

**BETA-ADRENERGIC FUNCTION IN JUVENILE SOCKEYE SALMON HEARTS**

by

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**Beta-adrenergic function in juvenile sockeye salmon hearts**

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## Abstract

Adult Fraser River sockeye salmon (*Oncorhynchus nerka*) show population level tailoring of their cardiorespiratory system specific to upriver migration challenges. My thesis sought to expand our knowledge to juvenile sockeye salmon populations by studying stimulation of their cardiac performance via  $\beta$ -adrenergic receptors.

I made measurements of myocardial cell-surface  $\beta_2$ -adrenoceptor density in fish hearts 14-times smaller than previously accomplished by modifying and validating the tritiated ligand technique. For the Chilko sockeye salmon population, smolts had about half the receptor density of adults, but still twice that of adult hatchery *O. mykiss*. With my new technique, cardiac receptor density can now be investigated in a much wider range of fish species and life stages.

I also investigated the effects of acclimation temperature on myocardial  $\beta$ -adrenergic stimulation ( $\beta$ AS) in juvenile sockeye by studying *in vitro* ventricular preparations that developed maximum isometric tension over a range of pacing frequencies (a force-frequency relationship – FFR) at both 5 °C (0.2 – 0.8 Hz) and 14°C (0.2 – 1.6 Hz). I compared juveniles from the Chilko River and Weaver Creek populations raised in a common-garden laboratory rearing environment (captive-reared) and wild Chilko juveniles captured and acclimated to the laboratory environment (wild-reared). Under tonic  $\beta$ AS (0.01  $\mu$ M isoprenaline), active tension at 5 °C was either unchanged by pacing frequency (both Chilko populations), or modestly biphasic FFR (Weaver), whereas at 14 °C all three study groups had a negative FFR. Maximal  $\beta$ AS (32  $\mu$ M isoprenaline) at 0.2 Hz doubled active tension in all study groups and at both temperatures, and all study groups had a negative FFR independent of temperature. However, only at 14 °C was active tension under maximal  $\beta$ AS greater than under tonic  $\beta$ AS for the highest and physiologically relevant pacing frequencies. Importantly, other than the modest biphasic FFR of

Weaver Creek, the FFR did not differ appreciably between juveniles of two Fraser River sockeye populations.

## **Lay summary**

The effects of temperature on the cardiovascular performance of adult Fraser River sockeye salmon is well studied, but similar information for the juvenile life stages is lacking. I filled this data gap by studying juveniles from two populations of Fraser River sockeye salmon showing that juveniles have about half the ventricular beta-adrenoceptors of an adult and that two populations with dissimilar adult river migrations had a similar force-frequency relationship for the maximum isometric force developed by the ventricular muscles independent of the water temperature they are acclimated to.

## **Preface**

This thesis is the original independent research of the author, Adam Taylor Goulding. All experimental work was conducted at the University of British Columbia. I designed and conducted all experiments, carried out all analyses, and wrote the manuscript under the supervision of Dr. Anthony Farrell.

A version of Chapter 2 has been published as Goulding, A. T., and Farrell, A. P. (2016) Quantification of Ventricular  $\beta^2$ -Adrenoceptor Density and Ligand Binding Affinity in Wild Sockeye Salmon *Oncorhynchus nerka* Juveniles Using a Novel Modification to the Tritiated Ligand Technique. *Journal of Fish Biology* 88, 2081–2087. I was responsible for all major areas including concept formation, data collection and analysis, and manuscript composition. Farrell A.P. was the supervisory author on this project and was involved throughout the project in concept formation and manuscript composition.

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## List of abbreviations

[ <sup>3</sup> H] CGP 12177	Tritiated 4-[3-[(1,1-Dimethylethyl)amino]2-hydroxypropoxy]-1,3-dihydro-2 <i>H</i> -benzimidazol-2-one hydrochloride
3N	Triploid
ACh	Acetylcholine
ANOVA	Analysis of variance
AR	Adrenergic receptor / Adrenoceptor
AS	Adrenergic Stimulation
BC	British Columbia
BM	Body mass
$B_{max}$	Ventricular cell surface $\beta$ -adrenoceptor density
$Ca^{2+}$	Calcium ion
$CaCl_2$	Calcium chloride
cAMP	Cyclic adenosine monophosphate
CF	Condition factor
cGMP	Cyclic guanosine monophosphate
CR <sub>C</sub>	Captive-reared Chilko River sockeye salmon
CR <sub>W</sub>	Wild-reared Chilko River sockeye salmon
$dT/dt$	Rate of contraction
$-dT/dt$	Rate of relaxation
DPM	Disintegrations per minute
EC <sub>50</sub>	Half maximal effective concentration
FFR	Force-frequency Relationship
HCN	Hyperpolarization-activated cyclic nucleotide-gated
$I_f$	Funny current
iTLT	image-based, tritiated ligand technique
$K^+$	Potassium ion
KCl	Potassium chloride
$K_d$	Dissociation constant
$L_{max}$	Stretched length at which active tension is maximal
$MgSO_4$	Magnesium sulfate
$Na^+$	Sodium ion
NaCl	Sodium chloride
NJ	New Jersey
NL	Newfoundland

NS	Nova Scotia
O <sub>2</sub>	Oxygen gas
ON	Ontario
otc	Off the coast
PKA	Protein Kinase A
QC	Quebec
RVM	Relative ventricular mass
SEM	Standard error of the mean
SR	Sarcoplasmic reticulum
<i>T</i>	Temperature
TES	N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid
TMAC	Transmembrane adenylyl cyclase
tTLR	traditional tritiated ligand technique
UBC	University of British Columbia
USA	United States of America
WC <sub>c</sub>	Captive-reared Weaver Creek sockeye salmon

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## **Dedication**

In loving memory of Christine Chuk. We started this journey together, but now the end is in sight and you are no longer here. I could not have done this with out you. I miss you.

## Chapter 1. Introduction

### 1.1 Overview

My thesis examines the interplay between temperature and  $\beta$ AS of the heart of the juvenile life stage of Fraser River sockeye salmon (*Oncorhynchus nerka*). Adults of this fish species can perform high levels of swimming performance and cardiac work over a range of temperatures (Eliason et al., 2011; 2013 a, b, c) and has an iconic status in British Columbia (BC) because of its social (Jones et al., 2004), economic (Williams, 2007), and biological importance (Finney et al., 2000; Helfield and Naiman, 2001; Johnston et al., 2004). The heart is the life-support organ that must power blood flow through fish during energetically taxing activities such as foraging, predator avoidance, and especially their upstream migration as adults to natal spawning grounds. These migrations vary among populations with some traveling upwards of 1000 km, maintaining swimming speeds of 20-40 km/day, and passing through many hydraulic challenges, all without feeding. Juvenile sockeye migration also varies among populations with some passively migrating downstream both to rearing areas after emergence as fry and to the ocean as smolts, while others, such as Weaver Creek and Chilko River population, migrate as fry partially or wholly upstream to reach rearing lakes (Brannon, 1972; Sopinka et al. 2013). While we have begun to understand cardiorespiratory function of adult sockeye salmon and how it is tailored to the migratory challenges these fish face (Eliason et al., 2011, 2013a,b,c; Anttila et al., 2014), the equivalent level of understanding is lacking for juvenile sockeye salmon whose population specific migratory challenges may vary from that of their adult counterparts. Therefore, the overarching goal of my thesis research was to study one aspect of cardiac function in juvenile fish: the  $\beta$ -adrenergic regulation of cardiac contractility, which is the primary means of stimulating and protecting cardiac performance in fishes (Farrell and Smith, 2017). To achieve

my goal, I first sought to quantify the cardiac  $\beta$ -adrenergic receptor density of juvenile Chilko sockeye salmon so that it may be compared to the elevated level of receptors found in the adults of the same population. To achieve this, I needed to validate a novel method of measuring  $\beta$ -adrenergic receptor density in the very small hearts juvenile fish by modifying an established method used for larger hearts. This new method and the resulting information about cardiac  $\beta$ -adrenergic receptor density of juvenile Chilko sockeye salmon is reported in Chapter 2. I next sought to examine the influence of  $\beta$ AS on cardiac contractility by measuring the maximum isometric force developed by isolated ventricular strips at different pacing and two levels of  $\beta$ AS. These measurements were performed at two acclimation temperatures and compared the Chilko sockeye salmon and Weaver sockeye salmon populations. The Weaver sockeye salmon population has a considerably shorter and less arduous migration as adults from the sea to their natal spawning area when compared with the Chilko sockeye salmon population (Eliason et al., 2011), whereas both populations migrate a similar distance upstream to reach their rearing areas as fry (Brannon, 1972; Sopinka et al., 2013). Despite this similar upstream migration distance as fry, burst swimming performance varies among populations of sockeye salmon and reflects the difficulty of adult reproductive migration more so than that of fry migration (Sopinka et al. 2013). Juvenile sockeye salmon from both populations, raised from eggs (obtained from geographically distinct spawning areas) in a common-garden rearing environment (Chen et al., 2013; Whitney et al., 2013), formed the basis of Chapter 3.

## **1.2 Background**

The vertebrate heart, in the most fundamental sense, is a variable output pump generating pressure that powers blood flow through a closed-loop vascular system. Cardiac output is the

product of heart rate and the volume of blood pumped with each heartbeat (stroke volume). This pump must adjust its output whenever an organism encounters novel or challenging conditions to ensure adequate delivery of oxygen and nutrients, and adequate removal of metabolic wastes. The volume of blood pumped by the heart every minute, i.e., cardiac output, is tightly regulated in an attempt to maintain an animal's physiological functions across the full spectrum of environmental conditions and activities.

Intrinsic heart rate is set by cardiac pacemaker cells, which are located in the sinoatrial node of a fish (Farrell and Smith, 2017). These pacemaker cells have a spontaneous depolarization of the cell membrane (a pacemaker potential), the speed of which sets the intrinsic heart rate. Intrinsic heart rate changes with temperature largely because of a change in speed of the pacemaker potential. Stroke volume is also intrinsically set both by the amount of cardiac filling (through the Frank-Starling mechanism; Starling and Visscher, 1927), which sets end-diastolic volume, and by the force of the resulting cardiac contraction (contractility), which is determined in large part by end-diastolic volume and sets end-systolic volume.

Although cardiac output can be modulated by changing stroke volume and heart rate in any combination, the relative reliance on either mechanism varies among taxa (Lillywhite et al., 1999). For example, while mammals invariably change heart rate to increase cardiac output during exercise, fishes are capable of much greater increases in stroke volume, especially during exercise (Farrell and Jones, 1992; Farrell and Smith, 2017). As a major determinant of stroke volume, contractility plays a critical role in determining cardiac output in salmon. For example, if cardiac contractility is impaired by extracellular acidosis or hypoxia, maximum cardiac output is impaired (Farrell and Smith, 2017).

Temperature is an external variable that has overarching effects on the fish heart. For example, the increase in cardiac output with acute warming of a fish is met primarily by an increase in heart rate. Temperature can also increase the maximum heart rate as well as the maximum force of cardiac contraction (maximum isometric force). However, maximum isometric force is not independent of heart rate, a relationship that is best characterized by measuring maximum isometric force while increasing the pacing frequency of an isolated strip of cardiac muscle, which produces a Force Frequency Relationship (FFR). As shown in Table 1.1, fishes typically have a negative FFR, but a few species have a biphasic one, with increasing contractility over low pacing frequencies and then decreasing contractility at high pacing frequencies (Shiels et al., 2002).

The autonomic nervous system can modify the performance of all vertebrate hearts by regulating heart rate and contractility (Nilsson, 1983). For example, catecholamines such as adrenaline and noradrenaline can independently increase heart rate and maximum contractile force via stimulation of  $\beta$ -adrenoceptors ( $\beta$ AR) found on the cell membranes of fish hearts (Farrell and Smith, 2017).  $\beta$ AS can even offset some of the negative effects of hypoxia and extracellular acidosis on cardiac contractility (Hanson et al., 2006; Hanson and Farrell, 2007). Therefore, adrenergic stimulation of the heart is central to the regulation of cardiac output in fishes. Since, the effects  $\beta$ AS and temperature on cardiac contractility can be explored with a FFR, my thesis uses the FFR of cardiac strips from juvenile sockeye salmon to examine the interplay between temperature acclimation and  $\beta$ AS. Furthermore, comparisons were made between sockeye salmon populations of different natal origins.

### 1.3 The Autonomic Control of the Teleost Heart

The autonomic innervation and regulation of vertebrate hearts was thoroughly reviewed by Nilsson (1983). Teleost hearts are characterized by inhibitory cholinergic (parasympathetic) components of the autonomic system and, in some species such as salmon, by excitatory adrenergic (sympathetic) components. Indeed, both the sympathetic and parasympathetic arms of the autonomic nervous system are routinely active in fishes and their relative tones set the routine values for heart rate, contractility and hence cardiac output (Sandblom and Axelsson, 2011).

The inhibitory parasympathetic action of the autonomic nervous system in the heart is a result of direct vagal stimulation through the release of the neurotransmitter acetylcholine (ACh; Holmgren, 1977). ACh binds to  $M_2$  muscarinic receptors, which activate an inhibitory G-protein coupled signalling pathway that inhibits transmembrane adenylyl cyclase (TMAC) and reduces the production of cyclic adenosine monophosphate (cAMP; Jakobs et al., 1979). Through this mechanism, ACh can decrease cardiac contractility (negative inotropy; George et al., 1973) and heart rate (negative chronotropy; Imbrogno et al., 2001). Interestingly, nanomolar concentrations of ACh produced positive inotropic effects mediated through  $M_1$  muscarinic receptors and a cyclic guanosine monophosphate (cGMP) mechanism in the European eel (*Anguilla anguilla*), while micromolar concentrations of ACh produced negative inotropic effects (Imbrogno et al., 2001).

The excitatory sympathetic action of the autonomic nervous system in the heart is a result of catecholamine release either into the venous blood from chromaffin tissue or from cardiac nerve terminals. Catecholamines (noradrenaline and adrenaline) that stimulate  $\beta$ AR on the heart are signalling molecules derived from tyrosine through a series of reactions in both

postganglionic nerve fibres and neuroendocrine chromaffin cells known as the Blaschko pathway (Blaschko, 1939; Randall and Perry, 1992). Noradrenaline and adrenaline convey sympathetic signals throughout an organism via the peripheral nervous and endocrine systems. As such, catecholamines can act as endocrine signalling molecules as well as neurotransmitters. Noradrenaline is released from the sympathetic nerve terminals in the heart (Yamauchi and Burnstock, 1968), with atrial muscle being more densely innervated than ventricular muscle (Vornanen, 2017). Adrenaline and noradrenaline are released into circulation from chromaffin tissues, which in teleosts are primarily found in the wall of the posterior cardinal vein as it passes through the head kidney (Reid et al., 1998). Catecholamines that are produced and stored in chromaffin cells are released into the circulatory system when the cells are subjected to cholinergic stimulation (Perry et al., 1991). The amount of circulating and stored levels of noradrenaline and adrenaline, and the ratio between the two catecholamines, vary considerably amongst teleosts, but adrenaline generally predominates (Randall and Perry, 1992; Reid et al., 1998), with as much as a 4:1 ratio of adrenaline to noradrenaline in the posterior cardinal vein of the rainbow trout (*Oncorhynchus mykiss*; Reid and Perry, 1994).

