Hybridization in Western Trout: spatial variation and the role of environmental factors

by

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Abstract

Hybridization and introgression with non-native salmonids is one of the greatest factors threatening native cutthroat trout species. Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WSCT) were recently listed under the Canadian *Species at Risk Act* (SARA) as "special concern" (British Columbia populations) and "threatened" (Alberta populations). I employed a 10 locus-microsatellite DNA assay to investigate levels of hybridization between westslope cutthroat trout and introduced rainbow trout (*O. mykiss*; RT) at 159 sampling locations in southwestern Alberta and parts of southeastern British Columbia. My results revealed that hybridization is extensive across the region sampled. Admixture levels (q_{wsct} of 0 = pure rainbow trout, 1.0 = pure westslope cutthroat trout) at sampling locations ranged from 0.01 to 0.99. An average q_{wsct} below 0.99 is a criterion that has been used in previous work to designate a population as "hybridized." Landscape genetic analysis using regression trees indicate that water temperature, elevation, distance to the nearest stocking site and distance to the nearest railway were significant components of a model that described 34% of the variation in q_{wsct} across 58 sites for which habitat variables were available. Building on this finding, I explored the role of water temperature, the best predictor of hybridization levels amongst the variables tested, in limiting the spread of admixture by evaluating cold tolerance in both species using critical thermal methods (CTM). Analysis of variance revealed a statistically significant difference between the critical thermal minima (CTMin) of WSCT and RT acclimated to 15 °C (1.0 ± 0.8 °C and 1.4 ± 1.0 °C, respectively). The heritability of cold tolerance observed in this study appears to be complex and does not seem to behave in a simple additive manner. The identification of water temperature as a major factor influencing admixture and subsequent test for physiological differences in cold tolerance provide evidence to support a hypothesis that cold water habitats act as a natural barrier to hybridization between WSCT and RT. This information provides insight into the evolutionary history of WSCT and RT and will be useful in assisting conservation efforts aimed at mitigating the wide-spread loss of WSCT to genomic extinction.

Preface

The experiments carried out in Chapter 3 were covered by Animal Care Certificate number A11-0009.

Chapter 2. A version of this chapter has been published as [Yau M. M. and Taylor E. B.. Environmental and anthropogenic correlates of hybridization between westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*O. mykiss*). Conservation Genetics 14:855-900, 2013]. I was responsible for data collection, data synthesis, concept formation and manuscript composition. Tissue samples were collected by Alberta Sustainable Resource Development and Parks Canada. Jen Gow and Eric Taylor developed the genetic methods that I employed in this study. Eric Taylor was the supervisory author on this project and was involved throughout the project in concept formation, statistical analysis and manuscript composition.

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Dedication

This work is dedicated to my grandfather, Yau Kwan Leung, as well as, Charlie Pacas and the biologists at Alberta Sustainable Resource Development and Parks Canada who caught the fish and collected tissue samples used in this thesis.

Chapter 1

Introduction

The introduction of exotic species is a critical issue in conservation biology (Rhymer and Simberloff 1996; Allendorf et al. 2001; Levin 2002). With marked growth in transoceanic and transcontinental transport and travel, non-native species are brought into new habitats and have the potential to disrupt natural ecosystems. The outcomes of introductions are hard to predict. Some taxa may never colonize the new environment, while others that do, may have positive, negative or zero impact on the system. Those that spread and yield negative consequences are termed 'invasive' and can generate massive, unforeseen costs (Dawson 2002; Pimentel et al. 2005). In the United States, the number of exotic species is estimated to be 50,000 and rising (Pimentel et al. 2005). This translates to almost $120 billion dollars (US) in environmental damages annually. In Canada, the same model estimates a loss of $7.5 billion (CDN) per annum (Dawson 2002).

Introductions may be deliberate, usually done in an attempt to enhance economic gain through agriculture or aquaculture, or unintentional. Regardless of the motivation behind the transfer, invasive species pose a serious threat to the environment and to biodiversity (Rhymer and Simberloff 1996). Native taxa, which are often already threatened by other stressors such as pollution and habitat destruction, can be pushed to extinction when an exotic species enters the system. Invasive taxa can impact an ecosystem through predation, habitat alteration, competition, infection and hybridization (Simberloff 2005). Introduced taxa are responsible for the 'endangered' status of nearly 49% of listed species in the United States and 22% of the endangered species in Canada (Fig. 1.1, Wilcove et al. 1998; Venter et al. 2006). Amongst the listed fish species, introduced species threaten 53% of freshwater and marine fishes in the United States and 25% of the endangered fishes of Canada (Fig. 1.2, Wilcove et al. 1998; Venter et al. 2006). Unlike other stressors that can be mitigated or restored, rarely is it possible to completely extirpate an invasive species (Allendorf et al. 2004).

Hybridization with introduced species can seriously threaten naturally evolved, regional taxa (Rhymer and Simberloff 1996; Allendorf et al. 2001; Levin 2002). Introductions and habitat modifications can bring previously

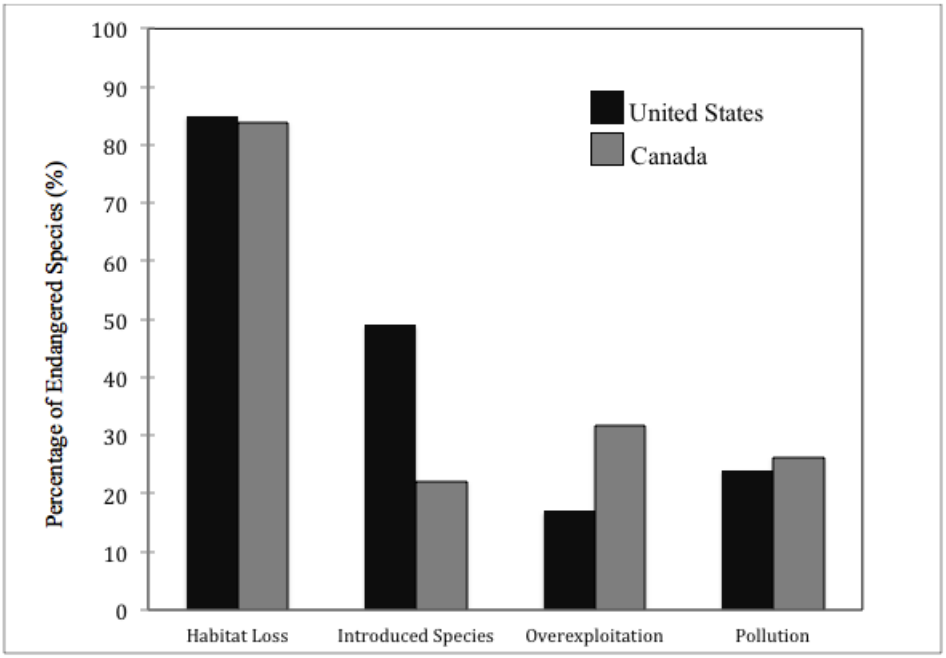

Figure 1.1: The percent of all listed endangered species threatened by habitat loss, introduced species, overexploitation and pollution in the United States (n=1880, Wilcove et al. 1998) and Canada (n=488, Venter et al. 2006). These values are not exclusive which is why they add to a value greater than 1.

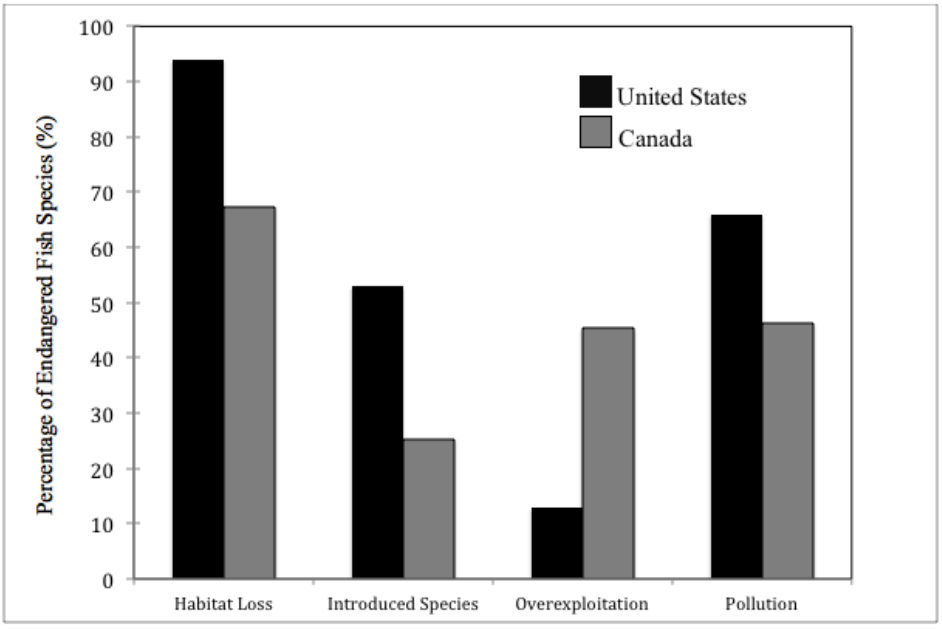

Figure 1.2: The percent of endangered fish species (marine and freshwater) threatened by habitat loss, introduced species, overexploitation and pollution in the United States (n=213, Wilcove et al. 1998) and Canada (n=95,Venter et al. 2006) These values are not exclusive which is why they add to a value greater than 1.

allopatric populations into contact creating an opportunity to hybridize (Allendorf and Leary 1988). These groups generally lack evolved characteristics that prevent hybridization and the production of viable, hybrid offspring. If the hybrids are fertile, they then act as a vector to introgressive hybridization by reproducing with each other or an individual from either parental species, moving alleles from one genetically distinct group to another. Uncontrolled hybridization can lead to the homogenization of locally adapted gene complexes into a hybrid swarm and the loss of native genotypes (Allendorf et al. 2001).

