Swimming in 'Thin Air': Evaluating the Combination of Hypoxic and Thermal Stress as an Additive or Synergistic Effect on Redband Trout (*Oncorhynchus mykiss gairdneri*)

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ABSTRACT

Climate warming causes both increases in extreme environmental water temperature and decreased dissolved oxygen (DO) concentrations (hypoxia) that induce stress in salmonid fishes including trout. Redband trout (*Oncorhynchus mykiss gairdneri*) have divergent ecotypes corresponding to a diverse range of thermal conditions from warm desert to cold montane ecosystems. Additionally, trout can alter their physiological phenotype to acclimate to environmental stressors. I predicted that warm acclimation would increase thermal tolerance, increase cardiac performance, and decrease hypoxia tolerance relative to the cooler acclimation temperature. Further, I anticipated that the impact of hypoxic and thermal stress would be lowest for the desert ecotype. The combination of hypoxia and acute temperature increase stress was postulated to have a synergistic effect on trout performance. Trout were collected from wild populations and acclimated to 21°C (near upper pejus temperature) and 15°C (within thermal optimum range) to model the desert and cold montane habitats, respectively, using two metric units. First, the time at loss of equilibrium (LOE) was recorded as a proxy for hypoxia tolerance where DO was decreased to ~12% saturation in an experimental tank. Second, cardiac phenotypic response, quantified by heart rate (f_H), measured using an electrocardiogram (ECG) during acute temperature increase with 50% DO saturation (hypoxia) or full DO saturation (normoxia). Warm acclimated trout had significantly shorter time to LOE, and thus inferior hypoxia tolerance relative to the cold-acclimated trout ($p \leq 0.001$). A significant interaction between ecotype and acclimation temperature took place at the 21°C acclimation temperature where the cool montane ecotype had significantly shorter time to LOE relative to the desert ecotype ($p \leq 0.05$). Warm acclimated fish exhibited a significantly higher temperature at peak heart rate (T_{peak}), and thus a superior thermal tolerance than cold-acclimated trout ($p \leq 0.001$). As expected, the T_{peak} was significantly greater for trout subjected to normoxia rather than hypoxia ($p \leq 0.05$). No significant difference was detected for T_{peak} response between ecotypes. The warm-acclimated trout displayed significantly lower f_H at

temperature increments 15-20°C ($p \leq 0.001$) and 21- 22°C ($p \leq 0.05$) relative to the cold-acclimated trout. Greater f_H values indicate greater bioenergetic cost. As T_{peak} increased with warm acclimation temperature, f_H had decreased, indicating that thermal tolerance can vary inversely with cardiac performance. The f_H values with both hypoxic and thermal stressors combined were not greater than the sum of f_H for each isolated stressor, thus the combination of hypoxic and thermal stress was seen to have an additive rather than synergistic effect on trout performance.

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DEDICATION

I would like to dedicate this thesis to my partner, grandparents and close family, close friends, and anyone around me who has shown a mild interest in Redband trout and/or fisheries because of my overt expression of these interests of mine.

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Chapter 1: Introduction

Environmental Thermal and Hypoxic Stress

Anthropogenic climate change is the main factor causing a shift in thermal regimes in river and stream ecosystems (Isaak et al., 2011; Fellman et al., 2018). Models of the earth's climate predict that the average global surface temperature could exceed 4°C during the 21st century with a likelihood of ≥ 50%. However, if greenhouse gas emissions decrease to net zero later this century, projected warming is limited to 1.5°C (IPCC, 2022). This drastic increase in temperature will continue to have negative effects on habitat quality in stream ecosystems (Fellman et al., 2018). As the environment continues to swiftly change from historical conditions, many aquatic organisms will need to explore nuanced mechanisms for their populations to persist. (Chevin and Lande, 2010). These populations will be forced to disperse to a more optimal environment, genetically evolve to new local conditions, utilize phenotypic plasticity, or face extinction (Chevin and Lande, 2010).

Thermal regimes in aquatic ecosystems are critically important for fish and other aquatic organisms. Most ectotherms have physiological processes that are directly influenced by the fluctuations of their ambient temperature (Isaak et al., 2012). Limitations of a species' realized niche are likely a result of its temperature-dependent biogeography and its response to warming (Pörtner et al., 2017). Short-wave solar radiation is the dominant thermal factor in stream heat budgets, and these budgets are often altered by human activities. Including increased solar gain by removal of riparian vegetation, diversion of water out of streams, thermal effluents from power plants, warm runoff from paved surfaces in urban areas, and storage of water in reservoirs. Further, air temperature can cause increased stream temperatures, which is a result of long-wave atmospheric radiation (Isaak et al., 2012). Atmospheric warming affects not only affects the thermal regime, but also the solubility of gases and the flow regime in many aquatic habitats (Fellman et al. 2018). Ectothermic animals have temperature limits and tolerance ranges that will determine their

sensitivity to climate change. Thermal tolerance can also vary depending on an individual's life stage; tolerance ranges generally become wider as a fish develops from embryo to larval and adult stages, after development of cardiorespiratory organs (Dahlke et al., 2020).

Lethal temperatures can limit species distributions. Additionally, chronically elevated temperatures still cause negative effects for ectotherms (Pörtner et al. 2017). The optimum temperature for fish is that at which maximum aerobic scope, or the oxygen consumption rate range, is reached (Antilla et al., 2013). There is a range of temperatures between optimal and lethal that a fish can still inhabit; however, the negative effects can entail increased mortality rates, reduced growth, and reduced abundance (Casselman et al., 2012). Optimum temperatures may vary for attributes such as optimal growth, swimming performance, and metabolic power (Gamperl et al., 2002). Individuals that do survive non-lethal thermal stressors may have increased bioenergetic costs, reduced growth and/or reproductive success. Thus, the population survival could, in turn, be jeopardized (Pörtner et al., 2017).

Acute heat stress is common for many fish in their native habitats as climate change causes temperature increase (Isaak et al., 2012). This stressor can enforce substantial phenotypic selection on fishes (Chen et al., 2018b). Metabolic rate increases as temperature increases acutely, in a proportional manner, following the Q_{10} effect (Callaghan et al., 2016). The Q_{10} effect is described as the degree of temperature dependence on a biological process (Bennet, A. F., 1984; Yaeger and Ultch, 1989; Mundim et al., 2020). As temperature increases, homeostasis of many physiological functions becomes more energetically costly for fish (Callaghan et al., 2016).

The frequency and magnitude of heat stress events are factors of concern regarding fish performance and survival. Research shows that both high and low temperatures may be stressful and even lethal with prolonged and intermittent exposure (Eldridge et al., 2015). In some desert streams in Idaho, diel temperature fluctuations can exceed 7.5-7.8°C (Chen et al., 2018a). While many fish can tolerate a wide range of temperatures within their upper and lower bounds of critical

temperature, any temperature fluctuations that are greater than 4°C can be incredibly stressful to a fish's metabolic, physiological, and cellular functions (Eldridge et al., 2015; Tunnah et al., 2016).

Aquatic hypoxia, or dissolved oxygen (DO) deficiency, has widely increased in frequency as climate change continues to negatively affect water quality (Antilla et al. 2013). DO levels bear a strong association with stream temperature; as stream temperature increases, DO systematically decreases (Fellman et al. 2018). As aquatic organisms encounter reduced habitat quality due to increased temperature, upward dispersal is common along latitudinal and altitudinal gradients. However, species are seen to have a dispersal pattern that is not congruent with temperature shifts. One possible explanation is that oxygen availability is scarcer at higher altitudes. Therefore, fish may not inhabit water within their thermal optimum range if the DO concentration is depleted from relatively lower partial pressure at higher altitudes. (Jacobsen, 2020). Hypoxia is a notable challenge for fishes, seeing as oxygen is a limiting factor for metabolic activity (Fellman et al., 2019). Multiple phenomena can increase the frequency of hypoxia in aquatic environments. Three of which are eutrophication, biological demand, and flow regime (Antilla et al., 2013).

Eutrophication, or the increased richness of nutrients, increases the prevalence of aquatic hypoxia. Eutrophication can result in dense plant growth and subsequent aquatic animal mortality due to hypoxic events (Antilla et al. 2013). Small introductions of nutrients can have large-scale impacts on the water quality, including DO concentration, and subsequently, local biota (Chiu et al., 2017). Nutrient enrichment increases the proliferation of phytoplankton and aquatic plants. The respiratory activity of these organisms depletes DO, particularly at night when there is no photosynthetic activity. Moreover, once phytoplankton expire, they emit more nutrients for bacterial decomposition, whose respiration further deoxygenates the water (McBryan et al., 2013). Although eutrophication occurs naturally, human activities such as agriculture, industry, and sewage disposal have accelerated the process (Chislock et al., 2014). The consequences of eutrophication are amplified in

low-velocity, warm conditions often seen in intermittent rivers and ephemeral streams (Chiu et al., 2017). The metabolic rate for microorganisms that are responsible for eutrophication-induced hypoxia has a positive relationship with temperature increase. Thus, as water temperature rises, these micro-organisms' demand for oxygen increases, which coincides with the conditions where fishes' demand for oxygen is greatest. Further, as oxygen solubility decreases with warmer temperatures, the problem becomes exacerbated (McBryan et al., 2013).

Biological demand and the respiration rate of aquatic species can greatly decrease DO levels and have been observed to cause pre-spawning mortality. High densities of fish can increase a habitat's biological demand, and subsequent availability of DO (Gamperl et al., 1998; Tillotson and Quinn, 2016). Oxygen demand can be density dependent with non-native population size. Increased respiration occurs with increased density, especially during spawning season (Tillotson and Quinn). The distribution and abundance of native cold-water fisheries in the western United States have been greatly reduced since European settlement (Tate et al. 2007). Non-native Brook trout (*Salvinus fontinalis*), hatchery Rainbow trout (*Oncorhynchus mykiss*), and nonnative Cutthroat trout (*Oncorhynchus clarki* spp.) have been stocked in the western United States, including Idaho (Miller et al., 2014; Neville and Dunham, 2011). Trout are generalists, and non-natives can occupy similar niches as native trout. Higher densities of non-native species in crucial niche space for native salmonids can lead to resource competition and depletion (Miller et al., 2014). Non-native species can introduce competition with native species for limiting factors, such as oxygen. This may exacerbate the decrease in oxygen concentration as non-native species colonize, establish populations, and expand their distribution range. Non-native species that are better adapted to hypoxic episodes could successfully exploit these events to outcompete native species (Nati et al., 2018).

As more pressure is put on water resources due to climate change and increased human consumption, intermittent streams will increasingly become more common and pose many management issues (Archdeacon and Reale, 2020). Increased

air temperature has resulted in more sporadic precipitation and lower average winter snowpack. A low snowpack can cause sizable shifts in flow regime for adjacent streams. Consequently, lower streamflow can elevate stream temperatures and decrease DO to a fatally low concentration for local biota (Fellman et al. 2019). Stream intermittency can vary regarding timing, duration, frequency, and magnitude. The lack of refuge during stream intermittency can result in mortality of aquatic organisms (Tate et al. 2007; Archdeacon and Reale, 2019). With an absence of barriers, many organisms can migrate toward a refuge and recolonize the intermittent area once a higher flow regime has returned (Archdeacon and Reale, 2020). Isolated pools, or pools that lack surface flow and connectivity, that are greater than 0.6 m in depth can be considered refuges for fish. They can provide protection from predators and lethal temperatures. However, as these pools lose volume, fishes can be exposed to multiple stressors including deoxygenation of the water and elevated water temperatures (Archdeacon and Reale, 2019).

Vertical thermal stratification can occur in large pool refuges, where surface temperatures can be as much as 7.6°C warmer than the bottom (Tate et al., 2007). There can be cool water refuge at the bottom of deep pools because of reduced heat diffusion or cold springs. Nevertheless, the benefits of this cool refuge could be muted due to the lack of oxygen mixing at greater depths (Blaszczak et al., 2019). Oxygen demands may exceed supply in cooler deep pools as well as warmer surface water. During times where the ambient stream temperature was at its peak, Redband trout (*Oncorhyncus mykiss gairdneri*) have been found in locations averaging 19.3°C, while there were colder temperatures available at the bottom of pools (Tate et al., 2007; Meyer et al., 2010). Another possibility for warmer habitat selection could be increased availability of invertebrate food sources (Meyer et al., 2010).

The combination of hypoxia and temperature stress could interact in a variety of ways. The effect of the combined stressors could simply be the sum of the two independent effects or an "additive" interaction. The combination of these stressors could have even more of an effect than the sum of the independent effects, where the

relationship would be described as "synergistic." When the combined effect is a product of the two effects it is "multiplicative," and when the combined effect is less than that of the two stressors independently it is "antagonistic" (McBryan et al., 2013). The relationship between temperature and hypoxia stressors can be hard to predict, but 30-50% of the studies have shown that interactions are evidently synergistic (McBryan et al., 2013). Further, the combination of these stressors may hold negligible effect until a certain critical threshold is met, or the combination may act additively until a threshold is met and then act synergistically after beyond the threshold (McBryan et al., 2013). Since elevated temperature and hypoxia can have synergistic effect on fishes, a significant increase in one stressor could have strong effects on the capacity for a fish to respond to either abiotic stress (Antilla et al., 2015).

Redband Trout Background

Shifting thermal regimes, chronic and acute heat stress, hypoxia-induced by eutrophication, biological demand, and flow regime are all environmental factors that are threatening the persistence of many aquatic species, including the Columbia River Redband trout (*O. mykiss gairdneri*), or "Redband trout" hereafter. Redband trout are an inland subspecies of rainbow trout that are native to the Columbia River basin east of the Cascade Mountains (Schill et al. 2007; Currens et al., 2009). Redband trout are morphologically diverse and can be found in pluvial lakes, or lakes formed by exorbitant rainfall, in Oregon, northern California, and the interior Columbia River Basin (Currens et al., 2009). Redband trout occupy a variety of natural environments across the Colombia River Basin including desert and montane habitats in lakes, rivers, and streams (Meyer et al., 2010). Redband trout are a polyphyletic group with multiple subspecies and genetic races (Currens et al., 2009).

Redband trout have been suggested to have three genetically divergent ecotypes dependent on geographic location and thermal regime. These include desert, cool montane, and cold montane ecotypes (Chen et al., 2018a, 2018b). Unambiguous evidence of genetic divergence exists between the desert and montane ecosystems,

likely due to dispersal barriers such as intermittent streams in desert sites. Furthermore, the cool montane and cold montane ecotypes exhibit an isolation-by-distance model between each other (Narum et al., 2010). Further, the lack of connectivity between Redband trout populations poses a risk of inbreeding depression and reduces the fish's adaptive capacity for coping with pervasive environmental stressors such as elevated temperatures and low DO concentrations (Chen and Narum, 2021).

Redband trout are listed as a sensitive species by the U. S. Fish and Wildlife Service because 11 populations have become extinct, and 10 others are at risk (Gamperl et al., 2002; Tate et al. 2007; Schill et al., 2007). In 1995, there was a petition to protect Redband trout in southwest Idaho under the Endangered Species Act (ESA), but the petition was deemed unwarranted. Later, in 2000, Oregon also petitioned for protection of Redband under ESA, but it again was found unwarranted (Shill et al., 2007).

The Columbia River Redband trout were a historically anadromous subspecies in many populations, however, today they remain sympatric and exhibit an adfluvial life history where they spawn in streams, but rear in larger bodies of water such as lakes, large rivers, or reservoirs (Holecek and Scarnecchia, 2013). A historically anadromous population of Redband trout persists above the Hells Canyon dam in the Snake River basin (Holecek and Scarnecchia, 2013). Today, this population is resident because of anthropogenic barriers that exclusively restrict Redband trout dispersal to freshwater habitat. Thus, these fish cannot reach the ocean and complete their anadromous life cycle. However, this population remains adfluvial and participates in sympatric migrations. Resident fish are observed to have relatively slower growth, earlier sexual maturity, and a reverse sex ratio (0.23 females per male) compared to adfluvial Redband populations. Adfluvial females maximize their fitness by increasing growth and fecundity while males sacrifice growth for survival (Holecek and Scarnecchia, 2013).

Redband trout have evolved to a cold-water habitat, but as stream temperature continues to rise, suitable temperatures may become fleeting in Redband trout's native range (Currens et al., 2009). The optimum temperature for fish is that at which maximum aerobic scope, or the difference of routine and maximum metabolic rates, is reached. This is the temperature where a fish has its best metabolic performance (Chen et al., 2018b; Antilla et al., 2013). When water increases with temperature, oxygen levels decrease slightly, however, organisms' demand for oxygen increases drastically (Chen et al. 2018b). Casselman et al. (2012) estimated that optimum temperature for aerobic scope in *Oncorhynchus* spp. juveniles is 17.0 ± 0.7°C. Chen et al. (2018b) studied Redband trout from streams ranging from 11.6 -18.8°C, which is within the species' thermal optima. The optimal thermal range for Redband trout for desert ecotypes is 3°C greater than montane ecotypes (Chen et al., 2018b). Desert ecotypes are not seen to have a decline in observed physiological functions until the water temperature reaches 24°C (Chen et al., 2018b). There is evidence that the preferred temperature of Redband trout is approximately 13°C (Gamperl et al., 2002). It is likely that the preferred temperature for Redband trout is closely related to their optimum temperature for growth. Another possibility is that Redband trout's preferred temperature is a compromise between optimum temperature for growth and the most advantageous temperature for maximum metabolic power (Gamperl et al., 2002). However, Redband trout individuals may prefer a different operational temperature than their optimum temperature. This could be a result of many distinct factors including food availability, oxygen availability, or refuge from predators, for example (Meyer et al., 2010).

