INTRASPECIFIC VARIATION AND PLASTICITY IN RAINBOW TROUT RESPONSES TO CLIMATE CHANGE STRESSORS

by

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B. Sc. Hons., Dalhousie University, 2017

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF

THE REQUIREMENTS FOR THE DEGREE OF

MASTER OF SCIENCE

in

THE FACULTY OF GRADUATE AND POSTDOCTORAL STUDIES

(Zoology)

THE UNIVERSITY OF BRITISH COLUMBIA

(Vancouver)

December 2020

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Intraspecific variation and plasticity in rainbow trout response to climate change stressors

submitted by Nicholas R Strowbridge in partial fulfillment of the requirements for

the degree of Master of Science

in Zoology

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Abstract

Global climate change threatens to reduce available habitat for cold-water fish such as rainbow trout (*Oncorhynchus mykiss*). Phenotypic plasticity might help individuals to cope, but may not be sufficient and survival could require adaptation in situ. Here, I assessed levels of phenotypic variation in thermal and hypoxia tolerance that could act as the substrate for adaptation. Furthermore, I also characterized the extent of thermal plasticity in these traits in multiple strains of rainbow trout. To characterize phenotypic variation, I used a common garden breeding approach with 25 family crosses in each of several strains of British Columbia rainbow trout, assessing critical thermal maximum (CT_{max}) and incipient lethal oxygen saturation (ILOS). Using California strains, I investigated the extent of thermal plasticity in CTmax, ILOS, and (in collaboration) standard and maximum metabolic rate, absolute aerobic scope (AAS), critical oxygen tension (Pcrit), and measures of cardiac performance such as maximum heart rate (f_{Hmax}) and cardiac gene expression. I found little among but large within-strain variation in CTmax and ILOS, whereas post-trial mortality clearly differentiated the strains. There was little correlation between upper thermal and hypoxia tolerance at the individual level. I observed significant plasticity in CTmax, with associated declines in AAS and f_{Hmax} and increases in the expression of stress-related genes. However, plasticity in all these measures reached a limit at a high but ecologically relevant temperature. Taken together, these findings suggest that thermal plasticity will not be sufficient to allow rainbow trout to cope with climate change, but that trout populations possess substantial phenotypic variation in climate-change relevant traits that may allow adaptation in situ. However, differences between strains were not evident for all traits, and managers will need to take a multifaceted approach when examining the effects of climate change on natural and stocked strains of rainbow trout.

Lay Summary

Human-caused climate change threatens freshwater fishes through increases in temperature and decreases in oxygen in freshwater systems. In this thesis, I investigate how well different strains of rainbow trout, a popular species for recreational fishing, may deal with these stressors. I show that rainbow trout strains differ in their mortality following exposure to high temperature, but that traditional metrics of thermal and hypoxia tolerance do not capture this difference. There is, however, substantial variation in these metrics within strains which may be important for adaptation in response to climate change. This is particularly important because I also show that ability of rainbow trout to change their sensitivity through acclimation is likely insufficient to deal with the high temperatures of climate change. My research shows that we need to tailor conservation management and stocking strategies to individual strains to ensure they survive well into the future.

Preface

This thesis represents the result of work in collaboration with the Freshwater Fisheries of British Columbia (FFSBC), the Fangue laboratory at the University of California, Davis, and the Farrell laboratory at the University of British Columbia. Both Chapters 2 and 3 are currently in-preparation for submission for publication. All experiments involving animals were done in accordance with the principles of the Canadian Council on Animal Care under the approved University of British Columbia Animal Care and Use protocol: A16-0329.

Chapter 2 of this thesis is co-authored by Nicholas Strowbridge and Patricia M. Schulte. In collaboration with the FFSBC, all data was collected by Nicholas Strowbridge and Sara Northrup (FFSBC). Data analysis was performed by Nicholas Strowbridge.

Chapter 3 of this thesis was performed in collaboration with the laboratories of Dr. Anthony Farrell (University of British Columbia) and Dr. Nann Fangue (University of California, Davis). Chapter 3 is co-authored by Matthew J. H. Gilbert, Yangfan Zhang, Jessica L. McKenzie, and Patricia Schulte. Patricia M. Schulte, Anthony P. Farrell, and Nann A. Fangue all conceived the experiment, with input from all other authors. All data was collected by Nicholas Strowbridge (Whole-animal tolerance), Matthew J. H. Gilbert (Cardiac performance), Yangfan Zhang (Metabolic rate), Jessica L. McKenzie (Gene expression), and Lais Lima (Fish sourcing and care). Data analysis was performed by Nicholas Strowbridge, Matthew J. H. Gilbert, Yangfan Zhang and Jessica L. McKenzie.

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List of Abbreviations

AAS	Absolute aerobic scope
ANOVA	Analysis of variance
BC	British Columbia
BW	Blackwater River
CA	California
CAD	Canadian dollar
CL	Carp Lake
CT_{max}	Critical thermal maximum
DO	Dissolved oxygen
EPOC	Excess post exercise oxygen consumption
FDR	False discovery rate
FFSBC	Freshwater Fisheries Society of British Columbia
f_{Hmax}	Maximum heart rate
FV	Fraser Valley
GO	Gene ontology
HCT	Hypoxia challenge test
HF	Horsefly River
HIF-1α	Hypoxia inducible factor-1α
ILOS	Incipient lethal oxygen saturation
IRAP	Integrated respiratory assessment paradigm
kPA	kilopascals
LOE	Loss of equilibrium

MMR	Maximum metabolic rate
$\dot{M}O_2$	Oxygen consumption
MS-222	Tricaine methanesulfonate
NaCl	Sodium chloride
$NaHCO_3$	Sodium carbonate
O_{2crit}	Critical oxygen tension
P_{crit}	Critical oxygen tension
PIT	Passive integrated transponder
PN	Pennask Lake
PVC	Polyvinyl chloride
RNA	Ribonucleic acid
RNAseq	Ribonucleic acid sequencing
rRNA	Ribosomal ribonucleic acid
SD	Standard deviation
SEM	Standard error of the mean
SMR	Standard metabolic rate
T_{arr}	Temperature at first cardiac arrhythmia
T_{peak}	Temperature at peak maximum heart rate

Acknowledgements

First and foremost, I want to thank my supervisor, Dr. Patricia Schulte, for taking a big chance by bringing me on as a Masters student in her laboratory. Trish has not only been a fantastic supervisor and scientific mentor but an incredibly kind person who has provided me with countless opportunities to further myself as a scientist, science communicator, professional and person. Also, I want to thank my lab mates Dr. Timothy Healy, Heather Bryant, Dr. Dillon Chung, Dr. Dave Metzger, Madison Earhart, Tessa Blanchard and Bea Rost-Komiya for their support and help with my various projects and talks. I want to specifically thank Dr. Jessica McKenzie for our many discussions and troubleshooting over the years. Additionally, I want to thank Yangfan Zhang and Dr. Matthew Gilbert for their collaboration on the California project.

I want to thank my committee of Dr. Colin Brauner and Dr. Jeffrey Richards for consistently providing support, insight, and ideas throughout the years. Furthermore, I want to thank my departmental examiner Dr. Christopher Harley for providing an outside perspective on the research I conducted over the last few years.

Importantly I want to thank the Freshwater Fisheries Society of British Columbia (FFSBC) for providing the fish, fish care, and a place to conduct my experiments for Chapter 2. In particular, I want to thank Dr. Sara Northrup at the FFSBC for her tough love, mentorship, and hard-work throughout the FFSBC project. Additionally, I want to thank Dusty Waite for his help, entertainment, and discussions throughout the trials.

Additionally, I want thank my family for supporting me throughout this journey and always pushing me to follow my dreams. Most importantly, I want to thank my partner Taylor van Mook for being by my side throughout my masters, celebrating my victories and pulling me

up when I struggle. Taylor has been my biggest supporter and has always been willing to listen when I needed to vent my frustrations or when I ramble on about my research.

Finally, thank you to our funders at Genome Canada and Genome British Columbia for the support to conduct these projects.

Chapter 1: General introduction

Freshwater habitat quality is declining rapidly due to human-mediated climate change (Eaton and Scheller, 1996; Ficke *et al.*, 2007; Comte and Olden, 2017). Both increasing temperatures and decreasing dissolved oxygen in freshwater habitats threaten to extirpate vulnerable freshwater fish populations (Sharma *et al.*, 2007, 2011). These stressors also affect where managers can successfully stock freshwater fish for recreational fishing (Cowx, 1994). To cope with these increasing temperatures and decreasing oxygen, freshwater fish must adapt, migrate, or exhibit phenotypic plasticity (Fuller *et al.*, 2010; Somero, 2010). Freshwater fish are likely limited in their ability to migrate due to the fragmented nature of freshwater habitats (Strayer and Dudgeon, 2010), thus, plasticity and adaptation are likely to be the important factors for their survival. In this introduction, I will 1) demonstrate the importance of recreational fishing, 2) lay out the effects of high temperature and hypoxia on freshwater ecosystems and freshwater fish, 3) examine the strategies within which freshwater fish may deal with climate change, 4) outline the measurements used to assess traits that may be associated with climate change resilience in fish, and finally 5) summarize the importance of rainbow trout and detail previous literature examining these impacts on rainbow trout.

In this thesis, I used rainbow trout strains from British Columbia to examine the extent of adaptive variation in upper thermal and hypoxia. My approach of common garden experimental design, large sample sizes, and consistent hatchery conditions provide a comprehensive examination of the extent of phenotypic variation in these traits. This approach allowed for a thorough investigation of within and among-strain variation in upper thermal and hypoxia tolerance across multiple strains of rainbow trout. Additionally, I used rainbow trout strains from California to examine the extent of plasticity across multiple levels of biological organization,

from whole-animal tolerance to metabolism, cardiac performance, and gene expression. I used multiple ecologically-relevant acclimation temperatures to characterize how rainbow trout may respond to the increasing temperatures of climate change.

1.1 Recreational fishing and freshwater stocking

Recreational fishing is an economically and socially important industry in North America. Annually in the United States recreational fishing contributes ~$50 billion (CAD) to the national economy while in Canada it contributes ~$8 billion (CAD), both through direct (e.g. licenses, tackle, and boats) and indirect (e.g. accommodations and travel) purchases (GSGislason & Associates Ltd., 2009; Fisheries and Oceans Canada, 2015; U.S. Fish & Wildlife Service, 2016; National Oceanic and Atmospheric Administration, 2018). Approximately 36 million anglers in the United States and ~3 million anglers in Canada participate in recreational fishing, catching a variety of species of fish including salmon, trout, bass, drum, walleye (Hughes, 2014; Fisheries and Oceans Canada, 2015; U.S. Fish & Wildlife Service, 2016). Within our study areas, California (CA) and British Columbia (BC), recreational fishing contributes approximately $1.7 billion (CAD) and $1 billion (CAD) respectively (Bailey and Sumaila, 2012; National Oceanic and Atmospheric Administration, 2018), and sustains approximately 2.8 million (CA) and 400,000 (BC) recreational fishers (Bailey and Sumaila, 2012; U.S. Fish & Wildlife Service, 2016).

To support recreational fisheries, both Canada and the United States employ extensive stocking programs. Stocking in North America has a long history, beginning in the mid-to-late 1800s with settlers bringing fish to stock in fish-less lakes as they moved west (Dill and Cordone, 1997; Pister, 2001; Kerr, 2006). By the inter-war period, both Canada and the United States were stocking millions of fish a year, mainly salmon and trout (Mulvey, 1921; Pister,

2001). Few standard policies existed in stocking until ~1960s which has led to negative consequences such as declines in native fish and invertebrate populations (Courtenay and Williams, 1992; Liss *et al.*, 1999; Kerr, 2006); however, legislation enacted by both Canada and the United States now stringently regulates fish stocking. Regulatory bodies currently assess risks associated with stocking such as disease introduction, predation impacts, and hybridization of native and invasive species (Fisheries and Oceans Canada, 2003; e.g. in the US "North Cascades National Park Service Complex Fish Stocking Act," 2014).

For part of this thesis (Chapter 2), I collaborated with the Freshwater Fisheries Society of British Columbia (FFSBC), a non-profit organization responsible for stocking freshwater fish in British Columbia. To support the demand of the hundreds of thousands of recreational fishers in British Columbia the FFSBC stocks ~800 lakes, ranging from completely isolated alpine lakes only accessible by hiking to metropolitan ponds, province-wide (Bailey and Sumaila, 2012; Northrup, 2017). Within these lakes the FFSBC stocks a variety of freshwater fish including white sturgeon, brook char, cutthroat trout, kokanee, and steelhead, but the vast majority (~5 million of 8 million total stocked annually) are rainbow trout (Northrup, 2017). To accomplish this goal the FFSBC operates six major fish hatcheries province-wide, within which they rear the fish from fertilization up to release, with released sizes for rainbow trout ranging from ~1g (beginning of fry stage) up to 250g (Northrup, 2017; FFSBC, 2020). The success of this stocking program depends on a variety of factors, but now, importantly, due to climate change managers need to tailor stocking strategies to ensure the continuation of these strains in the face of increasing temperatures (Cowx, 1994).

1.2 High temperatures and hypoxia in freshwater

Freshwater fish habitat and biodiversity are under threat due to elevated temperatures as a result of anthropogenic-mediated climate change, and large decreases in vertebrate biodiversity in freshwater compared to terrestrial and marine ecosystems have been documented (Eaton and Scheller, 1996; World Wild Fund for Nature, 2016; Comte and Olden, 2017; Reid *et al.*, 2019). Temperature increases are predicted to lead up to a 50% reduction in the habitat of cold-water fishes, compared to just 14% in warm-water fishes within the United States (Eaton and Scheller, 1996). The pervasive effects of temperature increases on fish are a direct result of temperature's influence on biological characteristics such as aerobic metabolism (Portner and Farrell, 2008), life-history (Dahlke *et al.*, 2020), growth (Morita *et al.*, 2010), and ecological interactions (Selbie *et al.*, 2011). The response of fish to these temperature increases is likely to determine their global distribution (Sunday *et al.*, 2012), and further studies into the effects of temperature on fish species, strains, and individuals are crucial to determine how they will respond to climate change (Harrod, 2016).

An indirect effect of climate change in freshwater ecosystems is the decline of dissolved oxygen. The cause of this decline is two-fold. Intrinsically, as temperature rises the solubility of oxygen in the water declines and reduces oxygen content. Furthermore, as temperatures increase so does the oxygen uptake rate of aquatic organisms, further decreasing dissolved oxygen (Diaz and Breitburg, 2009). Like temperature, oxygen availability plays a fundamental role in the physiology and performance of freshwater fish, affecting characteristics such as growth (Wang, et al., 2009), fecundity (Landry *et al.*, 2007), immune responses (Kvamme *et al.*, 2013), and ecological interactions (Pollock *et al.*, 2007). Together, high temperatures and low oxygen interact synergistically to impair organismal performance beyond that due to each stressor in

isolation. For example, when exposed to acute high temperatures, the time to LOE (hypoxia tolerance) of Atlantic Killifish declined by 20-fold, a significantly larger decline than what may be expected through the effect of temperature on metabolic rate alone (McBryan *et al.*, 2013). Clearly, organisms will need strategies to cope with these increased temperatures and decreased dissolved oxygen in freshwater ecosystems.

1.3 Strategies to deal with high temperatures and hypoxia

To survive climate change, organisms have few options: they can migrate to a more suitable environment, they can exhibit phenotypic plasticity and change their physiological response, or they can adapt over time (Fuller *et al.*, 2010; Somero, 2010). Unfortunately, freshwater fish are limited in their ability to migrate to new environments in two critical ways. Firstly, freshwater ecosystems are naturally fragmented as a result of biogeographic barriers such as waterfalls, mountain ranges and catchment divides, resulting in limited connectivity through streams and rivers (Rahel, 2007). Secondly, human alterations such as damming have led to even greater decreases in connectivity (Pringle, 2003; Strayer and Dudgeon, 2010). Clearly, phenotypic plasticity and adaptation will be particularly important for freshwater fish to cope with climate change.

Phenotypic plasticity is defined as the ability of an organism to change its phenotype depending on its environment (Schlichting, 1986). This change in phenotype is independent of a modification in genotype and occurs either within the life-span of an organism or trans-generationally (Cavieres *et al.*, 2019). Within the life-span of organisms, plasticity occurs at short-time scales with reversible changes or in early development leading to irreversible modifications (Munday *et al.*, 2013). Trans-generationally, phenotypic plasticity can occur irreversibly and reversibly, often through molecular (transcriptomic and epigenetic) modification

(Veilleux *et al.*, 2015; Donelson *et al.*, 2018). At the whole-animal level, phenotypic plasticity allows organisms to change their phenotype, for example, tolerance to external strngaessors to sustain performance. This process of phenotypic plasticity at the whole-animal level is likely underlaid by concomitant changes in underlying physiological function and gene expression (Logan and Somero, 2010; Anttila *et al.*, 2014a; Logan and Buckley, 2015; Muñoz *et al.*, 2015; Veilleux *et al.*, 2018).

Previous studies have shown significant plasticity in salmonids in response to increasing temperatures, with particularly large increases in thermal tolerance following acclimation to higher temperatures (e.g. Myrick & Cech, 2000). Similarly, McBryan *et al.*, (2016) has shown that acclimation to higher temperatures can improve hypoxia tolerance in a euryhaline fish species. Additionally, acclimation to hypoxia has also been shown to improve hypoxia tolerance (Borowiec, et al., 2020). Previous findings suggest that these shifts in whole-animal tolerance are associated with plastic changes in metabolic capacity and cardiorespiratory function (Logan and Somero, 2010; Anttila *et al.*, 2014a; Logan and Buckley, 2015; Muñoz *et al.*, 2015). Furthermore, many studies have detected a transcriptional response to thermal acclimation (e.g. Veilleux *et al.*, 2018). Thus, assessing acclimatization, and therefore plasticity, across biological organization is likely to be important in defining which species persist or perish through climate change (Somero, 2010).

Although these previous examples highlight important phenotypic plasticity that could buffer organisms in their response to anthropogenic mediated climate change, limits to plasticity have been illustrated in prior studies. For example, Sandblom *et al.*, (2016) note that acute warming tolerance or the difference between upper thermal tolerance and habitat temperature ($CT_{max} - T_{hab}$) is diminished at high temperatures. Furthermore, Myrick and Cech, (2000)

showed that both growth and routine metabolic rate declines at higher temperatures, even with an increase in CT_{max}. These results indicate that plasticity may fail to buffer fish from the effects of climate warming.

If plasticity fails to safeguard freshwater fish from the effects of climate change, then perhaps adaptation through natural selection may allow them to survive (Hoffmann and Sgró, 2011). Adaptation by natural selection can occur both within- and among-strains of the same species as a result of selection on new mutations or selection on standing genetic variation, both which could be particularly important for rapid adaptation to climate change (Barrett and Schluter, 2008). Furthermore, there is evidence for both local adaptation and artificial selection on hypoxia and/or thermal tolerance in fish (Faust *et al.*, 2004; Chen *et al.*, 2015; Verhille *et al.*, 2016), suggesting that there is genetic variation associated with these important traits. Consequently, comprehensively examining variation within- and among-strains is vital to predicting how species, strains, and individuals may adapt to climate change (Harrod, 2016).

1.4 Metrics of tolerance

Researchers have developed two main laboratory methods to examine whole-animal upper thermal tolerance in fish at acute timescales: incipient upper lethal temperature (IULT) and critical thermal maximum (CT_{max}). These methods differ in two critical ways, IULT is a static and lethal measurement of upper thermal tolerance, while CT_{max} is a dynamic and sublethal measurement of upper thermal tolerance (Beitinger and Lutterschmidt, 2011). IULT is defined as the static temperature at which 50% of the organisms being tested perish, while CT_{max} is the temperature at which an organism exhibits loss of equilibrium (LOE) or the inability to maintain dorsoventral equilibrium (Beitinger and Lutterschmidt, 2011). For this study, I chose to use CT_{max} as our measurement of upper thermal tolerance because of the dynamic nature of the

method, which allows us to measure variation within strains and across numerous individuals. Additionally, CT_{max} utilizes a sublethal endpoint which allows us to measure recovery from thermal stress. While the direct ecological relevance of CT_{max} is questionable, previous studies indicate that it is a predictor of the global distribution of fish and correlates with a more ecologically relevant, slow warming tolerance (Sunday *et al.*, 2011; Åsheim *et al.*, 2020). Furthermore, previous studies have indicated that cardiorespiratory function likely underlies an organism's response to elevated energy demand under high temperatures (Steinhausen *et al.*, 2008), as such we also used cardiac thermal tolerance as measurement of underlying organ system plasticity compared to whole-animal upper thermal tolerance.

While IULT, CT_{max}, and cardiac thermal tolerance are all measures of acute thermal tolerance, researchers have used several measures that aim to address the effects of chronic temperature on organismal performance. For example, previous work has examined the effects of chronic temperatures on traits such as growth, fecundity, swimming speed, and oxygen consumption rate to name a few (Taylor *et al.*, 1996; Myrick and Cech, 2000; Crozier and Hutchings, 2014). These methods attempt to characterize the effects of chronic temperature on basic biological processes that have thermal sensitivities below acute thermal tolerance. The results of these studies all indicate failure or limits to these processes at temperatures considerably lower than acute upper thermal tolerances; however, it remains unclear if upper thermal tolerance is correlated with individual, strain, and species-specific limits in these processes. For this thesis, I have used acute methods of thermal tolerance because of the ease of measuring numerous individuals to characterize within-strain variation as well as the lower time constraints associated with acute methods.

Like upper thermal tolerance, researchers have developed two main acute hypoxia tolerance measurements: critical oxygen tension (P_{crit}) and incipient lethal oxygen saturation (ILOS). P_{crit} is a measurement of the oxygen saturation at which fish are no longer able to maintain their basal oxygen consumption rate and oxygen consumption becomes dependent on the oxygen saturation of the water, while ILOS is the oxygen saturation at which an organism exhibits LOE (Claireaux *et al.*, 2013; Wood, 2018). Both P_{crit} and ILOS are sublethal measurements of hypoxia tolerance. For this thesis, I chose to use ILOS for the first data chapter, as this measurement allows us to comprehensively examine within-strain variation across numerous individuals, while we measured both ILOS and P_{crit} in the second data chapter to characterize the effects of chronic temperature acclimation on organismal oxygen consumption and performance.

Similar to thermal tolerance, researchers have also used methods to look at the effects of chronic hypoxia on basic biological processes that have sensitives below acute hypoxia tolerance. For example, previous work has examined the effects of chronic hypoxia on traits such as growth, fecundity, and oxygen consumption rate (Landry *et al.*, 2007; Pollock *et al.*, 2007). These results indicate limits at oxygen saturations far above P_{crit} and individual ILOS in at least some fish species; however, similar to upper thermal tolerance, it remains unclear whether both P_{crit} and ILOS are correlated with individual, strain, and species-specific limits in these processes.

1.5 Rainbow trout

Rainbow trout, *Oncorhynchus mykiss,* are a cold-water salmonid that spawn and develop in cold-water lakes, rivers, and streams ranging from Northern Mexico to Alaska, with strains also occurring in eastern Russia (MacCrimmon, 1972; Smith and Stearley, 1989). Rainbow trout

have substantial variation across strains in morphology and life-history, especially in their temperate ranges. This variation in their temperate range is likely due to local adaptation following population differentiation as the glaciers receded (Keeley *et al.*, 2005, 2007). The fundamental differentiation among strains in rainbow trout is across two major ecotypes, a resident ecotype which spends the whole life-cycle in freshwater and an anadromous ecotype (Steelhead) which migrates to the marine environment during maturity and returns to freshwater to spawn (Behnke, 1979). In this thesis, I focus on resident rainbow trout, which occupy freshwater with temperatures typically less than < 20 °C (MacCrimmon, 1972; Raleigh *et al.*, 1984). Rainbow trout are also considered a hypoxia sensitive species and require oxygenated waters > 30 % air saturation at 12°C (Raleigh *et al.*, 1984; Molony, 2001; Rytkönen *et al.*, 2007). These temperature and oxygen requirements are likely to be important factors establishing their native range and stocked ranges (Raleigh *et al.*, 1984; Sunday *et al.*, 2012), and some strains may already be at risk of extirpation due to elevated temperatures and other factors such as agricultural practices (e.g. COSEWIC, 2014). Recreational fish stocking has expanded the distribution of rainbow trout worldwide with historical and on-going stocking on six out of the seven continents (excluding Antarctica) of the world (MacCrimmon, 1972).

As a result of their importance in recreational fish stocking, there is substantial research into the tolerance of rainbow trout to high temperatures (e.g. Currie *et al.*, 1998; Myrick and Cech, 2000; LeBlanc *et al.*, 2011; Recsetar *et al.*, 2012; Chen *et al.*, 2015; Verhille *et al.*, 2016; Ekström *et al.*, 2019). Previous studies have indicated that rainbow trout have substantial plasticity in tolerance to high temperatures and can raise their upper thermal tolerance (CT_{max}) > 4 °C with acclimation to high temperatures (Myrick and Cech, 2000; Chen *et al.*, 2015). Furthermore, previous studies have suggested local adaptation in their tolerance to high

temperatures, with southern rainbow trout strains able to maintain absolute aerobic scope over a broader range of temperatures compared to northern strains (Verhille *et al.*, 2016). These results suggest the need to examine strain-specific responses when assessing climatic vulnerability of rainbow trout to climate change.

Similar to thermal tolerance, there is substantial interest in studying the impact of hypoxia on rainbow trout (e.g. Roze *et al.*, 2013; Scott *et al.*, 2015; Zhang *et al.*, 2018b; Williams *et al.*, 2019), especially as hypoxic episodes are becoming increasingly common in freshwater ecosystems (Jenny *et al.*, 2016). Hypoxia tolerance also has substantial plasticity in rainbow trout, with exposure to environmentally-relevant diel hypoxia cycling enhancing whole-animal oxygen regulation compared to acutely exposed fish (Williams *et al.*, 2019). Furthermore, hypoxia tolerance may also be locally adapted as strains differ significantly in cardiac and whole-animal hypoxia tolerance (Faust *et al.*, 2004; Zhang *et al.*, 2018b). Again, providing further evidence of the need to investigate strain-specific responses to predict the response of rainbow trout to increasing temperatures and decreasing dissolved oxygen.

