	2%	98%	0%	77%	23%
	2011	2568	14%	86%	0%	92%	8%
Н3	2010*	1296	4%	96%	0%	78%	22%
	2011	2568	15%	85%	0%	94%	6%
	11 '	(1 (0) 0	D' (D) D			7 1	

Table 4.1. Habitat thermal quality assessment of the Seymour River and Brothers Creek watersheds during the summer (1 June to 15 Sept). Data are presented as percentage of total time temperatures were within the designated range for different stream reaches.

Reaches are abbreviated; (S) Seymour River. (B) Brothers Creek. (H) Hadden Creek.

* 2010 data is from 24 July to 15 Sept.

Table 4.2. Environmental conditions, physical parameters, cardiac collapse, and breakpoint analysis of heart rate ($f_{\rm H}$) during an acute temperature change (10°C h⁻¹) in wild juvenile coho salmon (*Oncorhynchus kisutch*) sedated with 50 ppm MS-222. Maximum $f_{\rm H}$ was stimulated with atropine (1.2 mg kg⁻¹) and isoproterenol (4 µg kg⁻¹) injections. Fish were collected in 2011 from the Seymour River and Brothers Creek and field tested at the Seymour River Hatchery and Capilano Hatchery, respectively. Temperature adjustments to a lower initial temperature were performed in 0.5°C step-wise changes during the 1 h equilibration period.

	Seymour River	Seymour River	Brothers Creek
	N = 12	$N \equiv 8$	$N \equiv 9$
Protocol Procedures			
Dates of Testing	17 July – 19 July	7 Sept – 10 Sept	29 Aug – 1 Sept
Ambient Temperature (°C)	8.1 - 8.5	13.8 - 14.2	14.7 - 15.3
Temperature Adjustment (°C)	-	-	-2.0°C
Sampling			
Mass (g)	5.7 ± 0.3	7.1 ± 0.5	4.5 ± 0.3
Individual Breakpoint Analysis			
Arrhenius break temperature (°C)	14.3 ± 0.2	16.9 ± 0.4	18.2 ± 0.2
Range (Min-Max)	13.8 - 15.1	15.3 - 17.9	17.6 – 19.3
Breakpoint $f_{\rm H}$ (beats min ⁻¹)	110.5 ± 1.6	114.8 ± 3.6	117.3 ± 2.6
Cardiac Collapse			
Pre-arrhythmia $f_{\rm H}$ (beats min ⁻¹)	127.6 ± 10.4	138.1 ± 7.1	141.0 ± 4.3
Arrhythmia temperature (°C)	23.2 ± 0.5	22.9 ± 0.9	23.0 ± 0.5
All values are mean \pm S.E.			



Figure 4.1. (A) Polynomial quadratic regression of aerobic scope for juvenile (*Oncorhynchus kisutch*) over the measured temperature range (solid line) and extrapolation to zero aerobic scope at high temperatures (dashed line). (B) A Fry curve for aerobic scope for coho salmon presented as the percentage of maximum aerobic scope. Optimal temperature (T_{opt}) is 16.6°C. *Low* (<13°C), *Optimal* (13-20°C), and *High* (>20°C) temperature ranges were based on an estimated aerobic scope of 80% (dashed line) required for rearing success of juvenile coho salmon. The estimate was based on instream competition (Mason and Chapman 1965), habitat preference (Taylor 1991), and growth curves (Sullivan et al. 2000).

REFERENCES

Altimiras, J., Aissaoui, A., Tort, L., and Axelsson, M. 1997. Cholinergic and adrenergic tones in the control of heart rate in teleosts. How should they be calculated? Comp. Biochem. Physiol. A **118**(1): 131-139.

Altimiras, J., and Larsen, E. 2000. Non-invasive recording of heart rate and ventilation rate in rainbow trout during rest and swimming. Fish go wireless! J. Fish Biol. **57**(1): 197-209.

Anderson, W.G., McKinley, R.S., and Colavecchia, M. 1997. The use of clove oil as an anesthetic for rainbow trout and its effects on swimming performance. N. Am. J. Fish. Manage. **17**(2): 301-307.

Arrhenius, S. 1915. Quantitative Laws in Biological Chemistry. G. Bell and Sons, Ltd., London.

Axelsson, M., and Farrell, A.P. 1993. Coronary blood flow *in vivo* in the coho salmon (*Oncorhynchus kisutch*). Am. J. Physiol. **264**(5): R963-R971.

Berman, C.H., and Quinn, T.P. 1991. Behaviroal thermorgulation and homing by spring chinook salmon *Oncorhynchus tshawytscha* (Walbaum), in the Yakima River. J. Fish Biol. **39**(3): 301-312.

Blackman, F.F. 1905. Optima and limiting factors. Ann. Bot. 19(73-76): 281-296.

Booth, J.H. 1979. Circulation in trout gills: the relationship between branchial perfusion and the width of the lamellar blood space. Can. J. Zool. **57**(11): 2183-2185.