As for optimum DO content, mortality for most fishes occurs at DO levels between 1 to 3 mg/L. For rainbow trout in 24-hour DO tests, the median tolerance of low DO was between 2.6-2.7 mg/L, however, this is far below the optimum (Wagner et al., 2001). Many salmonid species excel in maximum performance and growth when exposed to 80% to 100% DO saturation (Waldrop et al., 2020; Antilla et al., 2015). Fish begin to lose their equilibrium if oxygen levels decrease below 13% saturation (Antilla

et al., 2015). Anywhere below this optimal DO saturation level can result in deleterious effects associated with stress, reduced growth performance, reduced wound healing, increased disease susceptibility, and mortality (Waldrop et al. 2020).

Redband trout's native range spans lower elevation sagebrush deserts as well as high-elevation montane forests where optimum conditions are not always available. In desert habitats, summer water temperature can reach 29°C in arid rangeland streams in southwest Idaho, which greatly exceeds the optimum temperature for Redband trout, and is near the critical thermal maximum (28-30°C) for Redband trout (Chen et al., 2018a). Late summer flow ranges from patchy to non-existent, and temperatures can fluctuate 8-12°C on a diel-cycle (Gamperl et al., 2002). In desert habitat, Redband trout are observed to persist in streams that have static flow conditions, DO levels as low as 2 mg/L, and alarming water temperatures (e.g., 29°C) (Tate et al., 2007). Shading is positively correlated with the occurrence and density of Redband trout in desert streams, while temperature and distance from headwater are negatively correlated (Meyer et al., 2010). Livestock grazing reduces stream shading and negatively affects Redband habitat (Meyer et al., 2010). Physiological adaptations and the presence of refuge for these fish are two mechanisms potentially sustaining the Redband population in high desert basins (Tate et al., 2007).

Conversely, water temperature in montane streams in Idaho generally remains below 20°C (Chen et al., 2018b). However, DO may be lower because oxygen availability varies along an altitudinal gradient (Jacobsen, 2020). Redband trout were more likely to occur in lower-elevation sites with a lower-gradient and cobble or boulder-like substrate. Redband in montane streams rarely undergo high summer water temperatures (Meyer et al., 2010). Shading is also seen to have a positive correlation with density in montane streams, but not a correlation with occurrence. This implies that shade improves habitat quality, but not necessarily habitability. (Meyer et al. 2010).

Because of their noteworthy advanced thermal tolerance, Redband trout are a species of interest for thermal and hypoxia tolerance. For example, Redband trout have been observed feeding at temperatures approaching 28°C , which are fatal to other salmonid species, although the preferred temperature for Redband trout populations is approximately 13°C. Optimal temperatures for Redband trout are estimated to be below 21°C (Wagner et al., 2000; Gamperl et al., 2002; Tate et al. 2007). This species exhibits robust performance in swimming, growth, reproduction, and predation under temperature stress, which suggests that they may utilize adaptations via various mechanisms for their perseverance in extreme environments (Chen and Narum, 2021).

Mechanisms of Hypoxic and Thermal Tolerance

Myocardial hypoxia tolerance exists in more than 20,000 species of fish (Faust et al., 2004). As described by Pörtner et al. (2017), organisms, including salmonids, have an oxygen- and capacity-limited thermal tolerance (OCLTT). This is a range of non-lethal elevated temperatures that verges on the limiting capacity of oxygen demand of an animal. For example, an animal's heart has functional limitations, which creates a limit for oxygen delivery capacity and a thermal optimum for the organism (Chen et al. 2018).

Hypoxia can negatively affect fish behavior, physiology, immunology, and growth (Antilla et al., 2013). As DO decreases, respiration and feed intake also systematically decrease. Prolonged exposure to hypoxia can consequently have a negative impact on physiological and metabolic activities such as cardiac function. Sub-lethal hypoxia can initiate complex primary and secondary stress responses in fishes. During a hypoxic event, fishes are observed to increase ventilation, anaerobic respiration, and hemoglobin affinity, but decrease metabolic rate. Several physiological consequences for fishes ensue after a hypoxic event, including increased cortisol levels, glucose levels, and spleen somatic index. Furthermore, fishes have been observed to have rapid and significant increases of red blood cell number and/or

hematocrit following hypoxia (Abdel-Tawwab et al., 2019). Hypoxia-tolerant species immensely decrease their metabolic rate and heat production when in the presence of a hypoxic stress event. The metabolic rate depression seen in these species is a product of the downregulation of ATP turnover to a point where all ATP production is independent of oxygen availability (Speers-Roche et al., 2010).

Perhaps Redband trout have genetic mechanisms to increase their OCLTT. Hypoxia tolerance has been seen to be heritable, among common carp (*Cyprinus carpio*), which insinuates that hypoxia tolerance may be at least partially determined in fishes. A significant correlation occurs between hypoxia and critical maximum temperature, time to loss of equilibrium, between Atlantic salmon (*Salmo salar*) families. (Antilla et al., 2013). These findings imply that there may be a genetic correlation between thermal tolerance and hypoxia tolerance in *Oncorhynchus* species as well (Antilla et al., 2013). The capacity for selection of hypoxia tolerant traits will rely on whether temperature tolerance traits and hypoxia tolerance traits are positively or negatively associated (Antilla et al., 2013). For example, if there were a functional trade-off for hypoxia tolerance at the expense of thermal tolerance, it would be difficult for selection to favor both traits (Gamperl et al., 1998).

Temperature-at-death is highly heritable among multiple fish species, which suggests a probable genetic component of maximum temperature tolerance with potential for selection (Antilla et al., 2013). Production of heat shock proteins is one mechanism of heat shock response. All organisms, including fish, are seen to increase the production of stress or heat shock proteins when exposed to adverse conditions (Gamperl et al., 1998, Narum et al., 2013). These proteins are crucial cellular mechanisms that repair the damaging effects of hot temperatures and protect myocytes from more acute stressors to come (Gamperl et al. 1998; Meyer et al., 2010). This biological phenomenon, "heat hardening," upregulates the production of heat shock protein 70. Heat hardening can be beneficial for acute diel temperature fluctuations that are seen to have detrimental cellular stress and damage (Tunnah et

al., 2016). In fact, myocytes observed with greater amounts of heat shock protein 70 are seen to survive much longer when exposed to hypoxemia (Gamperl et al. 1998).

Fish utilize energy stores to increase their plasma glucose levels proportionally in response to increases in temperature. If acute temperature stress ensues, fish may adapt to anaerobic metabolic processes if the blood becomes too hypoxemic. A fish may not be able to survive multiple consecutive high-temperature events during multiple diel cycles, for example (Callaghan et al., 2016). Proteolysis, the breakdown of proteins or peptides, may become an option to sustain a fish during extended periods of heat stress, however, this process is exceedingly unsustainable for multiple diel-cycle temperature fluctuations (Callaghan et al., 2016).

Desert Redband trout have a strong heat shock response to reduce the energetic demands of heat shock protein production, but because it is still energetically costly, these populations have a tradeoff of reduced growth and fitness (Narum et al., 2013). Although montane Redband trout have improved short-term survival after exposure to heat stress, demands of heat shock protein production may have led to high mortality with repeated and chronic exposure to heat stress (Narum et al., 2013). Adaptive capacity around heat shock response and survival have been observed for Redband trout and are associated with the frequency of a single nucleotide polymorphism (SNP) in the 3'-untranslated region (UTR) of the Hsp47 gene, among other genetic markers (Narum et al., 2013). Redband trout also possess a candidate gene, ceramide kinase (cerk) on chromosome 4, that widely differs between desert and montane populations (Chen and Narum, 2021). The cerk gene in Redband trout is significantly associated with chronic and acute thermal tolerance as well as cardiac performance under thermal stress (Chen and Narum, 2021). More research is necessary to determine whether the cerk gene association with cardiac performance can be linked to the functional thermal performance and hypoxia tolerance of Redband trout (Chen and Narum, 2021). For vertebrates broadly, the isoforms of cytochrome c oxidase (COX) are seen to be differentially expressed in response to hypoxia. One of these genes is COX6B with isoforms B1 and B2. COX6B2 is seen to be

upregulated in the desert strain of Redband trout in response to temperature, suggesting another candidate gene and a complex regulation of thermal tolerance. (Garvin et al., 2015).

Desert and montane Redband trout have locally adapted their respiratory response to heat shock in their respective, genetically divergent populations. Montane Redband may lack the capacity within their gill tissue to decrease respiration in response to temperature stress. Contrarily, desert Redband trout are seen to upregulate many genes involved in respiration control when responding to temperature stress, which requires coordination of the entire respiratory machinery (Garvin et al., 2015). Furthermore, genes that control respiration in Redband trout can express differently among fish acclimated to different environmental regimes due to phenotypic plasticity (Garvin et al. 2015).

Drastic changes in environmental conditions force species to respond in distribution and diversity**.** Phenotypic plasticity is often employed so species can shift their distribution and survive in habitats that, historically, they have not occupied (Chen et al., 2018). Fish can adjust to environmental variables such as temperature change and hypoxia via phenotypic plasticity (McBryan et al., 2013). There has been evidence of a cross-tolerance phenomenon with hypoxia and temperature stress. For example, tide pool sculpins (*Oligocottus maculosus*) are observed to have higher hypoxia tolerance after exposure to acute heat stress. Conversely, acclimation to hypoxic events can make the channel catfish (*Ictalurus punctatus*) more tolerant to normoxic heat stress events (McBryan et al., 2013).

Acclimation, a reversible form of phenotypic plasticity, is known to modify the maximum temperature and hypoxia tolerance in many fish species. Acclimation to warm temperatures and overnight hypoxia can nearly double hypoxia tolerance in Atlantic salmon (Antilla et al., 2015). Generally, salmonids possess a limited capacity for acclimation of temperature and hypoxia tolerance; thus, selection may act on these traits (Antilla et al., 2013). Moreover, intraspecific differences in DO tolerance have been observed in rainbow trout (Wagner et al., 2001).

Climate induced stressors such as hypoxia and increased water temperature can also initiate plastic effects on Redband trout's internal organs and initiate a physiological response. Two organs of interest for hypoxia and thermal tolerance are the gills and the heart. Teleost gills have multiple functions beyond respiratory gas exchange that include ionoregulation, nitrogenous waste excretion, acid-base balance, and water exchange (Onukwufor and Wood, 2018). Such functions of the gill have environmental conditions that facilitate optimum performance and may cause osmorespiratory compromise (Gilmour and Perry, 2018).

Osmorespiratory compromise is a trade-off between enabling osmoregulation and facilitating respiratory gas exchange via passive diffusion in the gills (Gilmour and Perry, 2018). When osmoregulatory demand is high, the gills exhibit thicker epithelial cell membranes that reduce permeability and surface area, which reduces ion and gas exchange (Dhillon et al. 2013). These enlarged epithelial cell membranes are called the interlamellar cell mass (ILCM) (Blair et al., 2016; Gilmour and Perry, 2018; Onukwufor and Wood, 2018). When ILCM is not expressed, the gills facilitate respiratory gas exchange (O_2 influx and CO_2 excretion). This means the epithelial membranes are thin, the surface area between lamellae is increased, diffusion distance is decreased, the blood-water flow rates are high, and the ion flux rates are high (Onukwufor and Wood, 2018). Generating the ILCM to enable osmoregulation can take several days to build up, but when a fish is exposed to an environmental signal, they can be eliminated in a matter of 30-35 minutes, as seen in the classic example of ILCM, the crucian carp (*Carassius carassius*)(Gilmour and Perry, 2018).

There are multiple mechanisms that cue physiological remodeling in gills. Chemoreception is one of these mechanisms that can allow teleosts to detect chemical changes in their environment, such as changes in DO or salinity, and adjust the presence of the ILCM (Blair et al., 2016; Gilmour and Perry, 2018). Teleosts from many families, including Salmonidae, can sense higher oxygen demand, like after physical exertion or when the ambient temperature rises (Gilmour and Perry, 2018). One example of signaling mechanisms used to detect oxygen demand is by monitoring

changes in arterial blood oxygen concentration (Gilmour and Perry, 2018). Since Redband trout are highly tolerant to thermal and hypoxic stress, it is likely that this species is also able to exhibit phenotypic plasticity within their gill morphology to combat adverse environmental changes (Garvin et al. 2015). Although, more research needs to be done on the specific mechanisms involved.

When Redband trout populations are exposed to adverse conditions such as hypoxia and/or elevated temperature, the gills may favor a state of heightened gas exchange where the ILCM is not present (Chen et al., 2018; Onukwufor and Wood, 2018). As a result, a loss of vital salts such as Na^+ and Cl^- may occur (Onukwufor and Wood, 2018; Blair et al., 2016). Compensation, or active salt absorption across gills, is necessary for salmonids living in diluted freshwater environments. Sodium-potassium ATPase is a vital component of systemic cellular ionic homeostasis maintenance (Blair et al. 2016). Increased lamellar perfusion, increased branchial perfusion, and recruitment of lamellae have been observed in rainbow trout in response to this Na^+ loss, increasing gill oxygen transfer. However, when hypoxia was maintained for prolonged periods of time and at elevated severity, Na^+ loss remained elevated as well (Onukwufor and Wood, 2018). If Redband trout are exposed to hypoxia periodically, they may be able to remodel their gills by lamellar recruitment and compensate for the osmorespiratory trade-off that is Na^+ loss to their dilute environment. Although, if they are exposed to severe hypoxia for extended periods of time, they may falter and suffer severe deleterious effects. However, more research is needed to understand the limitations of hypoxia-tolerant Redband trout subspecies in contrast with the hypoxia-sensitive rainbow trout.

Wagner et al. (2001) noted an increase of critical thermal maximum with Cutthroat trout acclimated at higher temperatures. Critical thermal maximum loss of equilibrium and critical thermal maximum at spasms were significantly higher for fish acclimated to 18°C than those acclimated to 13.6°C (Wagner et al., 2001). Phenotypic plasticity is a plausible explanation for intraspecific differences in thermal and hypoxic tolerance among *Oncorhynchus* species. Salmonids have been observed

to induce several molecular defenses in response to heat stress both during and following a stress event (Callaghan et al., 2016). One of these mechanisms is appointing cell survival pathways by inhibiting adenosine-monophosphate-activated protein kinase activity (Callaghan et al., 2016).

There is evidence of thermally mediated plasticity in fish cardiac function as well (Callaghan et al., 2016). Significant reductions in blood oxygen content and partial pressure are associated with environmental hypoxia. Hypoxia exposure reduces cardiac myocyte beta-adrenoreceptor density by 35-65% of the cell surface area. Loss of beta-adrenoreceptors can have ionotropic or chronotropic responsiveness of the myocardium to catecholamines, such as neurotransmitters like epinephrine. Fish with low beta-adrenoreceptor density or reduced binding affinity may have lower cardiac performance in presence of hypoxia (Gamperl et al. 1998)

The fish heart is observed to remodel itself via phenotypic plasticity in the presence of environmental stressors (Nørstrud et al., 2018). Seasonal temperature changes can drive cardiac remodeling in fish to establish appropriate output (Keen et al., 2016). As temperature decreases, fish hearts tend to become bradycardic, or slowed heart rate (f_H). This is a result of greater diastolic duration, while systolic duration remains constant. As diastolic duration decreases, stroke volume can decrease as well, leading to lower cardiac performance (Keen et al., 2017). These phenotypic responses can be a result of myocardial hypertrophy (Keen et al., 2017).

Hypertrophy, or the augmentation of a part or a whole organ by enlargement of constituent cells, is a common mechanism used to remodel the heart during shifting temperature regimes. In this case, the fish ventricle will increase in thickness in response to chronic cooling, allowing systolic pressure to be maintained (Keen et al., 2017). This hypertrophy in the ventricle is seen to regress during summer seasonal warming in rainbow trout (Keen et al., 2017). Moreover, rainbow trout that are warm acclimated have an overall reduction in ventricular mass, an increase in thickness of the compact layer, and a decrease in connective tissue content (Keen et al., 2017). With these phenotypes, the fish heart can have a larger luminal capacity in the ventricle,

which increases cardiac output (Keen et al. 2017). Contrarily, cold acclimated rainbow trout have greater collagen and connective tissue to protect the myocardium from hemodynamic stress of pumping viscous, cold blood. Increased collagen also increases passive stiffness and an increase in the spongy myocardium layer (Antilla et al., 2015; Keen et al., 2017).

The amount of the steroid stress hormone, cortisol, in the fish's system is another component that affects ventricular mass in Rainbow trout (Nørstrud et al., 2018). Nørstrud et al. (2018) explained that a 45-day treatment of cortisol feed resulted in a 34% increase in relative ventricular mass in rainbow trout. As a result, chronic cortisol exposure, myocardial hypertrophy, focal fibrosis, and impaired cardiac function could ensue (Nørstrud et al., 2018). There are many things that can cause a cortisol increase in salmonids, including spawning, migration, temperature stress, hypoxic stress, natural chronic stressors, and artificial chronic stressors (Norstrud et al., 2018; Kammerer and Heppel, 2013). While chronic stressors can cue phenotypic plasticity, acute stress is seen to trigger cardiac events and mass mortality (Nørstrud et al., 2018). Although warm acclimation has been observed to decrease ventricular mass, if salmonids have chronic stress and increased cortisol levels in addition to a warm acclimation temperature, there is a possibility that the ventricular mass could still increase. Thus, impairing cardiac performance in a high-stress environment (Antilla et al, 2015; Nørstrud et al. 2018).

More research is necessary to encompass the combined effects of hypoxia and thermal tolerance mechanisms in Redband trout. These are two of the most prominent challenges Redband trout will face during the climate crisis. A better understanding of the mechanisms behind Redband persistence in extreme thermal and hypoxic events may provide more tools for successful management or genetic rescue of small, isolated populations. If hypoxia and elevated temperatures do have a synergistic effect on Redband trout, the species may be more imperiled than previously understood. Although, if Redband are able to acclimate to water that is chronically

beyond their optimal thermal and oxygen concentration ranges, there may be more suitable habitat for Redband populations to inhabit for a longer period of time.