1.6 Thesis objectives

The overall goal of this thesis was to examine the evidence for adaptive variation in and plastic potential of one of the most economically important freshwater fisheries species in the world, the rainbow trout. To do this, I partnered with the FFSBC to examine strain-specific responses to high temperatures and low oxygen in multiple strains of rainbow trout from British Columbia. I also partnered with Dr. Fangue and her laboratory at the University of California, Davis, as well as Dr. Farrell and his laboratory at the University of British Columbia to examine plastic responses to temperature acclimation across multiple levels of biological organization in two strains of rainbow trout from California. My thesis addressed the following objectives:

1) Examine the evidence for phenotypic variation within and among rainbow trout strains currently used and/or being assessed for stocking in British Columbia

To do this, I examined upper thermal and hypoxia tolerance (as CT_{max} and ILOS) in five different strains of rainbow trout. Furthermore, I used large numbers of fish raised from fertilization under the same conditions in common garden experiments to comprehensively investigate the variation in these traits. I predicted that the strains would differ in upper thermal and hypoxia tolerance and these differences may be a result of their native environments.

2) Investigate the relationship between upper thermal and hypoxia tolerance in the same strains as above

To do this, I used passive integrated transponder (PIT) tags for individual identification and measured both upper thermal and hypoxia tolerance on the same individuals. I predicted that due to the intrinsic nature of the relationship between high temperatures and low oxygen in freshwater ecosystems that individuals and strains that are thermally tolerant will also tend to be hypoxia tolerant.

3) Characterize the thermal plasticity of rainbow trout across multiple levels of biological organization

To do this, I worked in collaboration to examine upper thermal and hypoxia tolerance, metabolic rate, cardiac performance, and gene expression in two strains of rainbow trout acclimated to three ecologically relevant temperatures for three weeks. I predicted I would find extensive plasticity in all traits examined, with compensatory changes in gene expression across

the three temperatures examined. In addition, I hypothesized that there would be limits to this plasticity at the highest temperature tested.

Chapter 2: Characterization of within- and among-strain variation in upper thermal and hypoxia tolerance in multiple stocked strains of rainbow trout (*Oncorhynchus mykiss)*

2.1 Introduction

Global climate change and other human-induced habitat alterations are causing major declines in freshwater biodiversity (Jenkins, 2003; Ficke *et al.*, 2007; Comte and Olden, 2017; Reid *et al.*, 2019). In particular, increasing temperatures are projected to contribute to drastic declines in suitable freshwater habitat for cold-water fish (Eaton and Scheller, 1996; Wenger *et al.*, 2011; Comte *et al.*, 2013). These increased temperatures are associated with declines in dissolved oxygen (hypoxia) because they have the dual effect of decreasing oxygen solubility and increasing oxygen consumption by microorganisms (Diaz and Breitburg 2009). High nutrient loading from agricultural activities exacerbates these effects through increasing microbial metabolism, which causes further decreases in dissolved oxygen levels. The resulting hypoxic episodes can be devastating for fish, particularly when increased temperatures has elevated their own oxygen demand (Diaz and Breitburg 2009). Episodes of high temperature and hypoxia are becoming increasingly frequent in freshwater ecosystems (Diaz and Breitburg, 2009; O' Reilly *et al.*, 2015; Jenny *et al.*, 2016), which presents a substantial challenge for the conservation and management of freshwater fish.

Organisms have only a few fundamental ways in which they can respond to the increasing temperatures and episodes of hypoxia in freshwater. They can migrate, exhibit phenotypic plasticity, or adapt *in situ* (Gienapp *et al.*, 2008). Phenotypic plasticity may be limited in its effectiveness in response to climate stressors (DeWitt *et al.*, 1998; Seebacher *et al.*,

2015), and I will not consider it further here. Similarly, migration is not an option for many freshwater organisms either because of the natural structure of the habitat or due to habitat fragmentation from damming (Strayer and Dudgeon, 2010). On the other hand, human-assisted migration, which involves passively or actively moving species or strains to areas outside their currently occupied ranges, has been proposed as a means of addressing the impacts of climate change (Vitt *et al.*, 2009; Hewitt *et al.*, 2011). This approach is not without risk and will require a deep understanding of the relative resilience of different species or strains within species to climate change stressors. Finally, evolutionary adaptation will likely play a key role in the survival of freshwater organisms into the future (Somero, 2010). Rapid adaptation typically occurs from standing genetic and phenotypic variation within strains (Barrett and Schluter, 2008), but for many species, we have little information regarding the extent of within-species genotypic and phenotypic variation for traits that are relevant to climate change resilience. Thus, in the context of both estimating the likelihood of adaptation *in situ*, and evaluating the need for assisted migration, characterizing both within and between strain variation in traits related to an organism's response to climate change will be vital for conservation and management programs going forward. Indeed, it is becoming increasingly clear that analyzing strain-specific responses to climate-change relevant stressors will be critical in developing appropriate mitigation and conservation efforts to protect species as our climate warms (e.g. Eliason *et al.*, 2011; Roze *et al.*, 2013; Chen *et al.*, 2015; Verhille *et al.*, 2016; Zhang *et al.*, 2018b).

Here, I examine within- and among-strain variation in thermal and hypoxia tolerance in rainbow trout (*Oncorhynchus mykiss*), a fish species that is widely stocked for recreational purposes globally and is now present in six out of seven continents (MacCrimmon, 1972; Stanković *et al.*, 2015). Rainbow trout are native to the western coast of North America. In

British Columbia, which encompasses most of the native range of rainbow trout in Canada, this species is widely stocked to support the recreational fishing industry. This industry is of substantial socio-economic importance, contributing ~$8 billion annually to the Canadian economy with close to $1 billion in British Columbia alone (GSGislason & Associates Ltd., 2009; Bailey and Sumaila, 2012; Northrup, 2017). In British Columbia, the Freshwater Fisheries Society of British Columbia (FFSBC), stocks ~800 lakes province-wide with approximately 8 million fish, of which 5 million are rainbow trout (Bailey and Sumaila, 2012; Northrup, 2017). Such stocking programs help to preserve recreational fisheries into the future and may also help promote the survival of natural strains through the alleviation of fishing pressure on wild stocks (Cowx, 1994; Cooke and Cowx, 2006; Froehlich *et al.*, 2017).

Oxygen availability and temperature are important limiting factors in the natural and stocked distributions of rainbow trout (MacCrimmon, 1972). Rainbow trout occupy lakes, rivers, and streams with temperatures ranging from 0 – 25 °C, with an optimal range of 12-18 °C (MacCrimmon, 1972; Raleigh *et al.*, 1984), and can tolerate oxygen levels from ~30 – 100 % air saturation with an optimum at ~75% air saturation at 12 °C (Raleigh *et al.*, 1984; Molony, 2001). Previous studies have demonstrated that there is variation among at least some strains of rainbow trout in their ability to cope with high temperature and hypoxia (Myrick and Cech, 2000; Chen *et al.*, 2015; Verhille *et al.*, 2016; Zhang *et al.*, 2018b), but little is known about the extent of within-strain variability in these traits, as most studies that compare tolerance among strains use relatively few individuals from each strain.

In this study, I examine variation in acute thermal and hypoxia tolerance (measured as CTmax and ILOS, respectively) within and among multiple strains of rainbow trout currently stocked in British Columbia or under consideration for stocking to identify strains that may be

resilient to high temperatures and low environmental oxygen. Furthermore, I also assess the extent of intra-strain variation to assess the potential for rapid adaptation. I address the following questions, 1) How much within-strain variation in CTmax and ILOS is present? 2) Is there a difference among the strains in these traits and among the strains in survival following hypoxic and thermal challenges? and 3) Are any observed differences consistent across life-stages and brood years? Additionally, as high temperatures and low environmental oxygen are intrinsically linked in freshwater ecosystems (Diaz and Rosenberg, 2008; McBryan *et al.*, 2013), I also assess the correlation between CTmax and ILOS within individuals.

Most studies in conservation physiology assess relatively few individuals per strain, which limits the ability to fully assess both within and between strain variation. Similarly, many studies are performed using wild-caught individuals, which makes it difficult to distinguish potentially genetically based differences from variation due to various forms of plasticity and epigenetic effects (Gienapp *et al.*, 2008; Crozier and Hutchings, 2014; Merilä and Hendry, 2014). In contrast, in this study I reared multiple strains of rainbow trout in common conditions from fertilization, thus minimizing the potential effects of phenotypic plasticity, used large numbers of families to capture a significant fraction of within-strain genetic variation, and used large numbers of individuals of each strain (~100 to ~500) to characterize the range of phenotypic variation among individuals, thus, allowing me to perform a robust assessment of factors influencing climate change resilience.

2.2 Methods

2.2.1 Experimental animals

I examined variation in upper thermal tolerance and hypoxia tolerance in five strains of rainbow trout currently being used or under consideration for use in stocking programs across

two brood-years (2017 and 2018) and two life-stages (fry and yearling). Strains assessed include the wild strains Blackwater River (BW), a riverine piscivorous strain (Scott *et al.*, 2015), Carp Lake (CL), a highly competitive lake-dwelling strain (FFSBC 2020), Pennask Lake (PN), an insectivorous, non-competitive lake-dwelling strain (FFSBC 2020), Horsefly River (HF), a large, late-maturing Quesnel Lake strain that utilizes the Horsefly River for spawning and rearing opportunities (Holmes, 2009), and Fraser Valley (FV), a domesticated strain thought to be of Californian origin, that has been used in the British Columbian recreational fish stocking program since the 1960s (FFSBC 2020). Not all strains were used for each brood year and/or life-stage (see Tables 2.1 and 2.2).

Following the acquisition of milt and eggs from adults residing in broodstock lakes (CL and BW; FV adults housed at FFSBC, Duncan, BC hatchery) or wild lakes (HF and PN), I bred 25 independent families (25 females and 25 males) per strain for the respective brood years of BW, CL, PN and FV, and 8 families (8 females and 7 males) for HF because of the difficulty of capturing wild individuals in Quesnel Lake. Following fertilization eggs were counted out evenly to represent all mothers then eggs were pooled for incubation by strain. All offspring were maintained as diploids (2n). These strains breed at different times of the year and thus crosses for BW, CL, and HF were performed in May, for PN in June, and for FV occurred in October of each brood year.

All fish were housed and experimented on at the Fraser Valley Trout Hatchery (Abbotsford, British Columbia). Following fertilization, eggs were raised in Heath trays until hatch (~6-8 weeks post-fertilization) with flow-through well water (10-12°C). After hatch, fry from each strain were housed separately in 2400 L tanks containing 10-12°C flow-through well water and kept under a natural photoperiod. Following yolk-sac absorption to ~5 grams the fish

were fed to satiation multiple times a day with Bio-Oregon BioVita #0 to #2 and with Bio-Oregon Bio-Clark's fry 1.2mm thereafter (Bio-Oregon 2020).

Some experiments were performed using a repeated measures design in which the same individual was tested for both upper thermal and hypoxia tolerance (see experimental design section). For these repeated measures trials, fish were individually tagged with Biomark GPT12 (12mm) passive integrated transponder (PIT) tags. Briefly, individual fish were anesthetized with 50-100 mg/L (depending on size) dose of MS-222 (Tricaine methanesulfonate; buffered 1:1.5 with $NaHCO_3$), length and weight were measured, then a small incision was made along the bottom of the fish slightly forward (towards the head) of the anal fins and a PIT tag was inserted, read for identification and the fish was returned to their holding tank (500 L).

All experiments were performed according to approved University of British Columbia animal use protocol A16-0329.

2.2.2 Hypoxia tolerance (measured as ILOS)

I measured incipient lethal oxygen saturation (ILOS) using a hypoxia challenge test (HCT) as an index of hypoxia tolerance, as outlined by Claireaux *et al.*, (2013). Note that ILOS is inversely related to hypoxia tolerance, with high ILOS indicating poor hypoxia tolerance. Briefly, fish were transferred from holding tanks to the assessment tank (215 L) and left for 30 minutes before the beginning of each trial. All hypoxia trials were conducted at the fish's holding temperature of 11°C ± 1°C. A small circulation pump was placed in the experimental tank to ensure consistent mixing of the added nitrogen. During each trial, the dissolved oxygen (DO) was lowered by bubbling nitrogen into the experimental tank at a rate of ~1.5% air saturation minute^{-1} until ~20% air saturation was reached. After ~20% air saturation the rate of decrease was lowered to 0.1% air saturation minute^{-1} until the end of the trial. Hypoxia tolerance was

determined as the air saturation (% air sat.) at which an individual fish experienced loss of equilibrium (LOE; i.e., the inability to maintain dorsoventral orientation). Once LOE was reached the fish was removed from the experimental tank, scanned for PIT tag identification where applicable, and placed into a recovery tank containing fresh, fully aerated water at their rearing temperature. Following measurement of hypoxia tolerance, the fish were either sacrificed and fin-clipped for fish involved in non-repeated measures trials, or allowed to recover for two or three weeks (depending on the experiment see experimental design section) before determination of upper thermal tolerance.

2.2.3 Upper thermal tolerance (measured as CT_{max})

I assessed upper thermal tolerance using a critical thermal maximum (CT_{max}) protocol modified from Beitinger *et al.*, (2000). Briefly, before each experimental day, a 500 L tank adjacent to the assessment tank was heated to ~40-45°C for use as a source of heated water. At the start of each trial, fish were transferred from their holding or rearing tanks to the assessment tank (250 L) and left undisturbed for 30 minutes to adjust to the testing apparatus. A small circulation pump was placed in the assessment tank to avoid thermal stratification of the water and to achieve consistent mixing of added water. Beginning at 11°C ± 0.5°C, water temperature was increased by pumping water from the hot-water tank into the assessment tank through a polyvinyl chloride (PVC) pipe fitted with a flow valve. Throughout each trial small adjustments were made to the flow to achieve a consistent ramping rate of 0.3 °C min^{-1} until the water reached 18 °C. At 18 °C the flow was slowed considerably to achieve a ramping rate of 0.1 °C min^{-1} for the remainder of the trial. Upper thermal tolerance (CT_{max}) was measured as the temperature at LOE. After LOE, each fish was removed from the experimental tank, scanned for PIT tag identification where applicable, and placed in a recovery tank containing fresh ~21 °C

water. Over the course of two hours post-trial, the temperature in the recovery tank was slowly brought down to the acclimation temperature (~11 °C). This gradual recovery protocol was adopted because preliminary experiments suggested that post-trial mortality was extremely high if fish were immediately transitioned from their temperature at CT_{max} to their acclimation temperature. Following measurement of upper thermal tolerance, the fish were either sacrificed and fin-clipped for non-repeated measures trials, allowed to recover for ~ one week to collect post-trial mortality data, or allowed to recover for three weeks and had their hypoxia tolerance measured (depending on the experiment, see below).

2.2.4 Experimental design

Experiment 1: This experiment utilized fry of the 2017 brood year from the BW, CL, and FV strains (Table 2.1). Fish were not tagged and different individuals were used for assessment of ILOS and CT_{max}. Each strain was tested in a different trial and either one trial of ~100 fish or two trials of ~50 fish each were performed per strain (Table 2.1). The FV strain was tested at a different time of year (Table 2.1) to allow testing at a similar body size across strains (Table and Figure A.1). Note that the hatchery has a constant-temperature water supply (10-12°C year-round), but natural photoperiod. The primary purpose of this experiment was to assess the feasibility of performing trials on large numbers of individuals simultaneously and to examine levels of variation within a strain, thus statistical comparisons among strains were not performed.

Experiment 2: At the yearling stage for the 2017 brood year, ILOS, CT_{max}, and post-trial mortality were assessed for tagged individuals from the BW, CL, PN, and FV strains (Table 2.1). The primary aim of these experiments was to determine whether hypoxia tolerance and thermal tolerance could be assessed on the same individuals and whether there were strong effects of trial on these traits. Prior to the beginning of the trials, all individuals were PIT-tagged and moved to

holding tanks (500 L), with each strain held separately. For BW, CL, FV, strains were tested in five separate trials for each strain (although only four trials were completed for CL because of equipment malfunction; Table 2.1). Only a single trial was performed for PN because this strain experienced unusually high mortality during rearing, and fish numbers were limited. ILOS was assessed first, then two weeks of recovery before the determination of CT_{max}, followed by one week of recovery to assess post-trial mortality. The FV and PN fish were tested at a different time of year than the other strains because of the difference in their spawn timing, and thus comparisons with these strains should be viewed with caution. First, the effect of trial group within a strain for CT_{max} and ILOS was analyzed using one-way analysis of variance (ANOVA). All data met the assumptions for this analysis. Then CT_{max} and ILOS were compared across strains at the 2017 brood yearling stage using linear mixed effects models with strain as a fixed factor and trial group (nested within strain) as random factor followed by Tukey pairwise comparisons for all strains except PN, as this strain was tested in only a single trial. Post-trial mortality was compared across strains using one-way ANOVA for all strains except PN again. Correlations between ILOS and CT_{max} were assessed for each strain using Kendall rank or Pearson correlations. All statistical analyses were carried out using RStudio (version 1.1.456; R Foundation for Statistical Computing, 2018). Alpha was set at 0.05 throughout.

Experiment 3: This experiment was designed to determine whether there was significant variation in tolerance among strains, using BW, CL, and HF strains from the 2018 brood year (Table 2.2). To facilitate this comparison, all individuals were PIT-tagged, held (130 L tanks for fry, 500 L tanks for yearling), and tested in common-garden, with ~33 individuals from each strain in a trial for a total of 400-500 fry per strain across 14 trials and ~100 yearlings per strain across 3 trials (Table 2.2). ILOS was determined first, then three weeks of recovery before the

determination of CT_{max}, followed by one week of recovery to assess post-trial mortality. The HF strain replaced the FV strain tested in the 2017 brood year to allow simultaneous testing. Differences in tolerance among strains were assessed using linear mixed effects models with strain as a fixed factor and trial group as a random factor with Tukey pairwise comparisons. Differences in post-trial mortality among strains within a brood year and life-stage were analyzed using one-way analysis of variance (ANOVA). ILOS, CT_{max}, and post-trial mortality were also assessed for the PN strain (at the fry stage only; ~100 fish across 5 trials, Table 2.2), but as these assessments were performed at a different time of year due to the difference in its spawning time, I did not make a statistical comparison of PN with the other strains. Correlations between ILOS and CT_{max} were assessed for each strain as described for Experiment 2.

Experiment 4: To assess the effects of the order of tolerance assessments I used additional individuals from the BW and CL strains at the 2018 brood yearling life-stage and assessed CT_{max} first, followed by three weeks of recovery before the determination of ILOS. Fish were assessed in two common garden trials consisting of ~50 individuals each, for a total of ~100 individuals per strain (see Figure 2.4 for exact sample sizes). These data were compared to data for the same strains from Experiment 3 (in which ILOS was determined first, followed by CT_{max}) using linear mixed effects models with trial order as a fixed factor and trial group as a random factor followed by Tukey pairwise comparisons.

2.3 Results

The mean masses of the fish for each strain, life stage, and tolerance assessment (for Experiment 1 when these assessments were done on different groups of fish) are provided in Figures A.1 and A.2, and Tables A.1 and A.2. There was no consistent effect of mass on either

CT_{max} or ILOS (Tables A.3 and A.4) and thus fish mass was not considered in subsequent analyses.

2.3.1 ILOS and CT_{max}

Figure 2.1 summarizes the extent of variation in both hypoxia and upper thermal tolerance at the fry (Figure 2.1A, C) and yearling (Figure 2.1B, D) stages for the 2017 brood year (Experiments 1 and 2), and data are also presented in Table A.5 along with data for the PN strain which was not included in the analysis in the main figures. Hypoxia tolerance varied by as much as 5% saturation between the best and worst-performing fish within a strain for the 2017 brood year (excluding outliers; Figure 2.1A, B). Taking the oxygen ramping rate into account, this translates into a difference of approximately 50 minutes before LOE occurs under extreme hypoxia. Thermal tolerance also exhibited substantial variation within strains at both life stages (Fig 2.1 C, D, Table A.5), varying by as much as 1.5 °C between high and low performers within a strain (excluding outliers), which translates into a difference of 15 minutes tolerated at high temperature.

Because trials at the fry stage in 2017 were conducted separately for each strain with only one or two trials per strain, it is not appropriate to make statistical comparisons among strains at this life stage, but at the yearling stage for the 2017 brood year where multiple trials per strain were performed (Table 2.1), there were significant differences across strains in upper thermal tolerance (Figure 2.1D; $p = 2.059 \times 10^{-3}$, Table A.5), with the BW strain having the greatest tolerance. In contrast, there were no significant differences among strains in hypoxia tolerance (Figure 2.1B; $p = 1.173 \times 10^{-1}$, Table A.5). Note that these comparisons should be viewed with caution, as the FV strain differed slightly in mass and was tested at a different time of year than the other two strains (Table 2.1 and Figure A.2). These analyses also revealed small but

significant differences in tolerance between trials within some strains (Table A.6 and A.7; **ILOS:** BW – $p = 4.35x10^{-2}$, CL – $p < 2.00x10^{-16}$, FV – $p = 2.14x10^{-2}$, CT_{max}**:** BW – $p = 1.23x10^{-1}$, CL – $p = 6.58x10^{-7}$, FV – $p = 2.85x10^{-4}$) and one ILOS trial for CL differed substantially from the rest, which may have been due to inaccurate calibration of the DO meter. These data emphasize the importance of testing the tolerance of all strains within the same trial to accurately assess differences among strains.

I used this common garden testing approach the 2018 brood year, removing the FV strain because of the difference in spawn timing and replacing it with HF (Table 2.2). I also tested the PN strain (at the fry stage only) in separate trials, and these data can be found in Table A.8. As was the case for the 2017 brood year, I detected substantial variation in both hypoxia tolerance and upper thermal tolerance within strains at both life stages (Figure 2.2 A-D).

I also found small but statistically significant differences among the BW, CL, and HF strains in upper thermal tolerance at both life stages and hypoxia tolerance at the yearling stage (Figure 2.2 A-D; Fry: ILOS – $p = 4.492^{-1}$, CT_{max} – $p = 3.085e^{-06}$ Yearling: ILOS – $p = 3.505e^{-10}$, CT_{max} – $p < 2.200e^{-16}$, Table A.8). The rank order of hypoxia tolerance across strains was consistent across life stages, with the HF strain having the lowest hypoxia tolerance (highest ILOS) for both fry and yearling. At the fry stage, mean ILOS differed by 0.4 % air saturation between the most and least tolerant strains and by 0.9% air saturation at the yearling stage. By contrast, the rank order of upper thermal tolerance (CT_{max}) across strains was not consistent across life stages. Although the CL strain had the highest CT_{max} across both life stages, the HF strain had the lowest CT_{max} at the yearling, but not at the fry life-stage. However, it is important to emphasize that differences in mean CT_{max} among strains were extremely small (0.1°C in fry

and 0.3°C in yearlings) relative to the large within-strain variation in this trait and were only detectable statistically because of the very large sample sizes analyzed here.

Although formal statistical comparisons are not appropriate for the PN strain, in general, this strain was somewhat less tolerant of both hypoxia and high temperature relative to the other strains across both brood years (Table A.5 and A.8), but any comparisons with this strain should be viewed with caution because these trials were performed separately and at a different time of year.

2.3.2 Post-trial mortality

I assessed mortality in the seven days following the upper thermal tolerance trials in experiments using tagged fish (yearlings in the 2017 brood year and both life stages in the 2018 brood year). In both fry and yearling there were significant differences in post-trial mortality between strains (Figure 2.3, Fry: 2018 $p < 2e^{-16}$, Yearling: 2017 $p = 3.6e^{-8}$, 2018 $p = 1.1e^{-3}$). At both the fry and yearling stages, HF had the greatest post-trial mortality (~55%), BW had intermediate mortality (20-25%), and the other strains had very low mortality. BW and CL were the only strains tested across both brood years (yearling life-stage) and post-trial mortality was consistent with BW incurring ~20% mortality and CL experiencing < 5%. This consistency is particularly striking as the experimental protocol differed between years, with two weeks in between ILOS and CT_{max} assessment in the 2017 brood year and three weeks in the 2018 brood year.

2.3.3 Effect of trial order

Trial order affected hypoxia tolerance in both strains assessed (BW and CL), with prior experience of a CT_{max} trial associated with decreased hypoxia tolerance (increased ILOS of 0.5-0.7% air saturation; Figure 2.4A, BW: $p = 3.97x10^{-2}$, CL: $p = 1.16e^{-5}$). With a ramping rate of

0.1% sat min^{-1} throughout the LOE period, this absolute difference between means accounts for a ~ 5-7 minute difference in tolerance under extreme hypoxia. In contrast, having first experienced a hypoxia tolerance trial did not significantly affect upper thermal tolerance in either the CL or BW strains (Figure 2.4B, BW**:** $p = 4.73x10^{-1}$, CL**:** $p = 4.14x10^{-1}$).

2.3.4 Correlation between hypoxia and upper thermal tolerance

Correlations between hypoxia and upper thermal tolerance were assessed for experiments involving tagged fish. For the fry life-stage (2018 brood year only) there was a weak but statistically significant correlation between hypoxia and upper thermal tolerance in the BW, CL and HF strains but not PN (Figure 2.5; BW: $p = 1.94x10^{-2}$, $r = -0.08$, CL: $p = 1.25x10^{-3}$, $r = -0.11$, HF: $p = 2.99x10^{-4}$, $r = -0.13$, PN: $p = 3.62x10^{-1}$), with individuals having a high tolerance to hypoxia (low ILOS) also tending to have a high upper thermal tolerance.

At the yearling stage of both the 2017 and 2018 brood years there were weak but statistically significant correlations between hypoxia and upper thermal tolerance in some, but not all, strains (2017 yearlings Figure 2.6A, C and E; BW: $p = 1.11x10^{-4}$, $r = -0.12$, CL: $p = 2.58x10^{-4}$, $r = -0.13$, FV: $p = 4.92x10^{-12}$, $r =-0.23$; 2018 yearlings Figure 2.6B, D and F; BW: $p = 2.06x10^{-2}$, $r = -0.17$, CL: $p = 9.13x10^{-1}$, HF: $p = 1.27x10^{-1}$). As was the case at the fry life-stage, where correlations were present, individuals with high tolerance to hypoxia (low ILOS) also tended to have a high upper thermal tolerance. However, in all cases the correlation coefficient was very small, indicating a weak correlation.

