THERMAL ACCLIMATION POTENTIAL OF AUSTRALIAN RAINBOW TROUT, 

ONCORHYNCHUS MYKISS 

by 

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The following individuals certify that they have read, and recommend to the Faculty of Graduate and Postdoctoral Studies for acceptance, a thesis entitled:

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Abstract

With impending global warming predictions, surviving animals will need to either escape increasing temperatures in their current biogeography, acclimate or adapt. As temperature increases above the optimal range of an animal, physiological performance is negatively affected, which results in decreased growth, feeding, reproduction and aerobic scope. My thesis studied a hatchery-raised strain of rainbow trout (Oncorhynchus mykiss, H-strain) previously selected for upper thermal tolerance at the Pemberton Freshwater Research Centre (PFRC) in Australia to understand their acclimation potential. This H-strain of rainbow trout was acclimated to six experimental temperatures (15, 17, 19, 21, 23, 25°C) for over one month before performing a range of tests at each acclimation temperature that determined the optimal temperatures, or acclimation potential, for growth, digestibility (specific dynamic action; SDA), feed conversion (feed conversion ratio; FCR), aerobic performance and the response of maximum heart rate ($f_{H_{\text{max}}}$) to acute warming. Intermittent-flow respirometry was used to determine respiratory oxygen uptake for analysis of SDA, standard metabolic rate (SMR), maximum oxygen uptake ($\dot{M}O_{2\text{max}}$), excess-post exercise oxygen consumption (EPOC) and hypoxia tolerance (ILOS). Growth, feed efficiency, SDA duration and $f_{H_{\text{max}}}$ had acclimation potential up to 23°C. $\dot{M}O_{2\text{max}}$ was also maintained up to 23°C, while SMR followed a typical exponential increase; the calculated difference between $\dot{M}O_{2\text{max}}$ and SMR is absolute aerobic scope (AAS), which had an acclimation potential up to 21°C. With acute warming, the critical thermal maximum ($CT_{\text{max}}$) plateaued at an acclimation temperature of 23°C (reaching 31.2°C), and the temperature of $f_{H_{\text{max}}}$ and arrhythmia had acclimation potential up to 23°C. This integrative approach in assessing physiological performance illustrates that this warm-tolerant strain of rainbow trout has a broad thermal range for performance and a large-scale consideration
of the thermal tolerance of other strains of rainbow trout is warranted given that rainbow trout are typically considered a cold-water species.
Lay Summary

Global warming is threatening many animal species, including fishes. When fish experience elevated temperature, many physiological functions become weakened including growth, movement and reproduction. My thesis studies a strain of rainbow trout, considered a cold-adapted species, that survived extreme summer temperatures after being introduced to Western Australia’s hot summer climate in the early 1900s. The offspring of these survivors are currently used to stock lakes and rivers for recreational fishing. By studying the energetic capacities of this strain of rainbow trout, I determined that these rainbow trout perform better at high temperatures than expected based on previous research with other strains of North American rainbow trout when acclimated to warm temperatures. My research shows that rainbow trout are able to acclimate to warmer temperatures, which is informative for the coming years as we deal with rising temperatures on this planet.
Preface

This thesis is a collaboration of work through the University of British Columbia, Department of Primary Industries and Regional Development (Western Australia, Australia) and the University of Western Australia (Australia). Experiments and procedures were approved by the Animal Care Committee of the University of British Columbia in accordance with the Canadian Council on Animal Care (A18-0340).

Chapters 2 and 3 are being drafted into a manuscript for publication. Dr. Anthony Farrell conceived the experiments, and he and I designed the procedures and experiments. I performed the experiments and analyzed the data. Terry Cabassi, Dr. Craig Lawrence and Dr. Mike Snow organized fish breeding and rearing.
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<th>Definition</th>
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<tr>
<td>AAS</td>
<td>Absolute aerobic scope</td>
</tr>
<tr>
<td>CT&lt;sub&gt;max&lt;/sub&gt;</td>
<td>Critical thermal maximum</td>
</tr>
<tr>
<td>ECG</td>
<td>Electrocardiogram</td>
</tr>
<tr>
<td>EPA</td>
<td>Environmental Protection Agency (United States)</td>
</tr>
<tr>
<td>EPOC</td>
<td>Excess post-exercise oxygen consumption</td>
</tr>
<tr>
<td>FAS</td>
<td>Factorial aerobic scope</td>
</tr>
<tr>
<td>f&lt;sub&gt;Hi&lt;/sub&gt;</td>
<td>Heart rate</td>
</tr>
<tr>
<td>f&lt;sub&gt;Hi&lt;/sub&gt;&lt;sup&gt;max&lt;/sup&gt;</td>
<td>Maximum heart rate</td>
</tr>
<tr>
<td>ILOS</td>
<td>Incipient lethal oxygen saturation</td>
</tr>
<tr>
<td>M&lt;sub&gt;O&lt;/sub&gt;&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Oxygen uptake</td>
</tr>
<tr>
<td>M&lt;sub&gt;O2&lt;/sub&gt;&lt;sup&gt;max&lt;/sup&gt;</td>
<td>Maximum oxygen uptake</td>
</tr>
<tr>
<td>MS222</td>
<td>Tricaine methanesulfonate</td>
</tr>
<tr>
<td>NaHCO&lt;sub&gt;3&lt;/sub&gt;</td>
<td>Sodium bicarbonate</td>
</tr>
<tr>
<td>O&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Oxygen</td>
</tr>
<tr>
<td>OCLTT</td>
<td>Oxygen- and capacity- limited thermal tolerance</td>
</tr>
<tr>
<td>Q</td>
<td>Cardiac output</td>
</tr>
<tr>
<td>P&lt;sub&gt;crit&lt;/sub&gt;</td>
<td>Critical oxygen tension</td>
</tr>
<tr>
<td>PFRC</td>
<td>Pemberton Freshwater Research Centre</td>
</tr>
<tr>
<td>PO&lt;sub&gt;2&lt;/sub&gt;</td>
<td>Partial pressure of oxygen</td>
</tr>
<tr>
<td>RMR</td>
<td>Routine metabolic rate</td>
</tr>
<tr>
<td>SDA</td>
<td>Specific dynamic action</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
</tr>
<tr>
<td>--------------</td>
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</tr>
<tr>
<td>$SDA_{dur}$</td>
<td>Duration of specific dynamic action</td>
</tr>
<tr>
<td>$SDA_{peak}$</td>
<td>Peak oxygen uptake value of specific dynamic action</td>
</tr>
<tr>
<td>sem</td>
<td>Standard error of the mean</td>
</tr>
<tr>
<td>SGR</td>
<td>Specific growth rate</td>
</tr>
<tr>
<td>SMR</td>
<td>Standard metabolic rate</td>
</tr>
<tr>
<td>$T_{arr}$</td>
<td>Temperature at which arrhythmia occurs</td>
</tr>
<tr>
<td>$T_{opt}$</td>
<td>Optimum temperature</td>
</tr>
<tr>
<td>$T_{peak}$</td>
<td>Temperature of peak maximum heart rate</td>
</tr>
<tr>
<td>7DADDM</td>
<td>7-day average daily water temperature maximum</td>
</tr>
<tr>
<td>% sat</td>
<td>Dissolved air saturation</td>
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</table>
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A sincere thank you goes to my supervisor, Dr. Tony Farrell, who has guided me throughout this process since the beginning. Tony trusted me to go to another country to run my own research project, giving me the opportunity to collaborate and go on an adventure I will never forget. His welcoming attitude for discussion has allowed me to feel confident in myself as a young scientist in this world. I will be forever thankful for the support and patience he has given me.

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Chapter 1: Introduction

1.1 Geographic range and temperature preferences of Rainbow trout

Rainbow trout (Oncorhynchus mykiss) have a native range along the Pacific coast and inland streams of North America and Asia. They are found in both coastal waters and streams along the Pacific Ocean and inland of the west coast of North America. Rainbow trout are members of the Salmonidae family, which diverged from ray-finned fishes 59 million years ago (Lecaudey et al., 2018). Another 30 million years later Salmo and Oncorhynchus split from each other and evolved in the Atlantic and Pacific Ocean, respectively (Lecaudey et al., 2018). About 2 million years ago a division of Oncorhynchus lines between the cutthroat (O. clarkii) and rainbow trout (O. mykiss) occurred, producing the current species of rainbow trout (Behnke, 2002). While the cutthroat trout occupied the areas surrounding the Columbia River, rainbow trout occupied more southern areas surrounding the Gulf of California. During a 50,000-year span, multiple ice ages occurred throughout North America, creating many shifts in watersheds and topography. Following the receding of glaciers of the last glacial era about 10,000 years ago, rainbow trout spread north and south as a native species in Canada, Mexico and the US (Behnke, 2002).

Following glaciation and the initial divergence in the Oncorhynchus line to produce rainbow trout, there have been many divergences into multiple rainbow trout subspecies associated with isolation along the Pacific coast (Behnke, 2010). Behnke (2010) classified rainbow trout into six subspecies based on colours and geographic distribution. Rainbow trout that first entered inland Columbia river systems are known as redband rainbow trout (O. mykiss gairdneri), while coastal rainbow trout (O. mykiss irideus) spread further north and later followed the redbands to inland river systems. The term redband encompasses trout that are
native to particular regions throughout North America. Other non-anadromous forms of rainbow trout include the redband trout of Northern Great Badin (*O. mykiss newberrii*), Sacramento redband trout (*O. mykiss stonei*), Kern river basin trout (*O. mykiss gilberti* and *O. mykiss aguabonita*), and Kamchatkan rainbow trout (*O. mykiss mykiss*) (Behnke, 1992). While debate continues over what is considered a subspecies of rainbow trout, such as the golden trout and rainbow-like trout in Mexico (Behnke, 2002, 2010), it is well known that there are many strains of rainbow trout that inhabit a wide biogeographic range along the Pacific Rim of North America.

While rainbow trout have differentiated as they invaded new river systems, certain subspecies differentiate in their life cycle and morphology, such as for the coastal rainbow trout (*O. mykiss irideus*). One life cycle involves rainbow trout remaining in fresh water for the entirety of its life (non-anadromous), remaining close to its spawning grounds. The other life cycle involves rainbow trout migrating to the Pacific Ocean following the first few years of its life, only for the rainbow trout to return a year or two later to spawn; this anadromous rainbow trout is also known as steelhead trout (Behnke, 2002). This life cycle of anadromous rainbow trout is similar to other Pacific salmon of *Oncorhynchus* genus, although some steelhead are iteroparous, meaning they are able to spawn more than once in their lifetime, although this is not common (<10%, Behnke, 2002). While steelhead and coastal rainbow trout have distinct life patterns, they are the same species. Previous studies have shown that they originated from one river, but diverged following the glaciation period (Behnke, 2002; Taylor et al., 2007).

In current day North America, rainbow trout are typically found west of the continental divide (Behnke, 1992). Many subspecies of rainbow trout are both morphologically distinguishable and have distinct populations such as the inland redband trout, coming from river
drainages such as the Sacramento, Columbia and Fraser watersheds, or the coastal rainbow trout. Another subspecies, the Athabasca rainbow trout population, has distinct characteristics such as a dark brassy colour and large spots (Behnke, 1992; Carl et al., 1994). Athabasca rainbow trout possess a different geographic separation from the other two North American *O. mykiss* (*gairdineri* and *irideus*) populations and is uniquely adapted to colder water and is geographically separated by the continental divide (Behnke, 1992).