Bouchard, P., and Guderley, H. 2003. Time course of the response of mitochondria from oxidative muscle during thermal acclimation of rainbow trout, Oncorhynchus mykiss. J. Exp. Biol. **206**(19): 3455-3465.

Brett, J.R. 1944. Some lethal temperature relations of Algonquin park fishes. Publ. Ontario Fish. Res. Lab. **63**: 1-49.

Brett, J.R. 1964. The respiratory metabolism and swimming performance of young sockeye salmon. J. Fish. Res. Bd. Can. **21**(5): 1183-1226.

Brett, J.R. 1965. The relation of size to rate of oxygen consumption and sustained swimming speed of sockeye salmon (*Oncorhynchus nerka*). J. Fish. Res. Bd. Can. **22**(6): 1491-1501.

Brett, J.R. 1971. Energetic responses of salmon to temperature. A study of some of the thermal relations in the physiology and freshwater ecology of sockeye salmon (*Oncorhynchus nerka*). Amer. Zool. **11**(1): 99-113.

Brett, J.R., Hollands, M., and Alderdice, D.F. 1958. The effect of temperature on the cruising speed of young sockeye and coho salmon. J. Fish. Res. Bd. Can. **15**(4): 587-605.

Brett, J.R., Shelbourn, J.E., and Shoop, C.T. 1969. Growth rate and body composition of fingerling sockeye salmon, *Oncorhynchus nerka*, in relation to temperature and ration size. J. Fish. Res. Bd. Can. **26**(9): 2363-2394.

Britton, S.W. 1924. The effects of extreme temperatures on fishes. Am. J. Physiol. **67**(2): 411-421.

Brodeur, J.C., Dixon, D.G., and McKinley, R.S. 2001. Assessment of cardiac output as a predictor of metabolic rate in rainbow trout. J. Fish Biol. **58**(2): 439-452.

Burka, J.F., Hammell, K.L., Horsberg, T.E., Johnson, G.R., Rainnie, D.J., and Speare, D.J. 1997. Drugs in salmonid aquaculture - A review. J. Vet. Pharmacol. Ther. **20**(5): 333-349.

Burton, T.M., and Likens, G.E. 1973. The effect of strip cutting on stream temperatures in Hubbard Brook experimental forest, New Hampshire. Bioscience **23**(7): 433-435.

Butterworth, J., James, R.L., and Grimes, J. 1997. Structure-affinity relationships and stereospecificity of several homologous series of local anesthetics for the beta(2)-adrenergic receptor. Anesth. Analg. **85**(2): 336-342.

Butterworth, J.F., and Strichartz, G.R. 1990. Molecular mechanisms of local anesthesia: a review. Anesthesiology **72**(4): 711-734.

Caissie, D. 2006. The thermal regime of rivers: a review. Freshw. Biol. 51(8): 1389-1406.

Carter, K.M., Woodley, C.M., and Brown, R.S. 2011. A review of tricaine methanesulfonate for anesthesia of fish. Rev. Fish. Biol. Fish. **21**(1): 51-59.

Clark, E., Webb, B.W., and Ladle, M. 1999. Microthermal gradients and ecological implications in Dorset rivers. Hydrol. Process. **13**(3): 423-438.

Clark, T.D., and Farrell, A.P. 2011. Effects of body mass on physiological and anatomical parameters of mature salmon: evidence against a universal heart rate scaling exponent. J. Exp. Biol. **214**(6): 887-893.

Clark, T.D., Sandblom, E., Cox, G.K., Hinch, S.G., and Farrell, A.P. 2008. Circulatory limits to oxygen supply during an acute temperature increase in the Chinook salmon (*Oncorhynchus tshawytscha*). Am. J. Physiol. **295**(5): R1631-R1639.

Clarke, A., and Fraser, K.P.P. 2004. Why does metabolism scale with temperature? Funct. Ecol. **18**(2): 243-251.

Cooke, S.J., Ostrand, K.G., Bunt, C.M., Schreer, J.F., Wahl, D.H., and Philipp, D.P. 2003. Cardiovascular responses of largemouth bass to exhaustive exercise and brief air exposure over a range of water temperatures. T. Am. Fish. Soc. **132**(6): 1154-1165.

Cotter, P.A., and Rodnick, K.J. 2006. Differential effects of anesthetics on electrical properties of the rainbow trout (*Oncorhynchus mykiss*) heart. Comp. Biochem. Physiol. A **145**(2): 158-165.

Crozier, W.J. 1926. The distribution of temperature characteristics for biological processes; Critical increments for heart rates. J. Gen. Physiol. **9**(4): 531-546.

Davis, L.E., and Schreck, C.B. 1997. The energetic response to handling stress in juvenile coho salmon. T. Am. Fish. Soc. **126**(2): 248-258.

Dejours, P. 1981. Principles of Comparative Respiratory Physiology. 2nd ed. Elsevier/North Holland Press, New York.