Upon visual inspection, there was no clear relationship between post-trial mortality and either CT_{max} or ILOS in any strain when individuals that died were indicated on graphs of CT_{max} and ILOS correlations (Figures A.3 and A.4).

2.4 Discussion

The results presented here clearly demonstrate that different measures of resilience to climate change stressors (CT_{max}, ILOS, post-trial mortality) lead to different pictures of the relative resilience of rainbow trout strains. Post-trial mortality clearly differentiated the strains I assessed and these patterns were consistent across life stages and brood years, despite the fact that measures of acute upper thermal (CT_{max}) and hypoxia tolerance (ILOS) differed only modestly among strains. These same measures exhibited substantial variation within strains at all life-stages and brood years assessed. Unlike other studies in salmonids that have detected a strong correlation between strain level CT_{max} and ILOS (e.g. Zhang *et al.*, 2018b), my results detect little or no relationship between these traits at the individual level, which suggests that different mechanisms underly these two traits.

Unlike previous studies of strain-level variation in thermal tolerance in salmonids (e.g. Stitt *et al.*, 2014; Chen *et al.*, 2015; Scott *et al.*, 2015; Zhang *et al.*, 2018b), I found limited and inconsistent differences in CT_{max} between strains and across life stages (Figure 2.1 and 2.2). For example, our strains differed in CT_{max} by a maximum of 0.4°C, on average. This contrasts with differences of as much as 2°C among strains of rainbow trout (for fish acclimated to 15°C) reported in a summary of previous studies (Chen *et al.*, 2015). One possible explanation for this difference is that these previous studies used a range of thermal ramping rates, were performed in different locations, at different times of the year, and on fish ranging in size from 2g to 140g, depending on the experiment. Alternatively, the limited variation in CT_{max} that I observed among our strains may be explained by the fact that, with the exception of FV, all of the strains I used are from a rainbow trout lineage from the interior of British Columbia (McCusker *et al.*, 2000; Pollard and Yesaki, 2004; Holmes, 2009; Tamkee *et al.*, 2010; Taylor *et al.*, 2011). However,

this cannot explain the observation of limited differences in CT_{max} between these strains and the FV domesticated strain, which is thought to be of California origin and might be expected to have higher thermal tolerance. However, comparisons with this strain must be viewed with caution because it was tested at a different time of year from the other strains in our experiments. Another important consideration is that the FV strain has been used in the BC stocking program since ~1960s and was initially domesticated in the 1940s (Northrup, 2017). The extent of introgression of alleles from other strains and the role of long-term selection in a constant-temperature hatchery environment in determining the CT_{max} of this strain remains unknown. The limited differences among strains in CT_{max} that I observe, taken together with the relatively limited differences in this trait across studies (reviewed in Chen *et al.*, 2015), suggests that assessment of CT_{max} is not likely to be the most useful tool for detecting strains that are particularly resilient to climate change stressors.

Similar to the case of CT_{max}, I also detected relatively small differences in ILOS among our strains of rainbow trout, with loss of equilibrium occurring at 10-11.4% air saturation in yearlings and 8.6-8.9% saturation in fry (Tables A.5 and A.8). Fewer studies have examined variation hypoxia tolerance among strains in rainbow trout, but Scott *et al.,* (2015) assessed time to loss of equilibrium at 10% air saturation, which is conceptually similar to our analysis of ILOS. They found differences between the strains of about 10 minutes in time to loss of equilibrium (on average). Taking into account the oxygen ramping rate in our trials, the differences in ILOS among strains translate to approximately 10 minutes difference in time to LOE for yearlings and 3 minutes difference for fry. This relatively small difference suggests rainbow trout strains may be similar in their acute hypoxia tolerance.

In contrast to the limited differentiation among strains in upper thermal and hypoxia tolerance, I found substantial and consistent differences in post-trial mortality among our strains. The FV, PN, and CL strains exhibited little post-trial mortality (between 1-4% depending on the strains), the BW strain exhibited intermediate mortality (~20%), whereas the HF strain exhibited extreme mortality (50-60%) depending on the life stage. This high level of mortality is unusual following a CT_{max} trial, as post-trial mortality is generally thought to be low (around 1-5%) in fish (Anttila *et al.*, 2013; Scott *et al.*, 2015; Morgan *et al.*, 2018; Zhang *et al.*, 2018b; Joyce and Perry, 2020). The main difference between our study and most others is that our fish were exposed to a hypoxia tolerance trial 2-3 weeks previous to the measurement of upper thermal, which might contribute to additional mortality. However, this cannot explain the substantial differences in mortality among strains. In general, the HF strain was a poor performer in all of the metrics I assessed, with relatively low CT_{max} and high ILOS (Figure 2.2 and Table A.8), although these differences were much less clear than the differences in post-trial mortality.

It is possible that the relatively poor performance of the HF strain at high temperature or low oxygen is a consequence of local adaptation to its native environment. HF spawn in the lower Horsefly River, but reside in Quesnel Lake, a very deep (~157m average depth) fjord lake which rarely experiences temperatures > 18°C (Petticrew *et al.*, 2015; Stiff *et al.*, 2018), while the other strains originate from or are kept as broodstock in lakes that regularly experience temperatures > 22°C with temperatures exceeding 25°C in some (data not shown). Furthermore, deep water in Quesnel Lake is near full air saturation (James *et al.*, 2004), therefore HF trout rarely experience extreme thermal or hypoxia exposure and may be less able to cope with these stressors, potentially explaining their high rates of mortality. Examination of the genetic differences between the strains may help to elucidate a molecular mechanism for differences in

recovery from extreme thermal stress. Overall, these data clearly demonstrate that measurements of ILOS or CT_{max} do not fully capture the variation in sensitivity among strains. These data add to the growing consensus that more nuanced approaches are required to assess the thermal niche of fish strains in the context of climate change (McKenzie *et al.*, 2016; Åsheim *et al.*, 2020).

In contrast to the limited differentiation among strains in CT_{max} and ILOS, there was substantial variation in these tolerances within strains. All strains were raised from fertilization in similar conditions and these inter-individual differences in CT_{max} and ILOS may be reflective of genetic differences (Garland and Adolph, 1991). Future examination of genetic variation across individuals may be able to establish the genetic architecture of these tolerances. Furthermore, if this variation is genetic then it may represent the standing genetic variation with which rainbow trout could adapt to future warming and declines in dissolved oxygen (Hoffmann and Sgró, 2011).

Our data suggests that there is little to no relationship between whole-animal upper thermal (assessed as CTmax) and hypoxia tolerance (assessed as ILOS) when compared across individuals within a strain. This is in contrast to previous studies, which have indicated that there is a relationship between these traits in salmonids (Anttila *et al.*, 2013; Zhang *et al.*, 2018b); however, these previous studies compared rank order across families or strain rather than across individuals. Similar to our findings, Joyce and Perry (2020) found that CT_{max} was not correlated with hypoxia tolerance across individuals in zebrafish, further supporting the lack of direct mechanistic linkage between these traits. Indeed, evidence is accumulating that the mechanisms underlying acute upper thermal and hypoxia tolerance are likely very different (Jutfelt *et al.*, 2019; Mandic *et al.*, 2020). The knockout of HIF-1α in zebrafish, a protein implicated in cellular metabolism (Semenza, 2012), clearly shows this difference as it affects hypoxia tolerance but not

thermal tolerance (Mandic *et al.*, 2020). It is likely that failure of neurological mechanisms, not oxygen transport (Joyce and Perry, 2020), may be responsible for setting acute thermal tolerance (Jutfelt *et al.*, 2019), whereas oxygen transport processes or metabolic regulation are likely critical in determining hypoxia tolerance.

Natural environments are complex and involve changes in multiple interacting stressors. It is becoming increasingly common to measure multiple traits in individual fish to obtain a multifaceted view of the responses of organisms to the environment (Gunderson *et al.*, 2016; Åsheim *et al.*, 2020; Joyce and Perry, 2020; Nudds *et al.*, 2020). However, measuring multiple traits in single individuals results in logistical challenges as prior exposure to a stressful environment has the potential to alter subsequent tolerance, either reducing tolerance due to accumulation of cellular or organismal damage or improving tolerance through phenomena such as heat-hardening and cross-tolerance (Todgham *et al.*, 2005; McBryan *et al.*, 2013; Morgan *et al.*, 2018; McArley *et al.*, 2020). For example, Todgham *et al.,* (2005) found that prior exposure to heat shock improved hypoxia tolerance in fish. However, McArley *et al.*, (2020) found opposite results, with a longer heat-shock impairing subsequent hypoxia tolerance. Similarly, it has been suggested that the interaction between temperature and hypoxia can impair tolerance (McBryan *et al.*, 2013). Few, if any, studies have examined the reciprocal effects of previous temperature or hypoxia exposure on these respective tolerances. Here, I show no effect of prior exposure to hypoxia on upper thermal tolerance, but significant decreases in hypoxia tolerance if individuals had previously experienced a thermal tolerance trial (Figure 2.4). These results emphasize the importance of careful experimental design in studies assessing multiple tolerance metrics in individual fish and provide important lessons for the design of studies in conservation physiology going forward.

2.4.1 Conclusion

Incorporating physiological information into fisheries management strategies is increasingly important in the context of the effects of anthropogenic climate change (Ficke *et al.*, 2007; Madliger *et al.*, 2016; McKenzie *et al.*, 2016). Moreover, it is becoming increasingly common for fishery and conservation managers to use physiological measurements to examine strain, individual, and species-specific responses (Harrod, 2016; Madliger *et al.*, 2016). Here I show that different metrics of tolerance (e.g. hypoxia or upper thermal tolerance vs survival) provide different information. This observation has important direct implications for the management of rainbow trout, an economically important freshwater fish, but are also generalizable across fish species and indeed are likely to be the case for the assessment of climate change resilience in a wide variety of organisms. Another important lesson from the data presented here is the power that can be obtained using a common garden approach and very large sample sizes. Taken together, these results are an important additional input into the multi-faceted decision-making process required to plan stocking programs in the face of climate change and to conserve strains of this important recreational fish species (Madliger *et al.*, 2016; McKenzie *et al.*, 2016; Reid *et al.*, 2019).

Table 2.1 Trial dates, sample sizes and trial numbers for all strains in the 2017 brood year

Strain	Trial dates (fry)	Sample size (fry)		Trial dates (yearling)	Sample size (yearling)	
		Hypoxia	Thermal		Hypoxia	Thermal
Blackwater River (BW)	**Thermal:** Nov./Dec. 2017	1 trial 100 fish/trial n = 100	2 trials 50 fish/trial n = 100	Apr./May 2018	5 trials 99-101 fish/trial n = 500	5 trials 99-100 fish/trial n = 500
	Hypoxia: Feb. 2018					
Carp Lake (CL)	**Thermal:** Nov./Dec. 2017	1 trial 99 fish/trial n = 99	2 trials 50 fish/trial n = 100	Apr./May 2018	4 trials 100 fish/trial n = 400	4 trials 100 fish/trial n = 400
	Hypoxia: Feb. 2018					
Fraser Valley (FV)	June 2018	1 trial 99 fish/trial n = 99	1 trial 99 fish/trial n = 99	Aug. 2018	5 trials 99-100 fish/trial n = 500	5 trials 99-100 fish/trial n = 500
Pennask Lake (PN)	N/A	N/A	N/A	June 2018	1 trial 100 fish/trial n = 100	1 trial 97 fish/trial n = 97

Table 2.2 Trial dates, sample sizes and trial numbers for all strains in the 2018 brood year

Strain	Trial dates (Fry)	Sample size (fry)		Trial dates (Yearling)	Sample size (yearling)	
		Hypoxia	Thermal		Hypoxia	Thermal
Blackwater River (BW)	Oct. - Dec. 2018	14 trials 28-38 fish/trial n=489	14 trials 25-37 fish/trial n=436	Apr./May 2019	3 trials 33-34 fish/trial n = 100	3 trials 33-34 fish/trial n = 100
Carp Lake (CL)	Oct - Dec. 2018	14 trials 29-34 fish/trial n=487	14 trials 28-34 fish/trial n=425	Apr./May 2019	3 trials 33-34 fish/trial n = 100	3 trials 33-34 fish/trial n = 100
Horsefly River (HF)	Oct - Dec. 2018	14 trials 30-36 fish/trial n=497	14 trials 28-36 fish/trial n=453	Apr./May 2019	3 trials 33-34 fish/trial n = 100	3 trials 33 fish/trial n = 99
Pennask Lake (PN)	Feb. 2019	5 trials 100 fish/trial n=500	5 trials 97-100 fish/trial n=495	N/A	N/A	N/A

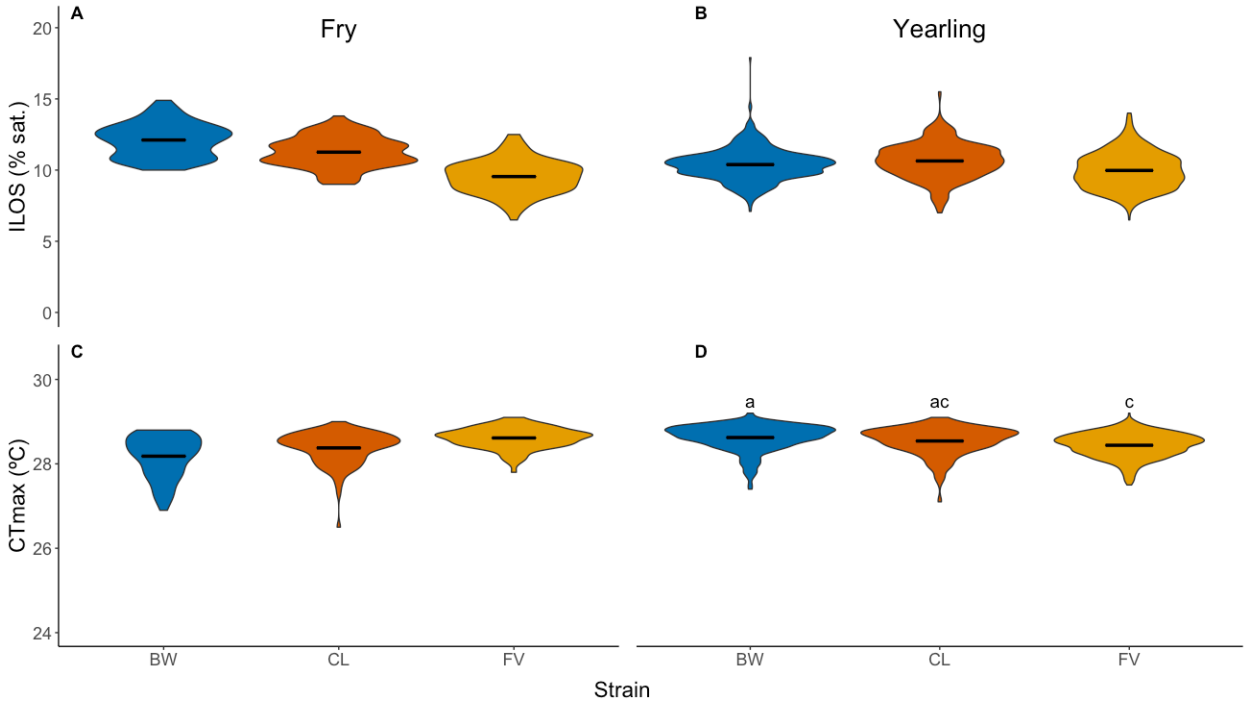

Figure 2.1 Variation in hypoxia tolerance (incipient lethal oxygen saturation (ILOS; **A** and **B**)) and upper thermal tolerance (CT_{max}; **C** and **D**) for the 2017 brood year (Fry; **A** and **C**; Yearling **B** and **D**).
Differences between strains were not statistically compared for fry because tolerance of each strain was assessed separately in either one or two trials (Table 2.1). For yearlings, where multiple trials were performed for each strain, data were analyzed using nested linear mixed effect models followed by Tukey pairwise comparisons ($\alpha = 0.05$). Significant differences are indicated by dissimilar letters. For sample sizes see Table 2.1. Data for the PN (yearling) are presented in Table A.5.

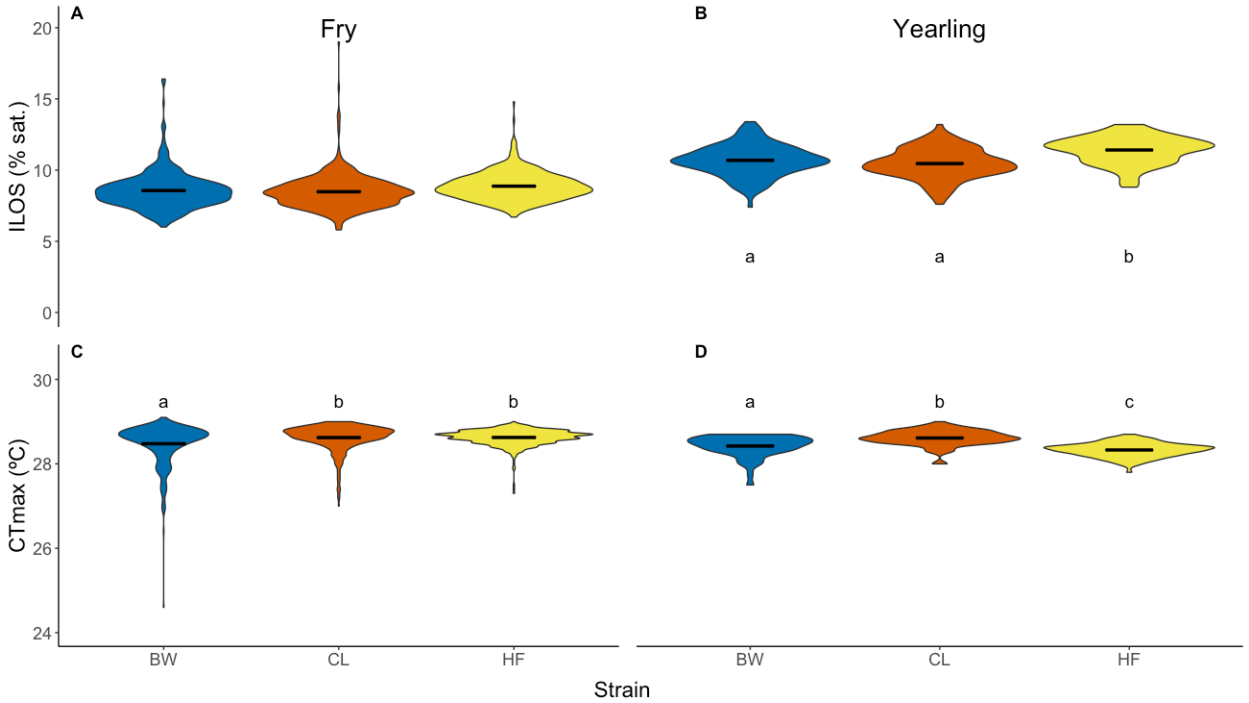

Figure 2.2 Hypoxia tolerance (incipient lethal oxygen saturation (ILOS; **A** and **B**)) and upper thermal tolerance (CT_{max}; **C** and **D**) for the 2018 brood year (Fry; **A** and **C**; Yearling **B** and **D**). Significant differences between strains are indicated by dissimilar letters. All data were analyzed using linear mixed effects models with Tukey pairwise comparisons ($\alpha = 0.05$). See Table 2.2 for sample sizes. Data for the PN (fry) are presented in Table A.8.

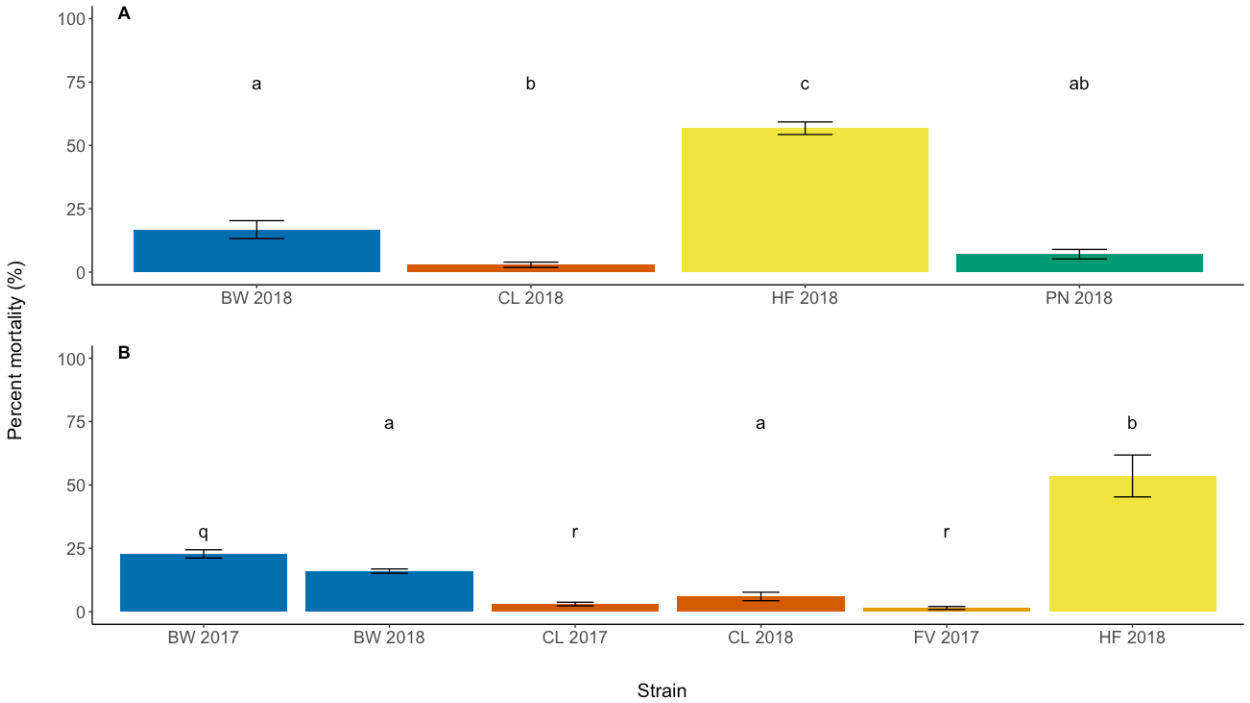

Figure 2.3 Percent mortality in fish that had experienced a hypoxia tolerance trial followed by an upper thermal tolerance trial at the fry (**A**) and yearling (**B**) life-stage.
Values are means across multiple trials ± SEM. At the fry life-stage (**A**), number of trials = 5 for PN, 14 trials for BW CL and HF. At the yearling life-stage (**B**), number of trials = 5 for BW and FV 2017, 4 for CL 2017, and 3 trials for BW, CL and HF 2018. Significant differences between strains are indicated by dissimilar letters, with q-r for the 2017 brood year and a-c for the 2018 brood year. Differences in mortality within a brood year were analyzed by one-way ANOVA with Tukey-HSD pairwise comparisons ($\alpha = 0.05$).

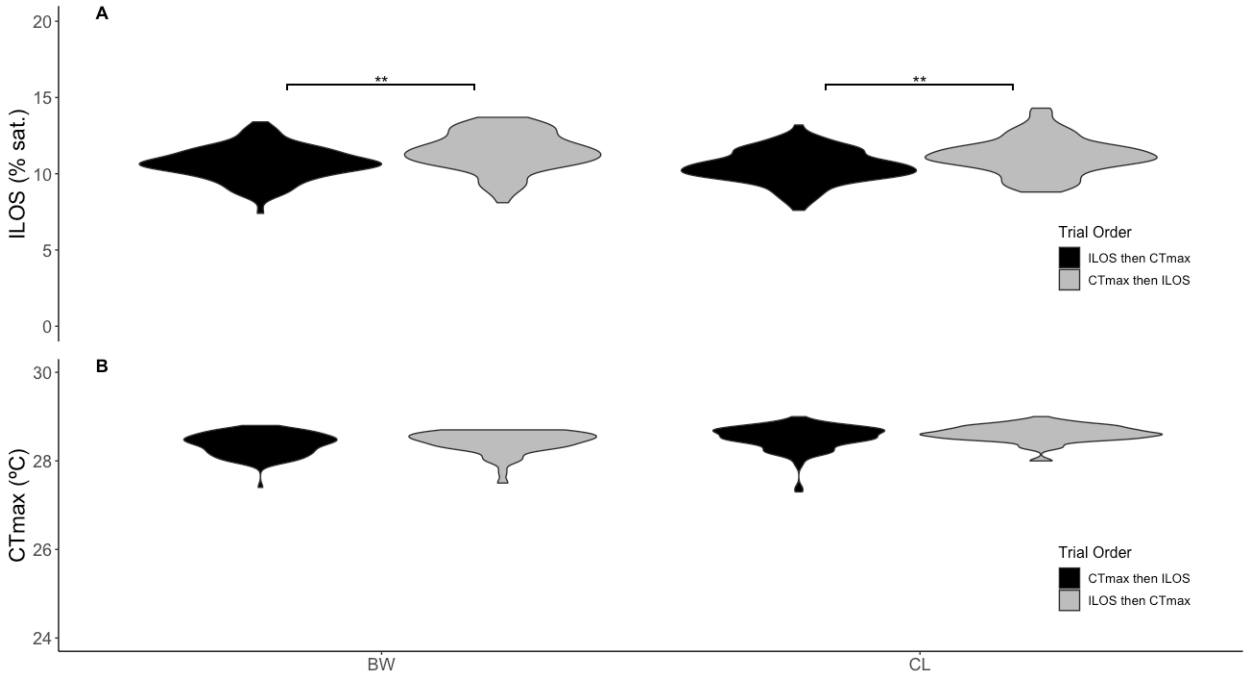

Figure 2.4 Trial order effect on critical upper thermal maximum (CT_{max}; **A)** and incipient lethal oxygen saturation (ILOS; **B**) for BW and CL 2018 brood yearling.
Sample sizes are as follows: **BW** (ILOS): ILOS then CT_{max}, n = 100, CT_{max} then ILOS, n = 87. **CL** (ILOS): ILOS then CT_{max}, n = 100, CT_{max} then ILOS, n = 99. **BW** (CT_{max}): n = 100 for both trial orders. **CL** (CT_{max}): n = 100 for both trial orders. Significant differences between trial orders are indicated by an asterisk (*). Trial order effect on ILOS and CT_{max} was analyzed using linear mixed effects models with Tukey pairwise comparisons ($\alpha = 0.05$). Note that data for trials with ILOS then CT_{max} are the same as those shown in Figure 2.2.