Rainbow trout generally prefer water temperatures below 20°C and are therefore considered a cold-water species. Optimum temperature of *O. mykiss* is 15-18.6°C for growth and is 13-16°C for feeding (Hokanson et al., 1977; Behnke, 2002). Steelhead undergo smoltification best at >13°C (Hoar, 1988). Adult migration of steelhead is typically blocked if temperature is >21°C (Coutant, 1977; Stabler, 1981). Spawning temperatures range from 2-15.5°C depending on sub-species and location, while egg incubation has a narrower optimal range of 5.5-12°C (Raleigh et al., 1984). The acute upper critical temperature (*CT*max) is typically above 24°C (Richter & Kolmes, 2005), but varies with acclimation temperature (Chen et al., 2015) and sub-species. With chronic temperature exposure, a growth study showed that temperatures above 24°C (tested up to 27°C) were lethal to rainbow trout even after acclimation to 22°C (Hokanson et al., 1977). Based on the growth study by Hokanson et al. (1977), the US Environmental Protection Agency (US Environmental Agency, 2003) implemented 18°C as the 7-day average daily water temperature maximum (7DADM) as a water temperature regulation for managed strains of rainbow trout in the Pacific Northwest. However, this EPA report summarized experiments on the thermal requirements for rainbow trout performed before 2000 and may not fully encompass the thermal range of other strains of rainbow trout (McCullough et al., 2001).
Despite being post-glaciation invaders and natives of cool, temperate habitat in North America, the native range of rainbow trout extends from California to Alaska, which represents a large latitudinal temperature gradient. Also, rainbow trout have managed to prosper after being introduced to every continent except Antarctica, including tropical locations at high elevations in Sri Lanka (Jinadasa et al., 2005) and Kenya (Ngugi & Green, 2007). An interesting example of such introductions is the movement of eggs derived from Sonoma Creek in San Francisco Bay which were transported to New Zealand and Australia in the 1890s to many thermally variable locations (Behnke, 2002; Morrisey et al., 2002). In New Zealand, rainbow trout became established in locations such as Lake Otamangakau, where summer surface water temperatures rarely exceed 20˚C (Dedual et al., 2000), and Lake Taupo, with even lower temperatures (<10˚C) throughout much of the year (McDowall, 2006). In Tasmania, similar introductions into cool environments occurred at the Liawenee Canal where the mean migration water temperature for rainbow trout was 8.1˚C (Davies & Sloane, 1987). Following many introductions into Western Australia from the late 19th century to the 1980s, cultured rainbow trout were successfully established in culture at the Pemberton Freshwater Research Centre (PFRC) in 1931 and used for lake stocking. PFRC has since maintained a continuous line of rainbow trout despite new environmental challenges, with the most prominent being a hot climate. Due to extremely hot summer water temperatures (>25˚C) at PRFC, Molony et al. (2004) suggested a heat-tolerant strain (the H-strain) had been unintentionally selected that has less genetic diversity compared with the now extinct (at PFRC) founder population. No new fish have been introduced to PFRC since 1972. Thus, the current lineage has undergone about 60 generations, being bred every two years. This H-strain of rainbow trout has survived and has since been used as an experimental tool to study thermal adaptation and intraspecific variation. For example, this hatchery line, when
compared to a “wild” line from a self-sustaining population in a nearby reservoir, was able to survive twice as long at 27.5˚C (Molony, unpublished data; Molony, 2001). While many factors likely contributed to the survival of this species, the persistence of rainbow trout in Western Australia’s extremely hot climate suggests that this strain has been selected for high-temperature tolerance.

Up until the late 1990s, very little research had been conducted to determine how Pacific Northwest rainbow trout strains respond to conditions above their optimum temperature. In fact, many stakeholders base their water management decisions on a collection of studies completed on Northern steelhead stocks from Oregon, Washington and British Columbia (Myrick & Cech, 2004). More recent studies with different strains have indicated that rainbow trout indeed can tolerate high temperatures, among other environmental variables, with survival over 26.5˚C and a growth range of 10-22˚C (Barton, 1996). In the 1980s, Catlow Valley redband trout (Threemile Creek) were shown to have maximum performance of feeding and growth at 19˚C (the highest temperature tested; Dwyer, 1981), while all other strains tested had maximum growth and feeding at temperatures lower than 18˚C (Schroeder & Hall, 2007; Hokanson et al., 1977). Along with rainbow trout in Western Australia, other introduced strains have survived at high temperatures, furthering the interest for studying acclimation and adaptation. In Japan, the Donaldson strain of rainbow trout were selectively bred at high temperatures for over 14 generations, resulting in higher death temperatures (30.9˚C) at 20˚C acclimation than the non-thermally selected strain (30.2˚C; Ineno et al., 2005). The higher temperatures experienced by rainbow trout introduced in different geographic locations indicate that this species has a thermal tolerance much greater than the thermal preference of the Pacific Northwest strains. Not only is
this indicative of intraspecific variation, but these high temperatures are of interest to study phenotypic plasticity and generational adaptation to temperature in rainbow trout.

1.2 Thermal ranges of freshwater fishes

Environmental conditions can result in a selective pressure for animals to either disperse, adapt, or acclimate to avoid extinction (Chevin et al., 2010; Hoffmann & Sgrò, 2011). Some animals are able to genetically adapt to rapid temperature changes over a few generations, while others can increase phenotypic plasticity to perform physiological functions over a larger thermal range. As fish are ectotherms, they match their body temperature to the surrounding water environment (Fry, 1947). While food availability, oxygenation, freezing and other factors make up a habitat, the ectothermic nature of fishes is what makes temperature a vital factor in the distribution and abundance of different species’ habitats. Thus, the thermal range of all fishes is bound by minimum and maximum temperature zones where fish can resist for a short period of time before succumbing to upper and lower critical temperatures. Within this temperature zone, also called an optimal thermal window, fish will have adapted to and are able to deal with associated costs for physiological performance through thermal tolerance (Hofmann & Todgham, 2010). Thus, to understand how an animal will respond to increasing temperature in the future, it is necessary to understand its range of thermal tolerance, optimal temperature for physiological performances within this tolerance range, and the tipping point temperature at which performance begins to fail.

A fish can respond to warming water in three ways that reflect different time scales and consequences: immediately relocate to cooler water (if it is available), acclimate to the warmer temperature over several weeks, or adapt at the population level over several generations
(Habary et al., 2017). This repertoire of options likely reflects the plasticity of a fish and the environmental factors they face. Consequently, juvenile steelhead from Mendocino county move away from sections of Big Sulfur Creek long periods during the summer when the creek reaches a temperature $>26^\circ\text{C}$ (Kubicek & Price, 1976). However, ectotherms can also change their physiology through thermal acclimation to compensate for the predicted increase in water temperatures in oceans and lakes with climate change (Stachowicz et al., 2002.; Hondzo & Stefan, 1993; Austin & Colman, 2007, Guderley, 1990). For example, warm acclimation in rainbow trout can increase their upper critical lethal temperature, indicating thermal compensatory mechanisms are in play (Currie et al., 1998, Brett, 1956). Ideally, thermal compensation would keep physiological rates constant across a thermal range, meaning that plasticity could maintain the same fitness across the temperature range (Wilson & Franklin, 2002). In reality, such phenotypic plasticity can have an energetic cost that can be constitutive, such as a reduction in fitness to maintain physiological machinery, or induced, which is a reduction in performance due to a constraint on energy (DeWitt et al., 1998). As such, plasticity rarely produces perfect compensation.

Many factors determine a fish’s fitness, especially since ecosystems are never static. Temperature, for example can drive ongoing responses to food availability, migration, population divergences or shifts in geographic location (Perry et al., 2005). These ecosystem level changes can have direct impacts on the physiology of individuals, which is the level at which Darwinian fitness acts (Pough, 1989). As adaptation requires the selection of beneficial alleles within a population, the expectation is that various physiological activities will have different temperature optima, while the ultimate thermal range must be no greater than the upper and lower critical temperature limits of an ectothermic fish (Beitinger & Lutterschmidt, 2011).
Consequently, characterizing the physiology of a fish helps inform us of how rainbow trout will perform at high temperatures and how this will change with phenotypic plasticity. A plastic response in a fish can shift performance along the thermal range to create a new optimum, which is why phenotypic plasticity is an essential component that will allow for species to survive rapidly changing environmental conditions. Sticklebacks show a heritable selection for cold tolerance (Barrett et al., 2011); coral reef fish have different thermal plasticity capacities (Veilleux et al., 2018). Redband trout (*O. mykiss gairdneri*) have been shown to adjust their physiological tolerance to temperatures of 29°C (Rodnick et al., 2004). These changes in physiological performance indicate the impact of temperature on phenotypic plasticity of fishes.

Intraspecific variation in the capacity to acclimate can indicate how species respond over time to thermal adaptation or other environmental stressors. For example, common killifish (*Fundulus heteroclitus*) have a distribution along the North American Atlantic coast and show variation in whole-organism thermal tolerance between northern and southern killifish (Fangue et al., 2006). Similarly, high- and low-altitude tropical killifish (genus *Aphyosemion*) have a divergent physiological response to temperature (McKenzie et al., 2013). Within the *Oncorhynchus* genus, adult sockeye salmon (*O. nerka*) have shown a local adaptation to the different river temperatures they experience during their migrations (Eliason et al., 2011). Two subspecies of redband trout, *O. mykiss gairdneri* and *O. mykiss newberrii*, show intraspecific variation in swimming ability (Rodnick et al., 2004). Likewise, two Californian strains of juvenile *O. mykiss* differ in their thermal performance curves despite having similar CTmax values (Myrick & Cech, 2000, 2004). Another Californian population of *O. mykiss* can maintain 95% of their aerobic scope up to 24.6°C, indicating a high thermal tolerance in comparison to their

To determine the thermal optimum, a fish’s physiological performance must be tested at different acclimation temperatures (Healy & Schulte, 2012) and test temperatures (Eliason et al., 2011; Chen et al., 2015; Clark et al., 2011). The former is useful when examining plasticity and the impacts of climate change because it identifies the limits of compensation for different performance indices, which can also be defined as the tipping point for optimal thermal ranges. As acclimation is associated with tradeoffs, high resolution is necessary to determine such tipping points, which requires narrow increments in acclimation temperature. By increasing the measured temperature points in a range, I hope to increase the resolution in determining the acclimation potential of rainbow trout and at what acclimation temperature do the costs apparently outweigh the benefits, leading to a decline in performance. I will use thermal acclimation to test and understand the performance of rainbow trout across an upper range of temperatures typically found at the PFRC.

1.3 Oxygen delivery capacity

Thermal performance curves showing a fitness tradeoff across a thermal range have been demonstrated by measuring the capacity for oxygen (O₂) delivery (Fry, 1947). A fish has a minimum O₂ requirement and a capacity to increase O₂ delivery to tissues beyond this. A Fry Curve indicates this capacity, the scope for activity, as a function of temperature (Farrell, 2009; Fry, 1947) and can identify optimal thermal ranges. The oxygen- and capacity-limited thermal tolerance (OCLTT) framework, developed over the past two decades, advanced this concept by hypothesizing that thermal tipping points reflect constraints in O₂ delivery to tissues (Pörtner,
An inability to keep up with a thermodynamically-driven increase in O$_2$ demand with temperature then will cause a decline in performance of key functions such as growth and locomotion at higher temperatures (Pörtner et al., 2017). The OCLTT hypothesis suggests that O$_2$ limitation at supra-optimal temperatures decreases aerobic scope (Pörtner, 2002; Pörtner & Farrell, 2008), thereby limiting growth, reproduction and locomotion at high temperatures (Fry, 1947; Brett, 1971; Pörtner & Knust, 2007; Wang & Overgaard, 2007). While there are limitations to this framework, as aerobic scope changes during development and among species and cannot be generalized (Clark et al., 2013), experimental quality control and interpretations within species and life cycles permit the use of this hypothesis to better understand how rainbow trout perform at warm acclimation temperatures. Ultimately, temperature limits physiological mechanisms through O$_2$ uptake and therefore thermal tolerance can limit the suitability of a fish’s environment.