Donaldson, M.R., Cooke, S.J., Patterson, D.A., Hinch, S.G., Robichaud, D., Hanson, K.C., Olsson, I., Crossin, G.T., English, K.K., and Farrell, A.P. 2009. Limited behavioural thermoregulation by adult upriver-migrating sockeye salmon (*Oncorhynchus nerka*) in the Lower Fraser River, British Columbia. Can. J. Zool. **87**(6): 480-490.

Dulvy, N.K., Rogers, S.I., Jennings, S., Stelzenmueller, V., Dye, S.R., and Skjoldal, H.R. 2008. Climate change and deepening of the North Sea fish assemblage: a biotic indicator of warming seas. J. Appl. Ecol. **45**(4): 1029-1039.

Dunham, J., Chandler, G., Rieman, B., and Martin, D. 2005. Measuring stream temperature with digital data loggers: A user's guide. United States Department of Agriculture, Forest Service, Rocky Mountain Research Station. GTR-150WWW. pp. 15.

Ebersole, J.L., Liss, W.J., and Frissell, C.A. 2003a. Cold water patches in warm streams: Physicochemical characteristics and the influence of shading. J. Am. Water Resour. Assoc. **39**(2): 355-368.

Ebersole, J.L., Liss, W.J., and Frissell, C.A. 2003b. Thermal heterogeneity, stream channel morphology, and salmonid abundance in northeastern Oregon streams. Can. J. Fish. Aquat. Sci. **60**(10): 1266-1280.

Edsall, T.A., Frank, A.M., Rottiers, D.V., and Adams, J.V. 1999. The effect of temperature and ration size on the growth, body composition, and energy content of juvenile coho salmon. J. Gt. Lakes Res. **25**(2): 355-362.

Ege, R., and Krogh, A. 1914. On the relationship between the temperature and the respiratory exchange in fishes. Int. Rev. Hydro. Hydrograph. 7: 48-55.

Eliason, E.J. 2011. Cardiorespiratory physiology and temperature tolerance among populations of sockeye salmon (*Oncorhynchus nerka*). PhD thesis. University of British Columbia, Vancouver, BC. pp. 197.

Eliason, E.J., Clark, T.D., Hague, M.J., Hanson, L.M., Gallagher, Z.S., Jeffries, K.M., Gale, M.K., Patterson, D.A., Hinch, S.G., and Farrell, A.P. 2011. Differences in thermal tolerance among sockeye salmon populations. Science **332**(6025): 109-112.

Elliott, J.M. 1975. The growth rate of brown trout (*Salmo trutta* L.) fed on reduced rations. J. Anim. Ecol. **44**(3): 823-842.

Elliott, J.M., and Elliott, J.A. 2010. Temperature requirements of Atlantic salmon *Salmo salar*, brown trout *Salmo trutta* and Arctic charr *Salvelinus alpinus*: Predicting the effects of climate change. J. Fish Biol. **77**(8): 1793-1817.

Elliott, J.M., and Hurley, M.A. 2000. Optimum energy intake and gross efficiency of energy conversion for brown trout, Salmo trutta, feeding on invertebrates or fish. Freshw. Biol. **44**(4): 605-615.

Environment Canada. 2011. National Climate Data and Information Archive. Available from www.climate.weatheroffice.gc.ca [Accessed 1 December 2011].

Erickson, T.R., and Stefan, H.G. 2000. Linear air/water temperature correlations for streams during open water periods. J. Hydrol. Eng. **5**(3): 317-321.

Everson, L.B. 1973. Growth and food consumption of juvenile coho salmon exposed to natural and elevated fluctuating temperatures. MSc thesis. Oregon State University, Corvallis, OR. pp. 68.

Farrell, A.P. 1991. From hagfish to tuna: a perspective on cardiac function in fish. Physiol. Zool. **64**(5): 1137-1164.

Farrell, A.P. 2009. Environment, antecedents and climate change: lessons from the study of temperature physiology and river migration of salmonids. J. Exp. Biol. **212**(23): 3771-3780.

Farrell, A.P., and Clutterham, S.M. 2003. On-line venous oxygen tensions in rainbow trout during graded exercise at two acclimation temperatures. J. Exp. Biol. **206**(3): 487-496.

Farrell, A.P., Daxboeck, C., and Randall, D.J. 1979. Effect of input pressure and flow on the pattern and resistance to flow in the isolated perfused gill of a teleost fish. J. Comp. Physiol. **133**(3): 233-240.

Farrell, A.P., Eliason, E.J., Sandblom, E., and Clark, T.D. 2009. Fish cardiorespiratory physiology in an era of climate change. Can. J. Zool. **87**(10): 835-851.

Farrell, A.P., Hinch, S.G., Cooke, S.J., Patterson, D.A., Crossin, G.T., Lapointe, M., and Mathes, M.T. 2008. Pacific salmon in hot water: Applying aerobic scope models and biotelemetry to predict the success of spawning migrations. Physiol. Biochem. Zool. **81**(6): 697-708.