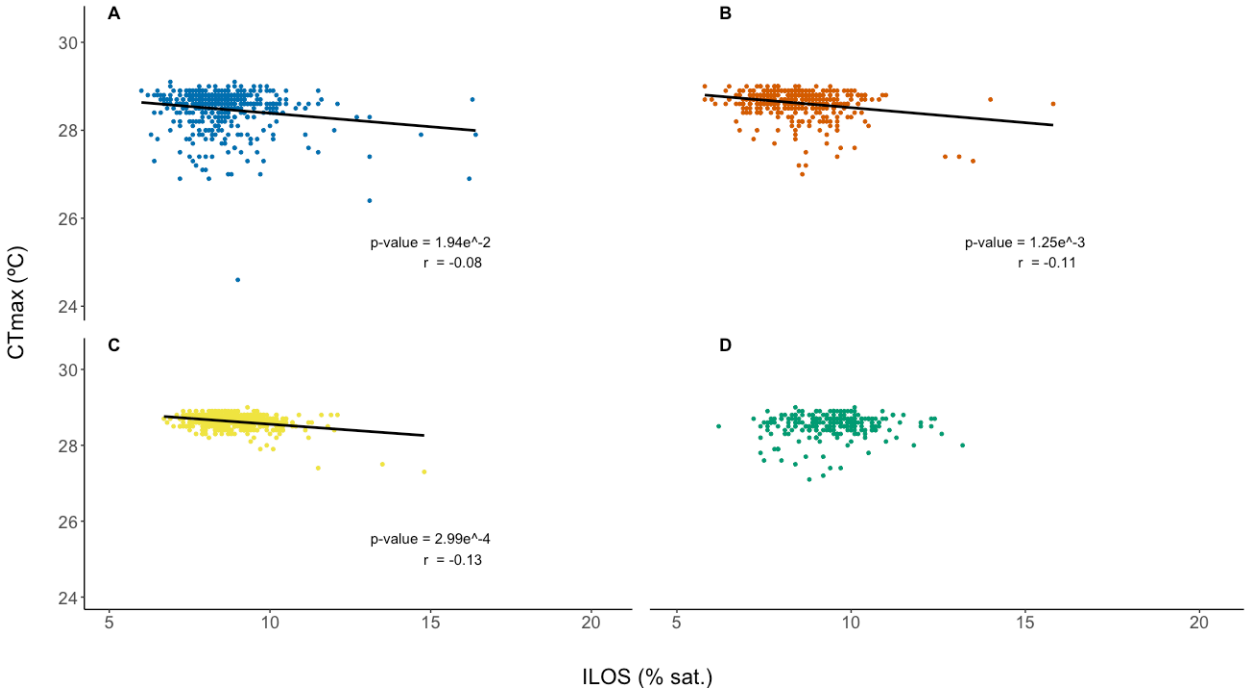

Figure 2.5 Correlation between critical thermal maximum (CT_{max}) and incipient lethal oxygen saturation (ILOS) for the fry life-stage for 2018 brood.
A BW, **B** CL, **C** HF, **D** PN. Sample sizes are as follows: **BW:** n = 435, **CL:** n = 426, **HF:** n = 453, **PN:** n = 253. All correlations were analyzed using Kendall rank or Pearson correlation (α = 0.05).

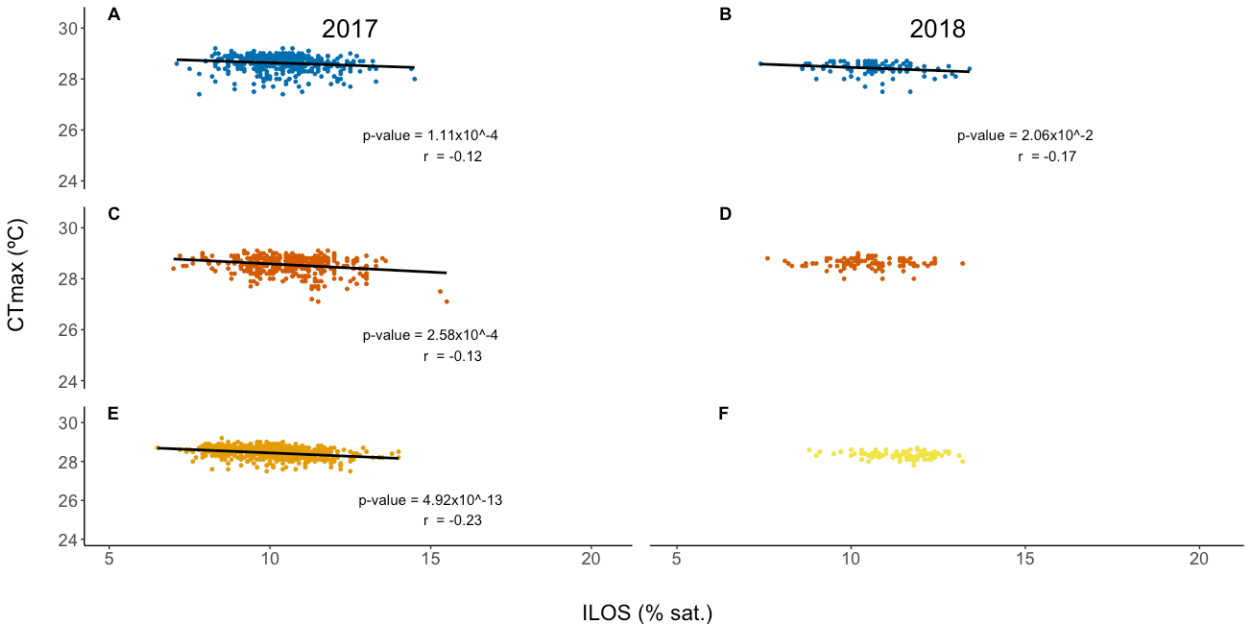

Figure 2.6 Correlation between critical thermal maximum (CT_{max}) and incipient lethal oxygen saturation (ILOS) for multiple strains across two brood years (2017, 2018) at the yearling life-stage.

A, B BW, **C,D** CL, **E** FV, **F** HF. Sample sizes are as follows: **BW (2017):** n = 499, **BW (2018):** n = 100, **CL (2017):** n = 400, **CL (2018):** n = 100, **FV:** n = 491, **HF:** n = 99. All correlations were analyzed using Kendall rank or Pearson correlation ($\alpha = 0.05$).

Chapter 3: Climate warming will test the limits of thermal plasticity in a globally distributed fish

3.1 Introduction

Global temperatures will continue to rise through the mid-21st century under all emission scenarios considered by the Intergovernmental Panel on Climate Change (IPCC, 2014). These increases in temperature also lead to decreases in dissolved oxygen (DO) within aquatic ecosystems, further challenging the survival of aquatic organisms (Ficke *et al.*, 2007). Together, these changes are having and will continue to have negative effects on biodiversity, particularly in freshwater ecosystems, which are experiencing extinctions at 4-6 times higher rates than terrestrial or marine habitats (Jenkins, 2003; Comte and Olden, 2017). To cope with rapid changes in global and local temperatures as well as concomitant changes in DO in aquatic systems, organisms have three options if they are to avoid local extinction: they can emigrate, adapt *in situ* or respond with plastic change (Fuller *et al.*, 2010; Somero, 2010). Indeed, organisms already appear to be escaping anthropogenic warming by either moving northwards or increasing their elevation to seek cooler habitats (Chen, et al., 2011). However, many organisms, and particularly those in freshwater ecosystems, cannot move to alternative habitats (Strayer and Dudgeon, 2010; Reid *et al.*, 2019). In addition, climate change may be proceeding too rapidly for many organisms to track environmental changes through adaptation (Visser, 2008; Radchuk, 2019). Thus the ability to recruit plastic responses may be an important determinant of organismal responses to anthropogenic environmental change (Somero, 2010; Swain *et al.*, 2018; Fox *et al.*, 2019).

Past research in many fish species has shown that phenotypic plasticity can markedly improve whole-animal tolerance to low oxygen (hypoxia) and high temperature. Yet, limits to this beneficial plasticity exist (Myrick and Cech, 2000; Fangue *et al.*, 2014; Sandblom *et al.*, 2016), and understanding these limits will likely be crucial for predicting how organisms will respond to rapid environmental change. What remains unclear is whether the limits of whole-animal plasticity reflect the failure of individual critical processes or limits that are integrated across levels of biological organization. Consequently, assessing plasticity across multiple biological levels within a single study, from the whole-animal through organ systems to individual tissues and gene expression is essential to determine the extent to which a species or population is likely to be a "winner" or a "loser" as climate change progresses (Somero, 2010).

To this end, I examined the thermal plasticity of two strains of rainbow trout (*Oncorhynchus mykiss)* across multiple levels of biological organization at a range of ecologically relevant temperatures. Rainbow trout, despite their native range being almost entirely in Western North America, are now the most commonly stocked freshwater fish on Earth. They are currently stocked, invasive, or farmed on all continents except Antarctica (Crawford and Muir, 2008). While their native range is contracting in response to habitat loss and climate change (Moyle, et al., 2017), hundreds of millions of rainbow trout are stocked globally each year and they are on the International Union for Conservation of Nature's top 100 list of most invasive species (Lowe *et al.*, 2000). Thus, we focus on rainbow trout because of their great ecological, economic, and cultural importance.

I hypothesized that despite their substantial capacity for acclimation, rainbow trout would exhibit upper limits to plasticity across multiple levels of biological organization at temperatures that are becoming increasingly common as climate change progresses. Beginning at the whole-

animal level, I estimated whole-animal thermal tolerance as critical thermal maximum (CT_{max}), which is the maximum temperature at which a fish can maintain equilibrium in response to acute temperature increases (Becker and Genoway, 1979), at three acclimation temperatures (12, 18 and 24°C), that span an ecologically relevant range. Furthermore, I assessed hypoxia tolerance at these acclimation temperatures, using an acute hypoxia challenge test (HCT) to measure incipient lethal oxygen saturation (ILOS), or the oxygen saturation at which a fish loses its ability to maintain equilibrium.

Shifts in whole-animal tolerances have been associated with metabolic and cardiorespiratory plasticity (Logan and Somero, 2010; Anttila *et al.*, 2014a; Logan and Buckley, 2015; Muñoz *et al.*, 2015). Therefore, I worked collaboratively to assess whole-animal metabolic capacity for acclimation through the measurement of standard metabolic rate (SMR) and maximum metabolic rate (MMR). These measures were then used to calculate absolute aerobic scope (AAS), or an animal's capacity to increase oxygen consumption beyond baseline requirements. To further examine the effects of hypoxia on the whole-animal, we also examined O_{2crit}, or the oxygen saturation at which fish are no longer able to maintain their SMR, a measure of the minimum amount of oxygen needed to sustain basic functions. To understand recovery from hypoxia, a measure of oxygen consumption during the recovery period following extreme exercise (the excess post-exercise oxygen consumption, EPOC) was also assessed across acclimation to estimate the oxygen consumption that is needed to restore physiological systems to homeostasis (Gaesser and Brooks, 1984; Zhang *et al.*, 2018a). At the organ system and tissue level, we assessed cardiac capacity for thermal acclimation through the measurement of maximum heart rate in warming, identifying several measures relevant to cardiac function such as peak maximum heart rate, and temperature at peak heart rate and arrhythmia. Previously

documented plasticity in these measures is associated with changes in gene expression (Veilleux *et al.*, 2015). Therefore, we examined the transcriptomic response to acclimation in the heart. As plasticity at lower levels of organization can reveal mechanisms underlying responses at higher levels (Bartholomew, 1982), our approach of assessing plasticity across multiple biological levels within a single study, from the whole-animal to organ systems, individual tissues, and gene expression, is crucial to understanding how organisms will respond to rapid environmental change. Furthermore, understanding the limits of plasticity and their mechanistic basis, has the potential to advance our ability to predict how rainbow trout and other fish species will respond to anthropogenic climate change.

3.2 Methods and materials

3.2.1 Experimental animals and acclimations

Two strains of rainbow trout currently used in California stocking programs were used in these experiments, including the Shasta strain from the American River Hatchery (Gold River, CA), a hatchery strain originating from a cross between the Hot Creek strain and Meader's Trout farm rainbow trout and the Coleman strain from the Moccasin Creek Hatchery (Moccasin, CA), a hatchery strain originating from the Central Valley in California (Busack and Gall, 1980; Garza and Pearse, 2008). Fish from the Shasta and Coleman strains differed in size by 10-fold (~ 50 vs ~ 5 g; Table 3.1). All sample sizes and fish mass and length for each experiment can be found in Table 3.1.

All rainbow trout were held at 11°C ± 1°C prior to experimental acclimation. Starting at 11°C temperature was increased by 1.5°C·day^{-1} till the temperature reached 12°C for the lowest acclimation temperature group, 18°C for the intermediate temperature group, and 24°C for the highest temperature group. Once the respective acclimation temperature was achieved the fish

were held at their acclimation temperature for three weeks prior to experimentation. These acclimation temperatures were selected because they represent current late winter/early spring mean (12°C), spring high/summer mean (18°C), and summer extreme (24°C) temperatures for central and southern California freshwater ecosystems (Verhille *et al.*, 2016; California Department of Fish & Wildlife, 2018; Ullrich *et al.*, 2018).

3.2.2 Whole-animal tolerance

At the beginning of each whole-animal tolerance trial fish were starved for 48 h in separate tanks before transfer to an experimental tank, where they were left undisturbed for 30 min before sampling or experimentation.

3.2.2.1 Upper thermal tolerance (Critical thermal maximum, CT_{max})

A CT_{max} trial began at the intermediate acclimation temperature (18°C). An aeration bar and circulation pumps were placed in the experimental tank to maintain > 75% air saturation and to prevent thermal stratification of the water. As previously described (section 2.2.3; Beitinger *et al.*, 2000), the water temperature was progressively increased by 0.1 °C min^{-1} until the fish was unable to maintain dorsoventral orientation (i.e., loss of equilibrium, LOE, which represents an inability to escape a life-threatening circumstance; Cowles, R.B., Bogert, 1944; Beitinger *et al.*, 2000). CT_{max} was determined as the temperature (°C) at LOE. After LOE the fish was removed from the experimental tank, euthanized, and measured for length and weight.

3.2.2.2 Hypoxia challenge test (Incipient lethal oxygen saturation, ILOS)

For hypoxia tolerance assessment, trout were either acclimated to the test temperature ("Acclimated" in Figure 3.1) or acclimated to 12°C and acutely warmed to 18 °C over one hour or 24°C over two hours to reveal any effect of warm acclimation ("Acute" in Figure 3.1). Water temperature for the hypoxia challenge test was maintained at the relevant temperature ± 1°C

throughout the trial. As previously described (section 2.2.2; Claireaux *et al.*, 2013) after fish were introduced into the experimental tank, dissolved oxygen (DO) was progressively decreased at a rate of ~1.5% air saturation min^{-1} from ~90% air sat. to ~20% air saturation by bubbling nitrogen into the experimental tank. Small circulation pumps in the experimental tank prevented water stratification during the imposed hypoxia. At ~20% air saturation the ramping rate was reduced to 0.1% air saturation min^{-1} until the fish experienced LOE. Hypoxia tolerance was determined as the air saturation (% air sat.) at LOE, or ILOS. After LOE the fish was removed from the experimental tank, euthanized, and measured for length and weight.

Differences in CT_{max} and ILOS among acclimation and/or test temperatures within a strain were assessed by ANOVA and Holm-Sidak adjusted pairwise comparisons. Differences between acutely warmed (see above) and acclimated ILOS within a test temperature were assessed by ANOVA and Holm-Sidak adjusted pairwise comparisons.

3.2.3 Respirometry

3.2.3.1 Standardized protocol for Integrated Respiratory Assessment Paradigm (IRAP)

The integrated respiratory assessment paradigm (IRAP) is a standardized experimental protocol that involves continuously monitoring $\dot{M}O_2$ over 3 days using automated intermittent-flow respirometry (Zhang et al., 2017). We simultaneously tested seven fish that were housed in individual respirometry chambers. The size of the chamber was matched to the fish size (water volume : fish ratio = 50:1 (Coleman) & 27:1 (Shasta)) to optimize detection sensitivity for the change in water DO inside of the respirometer due to fish oxygen uptake when the respirometer was in the closed mode (Claireaux *et al.*, 2013). All eight chambers (seven chambers containing fish and one blank chamber for background consumption) were immersed in a ~300 L bath

controlled at the acclimation temperature which was connected via a pump to a gas exchange column that delivered aerated water to the respirometers in the open mode. DO was maintained above 70 % saturation throughout the protocol except during a hypoxia challenge test (HCT). For a HCT, a controlled injection of nitrogen rather than air into the bottom of the exchange column progressively decreased DO in the outer bath in a controlled fashion.

All fish were fasted for at least 48 h before being placed in the respirometer, where oxygen uptake rate ($\dot{M}O_2$) was continuously monitored for at least 3 days during three states. The IRAP protocol started by individually chasing a fish to exhaustion. Fish were individually chased to exhaustion by hand in a 20 L bucket and given a standardized 1-min air exposure during the transfer to the respirometer. An $\dot{M}O_2$ measurement cycle consisted of flush, stabilization, and measurement periods. Only oxygen saturation values obtained during the measurement periods were used to calculate $\dot{M}O_2$. Oxygen saturation was then monitored using a measuring cycle comprised of a 20 s flush, a 40 s stabilization, and a 120 s measurement at the first 2 h to capture the maximum $\dot{M}O_2$ (MMR). By monitoring post-exhaustion $\dot{M}O_2$ over a 16-h recovery period under normoxic conditions estimates of MMR and excess post-exercise oxygen consumption (EPOC) with DO were generated.

As $\dot{M}O_2$ decreased toward SMR, the measurement cycle was changed to a 75 s flush, a 120 s stabilization and a 780 s measurement for Coleman stain and 90 s flush, a 95 s stabilization, and a 360 s measurement. This is to guarantee that oxygen saturation was 97% at the start of the measurement period, which assures a strong signal to noise ratio. Monitoring of $\dot{M}O_2$ then continued in an undisturbed, normoxic fish for at least a 2-day quiescent period to generate a reliable estimate of SMR and some behaviour-related traits (*e.g.* RMR). These

measurements of MMR and SMR were used to derive absolute aerobic scope (AAS) to quantify aerobic capacity (Zhang *et al.,* 2016).

The IRAP protocol ended with an HCT to assess hypoxia tolerance by estimating O_{2crit} and determining ILOS (the DO at a loss of equilibrium (Claireaux *et al.*, 2013). In HCT, each flushing cycle with deoxygenated water made the fish progressively hypoxic. Water DO in the outer bath was reduced over a 45 min period to a moderate level of hypoxia for that species (*i.e.* about twice O_{2crit}). The rate of deoxygenation then slowed (0.15 ± 0.02% sat. min^{-1}) until the fish lost its dorso-ventral equilibrium (LOE) to measure incipient lethal oxygen saturation (ILOS), after which the protocol was terminated. Fish were typically removed immediately from the respirometer chamber, recovered in fully aerated freshwater, and placed in a recovery tank for assessment prior to being returned to the holding tank. Background respiration of each empty respirometer chamber was measured throughout the experiment. The fish's $\dot{M}O_2$ was corrected by subtracting the background $\dot{M}O_2$ value. Background respiration was minimized by thoroughly disinfecting the entire apparatus with diluted household bleach for 30 min between IRAP trials.

3.2.3.2 Calculation $\dot{M}O_2$ and other IRAP indices

$\dot{M}O_2$ was continuously and automatically monitored using water DO measurements in the respirometers (a 1 Hz sampling rate) from an optical oxygen probe associated with each respirometer. The optodes were calibrated to 0% saturation (water saturated with sodium sulphite and bubbled with nitrogen gas) and 100% saturation (fully aerated water) at the start of each experiment.

Maximum oxygen uptake (MMR), was assigned to the peak $\dot{M}O_2$, which typically occurred during the first measurement cycle post-exhaustion. A sequential interval (120 s) regression algorithm was used to assign the highest $\dot{M}O_2$ value (provided R^2 is >0.9). MMR was

calculated after a conventional exhaustive chase outside the respirometer protocol using Eqn 1. The slope of the decrease in DO over time met a minimum requirement for linearity (*i.e.*, $R^2 > 0.95$) to calculate $\dot{M}O_2$ (a higher standard than the manufacturer's software).

$$\dot{M}O_2 = \left[\frac{d_{DO_{[i,(i+a)]}}}{d_{t_{[i,(i+a)]}}} * \left(V_r - V_f\right) * S_O\right] / (t * M_f) \qquad \text{Eqn. 1}$$

Where units for $\dot{M}O_2$ are mg O_2 h^{-1} kg^{-1}, d_{DO}/d_t is the change in O_2 saturation over time, V_r is the respirometer volume, V_f is the assumed fish volume, S_o is the solubility of O_2 in freshwater at 14°C at 1 atm, t is a time constant of 3600 s h^{-1}, M_f is fish mass, a is the sampling window duration (s), i is 1 DO sample forward from the end of previous sampling window, and n is 1 DO increment from the first DO measurement at a sampling frequency of 1 Hz.

SMR, the minimum maintenance metabolic rate, was calculated with an established quartile method (Chabot *et al.*, 2016), which reports SMR as the 20% quartile (q0.2) of all $\dot{M}O_2$ values recorded for two diurnal cycles after the first 12-h recovery period. Typically, SMR was estimated from > 300 $\dot{M}O_2$ values and therefore provided a reliable baseline for calculating other IRAP indices (AAS, total EPOC, and O_{2crit}). Aerobic capacity was assessed using two derived indices: absolute aerobic scope (AAS = MMR – SMR, Claireaux et al., 2005). Excess post-exercise oxygen consumption (EPOC), a measure of effort during exhaustive swimming, is the sum of all $\dot{M}O_2$ values less SMR during the entire recovery EPOC period – until $\dot{M}O_2$ first returned to SMR plus 10% (Zhang, et al., 2018).

The HCT generated two indices to assess anaerobic performance and anaerobic capacity with nearly zero mortality rate. The slower phase of progressive deoxygenation generated at least

10 $\dot{M}O_2$ data points before O_{2crit} (Claireaux *et al.*, 2013). A linear regression applied to these data allowed off-line determination of O_{2crit} (the DO when the regression line intercepts SMR; Claireaux & Chabot, 2016) Slope validation required that the line did not pass below any $\dot{M}O_2$ recorded in normoxia and the intercept at the y-axis was negative (Claireaux & Chabot, 2016).

All metabolic capacity metrics (SMR, MMR, AAS, O_{2crit}, EPOC) were analyzed by ANOVA with Holm-Sidak corrected pairwise comparisons.

3.2.4 Maximum heart rate and cardiac thermal tolerance

Cardiac performance during acute warming was assessed in both strains in anesthetized fish injected with two drugs to induce a maximum heart rate (f_{Hmax}). Fish were warmed until f_{Hmax} reached a peak or plateau and then the heart lost rhythmicity, as previously described (Chen *et al.*, 2015). Specifically, fish were anesthetized to stage III anesthesia (as per Canadian Animal Care Committee definitions) with Tricaine methanesulfonate (MS-222) (150 mg/L) and were transferred to a bath containing a maintenance dose of anesthetic (60 mg L^{-1} MS-222) that continuously irrigated the gills throughout the warming trial. Fish were restrained in individual foam troughs to maintain their position while two electrodes were placed ventrally over the heart, one near the left pectoral fin and one slightly more anterior on the right side of the body. An intra-peritoneal injection of atropine and isoproterenol (1.2 mg kg^{-1} and 4 ug kg^{-1}, respectively, in a total volume of 3 ml kg^{-1} 0.8% NaCl solution) produced f_{Hmax}. Rainbow trout were acutely warmed from 12°C, 15 min after these injections, in 1°C increments every 6 min (10°C h^{-1}) until an arrhythmic heartbeat developed, at which point fish were euthanized with an overdose of MS-222 followed by pithing. All MS-222 solutions were buffered with $NaHCO_3$ (1:1.5). Automated heart beat detection (Labchart v.8, ADInstruments) was used to calculate f_{Hmax} for the final 1-min period of each temperature increment. The peak f_{Hmax} reached during

acute warming, the temperature at peak f_{Hmax} (T_{peak}), and the temperature at the first cardiac arrhythmia (T_{arr}) were assessed as previously described (Chen *et al.,* 2015). The scope for f_{Hmax} during warming was calculated as the difference between f_{Hmax} at the initial test temperature (12°C) and peak f_{Hmax}. Differences in f_{Hmax} scope, peak f_{Hmax}, T_{peak}, and T_{arr} among acclimation temperatures within a strain were assessed by ANOVA and Holm-Sidak adjusted pairwise comparisons. Differences in f_{Hmax} during warming from 12-20°C between acclimation temperatures for each strain were assessed using linear mixed effect modelling (lme 4 package; (Bates *et al.*, 2015) followed by Holm adjusted post-hoc comparisons between acclimation temperatures (lsmeans package; Lenth, 2016).