1.4 Upper thermal tolerance and aerobic scope

Within a zone of thermal tolerance, an animal has an aerobic scope: the excess aerobic energy available above the energy needed for maintenance (Pörtner et al., 2017). Indices of the aerobic power budget are absolute aerobic scope (AAS) and factorial aerobic scope (FAS), which are calculated as the difference and ratio, respectively, between maximum metabolic rate (MMR, or maximum O$_2$ uptake rate, $\dot{M}$O$_{2\text{max}}$, as used throughout this thesis) and standard metabolic rate (SMR). Temperature directly influences the aerobic power budget. If temperature limits the aerobic power budget, tasks such as digestion and swimming, which are energy expenditures above SMR, must also become limited (Fry, 1947). SMR (the baseline energy
requirement for an animal without feeding, reproducing while remaining in a steady state) and MMR (the maximum energy expenditure) vary with temperature independently of each other and can both be estimated using respirometry. By measuring SMR and $\dot{M}O_2^{\text{max}}$, a Fry curve can be generated as a function of temperature to represent the scope for the physiological function of a fish. The thermal range for aerobic activity then is bounded by upper and lower critical temperatures ($T_{\text{crit}}$) where aerobic scope is zero (Fry, 1947). Fry observed that $\dot{M}O_2^{\text{max}}$ increased with increasing temperature until reaching an optimal temperature ($T_{\text{opt}}$) at which point $\dot{M}O_2^{\text{max}}$ fails to increase further (and may even decrease), while $\dot{M}O_2^{\text{min}}$ continued increasing exponentially with increasing temperature until the fish reached a lethal temperature (Fry, 1947). While Fry curves are species-specific and can vary depending on hypoxia, life stage or behavior, they indicate the optimal thermal range, which can be used as an important index in understanding resilience to climate change.

Fish digest food in order to obtain the energy required for metabolism and must do so within the aerobic power budget. Digestion encompasses ingestion, absorption and assimilation of a meal and the metabolic costs associated with digestion are encompassed by specific dynamic action (SDA, Jobling, 1995). While the concepts of SDA and bioenergetics can be first dated back to the late 1700s with the French chemist Antoine-Laurent Lavoisier, studies of SDA in fish were not conducted until the 1970s, when there was increasing interest in commercial and aquaculture fields to determine the ideal meal composition and size for fish (Secor, 2009). SDA is measured as the $\dot{M}O_2$ increase above SMR following feeding. Ideally, a smaller SDA for a given amount of food intake represents a higher efficiency for digestion. Many factors can impact SDA in fish such as meal size, composition and intake and fish have demonstrated physiological adaptations to minimize SDA (Fu et al., 2005a; Fu et al., 2005c; Fu et al 2005b),
swimming speed (Beamish, 1974) and fish density (Beamish & MacMahon, 1988). Following feeding, the body will increase $\dot{M}O_2$ to supply the energy required for digestion and biochemical processing of the meal (Secor, 2009). In fact, some fish species have been shown to have higher $\dot{M}O_{2\text{max}}$ levels following feeding than during swimming challenges (Fu et al., 2009), suggesting that using swimming to obtain $\dot{M}O_{2\text{max}}$ for a Fry curve in these species would be the wrong approach. Regardless, post-prandial $\dot{M}O_2$ must remain within the aerobic power budget.

Temperature directly affects SDA as well (Wang et al., 2002). For example, increasing temperature increases the peak in $\dot{M}O_2$ during SDA, but reduces the duration of SDA. Increasing temperature reduces $\dot{M}O_2$ and aerobic capacity, thereby creating a pressure to reduce SDA. It is of interest to determine at which temperature digestion is optimized and if it is limited by aerobic scope.

Growth also depends on the available aerobic scope for a fish. For some species, such as salmonids, growth has been shown to have a similar bell-shaped curve to the Fry curve for aerobic scope (Brett, 1971). In rainbow trout the optimal thermal range for aerobic scope (16.5-20.5°C, Anttila et al., 2013) is similar to that for growth rate (17-19°C, Hokanson et al., 1977). Growth, however, can be restricted at high acclimation temperatures even when a high aerobic scope exists, indicating that oxygen transport capacity does not necessarily limit growth (Healy & Schulte, 2012). Starvation, or a lack of energy intake, is a stressor for animals that can be based on food availability, seasonal or competitive factors and can result in variation in individual growth (Persson & Roos, 2006; Wang et al., 2002, Jobling, 2002). Food deprivation followed by a reintroduction of food can result in compensatory growth and can generate two phenotypic responses: phenotypes with high growth (they grow as quickly as possible to the detriment to other physiological characteristics), and phenotypes with high tolerance of feed
intake (they lower energy expenditure during deprivation) (Dupont-Prinet et al., 2010).

Regardless, increasing temperature increases SMR and unless $\dot{MO}_2_{\text{max}}$ increases proportionately (or processes become more efficient) the AAS available will decrease. Such decreases in AAS are important because temperature increases the peak in $\dot{MO}_2$ during SDA post-prandial peaks when digesting a larger meal (Norin & Clark, 2017). Therefore, peak SDA increasingly dominates AAS aerobic scope with increasing temperature. In this regard, comparing the ratio of peak SDA to SMR with FAS becomes particularly informative. Rainbow trout will be expected to have restricted growth when exposed to higher temperatures but understanding feeding mechanisms and energy expenditure will be important to build a full story of thermal potential.

1.4.1 **Beyond aerobic scope: anaerobic metabolism**

Environmental stressors that have synergistic interactions, where two stressors together are more than the individual stressors separately, can be hazardous for fish with rapid environmental change (McBryan et al., 2013). The OCLTT hypothesis of O$2$ limitation can suggest that temperature and hypoxia are linked as synergistic stressors, which inflates the need for fish to adapt or acclimate in these environmental conditions (Pörtner, 2001; Pörtner & Farrell, 2008). If fish are unable to partition $\dot{MO}_2$ or as aerobic scope is used up, ATP will be produced anaerobically without the use of O$2$ in a time-limited process. In fact, fish who use the majority of their aerobic scope may be restricted in growth and distribution due to hypoxic limitations (Claireaux et al., 2000; Claireaux & Chabot, 2016). In this thesis, a hypoxia challenge is used to test the tolerance of rainbow trout as a function of acclimation temperature.
1.5 Thermal performance with acute warming

During acute warming, fish are only able to survive at upper and lower critical temperatures for a short period of time, as they have reached the limits of their zone of resistance. Using the critical thermal maximum (CT\text{max}) methodology is a measurement for short-term survival that quantifies the temperature at which a fish becomes disoriented, thus their zone of resistance (Beitinger et al., 2000). At CT\text{max} life is limited and fish are unable to escape predators or perform locomotor functions. CT\text{max} varies interspecifically and intraspecifically among populations (Fangue et al., 2006). I measured CT\text{max} in rainbow trout as a function of acclimation temperature as a useful comparative indicator of their thermal limit to acute warming; it was not used as an indicator of physiological performance. Instead, I used the failure of a crucial part of the oxygen delivery system, maximally stimulated heart rate (f_{H\text{max}}), to assess the physiological consequences of acute warming.

The failure of the O\textsubscript{2} delivery system at extreme temperatures can be explored through the use of heart rate (f_{H}). In animals, understanding the link between O\textsubscript{2} uptake and cardiac function can be described through the Fick equation:

\[ \dot{M}_{O_2} = f_H \times V_s \times ([C_aO_2] - [C_vO_2]) \]

Where $\dot{M}_{O_2}$ is the rate of O\textsubscript{2} uptake (mg min\textsuperscript{-1}), $f_H$ is heart rate (beats min\textsuperscript{-1}), $V_s$ is stroke volume (ml beat\textsuperscript{-1}), $C_aO_2$ is arterial oxygen content (mg O\textsubscript{2} ml\textsuperscript{-1}) and $C_vO_2$ is venous oxygen content (mg O\textsubscript{2} ml\textsuperscript{-1}). As $Q$ and $f_H$ increase with increasing temperature, stroke volume is shown to stay the same or decrease (Gollock et al., 2006; Axelsson et al., 1992). The difference between $C_aO_2$ – $C_vO_2$ either stays the same, or it increases due to a decrease in $C_vO_2$ from increased tissue extraction (Sartoris et al., 2003) or an increase in $C_aO_2$ from increased gill ventilation (Sandblom & Axelsson, 2007). If there is a greater increase in $f_{H\text{rest}}$ than $f_{H\text{max}}$ during acute warming, then a
decrease in $f_{\text{Hoscope}}$ occurs (Farrell et al., 2009). With acute warming, an increase in $f_{\text{H}}$ and a decrease in $C_vO_2$ are the primary drivers to increase $\dot{M}O_2$. However, neither variable can change indefinitely: heart rate must reach a maximum and $C_vO_2$ cannot be lower than 0. Thus, circulatory oxygen delivery is a candidate for causing an oxygen deficiency in heat stress. I focused on one of these variables: maximum heart rate $f_{\text{Hmax}}$.

During acute warming, $f_{\text{H}}$ reaches a peak as the fish approaches the upper thermal limit then declines and exhibits arrhythmia (Anttila et al., 2014; Casselman et al., 2012; Eliason et al., 2011; Eliason & Farrell, 2014). Indeed, at supra-optimal temperatures, swimming sockeye salmon decrease rather than increase heart rate. The inability of $f_{\text{H}}$ to increase at this upper thermal limit is a constraint for $O_2$ delivery (Fry, 1947; Eliason et al., 2011; Steinhausen et al., 2008). This decline in performance is likely due to a protective autonomic response as rainbow trout exhibit a cholinergic mediated vagal depression at high temperatures (Ekström et al., 2016; Eliason & Anttila, 2017). This cholinergic depression of $f_{\text{H}}$ at high temperatures allows for the heart to recover while also decreasing cardiac oxygen demands (Eliason & Anttila, 2017). Within this autonomic response in teleost hearts, there is a combined $\beta$-adrenergic stimulation and cholinergic inhibition for regulation of its spontaneous depolarization (Sandblom & Axelsson, 2011). In other words, this combined regulatory effect allows for cardiac protection at high temperatures and increases acute heat tolerance (Gilbert et al., 2019). As acute temperature increases typically have a positive chronotropic (increased heart rate) and negative inotropic (decreased force) effect on the myocardium, the heart is unable to keep up with oxygen delivery to tissues at upper limits (Steinhausen et al., 2008). This negative effect of acute warming is seen as a decline in $f_{\text{Hmax}}$ after reaching peak $f_{\text{Hmax}}$, indicating that $O_2$ levels are insufficient for maintenance of cardiac demand at high temperatures and heart rate cannot continue to increase
(Franklin et al., 2007). In fact, temperature of $f_{H\text{max}}$ ($T_{\text{peak}}$) typically occurs at temperatures below $C_{T\text{max}}$ in rainbow trout (Chen et al., 2015, 2018; Gilbert et al., 2019). As rainbow trout use cholinergic and adrenergic regulation for cardiac protection, I blocked muscarinic cholinergic tone with atropine and stimulated beta-adrenergic antagonists with isoproterenol to understand when this cardiac limitation occurs.

The limits of energetics in the heart are shown through insufficient blood circulation at extreme temperatures, as shown by the OCLTT hypothesis (Farrell, 1997; Pörtner, 2002; Pörtner et al., 2017). Acute warming invariably increases heart rate ($f_{H}$) in fish and this physiological response is the primary mechanism to increase cardiac output (Steinhausen et al., 2008). However, $f_{H}$ has an upper limit at a given temperature, which has led some to suggest that $f_{H}$ limits aerobic scope, and thus, upper thermal tolerance (Steinhausen et al., 2008; Eliason et al., 2011). Put simply, once maximum heart rate ($f_{H\text{max}}$) is reached at a given temperature, then there is no more scope to increase cardiac output and deliver more $O_2$ to tissues becomes greatly diminished (Fry, 1947; Eliason & Anttila, 2017; Steinhausen et al., 2008). Acute thermal performance curves for $f_{H\text{max}}$ have been generated for various fish species, including salmonids (Verhille et al., 2016; Anttila et al., 2014). Thus, by generating acute thermal performance curves for $f_{H\text{max}}$ at different acclimation temperatures, I can determine whether or not acclimation affects the point at which tissue oxygen delivery becomes limiting during acute warming.