Farrell, A.P., and Jones, D.R. 1992. The heart. *In* Fish Physiology. Vol. 12A. *Edited by* W.S. Hoar, D. J. Randall, and A. P. Farrell. Academic Press, San Diego. pp. 1-88.

Farrell, A.P., Lee, C.G., Tierney, K., Hodaly, A., Clutterham, S., Healey, M., Hinch, S., and Lotto, A. 2003. Field-based measurements of oxygen uptake and swimming performance with adult Pacific salmon using a mobile respirometer swim tunnel. J. Fish Biol. **62**(1): 64-84.

Flebbe, P.A., Roghair, L.D., and Bruggink, J.L. 2006. Spatial modeling to project southern Appalachian trout distribution in a warmer climate. T. Am. Fish. Soc. **135**(5): 1371-1382.

Frederich, M., and Pörtner, H.O. 2000. Oxygen limitation of thermal tolerance defined by cardiac and ventilatory performance in spider crab, Maja squinado. Am. J. Physiol.-Regul. Integr. Comp. Physiol. **279**(5): R1531-R1538.

Fredricks, K.T., Gingerich, W.H., and Fater, D.C. 1993. Comparative cardiovascular effects of four fishery anesthetics in spinally transected rainbow trout, *Oncorhynchus mykiss*. Comp. Biochem. Physiol. **104C**(3): 477-483.

Fry, F.E.J. 1947. Effects of the environment on animal activity. Publ. Ontario Fish. Res. Lab. **68**: 1-52.

Fry, F.E.J., and Hart, J.S. 1948. The relation of temperature to oxygen consumption in the goldfish. Biol. Bull. **94**(1): 66-77.

Gallaugher, P., Axelsson, M., and Farrell, A.P. 1992. Swimming performance and hematological variables in splenectomized rainbow trout, *Oncorhynchus mykiss*. J. Exp. Biol. **171**: 301-314.

Gamperl, A.K., Swafford, B.L., and Rodnick, K.J. 2011. Elevated temperature, per se, does not limit the ability of rainbow trout to increase stroke volume. J. Therm. Biol. **36**(1): 7-14.

Gibson, E.S., and Fry, F.E.J. 1954. The performance of the lake trout, *Salvelinus namaycush*, at various levels of temperature and oxygen pressure. Can. J. Zool. **32**(3): 252-260.

Gillooly, J.F., Brown, J.H., West, G.B., Savage, V.M., and Charnov, E.L. 2001. Effects of size and temperature on metabolic rate. Science **293**(5538): 2248-2251.

Gollock, M.J., Currie, S., Petersen, L.H., and Gamperl, A.K. 2006. Cardiovascular and haematological responses of Atlantic cod (*Gadus morhua*) to acute temperature increase. J. Exp. Biol. **209**(15): 2961-2970.

Griffiths, J.S., and Alderdice, D.F. 1972. Effects of acclimation and acute temperature experience on swimming speed of juvenile coho salmon. J. Fish. Res. Bd. Can. **29**(3): 251-264.

Hague, M.J., Ferrari, M.R., Miller, J.R., Patterson, D.A., Russell, G.L., Farrell, A.P., and Hinch, S.G. 2011. Modelling the future hydroclimatology of the lower Fraser River and its impacts on the spawning migration survival of sockeye salmon. Global Change Biol. **17**(1): 87-98.

Hartman, G.F. 1965. The role of behavior in the ecology and interaction of underyearling coho salmon (*Oncorhynchus kisutch*) and steelhead trout (*Salmo gairdneri*). J. Fish. Res. Bd. Can. **22**(4): 1035-1081.

Haynie, D.T. 2008. Biological Thermodynamics. 2nd ed. Cambridge University Press, Cambridge.

Heath, A.G., and Hughes, G.M. 1973. Cardiovascular and respiratory changes during heat stress in rainbow trout (*Salmo gairdneri*). J. Exp. Biol. **59**(2): 323-338.

Hill, J.V., Davison, W., and Forster, M.E. 2002. The effects of fish anaesthetics (MS222, metomidate and AQUI-S) on heart ventricle, the cardiac vagus and branchial vessels from Chinook salmon (*Oncorhynchus tshawytscha*). Fish. Physiol. Biochem. **27**(1-2): 19-28.

Hill, J.V., and Forster, M.E. 2004. Cardiovascular responses of Chinook salmon (*Oncorhynchus tshawytscha*) during rapid anaesthetic induction and recovery. Comp. Biochem. Physiol. **137**(2): 167-177.

Hochachka, P.W., and Somero, G.N. 2002. Biochemical Adaptation: Mechanism and Process in Physiological Evolution. Oxford University Press, Oxford.

Holtby, L.B. 1988. Effects of logging on stream temperatures in Carnation Creek, British Columbia, and associated impacts on the coho salmon (*Oncorhynchus kisutch*). Can. J. Fish. Aquat. Sci. **45**(3): 502-515.