3.2.5 Cardiac transcriptomics

Whole-transcriptome effects were examined in cardiac tissues of the Shasta and Coleman strains of rainbow trout after acclimation for three weeks at 12°C, 18°C, and 24°C. At each acclimation temperature, six individuals from each strain were sampled by removing the heart following euthanization and storing it in RNA*later*® (Sigma-Aldrich, St. Louis, Missouri). RNA was isolated using a combined TRIzol® Reagent (Invitrogen, Carlsbad, California) and RNeasy Mini Kit (QIAGEN, Hilden, Germany) protocol. Extracted RNA was then sent to Genome Québec Innovation Centre (Montréal, Québec) for library preparation and sequencing. The resulting raw reads were assessed for quality using FastQC (Andrews, 2020), rRNAs were removed using SortMeRNA (Kopylova *et al.*, 2012), and quality-base trimming and adapter removal was performed with Trimmomatic (Bolger *et al.*, 2014). Reads were then aligned to a pre-assembled *rainbow trout* transcriptome (assembly Omyk_1.0; https://www.ncbi.nlm.nih.gov/genome/196) using Bowtie2 (Langmead and Salzberg, 2012) and raw read counts per transcript per sample were generated using RSEM (Li and Dewey, 2011). Read counts were then

filtered to exclude any transcript for which an individual(s) showed expression counts less than 10 and tests of differential expression were conducted using edgeR (Robinson *et al.*, 2009; McCarthy *et al.*, 2012). Gene enrichment analysis was performed by comparing the list of genes differentially expressed between two temperature treatments within a given strain to the total list of genes in the dataset using the online software Gorilla (Eden *et al.*, 2009).

3.3 Results and discussion

3.3.1 Whole-animal tolerance to warming

To assess whole-animal tolerance to warming, I used two strains (Coleman and Shasta) of rainbow trout widely used for stocking rivers and lakes in California, a biogeographic region near the southern limit of the species' natural range. Consequently, these strains are likely warm-adapted relative to those with a more northern origin. I estimated whole-animal thermal tolerance as critical thermal maximum (CT_{max}) and although the direct ecological relevance of CT_{max} is uncertain (Terblanche *et al.*, 2011), the measure is correlated with tolerance to more ecologically relevant rates of warming (Åsheim *et al.*, 2020), with species biogeographic boundaries, and sensitivity to climate change in a variety of taxa (Sunday *et al.*, 2011, 2012, 2019; Dahlke *et al.*, 2020).

CT_{max} increased with increasing acclimation temperature in both strains (Figure 3.1a, ANOVA coleman: $p = 7.64 \times 10^{-7}$, Shasta: $p = 1.54 \times 10^{-7}$), consistent with the results of previous studies, which suggest the existence of substantial plasticity in CT_{max} in rainbow trout (Anttila *et al.*, 2014a; Logan and Buckley, 2015; Muñoz *et al.*, 2015; Veilleux *et al.*, 2015). The increase in thermal tolerance between temperatures was greater at lower temperatures, indicating that at an environmentally relevant temperature of 24°C, rainbow trout are reaching a ceiling to their plasticity in CT_{max}.

I also measured the oxygen saturation at which fish are unable to maintain equilibrium (ILOS) as an indicator of hypoxia tolerance. Similar to thermal tolerance, warm acclimation can improve hypoxia tolerance in fishes (McBryan *et al.*, 2016), although whether the limits to this plasticity are aligned to the limits of plasticity in thermal tolerance is not known. When 12°C-acclimated fish from both strains were acutely exposed to an increase in temperature, ILOS increased markedly (Figure 3.1b, open symbols ANOVA Coleman: $p = 2.63 \times 10^{-7}$, Shasta: $p = 2.62 \times 10^{-7}$), demonstrating that acute increases in temperature decrease hypoxia tolerance. However, when fish were acclimated to and tested at 18°C they maintained their equilibrium down to oxygen levels similar to those tolerated by fish tested at 12°C, indicating a complete compensation of hypoxia tolerance with warm acclimation (Figure 3.1b Coleman_{12v18}: $p = 0.004$, Shasta_{12v18}: $p = 0.15$). Fish acclimated to 24°C could not achieve this complete compensation (Figure 3.1b Coleman_{12v24}: $p = 9.30 \times 10^{-5}$, Shasta_{12v24}: $p = 1.10 \times 10^{-4}$), indicating a ceiling to plasticity in hypoxia tolerance that is aligned with the ceiling in the plasticity of thermal tolerance.

There was a 10-fold mass difference between strains in the individuals used here (Table 3.1), thus any comparison between strains should be done with caution (Nilsson and Östlund-Nilsson, 2008; Recsetar *et al.*, 2012; Roze *et al.*, 2013; Hines *et al.*, 2019). Despite this mass difference, the overall patterns of plasticity in CT_{max} and hypoxia tolerance were remarkably similar across strains. These data suggest that there are clear limits on plasticity in both thermal and hypoxia tolerance at the whole-animal level and that these limits occur at a temperature that is environmentally relevant under current conditions and that will become more prevalent as climate change progresses (Ullrich *et al.*, 2018).

3.3.2 Plasticity in aerobic and anaerobic performance

Aerobic and anaerobic performance are hypothesized to be key mechanisms underlying the ability of ectotherms to tolerate high temperature and low oxygen, and therefore I assessed the effects of thermal acclimation on these system-level traits (Portner and Farrell, 2008; Eliason *et al.*, 2011; Clark *et al.*, 2013; Pörtner *et al.*, 2017; Jutfelt *et al.*, 2018). Standard metabolic rate (SMR) increased exponentially with temperature in both strains (Figure 3.2a,b ANOVA Coleman: $p < 0.001$, Shasta: $p < 0.001$), as expected due to Arrhenius effects on metabolic processes (Schulte, 2015). In contrast, although maximum metabolic rate (MMR) significantly increased at the 18ºC acclimation temperature in both strains (Figure 3.2a,b ANOVA Coleman: $p < 0.001$, Shasta: $p < 0.017$), between the 18ºC and 24°C acclimation temperatures MMR either did not change significantly (Coleman_{18v24}: $p = 0.089$) or decreased (Shasta_{18v24}: $p = 0.013$). As a result, the absolute aerobic scope (AAS = SMR – MMR), which reflects the scope to perform critically important ecological activities such as foraging, was lower at the highest temperature tested, although this effect was only statistically significant for the Shasta strain (Figure 3.2a,b Coleman_{18v24}: $p = 0.282$, Shasta_{18v24}: $p < 0.001$). These data indicate that rainbow trout do not have sufficient plasticity to maintain their aerobic metabolic scope when acclimated to 24°C, and this was true in both strains despite differences in body size. While mass-specific SMR and MMR were lower in the Shasta compared to the Coleman strain (Mean over all temperatures of -48 and 23% for SMR and MMR respectively), this was likely a result of body size differences as metabolic rates typically scale with mass with a slope <1.0 (negative allometry; Wieser, 1985).

The effects of thermal acclimation were also examined for several measures of performance under oxygen limiting conditions. O_{2crit} increased with temperature in both strains (Figure 3.2c ANOVA Coleman: $p < 0.001$, Shasta: $p < 0.001$), with a sharp increase at higher temperatures ($Coleman_{18v24}$: $p < 0.001$, $Shasta_{18v24}$: $p < 0.001$) indicating that both strains are reaching a limit to plasticity in this trait.

Measures of oxygen consumption during the recovery period following extreme exercise, or EPOC, may in part provide an estimate of the extent to which anaerobic processes contributed to maximum performance (Gaesser and Brooks, 1984). In both strains, there was a substantial but non-significant increase in EPOC following intense exercise at 18°C relative to exercise at 12°C. However, EPOC declined appreciably from 18 to 24 °C (Figure 3.2d $Coleman_{18v24}$: $p = 0.023$, $Shasta_{18v24}$: $p = 0.003$). Together, these data indicate that even in California strains of rainbow trout, 24°C thermal plasticity is insufficient to compensate for heat-induced impairments in hypoxia tolerance, aerobic capacity, and glycolytic performance (Figure 3.2a-d).

3.3.3 Maximum heart rate and cardiac thermal tolerance

Fishes increase heart rate to elevate cardiac output when acutely warmed (Steinhausen, et al., 2008), a response that is essential to meeting elevated metabolic demands that arise at increased temperatures. However, maximum heart rate (f_{Hmax}) can only increase with temperature to a point, beyond which it peaks or plateaus (T_{peak}) and the heartbeat eventually becomes arrhythmic (T_{arr}), which then constrains aerobic capacity (Farrell, 2016). Here, consistent with observations in other fish species (Badr *et al.*, 2016; Drost *et al.*, 2016; Safi *et al.*, 2019), both T_{peak} and T_{arr} increased substantially (~5°C) with increasing acclimation temperature in both strains (Figure 3.3a-c, ANOVA post-hoc T_{arr}: $p < 0.001$, T_{peak}: $p \leq 0.027$); however, the

difference in T_{arr} between 18 and 24°C was not significant in the Shasta strain (Shasta $T_{arr18v24}$: $p = 0.080$).

Across moderate temperatures (~12-20°C), f_{Hmax} was progressively lower with increasing acclimation temperature in both strains (Figure 3.3a; $p < 0.001$). Resetting of f_{Hmax} to lower levels following warm acclimation (thermal compensation) has been previously documented in fish (Badr *et al.*, 2016; Drost *et al.*, 2016); however, the extent to which f_{Hmax} was lowered at intermediate temperatures in rainbow trout is notable (e.g. -33% at 12°C). Conversely, the peak f_{Hmax} attained during acute warming increased with acclimation temperature from 12 to 18°C ($Coleman_{12v18}$: $p = 0.006$, $Shasta_{12v18}$: $p = 0.008$) but then either decreased ($Shasta_{18v24}$: $p = 0.029$) or was unchanged from 18 to 24°C (Figure 3.3d; $Coleman_{18v24}$: $p = 0.303$). The generally lower f_{Hmax} values in Shasta relative to Coleman trout were likely a result of negative scaling of f_{Hmax} with body mass (Table 3.1; Anttila *et al.*, 2014b). For example, average f_{Hmax} between 12 and 20°C was ~12% lower in Shasta than Coleman trout (mass scaling coefficient = -0.05). The observed resetting of f_{Hmax} and changes in peak f_{Hmax} meant that the absolute scope to increase f_{Hmax} (f_{Hmax} scope) during warming above 12°C increased with acclimation temperature from 12 to 18°C (ANOVA post-hoc tests $p < 0.001$), but became constrained at 24°C in both strains (Figure 3.3e; $Coleman_{18v24}$: $p = 0.229$, $Shasta_{18v24}$: $p = 0.749$); however, the relative scope (fold change) increased progressively with acclimation temperature (Figure 3.3e; ANOVA post-hoc tests $p < 0.001$). Consequently, the observed compensation in f_{Hmax} with warm acclimation, which helped maintain or increase the f_{Hmax} scope, would likely allow fish to better balance increases in cardiac stroke volume and heart rate during routine exercise at a given acclimation temperature.

Together, the observed thermal plasticity in cardiac thermal tolerance suggests that it reaches a ceiling at temperatures similar to those for whole-animal hypoxia and heat tolerance, as most cardiac metrics (e.g. f_{Hmax} scope, peak f_{Hmax}, and T_{arr} in Shasta trout) had reached or exceeded their capacity for thermal acclimation by 24°C (Figure 3.3). For example, individual trout acclimated to 12°C typically exhibited cardiac arrhythmia or a decline in f_{Hmax} at a temperature below 24°C and this cardiac limitation would help explain the observed impairment of hypoxia tolerance of 12°C-acclimated fish tested at 24°C.

3.3.4 Cardiac gene expression

Our results at the whole-animal and tissue level strongly suggest there is a limit on the plasticity of cardiac processes that may be important in determining the performance of trout in a changing environment. To further investigate the underlying basis of these effects, I performed whole-transcriptome analysis on cardiac tissue of acclimated trout sampled at rest under normoxic conditions. A clear whole-transcriptome response to acclimation temperature emerged for both Coleman (Figure 3.4a) and Shasta (Figure 3.4b) strains.

When comparing the Coleman strain acclimated to 12°C to those acclimated to 18°C, 626 genes were differentially expressed following correction for false discovery rate (FDR). The significant enrichment of 59 gene ontology (GO) terms associated with cardiac acclimation included regulation of cardiac muscle cell membrane potential and regulation of ryanodine-sensitive calcium-release channel activity (Figure 3.4). Specifically, expression of ATP2A2 (or SERCA), an important calcium pump in heart tissue (Dally *et al.*, 2006), decreased significantly in fish acclimated to 18°C when compared to fish acclimated to 12°C (Figure B.1). In the Shasta strain, more than 10-times more genes (9,923) were differentially expressed between the 12°C and 18°C acclimation groups, with enriched GO terms (n=97; Figure 3.4) primarily involving

tricarboxylic acid metabolic processes with genes such as malate dehydrogenase and succinate dehydrogenase (Figure B.1). Together, these data suggest that both strains demonstrated putatively beneficial changes in the regulation of genes involved in metabolism with acclimation to 18°C, which would help maintain function at higher temperatures.

In contrast, acclimation to a higher temperature (24°C) revealed an entirely different pattern. For 24°C-acclimated Coleman fish compared with 18°C, the 2,006 differentially expressed transcripts were enriched for 126 GO terms, including terms associated with a cellular stress response such as glial cell proliferation (Figure 3.4). Here, the gene Clusterin, known to be up-regulated during a stress response (Li *et al.*, 2013), showed a significant increase in expression among fish acclimated to 24°C when compared to the 18°C acclimation group (Figure B.1). For 24°C-acclimated Shasta fish compared with 18°C, nearly 4-times more genes (7,933) were differentially expressed. They represented significant enrichment of 87 GO categories, including genes related to "nonsense-mediated decay" (Figure 3.4), a GO term that describes the accidental generation of a stop codon in the mRNA, resulting in premature termination of translation, and the production of potentially harmful proteins (Hentze and Kulozik, 1999). At the gene level, there was significant upregulation of the gene nucleoporin 133, which is known to play a role in the regulation of cellular response to heat (Figure B.1; The UniProt Consortium, 2019). Thus, at a high but environmentally relevant acclimation temperature, cardiac gene expression patterns are reflective of a cellular stress response.

3.3.5 Implications for natural strains and stocking programs in the Anthropocene

Our data clearly show that rainbow trout have substantial plasticity in whole-animal upper thermal and hypoxia tolerance, which reflects changes in whole-animal metabolism, cardiac function, and ventricular gene expression. However, across all levels of organization, I

identified a ceiling to plasticity at an ecologically relevant warm temperature (24°C). In their current natural and stocked range in California, strains of rainbow trout routinely experience temperatures that exceed 20°C, with many streams, rivers, and creeks exceeding 24°C during the summer (California Department of Fish & Wildlife, 2018). As we proceed through this time of climatic uncertainty, the thermal plasticity of rainbow trout will no longer be sufficient to maintain adequate performance of these fish in their natural habitat. This will likely limit the waterbodies that can sustain rainbow trout for management and recreational purposes. Notably, I focused on California strains of rainbow trout which should be amongst the most warm adapted (Verhille *et al.*, 2016). Less warm-adapted strains, such as those in the Pacific Northwest, would likely encounter similar limitations to plasticity but at lower temperatures. Thus, a strain's thermal physiology may become increasingly important for its use in aquaculture and stocking programs. This work clearly demonstrates the importance of integrating measurements of plasticity across many levels of biological organization for predicting species response to climate change (Mykles *et al.*, 2010). Understanding the limits to plasticity is critically important for managers of freshwater fisheries, as well as those who control water use for agriculture and human consumption (Quiñones and Moyle, 2015), as water-use management and fisheries protection increasingly come into conflict.

Table 3.1 Sample size (n), mass and length for fish used in each experiment within the study. Data are shown as mean ± s.e.m.

		Shasta			Coleman		
	Temperature (°C)	n	Mass (g)	Length (mm)	n	Mass (g)	Length (mm)
CT_{max}	12	12	43.2 ± 4.8	156.4 ± 6.3	12	4.3 ± 0.2	73.2 ± 1.5
	18	12	56.4 ± 4.2	177.8 ± 2.8	12	4.7 ± 0.4	74.7 ± 2.3
	24	12	51.9 ± 4.3	167.1 ± 5.0	12	4.5 ± 0.4	70.2 ± 1.5
ILOS	12	12	53.3 ± 5.2	170.0 ± 6.1	12	4.1 ± 0.2	70.9 ± 1.4
	18	12	55.9 ± 4.5	174.7 ± 4.9	12	6.3 ± 0.6	80.8 ± 2.1
	24	12	49.5 ± 4.2	164.3 ± 5.3	12	3.6 ± 0.4	65.6 ± 2.2
Acute ILOS	12-18	12	50.3 ± 4.8	167.6 ± 5.9	12	4.8 ± 0.3	77.0 ± 1.5
	12-24	12	63.4 ± 6.1	182.1 ± 5.9	12	5.4 ± 0.4	76.6 ± 2.2
IRAP	12	13	41.4 ± 2.0	-	13	4.2 ± 0.3	-
	18	14	49.2 ±2 .0	-	14	4.8 ± 0.3	-
	24	14	48.5 ± 2.0	-	12	4.3 ± 0.3	-
Cardiac thermal tolerance	12	8	39.8 ± 3.5	153.9 ± 5.6	11	4.6 ± 0.4	71.4 ± 2.1
	18	8	46.4 ± 5.7	163.9 ± 7.1	10	5.5 ± 0.6	73.8 ± 2.8
	24	8	47.2 ± 4.8	164.5 ± 5.6	8	4.6 ± 0.5	70.3 ± 2.4
Cardiac RNAseq	12	6	57.9 ± 5.9	173.8 ± 5.7	6	4.9 ± 0.7	72.2 ± 3.2
	18	6	51.7 ± 8.3	169.7 ± 8.3	6	7.8 ± 1.1	82 ± 4.0
	24	6	42.2 ± 2.8	156.5 ± 4.6	6	5.0 ± 0.3	71 ± 1.6

CT_{max} : Critical thermal maximum
ILOS : Incipient lethal oxygen saturation
IRAP: Integrated respiratory assessment paradigm

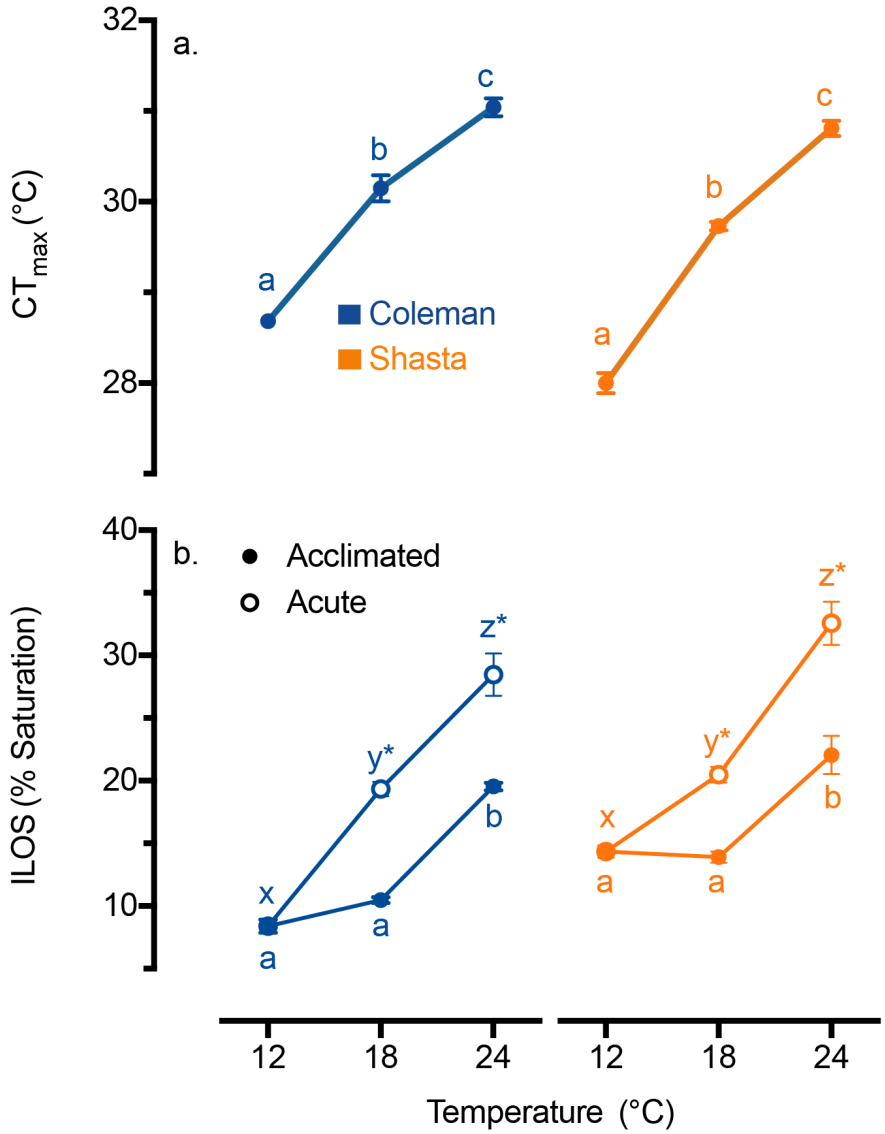

Figure 3.1 Critical thermal maximum (CT_{max}; upper thermal tolerance) and incipient lethal oxygen saturation (ILOS; hypoxia tolerance) in two strains of Californian rainbow trout: Coleman (blue) and Shasta (orange).
Temperature (x-axis) indicates acclimation temperature for CT_{max} data, and test temperature for ILOS data. For ILOS assessment, trout were either acclimated to the test temperature (acclimated) or to 12°C and acutely warmed to 18 °C over one hour or 24°C over two hours to reveal any effect of warm acclimation. Dissimilar letters indicate significant differences between acclimation or test temperatures (Acclimated: a-c, Acute: x-z) within a strain. Asterisks indicate differences between acute and acclimated for ILOS. Both CT_{max} and ILOS were analyzed by Kruskal-Wallis one-way ANOVA on ranks with Holm-Sidak corrected pairwise comparisons ($\alpha = 0.05$). Data are presented as mean±SEM.

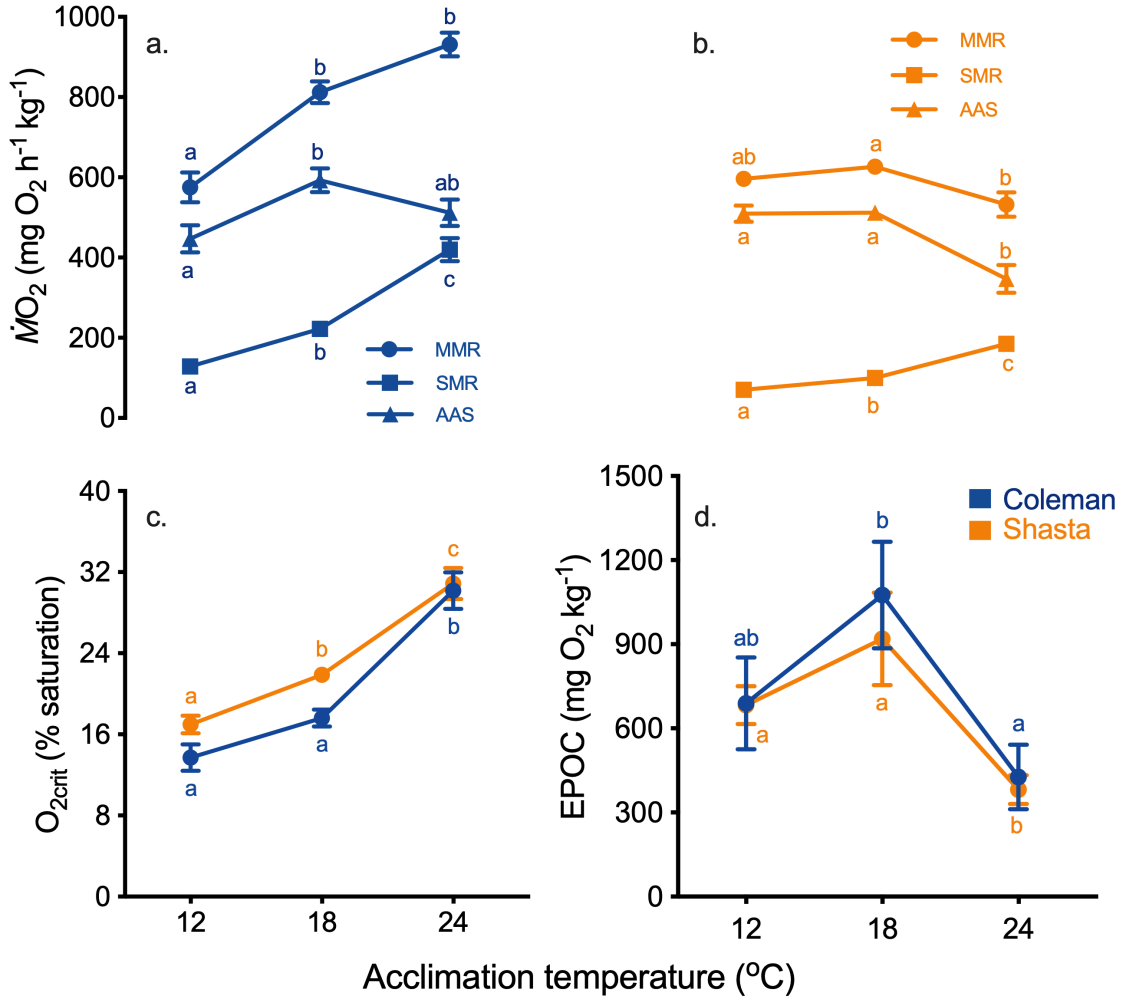

Figure 3.2 Aerobic and anaerobic metabolic performance for two strains of Californian rainbow trout: Coleman (blue) and Shasta (orange).
Rainbow trout were acclimated to 12, 18 and 24 °C and tested at the acclimation temperature to ensure a steady state for a reliable estimate of metabolic rate using oxygen uptake. Aerobic performance is assessed by maximum metabolic rate (MMR), standard metabolic rate (SMR) and absolute aerobic cope (AAS). Anaerobic performance is assessed by critical oxygen saturation (c. O_{2crit}) and excess post-exercise oxygen consumption (d. EPOC). Different letters denote statistical differences between temperatures within each strain and metric. All metrics were analyzed by ANOVA with Holm-Sidak corrected pairwise comparisons ($\alpha = 0.05$). Data are presented as mean±SEM.