1.6 Thesis objectives

The objective of my thesis was to determine the thermal acclimation potential of the H-strain of *Oncorhynchus mykiss* to elevated summer temperatures. I quantified physiological performance by measuring growth, digestive capacities, SMR, $\dot{M}O_2\text{max}$ and post-prandial $\dot{M}O_2$ as
a function of six acclimation temperatures. Also, heart performance was measured during acute warming for each temperature acclimation group. CT$_{\text{max}}$ was determined to set the upper thermal limit for each acclimation temperature. My experiments are divided into two sections:

Goal 1: Physiological limits in response to thermal acclimation

Performance was measured using physiological measurements (SMR, $\dot{M}O_2_{\text{max}}$, AAS, FAS and SDA), growth and feeding to assess and characterize thermal tolerance based on acclimation potential. The hypothesis was that phenotypic plasticity and adjustments to these parameters would occur in response to thermal acclimation. I predicted that rainbow trout acclimated to higher temperatures, within limits, would have a greater aerobic scope, growth and digestive capacity compared to rainbow trout not acclimated to higher temperatures.

Goal 2: Whole body and heart response to acute warming at different acclimation temperatures

In order to assess acute thermal tolerance, I used whole animal (CT$_{\text{max}}$) and organ (f$_{\text{Hmax}}$) approaches to look at the temperatures where the circulatory system starts to collapse. The hypothesis was that there would a cardiovascular adjustment to thermal performance during acute warming in response to thermal acclimation. I predicted that rainbow trout acclimated to higher temperatures, within limits, would have a higher CT$_{\text{max}}$ and a greater f$_{\text{Hmax}}$ than those not acclimated to higher temperatures.
Chapter 2: Effect of thermal acclimation on physiological performance

2.1 Introduction

In ectothermic fish, biochemical reaction rates are strongly influenced by ambient water temperature in ectothermic fish such as rainbow trout. Temperature is a controlling factor by placing a minimum and maximum bound on the physiological functions of an organism (Fry, 1971). As fish are predicted to be displaced with the global warming (Cheung et al., 2009), the ability to withstand increasing temperatures becomes vital for survival. Fish responses to increasing water temperatures have intraspecific variation, and recent studies have highlighted the importance of this local adaptation in heat stress of coral reef fish, cardiorespiratory capacity of sockeye salmon, and respiratory capacity of redband rainbow trout (Palumbi et al., 2014; Eliason et al., 2011; Chen et al., 2018). Rainbow trout have been distributed throughout every continent, except Antarctica, and thus are an interesting study animal for intraspecific variation. Even with a temperature preference below 20˚C, rainbow trout have survived and prospered with introductions to hot climates such as Australia. The Pemberton Freshwater Research Centre (PFRC) in Pemberton, Western Australia has established a brood stock of rainbow trout, despite hot summer temperatures reaching over 26˚C. A previous study on this PFRC strain, the H-strain, has furthered the support for intraspecific variation in rainbow trout (Chen et al., 2015).

To further understand this intraspecific variation, I use the hypothesis that O2 delivery can become limited at extreme temperatures, and this limitation can lead to an increased dependence on anaerobic metabolism or lead to mortality at these upper thermal limits also known as the oxygen- and capacity-limited thermal tolerance (OCLTT; Brett, 1971; Pörtner, 2002; Pörtner & Knust, 2007). Aerobic scope, which is the available capacity to provide O2 to supply any activities beyond basic needs, is determined as the difference between the baseline
energy requirement of an animal without any activity (standard metabolic rate, SMR), and the
maximum sustainable oxygen uptake ($\dot{M}O_{2\text{max}}$, Fry, 1971), which in salmonids is typically
measured in association with exercise. In this study, aerobic scope is used to assess available
energy for physiological requirements as a limitation can lead to a trade-off in energy, such as a
decrease in digestion, growth or activity. The aerobic capacity for a fish is maximal at an
optimum temperature and declines above this as $O_2$ delivery becomes limiting (Fry, 1947;
Pörtner, 2010; Anttila et al., 2014). As thermal tolerance limits are set at the highest complexity
level, oxygen limitations define the boundaries of thermal intolerance through a limited ability to
supply oxygen for mitochondrial respiration (Pörtner et al., 2017). The oxygen limitation of
thermal tolerance is shown as a limitation in aerobic scope (Pörtner, 2002).

Growth and digestive capacities are physiological processes that require the use of $O_2$ for
continued performance in fish. Growth can be most simply defined as the difference between the
mass of food consumed and waste output (Jobling, 1995) even though in reality growth is neither
that simple or monotonic. Food consumption, for example, increases with temperature until
peaking at an optimal temperature then decreases with further increasing temperature (Jobling,
2002). Thus, a plot of food consumption vs temperature can show a similar curve to aerobic
scope. While growth is a primary indicator of feed intake, other the physiological mechanisms
related to digestion can be studied to understand why a fish is growing better or worse. Specific
dynamic action (SDA) is the energy cost associated with ingestion, digestion, etc. of absorbed
nutrients (Jobling, 1981; Beamish & Trippel, 1990) and cannot exceed the aerobic power budget.
Increased oxygen intake, gut activity and blood flow to the gastrointestinal tract occurs following
feeding (Farrell et al., 2001; Axelsson et al., 2002; Eliason et al., 2008). Fish continue to grow
with hypoxic conditions, indicating that SDA does not necessarily cease during mild hypoxic
conditions and that the gastrointestinal system is prioritized in the circulatory system (Roze et al., 2013; Axelsson et al., 2002). The increase of $\dot{M}O_2$ and energy expenditure post-meal can last from hours to days depending on feed composition, quantity consumed, etc. (Tandler & Beamish, 1979; Farrell et al., 2001; Beamish & Trippel, 1990; Eliason et al., 2008). Peak postprandial metabolic response increases with temperature due to an elevation in both SMR and SDA, while the duration for SDA decreases with temperature (Wang et al., 2002, Frisk et al., 2013; Sandblom et al., 2014).

This chapter studies the upper thermal constraint on performance of growth, digestion and aerobic scope to determine the acclimation potential of the PFRC rainbow trout. I hypothesize that phenotypic plasticity will allow these performance parameters to be adjusted with increasing acclimation temperatures. Growth and appetite were measured. Four indicators of SDA are peak postprandial $\dot{M}O_2$, time-to-peak $\dot{M}O_2$, duration of SDA and total O$_2$ used beyond SMR during SDA, all of which will be used into this study to determine acclimation potential of digestion. I measured SMR and the maximum rate of oxygen consumption ($\dot{M}O_{2\text{max}}$) to generate absolute aerobic scope (AAS) and factorial aerobic scope (FAS) as an indicator of the aerobic capacity of these rainbow trout. Finally, excess-post exercise oxygen consumption (EPOC) was measured to quantify the ability of recovery, and incipient lethal oxygen saturation (ILOS) was used to characterize hypoxia tolerance as a function of acclimation temperature.

2.2 Methods

Procedures and protocols were approved by The University of British Columbia Animal Care Committee (A18-0340). A brood stock of rainbow trout maintained for over 30 years at the Pemberton Freshwater Research Centre (PFRC, Western Australia, Australia) originated from
Sonoma Creek in the San Francisco Bay area (Morrisey et al., 2002). The fish used for this study were bred in spring 2018 from the general fish population at the hatchery (~100 parents). Fish were raised for 9 months until experiments were ready to be performed. The ponds were aerated and received water from the Big Brook Dam at seasonal temperatures that also fluctuated daily. For the study, rainbow trout (n=3000) were transferred into indoor fiberglass holding tanks (250 l, N=50-80 fish per tank) containing a biological filter to remove particulate matter with a 12:12 h light/dark cycle. Fish were held for a minimum of 4 weeks at one of six acclimation temperatures (15, 17, 19, 21, 23 or 25˚C; see Figure 1) before any experimentation (performed from February to June 2019). The growth trial was performed in triplicate for each acclimation group (N=50 in each of 18 tanks). A separate additional tank for each acclimation temperature held fish (N=80) that were used for physiological analysis (SDA, aerobic scope, $f_{H\text{max}}$ and $C_{T\text{max}}$). This way fish in the growth trial were not disturbed when fish were being removed for the physiology tests (Figure 1). Each fish was used for only one physiological test (SDA, aerobic scope or $C_{T\text{max}}$), except that 6 of the 8 fish used for the aerobic scope tests were subsequently retested at each acclimation temperature to examine the response of $f_{H\text{max}}$ to acute warming (see chapter 3). Mortality was monitored in growth and physiological trials throughout the duration of experiments.

2.2.1 Growth trial

Fish were fed three times daily to satiation with commercial freshwater trout diet (Skretting, Cambridge, Tasmania, Australia), except on the day preceding and the day of weighing, when individual fish mass and length were measured at weeks 0, 2 and 4. Growth rate was calculated as specific growth rate (SGR) with the following equation:
\[ SGR \ (\% \ body \ weight \ gain/day) = \left( \frac{\log_{10}(final \ weight) - \log_{10}(initial \ weight)}{time} \right) \times 100 \]

where the time was 28 days (4 weeks), final weight was at week 4 and initial weight was at week 0. Feeding rate was calculated the same way and presented as % feed increase per day (Table 1). Daily feed mass and fecal output were measured for each tank to calculate feed conversion ratio:

\[ FCR \ (factorial) = \frac{total \ mass \ (g)}{total \ daily \ feed \ (g)} \]

expressed as an average for triplicate tanks at each temperature acclimation (N=3).

### 2.2.2 Respirometry system

I measured oxygen uptake ($\dot{M}O_2$) of individual fish at their acclimation temperature using eight intermittent flow-through respirometers (760 mL) submerged in a 200 L reservoir with recirculating chillers and heating rods according to established protocols and procedures (Chabot et al., 2016b; Zhang et al., 2016). Water from the Big Brook Dam supplies the hatchery directly, so a UV sterilizer light was placed in the reservoir to reduce microbial growth. A circulation loop continuously mixed the respirometry chamber water and contained a fiber optic oxygen probe (Firesting O₂, PyroScience GmbH, Aachen, Germany) to measure partial pressure of O₂ levels as % of air saturation (% air sat). The optodes were calibrated using 100% air saturation (aerated water) and 0% saturation (water and sodium sulfite). Each respirometer had a computer-controlled flush pump (Compact Pump 1000, Eheim, Germany) and relays (AquaResp, University of Copenhagen, Denmark) that controlled the 10-min measurement cycle (60 s flush period, 60 s stabilization, and 480 s measurement period). The fish $\dot{M}O_2$ values were corrected for background $\dot{M}O_2$, which was measured (for 30 min) without a fish in the respirometer, both
before and immediately after every trial. The respirometry system was thoroughly disinfected with 10% bleach for 1 h and thoroughly cleaned following each trial.

2.2.2.1 Specific dynamic action

Fish (N=16 per acclimation temperature, Table 2) were individually fed to satiation in the acclimation holding tank, weighed and transferred immediately with minimal air exposure (maximum 20 s) into a respirometer at their acclimation temperature, as described above. \( \dot{M}O_2 \) measurements started within 20 min following cessation of feeding and was terminated after 48-72 h depending on when SMR for specific dynamic action was reached. Fish were euthanized (80 mg l\(^{-1}\) MS222 buffered with 160 mg l\(^{-1}\) NaHCO\(_3\)) before weighing them and dissecting to weigh stomach and gut contents. Each SDA trace was analyzed as a fitted line (following Chabot et al., 2016a) to derive SMR as the 0.2 quantile of \( \dot{M}O_2 \) recordings following 48 h (SMR), peak SDA as the maximum value of the fitted line (SDA\(_{\text{peak}}\)), duration as the time between feeding when the fish reaches SMR (SDA\(_{\text{dur}}\), h), and the magnitude of SDA as the area under the SDA curve above SMR.