IPCC. 2007. Climate Change 2007: The Physical Science Basis: Intergovernmental Panel on Climate Change Fourth Assessment Report: URL

Isaak, D.J., Luce, C.H., Rieman, B.E., Nagel, D.E., Peterson, E.E., Horan, D.L., Parkes, S., and Chandler, G.L. 2010. Effects of climate change and wildfire on stream temperatures and salmonid thermal habitat in a mountain river network. Ecol. Appl. **20**(5): 1350-1371.

Iwama, G.K., McGeer, J.C., and Pawluk, M.P. 1989. The effects of five fish anesethetics on acid-base balance, hematocrit, blood gases, cortisol, and adrenaline in rainbow trout. Can. J. Zool.-Rev. Can. Zool. **67**(8): 2065-2073.

Iwaya-Inoue, M., Sakaguchi, K., and Kaku, S. 1989. Statistical studies using the AIC method to decide the question of "break" or "straight" in Arrhenius plots of water proton NMR relaxation times in chilling-sensitive *Vigna* and insensitive *Pisum* seedlings. Plant Cell Physiol. **30**(2): 309-316.

Jain, K.E., Hamilton, J.C., and Farrell, A.P. 1997. Use of a ramp velocity test to measure critical swimming speed in rainbow trout (*Oncorhynchus mykiss*). Comp. Biochem. Physiol. A **117**(4): 441-444.

Janz, D.M., Farrell, A.P., Morgan, J.D., and Vigers, G.A. 1991. Acute physiological stress responses of juvenile coho salmon (*Oncorhynchus kisutch*) to sublethal concnetrations of Garlon 4, Garlon 3A, and Vision herbicides. Environ. Toxicol. Chem. **10**(1): 81-90.

Jensen, F.B., Fago, A., and Weber, R.E. 1998. Hemoglobin structure and function. *In* Fish Respiration. Vol. 17. *Edited by* S.F. Perry, and B. Tufts. Academic Press, New York. pp. 1-40.

Johansen, J.L., and Jones, G.P. 2011. Increasing ocean temperature reduces the metabolic performance and swimming ability of coral reef damselfishes. Global Change Biol. **17**(9): 2971-2979.

Johnson, S.L. 2004. Factors influencing stream temperatures in small streams: substrate effects and a shading experiment. Can. J. Fish. Aquat. Sci. **61**(6): 913-923.

Johnson, S.L., and Jones, J.A. 2000. Stream temperature responses to forest harvest and debris flows in western Cascades, Oregon. Can. J. Fish. Aquat. Sci. **57**: 30-39.

Johnsson, J.I., Hojesjo, J., and Fleming, I.A. 2001. Behavioural and heart rate responses to predation risk in wild and domesticated Atlantic salmon. Can. J. Fish. Aquat. Sci. **58**(4): 788-794.

Johnston, I.A., Clarke, A., and Ward, P. 1991. Temperature and metabolic rate in sedentary fish from the Antarctic, North Sea, and Indo-West Pacific Ocean. Mar. Biol. **109**(2): 191-195.

Kiceniuk, J.W., and Jones, D.R. 1977. The oxygen transport system in trout (*Salmo gairdneri*) during sustained exercise. J. Exp. Biol. **69**: 247-260.

Knudsen, F.R., Enger, P.S., and Sand, O. 1992. Awareness and avoidance responses to sound in juvenile Atlantic salmon, *Salmo salar* L. J. Fish Biol. **40**(4): 523-534.

Lannig, G., Bock, C., Sartoris, F.J., and Portner, H.O. 2004. Oxygen limitation of thermal tolerance in cod, *Gadus morhua* L., studied by magnetic resonance imaging and on-line venous oxygen monitoring. Am. J. Physiol. **287**(4): R902-R910.

Lee, C.G., Farrell, A.P., Lotto, A., MacNutt, M.J., Hinch, S.G., and Healey, M.C. 2003. The effect of temperature on swimming performance and oxygen consumption in adult sockeye (*Oncorhynchus nerka*) and coho (*O. kisutch*) salmon stocks. J. Exp. Biol. **206**(18): 3239-3251.

Lillywhite, H.B., Zippel, K.C., and Farrell, A.P. 1999. Resting and maximal heart rates in ectothermic vertebrates. Comp. Biochem. Physiol. A-Mol. Integr. Physiol. **124**(4): 369-382.

Logan, S.R. 1982. The origin and status of the Arrhenius equation. J. Chem. Educ. **59**(4): 279-281.

Lynch, J.A., Rishel, G.B., and Corbett, E.S. 1984. Thermal alterations of streams draining clearcut watersheds: Quantification and biological implications. Hydrobiologia **111**(3): 161-169.

Mantua, N., Tohver, I., and Hamlet, A. 2010. Climate change impacts on streamflow extremes and summertime stream temperature and their possible consequences for freshwater salmon habitat in Washington State. Clim. Change **102**(1-2): 187-223.

Mason, J.C., and Chapman, D.W. 1965. Significance of early emergence, environmental rearing capacity, and behavioral ecology of juvenile coho salmon in stream channels. J. Fish. Res. Bd. Can. **22**(1): 173-190.