Adrenaline and noradrenaline are agonistic ligands for the adrenergic receptor (AR) class of transmembrane proteins. In general, ARs are G-protein coupled receptors that initiate a signal transduction cascade within the cell. There are two main groups of ARs,  $\alpha$  and  $\beta$ , each with several subtypes. Traditionally, adrenergic-mediated effects on vertebrate cardiac performance have been attributed to  $\beta$ -ARs, specifically subtypes  $\beta_1$  and  $\beta_2$  (Randall and Perry, 1992; Reid et al., 1998; Brodde et al., 2006). In fish,  $\beta_2$ -ARs are the dominant receptor subtype found in the cardiac tissue (Ask et al., 1981; Keen et al., 1993; Gamperl et al., 1994).

Stimulation of cardiac  $\beta$ -ARs leads to positive chronotropic and inotropic actions in the teleost heart by catecholamines binding to  $\beta$ -ARs on the cardiomyocyte cellular membrane and stimulating TMAC to produce cAMP (Sutherland et al., 1962). To increase heart rate, cAMP binds to the tail region of hyperpolarization-activated cyclic nucleotide-gated (HCN) channels on the pacemaker cells increasing the open probability of the channel (DiFrancesco and Tortora, 1991; Wainger et al., 2001). This increased open probability leads to greater  $\text{Na}^+$  and  $\text{K}^+$  influx into the cell through the HCN channels ( $I_f$ , the funny current) increasing the rate of depolarization of the pacemaker cell membrane thus increasing heart rate (DiFrancesco, 2010). Increased cAMP production following  $\beta$ AS also activates protein kinase A (PKA). PKA activation is known to occur both locally in the vicinity of L-type  $\text{Ca}^{2+}$  ( $\beta_2$ -ARs) and more globally throughout the cell ( $\beta_1$ -ARs) in frogs (Harvey and Hell, 2013); however, the presence of this spatial pattern of  $\beta$ -ARs in fish is unknown. PKA phosphorylation of the L-type  $\text{Ca}^{2+}$  channels increases  $\text{Ca}^{2+}$  influx into the cell increasing the contractility of ventricular cardiomyocytes (van der Heyden et al., 2005). This elevated cytosolic  $\text{Ca}^{2+}$  binds to troponin C enhancing myosin cross-bridge formation and contractility (Vornanen et al., 2002). PKA can further enhance cardiac contractility through the phosphorylation of cardiac myosin binding protein C (Yang et al., 2001), troponin I and phospholamban (Li et al., 2000) and type-2 ryanodine receptors (Marx et al., 2000). While myosin binding protein C and troponin I directly influence myosin cross-bridge cycling, phospholamban and ryanodine receptors act to increase  $\text{Ca}^{2+}$  release from the sarcoplasmic reticulum (SR), a mechanism whose importance is species and temperature dependant among fishes.

In rainbow trout hearts,  $\text{Ca}^{2+}$  release from the SR at 18 °C contributes twice as much to ventricular contractility than at 12 °C (Shiels and Farrell, 1997), while chronotropic depression

due to the inhibition of SR  $\text{Ca}^{2+}$  cycling with ryanodine was observed at 18 °C but not 11 °C (Haverinen and Vornanen, 2007). In general, fishes capable of high swimming activity such as the yellow fin tuna (*Thunnus albacares*; Shiels et al., 1999), skipjack tuna (*Katsuwonus pelamis*; Keen et al., 1992) and Pacific mackerel (*Scomber japonicas*; Shiels and Farrell, 2000) have a greater reliance on SR  $\text{Ca}^{2+}$  cycling than less active fishes such as the goldfish (*Carassius carassius*; Vornanen, 1989) and the hagfish (*Eptatretus stoutii*; Wilson and Farrell, 2013).

The positive chronotropic and inotropic effects of cardiac  $\beta$ -adrenergic stimulation have been elucidated in many fishes. Both chronotropic and inotropic effects were shown in the perfused hearts of lamprey (*Lamperta fluviatilis*; Falck et al., 1966) and plaice (*Pleuronectes platessa*; Cobb and Santer, 1973; Falck et al., 1966). Catecholamines and synthetic adrenergic agonists similarly increased the force and frequency of contraction in spontaneously beating *in vitro* atrial preparations from rainbow trout (Ask et al., 1981; Ask, 1983) and flounder (*Platichthys flesus*; Ask, 1983). Likewise, the addition of adrenaline to *in situ* perfused hearts from sea raven (*Hemitripterus americanus*; Farrell et al., 1982) and rainbow trout (Farrell et al., 1986) increased heart rate, cardiac output, power output and stroke volume.

In terms of the FFR, isoprenaline (a synthetic  $\beta$ -adrenergic agonist) increased the isometric force of contraction of isolated ventricular muscle strips in the common carp (*Cyprinus carpio*; Temma et al., 1986) and crucian carp (*Carassius carassius*; Vornanen, 1989). Adrenergic stimulation also increased maximum isometric force generation as well as the rates of force development and relaxation of *in vitro* ventricular muscle preparations of rainbow trout (Shiels and Farrell, 1997; Shiels et al., 1998), the blackfin icefish (*Chaenocephalus aceratus*) and cod icefish (*Notothenia coriiceps*; Skov et al., 2009) and the European eel (Methling et al., 2012).

#### 1.4 The Protective Effects Cardiac $\beta$ -Adrenergic Stimulation against Adverse Conditions

During strenuous exercise the cardiovascular system must increase its delivery of blood to working tissues. Thus, when a fish swims it increases cardiac output and stroke volume appreciably, and heart rate and arterial blood pressure modestly; it may even work at the maximum capacity of the cardiorespiratory system to deliver oxygen. Increased venous return of blood and autonomic nervous stimulation elicit the necessary increase in work output of the heart during swimming activity (Farrell and Smith, 2017). However, when activity becomes strenuous and anaerobically powered, the extracellular environment of the heart changes significantly and these changes can potentially impair maximum cardiac function. For example, strenuous exercise in rainbow trout decreases venous blood oxygen tension (Farrell et al., 2003) as a result of increased oxygen extraction by locomotory muscles, decreases blood pH (Holeton et al., 1983) due to an increased loading of carbon dioxide and hydrogen ions, and increases potassium ion concentrations (Holeton et al., 1983; Turner et al., 1983) due to loss from active skeletal muscles. Changes in venous blood composition can be particularly detrimental to salmonids as their inner spongy myocardium (which makes up the majority of the cardiac tissue) is supported and nourished by the venous blood as blood circulates through the heart (Farrell and Jones, 1992). Acidotic (low pH) and hyperkalemic (high  $K^+$ ) conditions also impair maximum cardiac performance, and even more so at elevated temperatures in rainbow trout (Hanson et al., 2006; Hanson and Farrell, 2007). Indeed, studies using *in vitro* cardiac muscle strips and *in situ* perfused hearts consistently demonstrate that acidotic, hypoxic (low  $O_2$ ), and hyperkalemic conditions have negative inotropic and chronotropic effects, that reduce maximum cardiac performance, and if severe, that can stop cardiac function completely.

Adrenergic stimulation is thought to be essential for maintaining cardiac performance during adverse conditions. For example, adrenaline prevented the loss of cardiac performance (both inotropic and chronotropic effects) associated with acidosis in *in situ* perfused hearts of the rainbow trout (Farrell and Milligan, 1986; Farrell et al., 1986, 1988) and the sea raven (Farrell et al., 1982). In comparative studies of rainbow trout and the red-eared slider turtle (*Trachemis scripta elegans*), adrenergic stimulation of both ventricular ring and strip preparations ameliorated the negative chronotropic and inotropic effects of high extracellular potassium (Nielsen and Gesser, 2001; Kalinin and Gesser, 2002). Likewise, adrenergic stimulation allowed *in situ* perfused rainbow trout hearts to maintain maximum cardiac output under simulated adverse hypoxic, hyperkalemic and acidotic extracellular conditions similar to those experienced during prolonged exercise (Hanson et al., 2006; Hanson and Farrell, 2007).

### **1.5 Temperature and Adrenergic Stimulation of the Teleost Heart**

Temperature acclimation is known to alter many aspects of a fish's physiology and biochemistry, including the heart's sensitivity to adrenaline (Ask et al., 1981; Graham and Farrell, 1989; Keen et al., 1993; Farrell et al., 1996; Aho and Vornanen, 2001). For example, an adrenergic tonus seems to be essential for the maintenance of routine cardiac function in rainbow trout acclimated to a very low temperature (5 °C), because a tonic level of adrenaline (levels similar to circulating catecholamine levels at rest) was needed to prevent deterioration and arrhythmia of an *in situ* perfused heart preparation (Graham and Farrell, 1989). Yet, at acclimation temperatures above 18 °C *in situ* perfused heart preparations had reduced adrenergic sensitivity, as evidenced by diminished positive chronotropic and inotropic effects, and reduced protective properties of adrenergic stimulation (Farrell et al., 1996; Hanson and Farrell, 2007).

This reduction in sensitivity with warm acclimation in rainbow trout has been attributed to a temperature-dependent decrease in myocardial cell surface  $\beta_2$ -AR density ( $B_{max}$ ), with  $B_{max}$  more than halving with increasing acclimation temperature from 8 °C to 18 °C (Keen et al., 1993) and from 8 °C and 14 °C (Gamperl et al., 1998).

Nevertheless, the thermal plasticity of cardiac  $B_{max}$  is not consistent across fishes or even across salmonid populations. In contrast to rainbow trout, the Chilko River population of sockeye salmon significantly increase  $B_{max}$  when acclimated from 13 °C to either 19 °C or 22 °C, perhaps reflecting their remarkably capacity to maintain maximum cardiac output and heart rate up to 22 °C (Eliason et al., 2011). Conversely, a co-migrating population of sockeye salmon from the Nechako watershed (Nechako) had no difference in  $B_{max}$  at the same acclimation temperatures, as well as lower maximum temperature for maximum cardiac output and heart rate (Eliason et al., 2011). Similarly, the tropical African catfish (*Claris gariepinus*) had no variation in  $B_{max}$  at acclimation temperatures of 15 °C, 22 °C and 32°C (Hanson et al., 2005). In an interspecific comparison, Olsson et al. (2000) suggested that species differences in  $B_{max}$  were associated with environmental temperature, but my review of this literature (Table 1.2) rejects this idea. Rather, when  $B_{max}$  and  $\beta_2$ -AR binding affinity ( $K_d$ ) are compared across all fish species tested to date (Table 1.2; Eliason and Anttila, 2017), the 24-fold variation in  $B_{max}$  and nearly eight-fold variation in  $K_d$  suggests a general trend of a higher  $B_{max}$  being found in fish that are capable of higher rates of activity, such as sockeye salmon, but such a trend has yet to be thoroughly investigated.

## 1.6 Chapter 2 and Chapter 3 Objectives

The objective of Chapter 2 was to first validate a modification to the tritiated ligand binding method of quantifying cardiac cell surface  $\beta$ AR density in fish to conduct measurements of hearts at least 14-times smaller than any heart previously tested. This novel modification was then used to test the hypothesis that an elevated level of cardiac  $\beta$ -adrenergic receptors discovered in adult Chilko sockeye salmon is also present in juvenile fish from the same population. This work has been published by Goulding and Farrell (2016). The objective of Chapter 3 was to investigate the effects cardiac  $\beta$ AR stimulation in juvenile sockeye salmon by measuring the maximum isometric force developed by isolated ventricular strips across different pacing frequencies at two levels of  $\beta$ AS and at two different acclimation temperatures. The hypothesis that the effects of cardiac  $\beta$ AR stimulation would differ between juveniles from the Chilko sockeye salmon and the Weaver sockeye salmon populations was then tested by comparing results from these two populations. Chapter 3 has been prepared for submission to the *J. Fish Biology*.

**Table 1.1** Myocardial force frequency relationship and peak tension in fishes

<b>Species</b>	<b>T (°C) Test (acclimation)</b>	<b>Tissue</b>	<b>Adrenergic stimulation</b>	<b>Peak tension @ 0.2 Hz (mN mm<sup>-2</sup>)</b>	<b>FFR</b>	<b>Reference</b>
3N Brown trout	14	Atrial strip	Tonic	34.5 ± 15.0	Negative	Mercier et al. (2002)
3N Brown trout	14	Ventricular strip	Tonic	31.7 ± 14.6	Negative	Mercier et al. (2002)
3N Brown trout	18	Atrial strip	Tonic	22.8 ± 12.5	Negative	Mercier et al. (2002)
3N Brown trout	18	Ventricular strip	Tonic	18.8 ± 10.6	Negative	Mercier et al. (2002)
Yellowfin tuna	15 (20)	Trabecula	Tonic	17.3 ± >2.7	Biphasic	Shiels et al. (1999)
Yellowfin tuna	18 (20)	Trabecula	Tonic	11.6 ± 1.8	Biphasic	Shiels et al. (1999)
Yellowfin tuna	25 (20)	Trabecula	Tonic	5.7 ± 1.1	Biphasic	Shiels et al. (1999)
Pacific mackerel	15	Trabecula	Tonic	2.4 ± 0.4	Flat	Shiels and Farrell (2000)
Pacific mackerel	20 (15)	Trabecula	Tonic	2.3 ± 0.8	Flat	Shiels and Farrell (2000)
European eel	0	Ventricular strip	Tonic	6.4 ± 1.8 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	0	Ventricular strip	Maximal	9.5 ± 2.9 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	10 (0)	Ventricular strip	Tonic	6.6 ± 1.3 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	10 (0)	Ventricular strip	Maximal	7.6 ± 1.7 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	10	Ventricular strip	Tonic	5.2 ± 1.3 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	10	Ventricular strip	Maximal	7.2 ± 1.8 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	10	Ventricular strip	Tonic	4.7 ± 1.1	Flat	Methling et al. (2012)
European eel	20 (10)	Ventricular strip	Tonic	4.5 ± 0.8 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	20 (10)	Ventricular strip	Maximal	5.1 ± 1.2 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	20 (10)	Ventricular strip	Tonic	4.5 ± 0.9	Flat	Methling et al. (2012)
European eel	20	Ventricular strip	Tonic	4.9 ± 0.6 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	20	Ventricular strip	Maximal	4.9 ± 0.7 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	20	Ventricular strip	Tonic	4.7 ± 0.5	Flat	Methling et al. (2012)