2.2.2.2 Aerobic and Anaerobic Capacities

The respiratory performance of individual fish (N=16 per acclimation temperature, Table 2) was characterized at each acclimation temperature using continuous \( \dot{M}O_2 \) measurements over 72 h and following established protocols and procedures (Zhang et al., 2016, 2017). Fish, netted from the acclimation holding tank following a 24 h fast, were weighed and placed in a respirometer at their acclimation temperature, where they were chased to exhaustion for 10 min.
$\dot{M}O_2$ was calculated as the slope of the decrease in dissolved oxygen (% sat) over time for each measurement cycle (Chabot et al., 2016b) with the following formula:

$$\dot{M}O_2 = \frac{([O_2]_{t_0} - [O_2]_{t_1}) \times V}{T \times M_b}$$

where $\dot{M}O_2$ is the $O_2$ uptake rate (mg O$_2$ kg$^{-1}$ h$^{-1}$), $[O_2]$ is oxygen concentration (mg O$_2$ L$^{-1}$), $t_0$ is time zero, $t_1$ is time 1, $V$ is respirometer volume minus volume of experimental animal (L), $T$ is $t_1$-$t_0$ (h) and $M_b$ is body mass (kg). The maximum rate of oxygen uptake ($\dot{M}O_{2\text{max}}$) was taken as the peak $\dot{M}O_2$ following the chase. I used the term $\dot{M}O_{2\text{max}}$ rather than maximum metabolic rate (MMR) because I measured oxygen removal from water. Following recovery, each fish was left undisturbed for 48 h, which generated ~150 continuous $\dot{M}O_2$ values from which I estimated standard metabolic rate (SMR) by applying a quantile method (q0.2; Chabot et al., 2016b).

Absolute aerobic scope (AAS) was calculated as the difference between $\dot{M}O_{2\text{max}}$ and SMR (Fry, 1971; Claireaux et al., 2005), while factorial aerobic scope (FAS) was calculated as the quotient of $\dot{M}O_{2\text{max}}$ to SMR (Clark et al., 2005). Excess post-exercise oxygen uptake (EPOC) was measured as the integral area from the first $\dot{M}O_2$ measurement following chase until three consecutive $\dot{M}O_2$ measurements had reached SMR+10% (Zhang et al., 2016). Characterization of the respiratory phenotype was completed with a hypoxia challenge test during which nitrogen was bubbled through the water such that the air saturation of the water decreased by 0.3% min$^{-1}$ until the fish lost equilibrium. This $O_2$ level was recorded as the incipient lethal oxygen saturation (ILOS). Fish were then immediately placed in aerated water and left to recover. By following $\dot{M}O_2$ during progressive hypoxia, it was possible to determine the water saturation at which SMR could no longer be maintained by the fish ($P_{\text{crit}}$).
2.2.3 Statistical Analysis

Data analysis was conducted in R (v.3.6.2, R Core Team, Austria) and Prism (v.8, GraphPad Software, USA). All values are presented as means ± standard error with statistical significances assigned when $\alpha=0.05$, unless otherwise indicated. For the growth study, a mixed effects analysis was performed on fish mass and length and a 2-way ANOVA was performed on feeding measurements; mass and length were fit with a Gompertz growth non-linear regression with a least square fit. Differences in SMR, $\dot{M}O_{2\text{max}}$, AAS, FAS, EPOC and ILOS were tested using a one-way ANOVA with Tukey’s post hoc. Data were normalized to fish mass for SMR, $\dot{M}O_{2\text{max}}$, AAS and FAS to analyze data as mass-independent values. Regression analysis was performed between the performance parameter and acclimation temperature to determine the line of best fit (quadratic polynomial for SMR and ILOS, Gaussian function for $\dot{M}O_{2\text{max}}$, AAS and FAS).

2.3 Results

Over a 4-week period, fish mortality in the growth trial for 25°C-acclimated fish was 21% (N=3), while all other acclimation temperatures had 3.2% mortality or less (Table 1). Mortalities occurred most often following weighing, even with minimal handling and air exposure. Three mortalities also occurred during aerobic scope trials for 25°C-acclimated fish. During SDA trials, six 23°C-acclimated fish and one 21°C-acclimated fish died.

2.3.1 Growth

Fish gained mass and length at all acclimation temperatures between weeks 0 and 2 as well as between weeks 2 and 4 ($P<0.001$), but the growth rate varied with acclimation
temperature (Table 1). Growth between weeks 0 and 2 was statistically indistinguishable among the acclimation groups from 15 to 23°C, but fish were significantly smaller for the 25°C-acclimation group (Figure 2). By week 4, all other acclimation groups had more than doubled body mass and length, whereas 25°C-acclimated fish had increased body mass by only 75%. These patterns in growth were mirrored in daily feed intake per tank, with appetite being almost completely suppressed in 25°C-acclimated fish (Table 1). Feed intake at week 4 in 23°C-acclimated fish was also significantly lower than all other acclimation groups. Feed intake also increased as the fish got bigger over time, except in 25°C-acclimated fish (Figure 2). Interestingly, FCR reached a numerical nadir in 19°C- and 21°C-acclimated fish, suggesting that feed utilization was best for fish acclimated between 17°C and 23°C and worst for fish acclimated to 25°C (Figure 3).

2.3.2 Specific Dynamic Action

As expected, SMR increased with increasing acclimation temperature (Figure 4A). Peak \( \dot{M}O_2 \) during SDA increased significantly with acclimation temperature, as did net peak \( \dot{M}O_2 \) (peak \( \dot{M}O_2 \) – SMR, Figure 8), illustrating that the increase in SMR with acclimation temperature alone did not drive the increase in peak \( \dot{M}O_2 \) (Figure 4B). Duration of SDA decreased significantly with acclimation temperature, plateauing at 21°C, but increasing at a 25°C acclimation temperature (Figure 4C). The magnitude of SDA increased with increasing acclimation temperature, except for the 21°C acclimation temperature (Figure 4D).
2.3.3 Aerobic and anaerobic performance

Again, SMR increased with acclimation temperature (Figure 5A). $MO_{2\text{max}}$, however, changed very little with acclimation temperature. Indeed, the only significant difference was between the 25°C-acclimated fish and the 19°C-acclimated fish, which had the maximum numerical value of 771±16 mg O$_2$ kg$^{-1}$ h$^{-1}$. As a result, AAS changed very little with acclimation temperature except for a significantly lower AAS with the 25°C-acclimated fish (Figure 5A). Factorial aerobic scope was similar for fish acclimated to 15°C, 17°C and 19°C, but then decreased significantly at higher acclimation temperatures (Figure 5B).

The ILOS increased with acclimation temperature from 15°C to 25°C (Figure 6). EPOC increase significantly from 17°C- to 19°C-acclimated fish, but there were no other significant changes (Figure 7). There was no significant different between the SMR curves in aerobic scope and SDA experiments and thus both are used interchangeably throughout the discussion.

2.4 Discussion

This chapter examined the acclimation potential of the H-strain of PFRC rainbow trout in Western Australia to determine the acclimation temperatures at which these rainbow trout were able to maintain growth, digestion and aerobic scope. Fish maintained growth (length and mass) and appetite when acclimated up to temperatures of 23°C. However, 25°C-acclimated fish were 1.3-1.5 times less efficient in feed conversion and lost appetite, indicating that the acclimation potential for growth performance peaked in the H-strain at around 23°C. These results are consistent with previous findings in the redband strain of rainbow trout, which were able to grow up to 22°C, but declined in growth at 25°C (Myrick & Cech, 2000). Regardless, SDA$_{\text{peak}}$ increased and SDA duration increased with acclimation temperature, suggesting a capacity to
digest a full meal still existed in warm conditions. However, AAS was maintained for fish acclimated between 17 and 21˚C, but declined in 23˚C-acclimated fish, while FAS declined in 21˚C-acclimated fish. The SMR increased with acclimation temperature, as expected, and SMR in the SDA experiment was almost indistinguishable from the SMR of the non-fed fish in the aerobic scope trials, which allowed a comparison between SDA_{peak} and \( \dot{M}O_{2\text{max}} \) (discussed later). There was no significant difference in EPOC at an acclimation of 25˚C, indicating there was no increase in repayment of an oxygen debt from going into anaerobic metabolism. Hypoxia tolerance decreased with increasing acclimation, indicating a tradeoff between hypoxia and thermal tolerance.

A Fry curve illustrates the thermal dependence of a fish to increase O\(_2\) delivery to tissues beyond basic metabolic needs (Fry, 1947; Farrell, 2009). If \( \dot{M}O_{2\text{max}} \) does not increase in proportion with the increase in SMR with temperature, AAS and FAS must decrease. This is the first study to measure a Fry curve as a function of acclimation temperature for PFRC rainbow trout, although an earlier study generated a Fry curve for 15˚C-acclimated fish that were acutely warmed (Chen et al., 2015). It is possible to compare my data for 15˚C-acclimated fish (574 mg O\(_2\) kg\(^{-1}\) h\(^{-1}\)) with the earlier study as aerobic scope was similar (544 mg O\(_2\) kg\(^{-1}\) h\(^{-1}\) as modelled on the average of the three family groups presented in Chen et al., 2015). Moreover, the acclimation potential is revealed by the difference in temperature for the peak AAS, where 15˚C-acclimated fish exposed to acute warming generated a peak of 550 mg O\(_2\) kg\(^{-1}\) h\(^{-1}\) at 15.8˚C, compared 643 mg O\(_2\) kg\(^{-1}\) h\(^{-1}\) recorded from fish acclimated to 19˚C. I showed that trout acclimated to 23˚C had an AAS that was just 19% lower than that at 19˚C, whereas acute warming of 15˚C-acclimated PFRC rainbow trout to 23˚C reduced their peak AAS by 36% (Chen et al., 2015). Further, 95% of maximum AAS was maintained with an FAS of 5.1 at an
acclimation temperature 21°C, compared to 90% of AAS maintained up to 20.5°C and an FAS of 3.1 in the non-acclimated rainbow trout. This increased range in the acclimated PFRC rainbow trout indicates that thermal acclimation allowed for a higher capacity of aerobic scope in rainbow trout.

Other North American rainbow trout strains show varying aerobic scope capacities. Similar $\dot{M}O_2$ results can be seen in redband trout from multiple fluctuating river temperatures ranging from 14-21°C (Rock Creek) and 19-29°C (12-mile Creek), as $\dot{M}O_2$ (determined from maximum swimming speed at 24°C) for all fish was 723±25 mg O2 kg$^{-0.83}$ h$^{-1}$ (Rodnick et al., 2004). The $\dot{M}O_2$ in the present study at 23°C was 713±23 mg O2 kg$^{-1}$ h$^{-1}$. The AAS was 533±22 mg O2 kg$^{-0.882}$ h$^{-1}$ and 530±21 mg O2 kg$^{-1}$ h$^{-1}$ in the present study (note: Rodnick et al., 2004 used routine metabolic rate (RMR) to include minor activity during field experiments rather than SMR). Rainbow trout from the Tuolumne River (California) maintain 95% AAS over a broad thermal range (17.8 to 24.6°C; RMR was used), although FAS was lower than my experiment (2.13 at 24°C vs. 2.9 at 25°C; Verhille et al., 2016). The Rock Creek, 12-Mile Creek and Tuolumne River rainbow trout strains, along with H-strain PFRC rainbow trout, show the ability for acclimation potential to temperatures higher than the current 7DADM listing of 18°C.