As discussed above, arid desert and rangeland streams can pose multiple threats to aquatic ecosystems including increased hypoxic and thermal stress, (Meyer et al., 2010). Because Redband trout are native to these streams, they have evolved several physiological mechanisms as well as shifted their phenotypic expression to survive in deteriorated realized niches (Narum et al., 2015; Chen et al., 2018a; Chen et al., 2018b; Chen and Narum, 2021; Pörtner et al., 2017). This research aims to investigate the plasticity of hypoxia and thermal tolerance in the presence of chronic thermal stress (21°C acclimation temperature) relative to optimum temperature (15°C acclimation temperature). Additionally, this research will show how cardiac performance during acute increases in temperature varies depending on Redband trout ecotype, ambient DO concentration, acclimation temperature. The following hypotheses were tested in the research summarized in this thesis. (H_1) The desert ecotype will have greater average hypoxia tolerance than montane ecotypes. (H_2) Redband trout acclimated to 21°C will have lower average hypoxia tolerance than Redband trout that are acclimated to 15°C. (H_3) The desert ecotype will have a higher temperature at peak heart rate (T_{peak}) and stronger cardiac performance in stressful temperatures and hypoxia, on average, than the montane ecotypes. (H_4) Redband trout acclimated to 21°C will display a higher T_{peak} and a stronger cardiac performance than Redband trout that are acclimated to a 15°C water when exposed to hypoxic and thermal stress. (H_5) The combination of hypoxic and thermal stress will have a synergistic effect regardless of ecotype and acclimation temperature.

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Chapter 2: Hypoxia Tolerance

Introduction

Climate change has increased the water temperature of many river and stream ecosystems (Fellman et al., 2019). Hypoxic and anoxic events, or low dissolved oxygen (DO) and lack of DO, respectively, can increase in prevalence and duration as water temperature increases (e.g., Henry's law) and other anthropogenically influenced factors such as eutrophication, biochemical oxygen demand, and low flow regime can also increase the prevalence of aquatic hypoxia (McBryan et al., 2013; Garvin et al., 2015; Antilla et al., 2013). Reduced flow regimes can be caused by habitat degradation from phenomena such as increased human water consumption, increased air temperature, lack of shaded areas, sporadic precipitation, and a low snowpack and are expected to increase in frequency and extent with climate change (Chiu et al., 2017; Archdeacon and Reale, 2020). Increased biological demand can also occur from high densities of a non-native species that deplete resources, such as oxygen (Miller et al., 2014). Eutrophication is characterized by excess nutrients as a byproduct of agriculture, industry, and sewage disposal in a water system. Excess nutrients such as nitrogen and phosphorus cause increased oxygen demand for microbial respiration as well as photosynthetic organisms to proliferate (Chislock et al., 2014), as these organisms respire throughout the diel cycle, and can deplete oxygen from the aquatic system (McBryan et al., 2013).

Chronic temperature stress may affect several physiological functions and have deleterious effects on aquatic ectotherms (Isaak et al., 2012; Pörtner et al., 2017). However, DO concentration may be the proximate physiological limiting factor for an ectotherm's persistence during a hypoxic and thermal stress event (Fellman et al., 2019). The combination of hypoxic and thermal stress is thought to have a synergistic interaction since hot temperatures increase the oxidative metabolic rate for an ectotherm, and hypoxia limits oxygen availability (McBryan et al., 2013). If evolutionary or plastic adaptation is slower than necessary to keep up with

environmental change, the lack of overlap between a species' physiological capacity and environmental conditions can lead to local extirpation (Isaak et al., 2012).

Redband trout are a temperature and hypoxia-tolerant subspecies of Rainbow trout that are native to the Columbia River Basin and can be said to have three genetically distinct subpopulations (Chen et al., 2018b) and some of the populations represent genetically divergent ecotypes associated with desert, cool montane, and cold montane habitats (Garvin et al., 2015; Schill et al. 2007; Currens et al., 2009; Chen et al., 2018a, 2018b). In the desert habitat, water temperatures can reach 29°C in summer months and can fluctuate 8-12°C on a singular diel cycle (Tate et al., 2007; Gamperl et al., 2002). High desert streams can also become intermittent in the summer months, which can decrease the DO levels to as low as 2 mg/L (Tate et al., 2007; Archdeacon and Reale, 2020). Conversely, the montane Redband trout habitat generally remains below 20°C and has perennial flow (Meyer et al., 2010). Genetic divergence of desert and montane Redband ecotypes is likely due to limited gene flow because of habitat fragmentation, relocation to new areas, or dispersal barriers such as intermittent streams in summer months and disconnected stream systems (Narum et al., 2010).

Redband trout have multiple heritable genes associated with chronic and acute thermal tolerance. These genes can be population-specific and can be differentially expressed in response to temperature (Garvin et al., 2015; Chen and Narum, 2021). Genetically based physiological responses to hypoxic stress are less well studied than responses to thermal stress, but many fishes may have developed heritable hypoxia and thermal tolerance. For example, the common carp (*Cyprinus carpio*) has been shown to have heritable hypoxia tolerance (Antilla et al., 2013). Further, hypoxia-induced genes show a common method of transcriptional regulation that depends upon the activation of an inducible transcription factor, like hypoxia-inducible factor 1 or vascular endothelial growth factor. Upregulation of these genes leads to the transcription induction of several other hypoxia-responsive genes for enhancing

overall hypoxia tolerance, as observed in extreme hypoxia-tolerant species like the Amazonian Oscar (*Astronotus ocellatus*) (Baptista et al., 2016).

Hypoxia and temperature tolerance have also been associated with phenotypic plasticity in physiology and behavior. Acclimation, a reversible form of phenotypic plasticity, has been widely observed (McBryan et al., 2013). Overnight hypoxia and increased water temperature can nearly double hypoxia tolerance in Atlantic salmon (*Salmo salar*) (Antilla et al., 2015).

Hypoxia-tolerant species are seen to increase ventilation, anaerobic respiration, and hemoglobin affinity, but decrease their metabolic rate and heat production in response to a hypoxic stress event. Myoglobin, an oxygen-binding hemoprotein, is seen to have an influential role in providing the heart with oxygen and can be expressed at the mRNA and protein levels of the heart during a hypoxic event (Abdel-Tawwab et al., 2019). Myoglobin is seen to have an influential role in providing the heart with oxygen (Abdel-Tawwab et al., 2019). Metabolism decrease is understood to result from the downregulation of ATP turnover. Hypoxia-tolerant species can effectively produce ATP independent of oxygen availability via anaerobic glycolysis (Speers-Roche et al., 2010; Abdel-Tawwab et al., 2019).

Although Redband trout have been understood to prefer temperatures near 13°C and have optimal temperatures below 21°C, they are known to withstand temperatures beyond this threshold, at least in part owing to phenotypic plasticity (Wagner et al., 2000; Gamperl et al., 2002; Tate et al, 2007; Chen et al., 2018b). In the context of OCLTT, hypoxia tolerance is expected to decrease with temperature stress, while thermal tolerance is expected to decrease with hypoxic stress on both chronic (e.g., days) and acute (e.g., hours) time scales. While chronic stress may decrease foraging ability, reproduction, or limit growth, acute stress may be critical to survival (McBryan et al., 2013). Optimal temperature can be defined as a range where maximum aerobic scope can be achieved. The maximum aerobic scope is when the difference between the maximum and routine metabolic rates is largest. Routine metabolic rate systematically increases with temperature increase (Antilla et al., 2013).

As for optimum DO content, many salmonid species excel in maximum performance and growth when exposed to DO saturation of 80% to 100% (Waldrop et al., 2020; Antilla et al., 2015). Environmental conditions below this DO saturation level can result in deleterious effects associated with stress, reduced growth performance, reduced wound healing, increased disease susceptibility, and mortality (Waldrop et al. 2020). Although Redband trout are seen to briefly persist in isolated pools with acute DO decrease reaching extremely low DO content (2 mg/L), salmonids can begin to lose their equilibrium near 13% saturation (Tate et al., 2007; Antilla et al., 2015). Such low DO concentrations may only occur for a short-term, acute stress event (Tate et al., 2007). Mortality for most fishes occurs at DO levels between 1 to 3 mg/L, which can occur in arid desert habitat for the Redband trout (Meyer et al., 2010; Tate et al., 2007; Wagner et al., 2001).

In this study, Redband trout were sampled from desert and montane streams throughout Idaho and were acclimated to near-optimal (15°C) and elevated (21°C) temperatures in a common garden environment similar to that used by Chen et al. (2018b). The objective was to compare the relative hypoxia tolerance of three ecotypes (desert, cool montane, and cold montane) at both acclimation temperatures. Intraspecific phenotypic differences may present themselves between ecotypes (cold montane, cool montane, and desert), acclimation temperatures, or both. The hypotheses for this study were: (H_1) The desert Redband trout ecotype will have greater average hypoxia tolerance than montane Redband trout ecotypes, and (H_2) Redband trout in each ecotype acclimated to 21°C will have lower average hypoxia tolerance than Redband trout acclimated to 15°C.

Methods

Fish Culture and Rearing Conditions

Age-0 (1–3-month-old fry) Redband trout were collected by electroshocking several desert and montane streams in Idaho. Little Jack's Creek (42.750433, −116.091990), William's Creek (42.833106, −116.932611), and Duncan Creek (42.4560069,

−116.0562117) provided the fish for the desert ecotype. Fish from the cool montane ecotype were collected from Keithley Creek (44.51787, −116.83524), Trail Creek (48.539, -116.359), and Whiskey Jack Creek (43.6537849, −115.3809219). Cold montane ecotype fish were collected from Fawn Creek (44.38714, −116.0615), Upper Mann Creek (44.57841, −116.95333), and Boulder Creek (48.8174265, −116.9496733) (Chen et al., 2018a; Chen et al., 2018b). Population sizes at the time of this experiment from each of the desert creeks were: Little Jack's (n = 12) William's (n = 3), and Duncan (n = 6). Population sizes for each of the cool montane creeks were: Keithley (n = 3), Trail (n = 14), and Whiskey Jack (n = 4). Population sizes for the cold montane creeks were: Fawn (n = 0), Upper Mann (n = 14), and Boulder (n = 2). For reference, temperature loggers were installed in the streams where sampling took place, and DO loggers were installed at Little Jack's Creek. Fish were held in a five-gallon bucket during sampling and transported to the University of Idaho Hagerman Fish Culture Experiment Station in a cooler with constant water aeration. Redband trout from each ecotype were acclimated for a minimum of 8 weeks in a common garden environment at 15°C (within thermal optimum range) or 21°C (near upper pejus temperature) water temperature to model the desert and cold montane habitats, respectively (Pörtner et al., 2017; Chen et al., 2018a). Fish from each population were acclimated in 50-gallon tanks on a diel photoperiod of 14 hours of light and 10 hours of dark (Chen et al., 2018b). All field collections were conducted under scientific collection permits issued by the Idaho Department of Fish and Game and University of Idaho Institutional Animal Care and Use Committee (IACUC) protocols.

Experimental Design

The fish used for the hypoxia tolerance experiment had not been handled for at least 4 weeks and were fasted 24 hours prior. Each trial consisted of 15-18 fish, which varied in sample size depending on acclimation temperature. Redband trout were placed in a cooler with water set to their acclimation temperature, covered, and secured with plexiglass. The fish were then acclimated to the conditions of the experimental tank

with oxygenated water for 1 hour while it was covered with an opaque top to avoid startling and stressing the fish. During acclimation, the water was saturated starting at an average of 86.2 % DO (7.81 mg/L) at 15°C and 74.8% DO (5.97 mg/L) at 21°C. DO was then systematically decreased by delivering N_2 gas into the water at a constant rate to reduce DO saturation by 1.5% per minute. This continued until each fish lost equilibrium or dorsoventral orientation. A fish was considered to have a loss of equilibrium (LOE) when they had rolled upside down for at least 5 seconds and had no sign of recovery and were oriented horizontally and not vertically (Antilla et al., 2013). Time at LOE was recorded for each fish and then the fish was taken out of the experimental tank and placed into a recovery tank with saturated oxygen their respective acclimation temperature. Once all fish had lost equilibrium and recovered, the experiment was completed.

Statistical Analysis

The effect of ecotype, acclimation temperature, and body mass on time at LOE were analyzed using a type II 3-way analysis of variance (ANOVA). Larger individuals have an increased ability to survive extreme hypoxia compared to smaller individuals or juveniles because they can adjust their standard metabolic rate (Baptista et al., 2016; Shi et al., 2018). Likewise, these similar statistical tests were used for DO concentration at LOE as the response variable. Values presented in the results are displayed as mean ± standard error of the mean unless stated otherwise. Tukey's honest significant difference (HSD) test was used in a post-hoc comparison of significant differences within interactions between main effects ecotype and acclimation temperature.

Results

We observed an overall effect of acclimation temperature on hypoxia tolerance (Figures 2.1, 2.2; Tables 2.1, 2.2). Fish that were acclimated under near-optimal temperature conditions (15°C) persisted significantly longer under acute hypoxic stress than Redband trout that were acclimated under chronic heat stress (21°C) (Figure 2.1; $p \leq 0.001$). All fish acclimated at 15°C ($n = 30$) had an average time at LOE

of 64.4 ± 0.67 minutes (min), while all fish that were acclimated to 21°C (n = 29) had an average time at LOE of 56.5 ± 0.75 min. LOE for fish acclimated to 15°C began at 13.2% DO concentration (1.19 mg/L) and ranged to 9.5% DO (0.86 mg/L), while time at LOE for fish acclimated to 21°C began 37.2% DO concentration (2.97 mg/L) and ranged to 11.2% DO (0.89 mg/L). These averages were not statistically different (Figure 2.2).

All fish acclimated to 15°C had a similar average time at LOE, regardless of ecotype (Figure 2.1). The cool (n = 10) and cold (n = 12) montane ecotypes persisted at averages of 64.7 ± 1.31 min and 64.6 ± 1.09 min, respectively. The desert ecotype (n = 8) average time at LOE was slightly lower at 63.7 ± 1.10 min; however, the averages for time at LOE for all ecotypes at 15°C were not statistically different (Figure 2.1). Among the fish acclimated to 21°C, the overall average time at LOE was 56.5 ± 0.75 min. The cool montane ecotype (n = 11) was seen to have significantly lower tolerance to hypoxic stress because their average time at LOE (53.6 ± 1.36 min) was significantly lower ($p = 0.048$) than that of the desert ecotype (n = 14) Thus, there was a significant interaction between acclimation temperature and ecotype for fish acclimated at 21°C and cold montane (n = 4) ecotypes (58.7 ± 0.52 min and 57.2 ± 1.99, respectively).

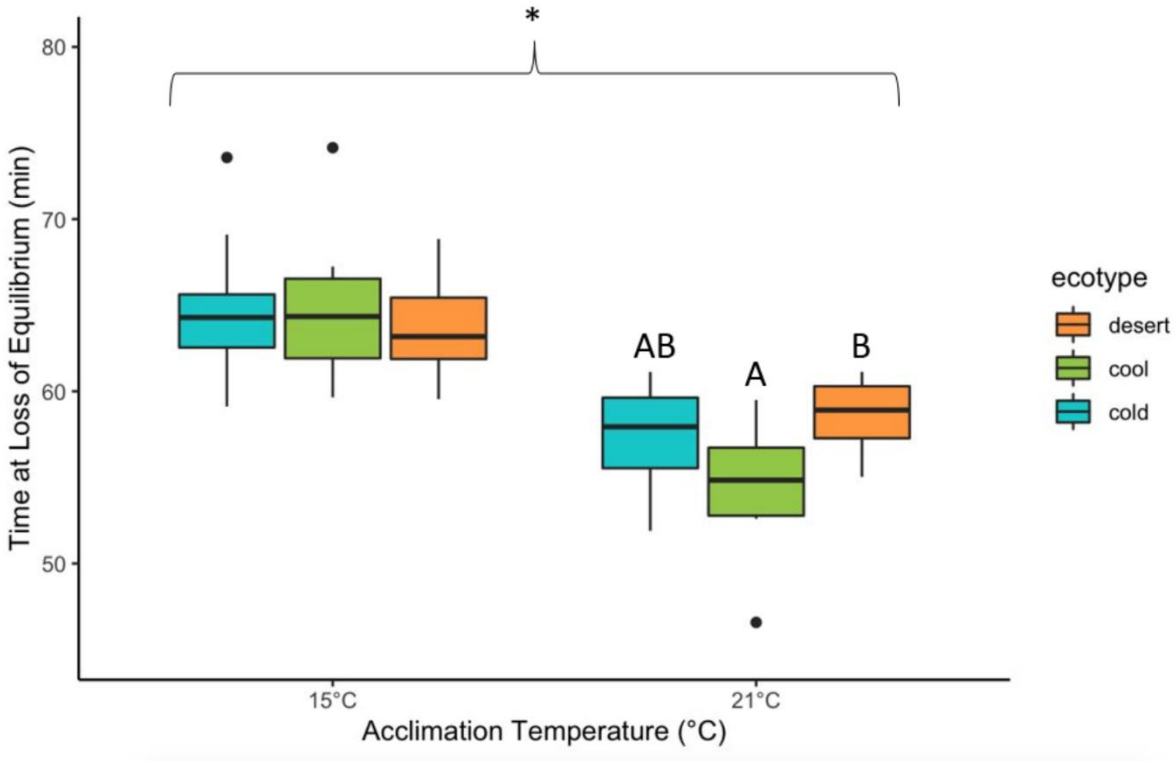

Figure 2.1 Time at LOE. Average time at LOE for Redband from each ecotype within each acclimation temperature. Redband trout ecotypes that were acclimated at 15°C had no significant differences in time at LOE. Redband trout acclimated at 21°C did have significant differences between time at LOE among ecotypes, as signified by letters ($p = 0.048$). Time until LOE was significantly longer for Redband trout that were acclimated to the near-optimal 15°C temperature compared to acclimation at 21°C, as indicated by an asterisk (ANOVA, Tukey's HSD, $p \leq 0.001$).