Species	<i>T</i> (°C) Test (acclimation)	Tissue	Adrenergic stimulation	Peak tension @ 0.2 Hz (mN mm <sup>-2</sup> )	FFR	Reference
European eel	10 (20)	Ventricular strip	Tonic	7.0 ± 1.1 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	10 (20)	Ventricular strip	Maximal	9.8 ± 1.4 (0.1 Hz)	Flat	Methling et al. (2012)
European eel	10 (20)	Ventricular strip	Tonic	7.0 ± 1.0	Flat	Methling et al. (2012)
European eel	18	Cardiac strip	-	%	Negative	Joyce et al. (2016)
Catfish	10 (20)	Ventricular strip	-	%	Negative	Mohamed et al. (2012)
Catfish	15 (20)	Ventricular strip	-	%	Negative	Mohamed et al. (2012)
Catfish	20	Ventricular strip	-	%	Negative	Mohamed et al. (2012)
Catfish	25 (20)	Ventricular strip	-	%	Negative	Mohamed et al. (2012)
Catfish	30 (20)	Ventricular strip	-	%	Negative	Mohamed et al. (2012)
Marbled swamp eel	15 (25)	Ventricular strip	Tonic	15.3 ± 1.8	Incomplete	Matheus et al. (2007)
Marbled swamp eel	25	Ventricular strip	Tonic	18.4 ± 1.9	Flat	Matheus et al. (2007)
Marbled swamp eel	35 (25)	Ventricular strip	Tonic	12.3 ± 1.3	Negative	Matheus et al. (2007)
Sea raven	-	Ventricular strip	-	%	Negative	Driedzic and Gesser (1988)
Atlantic cod	-	Ventricular strip	-	%	Negative	Driedzic and Gesser (1988)
White sturgeon	-	Ventricular strip	-	%	Biphasic	Driedzic and Gesser (1988)
Black dogfish	-	Ventricular strip	-	%	Biphasic	Driedzic and Gesser (1988)
Spiny dogfish	-	Ventricular strip	-	%	Biphasic	Driedzic and Gesser (1988)
Little skate	-	Ventricular strip	-	%	Biphasic	Driedzic and Gesser (1988)
Hagfish	-	Ventricular strip	-	%	Negative	Driedzic and Gesser (1988)

Species	<i>T</i> (°C) Test (acclimation)	Tissue	Adrenergic stimulation	Peak tension @ 0.2 Hz (mN mm <sup>-2</sup> )	FFR	Reference
Blackfin icefish	0	Atrial strip	Maximal	2.6 ± 0.4 (0.1 Hz)	Flat	Skov et al. (2009)
Blackfin icefish	0	Ventricular strip	Tonic	3.7 ± 1.1 (0.1 Hz)	Negative	Skov et al. (2009)
Black rockcod	0	Ventricular strip	Tonic	1.1 ± 0.1	Negative	Skov et al. (2009)
Curumbata	25	Cardiac ring	-	3.2 ± 0.2	-	Rivaroli et al. (2006)
Trahira	25	Cardiac ring	-	0.74 ± 0.07	-	Rivaroli et al. (2006)
Atlantic mackerel	10 – 13	Ventricular strip	-	%	Negative	Driedzic and Gesser (1985)
Atlantic salmon	10	Ventricular strip	-	%	Negative	Driedzic and Gesser (1985)
Sea raven	10 – 13	Ventricular strip	-	%	Negative	Driedzic and Gesser (1985)
Lumpfish	10 – 13	Ventricular strip	-	%	Negative	Driedzic and Gesser (1985)
Ocean pout	10 – 13	Ventricular strip	-	%	Negative	Driedzic and Gesser (1985)
Goosefish	10 – 13	Ventricular strip	-	%	Negative	Driedzic and Gesser (1985)
Longhorn sculpin	10 – 13	Ventricular strip	-	%	Negative	Driedzic and Gesser (1985)
Alewife	9 – 11	Ventricular strip	-	%	Negative	Driedzic and Gesser (1985)
Rainbow trout	15 – 20	Ventricular strip	-	%	Negative	Driedzic and Gesser (1985)
Rainbow trout	12	Trabecula	Tonic	1.6 ± 0.33	Negative	Shiels and Farrell (1997)
Rainbow trout	12	Trabecula	Maximal	%	Negative	Shiels and Farrell (1997)
Rainbow trout	12 (22)	Trabecula	Tonic	1.04 ± 0.08	Negative	Shiels and Farrell (1997)

Species	$T$ (°C) Test (acclimation)	Tissue	Adrenergic stimulation	Peak tension @ 0.2 Hz (mN mm <sup>-2</sup> )	FFR	Reference
Rainbow trout	12 (22)	Trabecula	Maximal	%	Negative	Shiels and Farrell (1997)
Rainbow trout	22	Trabecula	Tonic	2.5 ± 0.31	Negative	Shiels and Farrell (1997)
Rainbow trout	22	Trabecula	Maximal	%	Negative	Shiels and Farrell (1997)
Rainbow trout	22 (12)	Trabecula	Tonic	1.79 ± 0.17	Negative	Shiels and Farrell (1997)
Rainbow trout	22 (12)	Trabecula	Maximal	%	Negative	Shiels and Farrell (1997)

**Table 1.2** Ventricular  $\beta_2$ -adrenoceptor density ( $B_{max}$ ) and binding affinity ( $K_d$ ) as determined using the radio-labelled  $\beta_2$ -adrenoceptor ligand [<sup>3</sup>H] CGP-12177. Modified from Eliason and Anttila (2017)

Species	Population	$T$ (°C)	Acclimation period	$B_{max}$ (fmol mg protein <sup>-1</sup> )	$K_d$ (nM)	Reference
<i>T. bernacchii</i>	McMurdo Station (otc)	1	2 weeks	10.5	0.18	Olsson et al. (2000)
Rainbow trout	Hatchery (BC)	6	Months	36.3	0.23	Eliason et al. (2011)
Rainbow trout	Hatchery (NS)	8	Months	40	0.25	Gamperl et al. (1994)
Rainbow trout	Hatchery (BC)	12	3 weeks	22.6	0.13	Olsson et al. (2000)
Rainbow trout	Hatchery (BC)	14	Unknown	25	0.21	Gamperl et al. (1998)
Rainbow trout	Hatchery (BC)	14	3 weeks	26.4	0.19	Hanson et al. (2005)
Rainbow trout	Hatchery (BC)	14	Months	18.5	0.45	Goulding and Farrell (2016); Chapter 2
Sockeye salmon	Chilko smolts (BC)	8	4 days	54.2	0.43	Goulding and Farrell (2016); Chapter 2
Sockeye salmon	Chilko (BC)	13	4 days	78.5	0.18	Eliason et al. (2011)
Sockeye salmon	Chilko (BC)	19	4 days	123.5	0.2	Eliason et al. (2011)
Sockeye salmon	Chilko (BC)	21	4 days	128.2	0.22	Eliason et al. (2011)

Species	Population	$T$ (°C)	Acclimation period	$B_{\max}$ (fmol mg protein <sup>-1</sup> )	$K_d$ (nM)	Reference
Sockeye salmon	Nechako (BC)	13	4 days	46.8	0.27	Eliason et al. (2011)
Sockeye salmon	Nechako (BC)	19	4 days	66.2	0.21	Eliason et al. (2011)
Sockeye salmon	Nechako (BC)	21	4 days	57.7	0.23	Eliason et al. (2011)
Sockeye salmon	Stamp river (BC)—lab	10–13; then 16–17	3 weeks; 24–36 h	47.5	0.2	Olsson et al. (2000)
Sockeye salmon	Stamp river (BC)—wild	21	None	45.4	0.36	Olsson et al. (2000)
Chinook salmon	Robertson Creek (BC)	13	None	58	0.26	Gamperl et al. (1998)
Pacific mackerel	British Columbia (otc)	13	1 week	27.2	0.25	Olsson et al. (2000)
Winter flounder	Conception Bay (NL)	8	4 weeks	252.8	1.02	Mendonca and Gamperl (2009)
African catfish	Singapore	15	4 weeks	17.8	0.48	Hanson et al. (2005)
African catfish	Singapore	22	4 weeks	15.4	0.46	Hanson et al. (2005)
African catfish	Singapore	32	4 weeks	14.3	0.88	Hanson et al. (2005)
Yellowfin tuna	California (otc)	20	1 year	25.7	0.2	Olsson et al. (2000)
Mahi	Hawaii (otc)	25	None	46.9	0.2	Olsson et al. (2000)
Skipjack tuna	Hawaii (otc)	25	5 days	41.3	0.21	Olsson et al. (2000)

otc: off the coast; BC: British Columbia; NL: Newfoundland; NS: Nova Scotia

## **Chapter 2. Quantification of ventricular $\beta_2$ -adrenoceptor density and ligand binding affinity in wild sockeye salmon *Oncorhynchus nerka* smolts using a novel modification to the tritiated ligand technique**

### **2.1 Introduction**

Sockeye salmon *Oncorhynchus nerka* (Walbaum 1792) show remarkable fidelity for their natal stream and their once-in-a-lifetime spawning (semelparity) results in genetically and geographically distinct populations of *O. nerka* within the Fraser River watershed (Beacham et al., 2005). This genetic isolation would presumably allow strong selective pressures for adaptations that facilitate successful migration. Indeed, the hypothesis of intraspecific tailoring of the cardiorespiratory system to the upriver migration conditions was advanced for Fraser River *O. nerka* when it was found that aerobic and cardiac scope are positively associated with several migration difficulty indices (Eliason et al., 2011, 2013b). Among the Fraser River *O. nerka* populations, the Chilko population stand out with a ventricular cell surface  $\beta_2$ -adrenoceptor ( $\beta_2$ -AR) density ( $B_{max}$ ) at least twice that of other *O. nerka* populations and even more when compared with another fish species from the same genus, rainbow trout *O. mykiss* (Walbaum 1792) (Gamperl et al., 1994, 1998; Olsson et al., 2000; Hanson et al., 2005; Eliason et al., 2011). Stimulation of the salmonid heart via  $\beta_2$ -AR can enhance maximum cardiac performance both during exercise and at high temperature (Farrell et al., 1986; Randall and Perry, 1992; Nielsen and Gesser, 2001), as well as protect cardiac performance under conditions of low oxygen, low pH and high temperature (Hanson et al., 2006; Hanson and Farrell, 2007). Furthermore, adult Chilko *O. nerka* are the only fish known to increase ventricular  $B_{max}$  in response to increased acclimation temperature, nearly doubling their receptor density with an 8 °C change (Eliason et al., 2011). In contrast, *O. mykiss* decrease ventricular  $B_{max}$  in response to

warm acclimation (Keen et al., 1993), whereas adult Nechako *O. nerka* (Eliason et al., 2011) and the African catfish *Claris gariepinus* (Burchell, 1822) (Hanson et al., 2005) do not alter their  $B_{max}$  with temperature acclimation.

Thus far, ventricular  $B_{max}$  has only been measured in adults and so it remains unknown if this extraordinary  $B_{max}$  of Chilko *O. nerka* has a genotypic basis as well as phenotypic plasticity with temperature acclimation, in which case earlier developmental stages would have a similar high myocardial  $\beta_2$ -AR density as in the adult fish. As such, the objective of the current study was to quantify the myocardial  $B_{max}$  of 1+ year-old Chilko *O. nerka* smolts. However, to work with the relatively small heart of a smolt (about 15 mg here) when compared to adults (3.2 g; Eliason et al., 2011), the traditional ligand binding techniques used to measure ventricular  $B_{max}$  in adult fish hearts had to be modified.

## 2.2 Materials and Methods

This study was conducted in accordance with guidelines of the Canadian Council of Animal Care, as administered by the University of British Columbia (Animal Care # A10-0002). All values are presented as a mean value ( $\pm$  SEM). Wild, one-plus year-old Chilko *O. nerka* smolts ( $n = 161$ ; body mass (BM) =  $7.84 \pm 0.28$  g; condition factor (CF) =  $0.740 \pm 0.003$ ; relative ventricular mass (RVM) =  $0.194 \pm 0.003$  %) were captured on 8 May 2013 by dip-net at a government-run counting fence used to enumerate *O. nerka* passage as they leave Chilko Lake and enter the river. The counting fence was in place *c.* 1 km downstream from the Chilko Lake outflow between 22 April 2013 and 11 May 2013 when water temperatures ranged from 3 - 8 °C. Before beginning their migration, smolts rear in Chilko Lake, a deep (339 m) glacial-fed lake with summer temperatures ranging from 4 - 9 °C depending on depth (Desloges and Gilbert,

1998). Prior to sampling, smolts were held on-site for four days in a 500 L tank supplied with water ( $8.1 \pm 0.1$  °C) continuously pumped from the river. Water temperature in the holding tank was recorded each morning. *O. mykiss* were used to validate the assay techniques because previous studies have extensively studied ventricular  $B_{max}$  in this species. Adult female *O. mykiss* ( $n = 9$ ; BM =  $1.13 \pm 0.02$  kg; CF =  $1.51 \pm 0.03$ ; RVM =  $0.086 \pm 0.001$ ) were purchased from Miracle Springs Inc. (Mission BC, Canada) and sampled on site as a reference group. The *O. mykiss* had been maintained in outdoor raceways (14 °C) and fed to cessation twice daily with commercial trout pellets (EWOS Canada Ltd., BC, Canada). All fish were quickly euthanized by a blow to the head to measure fork length and body mass, and excise the ventricle, which was rinsed in TES (N-[Tris(hydroxymethyl)methyl]-2-aminoethanesulfonic acid) buffered saline (composition in mM: NaCl, 124.1; KCl, 2.5; MgSO<sub>4</sub> – 7H<sub>2</sub>O, 0.9; CaCl<sub>2</sub> – 2H<sub>2</sub>O, 2.5; D – glucose, 5.6; TES free acid, 3.9; TES Na salt, 6.1; pH 7.85 at 10 °C) before freeze-clamping (*O. mykiss*) or freezing on an aluminum plate (*O. nerka*) using liquid nitrogen. The tissues were stored at -80 °C until analysis.