Matthews, K.R., Berg, N.H., Azuma, D.L., and Lambert, T.R. 1994. Cool water formation and trout habitat use in a deep pool in the Sierra Nevada, California. T. Am. Fish. Soc. **123**(4): 549-564.

McMahon, T.E., Zale, A.V., Barrows, F.T., Selong, J.H., and Danehy, R.J. 2007. Temperature and competition between bull trout and brook trout: A test of the elevation refuge hypothesis. T. Am. Fish. Soc. **136**(5): 1313-1326.

Mendonça, P.C., and Gamperl, A.K. 2010. The effects of acute changes in temperature and oxygen availability on cardiac performance in winter flounder (*Pseudopleuronectes americanus*). Comp. Biochem. Physiol. A **155**(2): 245-252.

Moreira-Lobo, D.C.A., Linhares-Siqueira, E.D., Cruz, G.M.P., Cruz, J.S., Carvalho-De-Souza, J.L., Lahlou, S., Coelho-De-Souza, A.N., Barbosa, R., Magalhaes, P.J.C., and Leal-Cardoso, J.H. 2010. Eugenol modifies the excitability of rat sciatic nerve and superior cervical ganglion neurons. Neurosci. Lett. **472**(3): 220-224.

Munday, P.L., Crawley, N.E., and Nilsson, G.E. 2009. Interacting effects of elevated temperature and ocean acidification on the aerobic performance of coral reef fishes. Mar. Ecol.-Prog. Ser. **388**: 235-242.

Nielsen, J.L., Lisle, T.E., and Ozaki, V. 1994. Thermally stratified pools and their use by steelhead in northern California streams. T. Am. Fish. Soc. **123**(4): 613-626.

Nilsson, G.E., Crawley, N., Lunde, I.G., and Munday, P.L. 2009. Elevated temperature reduces the respiratory scope of coral reef fishes. Global Change Biol. **15**(6): 1405-1412.

Patterson, D.A., Macdonald, J.S., Skibo, K.M., Barnes, D.P., Guthrie, I., and Hills, J. 2007. Reconstructing the summer thermal history for the lower Fraser River, 1941 to

2006, and implications for adult sockeye salmon (*Oncorhynchus nerka*) spawning migration. Can. Tech. Rep. Fish. Aquat. Sci. **2724**: 1-43.

Perry, A.L., Low, P.J., Ellis, J.R., and Reynolds, J.D. 2005. Climate change and distribution shifts in marine fishes. Science **308**(5730): 1912-1915.

Plaut, I. 2001. Critical swimming speed: its ecological relevance. Comp. Biochem. Physiol. A **131**(1): 41-50.

Poole, G.C., and Berman, C.H. 2001. An ecological perspective on in-stream temperature: Natural heat dynamics and mechanisms of human-caused thermal degradation. Environ. Manage. **27**(6): 787-802.

Pörtner, H.O. 2001. Climate change and temperature-dependent biogeography: oxygen limitation of thermal tolerance in animals. Naturwissenschaften **88**(4): 137-146.

Pörtner, H.O. 2010. Oxygen- and capacity-limitation of thermal tolerance: a matrix for integrating climate-related stressor effects in marine ecosystems. J. Exp. Biol. **213**(6): 881-893.

Pörtner, H.O., Berdal, B., Blust, R., Brix, O., Colosimo, A., De Wachter, B., Giuliani, A., Johansen, T., Fischer, T., Knust, R., Lannig, G., Naevdal, G., Nedenes, A., Nyhammer, G., Sartoris, F.J., Serendero, I., Sirabella, P., Thorkildsen, S., and Zakhartsev, M. 2001. Climate induced temperature effects on growth performance, fecundity and recruitment in marine fish: developing a hypothesis for cause and effect relationships in Atlantic cod (*Gadus morhua*) and common eelpout (*Zoarces viviparus*). Cont. Shelf Res. 21(18-19): 1975-1997.

Pörtner, H.O., and Farrell, A.P. 2008. Ecology. Physiology and Climate Change. Science **322**(5902): 690-692.

Pörtner, H.O., and Knust, R. 2007. Climate change affects marine fishes through the oxygen limitation of thermal tolerance. Science **315**(5808): 95-97.

Priede, I.G. 1974. Effect of swimming activity and section of vagus nerve on heart rate in rainbow trout. J. Exp. Biol. 60(2): 305-319.

Priede, I.G., and Tytler, P. 1977. Heart rate as a measure of metabolic rate in teleost fishes; *Salmo gairdneri*, *Salmo trutta*, and *Gadus morhua*. J. Fish Biol. **10**(3): 231-242.

Quinn, T.P. 2005. The behavior and ecology of Pacific salmon and trout. University of Washington Press, Seattle.

Quinn, T.P., and Peterson, N.P. 1996. The influence of habitat complexity and fish sire on over-winter survival and growth of individually marked juvenile coho salmon (*Oncorhynchus kisutch*) in Big Beef creek, Washington. Can. J. Fish. Aquat. Sci. **53**(7): 1555-1564.