Table 2.1 Time at LOE ANOVA Table. Anova output with time at LOE as the response variable and acclimation temperature (Acc Temp), ecotype, and body mass as the indicator variables.

	Sum Sq.	Df	F value	Pr (>F)
Ecotype	79.18	2	3.0215	0.05753 .
Acc Temp	860.59	1	65.6807	9.982e-11 ***
Body Mass	2.89	1	0.2204	0.64070
Ecotype: Acc Temp	84.41	2	3.2212	0.04815 *
Residuals	668.23	51		

For Redband acclimated to a near-optimal temperature (15°C), dorsoventral orientation was maintained until the DO concentration was reduced to 1.00 ± 0.07 mg/L. On the contrary, when fish were acclimated to an elevated temperature (21°C), they maintained dorsoventral orientation only until DO concentration reached 1.42 ± 0.47 mg/L. In congruence with Figure 2.1, the mean effect of acclimation on DO

concentration at LOE between acclimation temperatures was statistically different ($p \leq 0.001$). The DO concentration at LOE response was also significantly different among ecotypes regardless of acclimation temperature ($p \leq 0.001$; Table 2.3). The means of DO concentration at LOE for cold montane (n = 16), cool montane (n = 21), and desert (n = 22) Redband trout were 1.056 ± 0.060 mg/L, 1.41 ± 0.11 mg/L, and 1.12 ± 0.052 mg/L, respectively. Trout acclimated to 21°C had larger standard errors than that of the trout acclimated within their optimal temperature range. In addition, the means of DO concentration at the time of LOE between ecotypes was also statistically different for the 21°C acclimation temperature, showing an interaction between acclimation temperature and ecotype factors ($p \leq 0.01$).

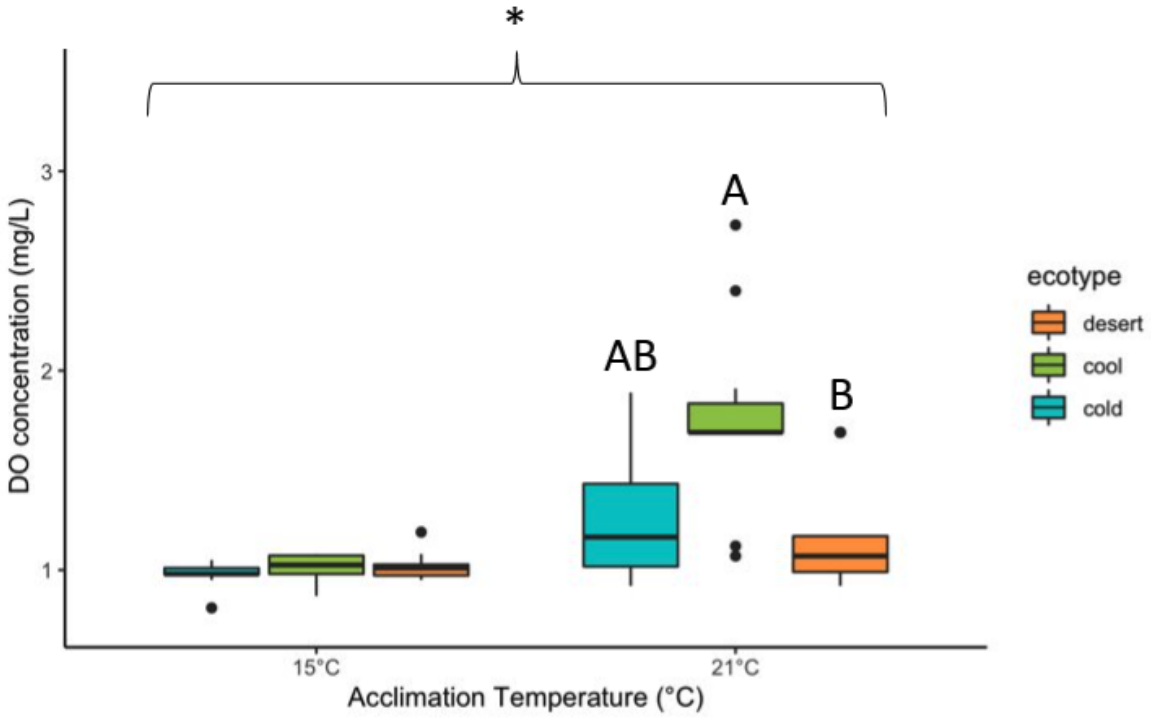

Figure 2.2 DO concentration at LOE. for Redband trout ecotypes acclimated to 15°C or 21°C. Redband trout that were acclimated at 15°C had no significant differences in time at LOE between ecotypes. Redband trout acclimated at 21°C did have a significant difference between DO concentration at LOE among ecotypes as signified ($p \leq 0.01$), by letters. Redband trout acclimated to 15°C exhibited LOE at a significantly lower DO concentration than those acclimated at 21°C, as indicated by the asterisk (ANOVA, Tukey's HSD, $p \leq 0.001$).

Table 2.2 [DO] at LOE ANOVA Table. Anova output with DO concentration at LOE as the response variable and acclimation temperature (Acc Temp), ecotype, and body mass as the indicator variables. Asterisks in the Pr (>F) column show level of significance (0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1).

	Sum Sq.	Df	F value	Pr (>F)
Ecotype	1.3018	2	8.2611	0.0007801 ***
Acc Temp	2.3971	1	30.4244	1.164e-06 ***
Body Mass	0.0030	1	0.0380	0.8462112
Ecotype: Acc Temp	0.9511	2	6.0356	0.0044398 **
Residuals	4.0183 51			

Discussion

This study established that the first hypothesis (H_1), desert Redband trout ecotype will have greater average hypoxia tolerance than montane Redband trout ecotypes, was not fully supported because desert Redband trout did not have a significantly greater time at LOE compared to both montane counterparts in either acclimation temperature. The second hypothesis (H_2), Redband trout in each ecotype acclimated to 21°C will have lower average hypoxia tolerance than Redband trout that are acclimated to 15°C, was confirmed by the results. These results show that a higher acclimation temperature decreases the Redband trout threshold for coping with acute hypoxic stress. If chronic thermal stress, as simulated with the 21^{o}C acclimation, is not a factor, a Redband trout's ecotype does not necessarily improve their hypoxia tolerance. When chronic temperature stress is a factor, it is seen that the desert ecotype has a significantly heightened hypoxia tolerance in comparison to the cool montane ecotype. This suggests that hypoxia tolerance, at least in part, may be a function of ecotype and, thus, genetically determined. An increased acclimation temperature did not appear to signal plastic acclimation to a hypoxic event, but it evidently increased the strain on the bioenergetic capacity of Redband trout. Hypoxia tolerance depends on how long a fish can maintain balance between ATP supply and demand when exposed to hypoxic stress. The LOE represents the point where fish can no longer meet the ATP demand via anaerobic metabolism (Rogers et al., 2016).

These results are important because fishes that are exposed to elevated temperatures outside of their optimal range may be more imperiled when hypoxic events occur than if they were in habitats within optimal temperature range. Often,

hypoxic events will occur in areas that already have increased temperature and degraded habitat because of reduced shading, intermittent flow, and eutrophication (Tate et al., 2007; Archdeacon and Reale, 2020; Chiu et al., 2017). Even a 6°C change in acclimation temperature can cause a substantial change in capacity for animals to respond to both hypoxia and temperature (Meyer et al., 2013). This is true for salmonids, where the higher end of tolerated temperatures have been shown to reduce hypoxia tolerance (Antilla et al., 2015).

The cool montane ecotype exhibited a significantly lower time at LOE ($p \leq 0.05$) than the desert and cold montane ecotypes acclimated to 21°C, which was an unexpected outcome. The cool montane ecotype was expected to perform better to hypoxic stress than the cold montane ecotype. Although the desert (n = 14) and cold montane (n = 4) ecotypes were seen to have similar hypoxia tolerance responses, this result must be treated with caution regarding the low cold montane ecotype sample size. Otherwise, the Redband of the desert ecotype may have some heritable genetic adaptation to hypoxia that the cool montane fish lack, perhaps because elevated temperatures and hypoxic stress that are associated with desert stream habitat.

Hypoxia tolerance was not significantly different for the divergent ecotypes when acclimated to 15°C. Because 15°C is within Redband trout thermal optima, hypoxic stress may not have the selective pressure that it does at an elevated temperature (Chen and Narum, 2021).

These results are limited because they do not show how differentially acclimated Redband trout would perform at a constant temperature. For example, from these results we cannot determine how fish acclimated to 21°C would perform during severe hypoxia in 15°C water, nor can we determine how fish acclimated to 15°C would react to severe hypoxia in 21°C water. This could tell us more about the phenotypic plasticity of Redband trout and just how much acclimation under chronic temperature stress improved or did not improve Redband trout performance during a hypoxic stress event. Certainly, the low time at LOE for Redband acclimated to 21°C is influenced by the lower amount of oxygen at the beginning at the experiment.

Having said that, because 21°C acclimated fish began to lose equilibrium at 37.2% DO saturation (2.97 mg/L) compared to 13.2% DO saturation (1.19 mg/L) like the 15°C acclimated fish, the chronic elevated temperature of 21°C appears to have impaired Redband trout persistence under oxygen stress. Seeing as the warm-acclimated cool montane ecotype had such a low hypoxia tolerance, there is a question of possible genetic component that decreases hypoxia tolerance further when fish are acclimated to an elevated temperature. However, there is no evidence for this notion and further research must be done.

More evidence about the mechanisms of hypoxia tolerance could be studied with an experiment investigating hypoxia tolerance of fishes acclimated to water with a low DO concentration, rather than a temperature acclimation. Low oxygen concentration may stimulate an accelerated adaptation by way of phenotypic plasticity to increase time until LOE and thus enhance persistence for fishes (Antilla et al., 2015). Further, another study with fish acclimated to both temperatures (15°C and 21°C) experiencing hypoxia at a constant temperature rather than their respective acclimation temperature would provide more evidence about the potential for phenotypic plasticity to hypoxia tolerance in Redband trout. This would be applicable to natural habitats providing a cool refuge, such as a deep pool or lake with low oxygen but near ideal temperatures. Further, more research needs to be conducted on the genetic components of hypoxia tolerance in Redband trout. Narum et al. (2015) described thermal tolerance as genetically derived, at least in part. However, there are few studies about the heritability of hypoxia tolerance, and further research is necessary to understand how Redband trout genotypes differ in hypoxia tolerance.

This study showed that greater acclimation temperature can decrease Redband trout's ability to tolerate environmental hypoxia. Further, ecotype can influence hypoxia tolerance when fish are warm-acclimated. In the field, fish that are already exposed to increased temperatures may experience more adverse effects if they encounter environmental hypoxia.

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Chapter 3: Maximum cardiac function under acute thermal and hypoxic stress

Introduction

Anthropogenic activity and climate change continue to contribute to habitat degradation for many aquatic species, including Redband trout (*Oncorhyncus mykiss gairdneri*), a sensitive species native to the Columbia River basin (Meyer et al., 2010). Human activity has increased thermal regimes and increased aquatic hypoxia, or dissolved oxygen (DO) deficiency, frequency, two significant threats to aquatic ecosystems (Isaak et al., 2011; Fellman et al., 2019). While chronic hypoxic or thermal stress can cue phenotypic plasticity, acute stress is seen to trigger cardiac events and mass mortality (Nørstrud et al., 2018). Hypoxia can be a result of eutrophication, biological demand, or low streamflow and intermittency (Antilla et al., 2013; Chiu et al., 2017). Elevated temperature and low oxygen availability are drivers for harsh selection in fishes because they are taxing on their bioenergetic homeostasis maintenance (Callaghan et al., 2016). To persist during drastic environmental shifts, Redband trout, and many other aquatic species, must disperse to more suitable habitats, utilize mechanisms of adaption such as genetic evolution or phenotypic plasticity, or combat extinction (Chevin and Lande, 2010).

Ectothermic animals, such as fishes, have temperature limits and tolerance ranges that will determine their sensitivity to climate change (Dahlke et al., 2020). The specific mechanism responsible for temperature-induced death has not been fully established. However, the oxygen- and capacity-limited thermal tolerance (OCLTT) concept is a widely accepted phenomenon that establishes an animal's fundamental and realized thermal niches. OCLTT posits that once an animal nears the limitations of their thermal tolerance, their ability to deliver oxygen to tissues largely declines, and they cannot meet the oxygen demand. Lack of oxygen can influence performance and individual survival, thus jeopardizing population survival (Pörtner et al., 2017). Many things can determine an animal's OCLTT including life stage, body size,

phenotypic plasticity, and genetic makeup (Dahlke et al., 2020). Abiotic factors, such as hypoxia, may lower performance maximum and narrow the OCLTT range in fishes (Pörtner et al., 2010).

Fish may have to engage in a physiological trade-off when encountering both thermal and hypoxic stressors (Gamperl et al., 1998). Optimal environmental conditions maximize fishes' aerobic scope, or the difference between routine metabolic rate and maximum metabolic rate. As environmental stressors strain a fish's metabolism, their maximum metabolic rate may not keep up with their routine metabolic rate, thus their aerobic scope diminishes (Casselman et al., 2012). The coalescence of two stressors, such as high temperature and hypoxia, can interact in a variety of ways, which can be "additive" or "synergistic." If the combination of the thermal and hypoxic stress is equal to the sum of the individual stressors, then the interaction is additive. If the combination of both stressors is greater than the sum of the individual stressors, then the interaction between thermal and hypoxic stress is synergistic (McBryan et al., 2013).

Redband trout are a species of interest for their impressive hypoxia and thermal tolerance (Gamperl et al., 2002). They occupy diverse habitats that can vary in climate, altitude, and resource availability (Meyer et al., 2010). Among the Columbia River basin Redband trout in Idaho, there are three divergent ecotypes of Redband trout: desert, cool montane, and cold montane, which are dependent on geographic location and thermal regime (Chen et al., 2018a; 2018b). These ecotypes are isolated by dispersal barriers such as fluvial distance and intermittent flow (Narum et al., 2010). These desert streams can reach 29°C and fluctuate 8-12 °C on a single diel cycle during the summer months (Gamperl et al., 2002). Desert habitats can also be prone to low flow velocity and intermittent streams, two factors that decrease oxygen availability (Meyer et al., 2010; Archdeacon and Reale, 2020). In contrast, high summer temperature rarely occurs in the montane Redband habitats (Meyer et al., 2010).

Since hypoxic and thermal stress are large drivers of phenotypic selection, it is no surprise that thermal performance and tolerance-related traits are seen to be heritable for salmonids (Muñoz et al., 2015). Intraspecific variation of thermal tolerance has been documented between Redband trout ecotypes. For example, desert Redband trout are observed to have a higher critical thermal maximum than their montane counterparts (Chen et al., 2018b). Redband trout genetic variation can determine a species' ability to adjust its physiological limitations (Chen et al., 2018a). Heat shock response and survival has been associated with several genetic markers such as heat shock protein genes (Chen et al., 2018b; Narum et al., 2013). In addition, the ceramide kinase (cerk) gene is understood to be responsible for cardiac performance as a function of thermal performance (Chen and Narum, 2021).

Besides the genetic adaptations that mitigate hypoxic and thermal stress, acclimation, a reversible form of phenotypic plasticity, may serve as a mechanism for more rapid adaptation of Redband trout to environmental stressors. Acclimation is known to alter the thermal and hypoxia tolerance of many fishes (Antilla et al., 2013). While warm-acclimated fish exhibit a higher thermal tolerance, but also have a lower hypoxia tolerance (Antilla et al., 2013; Antilla et al., 2015; Jung et al., 2020). This is likely a consequence of mismatching oxygen availability and demand. As water temperature increases, the oxygen solubility systematically decreases in accordance with Henry's law (McBryan et al., 2013; Garvin et al., 2015). Warm-acclimated fish already have a greater oxygen demand because of the temperature stress. When they are exposed to severe hypoxic conditions, they will likely perish sooner than fish acclimated to cooler temperatures (Jung et al., 2020).

Cardiac function for fishes is a limiting factor associated with thermal tolerance. The heart sustains life by circulating oxygen to all tissues and removing waste (Chen et al., 2018a; Chen et al., 2018b). Acute temperature change influences physiological processes and biological reaction rates in fish, following the Q_{10} effect (Keen et al., 2017). The temperature coefficient, or Q_{10}, is the rate ratio of a given process, such as f_H, taking place at temperatures differing by 10 units (Mundim et al.,

2020) As temperature increases, the heart must increase the rate of contractions to avoid ischemia, or inadequate blood supply (Keen et al., 2017; Overgaard et a., 2004). As temperature drops, fish hearts become bradycardic because of a longer diastolic duration and a less-affected systolic duration. Cardiac remodeling in all ventricular phases of the cardiac cycle is an example of reversible acclimation that fishes can use to preserve cardiac function across temperatures (Keen et al., 2017). When severe hypoxia is a factor, myocardial dysfunction could occur within the first 30 minutes of exposure (Faust et al., 2004). Greater than 20,000 species of fish are known to have myocardial hypoxia tolerance (Faust et al., 2004). Brief exposure to hypoxia, resulting from ischemia, can induce preconditioning on an animal's heart that can protect the myocardium from subsequent, longer hypoxic episodes which can cause myocardial necrosis and permanent damage. However, in rainbow trout (*O. mykiss*), preconditioning is only seen to influence populations with hypoxia-sensitive hearts, rather than the populations with hypoxia-tolerant hearts (Overgaard, et al., 2004).