The traditional tritiated ligand technique (tTLT) of Watson-Wright et al. (1989), as modified for fish hearts by Gamperl et al. (1994), is a well-established technique to measure ventricular cell-surface  $\beta_2$ -adrenoceptor density ( $B_{max}$ ) and binding affinity ( $K_d$ ) in adult teleost fishes (see Gamperl et al., 1994, 1998; Olsson et al., 2000; Hanson et al., 2005; Mendonca and Gamperl, 2009; Eliason et al., 2011). However, the ventricle of an *O. nerka* smolt was at least 14-times smaller ( $14.63 \pm 0.47$  mg) than any heart previously tested (Olsson et al., 2000) and this prevented using tissue punches, the standardizing unit of the tTLT. While alternative methods to measure cardiac  $B_{max}$  and  $K_d$  with less tissue exist, *e.g.*, the isolated sarcolemmal fraction technique used by Keen et al. (1993), questions remain about the usefulness of these techniques

as they may not be an accurate measure of cell-surface (*i.e.*, functional) receptors (Gamperl et al., 1994). The ventricle size limitation was overcome by developing a new, image-based, tritiated ligand technique (iTTLT) in which tissue punches were replaced with 350  $\mu\text{m}$  thick cross-sectional slices of the ventricle. Each smolt ventricle was sliced in its entirety from the apex to base with a McIlwain tissue chopper (Brinkman Rexdale, ON, Canada). Calibrated pictures were taken of each cross-section using a Canon Rebel T2i (Canon Canada Inc., ON, Canada) adapted to fit a Nikon PB-5 bellows and Micro-Nikkor 55mm f/2.8 macro lens (Nikon Canada Inc., ON, Canada) before they were incubated with a ligand in wells of a tissue culture plate. All subsequent steps of the assay technique followed that of the tTTLT and are described in detail elsewhere (Gamperl et al., 1994). Three to five slices from the centre of each ventricle were selected to maximize the tissue mass per heart (each heart yielded only 5-7 slices) and 30 – 33 individual hearts were pooled for each assay (*i.e.*,  $n = 5$  assay replicates using a total of 161 smolts). Pooling of ventricular tissue has been used previously to meet the tissue requirements of the tTTLT [e.g., Olsson et al. (2000) using *c.* 150 g *Trematomus bernachii* (Boulenger 1902) and Hanson et al. (2005) using 300 – 700 g African catfish *Clarias gariepinus* (Burchell 1822)]. The surface area ( $\text{mm}^2$ ) of individual ventricular cross-sections was determined using image analysis software (ImageJ), and multiplied by 0.35 mm (slice thickness) to determine tissue volume. The radioactivity of each ventricular cross-section, incubated with 500  $\mu\text{L}$  of several concentrations (0.05 – 3.5 nM) of the tritiated  $\beta$ -adrenoceptor ligand [ $^3\text{H}$ ] CGP 12177 (specific activity 37.7 Ci  $\text{mol}^{-1}$ ; PerkinElmer Inc.) in saline and counted with a liquid scintillation counter (LS 6500, Beckman Instruments, USA), was then expressed per unit volume (disintegrations per minute (DPM)  $\text{mm}^{-3}$ ) and used to generate a ligand-binding curve. The iTTLT was validated against the tTTLT in adult *O. mykiss*. To apply the iTTLT to adult *O. mykiss*, ventricles were sectioned 350

µm thick as in the tTLT then, instead of having tissue punches taken, slices were cut into pieces similar in size to the *O. nerka* smolt ventricular cross-sections. These ventricular pieces were then treated in the same manner as the cross-sections obtained from the smolt hearts. Ventricular punches and pieces for the *O. mykiss* groups were taken from both the compact and spongy myocardium, whereas the compact myocardium was not well defined in the ventricular cross-sections of the *O. nerka* smolts.

Binding parameters were determined using a Scatchard plot as described by Zivin and Waud (1982). The  $r^2$  values for CGP-binding curves ranged from 0.93 – 0.97. Protein content of representative punches for the tTLT (mg protein punch<sup>-1</sup>) and representative ventricular cross-sections for the iTLT (protein mm<sup>-3</sup> of tissue) were determined using the Better Bradford Protein Assay Kit (Bio Basic Inc., ON, Canada) so that  $B_{max}$  could be expressed in fmol mg protein<sup>-1</sup>.  $B_{max}$  and  $K_d$  values were compared between the three test groups (iTLT and tTLT for *O. mykiss*, and iTLT for Chilko smolts) using a one-way ANOVA (GraphPad Prism 6), followed by a Tukey's post-hoc test for multiple comparisons. The limit for statistical significance was set as  $p < 0.05$ .

### 2.3 Results and discussion

Ventricular cell-surface  $B_{max}$  for *O. mykiss* at 14 °C (tTLT = 18.5 fmol mg protein<sup>-1</sup> and iTLT = 21.1 fmol mg protein<sup>-1</sup>; Fig. 2.1A) was not significantly different for the two. Additionally,  $B_{max}$  for *O. mykiss* closely matched previous reports for *O. mykiss* acclimated to 14 °C. Hanson et al. (2005) reported a  $B_{max}$  of 26.4 fmol mg protein<sup>-1</sup> (included in Fig. 2.1A for comparison), and Gamperl et al. (1998) reported a  $B_{max}$  of 24.0 fmol mg protein<sup>-1</sup>. Given this

agreement, the new and the traditional methodologies used here to quantify myocardial  $\beta_2$ -AR density appear to be comparable.

$B_{max}$  for the Chilko *O. nerka* smolts (54.2 fmol mg protein<sup>-1</sup>) was significantly higher than *O. mykiss* (Fig. 2.1A).  $B_{max}$  for 8 °C acclimated Chilko smolts was ~60% of the  $B_{max}$  for adults acclimated to 13°C (78.5 fmol mg protein<sup>-1</sup>; included in Fig. 2.1A for comparison) and just ~30% for adults acclimated to 19 °C and 21 °C (123.3 and 128.2 fmol mg protein<sup>-1</sup>; Eliason *et al.*, 2011).  $B_{max}$  for Chilko *O. nerka* smolts was similar to  $B_{max}$  for adult Nechako *O. nerka* acclimated to 13 °C - 21 °C (46.8 - 66.2 fmol mg protein<sup>-1</sup>; Eliason *et al.*, 2011) and for adult Stamp River *O. nerka* sampled either at the riverside at 21 °C (45.2 fmol mg protein<sup>-1</sup>) or after being acclimated in captivity for 3 weeks to 20 °C (47.5 fmol mg protein<sup>-1</sup>; Olsson *et al.*, 2000). Like the Chilko population, the Nechako *O. nerka* are another long migrating Fraser River population, but the Stamp River *O. nerka* have a much shorter river migration.

$K_d$  did not differ significantly between the techniques or the species (Fig. 2.1B). This result is consistent with previous studies of  $K_d$  for several *Oncorhynchus spp.*, including *O. mykiss* and *O. nerka* (Gamperl *et al.*, 1998; Olsson *et al.*, 2000a; Eliason *et al.*, 2011). Numerically, the  $K_d$  values (0.39-0.45 nM) are roughly twice those previously reported for other salmonids (0.13-0.27 nM; Olsson *et al.*, 2000; Eliason *et al.*, 2011) and closer to that of 0.36 nM for Stamp River *O. nerka* sampled at riverside at 21°C, which then decreased to 0.20 nM after three weeks in captivity (Olsson *et al.*, 2000). Therefore, differences in how fish are handled and maintained before sampling may affect  $K_d$ . Also, small differences in saline composition (KCl was 3.1 mM vs 2.5 mM earlier) and pH (pH 7.85 at 10°C vs 7.85 at 15°C) could potentially have small effects on the dissociation constant of myocardial  $\beta_2$ -AR for the  $\beta$ -AR ligand CGP-12177. While it is known that  $\beta$ -AR ligand affinity for multiple ligands decreases at lower pH (Ijzerman

et al., 1984; Modest and Butterworth, 1995; Ghanouni et al., 2000), the specific effects of such small variations in pH on CGP-12177 binding are unknown.

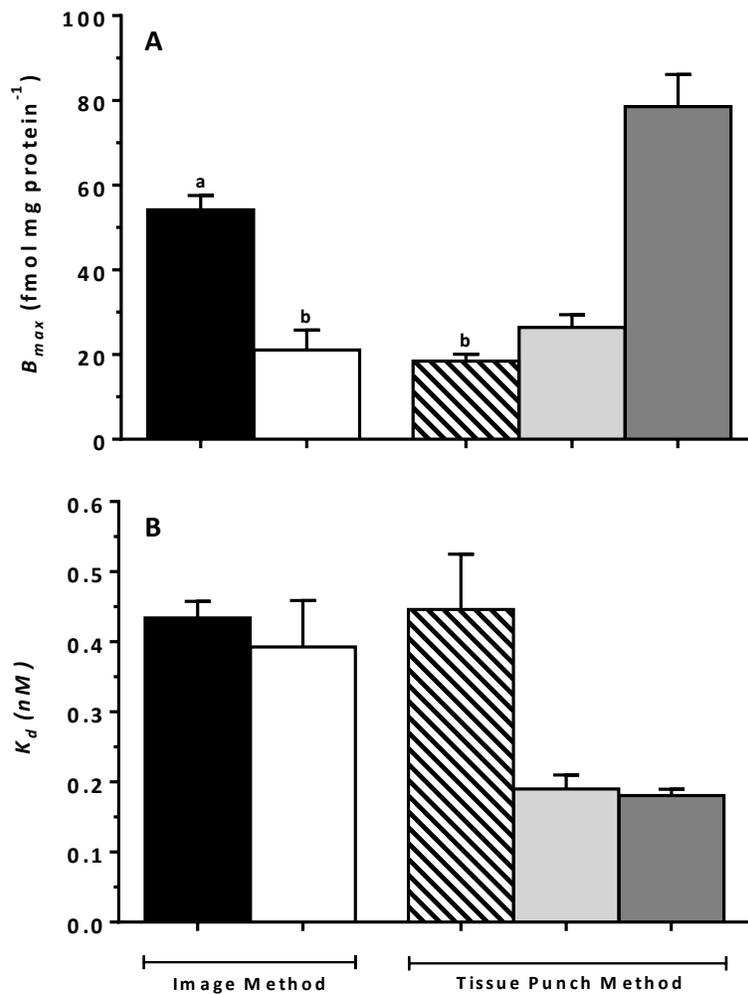
Beyond new information on the ventricular  $\beta_2$ -AR density for the smolt life stage of an interior population of *O. nerka*, these data support the hypothesis that a high ventricular  $B_{max}$  is a trait of the Chilko population (Eliason et al., 2011). However, more work will be needed to resolve the phenotypic and genotypic contributions. This is because the present study used an acclimation temperature of 8 °C for smolts, whereas  $B_{max}$  in adults increased between acclimation temperatures of 13 °C and 19 °C (Eliason et al., 2011). Thus, further work is needed with all life stages of Chilko population to test whether or not ventricular  $B_{max}$  responds similarly to temperature as in the adults and, more importantly, how the heart responds to adrenergic stimulation. Future research could test the possibility that the high cell surface expression of myocardial  $\beta_2$ -ARs is phenotypically enhanced in Chilko adults specifically when they leave cool seawater for warmer water in the Fraser River during their summer spawning migration. Future work could also test if the high constitutive expression of myocardial  $\beta_2$ -AR in the Chilko *O. nerka* population is related to juveniles rearing in a cold, alpine lake given that cold acclimation of *O. mykiss* increases cell surface expression of myocardial  $\beta_2$ -ARs (Keen et al., 1993) to potentially offset the effect of cold temperature on calcium delivery for excitation contraction coupling (Shiels et al., 2003).

The success of the image-based modification to the tritiated ligand technique opens the door to investigations of ventricular cell surface  $\beta_2$ -AR densities in a much wider range of fish and life stages. The technique is no longer limited by the previous need for a large ventricular size. Nevertheless, the iTLT still has limitations. Notably, *c.* 30 fish were pooled for each assay replicate; therefore, large numbers of fish are needed. Also, the compact and spongy

myocardium cannot be distinguished because entire cross-sections of ventricle were used.

Gamperl et al. (1998) showed  $B_{max}$  was 14 % higher in the spongy myocardium.

In conclusion, this study presents the first measurement of ventricular  $\beta_2$ -AR density and CGP binding affinity in a juvenile life stage of a fish, the 1+ year-old Chilko *O. nerka* smolt. Smolt  $B_{max}$  was elevated compared to adult *O. mykiss*, but did not approach values previously seen for adult Chilko fish acclimated to higher temperatures. It remains unclear exactly why Chilko smolts have a lower  $B_{max}$  than their adult counterparts and further studies utilizing the image-based tritiated ligand technique can now be used to better elucidate the relationship between  $B_{max}$  and temperature.



**Figure 2.1** Ventricular  $\beta_2$ -adrenoreceptor density ( $B_{max}$ ; A) and [ $^3\text{H}$ ] CGP-12177 dissociation constant ( $K_d$ ; B) of 1+ year-old Chilko *Oncorhynchus nerka* smolts at 8 °C measured using a novel image-based ligand binding assay (■). Measurements were also performed on adult *O. mykiss* acclimated to 14 °C with both the image-based (□) and traditional tissue punch-based techniques (▨) to validate the methodology. Values of adult *O. mykiss* (◻; 14 °C; Hanson et al., 2005) and return migrating Chilko *O. nerka* (◼; 13 °C; Eliason et al., 2011) are included for visual comparison only, and are excluded from the statistical analysis. Values are means  $\pm$  SEM. Dissimilar letters denote statistically significant differences at  $p < 0.0001$ .

## **Chapter 3. The effect of temperature acclimation on the force-frequency relationship and adrenergic sensitivity of the ventricle of juvenile sockeye salmon from populations with different migration distances between their natal spawning areas and the ocean**

### **3.1 Introduction**

While mammals primarily increase cardiac output during exercise by increasing heart rate, many fish increase stroke volume considerably (Farrell and Jones, 1992; Farrell and Smith, 2017). Thus, the force of cardiac contraction, which determines end-systolic volume of the ventricle and responds to the degree of cardiac filling (end-diastolic volume) via the Frank-Starling effect, has a central role in determining cardiac output when a fish exercises.

Temperature has an overarching effect on the force of contraction of fish hearts. Temperature-dependent changes are possible through direct effects of temperature, such as the pervasive increase in heart rate seen with acute warming seen in all fishes tested to date (Sandblom and Axelsson, 2011; Farrell, 2016), as well as the many changes known to occur with temperature acclimation (*e.g.* Eliason and Anttila, 2017; Farrell and Smith, 2017; Vornanen, 2017). However, there is an important trade-off between heart rate and contractile force, because higher heart rates have less time for mechanical restitution of the cardiomyocyte, preventing the heart from fully relaxing, thus leading to a lower developed force even though peak force may be unchanged (Aho and Vornanen, 1999; Shiels et al., 2002). This inter-dependence typically has been revealed by experimentally measuring the maximum isometric force developed by isolated ventricular strips, rings or trabeculae from various fish species (*e.g.*, Driedzic and Gesser, 1988; Driedzic and Gesser, 1985; Vornanen, 1989; Hartmund and Gesser, 1996; Shiels and Farrell, 1997, 2000; Shiels et al., 1998). As shown in Table 1.1, the vast majority of teleost species tested to date show a negative force-frequency relationship (FFR), although a few (generally the

more active species) have a biphasic FFR, with contractility increasing over low pacing frequencies and then decreasing at high pacing frequencies (Shiels et al., 2002).