In freshwater habitats, fishes may be brought in as an additional food source, but more often, non-native fishes are introduced in an attempt to enhance recreational fisheries (Allendorf and Leary 1988). Salmonid fishes (salmon, trout, char, grayling and whitefishes) are amongst the most widely stocked worldwide. They are generally excellent sport fishes, a natural source of protein, and can be reared and transported with relative ease. Fishes within this group that do not naturally co-occur, generally lack significant divergence in behaviour and spawning habitats (Allendorf and Leary 1988; Behnke 1992). This, combined with external fertilization, makes salmonids especially prone to hybridization (reviewed by Taylor 2004).

Over 65% of the invasive species affecting native fishes in Canada are the result of intentional introductions (Dextrase and Mandrak 2006). The earliest record of a species introduced outside their natural range in North America dates back to the late 1600s (DeKay 1842). In much of western North America, unexpected hybridization has become commonplace and at least two subspecies of inland cutthroat trout (Salmonidae: *Oncorhynchus clarkii*) are now genomically extinct (Behnke 2002). The common cause in both cases is hybridization with introduced rainbow trout (*Oncorhynchus mykiss* (Walbaum, 1792); RT) that are a staple in many hatcheries and are farmed worldwide. Rainbow trout are generally limited to freshwater systems, although anadromous populations are also represented in *O. mykiss* (steelhead trout). This species is broadly stocked and can be found on all continents with the exception of Antarctica (Welcomme 1992).

1.1 Westslope Cutthroat Trout

The westslope cutthroat trout (*Oncorhynchus clarkii lewisi* (Pratt and Graham, 1884); WSCT) is one of at least ten and perhaps as many as fourteen subspecies of cutthroat trout (Behnke 1992). It is native to the interior drainages of southeastern British Columbia, southwestern Alberta, Canada,

and adjacent watersheds in Idaho and Montana scattered areas in western Washington and Oregon in the United States (Fig. 1.3). The formation of waterfalls approximately 70,000 years ago in many of the large tributaries of the upper Columbia River appear to have shaped the natural distribution of WSCT (Figure 1.3, Behnke 1992). They were likely able to colonize waters above barrier falls due to high water levels produced by glacial melt, prior to isostatic rebound that follows glacial retreat (Behnke 1992). Rainbow trout found in this region are absent above the barrier falls and are believed to have been restricted to the lower Columbia River during the last glacial period, allowing WSCT to become established in large in-land regions of North America, geographically isolated from RT (Figure 1.4, Behnke 1992).

Westslope cutthroat trout evolved with few other fish species and their biology appears to be driven more by abiotic environmental factors rather than interspecific interactions (Griffith 1988). Fluvial and resident forms are common, while adfluvial populations are less so (Cleator et al. 2009). They are considered an indicator species for pristine environments, having a relatively narrow range of suitable living and spawning conditions. Adults make seasonal movements into spawning habitats that are characterized by silt-free, well-oxygenated water and clean gravel. Once developed, juvenile fish seek shallow pools with low water velocity. Adults require cool, clear water with in-stream structural complexity and riparian cover. In the summer, they are typically found in water temperatures between 9 and 12 °C. The adults are generalist predators, foraging on terrestrial and aquatic invertebrates available in cold and sometimes turbid water. Deep pools or areas with ground water discharge are needed to overwinter. Westslope cutthroat trout are typically not piscivorous even when forage fish are available (Cleator et al. 2009).

Westslope cutthroat trout typically spawn in late spring between May and June when water temperatures reach 10 °C (Nelson and Paetz 1992; Cleator et al. 2009). Females reach sexual maturity after 3-5 years and males between 2-4 years. Fry emerge from the gravel between early July and late August and can remain in their rearing habitats for 1-4 years depending on the productivity of the stream.

1.2 Hybridization with Rainbow Trout

Cutthroat trout and rainbow trout shared a common ancestor between 3.5-6 million years ago (McKay et al. 1996; Smith et al. 2002; Loxterman and Keeley 2012). Salmonids evolved approximately 100 million years ago, after

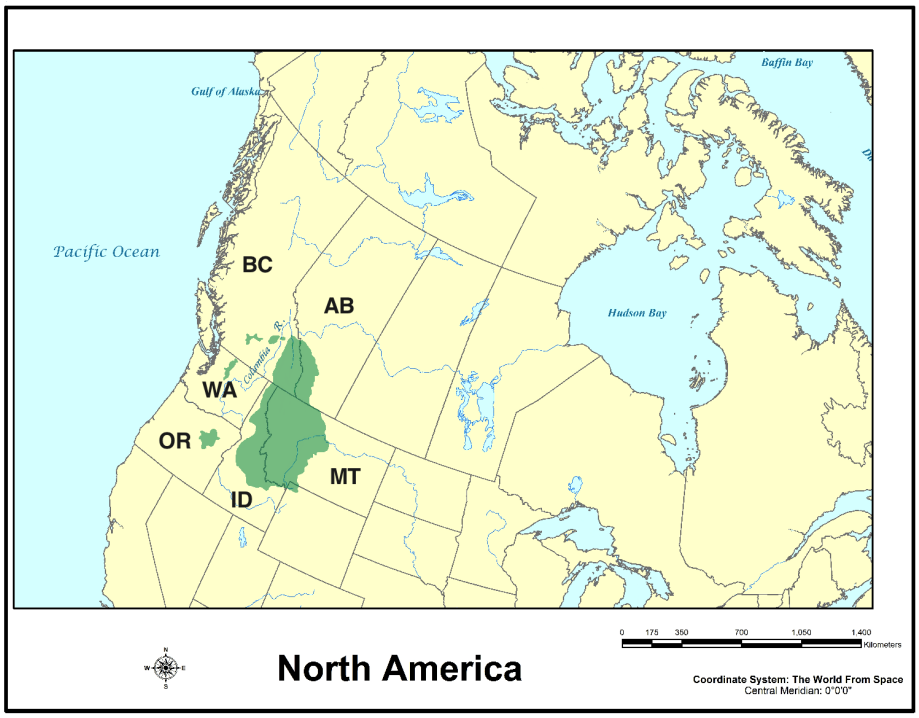

Figure 1.3: Native range of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). Modified from Behnke (2002).

Figure 1.4: Native range of rainbow trout (*O. mykiss*) in North America. Modified from Behnke (2002).

chromosome duplication (tetraploidy) separated them from most other fish species (Behnke 2002). Delineating the evolutionary history of WSCT and RT has proven difficult, due to one or more hybridization events that have occurred since their initial split (Allendorf and Waples 1996; Behnke 2002). Divergence in morphology, ecology and life history has also hampered efforts to define taxonomic groups. Both rainbow trout and cutthroat have significant intraspecific variation and local adaptation, represented by numerous proposed subspecies within each group (Behnke 2002).

Introduced RT are the greatest peril for WSCT (Allendorf and Leary 1988; COSEWIC 2006). They have restricted WSCT to the upper extremes of their range and will hybridize, and produce viable offspring (Fig. 1.5). Despite having different chromosome numbers (58-60 in RT, 66 in WSCT), WSCT and RT have the same number of chromosome arms (104) (Behnke 2002). This allows chromosome pairing to carry on as normal, resulting in hybrid offspring without major developmental deficiencies. Hybrids tend to be less fit (Allendorf and Leary 1988). Lab-spawned hybrids exhibit slower growth and post hatching survival. Recent work has also found that a hybrid individual will experience a 50% reduction in fitness with as little as twenty percent RT admixture (Muhlfeld et al. 2009a). First generation hybrids that survive to maturity, however, are highly fertile . They experience greater fertilization and hatching success than pure WSCT (Muhlfeld et al. 2009a). Under these circumstances, hybrid swarms will form as any progeny produced by a hybrid will be a hybrid and all subsequent offspring will also be hybrids, carrying some degree of RT admixture.

Introgression of RT genes into the WSCT genome can break up unique, locally adapted, gene complexes. Westslope cutthroat trout are characterized by their adaptation to local conditions (Behnke 1992). Vast stream networks subdivide populations and the genetic composition of a single population can be significantly different from adjacent populations (e.g., Taylor et al. 2003). Levels of genetic differentiation amongst WSCT tend to be higher within a watershed than between. Thus, the loss of a single population could eliminate a significant portion of the genetic diversity within a system. In addition, the presence of RT restricts pure populations of WSCT to high elevation, cold-water habitats, while RT and hybrids dominate downstream (Hitt et al. 2003; Weigel et al. 2003; Muhlfeld et al. 2009c; Rasmussen et al. 2010). This removes habitat connectivity and isolates pure WSCT populations from one another. These isolated populations tend to be small and vulnerable to inbreeding and stochastic events such as drift (Mayhood and Taylor 2011). If a population is lost, the lack of connectivity prevents WSCT from recolonizing the habitat.