The combination of acute temperature stress and hypoxic stress can lead to cardiac dysfunction, ischemia, and cardiac necrosis (Overgaard et al., 2004; Keen et al., 2017). Cardiac dysfunction can be monitored on an electrocardiogram (ECG) and characterized by arrhythmia, a skipped beat, or drastic deceleration of f_H (Chen et al., 2018b). This experiment investigates the maximum cardiac function under acute thermal and hypoxic stress for Redband trout as a product of genotype and/or phenotypic plasticity to the ambient thermal stress. Redband trout of each ecotype, desert, cool montane, and cold montane were acclimated to a common garden environment in 15°C water to model the cold montane habitat or 21°C water to model the desert habitat. Fish were then selected at random to undergo experimentation that monitored their maximum cardiac function during acute warming at a constant rate in either normoxic (full DO saturation) or hypoxic (~50% DO saturation) conditions. For this experiment, we hypothesized that (1) the desert Redband trout ecotype would have a higher temperature at peak heart rate (T_{peak}) and show stronger cardiac performance in stressful temperatures and hypoxia, on average, than the montane

ecotypes, and (2) Redband trout in each ecotype acclimated to 21°C water would display a higher T_{peak} and a stronger cardiac performance than those acclimated to 15°C water when exposed to thermal stress and hypoxia.

Methods

Fish Culture and Rearing Conditions

For this experiment, the Redband trout collection, culture, rearing and sampling were identical to that of Chapter 2 (See Chapter 2 Methods Fish Culture and Rearing conditions). Population sizes at the time of this experiment from each of the desert creeks were: Little Jack's (n = 24), Duncan (n = 15), and William's (n = 8). Population sizes for the cool montane creeks were: Trail (n = 36), Kiethley (n = 12), and Whiskey Jack (n = 11). Population sizes for the cold montane creeks were: Boulder (n = 8), Fawn (n = 3), and Upper Mann (n = 31).

Experimental Apparatus

The experimental apparatus was crafted from short sections of 6-inch PVC pipe that were capped at either end with an opening cut down the long way of the pipe. Each section of pipe was secured with primer and silicone glue. There was tubing at one endcap of the apparatus that infiltrated water from the temperature-controlled chiller into the system and had an elbow fitting that would fit into the fish's mouth to flux water over the gills. On the other side of the PVC section, there was tubing to return water into the chiller to complete the system. At this end, there was also a removable piece of PVC that was fashioned to fit onto the outlet tubing from within the chamber to adjust the water level. On each chamber, there was a metal bar that allowed the silver electrodes, secured in fastening brackets, to move freely along the side of each apparatus, such that they could be placed appropriately on the fish's body to produce the ECG. There were 4 apparatuses in this system, allowing 4 fish to be observed for one experiment (Casselman et al., 2012; Chen et al., 2018b).

Maximum Cardiac Output under Acute Temperature and Hypoxic Stress

Fish were fasted for 24 hours and selected at random before each experiment. To start each experiment, each fish was anesthetized individually for about 6 minutes (80 mg/L MS-222) to reach stage III sedation, or total loss of reactivity and slowed opercula contractions. Once each fish was properly sedated, they were then placed into the ECG-measuring experimental apparatus. This apparatus was part of a closed system and ensures that each fish is submerged in water and their gills are continuously supplied with aerated, anesthetized (80 mg/L MS-222), and temperature-controlled water.

Two silver electrodes (30-gauge, 5 cm long) are carefully placed on the ventral side of the fish's body to measure the fish's heart contractions to produce an ECG signal. The positive electrode is placed between the pectoral fins to measure ventricular contractions, and the negative electrode was placed near the pelvic fins. These electrodes were connected to an Animal Bio Amp (BioPac MP-150; BIOPAC Systems, Goleta, CA, USA) which amplified (1,000x) and filtered (60-Hz line filter; low pass: 30-50 Hz; high pass: 0.1-0.3 Hz). The temperature of the ambient water throughout the system was controlled with a heating and chilling unit (Chen et al., 2018b).

Anesthetized fish were stabilized at the initial conditions, 15°C and ≥ 95% DO saturation (8.59 mg/L) for 1 hour prior to temperature and oxygen manipulation. Intraperitoneal injection of atropine sulfate (2.7 mg/kg) blocked inhibitory vagal tonus to the heart (Chen et al., 2018b). Injections were given 15 minutes to take effect and resulted in elevated and stabilized maximum heart rate (f_{Hmax}) (Chen et al., 2018a). DO was then decreased by ~1.5% saturation per minute until 50% DO saturation (4.52 mg/L at 15°C) was attained for the treatment procedure. Fifty percent DO saturation was maintained throughout the experiment. For both the control and treatment procedures, temperature was acutely increased from 15°C at a constant rate of ~1°C every 7 minutes. During the acute temperature increase, each fish's cardiac

function was carefully monitored and measured using ECG outputs; cardiac dysfunction marked the endpoint of the experiment (Figure 3.1). Cardiac dysfunction was characterized by rapid slowing of f_H (> 5 bpm for 1°C warming), or the heartbeat developed an arrythmia (e.g., missed ventricular polarization) (Chen et al., 2018a). Once cardiac dysfunction was detected, T_{peak} (temperature at f_{Hmax}) was recorded, the fish was removed from the system, recovered, and returned to the common garden environment (Chen et al. 2018b; Casselman et al. 2012). This was repeated for each of the fish in the system as they reached f_{Hmax} (Chen et al. 2018b; Casselman et al. 2012).

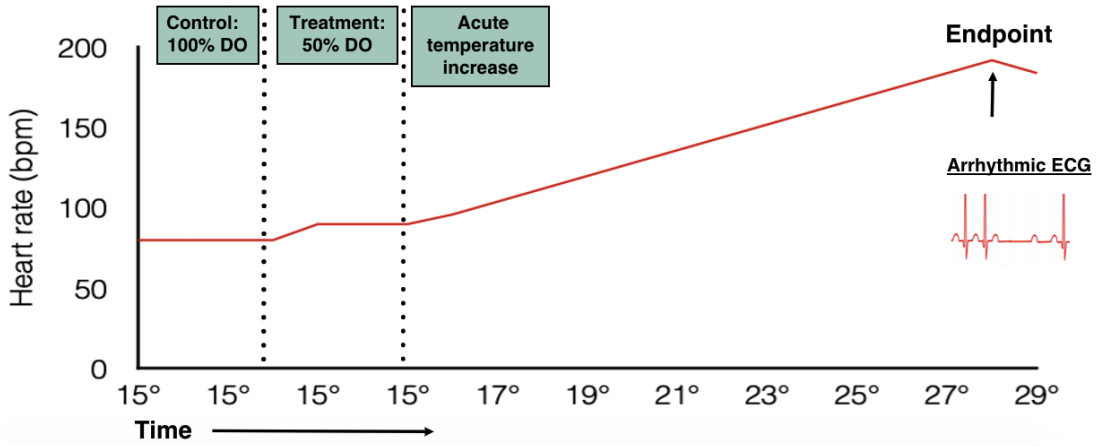


Figure 3.1 Maximum cardiac function under acute temperature stress. This experiment starts at normoxic conditions for all fish (control). For the treatment, the DO saturation then was decreased at a constant rate to 50% DO saturation (treatment). The ambient water temperature was then increased at a constant rate of 1°C every 7 minutes until each fish had reached cardiac dysfunction (e.g., arrhythmia). This response marked the endpoint of the experiment (acute temperature increase). The x-axis shows the water temperature (°C) as well as the progression of time, while the y-axis shows the relative heart rate in beats per minute (bpm). This graph is simply a visual aid for experimental trials and does not display empirical data.

Statistical Analyses

The T_{peak} represents the ultimate temperature a fish can withstand before its heart fails. T_{peak} is a metric that indicates the temperature at which a fish's heart fails, or becomes dysfunctional, during an acute temperature increase. T_{peak} can be used as a proxy for relative thermal tolerance. A three-way, type II analysis of variance (ANOVA) was used to understand the variance between Redband trout thermal tolerance depending on ecotype, acclimation temperature, and treatment (hypoxia or

normoxia) factors. Another statistic this research explores is the cardiac performance, determined by f_H, for each factor (ecotype, acclimation temperature, and DO concentration) of each experimental fish. The ECG output provided f_H values in seconds/10 beats. Heart rate was converted to bpm and corrected for each individual's body mass (corrected $f_H = f_H$*(body mass/median body mass)$^{0.1}$) (Chen et al., 2018a; Chen et al., 2018b). A three-way, type II ANOVA was run to statistically analyze the differences between factors for each temperature increment spanning the entire acute temperature increase ranging from the starting temperature 15°C to the temperature the fish's hearts failed, which in some cases, was greater than 28°C. The Q_{10} coefficient measures the degree of temperature dependence on f_H over the change of 10°C. Regressions of the Q_{10} coefficient were then taken for each factor and compared with an ANOVA. Values presented in the results are displayed as mean ± standard error of the mean, unless stated otherwise.

Results

Field DO Concentrations

DO loggers were deployed at Little Jack's Creek (42.7498, -116.0885), a warm desert site. The device was installed in a large pool with a high flow velocity regime. Results are shown in Figure 3.2.

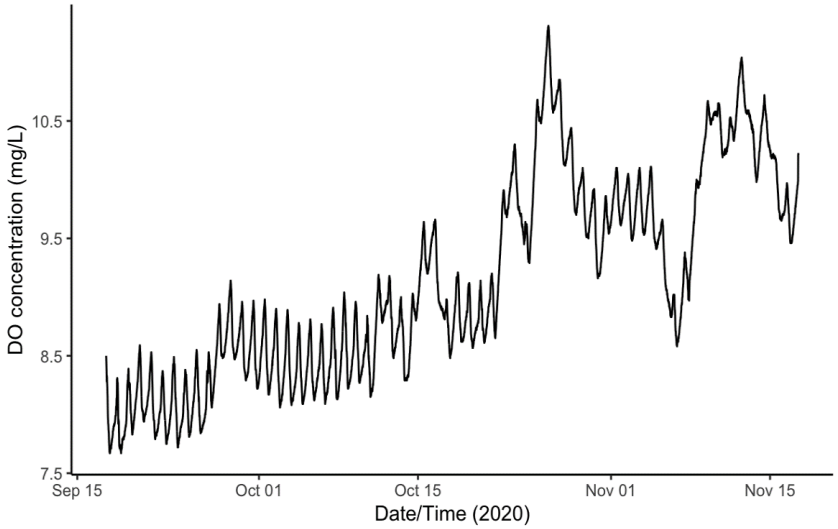


Figure 3.2 Desert stream DO concentrations. Late summer to late fall DO concentrations near the edge of a large pool in Little Jack's Creek (42.7498, -116.0885). Ambient DO concentration was recorded every 15 minutes over the span of about 2 months.

Temperature at Peak Heart Rate (T_{peak})

The T_{peak} measurements showed significantly different responses between the fish depending on acclimation temperature and the ambient DO saturation (Figure 3.3; Table 3.1). Fish acclimated to 21°C (n = 76) had a significantly higher T_{peak} (25.4 ± 0.15°C), and thus a superior thermal tolerance than fish acclimated to 15°C (n = 62; 23.7 ± 0.23°C) ($p \leq 0.001$). Across acclimation temperatures and ecotypes, fish that exposed to normoxia (n = 71) had a significantly greater T_{peak} (24.9 ± 0.21°C) than those that experienced hypoxic water (n = 67; 24.4 ± 0.20°C) ($p \leq 0.05$). The effect size of acclimation temperature was more than 3 times greater than oxygen treatment. Although there were no significant differences between ecotypes using our relatively small sample sizes, the desert ecotype acclimated to 21°C under normoxia had the strongest thermal tolerance performance with a mean T_{peak} of 26.1 ± 0.41°C.

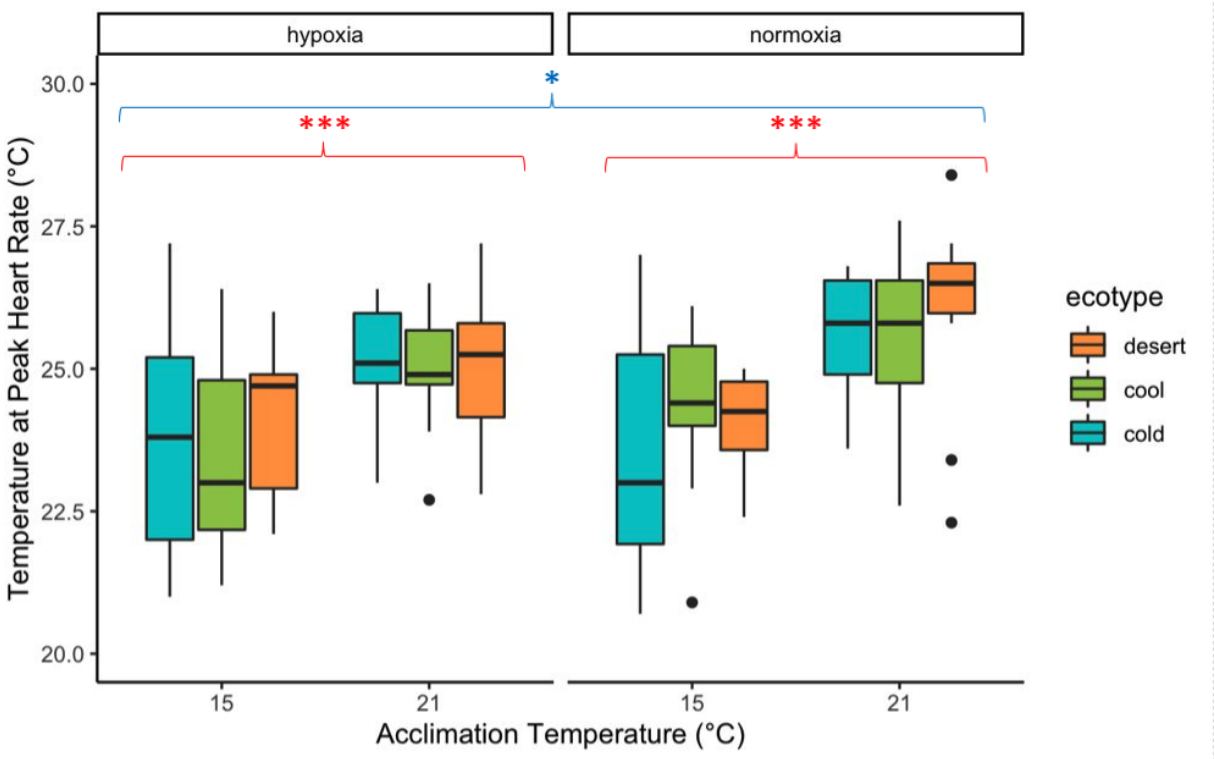


Figure 3.3 Temperature at peak heart rate. Temperature at peak heart rate (T_{peak}) is quantified on the y-axis (°C). Values are categorized by 2 matrix panels, "hypoxia" (~50% DO saturation) and

"normoxia" (~100% DO saturation) shown across the top of the graph. Overall, the T_{peak} response was significantly higher for fish in normoxic conditions compared to hypoxic conditions as shown with the blue bracket and single asterisk ($p \leq 0.05$ *). Both experimental treatments are then categorized by acclimation temperature (15 or 21°C), shown on the x-axis. T_{peak} response for fish acclimated to 21°C was significantly higher than that of the fish acclimated to 15°C, as indicated by the red brackets with triple asterisks ($p \leq 0.001$ ***). The legend on the right side of the graph depicts which ecotype the fill color of each boxplot represents. Orange symbolizes the warm desert ecotype, green symbolizes the cool montane ecotype, and blue symbolizes the cold montane ecotype. The T_{peak} response was not significantly different between ecotypes for any treatment.

Table 3.1 T_{peak} ANOVA Table. Analysis of variance with T_{peak} as the response variable and DO, acclimation temperature, ecotype, and body mass as the indicator variables.