Following digestion, peak metabolic rate increased above SMR from 187% for 17°C-acclimated fish to 167% for 25°C-acclimated fish, which is consistent with the suggestions that metabolic rate can double following a meal (Fu et al., 2005c; Eliason & Farrell, 2014). By comparison, FAS had a minimum of 2.9 times above SMR for 25°C-acclimated fish (Figure 8). Therefore, even the 25°C-acclimated PFRC rainbow trout had an aerobic power sufficient for peak digestion. Indeed, the Fry curve suggests that 25°C-acclimated fish had an excess oxygen delivery capacity of 204 mg O2 kg$^{-1}$ h$^{-1}$ above peak SDA. This has an important implication for
the interpretation of the feeding data. The overall feeding rate was significantly slower for 25°C-acclimated fish compared to all other acclimation groups, which significantly slowed but did not halt growth rate. While the FCR of 25°C-acclimated fish was poorer compared to all other groups, it seems that the primary reason for the poorer growth performance was that the 25°C-acclimated fish chose not to feed as indicated by a decline in average daily feed, despite having the aerobic capacity to digest a full meal. One explanation for growth occurring despite decreased feeding could be due to compensatory growth, a period where fish rapidly gain weight with refeeding following rapid weight loss (Dupont-Prinet et al., 2010). Decreased feeding could be a mechanism to deal with decreased aerobic scope. If there is a decreasing aerobic capacity reserve, these fish might make a voluntary decision to decrease feed intake in order to conserve energy for other required activities. Fish show a maximum efficiency of food use when there is a restriction, as shown with *Salmo trutta*, which will grow even with decreased food amounts (Brown, 1946b). Determining the mechanism for the relationship between appetite and growth in these upper acclimation temperatures will be important in future studies.

2.4.1 Conclusion

This chapter focused on determining the acclimation potential of the H-strain rainbow trout. I showed that temperature acclimation maintained physiological performance parameters such as aerobic scope, digestion and growth to temperatures at temperatures well above the 18°C recommended as optimum for North American rainbow trout. At a 25°C acclimation temperature, the H-strain rainbow trout clearly performed poorly compared to all other acclimation temperatures. Nevertheless, even with maximum performances occurring variably at 15°C to 23°C in these experiments, the absolute differences in performance measures in most
cases were rather small up to 23°C. Indeed, the minimum and maximum performances over the entire range of acclimation temperatures were 11% for MMR, 18% for FCR and 19% for AAS, a less than 20% difference for a thermal range of 8°C. The 25°C-acclimated rainbow trout were unable to maintain AAS, but had a longer duration for digestion, the lowest growth rate and the lowest feed intake. Thus, when a fish reaches its upper acclimation potential, instead of fully exploiting is aerobic power budget to feed and grow, it suppresses appetite to, in the present case, a near maintenance ration. This leaves more aerobic scope for other physiological activities and a FAS closer to 3, as peak SDA would otherwise consume most of FAS. The question remains now if there is another factor other than oxygen limitation that drives this behavior to not feed. In other words, what is the mechanism or why do these rainbow trout choose to not use the energetic capacity that is available to eat fully?
Table 1. Growth and feeding rates of PFRC rainbow trout. Values presented as mean ± SD, N=3. Letters indicate significant differences between acclimation groups.

<table>
<thead>
<tr>
<th>Acclimation group (˚C)</th>
<th>Growth rate (% body weight gain/day)</th>
<th>Feeding rate (% feed increase/day)</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>15</td>
<td>2.08±0.04a</td>
<td>1.42±0.04ab</td>
<td>3.2a</td>
</tr>
<tr>
<td>17</td>
<td>2.31±0.07b</td>
<td>1.5±0.3a</td>
<td>0.0a</td>
</tr>
<tr>
<td>19</td>
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<td>1.0±0.1bc</td>
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</tr>
<tr>
<td>21</td>
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<td>0.9±0.2c</td>
<td>0.0a</td>
</tr>
<tr>
<td>23</td>
<td>1.79±0.08d</td>
<td>0.66±0.04c</td>
<td>2.0a</td>
</tr>
<tr>
<td>25</td>
<td>0.81±0.07e</td>
<td>-0.61±0.03d</td>
<td>21.0b</td>
</tr>
</tbody>
</table>

Table 2. Body size of PFRC rainbow trout used in aerobic scope and specific dynamic action (SDA) experiments. Values presented as mean ± sem. Letters indicate significant differences between acclimation groups.

<table>
<thead>
<tr>
<th>Acclimation group (˚C)</th>
<th>Aerobic scope</th>
<th>SDA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>N</td>
<td>Mass (g)</td>
</tr>
<tr>
<td>15</td>
<td>16</td>
<td>18.5±0.6a</td>
</tr>
<tr>
<td>17</td>
<td>14</td>
<td>18.5±0.7a</td>
</tr>
<tr>
<td>19</td>
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<td>17.3±0.9a</td>
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<tr>
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<td>15</td>
<td>14.2±0.5b</td>
</tr>
<tr>
<td>23</td>
<td>15</td>
<td>14.1±0.4b</td>
</tr>
<tr>
<td>25</td>
<td>10</td>
<td>13.5±0.8b</td>
</tr>
</tbody>
</table>

Figure 1. Holding tank orientation for acclimation groups. Rainbow trout were held in three tanks per acclimation group for the growth experiment with randomized tanks (blue) and one
tank per acclimation group for physiological experiments (red). The number inside each tank represents the acclimation temperature.

Figure 2. Mass, length and average daily feed intake of PFRC rainbow trout.
A, Mass (g) measurements for acclimation groups. B, Length (mm) measurements for acclimation groups. C, Daily feed measured from each tank and averaged to number of fish in tank. Red circle = 25°C, pink diamond = 23°C, pink triangle = 21°C, purple triangle = 19°C, purple square = 17°C, blue circle = 15°C; Asterisk indicates significant differences of all acclimation groups between two time measurements; Letters indicates significant difference between acclimation groups at one time measurement. Presented as means ± sem, N=3 for triplicate tanks measured at week 0, 2 and 4.

Figure 3. Feed conversion ratio (FCR) of rainbow trout as a function of acclimation temperature.
Increasing FCR ratio indicates decreasing efficiency. Data points not sharing letters indicate significant differences between acclimation groups. All values are means ± sem, N=3.
Figure 4. Specific dynamic action (SDA) measurements for standard metabolic rate (SMR), peak SDA (SDA_{peak}), duration (SDA_{dur}) and magnitude of rainbow trout as a function of acclimation temperature.

A, SMR measured as oxygen uptake rate (\(\dot{MO}_2\)). B, SDA_{peak} measured as the highest \(\dot{MO}_2\) value following feeding. C, Duration of SDA, SDA_{dur}. D, Magnitude, or the area under the curve between postprandial \(\dot{MO}_2\), and SDA line. Data points not sharing letters indicate significant differences between acclimation groups. All values are means \(\pm\) sem, N=12-16.
Figure 5. Standard metabolic rate (SMR), maximum oxygen uptake (Ṁ\textsubscript{O}\textsubscript{2}\textsubscript{max}), aerobic (AAS) and factorial aerobic scope of rainbow trout as a function of acclimation temperature.

A, SMR, Ṁ\textsubscript{O}\textsubscript{2}\textsubscript{max} and AAS (difference between Ṁ\textsubscript{O}\textsubscript{2}\textsubscript{max} and SMR) of six acclimation groups (means ± SE, N=12-16). SMR (blue square) was fitted with a polynomial quadratic function and one-way ANOVA (Y=135.0 + 10.5*X + 1.35*X\textsuperscript{2}, R\textsuperscript{2}=0.67, F=31.14, P<0.0001), Ṁ\textsubscript{O}\textsubscript{2}\textsubscript{max} (red circle) and AAS (green triangle) were fit with a Gaussian distribution curve and one-way ANOVA (Y=760.2*exp(-0.5*((X-19.6)/9.89)\textsuperscript{2}), R\textsuperscript{2}=0.22, F=4.91 P=0.0006; Y=634.7*exp(-0.5*((X-18.6)/7.37)\textsuperscript{2}), R\textsuperscript{2}=0.42, F=15.40, P<0.0001).

B, FAS (green circle) was fitted with a Gaussian distribution curve and one-way ANOVA (Y=1.9*exp(-0.5*((X-2.0)/1.7)\textsuperscript{2}), R\textsuperscript{2}=0.73, F=43.41, P<0.0001). Corrected Ṁ\textsubscript{O}\textsubscript{2}\textsubscript{max} and SMR to mean body mass of acclimation group, presented per unit kg. Data points not sharing letters indicate significant differences between acclimation groups. All values are means ± sem, N=12-16.
Figure 6. Incipient lethal oxygen saturation (ILOS) of rainbow trout as a function of acclimation temperature.
Represented as loss of equilibrium (LOE) at PO$_2$ (% air sat). Curve was fit with polynomial quadratic function ($Y=15.2 + 0.55X + 0.04X^2$), $R^2=0.32$, $F=1.79$ $P<0.0001$. Data points not sharing letters indicate significant differences between acclimation groups, represented as means ± sem, N=12-16.

Figure 7. Excess-post exercise oxygen consumption (EPOC) following maximal $\dot{MO}_2$ of rainbow trout as a function of acclimation temperature.
EPOC is represented as the natural log of the area under the $\dot{MO}_2$ line and above SMR. Data points not sharing letters indicate significant differences between acclimation groups, represented as means ± sem, N=12-16.
Figure 8. Ratio of $\dot{M}O_2_{\text{max}}/\text{SMR}$ (FAS, aerobic scope experiment) and SDA$_{\text{peak}}$/SMR (SDA experiment) represented as factorials to show energetic scopes of PFRC rainbow trout as a function of acclimation temperature.

Data points not sharing letters indicate significant differences between acclimation groups, represented as means ± sem, N=12-16.
Chapter 3: Acute warming effects on maximum heart rate and critical thermal maximum

3.1 Introduction

In order to survive rapid environmental changes occurring due to global warming, animals must move to a different environment, acclimate or adapt. Temperature, an important environmental factor due to its large influence on ectotherms, can have negative consequences if too extreme for fish. An increase in water temperature decreases dissolved oxygen content in water and increases baseline energetic costs, disturbing the physiological balance of a fish by changing parameters in attempt to deal with this temperature stress (Dejours, 1975; Clarke & Johnston, 1999; Sartoris et al., 2003; Pörtner, 2001). Further, an O₂ limitation is hypothesized to characterize thermal intolerance (Pörtner, 2010). If a fish is unable to deliver sufficient O₂ to meet the needs of its tissues and organs, then to survive it must resort to anaerobic glycolysis, a time-limited process that is O₂-independent but produces ATP rapidly (Storey & Storey, 2005). With acute warming, cardiac output (Q) increases to deliver more O₂, paralleling the increase in whole animal \( \dot{M}O_2 \) and driven primarily by an increase in heart rate (\( f_H \)), (Brett, 1971; Gollock et al., 2006; Sandblom & Axelsson, 2007; Steinhausen et al., 2008; Clark et al., 2008; Ekström et al., 2016). With increasing acclimation temperature, ventricular contraction and relaxation rates become faster and ventricular mass may even decrease (Farrell et al., 1996). An increase in heart muscle mass can improve contractility and has been shown to compensate for temperature changes during cold acclimation (Graham & Farrell, 1989; Klaiman et al., 2011). Warm acclimation results in a decrease of connective tissue in the heart, indicating different heart remodeling responses for cold and warm acclimation (Klaiman et al., 2011).
The critical thermal maximum ($C_{\text{tmax}}$) is a metric used to define the upper thermal limit of an animal, and researchers use it to assess the effects of acute warming on locomotion and behavior in ectotherms (Lutterschmidt & Hutchison, 1997). A higher $C_{\text{tmax}}$ is indicative of a warm-tolerant phenotype even though $C_{\text{tmax}}$ typically occurs above the maximum daily temperature experienced a fish. In this experiment I examine variation of $C_{\text{tmax}}$ with acclimation temperature to define these limits. Moreover, I use it as a reference temperature to explore how heart performance might fail during acute warming before a fish reaches the upper limit of its thermal tolerance.