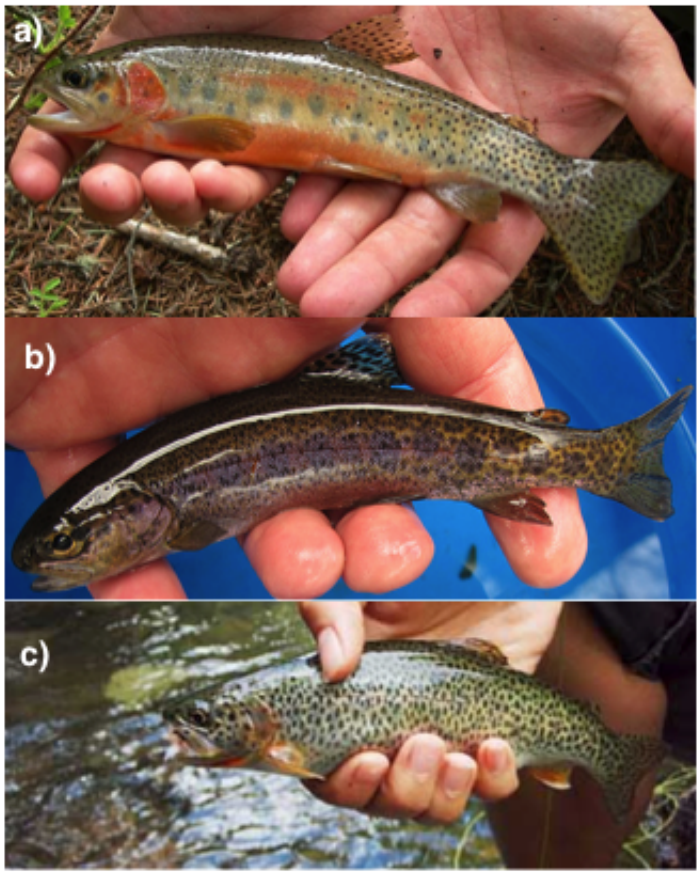

Figure 1.5: a) juvenile westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; note the orange slash on the underside of the mandible, body spots concentrated in the posterior region of the body), b) juvenile rainbow trout (*O. mykiss*; absence of orange slash, spots are uniform across the length of the body), c) westslope cutthroat trout x rainbow trout hybrid (orange slash on mandible present, spots have uniform distribution across the length of the body; the hybrid characters are clearly visible in this individual, but can be much harder to distinguish in other specimens. *Photo credit:* a) *US National Park Service,* b) *FISHBIO,* c) *Austin McPherson.*

1.3 Conservation Status of Westslope Cutthroat Trout

Widespread hybridization with RT is pervasive across the entire range of WSCT. In the United States, WSCT in Montana, Idaho, Washington and Oregon are recognized as a single population for conservation purposes and carry a status of 'special concern', occupying 59% of their historical distribution (Shepard et al. 2005). Between 65-85% of the remaining area no longer contain 'genetically-pure' WSCT populations. They are the state fish of Montana, but occupy only 72% of their historical range and 69% show some level of hybridization with RT. Westslope cutthroat trout fishing is regulated by catch limits and in some places is restricted to only catch-and-release angling. Westslope cutthroat trout also enjoy a level of protection as many "strong-holds" tend to be found in national parks. In addition, some of their distribution coincides with habitats of endangered species such as the bull trout (*Salvelinus confluentus*) and steelhead trout (*O. mykiss*), which are protected under the Endangered Species Act (US Fish and Wildlife Service. 1999). As a result, WSCT were denied listing under the ESA.

In Canada, the Committee on the Status of Endangered Wildilfe in Canada (COSEWIC) recognizes two designatable units (DUs) of WSCT, one in British Columbia and another in Alberta (COSEWIC 2006). The two DUs occupy separate ecozones and lack dispersal between them due to separation by the Rocky Mountains (COSEWIC 2006; The Alberta Westslope Cutthroat Trout Recovery Team 2013). There are also marked differences in the conservation status of each DU. In BC, WSCT maintain most of their historical distribution, but there is evidence of extensive hybridization with RT as illustrated in studies by Rubidge et al. (2001) and Bennett et al. (2010). In the upper Kootenay River drainage, however, WSCT were found in only 22% of their historical range (Rubidge et al. 2001). In 2010, the BC population of WSCT was federally listed as 'special concern' under the Canadian *Species at Risk Act* (SARA).

In southwestern Alberta, WSCT historically occurred, in abundance, in streams and rivers from the Bow River to the Alberta-Montana border (Fig. 1.3). Numbers began to decline following the construction of the Canadian Pacific Railway in 1883, which opened up access to the region. Overexploitation by early European settlers in the late 1880s and 1900s removed substantial numbers of fish from the system and likely caused the local extinction of many WSCT populations (Mayhood and Taylor 2011). The introduction of brook trout (*Salvelinus fontinalis*), rainbow trout and

brown trout (*Salmo trutta*) in large numbers further displaced native WSCT. These species are now common across the historical distribution of WSCT, threatening native populations with competition, hybridization, predation and disease (Mayhood and Taylor 2011).

Westslope cutthroat trout are the only native subspecies of cutthroat in Alberta (Behnke 1992). This DU, however, appears to be at an elevated level of risk and was designated as 'threatened' by COSEWIC in 2006 (COSEWIC 2006). Current estimates suggest WSCT occupy 5% of their historical distribution in the province and only 50 of approximately two hundred and seventy-four populations are considered 'genetically pure' (unhybridized) populations (Mayhood and Taylor 2011). These populations are said to have less than 5000 mature adults and 16% have a low chance of recovery. As of 2009, Alberta's populations are protected provincially under the Wildlife Act and in 2013, listed as 'threatened' under SARA. In March of the same year, the Alberta Species at Risk Program released a recovery plan for Alberta WSCT (The Alberta Westslope Cutthroat Trout Recovery Team 2013). The recovery plan highlights the importance of identifying genetically pure populations of WSCT with continual monitoring as well as "an evaluation of environmental and biological factors that promote and/or limit hybridization between westslope cutthroat trout and rainbow trout" as part of their recovery strategy (The Alberta Westslope Cutthroat Trout Recovery Team 2013).

1.4 Hybridization Studies

Studies to identify variables that influence levels of hybridization in salmonids have arisen in response to conservation concerns. Combinations of stocking history, habitat disturbance, water temperature, habitat connectivity (presence of barriers) and elevation appear to impact levels of hybridization; however, the relative influences of each vary across studies and geographic region. Rubidge and Taylor (2005) found that water temperature appeared to influence levels of admixture and that the Koocanusa Reservoir acted as a source of RT and, that hybridization decreased with increasing distance from the reservoir in the upper Kootenay River, British Columbia. Hitt et al. (2003) concluded that hybridization was spreading in an upstream manner in the Flathead River system in Montana, and agreed with Rubidge and Taylor (2005) that the presence of physical barriers was likely the only obstacle constraining the spread of RT and hybridization in the system. In contrast, hybridization was absent near areas of known stocking in the Clear-

water River Basin, Idaho, and locales in the Upper Oldman River, Alberta (Weigel et al. 2003; Rasmussen et al. 2010). In these systems, there were no physical barriers to movement and these authors hypothesized that the environment was exerting extrinsic control, primarily through changes in water temperature. Subsequently, Rasmussen et al. (2010) found evidence that parental habitat choice, likely driven by water temperature, and differential life history strategies contributed to hybrid zone structure. While in British Columbia and Montana, Muhlfeld et al. (2009c) determined that a mixture of stocking history, connectivity, anthropogenic disturbance, and water temperature all played major roles in predicting levels of hybridization in the upper Flathead River. These studies describe hybridization between the same species, but reveal high variability in individual conclusions. Consequently, the studies to date highlight the necessity of assessing local habitat conditions for effective conservation.

Despite geographic variation, one pattern appears to persist across most studies. A genotypic gradient is commonly reported with changes in elevation; genetically pure populations of WSCT are consistently reported at high elevations with increasing levels of admixture with RT downstream (Hitt et al. 2003; Weigel et al. 2003; Rubidge and Taylor 2005; Rasmussen et al. 2010). This pattern persists through time even when exotic fish are introduced to high elevation habitats (Weigel et al. 2003). Cold headwaters appear to be the only habitat in which genetically pure, indigenous salmonids experience apparent immunity from competition and hybridization with introduced taxa (Paul and Post 2001). This observation has lead to the 'elevation refuge hypothesis', where temperature-mediated competition dictates species dominance along an elevational gradient (Paul and Post 2001). Under this hypothesis, the invasive taxon appears to be a better competitor in warmer waters and the reverse is true at high elevations, where the native species is competitively superior.

Laboratory studies on a number of trout species support a shift in competitive ability (growth, survival and behaviour) as temperatures are altered (Taniguchi et al. 1998; Selong et al. 2001; Bear et al. 2007). Westslope cutthroat trout and rainbow trout, however, exhibit virtually identical optimal growth temperatures (WSCT: 13.6 °C; RT: 13.1 °C, Bear et al. 2007). Recent research by McHugh and Budy (2005), suggest clinal zonation may be driven not only by interspecific competition, but also physiological limitations. Specifically, at cold temperatures, typical of high elevation headwaters, where a reversal in competitive success favouring the native species is not observed. Work by Rasmussen et al. (2012) revealed metabolic differences between WSCT, RT and their hybrids. The authors suggest that cold,

headwater habitats are likely unable to support the energetic demands of pure RT and admixed offspring. These data suggest that temperature may be limiting the spread of hybridization in a way that is not solely based on competitive interaction, as suggested by the elevation refuge hypothesis, but also by way of physiological performance.

1.5 Research Objectives

There is an urgent need to mitigate the impacts of hybridization and protect species that are becoming threatened by it. A proper analysis to determine the levels of hybridization as well as the factors influencing hybridization is integral for an effective conservation programme. The aim of this research was to document the extent of hybridization between WSCT and introduced RT at sampling locations along the British Columbia-Alberta border, as well as to identify and further understand factors that may be driving or limiting hybridization between these species.

To do this, I conducted the following studies:

1. I evaluated the distribution of interspecific hybridization amongst populations of WSCT and introduced RT through DNA analysis of tissues collected across southwestern Alberta and southeastern British Columbia.

2. I used landscape data obtained from Alberta Sustainable Resource Development and other resources visualized in ArcGIS, to identify environmental factors that may be influencing rates of hybridization across the region.

3. I tested the elevation refuge hypothesis by conducting a laboratory study evaluating the cold tolerance of WSCT and RT. Here, I explored the possibility that cold-water temperatures may be limiting the spread of RT upstream to high elevation headwaters, where genetically pure populations of WSCT are characteristically found in watersheds with admixed populations further downstream.

Chapter 2

Distribution of hybridization and environmental factors

2.1 Introduction

Hybridization between native and introduced species is an on-going conservation issue (Rhymer and Simberloff 1996; Allendorf et al. 2001; Levin 2002). Uncontrolled gene flow between previously isolated groups disrupts local genotypes and can result in the genomic extinction of indigenous taxa (Epifanio and Philipp 2000; Allendorf et al. 2004; Muhlfeld et al. 2009a). In freshwater fishes, this problem has arisen in large part due to human efforts to enhance recreational fisheries (Larson and Moore 1985; Allendorf and Leary 1988) or by habitat alteration (e.g., Hubbs 1955; Vonlanthen et al. 2012). Many situations include fishes that have evolved in allopatry such that reproductive isolation is often incomplete (Allendorf and Leary 1988) and following introduction of non-indigenous species, widespread hybridization results (Rhymer and Simberloff 1996).