	Sum Sq.	Df	F value	Pr (>F)
DO	12.49	1	5.21	0.02421*
Acc_Temp	76.99	1	32.09	9.593e-08***
Ecotype	4.59	2	0.96	0.38678
Body Mass	4.54	1	1.89	0.1716
DO:Acc Temp	0.59	1	0.25	0.61980
DO: Ecotype	3.26	2	0.68	0.50823
AccTemp: Ecotype	0.27	2	0.06	0.94537
DO: Acc Temp: Ecotype	4.54	2	0.95	0.39114
Residuals	299.86	125	N/A	N/A

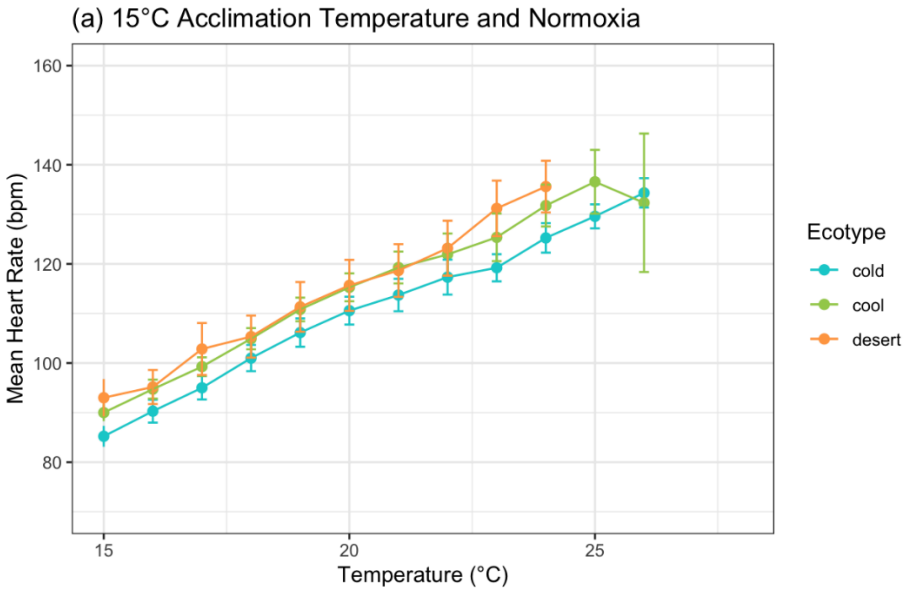
Maximum Heart Rate (f_{Hmax})

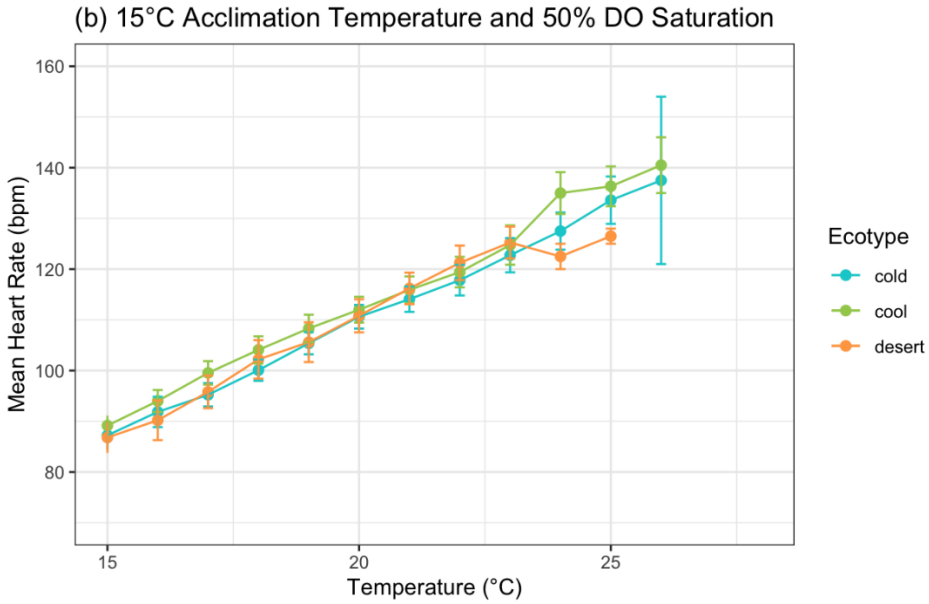
There was a significant difference between the f_H of fish depending on their acclimation temperature (15°C or 21°C) for each of the following temperature increments: 15°C ($p \leq 0.001$), 16°C ($p \leq 0.001$), 17°C ($p \leq 0.001$), 18°C ($p \leq 0.001$), 19°C ($p \leq 0.001$), 20°C ($p \leq 0.001$), 21°C ($p \leq 0.05$) and 22°C ($p \leq 0.05$). Fish acclimated to 15°C had significantly higher f_H until 23°C was reached. There was a significant interaction between acclimation temperature, DO concentration, and ecotype factors when the water temperature was 15°C ($p \leq 0.05$). Likewise, there was a significant interaction between acclimation temperature and ecotype at 20°C ($p \leq 0.05$). This was likely due to the low mean f_H of the cold montane ecotype (n = 20; 111.5 ± 1.98 bpm) relative to its cool montane (n = 18) and desert (n = 7) counterparts (113.72 ± 2.13 bpm and 113.1 ± 3.48 bpm, respectively) when acclimated to 15°C. Contrarily, when the fish were acclimated to 21°C, the cold montane ecotype had the greatest cardiac performance (n = 7; 106.6 ± 2.72 bpm) relative to the cool montane (n = 14) and desert (n = 15) ecotypes (104.5 ±1.60 bpm and 106.0 ± 2.24 bpm, respectively). Interestingly,

the cool montane ecotype had the opposite pattern and showed the highest average f_H in 15°C acclimation, while showing the lowest average fH when acclimated to 21°C.

As shown in Figure 3.4a, the desert and cool montane ecotypes acclimated to 15°C and experiencing normoxia had a higher mean f_H than the cold montane ecotype throughout the experiment. After 23°C was reached, the mean desert f_H was greater than the mean cool montane f_H. However, the desert ecotype did not persist past 25°C, but the cool and cold montane ecotypes persisted to 27°C. Figure 3.4b exhibits that all fish acclimated to 15°C and in hypoxic water had a similar cardiac performance regardless of ecotype, for their mean f_H did not have large, consistent differences.

However, the overall performance shows that hypoxia lowered the mean f_H for all ecotypes, meaning the lower oxygen concentration may lower cardiac performance, but this was not shown to be statistically significant. Figure 3.4c shows the effects of acute warming on fish acclimated to 21°C in normoxic water. There was not a major difference between ecotypes treated with normoxia and the 21°C acclimation temperature. All fish exposed to this treatment initially had a lower f_H compared to fish acclimated to 15°C until 23°C was reached. Although, fish acclimated to 21°C maintained a regular heartbeat longer at higher temperatures. Figure 3.4d shows Redband trout acclimated to 21°C and in hypoxic water. This graph shows a stark difference between the mean f_H of the cold montane fish and the superior performance of the cool montane and the desert Redband trout. The 21°C cold montane fish in hypoxic water have the lowest mean f_H compared to any other treatment group. Although there were no significant differences in mean f_H among the normoxia and hypoxia treatments ($p \geq 0.05$), the hypoxic f_H means were slightly higher than the normoxic f_H means, suggesting the potential for improved cardiac performance under hypoxic stress for the cold montane ecotype.


(a) 15°C Acclimation Temperature and Normoxia
Mean Heart Rate (bpm)
160
140
120
100
80
15
20
25
Temperature (°C)
Ecotype
cold
cool
desert


(b) 15°C Acclimation Temperature and 50% DO Saturation
Mean Heart Rate (bpm)
160
140
120
100
80
15
20
25
Temperature (°C)
Ecotype
cold
cool
desert

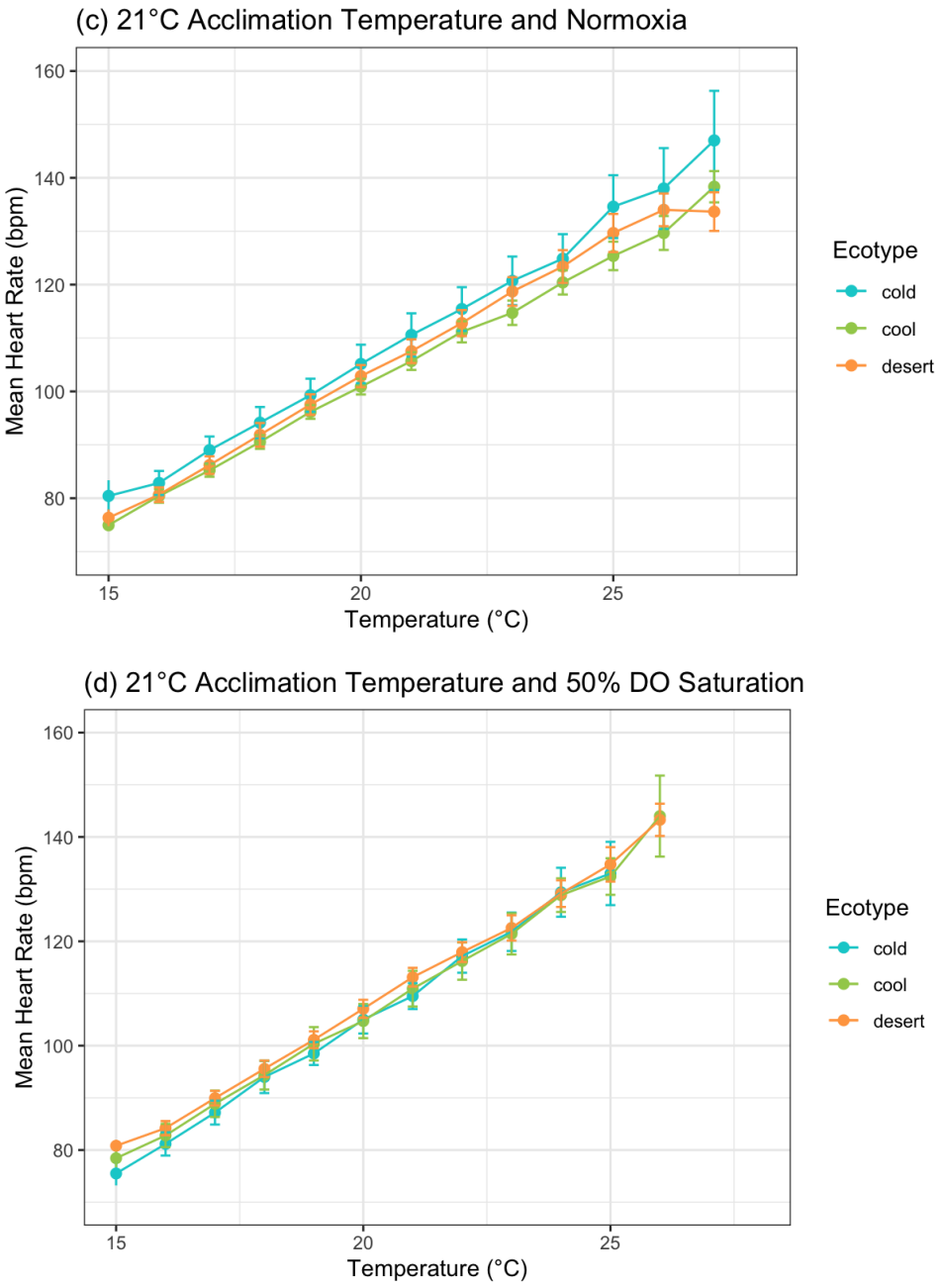


Figure 3.4 Mean heart rate during acute warming. Changes in *f*H during acute warming 1°C/every 7 min for redband trout for each of the 4 treatments: (a) 15°C acclimation temperature and normoxic water, (b) 15°C acclimation temperature and hypoxic water, (c) 21°C acclimation temperature and normoxic water, and (d) 21°C acclimation temperature and hypoxic water. For each graph, the blue line represents the cold montane ecotype, the green line represents the cool montane ecotype, and the orange line represents the warm desert ecotype. As the temperature increased past 21°C, many fish could not persist and were removed from the experiment, thus higher temperatures have larger confidence intervals.

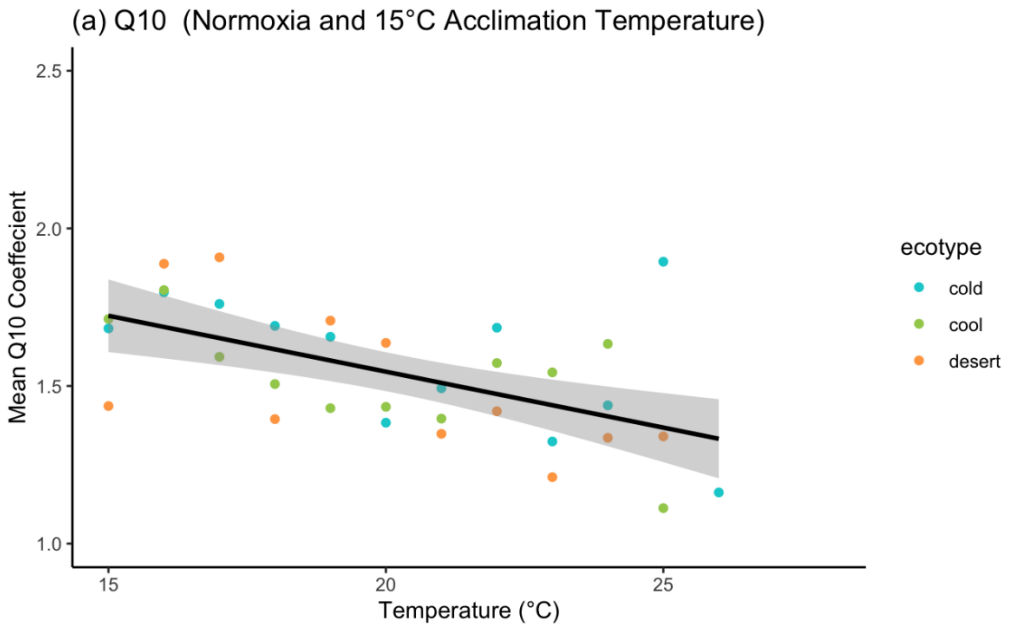
Temperature Coefficient (Q_{10})

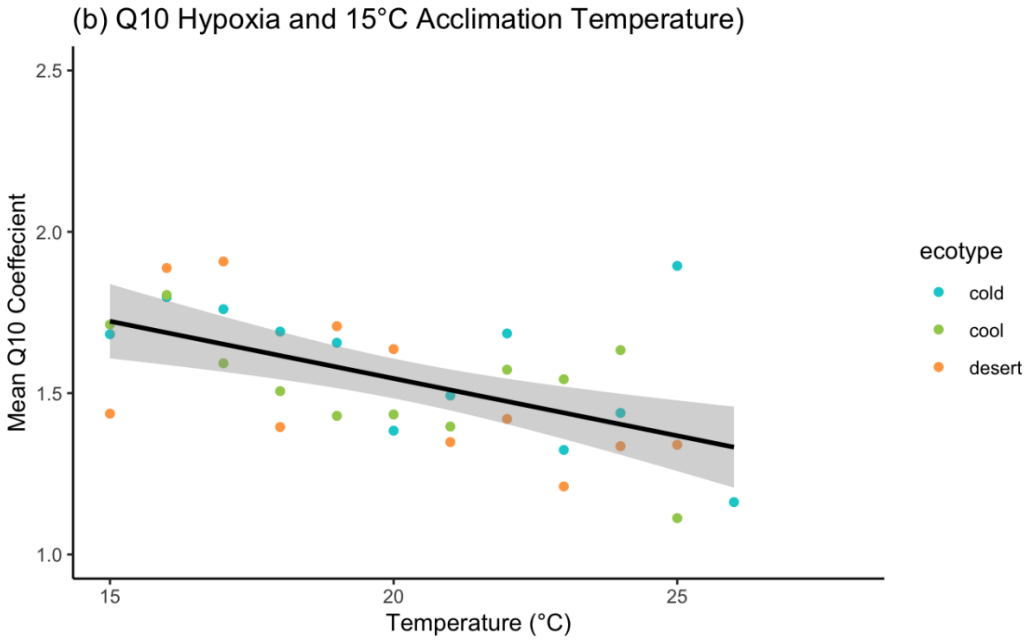
Mean Q_{10} at each 1°C temperature increase when the fish were exposed to acute warming was significantly different ($p \leq 0.001$; Figure 3.5). Q_{10} is negatively associated with temperature, and thus decreases as temperature increases. The rate of change in Redband trout heart rate is much larger as water temperature increases from 15°C to 16°C (1.77 ± 0.053) than when water temperature changes from 27°C to 28°C (1.13 ± 0.10). There was significant difference between the mean Q_{10} at each acclimation temperature ($p \leq 0.001$).

The mean Q_{10} for fish acclimated to 15°C was significantly lower (1.57 ± 0.019) than those acclimated to 21°C (1.69 ± 0.017). There was no significant difference between mean Q_{10} depending on ecotype. However, among fish acclimated to 15°C, the rate of change in heart rate was somewhat higher in Redband from cold montane habitats (1.61 ± 0.030) compared to the Q_{10} from cool montane (1.53 ± 0.025) and desert habitats (1.57 ± 0.051). The mean Q_{10} for the fish acclimated to 21°C was not significantly different between desert, cool montane, or cold montane ecotypes (1.67± 0.026, 1.69 ± 0.026, and 1.70 ± 0.030, respectively).

Table 3.2 Q_{10} ANOVA. Anova output with Q_{10} as the response variable and oxygen treatment (DO), acclimation temperature (Acc Temp), ecotype, and body mass as the indicator variables. Asterisks in the Pr (>F) column show level of significance (0 '***' 0.001 '**' 0.01 '*' 0.05 '.' 0.1 ' ' 1).