3.1.1 Acclimation influence on cardiac performance

Thermal plasticity is necessary for compensatory responses. The potential underlying mechanism of cardiac response to acclimation has indicated that there is an autonomic protection of cardiac performance in response to extreme warming (Gilbert et al., 2019). For example, an increase in cholinergic inhibition of $f_{i\text{rest}}$ and an initial increase in $f_{i\text{max}}$ is shown with warm acclimation in rainbow trout (Ekström et al., 2016). Thermal acclimation, by lowering $f_{i\text{rest}}$, can maintain scope to increase heart rate with acute warming to a certain degree. Atlantic salmon show thermal plasticity in $f_{i\text{max}}$ with long-term warm acclimation (Anttila et al., 2014). European perch (*Perca fluviatilis*, L.) show increased $f_{i\text{max}}$, not only with chronic warming, but also with acute exposure to warmer temperatures over 24 h when compared perch held at a constant temperature (Sandblom et al., 2016). With Antarctic fish (*Pagothenia borchgrevinki*), 4°C-acclimated fish have almost no scope for increases in $f_{i\text{t}}$ compared to -1°C acclimated fish, as $f_{i\text{max}}$ is similar to $f_{i\text{rest}}$ at acute upper temperatures (Franklin et al., 2007). Similarly, swimming sockeye salmon increase $f_{i\text{max}}$ with increasing temperature, but the overall scope decreases above
the optimal temperature (Eliason et al. 2013). These examples show that thermal acclimation can increase $f_{i \text{max}}$, which increases the scope for increasing heart rate. In this experiment, I examined the response $f_{i \text{max}}$ to acute warming as a function of acclimation temperature to determine the acclimation potential of heart rate relative to $C_T^{\text{max}}$. I predicted that $f_{i \text{max}}$ would increase with increasing acclimation temperature. My hypothesis was that acclimation temperature increase both $C_T^{\text{max}}$ and $f_{i \text{max}}$, and $f_{i \text{max}}$ would reset. As I did not measure $f_{i \text{rest}}$ I cannot determine $f_{i \text{scope}}$, although a decline in $f_{i \text{max}}$ would indicate that there is an inability of $O_2$ demand to meet with $\dot{M}O_2$ of a fish. By using the H-strain of PFRC rainbow trout, my goal is to determine the acclimation potential of cardiac performance and $C_T^{\text{max}}$.

3.2 Methods

Procedures and protocols were approved by the University of British Columbia Animal Care Committee (A18-0340). Animal holding and procedures were the same as Chapter 2. The H-strain of PFRC rainbow trout were used for these experiments, as previously described in Chapter 2.

3.2.1 Critical thermal maximum ($C_T^{\text{max}}$)

The PFRC rainbow trout ($N=10$, body mass in Table 3) were fasted for 24 h before being placed into the 200 mL aerated tank where $C_T^{\text{max}}$ was measured using a well-established methodology (Beitinger et al., 2000). Briefly, fish were left for 1 h at 12°C before water temperature was increased at 0.3°C min$^{-1}$ to 22°C and 0.1°C min$^{-1}$ above 22°C using two heating rods (100W Titanium Heater, Aquatop, Brea, CA, United States) As individual fish lost equilibrium, they were immediately removed and revived in a recovery bath at acclimation
temperature. The CT\textsubscript{max} was the temperature at which a fish first lost equilibrium and it is reported as the mean value for 10 individuals.

3.2.2 Heart rate (f\textsubscript{Hmax})

Measuring \( f_{H\text{max}} \) during acute warming followed the protocol developed by Casselman et al. (2012). I used 12 fish per acclimation temperature (Table 3), those that had recovered for 5 h following the aerobic performance test from Chapter 2. Briefly, fish were initially anesthetized (80 mg l\(^{-1}\) MS222 buffered with 160 mg l\(^{-1}\) NaHCO\(_3\)) and transferred to a 12°C water bath with recirculating water containing a maintenance anesthetic (65 mg l\(^{-1}\) MS222 buffered with 130 mg l\(^{-1}\) NaHCO\(_3\)) which continuously irrigated the gills. Two stainless steel electrodes, one inserted below the muscle layer near the heart and the second below the pelvic fin as a reference, were used to record an electrocardiograph (ECG) which was amplified and digitized (Animal BioAmp and a Powerlab 8/30, ADInstruments Inc., Bella Vista, NSW, Australia) for analysis using Labchart software (ADInstruments) that provided an on-line recording of heart rate (\( f_{H} \)). To obtain \( f_{H\text{max}} \), vagal tone was blocked by injecting fish intraperitoneally with atropine sulfate (1.8 mg kg\(^{-1}\)) and cardiac adrenergic β-receptors were maximally stimulated with an injection of isoproterenol (6 µg kg\(^{-1}\)) (Sigma Chemicals, Perth, Western Australia). Following a 30 min stabilization at 12°C, the water bath was heated by 1°C every 6 min which ensured that a new stable \( f_{H\text{max}} \) was generated for each temperature change. The experiment was terminated when acute warming generated a cardiac arrhythmia, after which the fish was euthanized with a lethal dose of anesthetic and ventricular mass was recorded. The temperature at which peak \( f_{H\text{max}} \) was reached (\( T_{\text{peak}} \)) and the temperature at which the first cardiac arrhythmia occurred (\( T_{\text{arr}} \)) were
determined for each fish and are reported as a mean value (N=12). For each acclimation group, the Q<sub>10</sub> of acute warming temperatures between 15 and 25°C were calculated as a ratio.

### 3.2.3 Statistics

Data analysis was conducted in R (v.3.6.2, R Core Team, Austria) and Prism (v.8, GraphPad Software, USA). All values are presented as means ± standard error with statistical significances assigned when α=0.05. Differences in CT<sub>max</sub>, T<sub>peak</sub> and T<sub>arr</sub> were also tested one-way ANOVA with Tukey’s post-hoc.

### 3.3 Results

#### 3.3.1 Upper thermal tolerance for the whole animal and for maximum heart rate during acute warming as a function of acclimation temperature

CT<sub>max</sub> increased significantly with acclimation temperature reaching a maximum of 31.1°C in the 23°C-acclimated fish and 31.2°C in the 25°C-acclimated fish (Figure 9).

Acute warming increased f<sub>Hmax</sub> in all acclimation groups until cardiac arrhythmia occurred. f<sub>Hmax</sub> at a given test temperature reset inversely by decreasing with increasing acclimation temperature. For example, f<sub>Hmax</sub> at 12°C was 80 bpm for 15°C-acclimated fish, but just 58 bpm for 25°C-acclimated fish (Figure 10). However, peak f<sub>Hmax</sub> was independent of acclimation temperature up to 23°C, ranging from 166±4 to 179±4 bpm, but in 25°C-acclimated fish f<sub>Hmax</sub> decreased significantly (151±6 bpm). Mean T<sub>arr</sub> increased by 3.4°C with acclimation temperature up to 23°C (29.1±0.5°C) but decreased significantly for 25°C-acclimated fish (27.2±0.6°C) (Figure 9). Similarly, T<sub>peak</sub> increased by 2.7°C up to 23°C (27.6±0.4°C) but decreased significantly for 25°C-acclimated fish (26.2±0.8°C) (Figure 9). Q<sub>10</sub> values for 15°C- to
25°C-acclimated fish in ascending order were: 1.70, 1.69, 1.81, 1.92, 1.93 and 1.79. No correlation existed between $f_{1\text{max}}$ and the $\dot{\text{M}}O_{2\text{max}}$ of individual rainbow trout at any temperature acclimation ($F=0.052$, $P=0.8028$).

3.4 Discussion

I assessed the effect of acclimation temperature on the response of heart rate to acute warming in PFRC rainbow trout and compared this with the effect of acclimation temperature on another acute response, $CT_{\text{max}}$. Previously determined $CT_{\text{max}}$ values for PFRC rainbow trout acclimated to 15°C ranged from 28.1-29.7°C (Chen et al., 2015), which is similar to my value for 15°C-acclimated fish (29.0±0.4°C). Another warm-tolerant strain selected to tolerate high temperatures in Japan had a similar $CT_{\text{max}}$ of 29.7°C when acclimated 15°C (Ineno et al., 2005). Redband rainbow trout experiencing daily temperatures ranging from 18-30°C also had a similar $CT_{\text{max}}$ in Oregon (29.7±0.3°C; Rodnick et al., 2004) and in Idaho (28.8-29.8°C; Chen et al., 2018).

$CT_{\text{max}}$ is however, dependent on acclimation temperature. By using 2°C increments in acclimation temperature, I could determine more precisely the temperature when acclimation potential decreased. I showed that PFRC rainbow trout reached a ceiling for $CT_{\text{max}}$ when acclimated to 23°C. $CT_{\text{max}}$ for Californian rainbow trout (Eagle Lake and Mt. Shasta strains) acclimated to 25°C was 31.8±0.1°C (Myrick & Cech, 2000), which is consistent with my ceiling for $CT_{\text{max}}$ of 31.2±0.3°C. The ability of PFRC rainbow trout to increase their upper thermal tolerance by 2.2°C over a 10°C acclimation range is indicative of their potential for surviving future temperature increases in their Western Australian habitats to which they are stocked on an annual basis.
As previously mentioned, $\dot{M}O_2$ is a product of heart rate and stroke volume (cardiac output) and tissue $O_2$ extraction. As temperature increases acutely, $O_2$ demand increases and increased cardiac output and $f_H$ are central to increasing $O_2$ delivery. Acclimation up to 23°C allowed rainbow trout to be more than 2°C warmer before their hearts first became arrhythmic ($T_{arr}$) and more than 1°C warmer before peak $f_H^{\text{max}}$ was reached ($T_{peak}$). However, both $T_{arr}$ and $T_{peak}$ declined in 25°C-acclimated fish without a change in their $C_T^{\text{max}}$. Heart arrhythmia occurred at least 2°C below the rainbow trout’s $C_T^{\text{max}}$ value, independent of acclimation temperature. The fact that $C_T^{\text{max}}$ is always several degrees Celsius higher than $T_{arr}$ means that cardiac failure precedes loss of orientation, suggesting two different mechanisms may be at play. The present work confirms previous studies in this regard (Chen et al., 2015, 2018; Gilbert et al., 2019).

In addition to an impending complete cardiac collapse at $T_{arr}$ with increasing temperature, the rate of increase in $f_H^{\text{max}}$ decreases as the fish approaches $T_{peak}$. This means that the potential contribution of increasing $f_H$ to supply more $O_2$ to tissues during acute warming must also decline before $T_{peak}$ is reached. Others have used the Arrhenius breakpoint and the instantaneous $Q_{10}$ to illustrate this point (Anttila et al., 2013; Chen et al., 2015; Ekström et al., 2017, 2016; Skeeles et al., 2020). Thus, the heart begins to falter in terms of $O_2$ delivery capacity with acute warming at a temperature below $T_{peak}$ and well below $C_T^{\text{max}}$. Further, the $Q_{10}$ values for acute warming in $f_H^{\text{max}}$ declined from 23 to 25°C-acclimated fish. Therefore, my results are in line with the OCLTT hypothesis that cardiac function may limit thermal tolerance (Farrell, 2009).
Table 3. Body size of PFRC rainbow trout used in acute heat exposure experiments.
Wet ventricle is presented as heart mass (mg) and relative ventricle mass (RVM, %). Letters represent statistically significant differences in means. All values are mean ± sem.