Salmonid fishes (trout, salmon and their relatives) are particularly susceptible to hybridization (Taylor 2004). Like most fishes, salmonids fertilize their eggs externally, however, they often lack significant divergence in spawning habitats and behaviour that results in incomplete reproductive isolation. Introgressive hybridization is so pervasive in freshwater salmonids, it has been called the most important factor responsible for the loss of native trout species (Allendorf and Leary 1988). Hybridization with stocked rainbow trout (*Oncorhynchus mykiss*; RT) has already claimed two subspecies of inland cutthroat trout (*O. clarkii*) by genomic extinction (Miller et al. 1989) and threatens many other species, including westslope cutthroat trout (*O. clarkii lewisi*; WSCT).

The abundance of WSCT has severely declined across its entire range. Westslope cutthroat trout are native to southeastern BC and southwestern Alberta, Canada and parts of the United States (Schmetterling 2001; Weigel et al. 2003; Rubidge and Taylor 2005; Mayhood and Taylor 2011). Westslope

cutthroat trout are the only trout native to Alberta and currently inhabit less than 20% of their historical range owing to overexploitation, habitat loss and habitat degradation (COSEWIC 2006). They are thought to number fewer than 5,000 adults (Mayhood and Taylor 2011). Populations of WSCT persist primarily in the headwater tributaries of the Oldman and Bow Rivers' drainage systems, which form part of the western headwaters of the South Saskatchewan River system (Mayhood and Taylor 2011). Early records of RT stocking date back to the mid-1920's, and some sixty million fish have been stocked since that time (Alberta Sustainable Resource Development, Airdree, Alberta, unpublished data). Hybridization with RT is considered to be widespread across extant populations (DFO 2009; Mayhood and Taylor 2011).

Although hybridization in freshwater fishes has a long history of study (e.g., Hubbs 1955; Taylor 2004; Hansen and Mensberg 2009), the biological, environmental, and anthropogenic factors that influence the extent and spatial distribution of hybridization are not well known. Heath et al. (2010) presented evidence that a combination of anthropogenic habitat alteration (e.g., logging and urbanization) and stocking intensity were important drivers of spatial variation in hybridization between rainbow trout and coastal cutthroat trout (*O. c. clarkii*) in southwestern BC. At the intraspecific level, Marie et al. (2012) demonstrated an effect of stocking intensity, habitat size, dissolved oxygen levels and pH among wild lake-dwelling populations of brook char (*Salvelinus fontinalis*) in Québec, Canada. Previous work on RT and WSCT hybridization has indicated that some environmental features, especially water temperature and elevation, seem to co-vary with admixture levels (e.g., Rubidge and Taylor 2005; Muhlfeld et al. 2009c; Rasmussen et al. 2010), while in other cases spatial arrangement of populations also seems to be important (e.g., Hitt et al. 2003; Boyer et al. 2008).

In this chapter, I report the results of an extensive survey of admixture levels across more than 150 localities with WSCT in southwestern, Alberta. I then use these data to investigate the key habitat and stocking variables that may influence spatial variation in observed levels of hybridization. By conducting this analysis, I hope to independently test the idea that certain environmental and anthropogenic factors (e.g., water temperature, human habitat disturbance) are important in influencing the degree of hybridization between RT and WSCT as has been suggested in other areas and species. The identification of natural variables influencing hybridization may also be relevant to understanding factors important in the evolution of reproductive isolation between these species (e.g., Culumber et al. 2012), while the identification both of natural and anthropogenic-related variables could be useful

when designing programs to limit the spread of hybridization or mitigate its effects.

2.2 Materials and Methods

2.2.1 Sampling

Tissue samples consisted of either fins clips stored in 95% ethanol or dried and stored in paper envelopes. All samples were obtained from populations of WSCT across localities in southwestern Alberta and a small adjacent region of British Columbia (BC) largely between 2006-2009, although a small number of samples dated to 1999 (Fig. 2.1, Appendix A Table. A.1). Most of my sampling localities spanned two major watersheds: the Bow and Oldman rivers and included samples from four national parks (Banff, Jasper, Kootenay and Yoho National Parks). The water that forms the Bow and Oldman rivers originate from glaciers in the Rocky Mountains near the BC border and the confluence of these rivers form the South Saskatchewan River (Hudson Bay drainage) representing the northern limit of the natural range of WSCT east of the Continental Divide. I also examined samples from several tributaries of the upper Kootenay River (Columbia River drainage) in BC. Altogether, I analyzed 159 sampling locations with a minimum of 15 fish sampled per site.

2.2.2 Genetic analyses

Genomic DNA was extracted from the fin samples using standard phenol-chloroform methods. Individuals were characterized for allelic variation using 10 microsatellite based markers: *Ssa*85 and *Ssa*197 (O'Reilly et al. 1996) *Ssa*456 (Slettan et al. 1995), *Ots*3, *Ots*4 (Olsen et al. 1996), *Ots*104, and *Ots*107 (Nelson and Beacham 1999), *Oki*3a (P. Bentzen, Dept.of Biology, Dalhousie University, Halifax, NS, unpublished data), *Omy*77 (Morris et al. 1996), and *Occ*16 (Ostberg and Rodriguez 2002). Only *Occ*16 appears to be strictly diagnostic between the two species (Ostberg and Rodriguez 2002 and Taylor unpubl. data), but all other loci (except *Ssa*197) showed major differences in allele size ranges and frequencies. For instance, in my final learning dataset (see below under Admixture analyses) F_{ST} (θ, Weir and Cockerham 1984) between the species averaged 0.50 (SD = 0.08) and ranged from 0.17 (*Ssa*197) to 1.0 (*Occ*16). These loci were also scored in 150 "learning samples" of allopatric RT and 75 WSCT that previous analyses indicated had no detectable admixture and that represented a range of

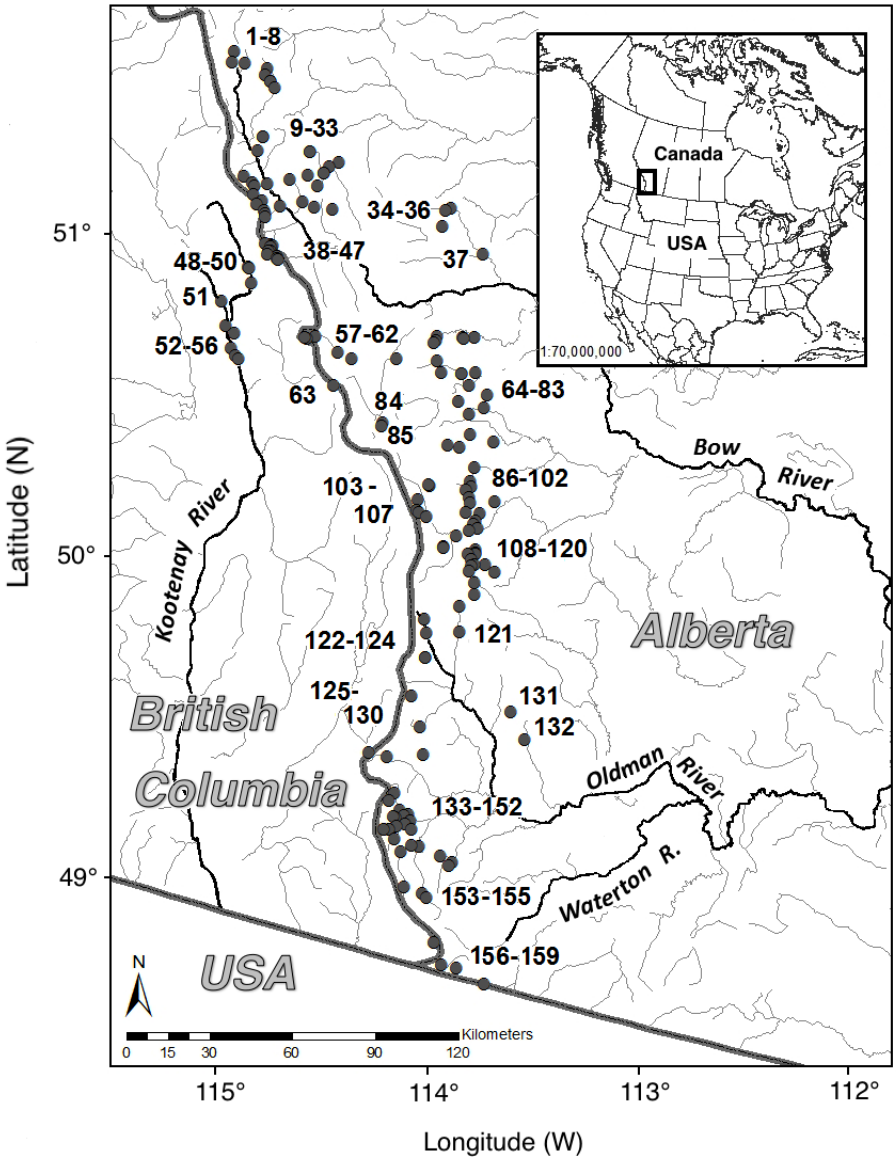

Figure 2.1: Map of 159 localities in southwestern Alberta and southeastern British Columbia, Canada, which were sampled for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and subject to microsatellite DNA analyses.

populations from BC and Alberta (Taylor et al. 2003, 2007; Tamkee et al. 2010).

Polymerase chain reactions were performed in 20 μl total volumes using the Qiagen Multiplex PCR Kit following the manufacturer's instruction. An average of 30 individuals were assayed per sampling location and PCR products were evaluated using fluorescently labeled primers and assayed on a Beckman-Coulter CEQ 8000 automated genotyper.