	Sum Sq.	Df	F value	Pr (>F)
DO	0.208	1	1.0018	0.3171
Acc_Temp	4.407	1	21.2563	4.405e-06***
Ecotype	0.436	2	1.0518	0.3496
Body Mass	0.066	1	0.3186	0.5725
DO:Acc Temp	0.078	1	0.3760	0.5398
DO: Ecotype	0.358	2	0.8632	0.4220
AccTemp: Ecotype	0.298	2	0.7185	0.4877
DO: Acc Temp: Ecotype	0.276	2	0.6648	0.5145
Residuals	275.137	1327	N/A	N/A


(a) Q10 (Normoxia and 15°C Acclimation Temperature)
Mean Q10 Coeffecient
2.5
2.0
1.5
1.0
15
20
25
Temperature (°C)
ecotype
cold
cool
desert


(b) Q10 Hypoxia and 15°C Acclimation Temperature)
Mean Q10 Coeffecient
2.5
2.0
1.5
1.0
15
20
25
Temperature (°C)
ecotype
cold
cool
desert

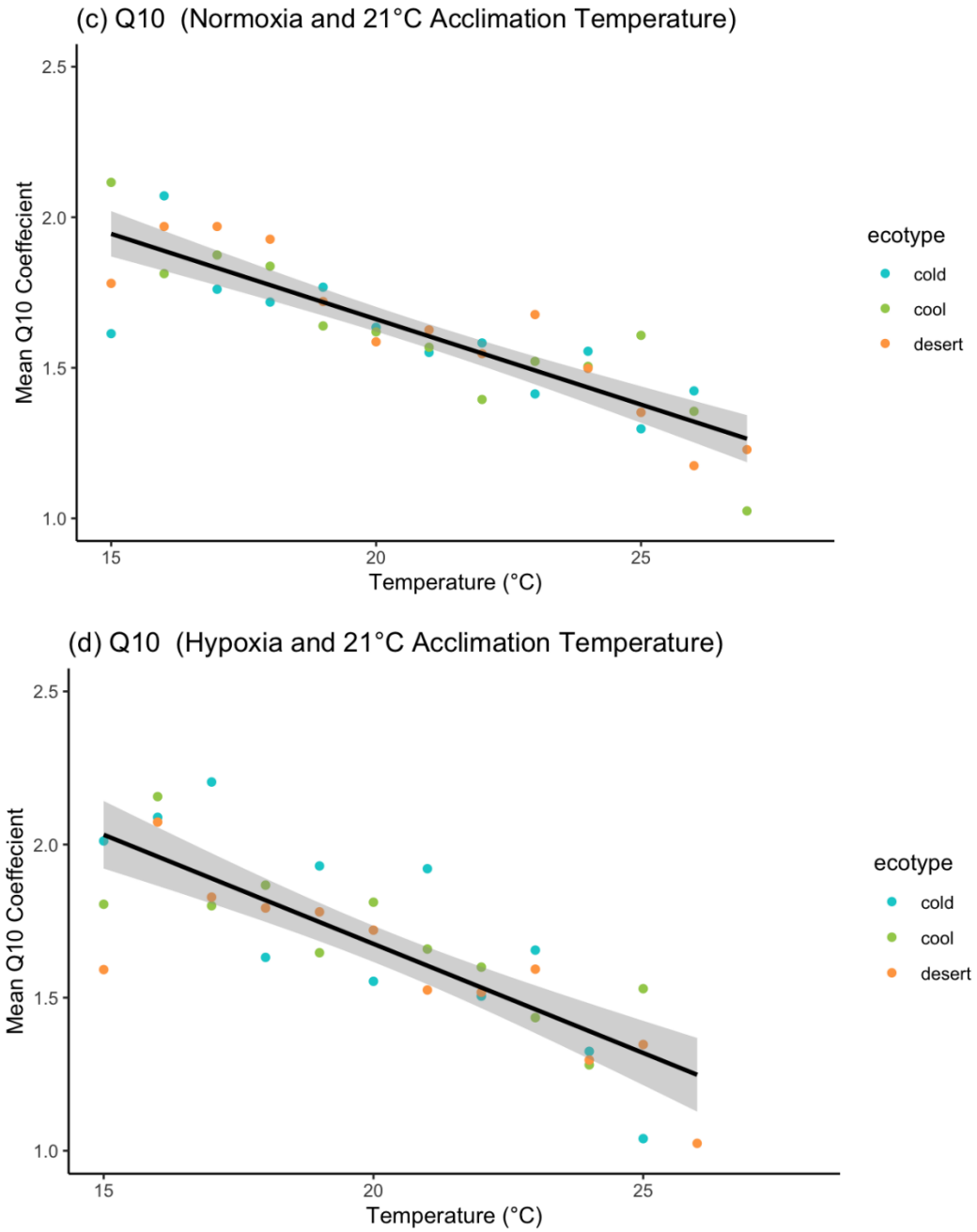


Figure 3.5 Mean temperature coefficient (Q10) during acute warming. The mean Q_{10} coefficient values are shown on the y-axis and the temperature ramp is shown on the x-axis for all treatments (a) normoxia (~100% DO saturation) and 15°C acclimation temperature, (b) hypoxia (~50% DO saturation) and 15°C acclimation temperature, (c) normoxia and 21°C acclimation temperature, and (d) hypoxia and 21°C acclimation temperature. Ecotype is indicated by the color of the datapoint. Cold montane values are blue, cool montane values are green, and warm desert values are orange, as indicated in the legend on the right.

Discussion

Temperature at Peak Heart Rate (T_{peak})

Aligned with the predictions, Redband trout in each ecotype acclimated to 21°C water exhibited a higher T_{peak} than those acclimated to 15°C water when exposed to thermal stress and hypoxia. The results of this experiment demonstrated that the desert Redband trout ecotype did show a higher mean T_{peak} than the montane ecotypes when fish were acclimated to 21°C. Additionally, the desert Redband trout had a higher mean T_{peak} when acclimated to 15°C. However, they only had a higher mean T_{peak} when exposed to a hypoxic treatment and not in the normoxic treatment, which was an unexpected outcome. The results confirmed that Redband trout in each ecotype acclimated to 21°C water displayed a higher T_{peak} than those acclimated to a 15°C.

T_{peak} represents upper limitations for circumstances that require the fish heart to function at its maximum capacity. Although conditions like this may be rare, this trait could be the reason for persistence during a high stress heat event (Chen et al., 2018a). Average temperature in montane streams remains below 20°C, so it is unlikely that montane trout will experience a heat event nor reach their T_{peak} in the wild. On the other hand, average stream temperature in desert habitats can reach 23°C and the maximum temperature in summer months can reach 29°C (Gamperl et al., 2002; Meyer et al., 2010, Chen et al., 2018b). These temperatures imply that desert Redband trout may be at risk of temperatures beyond their maximum cardiac limits in their native range. Yet, because there were no significant differences in T_{peak} among ecotypes, this trait does not appear to be under selection. However, the results showed that fish acclimated to 21°C persisted significantly longer than those fish acclimated to 15°C. This could imply that T_{peak} is a phenotype that is plastic and can be shifted when Redband trout are exposed to chronic temperature stress. Cardiac morphology remodeling in response to warm acclimation temperature could increase ventricular luminal capacity and the compact myocardium thickness. As a result, stroke volume could increase while cardiac performance may decrease (Antilla et al., 2015). This would also suggest that although stream temperature is increasing, Redband may

have the ability to acclimate to the warmer temperatures and persevere extreme heat stress events. These temperatures imply that desert Redband trout may be at risk of temperatures beyond their maximum cardiac limits in their native range.

Fish exposed to hypoxia had a significantly lower T_{peak} in comparison with those that performed in normoxic water. Redband trout exposed to low oxygen conditions may not be able to survive as long during acute warming as fish in normoxic water conditions. As shown in Figure 3.2, the ambient DO remains greater than 7.5 mg/L in Little Jack's desert habitat. Redband trout in desert sites may not encounter the same hypoxic stress from the maximum cardiac output experiment (50% saturation, or 4.52 mg/L - 3.5 mg/L) in flowing portions of streams. However, if the stream becomes intermittent and has a low flow regime, these fish may perish at a lower temperature than if they were in a normoxic environment. More data needs to be collected on the DO concentration in the intermittent and low flow regime stretches of desert stream in the summer months to understand the ultimate DO risk that Redband trout encounter in their native range.

Maximum Cardiac Performance (f_{Hmax})

In concordance with the prediction, the mean f_H for all fish acclimated to 21°C was lower than that of the fish acclimated to 15°C, indicating that fish acclimated within their optimal temperature range show a greater cardiac performance when experiencing an acute heat stress event. Contrary to the second hypothesis, the desert and cool montane ecotypes displayed similar mean cardiac outputs throughout all factors of the experiment. When fish were acclimated to 15°C and had the normoxic treatment, the desert and the cool montane trout showed significantly elevated cardiac performance compared to the cold montane trout. Likewise, the desert and cool montane ecotypes had superior cardiac performance in comparison with the cold montane ecotype when fish were acclimated to 21°C and treated with hypoxic water.

The significant differences between the f_H in fish from either acclimation temperature diminished as temperature acutely increased. The effect of acclimation

temperature and the consequential phenotypic plasticity is more prominent when temperatures are relatively lower. Once the temperature rose above 22°C, there were no significant differences between the f_H for fish at any of the observed factors: ecotype, acclimation temperature, nor DO concentration. The heart must increase the rate of contractions as temperature increases to avoid ischemia (Keen et al., 2017; Overgaard et al., 2004). It seems that although the fish acclimated to 21°C had a significantly inferior cardiac performance for each degree increment from 15- 22°C, their f_H appeared to increase at a faster rate, so the fish acclimated to the higher temperature (21°C) were able to increase their f_H to function at an elevated level that was similar to fish acclimated to the cooler temperature (15°C). The initial lower cardiac performance of fish acclimated to the warmer temperature could be a result of a lower stress response due to phenotypic plasticity (e.g., cardiac remodeling) resulting from acclimation to 21°C.

Greater cardiac performance under hypoxic stress compared to the normoxic treatment was observed (Figure 3.4) for both acclimation temperatures. A higher f_H indicates that the hypoxic treatment was more stressful for the fish than normoxia. Because of the lack of oxygen, the Redband trout heart had to increase performance during experimentation to maintain oxygen demand for the rest of the body. The significant interaction between the f_H among the three factors (acclimation temperature, ecotype, and DO concentration) at 15°C indicates that there is a combined effect of these factors on the f_H of Redband trout at this temperature. Acclimation temperature certainly stratified the cardiac performance of these groups. The interaction influenced the cold montane ecotype acclimated to 21°C and hypoxic treatment to have the lowest of f_H and cardiac performance, while the desert ecotype acclimated to 15°C and normoxic treatment had the greatest f_H and cardiac performance at 15°C, or the beginning of the experiment with no temperature stress (Figure 3.4). Likewise, the significant interaction between fish at different acclimation temperatures and oxygen treatments at 20°C could indicate that there were some combined effects of these treatments that influenced the differentiation of their f_H.

The ecotype did not have a significant interaction here, but it is apparent that the acclimation temperature compounded with the hypoxic treatment decreased the f_H for fish treated with more stressful conditions. If there are compounded stressors in natural settings, Redband trout may have decreased cardiac function as a result. Appendix A (Figure 4.1) shows that there is not a significant effect of hypoxia on f_H when high temperature is not a factor. These results support the hypothesis that hypoxia and temperature stress have a synergistic effect on Redband trout cardiac function.

Temperature Coefficient (Q_{10})

Q_{10} is the rate ratio of a given process, such as f_H, taking place at temperatures differing by 10 units (Mundim et al., 2020). Larger values indicate a greater rate of change in heart rate at the temperature increase of 1°C. The results show that the temperature increase of 1°C has a higher impact on the rate of change of f_H when the temperature increase is at a lower temperature. The Q_{10} coefficient decreases until it nears maximum performance. This means the change in f_H is more dependent on temperature increase when the water temperature is less stressful. Once the water reaches stressful temperatures, the f_H of Redband trout is less dependent on the temperature change. The f_H of fish acclimated to 15°C was significantly less affected by a change in water temperature than the f_H of fish acclimated to 21°C. Thus, the f_H of fish acclimated to 21°C is much more dependent on water temperature and has lower cardiac performance than the fish acclimated to 15°C. Because Redband trout acclimated to 21°C are more dependent on the temperature and have lower cardiac performance compared to fish acclimated to 15°C, they likely will experience more deleterious effects from ischemia and lack of efficiency.

There were some potential limitations in this experimental design that could have affected the results. The population size of the desert ecotype acclimated at 15°C was 11 fish (5 hypoxia, 6 for normoxia), which was much smaller than the other population sizes that had at least 20 fish per ecotype and acclimation temperature,

leaving at least 10 fish for either treatment (hypoxia or normoxia). Although the desert ecotype was expected to have superior cardiac performance and persist longer in all subgroups, the 15°C acclimated desert fish appeared to have no individuals persist in temperatures above 25°C, unlike their montane counterparts. It is possible that this is a result of the desert ecotype being acclimated to a temperature regime that did not represent their native range, although it was within their thermal optima of 10-15°C (Gamperl et al., 2002; Meyer et al., 2010; Chen et al., 2018b).

Conclusion

As specified above, the fish that were acclimated to 21°C exhibited a lower f_H, than fish acclimated to 15°C. Although the cardiac performance of the 21°C fish is noticeably inferior when exposed to 15°C up until 22°C, these fish did have a significantly higher T_{peak} than fish acclimated to optimal temperature, exhibiting greater persistence and chance of survival during a compounded thermal and hypoxic event. The lower cardiac performance of the warm acclimated Redband trout during these temperatures implies that there is an acclimation effect. Because these fish maintained homeostasis in 21°C water for at least 8 weeks, they seemed to experience less stress when introduced to lower temperatures in this experiment, thus showing a lower f_H for the 15-22°C temperature range. Although cardiac performance was lower for warm acclimated fish in this temperature range, they were able to increase their performance at a faster rate to reach sufficient cardiac performance as temperatures became more stressful. Decreased DO concentration appeared to significantly decrease the T_{peak} of all fish in this experiment in concert with acute heat stress. Because hypoxia alone did not induce cardiac stress or arrhythmia (Appendix A; Figure 4.1), this evidence supports the hypothesis that the combination of hypoxic and thermal stress is synergistic. Fish that have acclimated to a higher temperature are certainly at risk of deleterious effects as a result of chronic heat stress and lower DO concentration, which could lower the Redband trout's life expectancy and reproduction capacity (Abdel-Tawwab et al., 2020). These results support the

observations of Chen et al. (2018b), that utilization of phenotypic plasticity and cardiac remodeling from acclimation to warmer temperatures could save their life during an acute heat stress event, yet demonstrate a challenge for Redband trout acclimated to a higher temperature (21°C), as indicated by greater temperature dependance and lower cardiac performance compared to fish acclimated to 15°C.

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Chapter 4: Conclusion

Synthesis of Research Findings and Collective Implications

Fully understanding the combined effects of hypoxia and thermal stress on fishes is a challenging endeavor. There has been some evidence of plasticity and heritable genetic variation that increase hypoxic and thermal tolerance in fishes. Although much research has been done on the hypoxic and thermal stress on fishes, there have been few studies on plastic response of both hypoxic and thermal stress in concert (McBryan et al., 2013). This study sought to uncover the thermal and hypoxic tolerance of Redband trout in multiple circumstances. Acclimation temperature and ecotype were analyzed as factors that could influence the tolerance levels of Redband trout for both experiments. The hypoxia tolerance study focused on the effects of hypoxia with chronic temperature stress, while the maximum cardiac function study focused on the consequences of acute temperature stress both with and without hypoxic influence. Each study had noteworthy findings, and the amalgam of these studies revealed original implications that can be applied to multiple areas of research, including salmonid conservation.

Each experiment had some overarching messages about hypoxia and thermal tolerance in Redband trout. As discussed in Chapter 2, an elevated acclimation temperature (21^{o}C) was significantly detrimental to the hypoxia tolerance of Redband trout compared to a cooler acclimation temperature (15^{o}C). The integration of this chronic thermal stress and acute hypoxic stress had fish experience LOE almost 8 minutes earlier with more DO, on average, than those solely subjected to acute hypoxic stress. In Chapter 3, it was revealed that fish acclimated to 21°C had impaired cardiac performance in 15°C-22°C water in comparison to fish acclimated within their optimal temperature range (15°C) but had an amplified ability to persist longer in the presence of an acute heat stress event when compared to fish acclimated to a cooler temperature. During the acute temperature increase, it seemed that fish acclimated 21°C temperature expended less energy to maintain cardiac performance but

increased their heart rate at a faster rate to reach maximum performance similar to those acclimated at 15°C. This discussion will briefly review each of the key findings about each of the factors studied that influenced hypoxia and thermal tolerance and then relate the findings back to fish physiology and implications for the future of the species in the wild.

Hypoxia Tolerance Study

The hypoxia tolerance study shown in Chapter 2 showed the effect of acclimation temperature and Redband trout ecotype on persistence during an extreme hypoxic event. The main takeaway from this experiment was that increased acclimation temperature decreases hypoxia tolerance in Redband trout. As seen in Figures 2.1 and 2.2, regardless of ecotype, Redband trout are seen to be less tolerant of hypoxia when they are acclimated to 21°C. The other main point was that the Redband ecotype does not necessarily determine hypoxia tolerance. Unexpectedly, the cool montane ecotype had significantly inferior hypoxia tolerance when warm-acclimated. One possible explanation might be introgression with hatchery rainbow trout. The low time at LOE for the warm-acclimated cool montane ecotype is comparable to the hypoxia tolerance of a diploid hatchery rainbow trout strain from Fraser Valley (Scott et al. 2015). This strain had an average of about 40 minutes until LOE when DO concentration was at 10% and the temperature was 12°C, while the cool montane had an average of 53.7 ± 1.50 minutes until LOE when DO concentration was 13% and the temperature was 21°C. Perhaps the cool montane ecotype has progeny of stocked rainbow trout and the native Redband trout in some of the study sites and that is what decreased the average time at LOE relative to the other ecotypes. Another possibility is that there could have been some isolated sub-population that does not have the propensity to tolerate severe hypoxia under chronic heat stress like the other strains of Redband trout. Further research on the likelihood of these notions and the genetics of these individuals is necessary for more conclusive implications from these results.

When fish are introduced to hypoxic conditions, there are several physiological responses that occur to maintain homeostasis (Abdel-Tawwab et al., 2019). Oxygen consumption increases with temperature, which explains the Redband trout's lower hypoxia tolerance at a warm-acclimation relative to the cold-acclimated fish (Antilla et al., 2015). Fish behavior can also vary during hypoxic stress. Fishes have often been observed to rise to the water surface and try to escape or breathe air, or fishes are known to concede to the bottom of the experimental tank to reserve energy. Hypoxic stress also induces an endocrine stress response and is correlated to a rapid and steep increase of cortisol levels (Abdel-Tawwab et al., 2019). When fishes have a single or repeated exposure to hypoxia, they initially have an increase in cortisol levels, glucose levels, and spleen somatic index (Abdel-Tawwab et al., 2019). Further, morphological changes, such as gill reconstruction to reduce the gas diffusion distance on the lamellae, can also take place. For example, the ILCM can be shed during hypoxic events to decrease diffusion distance (Gilmour and Perry, 2018). As the gills are a multi-purpose organ, the loss of the ILCM could result in a loss of osmoregulatory control, and ion retention may be impaired (Gilmour and Perry et al., 2018; Abdel-Tawwab et al., 2019).

Hypoxia and low dissolved oxygen in isolated pools in intermittent streams negatively affect fish growth (Love et al., 2005). Impaired growth can occur in fishes when DO levels are <4-5 mg/L. The minimum DO requirements for cold-water fishes are 6 mg/L for healthy growth, tissue repair, and reproduction (Abdel-Tawwab et al., 2019). The population structure of fishes exposed to hypoxia could be diminished. There is ample evidence of reductions in gonadosomatic index, tertiary oocyte number, and motile spermatozoa due to hypoxia (Abdel-Tawwab et al., 2019). Like the gills, the fish heart is extremely sensitive to oxygen deficiency, with necrosis being a likely outcome due to the inability to match ATP supply and demand (Speers-Roche et al., 2010). When fishes have a single or repeated exposure to hypoxia, they initially have an increase in cortisol levels, glucose levels, and spleen somatic index (Abdel-Tawwab et al., 2019).