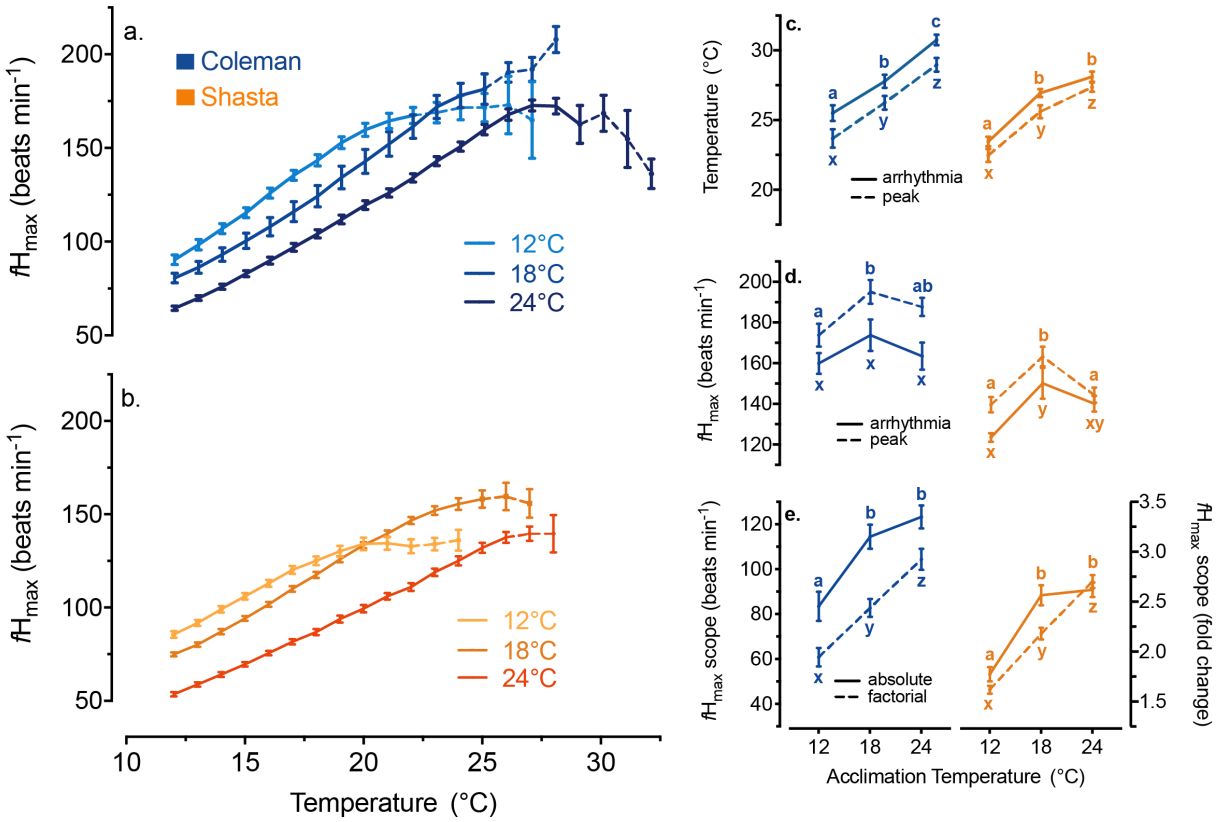

Figure 3.3 Maximum heart rate (f_{Hmax}) and cardiac thermal tolerance during acute warming in two strains of Californian rainbow trout: Coleman (blue) and Shasta (orange). f_{Hmax} in Coleman (a.) Shasta (b.) trout increased during acute warming and was reset to lower rates over moderate (12-20°C) temperatures (n= 8-11 per treatment group; Table S1). Broken lines in f_{Hmax} curves indicate that individual fish had become arrhythmic and were thus removed from the average. Temperature (c) and f_{Hmax} (d) at peak heart rate and at arrhythmia and significant differences between acclimation temperature with a strain are indicated by dissimilar letters (a,b,c or x,y,z). Absolute and relative (factorial) scope for f_{Hmax} above 12°C are also shown (e) with significant differences between acclimation temperatures indicated by dissimilar letters and numbers respectively. The resetting of f_{Hmax} between acclimation temperatures within a strain was assessed using linear mixed effect modelling and all other metrics were analyzed by ANOVA with Holm-Sidak corrected pairwise comparisons ($\alpha = 0.05$). Data are presented as mean±SEM.

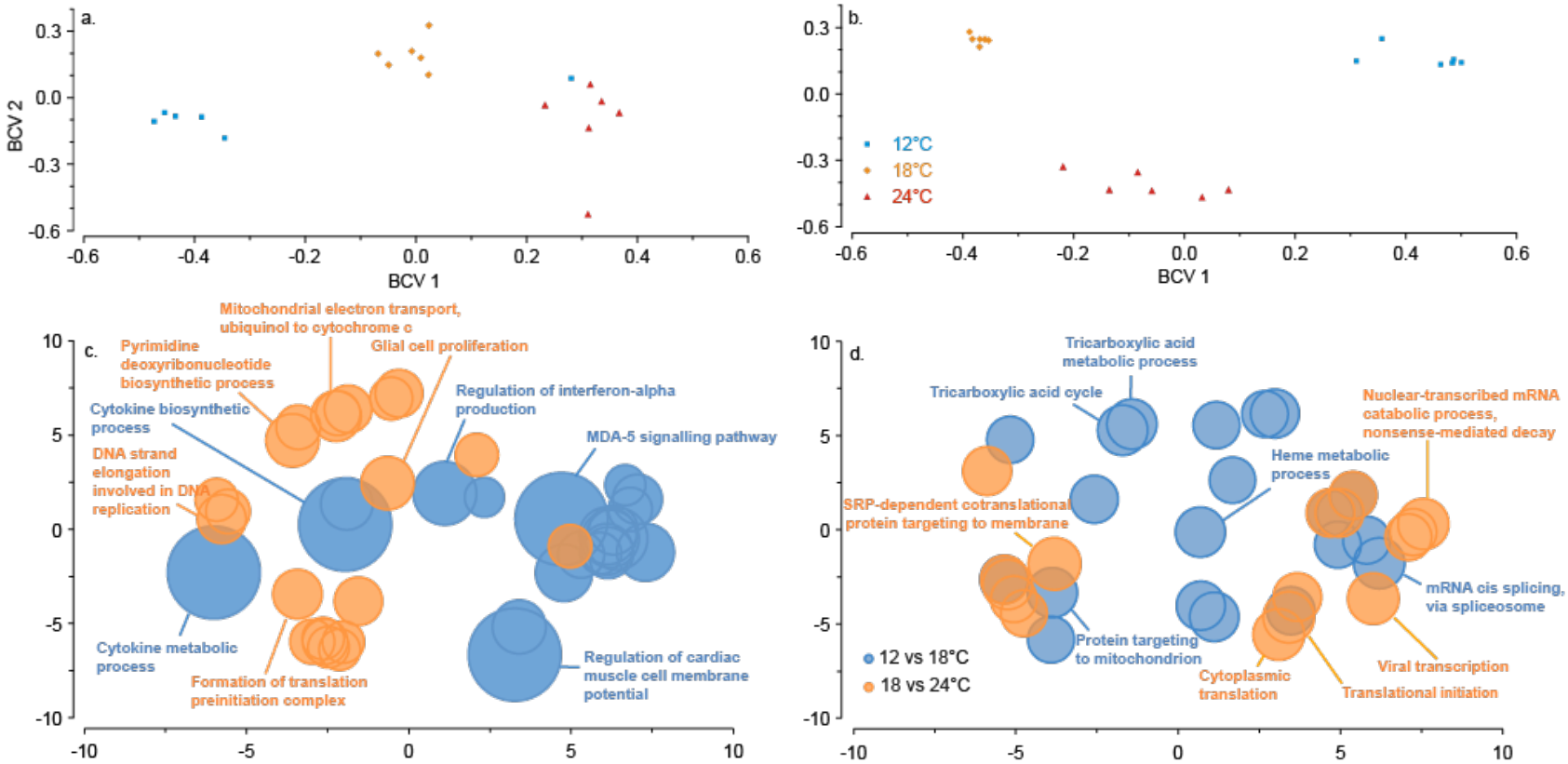

Figure 3.4 Multidimensional scaling plot illustrating whole-transcriptome response to three acclimation temperatures in (a) Coleman (14,753 transcripts) and (b) Shasta strain rainbow trout (17,987).
Bubble plots emphasizing shift in gene enrichment between treatment group comparisons: within (c) Coleman and (d) Shasta strain fish. 20 most significantly enriched GO categories shown in semantic space, 5 most highly enriched categories described.

Chapter 4: Conclusion

To cope with the rapid environmental alteration associated with anthropogenic climate change, organisms have three main options: adapt, migrate, or exhibit phenotypic plasticity (Fuller *et al.*, 2010; Somero, 2010). Freshwater fish, including rainbow trout, are constrained in migration because of the naturally low connectivity of their ecosystem, as well as the impacts of damming (Strayer and Dudgeon, 2010; Reid *et al.*, 2019). As such, adaptation and plasticity will be particularly important for freshwater fish to deal with the stressors of climate change. Therefore, the objectives of this thesis were to: 1) assess the extent of phenotypic variation in several rainbow trout strains that are stocked in British Columbia through the characterization of within- and among-strain variation in upper thermal and hypoxia tolerance, 2) investigate the relationship between upper thermal and hypoxia tolerance in these strains, 3) characterize the extent of thermal plasticity across levels of biological organization in two rainbow trout strains used to stock Californian lakes.

In this chapter, I highlight my main findings, indicate the strengths and potential limitations of this work, identify future directions, and provide general conclusions that may be useful for the management of freshwater fish and fisheries.

4.1 Within- and among-strain variation in upper thermal and hypoxia tolerance (Chapter 2)

One particularly important way an organism can adapt to environmental change is through standing genetic variation (Barrett and Schluter, 2008), this variation can exist within or among strains of the same species. I did not directly assess genetic variation and instead concentrated on characterizing phenotypic variation, but all individuals were hatched and raised in the same environment, therefore the phenotypic variation observed potentially reflects genetic

variation (Garland and Adolph, 1991). Furthermore, the large sample sizes and common garden design of my experiments suggest I have captured a fairly accurate picture of within- and among-strain variation in the traits that I assessed. My data indicate that there is substantial within-strain variation in upper thermal and hypoxia tolerance (measured as CT_{max} and ILOS, respectively) within all British Columbian stocked strains assessed; however, there is little evidence for differentiation among strains and detected differences were inconsistent across life stages and brood years. This suggests that within-strain variation may be particularly important for the future of rainbow trout within British Columbia, with the potential for selection to act upon this variation to allow rainbow trout to adapt to a changing climate. Previous research indicates that rainbow trout do have adaptive potential, with southern and transplanted strains exhibiting tolerance to increased temperatures (Chen *et al.*, 2015; Verhille *et al.*, 2016). However, it remains unclear whether the time-scale of climate change will allow for this adaptation to occur in affected strains.

4.2 Extreme post-trial mortality differences among strains (Chapter 2)

In contrast to the limited among strain variation in CT_{max} and ILOS that I observed, there were extreme differences in post-trial mortality among strains (from ~1% up to ~60%, depending on the strains). I hypothesize that strain origin explains these differences, with the most sensitive strain Horsefly originating from a habitat with moderate temperatures and in well-aerated water. These findings contrast to previous literature on post-trial mortality following upper thermal tolerance measurements, which suggests that mortality is generally low (Anttila *et al.*, 2013; Scott *et al.*, 2015; Morgan *et al.*, 2018; Zhang *et al.*, 2018b; Joyce and Perry, 2020); however, only a few of these studies examined multiple traits on the same individual. As such, my results suggest that it may be important to assess post-stress survival over an extended period when

attempting to characterize climate resilience using acute tests of tolerance, especially when examining repeated measures or multiple traits on individuals. Moreover, these findings suggest that examining upper thermal and hypoxia tolerance likely does not fully capture all relevant variation when assessing among-strain resilience.

4.3 Relationship between upper thermal and hypoxia tolerance within individuals (Chapter 2)

Increased temperatures and decreased dissolved oxygen are intrinsically linked in freshwater ecosystems through increased metabolism of aquatic organisms and decreased oxygen solubility (Ficke *et al.*, 2007; Diaz and Breitburg, 2009). Therefore, we might expect that resilience to these two stressors may have been selected for in parallel in aquatic ectotherms. However, my data provides little evidence for a relationship between upper thermal and hypoxia tolerance within individuals in all of the rainbow trout strains I assessed. Previous studies have suggested freshwater fish may use very different mechanisms with regards to upper thermal and hypoxia tolerance (Jutfelt *et al.*, 2019). For example, a set of studies have shown that knocking out HIF1α, a protein responsible for controlling expression genes involved in numerous cellular processes associated with metabolism (Semenza, 2012), drastically decreased hypoxia tolerance but not upper thermal tolerance (Joyce and Perry, 2020; Mandic *et al.*, 2020). Hence, this difference in underlying molecular mechanism may have contributed to the lack of relationship in upper thermal and hypoxia tolerance that I observed.

4.4 Thermal plasticity across biological organization in rainbow trout (Chapter 3)

As mentioned above, plasticity is one of the key mechanisms with which organisms may deal with climate change (Fuller *et al.*, 2010; Somero, 2010). In the collaborative study reported in Chapter 3, my collaborators and I found evidence for substantial plasticity in upper thermal

and hypoxia tolerance, metabolic rate, cardiac performance, and gene expression; however, I observed limits to this plasticity across all traits examined at a high, but ecologically relevant temperature. Declines in the extent of plasticity at the whole-animal level (upper thermal and hypoxia tolerance), reflected declines in aerobic scope, drop in cardiac performance, and increase in cellular stress response at the warmest acclimation temperature. Notably, across the two highest temperatures there was still an increase in upper thermal tolerance, albeit not as large an increase as at lower temperatures, whereas there were marked declines in performance at lower levels of biological organization. These data suggest that measures such as CT_{max} are not necessarily sensitive indicators of thermal limits and emphasize the need to incorporate responses across biological organization when investigating climate change resilience within aquatic ectotherms (Mykles *et al.*, 2010).

4.5 Strengths and limitations

One of the major strengths of this thesis was the careful approach within which I investigated within- and among-strain variation in upper thermal and hypoxia tolerance. By using 25 breeding pairs to establish each of my study strains (with exception of HF), large sample sizes (~100 to ~500 fish per strain), a common garden experimental design (in the 2018 brood year), and careful consideration of recovery protocol I was able to detect significant within-strain variation in tolerances as well as clear among-strain differences in post-trial mortality. This is the first study to my knowledge to examine such large numbers of individuals to fully characterize the within- and among-strain responses to acute thermal and hypoxia exposure.

Another major strength of this thesis was the characterization of plasticity across levels of biological organization. Several previous studies have examined plastic responses of whole-

animal tolerance and its association with underlying system effects, tissue-level effects, and/or molecular responses (e.g. Logan and Somero, 2010; Anttila *et al.*, 2014; Logan and Buckley, 2015; Muñoz *et al.*, 2015; Veilleux *et al.*, 2015); however, few, if any, of these studies have examined plasticity across all these levels (whole-animal, system-level, tissue and molecular). Integrating these effects will allow for better predictions of how organisms will physiologically cope with climate change stressors.

Despite these strengths, this thesis had some limitations that should be considered. One major limitation is the absence of genetic data in Chapter 2. I have assumed, due to high sample size, breeding pair numbers, and common rearing environment, that the observed variation may reflect genetic variation. However, it is conceivable that not all parents contributed equally to the progeny, therefore I may not have captured all relevant variation. Also, previous work has shown that there are maternal effects on thermal tolerance in salmonids and my breeding design did not control for this, which could result in attributing differences to the wrong mechanisms (Muñoz *et al.*, 2014). Additionally, previous work suggests seasonality in thermal tolerance (Bulger and Tremaine, 1985), therefore the different spawn times between our strains may have contributed to observed differences in tolerances. However, it is important to note that the common-garden experiments on which my main conclusions about differences among strains are based were conducted using fish that spawn at approximately the same time of year.

Another limitation of my thesis is the use of only three acclimation temperatures in Chapter 3. Although I was able to characterize an upper limit (24 °C) to plasticity, the large difference between the intermediate and upper acclimation temperature (18 vs. 24 °C) does not allow us to identify the temperature at which this decline in plasticity happens. Additionally, the use of gene expression data (transcriptomic response) does not fully allow us to identify

underlying molecular mechanisms of plasticity, as further characterization of these mechanisms should include measures of protein abundance and activity.

4.6 Future directions

Although this thesis has several limitations, these limitations provide future research questions to build upon our findings. For instance, to fully characterize the adaptive potential of BC interior rainbow trout strains future work should include individual genetic sequencing data to examine the relationship between genotype and phenotype with regards to upper thermal and hypoxia tolerance. Furthermore, as I have clearly shown large among-strain variation in post-trial mortality, future studies could use genetic sequencing of individuals that perished following upper thermal tolerance tests and those that did not to examine the underlying molecular mechanisms of acute thermal recovery. Also, further investigation into non-acute, ecological relevant measures of thermal and hypoxia tolerance may help identify the true relationship between these two concomitant stressors of climate change. Additionally, with regards to thermal plasticity, future studies should examine multiple acclimation temperatures to characterize where the limit to plasticity occurs.

4.7 Conclusions

More effort is being put into using physiological measurements to examine individual, species, and population-specific responses to anthropogenic mediated climate change (Harrod, 2016; McKenzie *et al.*, 2016). Our findings present important information that is not only useful to our collaborators, the FFSBC, in distinguishing between their strains for stocking in the face of increased temperature and decreased dissolved oxygen but also to fisheries and conservation managers as a whole looking to address within- and among-strain climate resilience. My data clearly demonstrate that simply measuring upper thermal and hypoxia tolerance (CT_{max} and

ILOS) is insufficient to identify resilient strains and that more nuanced approaches such as assessing post-trial recovery are also vitally important. Without measuring post-trial mortality, I would have potentially recommended that the FFSBC need not choose between strains for stocking in affected lakes; however, it is clear that exposure to acute high temperatures may affect the stocking success of HF. Furthermore, I have shown the importance of measuring plasticity across biological organization. For example, I have shown that performance at lower levels of biological organization, such as metabolic capacity, cardiac performance, and gene expression may decline or show stress without a decline in whole-animal performance (CT_{max}). Taken together, this thesis provides clear evidence for the need to carefully consider approaches when examining the effects of climate change on aquatic organisms and suggests that plasticity alone may not be sufficient for rainbow trout survival in the face of climate change.

References

Andrews S (2020) Babraham bioinformatics - FastQC a quality control tool for high throughput sequence data. https://www.bioinformatics.babraham.ac.uk/projects/fastqc/

Anttila K, Couturier CS, Øverli Ø, Johnsen A, Marthinsen G, Nilsson GE, Farrell AP (2014a) Atlantic salmon show capability for cardiac acclimation to warm temperatures. *Nat Commun* 5: 6–11.

Anttila K, Dhillon RS, Boulding EG, Farrell AP, Glebe BD, Elliott JAK, Wolters WR, Schulte PM (2013) Variation in temperature tolerance among families of Atlantic salmon (*Salmo salar*) is associated with hypoxia tolerance, ventricle size and myoglobin level. *J Exp Biol* 216: 1183–1190.

Anttila K, Jørgensen SM, Casselman MT, Timmerhaus G, Farrell AP, Takle H (2014b) Association between swimming performance, cardiorespiratory morphometry, and thermal tolerance in Atlantic salmon (*Salmo salar*). *Front Mar Sci* 1: 1–10.

Åsheim E, Andreassen A, Morgan R, Jutfelt F (2020) Rapid-warming tolerance correlates with tolerance to slow warming but not growth at non-optimal temperatures in zebrafish. *J Exp Biol*.

Badr A, El-Sayed MF, Vornanen M (2016) Effects of seasonal acclimatization on temperature dependence of cardiac excitability in the roach, *Rutilus rutilus*. *J Exp Biol* 219: 1495–1504.

Bailey M, Sumaila RU (2012) Freshwater angling and the B.C. economy. Report prepared for Freshwater Fisheries Society of British Columbia.

Barrett RDH, Schluter D (2008) Adaptation from standing genetic variation. *Trends Ecol Evol* 23: 38–44.

Bartholomew GA (1982) Scientific innovation and creativity: a zoologist's point of view. *Am*

Zool 22: 227–235.

Bates D, Mächler M, Bolker BM, Walker SC (2015) Fitting linear mixed-effects models using lme4. *J Stat Softw* 67.

Becker CD, Genoway RG (1979) Evaluation of the critical thermal maximum for determining thermal tolerance of freshwater fish. *Env Biol Fish* 4: 245–256.

Behnke RJ (1979) Monograph of the native trouts of the genus *Salmo* of Western North America. Lakewood, CO: U.S. Fish and Wildlife Service & U.S. Forest Service.

Beitinger TL, Bennet WA, McCauly RW (2000) Temperature tolerances of North American freshwater fishes exposed to dynamic changes in temperature. *Environ Biol Fishes* 58: 237–275.

Beitinger TL, Lutterschmidt WI (2011) Measures of thermal tolerance. Encyclopedia of Fish Physiology. Elsevier Inc.

Bolger AM, Lohse M, Usadel B (2014) Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30: 2114–2120.

Borowiec BG, Hoffman RD, Hess CD, Galvez F, Scott GR (2020) Interspecific variation in hypoxia tolerance and hypoxia acclimation responses in killifish from the family *Fundulidae*. *J Exp Biol* 223.

Bulger A, Tremaine S (1985) Magnitude of seasonal effects on heat tolerance in Fundulus heteroclitus. *Physiol Zool* 58: 197–204.

Busack CA, Gall GAE (1980) Ancestry of artificially propagated California rainbow trout strains. *Calif. Fish Game* 66: 17-24.

California Department of Fish & Wildlife (2018) Statewide drought response: stressor monitoring.

Cavieres G, Alruiz JM, Medina NR, Bogdanovich JM, Bozinovic F (2019) Transgenerational and within-generation plasticity shape thermal performance curves. *Ecol Evol* 9: 2072–2082.

Chabot D, Steffensen JF, Farrell AP (2016) The determination of standard metabolic rate in fishes. *J Fish Biol* 88: 81–121.

Chen IC, Hill JK, Ohlemuller R, Roy DB, Thomas CD (2011) Rapid range shifts of species of climate warming. *Science* 333: 1024–1026.

Chen Z, Snow M, Lawrence CS, Church AR, Narum SR, Devlin RH, Farrell AP (2015) Selection for upper thermal tolerance in rainbow trout (*Oncorhynchus mykiss* Walbaum). *J Exp Biol* 218: 803–812.

Claireaux G, Chabot D (2016) Responses by fishes to environmental hypoxia: integration through Fry's concept of aerobic metabolic scope. *J Fish Biol* 88: 232–251.

Claireaux G, McKenzie DJ, Genge AG, Chatelier A, Aubin J, Farrell AP (2005) Linking swimming performance, cardiac pumping ability and cardiac anatomy in rainbow trout. *J Exp Biol* 208: 1775–1784.

Claireaux G, Théron M, Prineau M, Dussauze M, Merlin FX, Le Floch S (2013) Effects of oil exposure and dispersant use upon environmental adaptation performance and fitness in the European sea bass, *Dicentrarchus labrax*. *Aquat Toxicol* 130–131: 160–170.

Clark TD, Sandblom E, Jutfelt F (2013) Aerobic scope measurements of fishes in an era of climate change: respirometry, relevance and recommendations. *J Exp Biol* 216: 2771–2782.

Comte L, Buisson L, Daufresne M, Grenouillet G (2013) Climate-induced changes in the distribution of freshwater fish: observed and predicted trends. *Freshw Biol* 58: 625–639.

Comte L, Olden JD (2017) Climatic vulnerability of the world's freshwater and marine fishes.

Nat Clim Chang 7: 718–722.

Cooke SJ, Cowx IG (2006) Contrasting recreational and commercial fishing: searching for common issues to promote unified conservation of fisheries resources and aquatic environments. *Biol Conserv* 128: 93–108.

COSEWIC (2014) Assessment and status report on the rainbow trout *Oncorhynchus Mykiss* in Canada.

Courtenay WRJ, Williams JD (1992) Dispersal of exotic species from aquaculture sources, with emphasis on freshwater fishes. In: Rosenfield A, Mann R, eds. Dispersal of Living Organisms into Aquatic Ecosystems. pp 49–81.

Cowles, R.B., Bogert CM (1944) A preliminary study of the thermal requirements of desert reptiles. *Bull Am Museum Nat Hist* 83.

Cowx IG (1994) Stocking strategies. *Fish Manag Ecol* 1: 15–30.

Crawford SS, Muir AM (2008) Global introductions of salmon and trout in the genus *Oncorhynchus*: 1870-2007. *Rev Fish Biol Fish* 18: 313–344.

Crozier LG, Hutchings JA (2014) Plastic and evolutionary responses to climate change in fish. *Evol Appl* 7: 68–87.

Currie RJ, Bennett WA, Beitinger TL (1998) Critical thermal minima and maxima of three freshwater game-fish species acclimated to constant temperatures. *Environ Biol Fishes* 51: 187–200.

Dahlke FT, Wohlrab S, Butzin M, Pörtner HO (2020) Thermal bottlenecks in the life cycle define climate vulnerability of fish. *Science* 369: 65–70.

Dally S, Bredoux R, Corvazier E, Andersen JP, Clausen JD, Dode L, Fanchaouy M, Gelebart P, Monceau V, Del Monte F, *et al.* (2006) Ca^{2+}-ATPases in non-failing and failing heart:

Evidence for a novel cardiac sarco/endoplasmic reticulum Ca^{2+}-ATPase 2 isoform (SERCA2c). *Biochem J* 395: 249–258.

DeWitt TJ, Sih A, Wilson DS (1998) Costs and limits of phenotypic plasticity. *Trends Ecol Evol* 63: 1508–1518.

Diaz RJ, Breitburg DL (2009) The hypoxic environment. In: Fish Physiology. Elsevier Inc., pp 1–23.

Diaz RJ, Rosenberg R (2008) Spreading dead zones and consequences for marine ecosystems. *Science* 321: 926–929.

Dill WA, Cordone AJ (1997) History and status of introduced fishes in California. California Department of Fish & Game Inland Fisheries Division.

Donelson JM, Salinas S, Munday PL, Shama LNS (2018) Transgenerational plasticity and climate change experiments: where do we go from here? *Glob Chang Biol* 24: 13–34.