The autonomic nervous system modifies cardiac performance in all vertebrates, by regulating heart rate and contractility (Nilsson, 1983). In particular, catecholamines (adrenaline and noradrenaline) via stimulation of  $\beta$ AR found on the cell membranes of fish hearts can independently increase heart rate and maximum contractile force (*e.g.*, Shiels and Farrell, 1997; Shiels et al., 1998; Skov et al., 2009; Methling et al., 2012; Farrell and Smith, 2017).  $\beta$ AR can even protect rainbow trout cardiac contractility from the negative effects of hypoxia, extracellular acidosis, and cold temperature (Graham and Farrell, 1989; Hanson et al., 2006; Hanson and Farrell, 2007). In rainbow trout adrenergic stimulation produces positive inotropic and chronotropic effects (Ask, 1983; Farrell et al., 1986), and can protect the heart under the combined challenges of hypoxia and extracellular acidosis (Hanson et al., 2006). Rainbow trout, however, lose the protective effect of adrenaline at elevated temperatures (Hanson and Farrell, 2007) possibly due to the reduction in cardiac adrenergic sensitivity and ventricular  $\beta$ -adrenoceptor density ( $B_{max}$ ) measured by Keen et al. (1993). These effects occur through enhancements to both the delivery and removal of calcium during excitation-contraction coupling, leading to stronger and faster contractions (see Shiels et al., 2002). Therefore, adrenergic stimulation of the heart is central to the regulation of cardiac output in fishes.

However, temperature acclimation variably modulates the density of  $\beta$ AR in fish hearts. In this regard, adult Chilko River sockeye salmon stand out as their  $B_{max}$  is at least twice that of Nechako sockeye, another *O. nerka* population from the same watershed (Table 1.2). Adult Chilko sockeye salmon also perform a very difficult upriver migration, can maintain a high aerobic scope over a broader temperature range and have a large relative ventricular mass

compared to other sockeye salmon within the Fraser River watershed that perform less challenging migrations, particularly the Weaver Creek population which represents the opposite extreme (Eliason et al., 2011, 2013b). Furthermore, of the limited number of species measured to date, adult Chilko river sockeye are the only fish known to increase ventricular  $B_{max}$  in response to increased acclimation temperature, nearly doubling  $B_{max}$  at 21 °C compared with 13 °C (Eliason et al., 2011). In contrast, rainbow trout more than halved  $B_{max}$  with increasing acclimation temperature from 8°C to 18°C (Keen et al., 1993) and from 8°C and 14°C (Gamperl et al., 1998). Also, ventricular  $B_{max}$  did not change with acclimation temperature in adults from the co-migrating Nechako population of sockeye salmon (Eliason et al., 2011) and the tropical African catfish (*Claris gariepinus*; Hanson et al., 2005). . Nevertheless, ventricular  $B_{max}$  of juvenile Chilko smolts acclimated to 8 °C (Chapter 2; Goulding and Farrell, 2016) was just ~60 % of the  $B_{max}$  measured for adults at 13 °C and just ~30 % for adults at 19 °C and 21 °C, but similar to adult Nechako *O. nerka* acclimated to 13 – 21 °C (Eliason et al., 2011).

Since the effects of  $\beta$ AR stimulation and temperature on cardiac contractility can be explored by measuring the FFR of isolated ventricular strips, the present study examined the interplay between temperature acclimation and  $\beta$ AR stimulation for juvenile sockeye salmon to test the hypothesis that the effects of  $\beta$ AR stimulation differed between juvenile Fraser River sockeye salmon populations. The Chilko and Weaver populations were chosen for comparison given that, as adults, the Weaver population has a considerably shorter (117 km vs. 642 km) and less arduous (180x lower and 10x lower indices of migratory work and migratory effort, respectively) migration from the sea to their natal spawning area when compared with the Chilko population with differences in migratory effort being linked to enhanced cardiovascular performance (Eliason et al., 2011). The elevated  $B_{max}$  seen in both juvenile and adult Chilko

sockeye (Chapter 2; Goulding and Farrell, 2016; Eliason et al., 2011) compared to adults of the relatively short migrating Stamp River Sockeye (Olsson et al., 2000) further supports this link. Additionally, the Weaver and Chilko populations have similar (*c.* 5-10 km) upstream migrations as fry (Brannon, 1972; Sopinka et al., 2013) and juvenile burst swimming performance varies among Fraser River sockeye salmon populations reflecting the difficulty of adult reproductive migration rather than juvenile migration (Sopinka et al. 2013). However, burst swimming is a measure of anaerobic capacity and juvenile Weaver sockeye perform better in endurance swimming tests than do Chilko sockeye, a measure of aerobic capacity, with these differences being partly attributed to differences in abiotic and biotic factors in natal rearing lakes (Eliason et al., 2017). Additionally, Weaver fry have higher mass-specific activities of the mitochondrial (aerobic) enzymes citrate synthase and cytochrome *c* oxidase and the glycolytic (anaerobic) enzyme lactate dehydrogenase than fry from the Gates Creek population (Patterson et al., 2004), a population with a more difficult adult migration (Eliason et al., 2011) and less difficult juvenile migration (Brannon, 1972; Patterson et al., 2004; Sopinka et al., 2013) than the Weaver population, and a similar natal rearing lake to the Chilko population (Eliason et al., 2017). Furthermore, the differences in RVM between populations seen in adult Fraser River sockeye are not present at the parr life stage (Eliason et al., 2017). To test this hypothesis, juvenile sockeye salmon from both populations were raised from eggs in a common-garden rearing environment before measuring and comparing the maximum isometric force developed at different pacing frequencies and at two different temperatures and two levels of  $\beta$ AR stimulation. I predicted that ventricular strips from the Chilko juveniles would have a greater adrenergic responsiveness than the Weaver juveniles, and that differences between the two populations would be amplified at the higher acclimation temperature.

## 3.2 Materials and methods

### 3.2.1 Fish origin and maintenance

All procedures were approved by the UBC Animal Care Committee (Animal Use Protocol A10-0002) in accordance with the guidelines of the Canadian Council of Animal Care. I compared hatchery-sourced *O. nerka* populations from the Chilko and Weaver systems, as well as wild-reared Chilko collected directly from the Chilko River. For the hatchery-sourced fish, eggs were fertilized in the fall of 2010 and incubated until hatch at 10 °C at UBC (see Whitney et al., 2013 for details), after which they were raised under common-garden conditions on a diet of commercial salmon feed (EWOS Canada Ltd., Surrey British Columbia, Canada) at 5 – 7°C under a simulated natural photoperiod that varied with season in the Pacific Salmon Ecology and Conservation Laboratory at UBC (see Chen et al., 2013 for details) until they had reached 2+ years old: Captive-reared Chilko River *O. nerka* (CR<sub>C</sub>):  $n = 12$ ; body mass =  $13.78 \pm 0.58$  g; fork length =  $12.27 \pm 0.09$  cm; condition factor =  $0.74 \pm 0.03$  and captive-reared Weaver Creek *O. nerka* (WC<sub>C</sub>):  $n = 12$ ; body mass =  $13.13 \pm 0.68$  g; fork length =  $11.76 \pm 0.21$  cm; condition factor =  $0.80 \pm 0.01$ ). Age 2+ Wild-reared Chilko River *O. nerka* (estimated based on fish size; CR<sub>W</sub>):  $n = 12$ ; body mass =  $11.51 \pm 0.61$  g; fork length =  $11.76 \pm 0.20$  cm; condition factor =  $0.70 \pm 0.01$ ; Irvine and Akenhead, 2013) were captured between 22 April 2013 and 11 May 2013, when water temperatures ranged from 3 to 8 °C, by dip-net at a government-run counting fence used to enumerate *O. nerka* passage during their migration to the sea from Chilko Lake into Chilko River. . The counting fence was located about 1 km downstream from the Chilko Lake outflow. Juveniles were held for four days in a 500 L tank supplied with river water (8 °C) prior to being transported to the Zoology Aquatic Facility at UBC where they were held in 80 L

fiberglass tanks receiving aerated, recirculated, dechlorinated municipal water. Prior to the force-frequency trials, all fish were acclimated (at least three weeks) to  $5.0 \pm 0.5$  °C in the Zoology Aquatic Facility. After the 5 °C force-frequency trials were completed the remaining fish were acclimated (at least three weeks) to  $14.0 \pm 0.5$  °C before force-frequency trials at 14 °C began. Temperature was maintained by housing the recirculation system inside a temperature-controlled environmental chamber with a 12 h L: 12 h D photoperiod. Tanks were cleaned daily before a 50 % water change.

### **3.2.2 Myocardial strip preparation and experimental protocol**

Fish were mechanically euthanized by a sharp blow to the head and the heart was excised and placed in ice-chilled TES-buffered saline (composition in mM: NaCl, 124.1; KCl, 2.5; MgSO<sub>4</sub>·7H<sub>2</sub>O, 0.9; CaCl<sub>2</sub>·2H<sub>2</sub>O, 2.5; D-glucose, 5.6; TES free acid, 3.9; TES Na salt, 6.1; pH 7.85 at 10 °C; Hanson et al., 2005, 2006). All chemicals were purchased through either Sigma-Aldrich (Oakville, ON, Canada) or Fisher Scientific (Ottawa, ON, Canada). Myocardial strips (around 1 mm thick) were obtained and tested following well-accepted techniques and protocols (Gesser, 1977; Driedzic and Gesser, 1985, 1988; Vornanen, 1989; Aho and Vornanen, 1999; Mercier et al., 2002; Skov et al., 2009; Methling et al., 2012; Larsen et al., 2016). Strips were obtained from the edge of the pyramidal ventricle closest to the atrium running from apex to base. One end of a ventricular strip was tied to a fixed post and the other end was tied to an isometric force transducer [model UTC 2, Gould and Statham, or model SS2, Sherborne Sensors (Wyckoff, NJ, USA)] using single strands from 6-0 silk. The preparation was placed inside a water-jacketed organ bath (25 mL volume), which contained saline (as above) bubbled with either medical grade 100% oxygen (at 14 °C) or air (at 5 °C). Preliminary trials showed no

difference between the use of air and 100% oxygen at 5°C. The muscle was electrically paced by a Grass SD9 Student stimulator that delivered current (5 V, 0.2 – 1.6 Hz and 10 ms duration) via two flat stainless-steel electrodes positioned on either side of the ventricular strip. Muscle strips were initially paced at 0.2 Hz while being stretched to  $L_{\max}$  (the length at which active tension is maximal) and left to stabilize for 1 h before recording the initial tension for each muscle preparation and starting the experiment. Signals from the force transducer were amplified (Gould 4600 series transducer bridge amplifier) and then recorded in AcqKnowledge 3.9.1 via a MP150 data acquisition system (Biopac Systems Inc., Montreal, QC, Canada).

Each muscle preparation underwent two force-frequency trials. The initial, control force-frequency trial used 10 nM of the  $\beta$ -adrenergic agonist isoproterenol hydrochloride (Shiels and Farrell, 1997) in the saline because catecholamines are present at nanomolar concentrations in the circulation of resting salmonids (Milligan et al., 1989) and these levels generate a resting cardiac tonus *in vitro*. After returning the preparation to and stabilizing it at the initial contraction rate of 0.2 Hz, cumulative additions of isoproterenol (final concentrations from 0.01 to 32  $\mu$ M) were made. Stable responses to isoproterenol typically occurred after 10 – 15 min with each addition of isoproterenol. Maximal  $\beta$ AR stimulation was achieved because the highest dose tested (32  $\mu$ M) produced no change active tension relative to the previous concentration. The force-frequency trial was then repeated for maximal  $\beta$ AR stimulation using 32  $\mu$ M isoproterenol. For 5 °C-acclimated fish, stimulation frequency was increased in 0.1 Hz increments until alternans, characterized by alternating high and low force of contraction readings, developed, typically occurring between 0.7 Hz and 0.9 Hz (the data for 0.9 Hz were not included because no more than 3 fish per group reached this frequency without alternans). For 14 °C-acclimated fish, stimulation frequency was increased in 0.2 Hz increments to either the onset of alternans or 1.6

Hz, thereby maintaining either the same end-point or a similar total duration for the force-frequency trial at both acclimation temperatures. Also, preliminary trials revealed an irreversible tissue rundown after alternans occurred at a higher frequency (2.0 Hz), which prevented further testing of that strip. At the end of the experiment, mean cross-sectional area of the strip was calculated using the length and wet mass of the muscle strips, and an assumed muscle density of  $1.06 \text{ g cm}^{-3}$  (Layland et al., 1995).

### 3.2.3 Data analysis

Tension is expressed as  $\text{mN mm}^{-2}$ . Measurements of active tension (peak tension – resting tension), the rate of tension development during contraction ( $dT/dt$ ) and relaxation ( $-dT/dt$ ) were obtained using a custom script (kindly provided by Dr Richard Brill) supported by Matlab R2015a (MathWorks, Natick, MA, USA).

All data are presented as mean values ( $\pm$ SEM) and were analysed using either Sigmaplot 12 or Graphpad Prism 6 at a significance level of  $P < 0.05$ . Concentration-response curves with variable Hill slopes were analysed using an exact sum-of-squares F-test. A two-factor analysis of variance followed by a Holm-Sidak multiple comparisons tests was used for each study group to assess the effects of both pacing frequency and  $\square$ -AR stimulation on active tension,  $dT/dt$  and  $-dT/dt$ . Within each study group, Student's  $t$ -tests were used to test for significant differences between the two acclimation temperatures at 0.2 Hz. Factorial analysis of variance followed by a Turkey's *post-hoc* test was used to test for significant differences among study groups for the initial and final pacing frequencies.

### 3.3 Results

#### 3.3.1 Active tension

##### *α-adrenergic sensitivity*

Active tension at 0.2 Hz increased with increasing  $\alpha$ AR stimulation across all three fish groups and acclimation temperatures, but the sensitivity to  $\alpha$ AR stimulation did not differ significantly among them (Fig. 3.1). As a result, a single common curve described the concentration-response with a Hill-slope of  $1.04 \pm 0.12$  and a  $\log EC_{50}$  of  $-6.18 \pm 0.05$  M ( $EC_{50}$  of  $0.67 \mu\text{M}$ ; Exact sum-of-squares F-test:  $P = 0.18$ ; Fig. 3.1). While two of the test groups tended to be slightly less sensitive to isoproterenol, this subtle difference was inconsistent across the acclimation temperatures. While maximal stimulation of active tension at this pacing frequency was clearly produced with  $32 \mu\text{M}$  isoproterenol, tonic stimulation of active force with  $0.01 \mu\text{M}$  isoproterenol was likely not great for any population or acclimation temperature tested given the sigmoidal nature of each concentration-response curve.