Each treatment group withstood severe hypoxia levels that rarely occur in the field before time at LOE. In Little Jack's creek, DO concentration fluctuates on a diel cycle and could decrease as low as 7.67 mg/L and as high as 9.06 mg/L in September of 2020 (Figure 3.2). Because stream temperatures peak in August, Little Jack's likely has lower DO concentrations earlier in the summer, however these are probably not the most stressful DO concentrations Redband trout will encounter. Some arid rangeland streams that are Redband trout habitats are characterized by low flow and even static water conditions. Redband trout can persist in some low flow or isolated pools that have DO concentrations as low as 2 mg/L (Tate et al., 2007). Redband trout acclimated to 15°C were seen to withstand acute hypoxia until an average of 1.00 ± 0.012 mg/L is reached, and Redband acclimated to 21°C could withstand acute hypoxia until an average of 1.42 ± 0.087 mg/L was reached (Figure 2.2). Although Redband are seen to persist in extreme hypoxic waters, water temperature will certainly influence how long they will be able to persist. Because hypoxia is more prevalent in low flow regimes associated with seasonal droughts, Redband are more likely to be in suboptimal water temperatures when they encounter extreme hypoxia, as modeled in this study (Archdeacon and Reale, 2019).

Maximum Cardiac Function Study

The maximum cardiac output experiment tested Redband trout thermal tolerance to acute temperature increase depending on the levels of three factors: acclimation temperature, ecotype, and oxygen saturation. This experiment has illuminated some key avenues for resilience of this species. For example, the desert and cool montane ecotypes had elevated mean f_H during acute temperature stress when compared to the cold montane ecotype for the experimental trials: 15°C acclimation temperature with normoxic treatment and the 21°C acclimation temperature with hypoxic treatment. Because f_H is a proxy for cardiac performance, the results imply that desert and cool montane ecotypes are more efficient at cardiac function under temperature stress, even when they are not acclimated to an elevated temperature. However, when

these ecotypes are acclimated to 21°C, they seem to have increased cardiac performance contrasting with the cold montane populations. This could be a result of specific adaptations to local habitats and divergence from the cold montane gene pool.

In addition, as shown in Figure 3.3, the desert populations displayed a modestly higher average T_{peak} relative to the montane populations in the following treatments under acute warming: cold-acclimation hypoxia, warm-acclimation hypoxia, and warm acclimation normoxia, although none of these differences between populations were statistically significant. The peak f_H for each of these populations had values ranging from 19.9°C to 28.4°C. The lowest T_{peak} values were observed in hypoxic conditions when fish were cold-acclimated. Likewise, fish were less tolerant of temperature when exposed to hypoxic conditions, as shown in Chapter 3 (Antilla et al., 2015).

Fortunately, for the montane ecotypes, the stressful conditions seen in these experiments will likely not be experienced in their native range. As acknowledged in the literature review, the montane habitats thermal regime rarely exceeds 20°C in the summer months (Chen et al., 2018a). Although thermal tolerance was shown to be relatively lower for the montane populations, thermal stress for montane populations is a rare occurrence (Chen et al., 2018b). In 2015, average stream temperatures for cool montane streams did not exceed 20°C, but maximum stream temperatures rose to about 22°C, which was above some of the T_{peak} values observed in the cool montane ecotype with cold acclimation temperatures and hypoxic treatment (Chen et al., 2018b). Although some of the cool montane individuals' T_{peak} was below these maximum temperatures, it is unlikely that they will experience 50% saturation hypoxic conditions in the field, considering their native streams do not have evidence of intermittency, and are thus not at risk for low oxygen infiltration and extirpation because of acute temperature increases (Meyer et al., 2010). Fortunately, the cold montane habitat did not exceed an average nor a maximum temperature of 20°C in 2015, which was below the peak f_H for all treatments of the cold montane populations (Chen et al., 2018b). Thus, the cold montane ecotype has minimal risk of extirpation

for now. Contrarily, the desert Redband trout populations seem to be at considerable risk of local extinction. In 2015, the average desert stream temperature was about 23°C and the highest stream temperatures were 26°C. The cold-acclimated desert fish are seen to have the lowest peak f_H, and most individuals had cardiac dysfunction before 26°C. Even when fish were acclimated to 21°C, the hypoxic conditions kept the average T_{peak} below 26°C.

The warm-acclimated desert Redband that were subjected to hypoxia did have an average T_{peak} 25.14 ± 0.32°C. Although, for fish acclimated to 21°C under normoxic conditions, the mean T_{peak} was 26.16 ± 0.45°C, which was the highest average for this statistic, there were still multiple fish that expired before that point, meaning the population size could diminish. Successful oxygen uptake is the outcome of cardiac output and tissue oxygen extraction from the blood. The physiological response to acute warming includes increased gill ventilation and cardiac output to meet elevated oxygen demands (Farrell et al., 2009). Cardiac function is the primary limiting factor in thermal tolerance (Chen et al, 2018b). Fish can limit their oxygen consumption by limiting certain activities such as feeding and digestion (Chen et al., 2018a). Critical thermal maximum is about 28-30°C for Redband trout, which is generally 5-6°C above the T_{peak}. The critical thermal maximum for Redband is above the peak summer temperatures in the desert streams, but only by about 2-4°C (Chen et al., 2018a).

Phenotypic plasticity is a crucial mechanism that Redband trout are observed to utilize that can increase their performance and persistence when exposed to thermal stress. Regardless of ecotype, warm-acclimated fish had a significantly greater T_{peak}, and thus, were able to maintain rhythmic cardiac function under higher temperatures relative to cold-acclimated fish (Figure 3.3). As a response to oxygen demands, warm-acclimated fishes are seen to have a decrease in overall ventricular mass, decrease of connective tissue, and an increase in ventricular luminal capacity to maximize stroke volume. This is reported to be the outturn of decreased size of myocytes in the spongy myocardium and an increase of thickness in the compact myocardium layer (Keen et al., 2017).

As the climate crisis transpires, the Redband trout will inevitably be subjected to increasingly more hypoxic and thermal stress in their native range, especially in desert habitats (Meyer et al., 2010). Redband trout are known for their persistence in water that exceed their thermal optima, with temperatures as high as 29°C (Gamperl et al., 2002). Because cardiac function is the primary limiting factor of thermal tolerance, the increased thermal tolerance and cardiac performance of Redband trout that were acclimated to 21°C could imply that this species is capable of seasonal cardiac remodeling when chronically exposed to warmer temperatures (Chen et al., 2018b). If Redband trout populations are to persist in changing thermal regimes, they must not only survive heat stress events, but they must perform activities such as growth and development at high summer temperatures (Chen et al., 2018b).

The purpose of these experiments was to further our understanding of the relationship between hypoxic and thermal stress as an additive or a synergistic effect. As discussed in Chapter 2, Redband trout were able to withstand an acute hypoxic stress event for a significantly ($p \leq 0.001$) longer amount of time with less oxygen when there was no heat stress component. Redband had survived in 21°C water for several weeks, but when acute hypoxia is a factor, fish will lose equilibrium much faster than those fish in 15°C water. Although hypoxic and thermal tolerance decreases with the combination (as expected with OCLTT) of both hypoxic and thermal stress, the results do not support a synergistic response. T_{peak} was analyzed in Chapter 3, where both acclimation temperature and oxygen treatment had significant effects on the longevity of cardiac function for Redband trout. However, acclimation temperature ($p \leq 0.0001$) had almost 3 times the effect on T_{peak} response than oxygen treatment ($p \leq 0.05$). DO concentration does significantly affect thermal tolerance, but the combination is likely additive. Further, an additive effect is evident because, in both experiments, the compounded effect of hypoxia and thermal stress was not significantly greater than the sum of responses from each isolated stressor. The f_H was not significantly affected when fish were exposed to hypoxic stress alone (Appendix A, Figure 4.1). However, when Redband trout were subjected to hypoxic

and thermal stress in concert (Figure 3.4b and 3.4d), the f_H was not significantly different from when trout were exposed to acute thermal stress alone (Figure 3.4a and 3.4c). Thermal conditions may affect Redband trout phenotypic response more than oxygen conditions. The amalgamation of all these findings does not support the hypothesis that the compounded stress of hypoxia and high temperature has a synergistic effect on Redband trout (Antilla et al, 2015; McBryan et al, 2013).

Future Research

More data needs to be collected on the amount of oxygen in low velocity regimes and isolated pools in intermittent streams to understand the risk of severe hypoxia for desert populations. Incorporating a hypoxia component to acclimation time would be an interesting avenue to pursue to understand how Redband trout might utilize phenotypic plasticity to cope with hypoxia in the desert study sites. Antilla et al. (2015) has shown that both warm acclimation and overnight hypoxia increased hypoxia tolerance in salmonids. These responses are species-specific, but these treatments could happen in the field and thus could evoke a significant response from Redband and show resilience to hypoxia.

Another helpful avenue for future research could be identifying the Redband populations that are at the greatest risk of extirpation. Local extinction could be a result of several factors. These may include lack of genetic diversity, lack of adaptive capacity, lack of suitable habitat, and lack of the ability to acclimate to poor environmental conditions (Chevin and Lande, 2010). Chen et al. (2018b) illustrates that there are candidate genes under selection for the upper thermal tolerance of Redband trout. These genes under selection may have enabled Redband trout to live in the harsh thermal conditions of desert streams. Although desert populations seem to have some adaptive capacity to reach a higher critical thermal maximum based on the existing genetic variation, Chen et al. (2018b) predicts that this adaptive capacity has a narrow margin of long-term success. These populations in warm environments

are still vulnerable to extirpation and will still need conservation attention through management.

Although many desert stream habitats have undesirable conditions for Redband trout, they may be able to recover from conditions outside their optima if thermal refugia with optimal temperatures are present. Refugia can include large pools in streams or groundwater upwellings. In which case, Redband trout can avoid chronic exposure to hypoxic and thermal stress and minimize the negative repercussions via behavioral thermoregulation (Tate et al., 2007; Chen et al., 2018b). Thermal refuge in large stream pools have been seen to provide critical habitat for Redband trout. Depending on the frequency and density of trout, these stream refuges may allow persistence and survival of Redband trout (Tate et al., 2007). Cold water refugia may not always be available in desert Redband trout habitat, thus leaving the fish at a greater risk. Pinpointing Redband trout access to thermal refugia in their habitat range could provide useful information for populations' ability to persist as thermal regimes continue to shift. Increased number of thermal refuges could suggest more protection from adverse temperatures, while habitats with few refugia sites that do not have much capacity to sustain sufficient biomass of Redband trout could indicate which populations need more conservation attention. Populations with lack of refugia or dispersal barriers can suffer mass mortality and local extirpation without management action, such as translocation (Archdeacon and Reale, 2020).

Management Suggestions

For Redband trout populations to survive changing environmental conditions, management action is necessary. If thermal refugia can be identified and located across the Redband trout native range, such information might assist with identifying regions where Redband trout could be successfully translocated or stocked. Identifying stretches of river that are prone to intermittency and have a low flow regime would be helpful as well. Populations in these locations could be closely monitored and relocated, if necessary.

Imperiled habitat for Redband could be improved with an increase of shading, so streams are less likely to dry up during the summer months. Shading is not only known to lower stream temperature, but it also increases invertebrate food supply for Redband. Livestock presence not only decreases the amount of shade near streams, but also decreases the amount of water in the stream system, increases the concentration of nutrients in the system, and increases sedimentation (Meyer et al., 2010). As discussed in the introduction, increased nutrients can increase aquatic vegetation and thus, decrease DO saturation in ambient water (Antilla et al., 2013). Limiting or eliminating livestock access to imperiled streams inhabited by Redband trout would improve their habitat quality.

Redband trout hybridization with other nonnative trout species such as hatchery rainbow trout (*O. mykiss irideus*) and cutthroat trout (*Oncorhynchus clarkii*) has been a threat to the integrity of the Redband trout gene pool. The Idaho Department of Fish and Game has halted stocking of cutthroat trout (Neville and Dunham, 2011). Further protection of Redband populations from further introgression could be achieved by stocking triploid, sterile rainbow trout (Meyer et al., 2010). Hybridized Redband trout can also pose a risk to the Redband trout population. Monitoring and minimizing the dispersal of these individuals would be beneficial for the resilience of the species (Neville and Dunham, 2011).

Isolation of the purest Redband trout populations is another notable risk that threatens the survival of the native populations. Many barriers to stream connectivity exist, such as culverts, dams, road crossings, and disconnected stream channels (Narum et al., 2010; Chen and Narum, 2021). Isolated, small Redband populations are threatened by possible inbreeding depression as a result (Chen and Narum et al., 2021). Increasing connectivity of the native streams could improve genetic diversity among populations and produce more viable generations of fish. Management might consider maintaining and restoring stream flow in smaller streams and eliminating barriers to connectivity. These techniques could allow Redband to shift their distributions and track thermal habitat as needed (Isaak et al., 2012).

Although the combination of hypoxia and thermal stressors may have a synergistic effect on Redband trout, both genetic and plastic mechanisms can be utilized by populations to survive extreme events. It seems if there are stochastic heat events when Redband have not had time to acclimate, mortality could ensue. However, in order to have long term population survival and persistence of the species, further research, monitoring and management are necessary.

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Appendix A

Cardiac Function under Acute DO decrease and Constant Temperature

To understand the stress of decreased DO concentration alone without the acute temperature increase, an experimental trial was conducted. Four fish were anesthetized and placed into the cardiac function apparatus seen in chapter 3. Three fish from the desert ecotype and one fish from the cold montane ecotype were tested from the 21°C acclimation temperature. These fish were selected because they were available after much of the acute temperature increase experiments were completed. Water temperature remained at a constant 15°C because it was deemed near the optimal temperature for Redband trout, and thus removed temperature stress from the experiment. Dissolved oxygen began at normoxia 87.5% saturation (7.91 mg/L). During normoxia, fish were injected with atropine, to see an effect of heart rate increase to avoid bradycardia, just as in the cardiac function experiment in chapter 3. Isoproterenol was also injected to show that there was not a large effect on heart rate beyond what the atropine solution had already done. This justifies only using atropine throughout the maximum cardiac function experiments. After a waiting period of 15 minutes after the atropine injection and 15 minutes after the isoproterenol injection, DO saturation was decreased at a rate of about 1% saturation every minute. However, because of the open design of the system, it was impossible to decrease the DO below 16% saturation (1.45 mg/L).

The results of this experimental trial show that hypoxia alone with no temperature decrease does not have extreme or adverse effects on the cardiac function of Redband trout. After the DO decreases below 50% saturation (4.52 mg/L), the desert fish had a slightly lower cardiac performance throughout the hypoxia experiment, perhaps because desert fish have a lower stress level when subjected to low oxygen environments relative to the cold montane ecotype due to their

adaptations to extreme desert environments. However, larger sample sizes and further investigation is needed to reinforce this claim. Regardless of ecotype, these fish did not seem to have significantly increased cardiac performance ($P > 0.05$; $P = 0.4891$), and were not stressed in the presence of hypoxia at a constant temperature of 15°C.

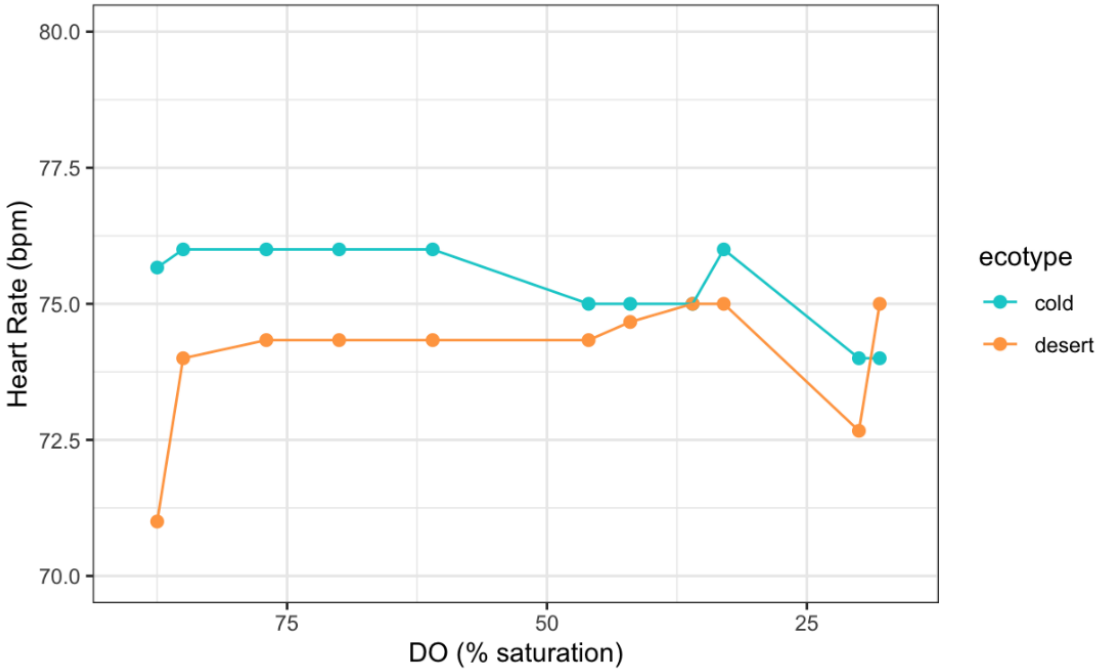

Figure 4.1 Cardiac function during DO decrease at 15°C. Change in heart rate under DO decrease at constant temperature (15°C). Experimental trial with a small population size (n=4). Desert Redband trout (n=3), cold montane trout (n=1). Initial increase of heart rate can be attributed to atropine and isoproterenol injections. The open system only allowed for DO saturation as low as 16% (1.45 mg/L).