2.2.3 Population genetic analyses

I used MICRO-CHECKER (van Oosterhout et al. 2004) to check for the presence of null alleles or PCR artifacts that could compromise subsequent analyses. Thereafter, basic descriptive statistics of sample size (N), number of alleles (N_A), observed (H_O) and expected (H_E) heterozygosity were compiled using FSTAT ver 2.9 (Goudet 1995). Tests for deviations from Hardy-Weinberg equilibrium were performed for each locus-population combination using an exact test in which probability values were estimated using a Markov chain method using GENEPOP ver. 3.3 (Rousset 2008). Tests for genotypic linkage disequilibrium for all combinations of locus pairs within a population were also made using a Markov chain method with GENEPOP default values.

2.2.4 Admixture analyses

Individual admixture values (q-values) and posterior probability intervals were estimated for each locality using STRUCTURE (Pritchard et al. 2000). I set K (number of genetic populations) to $K = 2$ to represent the two species which were clearly distinct from one another in preliminary analyses (e.g., ordination analyses, assignment tests). Models were run under the admixture model and assuming correlated allele frequencies with a burn-in of 100,000 steps and subsequent runs of 450,000 steps. I also calculated admixture values for a sample of 100 simulated hybrids between the two species. The simulated hybrid genotypes were generated by using the HYBRIDLAB (Nielsen et al. 2006) program by randomly selecting alleles for each locus from the allopatric, non-admixed populations of RT and WSCT. I performed admixture analyses in two steps. First, I ran simulations using the allopatric, non-admixed populations ("learning samples") and all other population samples using five replicated analyses. Here, the non-admixed samples were used as priors in the model, i.e., the program was forced to consider these individuals as known RT and WSCT by invoking the USE-

POPINFO model in STRUCTURE (Pritchard et al. 2000). The admixture value, q, was expressed as the proportion of the genome estimated to stem from WSCT, q_{wsct} (0 =pure rainbow trout, 1.0 =pure westslope cutthroat trout). The mean q_{wsct} value across the five replicate analyses was then calculated for all of the non-learning samples. From this analysis, three populations of WSCT for which all individuals had q_{wsct} values of ≥ 0.99 were added to the learning sample group and the admixture analyses was rerun across five further replicates. I conducted the admixture analyses in two steps because: (i) the initial learning sample of WSCT was much smaller (75) than that of the RT, and (ii) the initial learning sample contained no non-admixed populations from Alberta and I wanted to account for possible genetic differentiation between BC and Alberta populations of WSCT which are separated by the continental divide. The final learning sample set consisted of 150 RT and 165 WSCT. The final values of q_{wsct} for Alberta populations of WSCT represent the averages calculated across the five replicate analyses.

2.2.5 Stream characteristics and anthropogenic variables

Variables were organized into three broad categories: stream geomorphology/environments, stocking history, and variables representing aspects of anthropogenic-based habitat disturbance (Table 2.1). Environmental variables were chosen based on current indicators considered to influence hybridization in western trout (Hitt et al. 2003; Weigel et al. 2003; Rubidge and Taylor 2005; Muhlfeld et al. 2009c; Heath et al. 2010). Also, the number of stocking events and the total number of RT introduced both represent aspects of stocking intensity that may influence the potential for admixture (e.g., Ruzzante et al. 2004; Hansen and Mensberg 2009; Marie et al. 2010). Finally, habitat disturbance from human development has long been considered an important variable influencing interspecific hybridization in fishes (Hubbs 1955). More recently, Heath et al. (2010) found evidence of a positive association between logging activity and urban development and hybridization between naturally sympatric rainbow trout and coastal cutthroat trout (*O. c. clarkii*) in southwestern BC.

Locality and stocking data were available for a total of 58 streams (Alberta Sustainable Resource Development, Airdrie, AB; Appendix A Table. A.2). These data were mapped using ArcGIS 10.0 (ESRI, Redlands, CA, USA). Elevation data were extracted using a 25-metre Digital Elevation Model and stream order, a measure of stream branching, was assessed in ArcGIS using the Strahler method (Horton 1945; Strahler 1952).

Spot water temperature was recorded with hand-held thermometers at the time of fish sampling. To assess how well such instantaneous measures might reflect longer term, relative differences between localities, I obtained longer-term measures of air temperature near each locality through WorldClim (www.worldclim.org). These data layers are generated by interpolating monthly climate data from the Global Historical Climatology Network (GHCN), the Food and Agriculture Organization of the United Nations, and the World Meteorological Organization, and several other sources of climate data for the period 1950-2000 (see Hijmans et al. 2005). The locality water temperatures obtained during daytime fish sampling were positively correlated with mean annual air temperature ($r = 0.31$, df = 57, $P = 0.002$), maximum air temperature of the warmest month ($r = 0.26$, $P = 0.01$) as well as average air temperature over the warmest three month period ($r = 0.40$, $P < 0.0001$). Further, elevation and water temperature are expected to be negatively correlated with one another in natural systems (e.g., Paul and Post 2001; Rasmussen et al. 2010) as was observed in my data ($r =$ -0.51, $P < 0.001$). These associations with elevation and longer term temperature trends suggest that locality water temperatures recorded at time of fish sampling represent a reasonable proxy for relative differences between localities. Human impact variables (e.g., distance of sample site to nearest road) were measured as Euclidean distances and distance from the sample site to the stocking location (stockD) was calculated as the fluvial distance in ArcGIS (Table 2.1). In some streams, stocking of RT took place in tributaries other than that in which genetic samples were obtained. In others, apparent upstream migration barriers (mapped in ArcGIS) separated stocking and genetic sample localities within tributaries. Preliminary analyses, however, indicated no difference in average q_{wsct} values between localities where stocking took place in the same or different tributaries or separated by potential upstream migration barriers (Yau and Taylor, unpubl. data) so I did not include these potential effects in my analysis.

Stocking intensity was represented by the number of stocking events at the stocking location nearest to the genetic sample locality. Other variables, included the total number of RT stocked at each locality, and the year of last stocking, which was assessed to investigate how time scale may influence admixture levels.

2.2.6 Statistical analysis

Differences in the mean values (calculated across samples with a sample size of ≥ 10) of q_{wsct} were tested for significance using using variants of one-way

Table 2.1: Definition of variables potentially explaining variation in admixture levels between native westslope cutthroat trout and introduced rainbow trout.

Variable	**Definition**
Stream Variables	
Depth1	Maximum water depth at sample site
Depth2	Mean water depth at sample site
Water temperature	Water temperature at time of sampling
Order	Strahler stream order of sample site (1-5)
Elevation	Elevation in meters at sample site
Anthropogenic disturbance variables	
Road (RoadD)	Euclidean distance from sample site to nearest road
Pipeline (PipeD)	Euclidean distance from sample site to nearest gas/oil pipeline
Railway (RailD)	Euclidean distance from sample site to nearest rail line
Power (PowerD)	Euclidean distance from sample site to nearest power line
Stocking variables	
Distance (StockD)	Fluvial distance to nearest stocking site
Stocking intensity (StockI)	Total number of stocking events at the stocking site
Number of fish stocked (StockN)	Total number of rainbow trout stocked at the stocking site
Year of last stocking (StockY)	Total years between 2010 and year of last stocking at stocking site

ANOVA and subsequent post-hoc tests suitable for samples with unequal variances using PAST (version 2.12), a general spreadsheet-based statistical package (Hammer et al. 2001).

To identify habitat and human impact variables that might influence admixture levels, I used a nonlinear, regression tree approach implemented in the Random Forest algorithm described by Breiman (2001). Regression trees use decision tree analysis to help to resolve relationships between a response variable (in my case mean admixture level) and several potential predictor variables that may behave in a non-linear fashion and have multiple interactions amongst them. Regression trees and their use in ecological data analyses have been described by De'ath and Fabricius (2000) and Moisen (2008) have seen recent application in landscape genetics (e.g., Murphy et al. 2010; Hether and Hoffman 2012). Random Forest analysis uses bootstrapped, learning datasets to build an assemblage of regression trees each of which generates predictions of the dependent variable (q_{wsct}) based on the independent variables. These predictions are then averaged across all bootstrapped iterations to yield a final prediction. Examining many bootstrapped samples coupled with random sampling of subsets of predictor variables during tree construction helps to reduce the variance amongst different regression tree results. Random forests also have the desirable qualities of being insensitive to autocorrelation, are distribution free, do not require transformation of original variables, and they can assess complex interactions amongst many variables (De'ath and Fabricius 2000; Moisen 2008). I combined the approaches of Murphy et al. (2010) and Hether and Hoffman (2012) by first analyzing a full model incorporating all nine potential predictor variables, evaluating sub-models with subsets of predictor variables, running a final "best" sub-model and assessing the relative importance of each predictor variable and overall model significance using the RANDOMFOREST package v 4.5-28 with R (Liaw and Wiener 2002; R Development Team 2012) under the regression mode with 10,000 trees.

I converted the measures of variable importance (I_p) that are provided in RANDOMFOREST to model improvement ratios (MIRs) by dividing each I_p by the maximum I_p observed (Hether and Hoffman 2012). I then used the MIR to iteratively select the best sub-models from the full model. First, I ran a sub-model that incorporated only those variables that had MIR of at least 0.3 (i.e., they improved the model to a degree of at least 30% of the best variable). Next, I ran 20 independent RANDOMFOREST analyses for each of a series of sub-models that had one of the predictor variables removed, starting with the variable with the lowest MIR. I calculated the mean and 95% confidence intervals for the resulting pseudo-R^2 (Liaw and Wiener

2002) values for each such sub-model. I then chose a final sub-model that had the fewest predictor variables, but whose mean pseudo-R^2 had a 95% confidence interval overlapping that of the best sub-model (Hether and Hoffman 2012). I used partial dependence plots to assess the marginal effects of each retained predictor variable to admixture (i.e., the effect not explained by other predictor variables, Liaw and Wiener 2002; Cutler et al. 2007). Finally, I tested the significance of the derived sub-model following the randomization (of admixture values, N = 1,000) procedure and examining the randomized distribution of the simulated pseudo-R^2 values relative to the observed value as detailed in Hether and Hoffman (2012). I also explored alternative methods of analysis of the data (e.g., standard multiple regression on the original data or on scores from a principal components analyses of correlation matrices), but the RANDOMFOREST procedure consistently produced lower mean square errors, higher R^2, simpler models, identified the same major variables as predictors, and had the added advantage of being able to analyze the original variables without the need for transformations. This analyses employed the mean q-value for each stream which is uninformative concerning the variation amongst individuals within streams in admixture level. I also analysed the data using the median q-value (which as expected showed a correlation r of > 0.9 with the mean q-value) and very similar results were obtained (i.e., overall R^2 value of 0.32, same five variables chosen in same relative ranking of explanatory power see below).