##### *Population comparisons when paced at 0.2 Hz*

Active tension did not significantly differ among study groups when tested at acclimation temperatures of either  $5^\circ\text{C}$  or  $14^\circ\text{C}$  with  $0.01 \mu\text{M}$  isoproterenol (Table 3.1). Also, active tension under these conditions was similar at both acclimation temperatures. Maximal  $\alpha$ AR stimulation ( $32 \mu\text{M}$  isoproterenol) nearly doubled active tension at 0.2 Hz in all study groups and was similar among study groups at both  $5^\circ\text{C}$  ( $P \leq 0.01$ ; Fig. 3.2) and  $14^\circ\text{C}$  ( $P < 0.001$ ; Fig. 3.2).

### 3.3.2 Force-frequency responses at 5°C acclimation

Increasing pacing frequency (from 0.2 Hz to 0.8 Hz) with tonic  $\square$ AR stimulation had no significant effect on active tension in either Chilko population (Fig. 3.2A for CR<sub>W</sub> and Fig. 3.2B for CR<sub>C</sub>); but produced a weak biphasic FFR for the Weaver population, with active tension peaking at 0.5 Hz and decreasing significantly at 0.8 Hz ( $P \leq 0.045$ ; Fig. 3.2C).

Maximal  $\beta$ AR stimulation significantly increased active tension at pacing frequencies from 0.2 – 0.5 Hz in CR<sub>W</sub> and WC<sub>C</sub> and from 0.2 – 0.6 Hz in CR<sub>C</sub>, with a doubling at 0.2 Hz for all three study groups ( $P \leq 0.01$ ; Fig. 3.2). In contrast to tonic  $\square$ AR stimulation, a negative FFR was seen for all three study groups, and the active tension developed at higher pacing frequencies ultimately became not significantly different to that with tonic  $\square$ AR stimulation (Figure 3.2). Thus, with 5 °C acclimation and independent of the population tested, the considerable benefit of  $\square$ AR stimulation at low to moderate pacing frequencies was completely lost at the highest pacing frequency.

### 3.3.3 Force-frequency responses at 14°C acclimation

At 14 °C acclimation and unlike at 5 °C acclimation, increasing pacing frequency (up to 1.6 Hz) with tonic  $\square$ AR stimulation resulted in a modest, negative FFR for all three study groups ( $P \leq 0.043$ ; Fig. 3.2). While increasing the pacing frequency with maximal  $\square$ AR stimulation similarly resulted in a negative FFR in all three study groups ( $P \leq 0.040$ ; Fig. 3.2), it did not attenuate active tension as much as with 5 °C acclimation. As such, the benefit of maximal  $\square$ AR stimulation was at least 1.5-fold improvement compared with tonic  $\square$ AR stimulation across all pacing frequencies and study groups ( $P \leq 0.008$ ; Fig. 3.2).

### 3.3.4 Rate of isometric contraction

#### *Population comparison at 0.2 Hz*

The rate of contraction of ventricular strips ( $dT/dt$ ) with either tonic or maximal  $\square$ AR stimulation did not significantly differ among the three study groups when tested at either acclimation temperature (Table 3.1). As might be expected,  $dT/dt$  was almost twice as fast at 14 °C compared with 5 °C for all three study groups and at least 50 % faster with maximal  $\square$ AR stimulation (Table 3.1). Notably,  $dT/dt$  at 0.2 Hz for maximal  $\square$ AR stimulation at 5°C was similar to  $dT/dt$  at 0.2 Hz for tonic  $\square$ AR stimulation at 14 °C (Fig. 3.3).

#### *Force-frequency responses of $dT/dt$ at 5 °C acclimation*

Increasing the pacing frequency with tonic  $\square$ AR stimulation at 5 °C produced a significant increase in  $dT/dt$  of 50 – 70 % at 0.2 Hz in all three study groups ( $P \leq 0.008$ ; Fig. 3.3).  $dT/dt$  remained significantly faster up to 0.5 Hz for both CR<sub>w</sub> and WC<sub>C</sub> ( $P \leq 0.018$ ; Fig. 3.3A and C), and up to 0.6 Hz for CR<sub>C</sub> ( $P = 0.006$ ; Fig. 3.3B). Both CR<sub>C</sub> and WC<sub>C</sub> then showed a biphasic response with  $dT/dt$  decreasing significantly at 0.8 Hz ( $p \leq 0.040$ ; Fig. 3.3B and C), unlike CWR<sub>C</sub>. With maximal  $\square$ AR stimulation, a significant increase in  $dT/dt$  was seen only at the lower pacing frequencies in all three study groups (Fig. 3.3).

#### *Force-frequency responses of $dT/dt$ at 14 °C acclimation*

Increasing the pacing frequency with tonic  $\square$ AR stimulation at 14 °C produced no significant effect on  $dT/dt$  in WC<sub>C</sub> (Fig. 3.3C), but significantly increased  $dT/dt$  in both CR<sub>w</sub> and CR<sub>C</sub>, peaking at 1.4 Hz ( $P \leq 0.029$ ; Fig. 3.3A and B). Maximal  $\square$ AR stimulation significantly increased  $dT/dt$  by 60 – 80 % at 0.2 Hz in all three study groups compared with tonic  $\square$ AR

stimulation ( $P \leq 0.030$ ; Fig. 3.3). This difference was maintained until a pacing frequency of 1.4 Hz for both CR<sub>C</sub> and WC<sub>C</sub> which had a peak  $dT/dt$  at 1.2 Hz ( $P \leq 0.047$ ; Fig. 3.3B and C), unlike CR<sub>W</sub> where  $dT/dt$  was independent of pacing frequency up to 1.2 Hz (Fig. 3.3A), above which  $dT/dt$  decreased significantly just like CR<sub>C</sub> ( $P \leq 0.002$ ; Fig. 3.3A and B), but unlike WC<sub>C</sub> (Fig. 3.3A).

### 3.3.5 Rate of isometric relaxation

#### *Population comparison at 0.2 Hz*

The rate of relaxation of ventricular strips ( $-dT/dt$ ) with tonic  $\square$ AR stimulation did not differ significantly among the three study groups when tested at either acclimation temperature (Table 3.1). Similar to  $-dT/dt$ ,  $-dT/dt$  was again almost twice as fast at 14 °C for all three study groups and at least 50 % faster with maximal  $\square$ AR stimulation (Table 3.1). Notably,  $dT/dt$  at 0.2 Hz with maximal  $\square$ AR stimulation at 5 °C was similar to  $dT/dt$  at 0.2 Hz with tonic  $\square$ AR stimulation at 14 °C (Fig. 3.4).

#### *Force-frequency responses of $-dT/dt$ at 5 °C acclimation*

With tonic  $\square$ AR stimulation,  $-dT/dt$  was independent of pacing frequency stimulation for both CR<sub>W</sub> and CR<sub>C</sub> at 5 °C acclimation (Fig. 3.4A and B), but  $-dT/dt$  significantly increased in WC<sub>C</sub>, peaking at 0.4 Hz and remaining elevated up to 0.8 Hz ( $P \leq 0.033$ ; Fig. 3.4C). With maximal  $\square$ AR stimulation,  $-dT/dt$  increased by 50 – 70 % at 0.2 Hz in all three study groups ( $P \leq 0.002$ ; Fig. 3.4), remaining significantly faster up to 0.4 Hz in CR<sub>W</sub> ( $P \leq 0.004$ ; Fig. 3.4A), 0.5 Hz in CR<sub>C</sub> ( $P \leq 0.014$ ; Fig. 3.4B), and 0.6 Hz in WC<sub>C</sub> ( $P \leq 0.038$ ; Fig. 3.4C) before significantly

( $P \leq 0.045$ ) decreasing in all three study groups to a rate the same as that under tonic  $\square$ -adrenergic stimulation, *i.e.*, a biphasic response.

#### *Force-frequency responses of $-dT/dt$ at 14 °C*

With tonic  $\square$ AR stimulation,  $-dT/dt$  was independent of pacing frequency stimulation in all three study groups at 14 °C acclimation (Fig. 3.4). With maximal  $\square$ AR stimulation,  $-dT/dt$  increased by 50 – 80 % at 0.2 Hz in all three study groups (Fig. 4), and remained significantly elevated at all pacing frequencies in both CR<sub>C</sub> and WC<sub>C</sub> ( $P \leq 0.010$ ; Fig. 3.4B and C) and all frequencies in CR<sub>W</sub> ( $P \leq 0.028$ ; Fig. 3.4A) except the final 1.6 Hz when  $-dT/dt$  decreased significantly at 1.6 Hz in CR<sub>C</sub> ( $P \leq 0.004$ ; Fig. 3.4B).

### **3.4 Discussion**

#### **3.4.1 The force-frequency relationship**

*In vivo* heart rate data are unavailable for the size of sockeye salmon used in the current study. For adult fish at 15 °C, heart rate ranges from around 64 bpm at rest to 100 bpm while swimming (Steinhausen et al., 2008; Eliason et al., 2013a), a similar range to that seen in adult rainbow trout at 15 °C (around 30 bpm at rest to 100 bpm while swimming maximally; Priede, 1974; Gallagher et al., 1995; Altimiras and Larsen, 2000; Ekstrom et al., 2016). For ~1.5 g fry, maximum heart rate measured during Arrhenius breakpoint temperature measurements were 54 bpm and 51 bpm at 7 °C and 96 bpm and 94 bpm at 14 °C for Weaver Creak and Chilko River fish respectively (Chen et al., 2013). As such, the most physiologically relevant range for pacing frequencies in the present study is 0.5 Hz to the maximum frequency tested. Thus, the minor differences that did emerge in the FFR among the three test study groups at the 5 °C acclimation

temperature were at physiologically relevant pacing frequencies (0.4-0.5 Hz), but the consequences of these small differences may also be small.

An unexpected discovery was the limited dependence of active tension on pacing frequency, particularly the rather flat FFR with tonic adrenergic stimulation. Only the strongly negative FFR seen with maximum adrenergic stimulation at 5 °C was comparable to that reported for a wide variety of fish species (Driedzic and Gesser, 1985, 1988; Vornanen, 1989; Mercier et al., 2002; Skov et al., 2009; Joyce et al., 2016; Larsen et al., 2016). Indeed, the vastly different effects of maximal  $\square$ AR stimulation on the FFR at the two acclimation temperatures revealed complex interactions among pacing frequency, acclimation temperature and maximal  $\square$ AR stimulation that have clear physiological relevance because appreciable differences became fully expressed at the highest pacing frequency. Thus, unlike at the lowest pacing frequency when maximal  $\square$ AR stimulation doubled active tension regardless of acclimation temperature and population, maximal  $\square$ AR stimulation for pacing frequencies similar to maximum heart rate in swimming salmonids still almost doubled active force at 14 °C, but it was no better than tonic  $\square$ AR stimulation at 5 °C. Thus, given that  $\square$ AR stimulation would have greater benefits for fish swimming at the warmer acclimation temperature, the mechanistic basis for this temperature effect would be worth studying. Perhaps the temperature dependence of  $B_{max}$  for  $\square$ AR shown previously for Chilko sockeye salmon between 14 °C and 19 °C (Eliason et al., 2011) extends to a lower acclimation temperature and more generally among sockeye salmon populations. Interestingly, while all study groups increased (roughly doubled) both  $dT/dt$  and  $-dT/dt$  with acclimation temperature, active tension did not change appreciably at 0.2 Hz with either tonic or maximal  $\square$ AR stimulation. Unfortunately, the design of the present study cannot resolve between the direct effects of temperature and any changes due to thermal acclimation processes.

Further study into the difference in maximal  $\square$ AR stimulation at the highest pacing frequencies should try to make such a distinction.

### **3.4.2 Population comparison**

I used the FFR of ventricular strips to compare the cardiac  $\square$ -adrenergic responsiveness of 2+ year old juveniles from two populations of Fraser River sockeye salmon (Chilko River and Weaver Creek). The basis for my population comparison was that the adults have very distinct and different migratory challenges when returning to their natal spawning area (Crossin et al., 2004) and aspects of the adult cardiac anatomy and physiology differ appreciably (Eliason et al., 2011, 2013b). Both populations have similar upstream migrations as juveniles (Brannon, 1972; Sopinka et al., 2013), and inter-population differences in burst swimming performance reflect the difficulty of adult reproductive migration more so than that of fry migration (Sopinka et al., 2013). The typical thermal regime experienced by juvenile Chilko sockeye is colder than that of their Weaver counterparts with river temperatures as low as 3.3 °C during the Chilko fry upstream migration compared to 4.4 °C for Weaver fry (Bannon, 1972). Rearing lake epilimnetic temperatures rarely exceed 14 °C in Chilko Lake (Hume et al., 1996) with an average of 8.2 °C during the summer growth season (Goodlad et al., 1974; Shortreed et al., 2001), while Harrison Lake (the rearing lake of the Weaver population) can reach ~17 °C (Mathes et al., 2009) with an average epilimnetic temperature of 12.6 °C over the same period (Shortreed et al., 2001). While juvenile Chilko sockeye may be exposed to generally colder temperatures in the wild, acute warming up to as high as 22 °C of the same brood of sockeye I used here resulted in a reduction in endurance swim performance in the Weaver population, while Chilko parr were able to maintain their endurance swimming ability (Eliason et al., 2017). Given the above, and because

ventricular  $B_{max}$  is known to be very high for adult Chilko River sockeye salmon when compared with other sockeye salmon and salmonid populations (Olsson et al., 2000; Eliason et al., 2011), and to increase with increased acclimation temperature (Eliason et al., 2011), my expectation was that the Chilko River juveniles would have a greater  $\alpha$ -adrenergic responsiveness at one or both of the acclimation temperatures (5 °C and 14 °C).

Contrary to my hypothesis, no substantive differences emerged between the study populations at either acclimation temperature.  $\alpha$ AR sensitivity ( $EC_{50}$  and Hill slope values) and the effects of tonic and maximum adrenergic stimulation on the FFR (active tension, contraction rate and relaxation rate at either 0.2 Hz or the highest test frequency; Table 3.1) did not differ between the two populations of sockeye salmon and were independent of whether the Chilko population grew in their natal lake or in captivity. These results are consistent with  $B_{max}$  of Chilko smolts being more similar to that of adults from the Nechako River rather than Chilko Lake (Chapter 2; Goulding and Farrell, 2016). Thus, while sockeye salmon consistently show an age-independent higher ventricular  $B_{max}$  than rainbow trout, the previously reported elevated  $B_{max}$  for adult Chilko versus Nechako (Eliason et al., 2011) and Stamp River populations (Olsson et al., 2000) may reflect a population specific adaption that prepares this population for a more arduous upriver migration to the natal spawning area as adults (note: adult fish were sampled before the arduous part of their river migration had begun; Eliason et al., 2011), and a similar response of  $B_{max}$  to temperature may not be present in juvenile Chilko sockeye. Further support for this idea comes from a study showing that the differences in upper thermal tolerance among nine different populations of age-matched sockeye salmon fry disappeared when they were size matched (Chen et al., 2013). Also, the Chilko River and Weaver Creek populations did not differ significantly in the response of maximum heart rate to acute warming (Chen et al., 2013).