<table>
<thead>
<tr>
<th>Acclimation group (˚C)</th>
<th>CT&lt;sub&gt;max&lt;/sub&gt;</th>
<th>fH&lt;sub&gt;max&lt;/sub&gt;</th>
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<tr>
<td></td>
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<td>Mass (g)</td>
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<td>18.4±1.1&lt;sup&gt;a&lt;/sup&gt;</td>
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<td>25</td>
<td>9</td>
<td>23.0±1.4&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
</tbody>
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Figure 9. Critical thermal maximum (CT$_{\text{max}}$) and maximum heart rate ($f_{\text{Hmax}}$) in response to acute warming for rainbow trout as a function of acclimation temperature. A, Temperature at which each acclimation group reaches CT$_{\text{max}}$, cardiac arrhythmia temperature ($T_{\text{arr}}$), and maximum $f_{\text{Hmax}}$ ($T_{\text{peak}}$). B, Maximum $f_{\text{Hmax}}$ for each acclimation group. Letters represent statistically significant differences in means. Values are presented as means ± sem.
Figure 10. Maximum heart rate ($f_{\text{Hmax}}$) during acute warming of rainbow trout as a function of acclimation temperature. Sample size indicates decreasing numbers of rainbow trout as they reach cardiac arrhythmia and were not included in remainder of experiment. Temperatures in order of increasing acclimation group: 15°C ( ), 17°C ( ), 19°C ( ), 21°C ( ), 23°C ( ), 25°C ( ). Dotted line represents first fish reaching arrhythmia and removal from mean $f_{\text{Hmax}}$. 
Chapter 4: General Discussion

My thesis objective was to determine the acclimation potential of the H-strain of PFRC rainbow trout by studying whole-organism and organ functions. I measured growth, appetite, SDA, aerobic scope, CT\text{max} and fH\text{max}. The acclimation temperatures used were chosen from a common range of summer temperatures experienced at the PFRC hatchery. I hypothesized that rainbow trout would be able to maintain or improve physiological function up to a certain acclimation temperature, above which performance would decline. My integrative approach coupled with small increments in the acclimation temperature allowed for tipping points in the performance measures to be determined with a 2°C resolution. When fish were acclimated to 25°C, no physiological performance metric was maintained except for CT\text{max}, which increased up to 23°C acclimation but no further change with 25°C acclimation. Yet growth, appetite and feed conversion ratio (FCR) were maintained up to a 23°C acclimation temperature, while AAS was maintained up to 21°C. Likewise, Tarr and peak fH\text{max} were maximal at a 23°C acclimation temperature, indicating a cardiac limitation. These tipping points in performance should be useful in better formulating what long-term temperature thresholds exist for rainbow trout. Further, the parallels between aerobic, cardiac and growth performance curves should be further studied in regard to acclimation temperatures to understand strain specific differences in rainbow trout.

4.1 Thermal adaptation

In order to remain in a habitat with thermal fluctuations, an animal must have a thermal window that is broad enough to encompass that temperature range. Thermal acclimation may extend or move the zone of optimal performance within the limits of any animal. In this optimal
zone, fish maintain peak physiological performance required to grow, reproduce and swim because there is sufficient availability within the aerobic scope. Outside of this optimal temperature range, the metabolic scope available will decrease until it reaches zero at which point a fish will resort to anaerobic metabolism which is unsustainable. The PFRC rainbow trout clearly experience temperatures well above what is considered to be the thermal maximum of North American rainbow trout (18˚C, 7DADM; US Environmental Protection Agency, 2003) and my thesis showed they maintained optimal growth up to acclimation temperatures of 23˚C, suggesting that thermal adaptation may have occurred in PFRC rainbow trout. PFRC rainbow trout, which originally came from California, have higher thermal optima compared with other strains in the Pacific Northwest (Hokanson et al., 1977). In fact, the PFRC rainbow trout are more similar to the redband rainbow trout that also experience high thermal fluctuations (Rodnick et al., 2004). Thus, prolonged exposure to hot environments may have resulted in thermal adaptation to survive higher temperatures when compared to their Pacific Northwest counterparts. Interestingly, the mechanism for this purported adaptation occurred in different ways: the redband trout are thought to have migrated upstream to their current and now geographically isolated desert habitat, whereas domesticated PFRC rainbow trout were subjected to severe upper temperatures in a hatchery setting. If this strain is truly warm-adapted, then the various performance curves established in my thesis should be right-shifted as a function of acclimation temperature when compared with other strains of North American rainbow trout.

4.2 Partitioning of oxygen uptake

I hypothesized that at upper acclimation temperatures, physiological performances of PFRC rainbow trout would decline due to O₂ limitation in accordance with the OCLTT
hypothesis. Indeed, the H-strain had a broad thermal range peak AAS (17-23°C) that was similarly mirrored with other aerobic performances such as SDA and growth. Interestingly, the peak and decline of AAS occurred at a lower acclimation temperature (21°C) than peak $f_{Hmax}$ (23°C). This indicates that $f_{Hmax}$ is still increasing with temperature, but O$_2$ demand is increasing to the point where this increased $f_{Hmax}$ is not sufficient to deliver O$_2$ to tissues. Therefore, we see a decline in $\dot{M}O_2$ and AAS for the 21°C-acclimated fish before a decline in peak $f_{Hmax}$. As peak $f_{Hmax}$ is lower for the 25°C-acclimated fish, we can interpret that the demand for O$_2$ has exceeded the ability of $f_H$ to keep up for maintenance of $\dot{M}O_2$. Further, by comparing the Q$_{10}$ values for SMR (Q$_{10}$=1.98) over the range of acclimation temperatures for 15°C to 25°C with those for $f_{Hmax}$ during acute warming over the same temperature range, it is only the 21°C- and 23°C-acclimated rainbow trout that could match the doubling of the rate function. At the other acclimation temperatures, the Q10 for $f_{Hmax}$ was between 1.7 and 1.8.

The decrease in aerobic scope with acclimation to 25°C creates a trade-off of functions for these rainbow trout, specifically with growth. After eating food, fish must increase their $\dot{M}O_2$ as metabolic processes associated with digestion increase, yet AAS declined with 25°C-acclimation in PFRC rainbow trout. This decline corresponds to a decrease in growth rate, appetite, food conversion efficiency and peak SDA, as well as a decrease in T$_{peak}$ and T$_{arr}$ for $f_{Hmax}$. How interrelated these changes are is unclear from my study and further studies are warranted. Nonetheless, teleosts respond to seasonal food deprivation through compensatory growth, a period of more rapid growth once feeding resumes (Wang et al., 2006; Dupont-Prinet et al., 2010). The ability for the 25°C fish to choose to eat sufficiently enough to maintain weight, rather than grow, may be a short term (months rather than years) survival strategy. Eagle Lake and Mt. Shasta rainbow trout at 25°C also showed maintenance growth with decreased
feeding rates (Myrick & Cech, 2000). I showed the 25°C-acclimated fish ate less in the growth study and had a longer and more expensive digestion period than the other acclimation groups, as well as a greater peak SDA. This indicates a tradeoff between the available O$_2$ for tissues and O$_2$ required to maintain respiratory demands. Intraspecific variation of brown trout (*Salmo trutta*) shows individual variability in feeding capacity and growth where fish that ate less grew slower and exhibited lower mitochondrial respiration capacities (Salin et al., 2016). Similar to Salin et al (2016), the PFRC rainbow trout at high temperatures were unable to maintain growth despite having the ideal environmental conditions (no competition, feed *ad libitum*), and presented with decreased locomotor skills based on visual observation. This could be due to the partitioning of aerobic scope to digest enough feed to maintain growth.

The ratio of SDA$_{\text{peak}}$ to SMR (similar to FAS) following feeding indicates an $\dot{M}O_2$ requirement range of 1.5-1.9 for digestion (Figure 8). There is very little variation in this allocation of the aerobic energy budget even when compared to FAS which peaks at a ratio of 6 (17°C-acclimation) and is lowest at 2.9 (25°C-acclimation). The PFRC rainbow trout consistently used around 70% above their SMR at each acclimation temperature to digest food but had different scope remaining for growth, locomotion and any other aerobic activities based on their FAS. It would seem that supplying this fixed allocation for SDA$_{\text{peak}}$ requires an AAS that is greater than 3, otherwise appetite will be suppressed. How a fish might sense the available aerobic energy budget and partition it into different activities, including digestion, is unknown at this time.
4.3 Trade-off behavior

We know that sub- and supra-optimal temperatures reduce the energetic capacity for physiological activities (Pörtner & Knust, 2007; Pörtner & Farrell, 2008). To deal with this reduction in energetic cost, do fish reduce or eliminate certain activities to improve efficiency or further tradeoffs? These reductions in food intake combined with a low aerobic scope was unsustainable for long-term growth, fitness and survival in this experiment. This is common with fish facing extreme temperature conditions as they deal with decreased energy for maintenance, reproduction, growth and survival (Pörtner et al., 2010; Chase et al., 2018; D’Agostino et al., 2019). Indeed, when faced with supra-optimal temperatures, coral reef fish show behavioral plasticity when faced with seasonal temperature changes through a reduction in costly activities and a decrease in their diet (D’Agostino et al., 2019; Nowicki et al., 2012; Chase et al., 2018). The PFRC rainbow trout held at high temperatures also reduced their feed intake at a supra-optimal temperature. Fish size relative to other fish in a group is shown to have the biggest influence on growth of early fry (Brown, 1946a). Even when feeding fish ad libitum, there can be a social hierarchy that creates different specific growth rates of individuals (Brown, 1946a). Indeed, the larger 25°C-acclimated rainbow trout in my study would nip smaller fish who were not growing as fast. This behavioral aspect in regulating daily activities may be a method in dealing with a limited energetic scope as temperatures increase.

4.4 Conclusions

A major takeaway message from my thesis is for the management of domesticated and hatchery-produced rainbow trout. PFRC rainbow trout are an example of a strain that is likely thermally adapted and has a right-shifted optimal temperature range when compared to rainbow
trout from the Pacific Northwest. This adaptation provides acclimation plasticity and the potential to deal with impending climate change. It also indicates an interesting strain-specific variation among rainbow trout populations. More research into these different adaptations is important to understand the divergence of rainbow trout strains. The current EPA guidelines for hatchery management of North American rainbow trout indicate a 7DADM maximum of 18°C (U.S. Environmental Protection Agency, 2003). Based on this experiment and research on Californian and redband rainbow trout, a maximum temperature of 18°C is below the optimum temperature for different physiological performances (Chen et al., 2018; Rodnick et al., 2004; Verhille et al., 2016). This study further presses upon the idea that thermal tolerance defines a unique thermal niche for strain-specific temperature zones.

For the PFRC rainbow trout, temperature is one of the only extreme environmental factors they face on a day-to-day basis. This creates a limitation in comparing to wild populations of rainbow trout, as the hatchery environment eliminates many variables. As Lutterschmidt & Hutchison (1997) stated, “The tolerance of animals toward a single environmental factor such as temperature is typically greater than their tolerance of combinations of environmental factors studied simultaneously”. With no competition or predators, aside from the occasional water rat that can sneak into the hatchery, and sufficient feed, there are very few environmental factors that these rainbow trout have to contend with. While these results are useful for this hatchery setting and other hatcheries with different strains around the world, they cannot be directly related to wild rainbow trout strains experience more complex environmental conditions.

While this study only covered the upper thermal range that these trout are typically exposed to throughout the year, I showed that there is an available aerobic scope from 15 to
25°C. This availability of this scope is vital for maintenance of aerobic performances, such as growth, reproduction and locomotion. Even though the PFRC rainbow trout survivors performed at all acclimation temperatures, acclimation potential certainly decreased above 23°C. As the PFRC rainbow trout in the hatchery are only exposed to water temperatures above 23°C for a few hours during hot summer afternoons, a feed reduction strategy at these high temperatures still allows for growth maintenance without facing O2 limitations to other tissues throughout the body. These rainbow trout clearly show acclimation potential to temperatures higher than previously documented and the use of these results will allow for further study of strain-specific differences through the integration of a mechanistic study framework.
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