Where appropriate, adjustments for multiple simultaneous statistical tests incorporated the false discovery rate procedure of Narum (2006).

2.3 Results

2.3.1 Genetic analyses

Analysis of genetic data for all localities with at least 15 fish sampled indicated that evidence for null alleles was rare and scattered across individual loci and populations. Of 1,590 analyses (159 samples x 10 loci), five suggested that null alleles might be present at *Ots*107 in some samples. I found, however, no individuals that were null-null homozygotes at *Ots*107 suggesting that null alleles would not be a significant factor in subsequent analyses (cf. Taylor et al. 2003; Tamkee et al. 2010).

Across localities and loci, observed and expected heterozygosities, and the number of alleles averaged (SD) 0.32 (0.15), 0.34 (0.16), and 3.2 (1.4), respectively, and F_{IS} averaged 0.072 (0.14) and 29 of 159 permutation tests indicated F_{IS} values significantly greater than 0 (Appendix A Table. A.1).

Of the 159 samples examined, there was a broad range of estimated admixture levels ranging from q_{wsct} = 0.010 to 0.994. There was a strong right skew to the data with most localities having q_{wsct} values > 0.90. Pure RT (i.e., $q_{wsct} < 0.05$), however, were found at 12 localities, six localities had average q_{wsct} values of < 0.10 and 34 of the localities exhibited extensive hybridization with q-values below 0.90 (Appendix A Table. A.1). Three localities were identified from admixture analysis and stocking records as Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) or Yellowstone cutthroat trout x westslope cutthroat hybrids and removed from further analyses.

Of those localities with q_{wsct} values between 0.10 and 0.90 (N = 29), 24 had F_{IS} values not significantly different from 0, while five had significantly positive F_{IS} values associated with heterozygote deficiencies (e.g., Haiduk Lake, Smuts Creek, Fisher Creek, Spotted Wolf Creek, Appendix A Table. A.1). The estimated value of q_{wsct} was negatively correlated with the average (across loci) number of alleles observed; localities with high levels of admixture with RT (low q_{wsct}) tended to have a higher average number of alleles (r_s = -0.73, $P < 0.0001$). The simulated hybrids (N = 100 individuals) had a mean q_{wsct} value of 0.51 (SD = 0.053).

2.3.2 Influences on admixture values

Incorporation of all predictor variables into the RANDOMFOREST regression tree analysis resulted in a pseudo-R^2 value of 23.7% and a mean square error (MSE) of 0.059. The variable importances (I_p) for admixture prediction ranged from a low of 0.037 for stream order to a high of 1.019 for water temperature. Model improvement ratios relative to the best predictor (1.0 = water temperature) ranged from 0.036 (stream order) to 0.654 (elevation). Five variables, water temperature, elevation, stockD, powerD and railD were selected for sub-model analyses based on MIRs of at least 0.3. A model incorporating just these variables produced a pseudo-R^2 = 33.2% and a MSE = 0.052 ($P < 0.001$). Analyses of the various sub-models (Table. 2.2) indicated that a four variable sub-model incorporating water temperature (MIR = 1.0), elevation (MIR = 0.824), railD (MIR = 0.717) and stockD (MIR = 0.612) (but removing powerD, MIR = 0.632) had a mean pseudo-R^2 (34.4%, MSE = 0.051, $P < 0.001$ from randomization test) that was not significantly worse than the full five variable sub-model, but that removing any other single variable resulted in significantly lower pseudo-R^2 values (minimum value = 26.3% after removing temperature). This final, four variable model was used to predict admixture levels based on 10,000

Table 2.2: Variable importance values (I_p) and model improvement ratios (MIR) for the full and final, four variable (in boldface) models determined from randomForest analysis of the relationship between admixture values (q_{wsct}) between westslope cutthroat trout and rainbow trout and stream variables for 58 localities, in southwestern Alberta.

Variable	I_p-full	MIR-full	I_p-final	MIR-final
Mean depth	0.1378	0.1351	NA	NA
Maximum depth	0.1511	0.1482	NA	NA
Temperature	1.0294	1.0000	1.0730	1.000
Elevation	0.6672	0.6545	0.8848	0.8247
Stream order	0.0373	0.0365	NA	NA
RoadD	0.2567	0.2498	NA	NA
PipeD	0.2000	0.1964	NA	NA
PowerD	0.3364	0.3300	NA	NA
RailD	0.3762	0.3691	0.7697	0.7172
StockI	0.0833	0.0788	NA	NA
StockN	0.1751	0.1717	NA	NA
StockY	0.2130	0.2089	NA	NA
StockD	0.3297	0.3234	0.6121	0.6122

bootstrapped regression trees.

The correlation between observed admixture values and those predicted by the randomForest model was 0.61 ($\mathrm{df} = 58$, $P < 0.001$). The distributions of observed and predicted admixture levels mirrored each other reasonably closely, but the greatest deviations were observed at very low and very high q_{wsct} levels; about 21% of the samples had an observed q_{wsct} of > 0.99, but the RANDOMFOREST model predicted that the highest q_{wsct} was 0.96 (Fig. 2.2). Partial dependence plots indicated the effect of each retained predictor variable after averaging out the effects of all other predictor variables. The response of q_{wsct} to changes in water temperature suggested a step-like response as q_{wsct} decreased abruptly between about 11 °C and 13 °C (Fig. 2.3). Conversely, q_{wsct} increased steadily as elevations rose above 1300 m (Fig. 2.3). The q_{wsct} also tended to increase with increasing stockD, although somewhat more irregularly and leveled off after about 4000 m (Fig. 2.3). Finally, distance to the nearest railway (RailD) was the most irregular showing variable responses across different distances (Fig. 2.3).

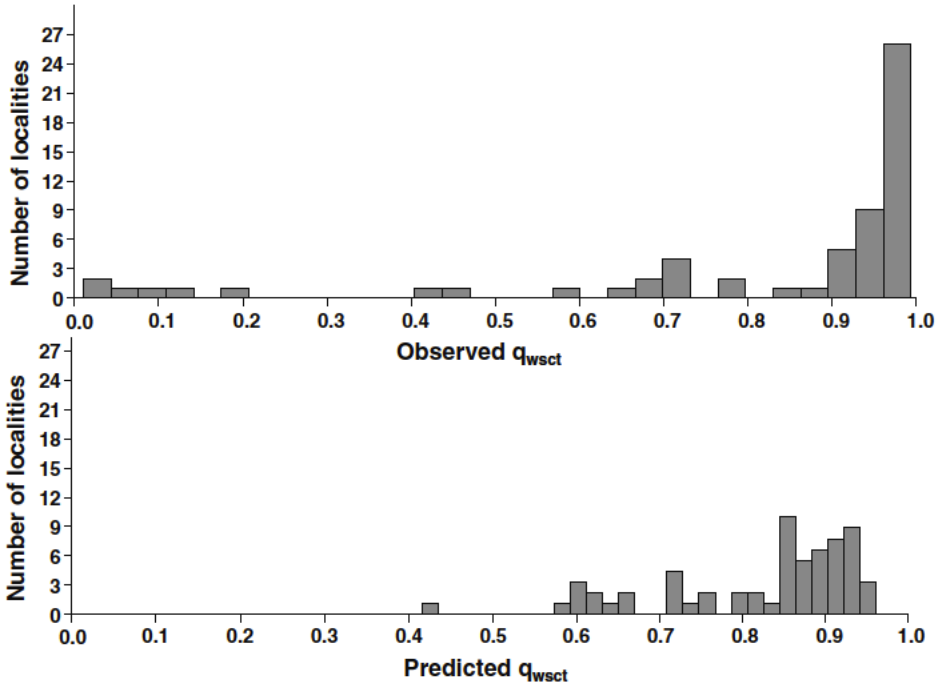

Figure 2.2: Observed (*upper*) and predicted (from RANDOMFOREST derived relationships, *lower*) admixture values (q_{wsct}, 0 = rainbow trout, 1 = westslope cutthroat trout) as a function of variation in water temperature, elevation, distance from sample site to stocking site and distance from sample site to the nearest railway line for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) sampled across 58 localities in southwestern Alberta, Canada, and assayed at 10 microsatellite DNA loci.