While the comparison between juvenile Chilko and Weaver sockeye salmon was based primarily on known cardiac differences in adults of these populations, the juveniles have similar migrations to their rearing lakes (Brannon, 1972; Sopinka et al., 2013). Therefore, the similarities in the FFR seen here for juvenile Chilko and Weaver sockeye salmon could reflect this life history similarity. This would mean that the population differences seen in the adult cardiac phenotype would be expressed later in life. Indeed, the RVM of nine populations of sockeye salmon did not differ significantly as parr (Eliason et al., 2017), while the RVM of Chilko adults was significantly greater than Weaver adults (Eliason et al. 2011). Differences do still persist between juveniles of various sockeye salmon populations, however. Juveniles from populations that rear in deep, clear, and comparatively unproductive lakes, such as the Gates Creek and Chilko River sockeye, had better burst swimming but worse endurance swimming performance than juveniles that rear in warmer, more productive, and often more turbid environments, such as Adams River and Weaver Creek sockeye (Eliason et al., 2017; Sopinka et al., 2013). These differences could be explained by differences in foraging and predator avoidance strategies (Eliason et al., 2017), or possibly from differences in enzyme activity (e.g. citrate synthase, cytochrome *c* oxidase, and lactate dehydrogenase; Patterson et al., 2004), and further research is needed.

In conclusion, the present study examined the interplay between temperature acclimation and  $\beta$ AR stimulation on the FFR of ventricular muscle from juvenile sockeye salmon and rejected the hypothesis that the effects of  $\beta$ AR stimulation differed substantively between Chilko and Weaver sockeye salmon. Instead, it was discovered that the two sockeye salmon populations had a rather flat FFR with tonic  $\beta$ AR stimulation that was independent of acclimation temperature and with maximal  $\beta$ AR stimulation at only 14 °C. Thus, while I predicted a greater

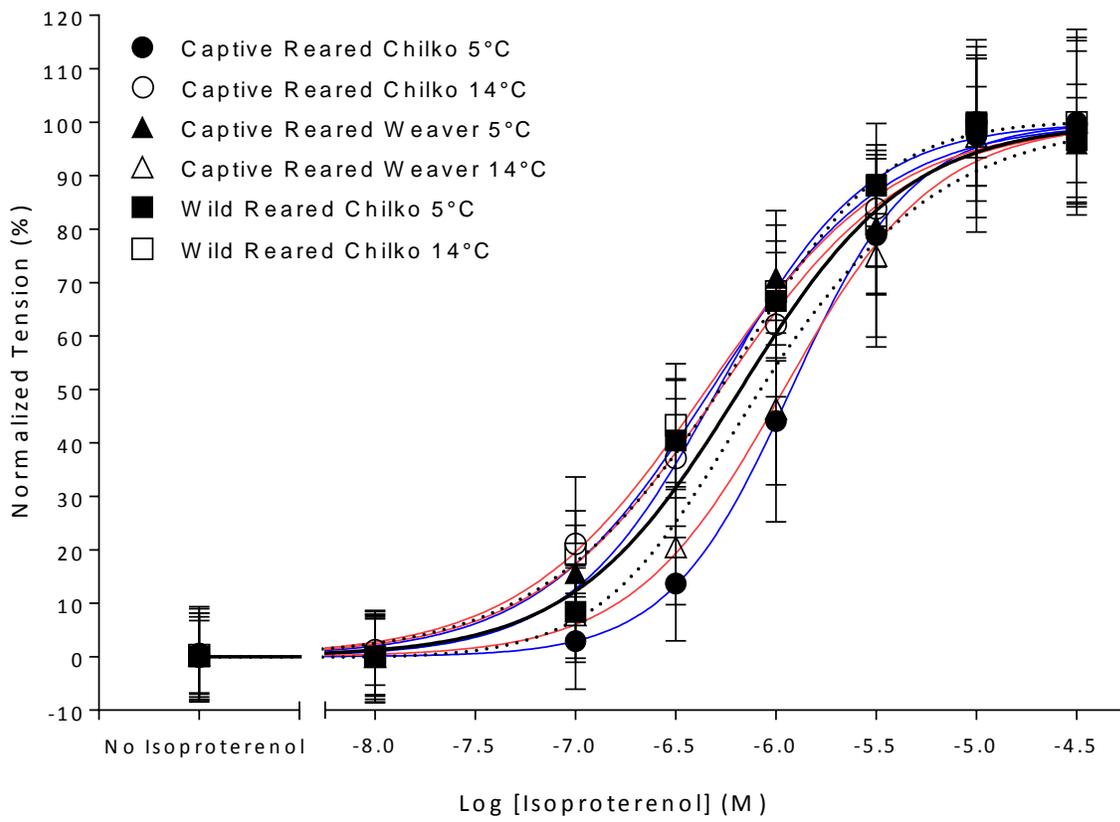
adrenergic responsiveness for Chilko sockeye that would be amplified at the higher acclimation temperature, the benefits of maximal  $\square$ AR stimulation (an almost doubling of active force at low pacing frequencies) was maintained in both populations at pacing frequencies similar to maximum heart rate in swimming salmonids at 14 °C, but not at 5 °C.

**Table 3.1** A summary of the effect of acclimation temperature at selected pacing frequencies on active tension, contraction rate and relaxation rate developed by isolated ventricular muscle strips with 10 nM (tonic stimulation) and 32  $\mu$ M (maximal stimulation) isoproterenol hydrochloride for wild-reared Chilko River ( $CR_w$ ), captive-reared Chilko River ( $CR_c$ ), and captive-reared Weaver Creek ( $WC_c$ ) *Oncorhynchus nerka* juveniles.

Variables	Study Group	Tonic Adrenergic Stimulation				Maximal Adrenergic Stimulation			
		0.2 Hz		0.8 Hz	1.6 Hz	0.2 Hz		0.8 Hz	1.6 Hz
		5 °C	14 °C	5 °C	14 °C	5 °C	14 °C	5 °C	14 °C
Active Tension (mN mm <sup>-2</sup> )	$CR_w$	6.35 ± 0.41	6.04 ± 0.41	5.22 ± 0.40 (4)	4.98 ± 0.40	10.19 ± 0.48	11.35 ± 0.60	<sup>†</sup> 5.01 ± 0.34	8.09 ± 0.69
	$CR_c$	5.54 ± 0.53	6.13 ± 0.31	5.47 ± 0.80	4.64 ± 0.49	10.99 ± 0.97	10.50 ± 0.74	5.81 ± 0.53	7.24 ± 0.62
	$WC_c$	5.76 ± 0.30	5.95 ± 0.36	5.65 ± 0.45	4.51 ± 0.28 (5)	9.85 ± 0.93	10.17 ± 0.66	6.45 ± 0.59	8.03 ± 1.02 (5)
Contraction Rate (mN s <sup>-1</sup> )	$CR_w$	12.83 ± 1.11*	26.31 ± 2.08	16.22 ± 1.54 (4)	31.71 ± 2.61	21.39 ± 1.60*	43.99 ± 3.88	16.40 ± 1.74	41.83 ± 5.18
	$CR_c$	15.09 ± 1.69*	28.68 ± 2.65	19.14 ± 1.84	33.88 ± 4.03	25.45 ± 2.30*	45.40 ± 3.35	21.25 ± 2.02	43.94 ± 2.74
	$WC_c$	15.25 ± 0.86*	26.32 ± 1.48	18.70 ± 1.40	28.02 ± 2.05 (5)	23.72 ± 2.86*	46.65 ± 5.44	21.66 ± 2.25	51.40 ± 5.51 (5)
Relaxation Rate (mN s <sup>-1</sup> )	$CR_w$	-16.80 ± 1.71*	-34.61 ± 3.93	-17.17 ± 1.44 (4)	-32.01 ± 2.38	-25.60 ± 1.92*	-51.43 ± 5.76	-16.94 ± 1.62	-44.69 ± 6.57
	$CR_c$	-18.17 ± 2.02*	-31.08 ± 2.17	-21.43 ± 2.14	-31.92 ± 3.90	-31.22 ± 2.33*	-55.74 ± 4.02	-22.60 ± 2.10	-46.63 ± 3.78
	$WC_c$	-17.66 ± 1.29*	-31.62 ± 1.25	-21.19 ± 1.75	-30.05 ± 2.18 (5)	-30.53 ± 3.60*	-57.28 ± 6.68	-24.30 ± 2.76	-58.44 ± 7.88 (5)

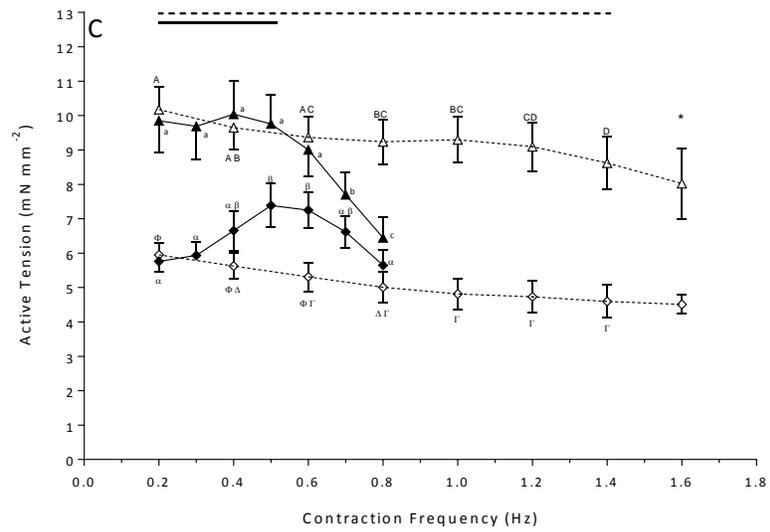
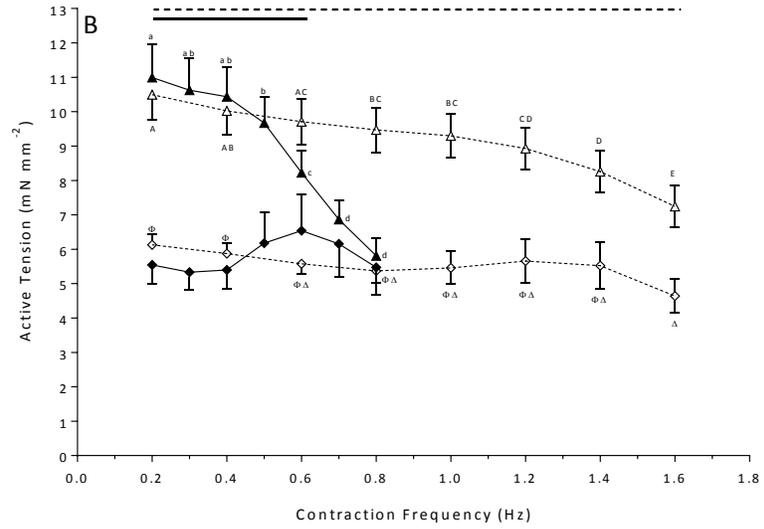
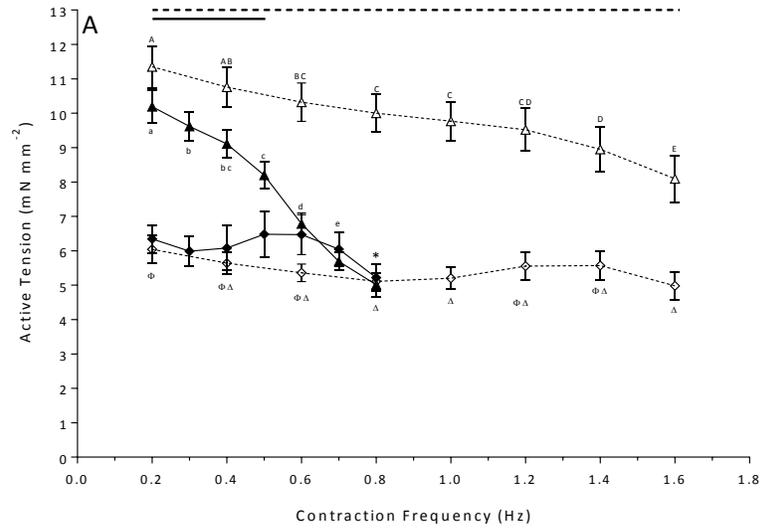
Values are group means ± S.E.M. (N)=6 unless otherwise noted.