Randall, D., and Brauner, C. 1991. Effects of environmental factors on exercise in fish. J. Exp. Biol. **160**: 113-126.

Randall, D.J. 1970. The circulatory system. *In* Fish physiology. Vol. 4. *Edited by* W.S. Hoar, and D. J. Randall. Academic Press, New York. pp. 133-172.

Randall, D.J., and Daxboeck, C. 1982. Cardiovascular changes in the rainbow trout (*Salmo gairdneri* Richardson) during exercise. Can. J. Zool. **60**(5): 1135-1140.

Randall, D.J., Holeton, G.F., and Stevens, E.D. 1967. The exchange of oxygen and carbon dioxide across the gills of rainbow trout. J. Exp. Biol. **46**(2): 339-348.

Reidy, S.P., Nelson, J.A., Tang, Y., and Kerr, S.R. 1995. Post-exercise metabolic rate in Atlantic cod and its dependence upon the method of exhaustion. J. Fish Biol. **47**(3): 377-386.

Rieman, B.E., Isaak, D., Adams, S., Horan, D., Nagel, D., Luce, C., and Myers, D. 2007. Anticipated climate warming effects on bull trout habitats and populations across the interior Columbia River basin. T. Am. Fish. Soc. **136**(6): 1552-1565.

Rishel, G.B., Lynch, J.A., and Corbett, E.S. 1982. Seasonal stream temperature changes following forest harvesting. J. Environ. Qual. **11**(1): 112-116.

Rodnick, K.J., Gamperl, A.K., Lizars, K.R., Bennett, M.T., Rausch, R.N., and Keeley, E.R. 2004. Thermal tolerance and metabolic physiology among redband trout populations in south-eastern Oregon. J. Fish Biol. **64**(2): 310-335.

Rommel, S.A. 1973. A simple method for recording fish heart and operculum beats without the use of implanted electrodes. J. Fish. Res. Bd. Can. **30**(5): 693-694.

Rosenzweig, C., Karoly, D., Vicarelli, M., Neofotis, P., Wu, Q.G., Casassa, G., Menzel, A., Root, T.L., Estrella, N., Seguin, B., Tryjanowski, P., Liu, C.Z., Rawlins, S., and Imeson, A. 2008. Attributing physical and biological impacts to anthropogenic climate change. Nature **453**(7193): 353-357.

Ryan, S.N., Davie, P.S., Gesser, H., and Wells, R.M.G. 1993. The effect of MS-222 on paced ventricular strips and the perfused heart of rainbow trout, *Oncorhynchus mykiss*. Comp. Biochem. Physiol. **106C**(2): 549-553.

Sandblom, E., and Axelsson, M. 2007. Venous hemodynamic responses to acute temperature increase in the rainbow trout (*Oncorhynchus mykiss*). Am. J. Physiol. **292**(6): R2292-R2298.

Scarabello, M., Heigenhauser, G.J.F., and Wood, C.M. 1991. The oxygen debt hypothesis in juvenile rainbow trout after exhaustive exercise. Respiration Physiology **84**(2): 245-259.

Scarabello, M., Heigenhauser, G.J.F., and Wood, C.M. 1992. Gas exchange, metabolite status and excess post-exercise oxygen consumption after repetitive bouts of exhaustive exercise in juvenile rainbow trout. J. Exp. Biol. **167**: 155-169.

Scruton, D.A., Clarke, K.D., and Cole, L.J. 1998. Water temperature dynamics in small forested headwater streams of Newfoundland, Canada: quantification of thermal brook trout habitat to address initial effects of forest harvesting. *In* Proceedings of the forest-fish conference: land management practices affecting aquatic ecosystems. *Edited by* M.K. Brewin, and D.M.A. Monita, Calgary, AB. pp. 325-336.

Selong, J.H., McMahon, T.E., Zale, A.V., and Barrows, F.T. 2001. Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerance in fishes. T. Am. Fish. Soc. **130**(6): 1026-1037.

Sensch, O., Vierling, W., Brandt, W., and Reiter, M. 2000. Effects of inhibition of calcium and potassium currents in guinea-pig cardiac contraction: comparison of beta-caryophyllene oxide, eugenol, and nifedipine. Br. J. Pharmacol. **131**(6): 1089-1096.

Shepherd, B.G., Hartman, G.F., and Wilson, W.J. 1986. Relationships between stream temperature and intragravel temperatures in coastal drainages, and some implications for fisheries workers. Can. J. Fish. Aquat. Sci. **43**(9): 1818-1822.

Sinokrot, B.A., and Gulliver, J.S. 2000. In-stream flow impact on river water temperatures. J. Hydraul. Res. **38**(5): 339-349.

St-Pierre, J., Charest, P.M., and Guderley, H. 1998. Relative contribution of quantitative and qualitative changes in mitochondria to metabolic compensation during seasonal acclimatisation of rainbow trout Oncorhynchus mykiss. J. Exp. Biol. **201**(21): 2961-2970.