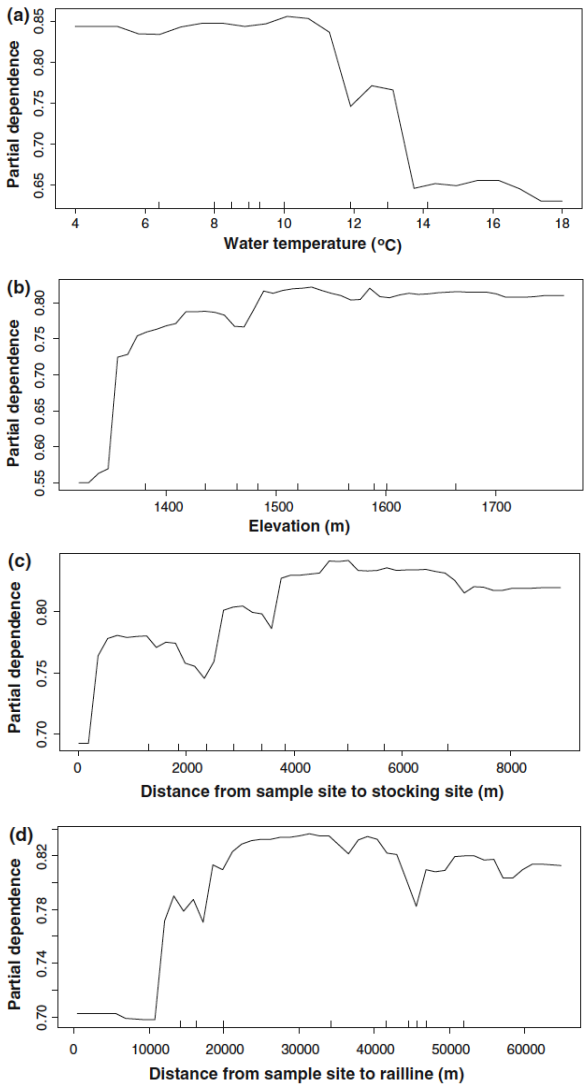

Figure 2.3: Partial dependence plots showing the relationship between a) water temperature, b) elevation, c) distance from sample site to stocking site, and d) distance from sample site to nearest railway line and admixture values (q_{wsct}, 0 = rainbow trout, 1 = westslope cutthroat trout) for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) sampled from 58 localities in southwestern Alberta, Canada, and assayed at 10 microsatellite DNA loci.

2.4 Discussion

Hybridization and introgression have become critical concerns in the conservation of biodiversity as genomic extinction and admixture have led to demographic declines (Rhymer and Simberloff 1996; Huxel 1999; Muhlfeld et al. 2009a). The study of admixture in cutthroat trout and other taxa has been widespread throughout its native range and dates back to the early 1980s (e.g., Leary et al. 1984; Allendorf and Leary 1988). All together, it has been estimated that genetically non-admixed WSCT may exist in less than 10% of the total historical native range (Trotter 2008). In western Alberta, WSCT and RT hybrids had been first recognized, morphologically, as early as 1947 and were considered widespread by 1950 (Mayhood and Taylor 2011). It is now estimated that fewer than 10% of populations of WSCT in western Alberta have no detectable introgression with RT and that many of these populations are relatively isolated in headwater reaches (Mayhood and Taylor 2011). The perilous state of these populations over the medium to long term was a major factor leading to their assessment as 'threatened' by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC 2006) and later listing under the federal *Species at Risk Act* (SARA). By contrast, there have been very few studies that have sought to understand the fate of hybrids or general explanations for spatial variation in admixture between native WSCT and introduced RT. Some laboratory-based (Leary et al. 1984; Ferguson et al. 1985) and, more recently, field-based studies (Muhlfeld et al. 2009a; Rasmussen et al. 2012) have shed light on the performance and fate of hybrids and in some cases have demonstrated that even small amounts of admixture from RT can influence physiology or reduce survival of hybrid offspring. The demonstration of real fitness costs to admixture stresses the importance of understanding what influences spatial variation in admixture in nature. Understanding the factors that explain this variation can aid our understanding of the biology of the hybridizing taxa, if and how admixture spreads, and what strategies might be best to deal with admixture.

2.4.1 Spatial variation in admixture

My survey indicated that while some localities showed considerable admixture with RT (i.e., $q_{wsct} < 0.75$), most areas showed q_{wsct} levels of between 0.85 and 0.99. I am unaware of any study of admixture between RT and WSCT trout that is as geographically extensive as the present study, but even on smaller geographic scales a similar pattern of spatially variable ad-

mixture is usually found. For instance, Hitt et al. (2003) documented a few heavily admixed populations (i.e., > 85% genetic contribution from RT) and a majority of localities with 1-27% admixture from RT across 42 samples from the upper Flathead River drainage of Montana. Such variation might be expected given differences in various aspects of introduction intensity and history across areas. For instance, both Rubidge et al. (2001) and Hitt et al. (2003) reported that admixture with RT decreased upstream from sources of introduction of RT. By contrast, Bettles et al. (2005) and Heath et al. (2010) reported extensive, but less structured spatial variation in the extent of natural admixture between native populations of rainbow trout and coastal cutthroat trout (*O. c. clarkii*). The finding that spatial variation in admixture does not appear to be governed simply by idiosyncrasies of specific artificial introduction programs suggests that local conditions also appear to play an important role (Aldridge and Campbell 2008). Regardless of the geographic pattern of variation in admixture levels, my results demonstrate that admixture with RT is widespread in southwestern Alberta. There has been no comparably extensive survey in British Columbia (BC), but surveys by Rubidge et al. (2001), Taylor et al. (2003) and Rubidge and Taylor (2005) indicate that many geographic localities in the upper Kootenay River system in BC are similarly significantly admixed.

2.4.2 Environmental correlates of admixture

The landscape genetic approach to studies of the spatial distribution of genetic variability (e.g., Manel et al. 2003; Van Houdt et al. 2005; Holderegger and Wagner 2006; Tamkee et al. 2010) can also clearly apply to understanding how admixture between species is distributed and spreads across a landscape. My analyses were conducted over a series of watersheds and thus add generality to the observations made in smaller scale studies across western North America suggesting that a consistent set of physical factors influence the extent of admixture between WSCT and RT (e.g., Weigel et al. 2003; Rubidge and Taylor 2005; Muhlfeld et al. 2009b). Specifically, water temperature and elevation are consistently associated with admixture levels; admixture between the species is consistently lowest in high elevation, low order and cooler streams. In particular, all three of these variables tend to be intercorrelated, but water temperature was the most important factor associated with admixture in my regression tree models. The influence of water temperature observed in my study is consistent with previous studies of hybridization in WSCT and other species of salmonids that typically report a gradient of admixture with genetically pure, native trout at the highest

elevations, increasing levels of admixture downstream and pure genotypes of the introduced species in the lowest reaches (Larson and Moore 1985; Paul and Post 2001; Hitt et al. 2003; Rubidge and Taylor 2005; Muhlfeld et al. 2009c; Rasmussen et al. 2010, 2012). Westslope cutthroat trout and rainbow trout exhibit very similar optimal temperatures (13.6 °C and 13.1 °C, respectively), at least as inferred by limited laboratory study, but RT displayed better growth at higher water temperatures and a broader range of growth at warmer temperatures (Bear et al. 2007). Rainbow trout may also be better adapted to warmer temperatures as suggested by having an upper incipient lethal temperature that is 4.7 °C greater than in WSCT (Bear et al. 2007). These trends in the elevational distribution of admixture and observations of different thermal characteristics of hybridizing species have led to the development of the 'elevation refuge hypothesis' (Fausch 1989; Paul and Post 2001; Rasmussen et al. 2010). Here, habitats at high elevations may provide a refuge for cold water-adapted, native trout, at temperatures that introduced species are unable to exploit as effectively.

A number of laboratory studies support this hypothesis, demonstrating a shift in growth rates and survival as water temperatures change (e.g., Reese and Harvey 2002; McMahon et al. 2007). Recent work in bull trout (*Salvelinus confluentus*) and brook trout (*S. fontinalis*) showed a competitive advantage of introduced brook trout at warm temperatures reared in sympatry with bull trout (McMahon et al. 2007), but no significant differences were observed when these species were grown in allopatry. Further, Rasmussen et al. (2010, 2012) found significant associations among admixture gradient, elevation, and difference in life history characters and metabolism between RT and WSCT and hybrids in the upper Oldman River, Alberta. Westslope cutthroat trout with low levels of admixture with RT tended to predominate in headwater reaches of the upper river and were also older and grew more slowly than hybrids and pure RT which tended to predominate in lower elevation and warmer reaches. Hybrids were found in mid-elevation reaches and exhibited intermediate metabolic rates that seem well-suited to such ecotonal habitats (Rasmussen et al. 2012).

Weigel et al. (2003) proposed one mechanism by which cold summer temperatures may impede the establishment of RT alleles. Cold summer temperatures can delay egg production and prolong incubation of early spring spawning RT compared to WSCT that spawn later in the season (Hubert et al. 1994; Stonecypher et al. 1994). Such a developmental delay may act to compound a decrease in growth rate at cooler water temperatures and also reduce overwinter survival of RT and hybrid fry. Indeed, Culumber et al. (2012) presented evidence that physiological adaptation to different ther-

mal niches could explain, in large part, the elevational pattern of genotypic distribution of two species of swordtails (*Xiphophorus*) and their hybrids in southeastern Mexico.

My analysis suggested, from the partial dependence plots, that the effect of water temperature was non linear and that a temperature of between 11-13 °C might represent a threshold at which the likelihood of admixture changes abruptly. This temperature range as a specific threshold must clearly be interpreted with caution because my temperature sampling was superficial and denser sampling, spatially and temporally, must be completed to see if such a threshold has any veracity. Interestingly, however, Rasmussen et al. (2010) proposed that a temperature threshold of about 7.3 °C marks a point at or below which admixture was low to absent within one tributary of the Oldman River system, Alberta. Of course, even with such more intensive sampling of water temperature, Rasmussen et al. (2010) cautioned that its extension to other streams was problematic given the many features that can vary between streams and influence admixture (e.g., barriers). Further, there is evidence that stream bed temperatures can vary significantly across a stream channel within a site (Webb et al. 2008) and that small temperature changes of 1 °C can alter salmonid distributions (Fausch et al. 1994). If these temperature thresholds to admixture exist, recent advances in the ability to accurately measure stream and river temperatures (Webb et al. 2008) can perhaps help to predict responses to environmental changes (e.g., long-term climate change, land use changes) that can influence stream water temperatures in terms of changes in the levels of genetic admixture, its geographic distribution, and the speed at which such changes might occur (cf. Rasmussen et al. 2010; Isaak et al. 2012). There is some evidence of spatial segregation of RT and WSCT and differences in growth potential as a function of water temperature (e.g., Bozek and Hubert 1992; Sloat et al. 2005; Bear et al. 2007), but McHugh and Budy (2005) reviewed several studies and suggested that, overall, condition (temperature)-dependent competition is an unlikely explanation for all salmonid zonation patterns. A better understanding of the physiological performance of these species and their hybrids across multiple life stages, particularly at low water temperatures is needed and I address this need in the next chapter of my thesis.