Drost HE, Lo M, Carmack EC, Farrell AP (2016) Acclimation potential of Arctic cod (*Boreogadus saida*) from the rapidly warming Arctic Ocean. *J Exp Biol* 219: 3114–3125.

Eaton JG, Scheller RM (1996) Effects of climate warming on fish thermal habitat in streams of the United States. *Limnol Oceanogr* 41: 1109–1115.

Eden E, Navon R, Steinfeld I, Lipson D, Yakhini Z (2009) GOrilla: a tool for discovery and visualization of enriched GO terms in ranked gene lists. *BMC Bioinformatics* 10: 1–7.

Ekström A, Gräns A, Sandblom E (2019) Can´t beat the heat? Importance of cardiac control and coronary perfusion for heat tolerance in rainbow trout. *J Comp Physiol B Biochem Syst Environ Physiol* 189: 757–769.

Eliason EJ, Clark TD, Hague MJ, Hanson LM, Gallagher ZS, Jeffries KM, Gale MK, Patterson DA, Hinch SG, Farrell AP (2011) Differences in thermal tolerance among sockeye salmon

populations. *Science* 1861: 1–4.

Fangue NA, Wunderly MA, Dabruzzi TF, Bennett WA (2014) Asymmetric thermal acclimation responses allow sheepshead minnow *Cyprinodon variegatus* to cope with rapidly changing temperatures. *Physiol Biochem Zool* 87: 805–816.

Farrell AP (2016) Pragmatic perspective on aerobic scope: peaking, plummeting, pejus and apportioning. *J Fish Biol* 88: 322–343.

Faust HA, Gamperl KA, Rodnick KJ (2004) All rainbow trout (*Oncorhynchus mykiss*) are not created equal: intra-specific variation in cardiac hypoxia tolerance. *J Exp Biol* 207: 1005–1015.

Ficke AD, Myrick CA, Hansen LJ (2007) Potential impacts of global climate change on freshwater fisheries. *Rev Fish Biol Fish* 17: 581–613.

Fisheries and Oceans Canada (2003) National code on introductions and transfers.

Fisheries and Oceans Canada (2015) Survey of recreation fishing in Canada, 2015.

Fox RJ, Donelson JM, Schunter C, Ravasi T, Gaita JD, Fox RJ (2019) Beyond buying time: the role of plasticity in phenotypic adaptation to rapid environmental change. *Philos Trans R Soc B Biol Sci* 374: 1–9.

Freshwater Fisheries Society of British Columbia (2020) Stocked Strains. https://www.gofishbc.com/Stocked-Fish.aspx

Froehlich HE, Gentry RR, Halpern BS (2017) Conservation aquaculture: shifting the narrative and paradigm of aquaculture's role in resource management. *Biol Conserv* 215: 162–168.

Fuller A, Dawson T, Helmuth B, Hetem RS, Mitchell D, Maloney SK (2010) Physiological mechanisms in coping with climate change. *Physiol Biochem Zool* 83: 713–720.

Gaesser GA, Brooks GA (1984) Metabolic bases of excess post-exercise oxygen consumption: a

review. *Med Sci Sports Exerc* 16: 29–43.

Garland TJ, Adolph SC (1991) Physiological differentiation of vertebrate populations. *Annu Rev Ecol Syst* 22: 193–228.

Garza JC, Pearse DE (2008) Population genetic structure of *Oncorhynchus mykiss* in the California Central Valley. California Department of Fish & Game.

Gienapp P, Teplitsky C, Alho JS, Mills JA, Merilä J (2008) Climate change and evolution: disentangling environmental and genetic responses. *Mol Ecol* 17: 167–178.

GSGislason & Associates Ltd. (2009) Freshwater sport fishing in British Columbia.

Gunderson AR, Armstrong EJ, Stillman JH (2016) Multiple stressors in a changing world: the nee for an improved perspective on physiological responses to the dynamic marine environment. *Ann Rev Mar Sci* 8: 357–378.

Harrod C (2016) Climate change and freshwater fisheries. In: John CF, ed. Freshwater Fisheries Ecology. John Wiley & Sons, Ltd., pp 641–694.

Hentze MW, Kulozik AE (1999) A perfect message: RNA surveillance and nonsense-mediated decay. *Cell* 96: 307–310.

Hewitt N, Klenk N, Smith AL, Bazely DR, Yan N, Wood S, MacLellan JI, Lipsig-Mumme C, Henriques I (2011) Taking stock of the assisted migration debate. *Biol Conserv* 144: 2560–2572.

Hines CW, Fang Y, Chan VKS, Stiller KT, Brauner CJ, Richards JG (2019) The effect of salinity and photoperiod on thermal tolerance of Atlantic and coho salmon reared from smolt to adult in recirculating aquaculture systems. *Comp Biochem Physiol -Part A Mol Integr Physiol* 230: 1–6.

Hoffmann AA, Sgró CM (2011) Climate change and evolutionary adaptation. *Nature* 470: 479–

485.

Holmes R (2009) The Horsefly River state of the watershed report.

Hughes RM (2014) Recreational fisheries in the USA: economics, management strategies, and ecological threats. *Fish Sci* 81: 1–9.

Intergovernmental Panel on Climate Change (2014) Climate change 2014 synthesis report summary chapter for policymakers.

James C, Laval B, Carmack E, Pieters R (2004) Analysis of water samples for determining salinity from CTD measurements in Quesnel Lake, British Columbia. In: Engineering Mechanics Conference. pp 1–8.

Jenkins M (2003) Prospects for biodiversity. *Science* 302: 1175–1177.

Jenny JP, Francus P, Normandeau A, Lapointe F, Perga ME, Ojala A, Schimmelmann A, Zolitschka B (2016) Global spread of hypoxia in freshwater ecosystems during the last three centuries is caused by rising local human pressure. *Glob Chang Biol* 22: 1481–1489.

Joyce W, Perry SF (2020) Hypoxia inducible factor-1α knockout does not impair acute thermal tolerance or heat hardening in zebrafish. *Biol Lett* 16: 20200292.

Jutfelt F, Norin T, Ern R, Overgaard J, Wang T, McKenzie DJ, Lefevre S, Nilsson GE, Metcalfe NB, R Hickey AJ, *et al.* (2018) Oxygen-and capacity-limited thermal tolerance: blurring ecology and physiology. *J Exp Biol* 221: 1–4.

Jutfelt F, Roche DG, Clark TD, Norin T, Binning SA, Speers-Roesch B, Amcoff M, Morgan R, Andreassen AH, Sundin J (2019) Brain cooling marginally increases acute upper thermal tolerance in Atlantic cod. *J Exp Biol* 222: 1–5.

Keeley ER, Parkinson EA, Taylor EB (2005) Ecotypic differentiation of native rainbow trout (*Oncorhynchus mykiss*) populations from British Columbia. *Can J Fish Aquat Sci* 62: 1523–

1539.

Keeley ER, Parkinson EA, Taylor EB (2007) The origins of ecotypic variation of rainbow trout: A test of environmental vs. genetically based differences in morphology. *J Evol Biol* 20: 725–736.

Kerr SJ (2006) An historical review of fish culture, stocking and fish transfers in Ontario, 1865-2004. Ontario Ministry of Natural Resources.

Kopylova E, Noé L, Touzet H (2012) SortMeRNA: fast and accurate filtering of ribosomal RNAs in metatranscriptomic data . *Bioinformatics* 00: 1–7.

Kvamme BO, Gadan K, Finne-Fridell F, Niklasson L, Sundh H, Sundell K, Taranger GL, Evensen Ø (2013) Modulation of innate immune responses in Atlantic salmon by chronic hypoxia-induced stress. *Fish Shellfish Immunol* 34: 55–65.

Landry CA, Steele SL, Manning S, Cheek AO (2007) Long term hypoxia suppresses reproductive capacity in the estuarine fish, *Fundulus grandis*. *Comp Biochem Physiol Part A* 148: 317–323.

Langmead B, Salzberg SL (2012) Fast gapped-read alignment with Bowtie 2. *Nat Methods* 9: 357–359.

LeBlanc S, Middleton S, Gilmour KM, Currie S (2011) Chronic social stress impairs thermal tolerance in the rainbow trout (*Oncorhynchus mykiss*). *J Exp Biol* 214: 1721–1731.

Lenth R V. (2016) Least-squares means: the R package lsmeans. *J Stat Softw* 69.

Li B, Dewey CN (2011) RSEM: Accurate transcript quantification from RNA-seq data with or without a reference genome. *BMC Bioinformatics* 12: 323.

Li N, Zoubeidi A, Beraldi E, Gleave ME (2013) GRP78 regulates clusterin stability, retrotranslocation and mitochondrial localization under ER stress in prostate cancer.

Oncogene 32: 1933–1942.

Liss WJ, Larson GL, Tyler TJ, Ganio L, Hoffman R, Deimling E, Lomnicky G, McIntire CD, Truitt R (1999) Ecological effects of stocked trout in naturally fishless high-elevation lakes, North Cascades National Park Service Complex, WA, USA: Phase II.

Logan CA, Buckley BA (2015) Transcriptomic responses to environmental temperature in eurythermal and stenothermal fishes. *J Exp Biol* 218: 1915–1924.

Logan CA, Somero GN (2010) Transcriptional responses to thermal acclimation in the eurythermal fish *Gillichthys mirabilis* (Cooper 1864). *Am J Physiol - Regul Integr Comp Physiol* 299: R843–R852.

Lowe S, Browne M, Boudjelas S, De Poorter M (2000) 100 of the world's worst invasive alien species. The Invasive Species Specialist Group (ISSG) a specialist group of the Species Survival Commission (SSC) of the World Conservation Union (IUCN).

MacCrimmon HR (1972) World distribution of rainbow trout (*Salmo gairdneri*). *J Fish Res Board Canada* 28: 663–704.

Madliger CL, Cooke SJ, Crespi EJ, Funk JL, Hultine KR, Hunt KE, Rohr JR, Sinclair BJ, Suski CD, Willis CKR, *et al.* (2016) Success stories and emerging themes in conservation physiology. *Conserv Physiol* 4: 1–17.

Mandic M, Best C, Perry SF (2020) Loss of hypoxia-inducible factor 1α affects hypoxia tolerance in larval and adult zebrafish (*Danio rerio*). *Proc R Soc B Biol Sci* 287.

McArley TJ, Hickey AJR, Herbert NA (2020) Acute high temperature exposure impairs hypoxia tolerance in an intertidal fish. *PLoS One* 15: 1–11.

McBryan TL, Anttila K, Healy TM, Schulte PM (2013) Responses to temperature and hypoxia as interacting stressors in fish: implications for adaptation to environmental change. *Integr*

Comp Biol 53: 648–659.

McBryan TL, Healy TM, Haakons KL, Schulte PM (2016) Warm acclimation improves hypoxia tolerance in *Fundulus heteroclitus*. *J Exp Biol* 219: 474–484.

McCarthy DJ, Chen Y, Smyth GK (2012) Differential expression analysis of multifactor RNA-Seq experiments with respect to biological variation. *Nucleic Acids Res* 40: 4288–4297.

McCusker MR, Parkinson E, Taylor EB (2000) Mitochondrial DNA variation in rainbow trout (*Oncorhynchus mykiss*) across its native range: testing biogeographical hypotheses and their relevance to conservation. *Mol Ecol* 9: 2089–2108.

McKenzie DJ, Axelsson M, Chabot D, Claireaux G, Cooke SJ, Corner RA, de Boeck G, Domenici P, Guerreiro PM, Hamer B, *et al.* (2016) Conservation physiology of marine fishes: state of the art and prospects for policy. *Conserv Physiol* 4: 1–20.

Merilä J, Hendry AP (2014) Climate change, adaptation, and phenotypic plasticity: the problem and the evidence. *Evol Appl* 7: 1–14.

Molony B (2001) Environmental requirements and tolerances of rainbow trout (*Oncorhynchus mykiss*) and brown trout (*Salmo trutta*) with special reference to Western Australia: a review. Department of Fisheries, Government of Western Austrlia.

Morgan R, Finnøen MH, Jutfelt F (2018) CTmax is repeatable and doesn't reduce growth in zebrafish. *Sci Rep* 9: 7099.

Morita K, Fukuwaka M, Tanimata N, Yamamura O (2010) Size-dependent thermal preferences in a pelagic fish. *Oikos* 1265–1272.

Moyle PB, Lusardi RA, Samuel PJ, Jacob MA, Katz VE (2017) State of the salmonids: status of California's emblematic fishes 2017.

Mulvey T (1921) Fifty-third annual report of the fisheries branch.

Munday PL, Warner RR, Monro K, Pandolfi JM, Marshall DJ (2013) Predicting evolutionary responses to climate change in the sea. *Ecol Lett* 16: 1488–1500.

Muñoz NJ, Anttila K, Chen Z, Heath JW, Farrell AP, Neff BD (2014) Indirect genetic effects underlie oxygenlimited thermal tolerance within a coastal population of chinook salmon. *Proc R Soc B Biol Sci* 281.

Muñoz NJ, Farrell AP, Heath JW, Neff BD (2015) Adaptive potential of a Pacific salmon challenged by climate change. *Nat Clim Chang* 5: 163–166.

Mykles DL, Ghalambor CK, Stillman JH, Tomanek L (2010) Grand challenges in comparative physiology: integration across disciplines and across levels of biological organization. *Integr Comp Biol* 50: 6–16.

Myrick CA, Cech JJ (2000) Temperature influences on California rainbow trout physiological performance. *Fish Physiol Biochem* 22: 245–254.

National Oceanic and Atmospheric Administration (2018) Fisheries economics of the US, 2016.

Nilsson GE, Östlund-Nilsson S (2008) Does size matter for hypoxia tolerance in fish? *Biol Rev* 83: 173–189.

North Cascades National Park Service complex fish stocking act (2014). United States Congress, United States of America.

Northrup SL (2017) Contributions of genetic variation and phenotypic plasticity to variation in high pH tolerance in rainbow trout. University of British Columbia.

Nudds RL, Ozolina K, Fenkes M, Wearing OH, Shiels HA (2020) Extreme temperature combined with hypoxia, affects swimming performance in brown trout (*Salmo trutta*). *Conserv Physiol* 8: 1–11.

O' Reilly CM, Rowley RJ, Schneider P, Lenters JD, Mcintyre PB, Kraemer BM, Weyhenmeyer

GA, Straile D, Dong B, Adrian R, *et al.* (2015) Rapid and highly variable warming of lake surface waters around the globe. *Geophys Res Lett* 1–9.

Petticrew EL, Albers SJ, Baldwin SA, Carmack EC, Déry SJ, Gantner N, Graves KE, Laval B, Morrison J, Owens PN, *et al.* (2015) The impact of a catastrophic mine tailings impoundment spill into one of North America's largest fjord lakes: Quesnel Lake, British Columbia, Canada. *Geophys Res Lett* 42: 3347–3355.

Pister EP (2001) Wilderness fish stocking: history and perspective. *Ecosystems* 4: 279–286.

Pollard S, Yesaki T (2004) Rainbow trout strains currently stocked in BC waters.

Pollock MS, Clarke LMJ, Dubé MG (2007) The effects of hypoxia on fishes: from ecological relevance to physiological effects. *Environ Rev* 15: 1–14.

Portner H, Farrell A (2008) Physiology and climate change. *Science* 322: 690–692.

Pörtner HO, Bock C, Mark FC (2017) Oxygen- & capacity-limited thermal tolerance: bridging ecology & physiology. *J Exp Biol* 220: 2685–2696.

Pringle C (2003) What is hydrologic connectivity and why is it ecologically important? *Hydrol Process* 17: 2685–2689.

Quiñones RM, Moyle PB (2015) California's freshwater fishes: status and management. *Fishes Mediterr Environ* 2015: 1–20.

Radchuk V (2019) Adaptive responses of animals to climate change are most likely insufficient. *Nat Commun* 10: 1–14.

Rahel FJ (2007) Biogeographic barriers, connectivity and homogenization of freshwater faunas: It's a small world after all. *Freshw Biol* 52: 696–710.

Raleigh RF, Hickman T, Solomon RC, Nelson PC (1984) Habitat suitability information: rainbow trout. Fish and Wildlife Service.

Recsetar MS, Zeigler MP, Ward DL, Bonar SA, Caldwell CA (2012) Relationship between fish size and upper thermal tolerance. *Trans Am Fish Soc* 141: 1433–1438.

Reid AJ, Carlson AK, Creed IF, Eliason EJ, Gell PA, Johnson PTJ, Kidd KA, MacCormack TJ, Olden JD, Ormerod SJ, *et al.* (2019) Emerging threats and persistent conservation challenges for freshwater biodiversity. *Biol Rev* 94: 849–873.

Robinson MD, McCarthy DJ, Smyth GK (2009) edgeR: A Bioconductor package for differential expression analysis of digital gene expression data. *Bioinformatics* 26: 139–140.

Roze T, Christen F, Amerand A, Claireaux G (2013) Trade-off between thermal sensitivity, hypoxia tolerance and growth in fish. *J Therm Biol* 38: 98–106.

Rytkönen KT, Vuori KAM, Primmer CR, Nikinmaa M (2007) Comparison of hypoxia-inducible factor-1 alpha in hypoxia-sensitive and hypoxia-tolerant fish species. *Comp Biochem Physiol - Part D* 177–186.

Safi H, Zhang Y, Schulte PM, Farrell AP (2019) The effect of acute warming and thermal acclimation on maximum heart rate of the common killifish *Fundulus heteroclitus*. *J Fish Biol* 95: 1441–1446.

Sandblom E, Clark TD, Gräns A, Ekström A, Brijs J, Sundström LF, Odelström A, Adill A, Aho T, Jutfelt F (2016) Physiological constraints to climate warming in fish follow principles of plastic floors and concrete ceilings. *Nat Commun* 7: 1–8.

Schlichting C (1986) The evolution of phenotypic plasticity in plants. *Annu Rev Ecol Syst* 17: 667–693.

Schulte PM (2015) The effects of temperature on aerobic metabolism: towards a mechanistic understanding of the responses of ectotherms to a changing environment. *J Exp Biol* 218: 1856–1866.

Scott MA, Dhillon RS, Schulte PM, Richards JG, Magnan P (2015) Physiology and performance of wild and domestic strains of diploid and triploid rainbow trout (*Oncorhynchus mykiss*) in response to environmental challenges. *Can J Fish Aquat Sci* 72: 125–134.

Seebacher F, White CR, Franklin CE (2015) Physiological plasticity increases resilience of ectothermic animals to climate change 5: 61–66.

Selbie DT, Sweetman JN, Etherton P, Hyatt KD, Rankin DP, Finney BP, Smol JP (2011) Climate change modulates structural and functional lake ecosystem responses to introduced anadromous salmon. *Can J Fish Aquat Sci* 68: 675–692.

Semenza GL (2012) Hypoxia-inducible factors in physiology and medicine. *Cell* 148: 399–408.

Sharma S, Jackson DA, Minns CK, Shuter BJ (2007) Will northern fish populations be in hot water because of climate change? *Glob Chang Biol* 13: 2052–2064.

Sharma S, Vander Zanden MJ, Magnuson JJ, Lyons J (2011) Comparing climate change and species invasions as drivers of coldwater fish population extirpations. *PLoS One* 6.

Smith GR, Stearley RF (1989) The classification and scientific names of rainbow and cutthroat trouts. *Fisheries* 14: 4–10.

Somero GN (2010) The physiology of climate change: how potentials for acclimatization and genetic adaptation will determine “winners” and “losers.” *J Exp Biol* 213: 912–920.

Stanković D, Crivelli AJ, Snoj A (2015) Rainbow trout in Europe: introduction, naturalization, and impacts. *Rev Fish Sci Aquac* 23: 39–71.

Steinhausen MF, Sandblom E, Eliason EJ, Verhille C, Farrell AP (2008) The effect of acute temperature increases on the cardiorespiratory performance of resting and swimming sockeye salmon (*Oncorhynchus nerka*). *J Exp Biol* 211: 3915–3926.

Stiff H, Hyatt K, Cone T, Patterson D, Benner K (2018) Water temperature, river discharge, and

adult sockeye salmon migration observations in the Quesnel/Horsefly watershed. Fisheries and Oceans Canada.

Stitt BC, Burness G, Burgomaster KA, Currie S, Mcdermid JL, Wilson CC (2014) Intraspecific variation in thermal tolerance and acclimation capacity in brook trout (*Salvelinus fontinalis*): physiological implications for climate change. *Physiol Biochem Zool* 87: 15–29.

Strayer DL, Dudgeon D (2010) Freshwater biodiversity conservation: recent progress and future challenges. *J North Am Benthol Soc* 29: 344–358.

Sunday JM, Bates AE, Dulvy NK (2011) Global analysis of thermal tolerance and latitude in ectotherms. *Proc R Soc B Biol Sci* 278: 1823–1830.

Sunday JM, Bates AE, Dulvy NK (2012) Thermal tolerance and the global redistribution of animals. *Nat Clim Chang* 2: 686–690.

Sunday JM, Bennett JM, Calosi P, Clusella-Trullas S, Gravel S, Hargreaves AL, Leiva FP, Verberk WCEP, Olalla-Tárraga MÁ, Morales-Castilla I (2019) Thermal tolerance patterns across latitude and elevation. *Philos Trans R Soc B Biol Sci* 374: 1–10.

Swain DL, Langenbrunner B, Neelin JD, Hall A (2018) Increasing precipitation volatility in twenty-first-century California. *Nat Clim Chang* 8: 427–433.

Tamkee P, Parkinson E, Taylor EB (2010) The influence of Wisconsinan glaciation and contemporary stream hydrology on microsatellite DNA variation in rainbow trout (*Oncorhynchus mykiss*). *Can J Fish Aquat Sci* 67: 919–935.

Taylor EB, Tamkee P, Keeley ER, Parkinson EA (2011) Conservation prioritization in widespread species: the use of genetic and morphological data to assess population distinctiveness in rainbow trout (*Oncorhynchus mykiss*) from British Columbia, Canada. *Evol Appl* 4: 100–115.

Taylor SE, Egginton S, Taylor EW (1996) Seasonal temperature acclimatisation of rainbow trout: cardiovascular and morphometric influences on maximal sustainable exercise level. *J Exp Biol* 199: 835–845.

Terblanche JS, Hoffmann AA, Mitchell KA, Rako L, Roux PC, Chown SL (2011) Ecologically relevant measures of tolerance to potentially lethal temperatures. *J Exp Biol* 214: 3713–3725.

The UniProt Consortium (2019) UniProt: A worldwide hub of protein knowledge. *Nucleic Acids Res* 47: D506–D515.

Todgham AE, Schulte PM, Iwama GK, April NM, Schulte PM, Iwama GK (2005) Cross-tolerance in the tidepool sculpin: the role of heat shock proteins. *Physiol Biochem Zool* 78: 133–144.

U.S. Fish & Wildlife Service (2016) National survey of fishing, hunting, and wildlife-associated recreation.

Ullrich PA, Xu Z, Rhoades AM, Dettinger MD, Mount JF, Jones AD, Vahmani P (2018) California's drought of the future: a midcentury recreation of the exceptional conditions of 2012–2017. *Earth's Futur* 6: 1568–1587.

Veilleux HD, Ryu T, Donelson JM, Ravasi T, Munday PL, Reitzel AM (2018) Molecular response to extreme summer temperatures differs between two genetically differentiated populations of a coral reef fish. *Front Mar Sci* 5: 349.

Veilleux HD, Ryu T, Donelson JM, Van Herwerden L, Seridi L, Ghosheh Y, Berumen ML, Leggat W, Ravasi T, Munday PL (2015) Molecular processes of transgenerational acclimation to a warming ocean. *Nat Clim Chang* 5: 1074–1078.

Verhille CE, English KK, Cocherell DE, Farrell AP, Fangue NA (2016) High thermal tolerance

of a rainbow trout population near its southern range limit suggests local thermal adjustment. *Conserv Physiol* 4: 1–12.

Visser ME (2008) Keeping up with a warming world; assessing the rate of adaptation to climate change. *Proc R Soc B Biol Sci* 275: 649–659.

Vitt P, Havens K, Hoegh-Guldberg O (2009) Assisted migration: part of an integrated conservation strategy. *Trends Ecol Evol* 24: 473–474.

Wang T, Lefevre S, Thanh Huong DT, Cong N van, Bayley M (2009) The effects of hypoxia on growth and digestion. In: Fish Physiology. Elsevier Inc., pp 361–396.

Wenger SJ, Isaak DJ, Luce CH, Neville HM, Fausch KD, Dunham JB, Dauwalter DC, Young MK, Elsner MM, Rieman BE, *et al.* (2011) Flow regime, temperature, and biotic interactions drive differential declines of trout species under climate change. *Proc Natl Acad Sci* 108: 14175–14180.

Wieser W (1985) Developmental and metabolic constraints of the scope for activity in young rainbow trout (*Salmo gairdneri*). *J Exp Biol* 118: 133–142.

Williams KJ, Cassidy AA, Verhille CE, Lamarre SG, MacCormack TJ (2019) Diel cycling hypoxia enhances hypoxia tolerance in rainbow trout (*Oncorhynchus mykiss*): evidence of physiological and metabolic plasticity. *J Exp Biol* 222. doi:10.1242/jeb.206045

Wood CM (2018) The fallacy of the Pcrit - are there more useful alternatives? *J Exp Biol* 221. doi:7

World Wild Fund for Nature (2016) Living planet report 2016: Risk and resilience in a new era. WWF International.

Zhang Y, Claireaux G, Takle H, Jørgensen SM, Farrell AP (2018a) A three-phase excess post-exercise oxygen consumption in Atlantic salmon *Salmo salar* and its response to exercise

training. *J Fish Biol* 92: 1385–1403.

Zhang Y, Healy TM, Vandersteen W, Schulte PM, Farrell AP (2018b) A rainbow trout *Oncorhynchus mykiss* strain with higher aerobic scope in normoxia also has superior tolerance of hypoxia. *J Fish Biol* 92: 487–503.

Zhang Y, Mauduit F, Farrell AP, Chabot D, Ollivier H, Rio-Cabello A, Le Floch S, Claireaux G (2017) Exposure of European sea bass (*Dicentrarchus labrax*) to chemically dispersed oil has a chronic residual effect on hypoxia tolerance but not aerobic scope. *Aquat Toxicol* 191: 95–104.