Stecyk, J.A.W., and Farrell, A.P. 2006. Regulation of the cardiorespiratory system of common carp (*Cyprinus carpio*) during severe hypoxia at three seasonal acclimation temperatures. Physiol. Biochem. Zool. **79**(3): 614-627.

Steffensen, J.F. 1985. The transition between branchial pumping and ram ventilation in fishes: energetic consequences and dependence on water oxygen tension. J. Exp. Biol. **114**: 141-150.

Steffensen, J.F. 1989. Some errors in respirometry of aquatic breathers: how to avoid and correct for them. Fish. Physiol. Biochem. 6(1): 49-59.

Steinhausen, M.F., Sandblom, E., Eliason, E.J., Verhille, C., and Farrell, A.P. 2008. The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). J. Exp. Biol. **211**(24): 3915-3926.

Stenseng, E., Braby, C.E., and Somero, G.N. 2005. Evolutionary and acclimation-induced variation in the thermal limits of heart function in congeneric marine snails (Genus *Tegula*): Implications for vertical zonation. Biol. Bull. **208**(2): 138-144.

Stevens, B.S., and DuPont, J.M. 2011. Summer use of side-channel thermal refugia by salmonids in the North Fork Coeur d'Alene River, Idaho. N. Am. J. Fish. Manage. **31**(4): 683-692.

Stevens, E.D., Shelton, G., Randall, D.J., and Bennion, G.R. 1972. Factors affecting arterial pressures and blood flow from the heart in intact unrestrained lingcod, *Ophiodon elongatus*. Comp. Biochem. Physiol. **43**: 681-695.

Stewart, I.T., Cayan, D.R., and Dettinger, M.D. 2005. Changes toward earlier streamflow timing across western North America. J. Clim. **18**(8): 1136-1155.

Story, A., Moore, R.D., and Macdonald, J.S. 2003. Stream temperatures in two shaded reaches below cutblocks and logging roads: downstream cooling linked to subsurface hydrology. Can. J. For. Res. **33**(8): 1383-1396.

Sullivan, K., Martin, D.J., Cardwell, R.D., Toll, J.E., and Duke, S. 2000. An analysis of the effects of temperature on salmonids of the Pacific Northwest with implications for selecting temperature criteria. pp. 192.

Summerfelt, R.C., and Smith, L.S. 1990. Anesthesia, surgery, and related techniques. American Fisheries Society, Bethesda, MD.

Taylor, E.B. 1991. Behavioral interaction and habitat use in juvenile chinook, *Oncorhynchus tshawytscha*, and coho, *O. kisutch*, salmon. Anim. Behav. **42**: 729-744.

Taylor, S.E., Egginton, S., and Taylor, E.W. 1996. Seasonal temperature acclimatisation of rainbow trout: Cardiovascular and morphometric influences on maximal sustainable exercise level. J. Exp. Biol. **199**(4): 835-845.

Thorarensen, H., Gallaugher, P.E., and Farrell, A.P. 1996. The limitations of heart rate as a predictor of metabolic rate in fish. J. Fish Biol. **49**(2): 226-236.

Tiffan, K.F., Kock, T.J., Connor, W.P., Steinhorst, R.K., and Rondorf, D.W. 2009. Behavioural thermoregulation by subyearling fall (autumn) Chinook salmon *Oncorhynchus tshawytscha* in a reservoir. J. Fish Biol. **74**(7): 1562-1579.

Torgersen, C.E., Price, D.M., Li, H.W., and McIntosh, B.A. 1999. Multiscale thermal refugia and stream habitat associations of chinook salmon in northeastern Oregon. Ecol. Appl. **9**(1): 301-319.

US EPA. 2003. EPA Region 10 Guidance for Pacific Northwest State and Tribal Temperature Water Quality Standards. Region 10 Office of Water, U.S. Environmental Protection Agency. EPA 910-B-03-002. pp. 49.

Webb, B.W., and Zhang, Y. 1997. Spatial and seasonal variability in the components of the river heat budget. Hydrol. Process. 11(1): 79-101.

Williams, J.E., Haak, A.L., Neville, H.M., and Colyer, W.T. 2009. Potential consequences of climate change to persistence of cutthroat trout populations. N. Am. J. Fish. Manage. **29**(3): 533-548.

Wood, C.M., Pieprzak, P., and Trott, J.N. 1979. Influence of temperature anaemia on the adrenergic and cholinergic mechanisms controlling heart rate in the rainbow trout. Can. J. Zool. **57**(12): 2440-2447.

Yeager, D.P., and Ultsch, G.R. 1989. Physiological regulation and conformation: a BASIC program for the determination of critical points. Physiol. Zool. **62**(4): 888-907.

Zeng, L.-Q., Zhang, Y.-G., Cao, Z.-D., and Fu, S.-J. 2010. Effect of temperature on excess post-exercise oxygen consumption in juvenile southern catfish (*Silurus meridionalis* Chen) following exhaustive exercise. Fish. Physiol. Biochem. **36**(4): 1243-1252.