Significant differences within study groups between acclimation temperatures at 0.2 Hz are denoted by \* (student's T-test's;  $P < 0.05$ )

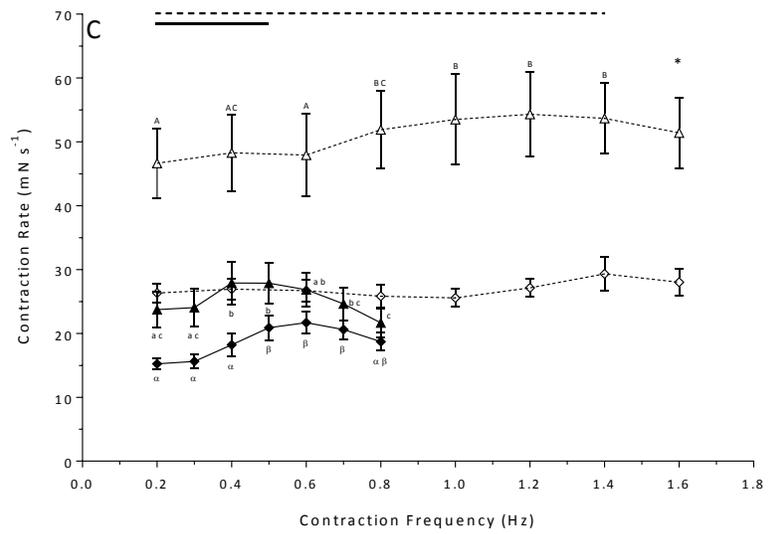
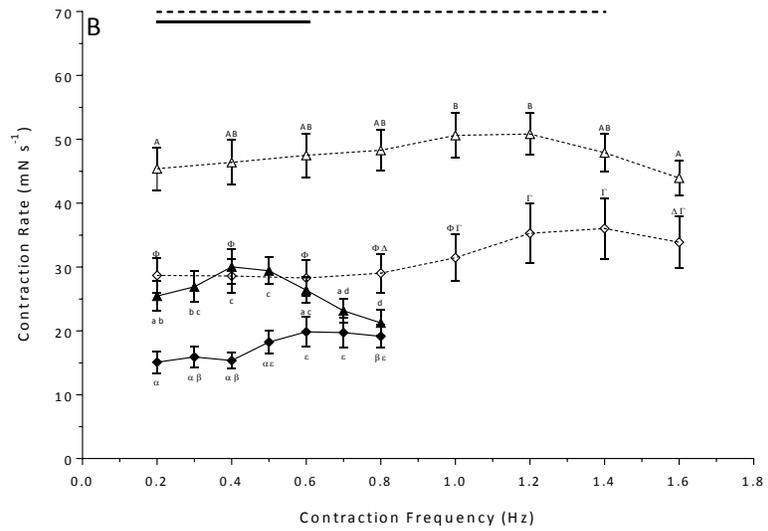
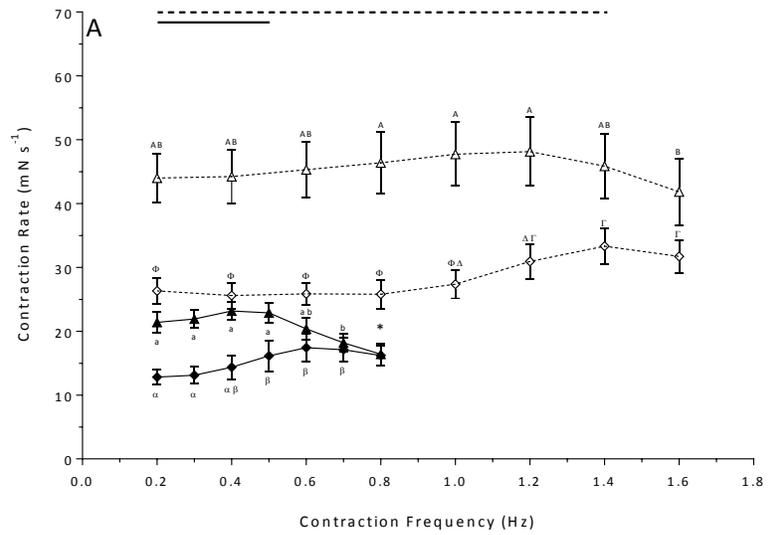


**Figure 3.1** The concentration-response relationship for isoproterenol hydrochloride (solid black line; Hill-slope =  $1.04 \pm 0.12$ ;  $\text{LogEC}_{50} = -6.18 \pm 0.05$  M) with 95% confidence bands (dashed lines) of isometrically contracting ventricular muscle strips isolated from wild-reared Chilko River, from captive-reared Chilko River, and from captive-reared Weaver Creek *Oncorhynchus nerka* juveniles acclimated to either 5 °C or 14 °C ( $n = 6$  for all data). Myocardial strips were electrically paced at 0.2 Hz *in vitro* in temperature-controlled tissue baths. Active tension is presented as a normalized value between 0 and 100% for each experimental group (%; mean  $\pm$  SEM). Concentration-response curves for individual experimental groups at either temperature

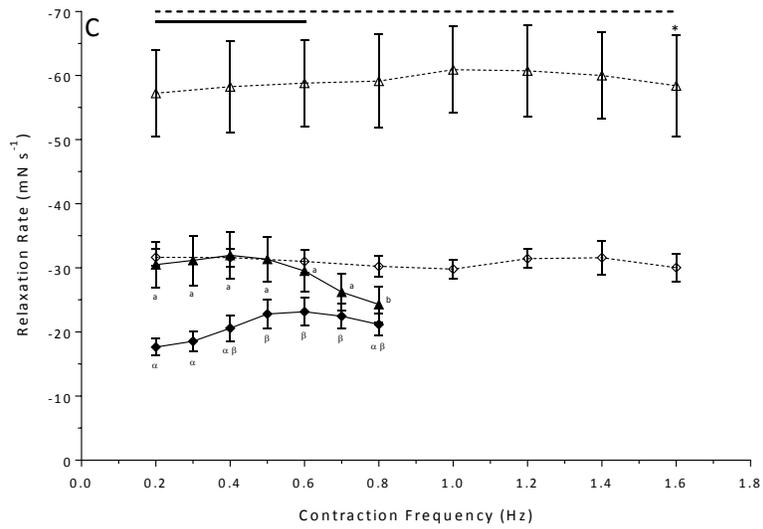
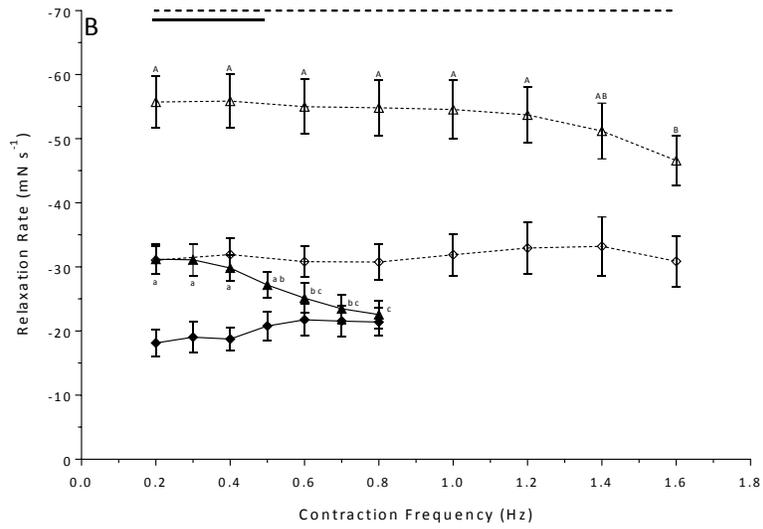
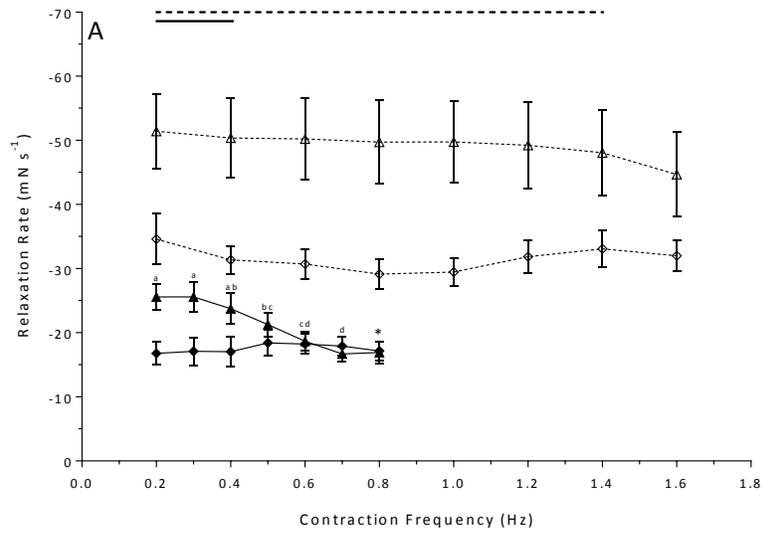
did not significantly differ (Exact sum-of-squares F-test:  $p = 0.18$ ; Red and blue curves for warm- and cold-acclimated groups, respectively).



**Figure 3.2** Active tension ( $\text{mN mm}^{-2}$ ; mean  $\pm$  SEM) of ventricular muscle strips as a function of pacing frequency from (A) wild-reared Chilko River, (B) captive-reared Chilko River, and (C) captive-reared Weaver Creek *Oncorhynchus nerka* juveniles acclimated to either 5 °C or 14 °C (solid or hollow symbols, respectively) while exposed to either tonic ( $\blacklozenge$   $\lozenge$ ) or maximal  $\beta$ AS ( $\blacktriangle$   $\triangle$ ) ( $n = 6$  for all data except where marked with an asterisk when  $n = 5$ ). Significant differences were assessed using two-way repeated measures ANOVAs followed by Holm-Sidak multiple comparisons tests ( $P \leq 0.05$ ). Dissimilar Latin and Greek letters denote significant differences between frequencies within an acclimation temperature and level of adrenergic stimulation. Solid and dashed lines (5 °C and 14 °C, respectively) denote frequencies where active tensions at tonic and maximal adrenergic stimulations were significantly different.



**Figure 3.3** Rate of contraction ( $dT/dt$ ;  $\text{mN s}^{-1}$ ; mean  $\pm$  SEM) of isometric ventricular muscle strips as a function of pacing frequency from (A) wild-reared Chilko River, (B) captive-reared Chilko River, and (C) captive-reared Weaver Creek *Oncorhynchus nerka* juveniles acclimated to either 5 °C or 14 °C (solid or hollow symbols, respectively) while exposed to either tonic ( $\blacklozenge$  $\lozenge$ ) or maximal  $\beta$ AS ( $\blacktriangle$  $\triangle$ ) ( $n = 6$  for all data except where marked with an asterisk when  $n = 5$ ). Significant differences were assessed using two-way repeated measures ANOVAs followed by Holm-Sidak multiple comparisons tests ( $P \leq 0.05$ ). Dissimilar Latin and Greek letters denote significant differences between frequencies within an acclimation temperature and level of adrenergic stimulation. Solid and dashed lines (5 °C and 14 °C, respectively) denote frequencies where relaxation rates at tonic and maximal adrenergic stimulations were significantly different.



**Figure 3.4** Rate of relaxation ( $-dT/dt$ ;  $\text{mN s}^{-1}$ ; mean  $\pm$  SEM) of isometric ventricular muscle strips as a function of pacing frequency from (A) wild-reared Chilko River, (B) captive-reared Chilko River, and (C) captive-reared Weaver Creek *Oncorhynchus nerka* juveniles acclimated to either 5 °C or 14 °C (solid or hollow symbols, respectively) while exposed to either tonic ( $\blacklozenge$ ) or maximal  $\beta$ AS ( $n = 6$  for all data except where marked with an asterisk when  $n = 5$ ). Significant differences were assessed using two-way repeated measures ANOVAs followed by Holm-Sidak multiple comparisons tests ( $P \leq 0.05$ ). Dissimilar Latin and Greek letters denote significant differences between frequencies within an acclimation temperature and level of adrenergic stimulation. Solid and dashed lines (5 °C and 14 °C, respectively) denote frequencies where contraction rates at tonic and maximal adrenergic stimulations were significantly different.

## Chapter 4. Conclusions

The overall objective of my thesis was to begin to fill the relative void in understanding of the cardiac physiology of juvenile sockeye salmon and to investigate the possibility of inter-population differences in said physiology. The primary reasons for focusing on *O. nerka* were the relatively recent discovery of remarkable differences in cardiac phenotypes of different populations of adult Fraser River sockeye salmon (Eliason et al., 2011; 2013 a, b, c), coupled with Sockeye salmon's immense social (Jones et al., 2004), economic (Williams, 2007), and biological importance (Finney et al., 200; Heldfield and Naiman, 2001; Johnston et al., 2004). My central underlying question was whether or not juvenile sockeye salmon show similar inter-population cardiac differences as previously shown for adults. I utilized two well-established techniques to explore fundamental aspects of cardiac physiology that had not previously been studied in juvenile sockeye salmon. Specifically, in Chapter 2 I used a modified tritiated ligand binding assay to measure the ventricular  $\beta$ AR density of juvenile sockeye salmon from the Chilko River population, while for Chapter 3 I measured the active force developed by ventricular myocardial tissue during isometric contractions and as a function of pacing frequency for juveniles from both the Weaver Creek and Chilko River populations.

To make my receptor density measurements in Chapter 2, I developed and validated a novel variation to the traditional tritiated ligand binding assay, one that can now be widely used on fishes 14-times smaller than had been previously accomplished. I discovered that juvenile Chilko sockeye salmon acclimated to 8 °C have ventricular  $B_{max}$  values similar to adult sockeye from the Nechako River acclimated to 13 °C – 21 °C (Eliason et al., 2011) and Stamp River populations acclimated to 10 °C – 21 °C (Olsson et al., 2000), but *c.* 30% lower than adult Chilko sockeye acclimated to 13 °C and *c.* 60% lower than adults acclimated to 19 °C and 21 °C

(Eliason et al., 2011). Clearly these data are confounded by temperature as the lower  $B_{max}$  of the juvenile Chilko sockeye may be either the result of lower acclimation temperature when compared to the adults or represent a clear difference between life stages in the Chilko population. To provide greater insight into this issue, future research should test the possibility that the high cell surface expression of myocardial  $\beta_2$ -ARs is phenotypically enhanced in Chilko adults specifically when they leave cool seawater for warmer water in the Fraser River during their summer spawning migration. All life stages of Chilko sockeye should also be tested to investigate whether or not ventricular  $B_{max}$  responds similarly to temperature as in the adults.

Although cardiac stimulation by catecholamines is the primary mechanism by which fish hearts are stimulated to beat both stronger and faster, knowing only ventricular  $\beta$ -AR density does not provide a full insight into the functional aspects of said stimulation. Therefore, in Chapter 3 I measured the FFR of ventricular strips from juvenile sockeye salmon. This approach allowed me to consider the important role that  $\beta$ -adrenergic stimulation and heart rate play in the force that the myocardium can develop. In addition, I compared juvenile Chilko and Weaver sockeye salmon at two acclimation temperatures with the expectation that Chilko River juveniles would have a greater response to  $\beta$ -adrenergic stimulation at one or both acclimation temperatures (5 °C and 14 °C). Instead, I discovered no substantive differences in active tension, contraction rate, relaxation rate, or  $\beta$ -adrenoceptor sensitivity among the study populations at either acclimation temperature. Maximal  $\beta$ AS increased all variables measured at the control pacing frequency of 0.2 Hz (12 bpm) and flat FFRs were seen under tonic  $\beta$ AS at both acclimation temperatures and under maximal  $\beta$ AS at 14 °C. Interestingly, negative FFRs were seen under maximal  $\beta$ AS at 5 °C such that by physiologically relevant pacing frequencies, there were no longer any differences between measurements taken under tonic and maximal  $\beta$ AS.

These data provide insight into the cardiac physiology of juvenile sockeye salmon, but the complexity and variation in the life cycle of *O. nerka* leaves many avenues of investigation open for future discovery. I compared juveniles from only two of the more than 100 genetically and geographically distinct Fraser River sockeye populations acclimated to two temperatures. Future research should investigate a broader spectrum of sockeye salmon populations, not only from the Fraser River, but from across the full habitat range of the species. In the wild, Fraser River sockeye salmon often rear in stratified lakes where they find thermal refuges in cooler deeper water during the day and migrate to the surface for feeding at night (Goodlad et al., 1974). Given this diel migration from cooler to warmer waters and back, future studies should also investigate the effects of acute temperature changes on juvenile sockeye cardiac physiology.

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