Zhang Y, Timmerhaus G, Anttila K, Mauduit F, Jørgensen SM, Kristensen T, Claireaux G, Takle H, Farrell AP (2016) Domestication compromises athleticism and respiratory plasticity in response to aerobic exercise training in Atlantic salmon (*Salmo salar*). *Aquaculture* 463: 79–88.

Appendices

Appendix A - Variation in upper thermal and hypoxia tolerance in rainbow trout (*Oncorhychus mykiss)*

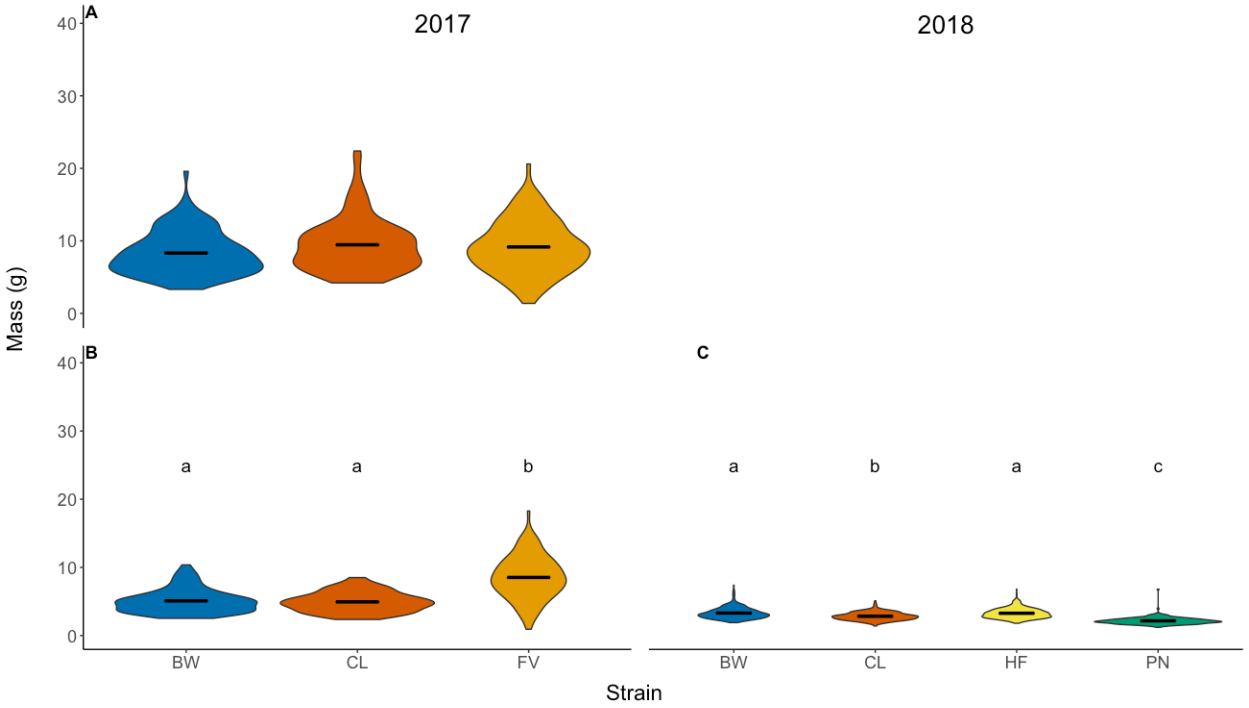

Figure A.1 Weight for individual strains at the 2017 (**A:** ILOS, **B:** CT_{max}) and 2018 (**C:** ILOS and CT_{max}) brood fry life-stage.
Significant differences between strains are indicated by dissimilar letters. All data were analyzed one-way ANOVA with Tukey-HSD pairwise comparisons ($\alpha = 0.05$). See Tables 2.1 and 2.2 for sample sizes.

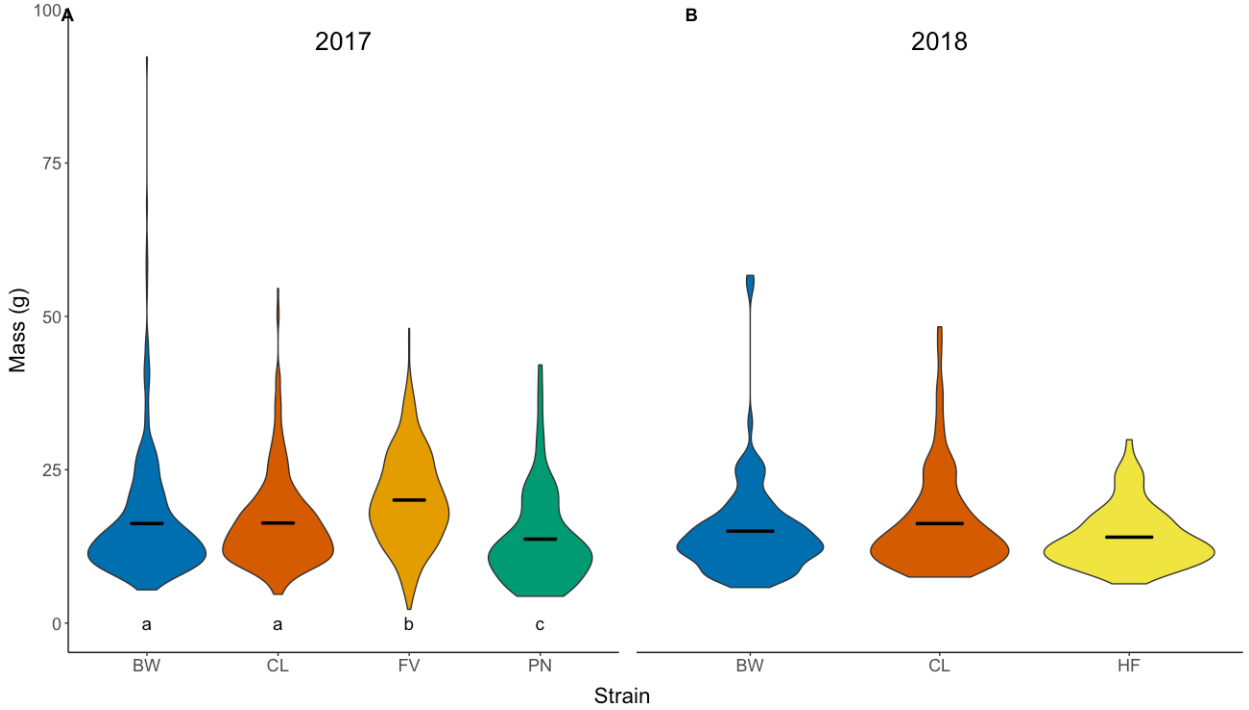

Figure A.2 Weight for individual strains at the 2017 (**A:** ILOS and CT_{max}) and 2018 (**B:** ILOS and CT_{max}) brood yearling life-stage.
See Table 2 for sample sizes. All data were analyzed one-way ANOVA with Tukey-HSD pairwise comparisons ($\alpha = 0.05$). See Tables 2.1 and 2.2 for sample sizes.

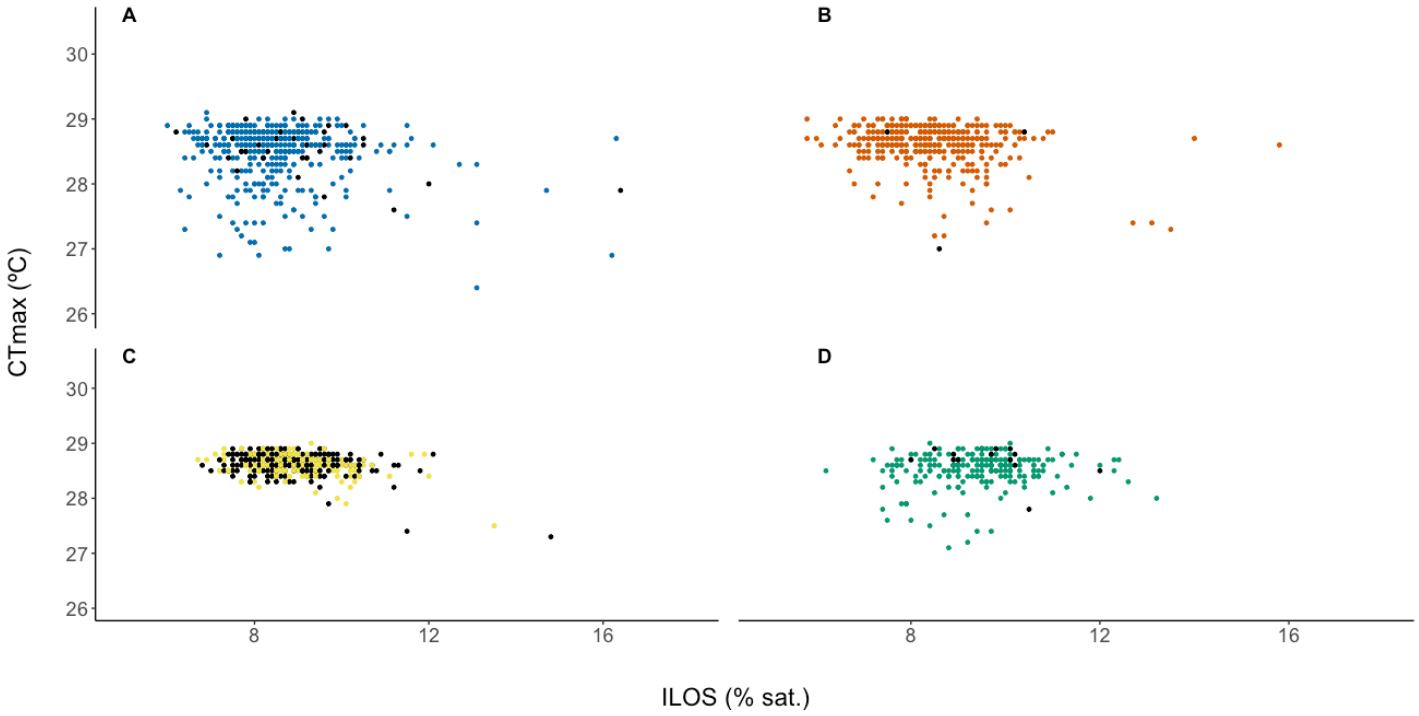

Figure A.3 Correlation between critical thermal maximum (CT_{max}) and incipient lethal oxygen saturation (ILOS) for the fry life-stage for 2018 brood.
A BW, **B** CL, **C** HF, **D** PN. Sample sizes are as follows: **BW:** n = 435, **CL:** n = 426, **HF:** n = 453, **PN:** n = 253. Individuals that perished following CT_{max} are coloured black.

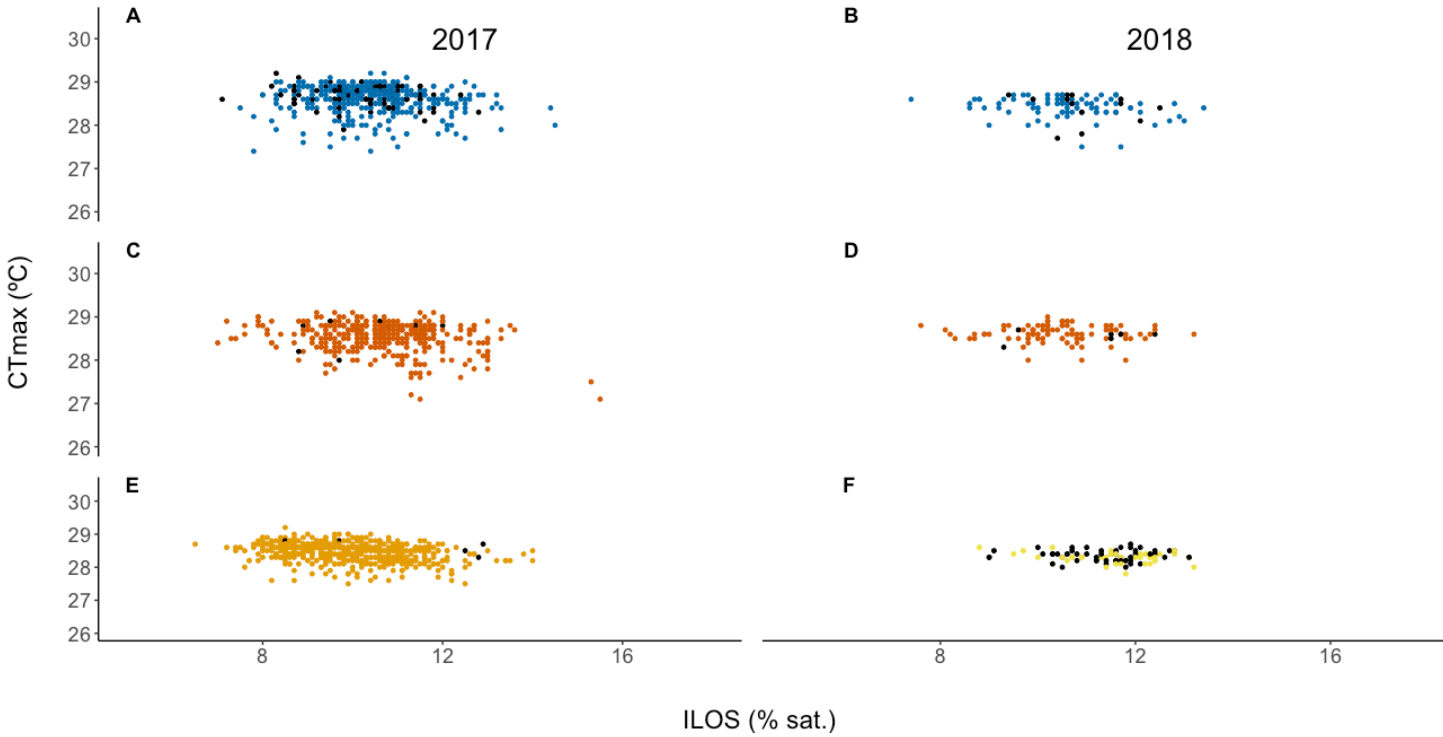

Figure A.4 Correlation between critical thermal maximum (CT_{max}) and incipient lethal oxygen saturation (ILOS) for multiple strains across two brood years (2017, 2018) at the yearling life-stage.
A, B BW, **C,D** CL, **E** FV, **F** HF. Sample sizes are as follows: **BW (2017):** n = 499, **BW (2018):** n = 100, **CL (2017):** n = 400, **CL (2018):** n = 100, **FV:** n = 491, **HF:** n = 99. Individuals that perished following CT_{max} are coloured black.

Table A.1. Mean ± standard deviation of weight for strains in the 2017 brood year

Strain	Fry (grams)		Yearling (grams)
	Hypoxia	Thermal	
Blackwater River (BW)	8.32 ± 2.99	5.08 ± 1.74[a]	16.20 ± 9.34[a]
Carp Lake (CL)	9.45 ± 3.67	4.94 ± 1.46[a]	16.30 ± 7.32[a]
Fraser Valley (FV)	9.16 ± 3.65	8.53 ± 3.17[b]	20.10 ± 7.50[b]
Pennask Lake (PN)	NA	NA	13.70 ± 7.52[c]

Significant differences between groups within a strain are indicated by dissimilar letters

Table A.2 Mean ± standard deviation of weight for strains in the 2018 brood year

Strain	Fry (grams)	Yearling (grams)
Blackwater River (BW)	3.31 ± 0.82[a]	15.00 ± 7.88
Carp Lake (CL)	2.83 ± 0.59[b]	16.20 ± 7.90
Horsefly River (HF)	3.28 ± 0.72[a]	14.00 ± 4.83
Pennask Lake (PN)	2.14 ± 0.49[c]	NA

Significant differences between groups within a strain are indicated by dissimilar letters

Table A.3 Correlation of CT_{max} or ILOS vs. mass statistical results for all strains at the fry stage

Strain		**p-value**		**Correlation coefficient**	
		CT_{max}	ILOS	CT_{max}	ILOS
Blackwater River:	**2017**	0.976	0.938	0.002	-0.005
	2018	0.316	0.112	-0.034	-0.050
Carp Lake:	**2017**	0.190	0.594	0.093	0.037
	2018	0.102	0.876	0.058	-0.005
Fraser Valley:	**2017**	0.002*	0.005	-0.310	0.281
	2018	-	-	-	-
Horsefly River:	**2017**	-	-	-	-
	2018	0.003*	0.008	0.104	-0.082
Pennask Lake:	**2017**	-	-	-	-
	2018	0.467	0.187	0.033	0.041

*indicates significant correlation after correcting for multiple comparisons

Table A.4 Correlation of CT_{max} or ILOS vs. mass for all strains at the yearling stage

Strain		**p-value**		**Correlation coefficient**	
		CT_{max}	ILOS	CT_{max}	ILOS
Blackwater River:	**2017**	0.515	0.027	-0.021	-0.067
	2018	0.088	0.128	-0.123	0.104
Carp Lake:	**2017**	0.056	0.002*	-0.0674	0.106
	2018	0.531	0.006	-0.046	0.188
Fraser Valley:	**2017**	1.01×10^{-7}*	5.30×10^{-4}*	-0.236	0.154
	2018	-	-	-	-
Horsefly River:	**2017**	-	-	-	-
	2018	0.327	0.788	0.072	-0.019
Pennask Lake:	**2017**	0.005	0.015	0.203	-0.168
	2018	-	-	-	-

*indicates significant correlation after correcting for multiple comparisons

Table A.5. Table 3. Mean ± standard deviation of ILOS and CT_{max} for strains in the 2017 brood year

Strain	Fry		Yearling	
	Hypoxia (% sat.)	Thermal (°C)	Hypoxia (% sat.)	Thermal (°C)
Blackwater River (BW)	12.1 ± 1.2	28.2 ± 0.5	10.4 ± 1.1	28.6 ± 0.3
Carp Lake (CL)	11.3 ± 1.1	28.4 ± 0.4	10.6 ± 1.3	28.5 ± 0.3
Fraser Valley (FV)	9.54 ± 1.2	28.6 ± 0.2	10.0 ± 1.3	28.4 ± 0.3
Pennask Lake (PN)	NA	NA	10.3 ± 1.3	28.3 ± 0.6

Table A.6 Individual group incipient lethal oxygen saturation comparisons for strains at the 2017 brood year yearling stage

Trial #	Blackwater River (mean ± sd)	Carp Lake (mean ± sd)	Fraser Valley (mean ± sd)
1	10.6 ± 1.4 % sat.	11.2 ± 0.8 % sat.[a]	9.8 ± 1.4 % sat.[a]
2	10.3 ± 1.1 % sat.	10.7 ± 1.0 % sat.[b]	9.8 ± 1.1 % sat.[a]
3	10.3 ± 1.0 % sat.	11.2 ± 1.2 % sat.[a]	10.0 ± 1.2 % sat.[ab]
4	10.2 ± 1.0 % sat.	9.5 ± 1.1 % sat.[c]	10.3 ± 1.2 % sat.[b]
5	10.5 ± 1.0 % sat.	NA	10.1 ± 1.3 % sat.[a]

Significant differences between groups within a strain are indicated by dissimilar letters

Table A.7 Individual group critical thermal maximum comparisons for strains at the 2017 brood year yearling stage

Trial #	Blackwater River (mean ± sd)	Carp Lake (mean ± sd)	Fraser Valley (mean ± sd)
1	28.6 ± 0.3 °C	28.5 ± 0.3 °C[a]	28.5 ± 0.3 °C[a]
2	28.6 ± 0.3 °C	28.4 ± 0.3 °C[a]	28.5 ± 0.3 °C[a]
3	28.6 ± 0.3 °C	28.6 ± 0.3 °C[b]	28.4 ± 0.3 °C[ab]
4	28.6 ± 0.3 °C	28.6 ± 0.3 °C [b]	28.4 ± 0.3 °C[ab]
5	28.7 ± 0.3 °C	NA	28.3 ± 0.3 °C[b]

Significant differences between groups within a strain are indicated by dissimilar letters

Table A.8 Mean ± standard deviation of ILOS and CT_{max} for strains in the 2018 brood year

Strain	Fry		Yearling	
	Hypoxia (% sat.)	Thermal (°C)	Hypoxia (% sat.)	Thermal (°C)
Blackwater River (BW)	8.6 ± 1.3	28.5 ± 0.5	10.7 ± 1.1	28.4 ± 0.3
Carp Lake (CL)	8.5 ± 1.2	28.6 ± 0.3	10.5 ± 1.1	28.6 ± 0.2
Horsefly River (HF)	8.9 ± 1.0	28.6 ± 0.2	11.4 ± 1.0	28.3 ± 0.2
Pennask Lake (PN)	9.5 ± 1.2	28.5 ± 0.3	NA	NA

Appendix B - Anthropogenic warming will test the limits of thermal plasticity in rainbow trout (*Oncorhychus mykiss)*

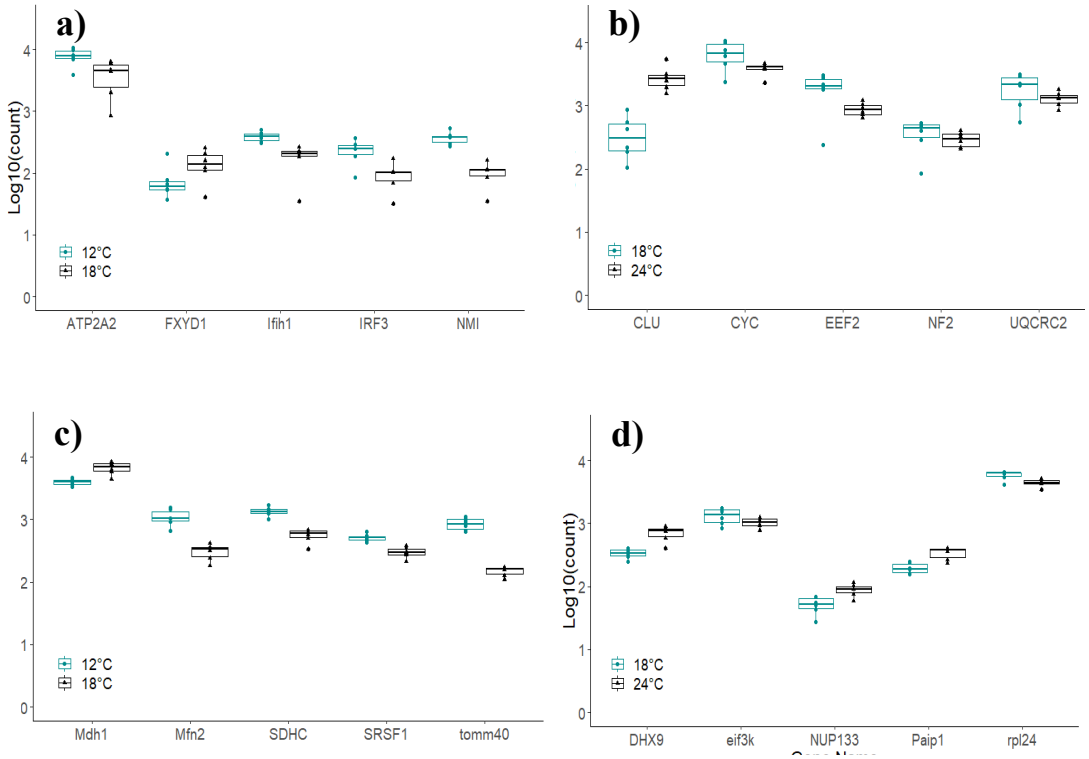

Figure B.1 Examples of differentially expressed genes among different acclimation groups.
(**a) Differentially expressed genes (DEGs) among Coleman fish acclimated to 12°C versus 18°C include:** ATP2A2 or SERCA (Sarcoplasmic/endoplasmic reticulum calcium ATPase 2) catalyzes the movement of Ca+2 ions from the cytosol to the sarcoplasmic reticulum during the contraction/relaxation cycle of the heart; FXYD1 (Phospholemman) inhibits sodium/potassium-transporting ATPase activity when unphosphorylated and stimulates activity when phosphorylated; IFIH1 (Interferon-induced helicase C domain-containing protein 1) is an immune receptor that plays an integral role in sensing viral infection; NMI (N-myc-interactor) increases transcription of cytokine-mediated signal transducers and activators of transcription. **(b) DEGs among Coleman 18°C versus 24°C include:** CLU (Clusterin) prevents clustering of proteins in response to stress (i.e. heat shock); CYC1 (Cytochrome c) is an electron transporter in the electron transport chain (ETC); EEF2 (Elongation factor 2) catalyzes movement of 2 tRNA molecules, mRNA, and conformational changes of the ribosome during translation elongation; NF2 (merlin or neurofibromin 2) functions to supress cell proliferation; UQCRC2 (Cytochrome b-c1 complex subunit 2, mitochondrial) is a required component of the ubiquinol-cytochrome c reductase complex of ETC. **(c) DEGs among Shasta fish acclimated to 12°C versus 18°C include:** MDH1 (malate dehydrogenase) catalyzes the conversion of malate to oxaloacetate in the citric acid cycle and other metabolic pathways; MFN2 (mitofusin-2) triggers mitochondrial clustering and fusion; SDHC (Succinate dehydrogenase cytochrome b560 subunit, mitochondrial) is a subunit of succinate dehydrogenase that ultimately transfers electrons from succinate to ubiquinone in the ETC; SRSF1 (Serine/arginine-rich splicing factor 1) prevents exon skipping, providing quality control for splicing and alternative splicing of mRNA; TOMM40 (Mitochondrial import receptor subunit TOM40 homolog) is a channel-forming protein with an essential role in importing protein precursors into the mitochondria. **(d) DEGs among Shasta 18°C versus 24°C included:** DHX9 (ATP-dependent RNA helicase A) unwinds DNA and RNA and plays a crucial role in many transcription and translation processes; EIF3K (Eukaryotic translation initiation factor 3 subunit K) is a component of a larger protein complex required for the initiation of protein synthesis; NUP133 (Nuclear pore complex protein Nup133) is involved in the transport of polyadenylated RNA; PAIP1 (Polyadenylate-binding protein-interacting protein 1) is a coactivator that regulates translation initiation of polyadenylated mRNAs; RPL24 (60S ribosomal protein L24) is a protein that is part of the eukaryotic large ribosomal subunit (60S).