My analyses also suggest that elevation plays a role in influencing admixture levels (cf. Weigel et al. 2003; Rasmussen et al. 2012). There is a clear negative association between water temperature and elevation (this study, see also Rasmussen et al. 2010; Culumber et al. 2012), but the partial dependence plots suggest an effect of elevation in excess of that which can be accounted for by its association with water temperature. Rainbow trout tend

to be more abundant in wider, lower elevation, lower gradient stream reaches (MacCrimmon 1971; Gard and Flittner 1974; Bozek and Hubert 1992; Paul and Post 2001). Henderson et al. (2000) and Muhlfeld et al. (2009b) found that upstream-downstream segregation in WSCT and RT, respectively, may also include spawning habitats, but that they also overlapped in some tributaries. Given that gradient increases and stream width generally declines as elevation increases, the physically smaller and more energetic higher elevation habitats may be less preferred by RT regardless of water temperature and reduce the incidence of interactions and hybridization with WSCT. In addition, given that stocking of RT was characteristically in the lower portions of my study streams, the negative association between admixture level and elevation (and its association with water temperature) may result from non-equilibrium conditions in which invasive RT and admixture are still in the process of extending in upstream directions (Rubidge et al. 2001; Hitt et al. 2003; Boyer et al. 2008; Muhlfeld et al. 2009c). My data can serve as a useful baseline to monitor changes in admixture with time and/or in response to habitat disturbances such as fires or floods (e.g., Isaak et al. 2012).

2.4.3 Anthropogenic correlates of admixture

A number of anthropogenic factors related to stocking operations and environmental disturbance were found to be related to admixture with RT amongst my samples. For instance, the level of admixture has been often observed to decline with distance from the point(s) of stocking (Rubidge et al. 2001; Hitt et al. 2003; Rubidge and Taylor 2005; Muhlfeld et al. 2009c). My results are in agreement with these findings and emerged despite the likelihood that all such comparisons suffer to varying degrees from incomplete stocking histories, undocumented movement of trout by the public, failure to establish spawning populations, and migration of stocked fish from sampling location (Moring 1993; Weigel et al. 2003). I expected that the number of fish stocked and stocking intensity would increase propagule pressure of RT and be associated with increasing admixture levels as has been reported in other salmonid systems (Lockwood et al. 2009; Muhlfeld et al. 2009b; Marie et al. 2010, 2012). I also expected an increase in number of years elapsed since the last stocking and increasing isolation (a combination of stream distance and presence of upstream migration barriers) from the stocking site would be associated with low levels of admixture (e.g., Rubidge et al. 2001; Ruzzante et al. 2004). Only the latter variable, however, was identified as an important variable in the RANDOMFOREST analysis.

Two streams, Loomis Creek in the Bow River and South Castle Creek in the Oldman River had unusually high numbers of fish stocked and stocking intensities. These streams had between five and 30 times as many fish stocked (up to 3 million in South Castle Creek) and between six and 10 times the stocking intensity (up to 64 years in South Castle Creek) as the average values for all other localities (Appendix A Table. A.2). Despite such high stocking levels, these two streams had levels of admixture with RT that were negligible in Loomis Creek ($q_{wsct} = 0.97$) to about the average ($q_{wsct} = 0.88$) in South Castle Creek ($q_{wsct} = 0.87$). Loomis Creek had one of the coolest water temperatures (7 °C which was in the lowest 15th percentile of all streams) and was almost 5 km upstream of the stocking site which was below a migration barrier and in a different tributary. Further, the water temperature of South Castle Creek (9.2 °C) was below the average value of 10 °C for all streams. Thus if the spot water temperatures that I used do accurately reflect cooler than average conditions in these streams, this factor combined with the location of stocking sites in terms of distance and in relation to migration barriers, and other aspects of habitat that may be unsuitable for RT may help to explain the relatively low admixture levels despite high levels of stocking of RT in some systems. More generally, these results suggest that the numbers and intensity of stocking interact with local environmental conditions to influence admixture levels (Weigel et al. 2003; Taylor et al. 2007; Muhlfeld et al. 2009c). Certainly, other cases of intraspecific salmonid supplementation programs have illustrated the idiosyncratic nature of the outcome of stocking that is dependent on more than just the numbers and intensity of non-native fish stocked (e.g., Krueger and Menzel 1979; Largiadèr and Scholl 1995; Taylor et al. 2007; Halbisen and Wilson 2009; Marie et al. 2012). My analysis reinforces the importance of connectivity between streams (as influenced by fluvial distance and migration barriers) as a critical factor influencing admixture in many situations (Rubidge et al. 2001; Gunnell et al. 2008; Muhlfeld et al. 2009c).

The effects of human disturbance factors on the spread of non-native species and hybridization in stream fishes may be direct, from increasing access to streams through road or railway construction, or indirect, from landscape developments that influence key features such as water temperature which themselves influence dispersal of non-native species and hybridization (e.g., Dunham et al. 2002; McMahon et al. 2007; Heath et al. 2010). For instance, Muhlfeld et al. (2009c) found that the number of upstream road crossings was positively correlated with levels of admixture between native WSCT and invasive RT in the upper Flathead River in British Columbia and Montana. In my study systems, only the distance to the nearest rail-

way appeared to influence admixture levels, but in an erratic manner. The apparent lack of an influence of roads, pipelines, and powerlines should be interpreted cautiously as they may not have represented measures of human disturbance per se particularly well. For instance, Muhlfeld et al. (2009c) found that road density did not have a significant effect in their study, but that number of upstream road crossings did. In this case, the latter variable is perhaps a more direct (and hence more sensitive) measure of potential habitat disturbance for streams. Similarly, my measures of disturbance were expressed only as distance to the nearest anthropogenic structure and did not incorporate density nor actual crossings. Interestingly, railways were historically the source of much stocking pressure (Mayhood 1999), but my analysis revealed a complex interaction between distance to the nearest railway (RailD) and admixture. This result may stem from the highly bimodal distribution of RailD values I obtained; one mode was found at about 16 km and the other at about 46 km (Appendix A Table. A.2). Still, I could not resolve any consistent directionality to the influence RailD and admixture. Finally, other human disturbance factors such as logging activity, recreational land use including angling activity or natural factors such as seasonal flood dynamics, and their interactions, may play roles in admixture and should be investigated (e.g., Fausch et al. 2001; Heath et al. 2010).

In summary, the analysis of variation in admixture levels between WSCT and RT in western Alberta further emphasize what appears to be a basic spatial pattern; admixture levels tend to be low in cooler, high order, high elevation streams (cf. Paul and Post 2001; Rubidge et al. 2001; Weigel et al. 2003; Rubidge and Taylor 2005; Muhlfeld et al. 2009b). This pattern has led to the temperature/elevation refuge hypothesis that suggests that native population of salmonid fishes in mountainous areas may be less susceptible to invasion of non-natives if native fishes have a physiological or behavioural advantage over non-native and hybrids at cooler water temperatures (Paul and Post 2001; McMahon et al. 2007; Rasmussen et al. 2010). In addition, the characterization of WSCT and RT as relatively cool-water and warm-water adapted, respectively, may be considered consistent with their evolutionary and biogeographic history given the concentration of the former in high elevation areas of the Rocky Mountains and adjacent mountain ranges. Still, the relative performance of either species at low water temperatures, a key component of the temperature refuge hypothesis, has been little explored, as has the potential role of thermal adaptation in speciation in fishes (cf. Culumber et al. 2012; Keller and Seehausen 2012). My results and those of others (e.g., Rubidge et al. 2001; Fausch 2008; Muhlfeld

et al. 2009b; Marie et al. 2012) clearly indicate, however, that factors other than water temperature (e.g., habitat area, stocking practices, migration barriers) can also influence admixture levels and that the influence of human disturbance factors (resource extraction, road and pipeline density and routes) will often be contingent on the local regulatory regime.

2.4.4 Implications for recovery planning

My comprehensive survey of Alberta populations provides insights into the interaction between hybridizing species and their environment that are complementary to previous, more localized studies, and can focus future research to assist with recovery planning for threatened WSCT (COSEWIC 2006). For instance, my results can help to prioritize populations for conservation during the assessment of recovery potential. Populations with little to no detectable admixture may be the highest priority for conservation and perhaps as sources of fish for recovery in more affected streams (Muhlfeld et al. 2009c; Mayhood and Taylor 2011). Second, the RANDOMFOREST analysis performed reasonably well in terms of predicting admixtures levels in bootstrapped samples and could used as an initial "triage" procedure to choose amongst a series of localities with unknown admixture levels as to which may be more or less likely to exhibit admixture with RT. The use of this model would, however, prove conservative because it was unable to accurately predict q_{wsct} values of > 0.96. This would be problematic for correctly identifying genetically non-admixed populations of WSCT under a proposed threshold value of $q_{wsct} > 0.99$ (Allendorf et al. 2004). Finally, more intensive sampling of stream water temperatures through time could help better assess the role of low water temperature on relative performance of genotypes and its effects on reproductive isolation and hybridization between species. My results also support the idea that higher order, lower elevation, and warmer streams appear to be favourable environments for hybridization and provide sources for the spread of admixture between WSCT and RT. In addition, physical stream characteristics, stocking practices, anthropogenic habitat modifications, and the local regulatory regime (e.g., within or outside protected areas) all interact to influence admixture levels in salmonid fishes in diverse ways which emphasizes the need for context-specific solutions.

The growing body of work both on intraspecific and interspecific hybridization in salmonid fishes (e.g., Rubidge and Taylor 2005; Halbisen and Wilson 2009; Muhlfeld et al. 2009b; Heath et al. 2010; Marie et al. 2012) suggests that admixture levels are somewhat predictable although much of

the variation remains unexplained. Even some small understanding of what environmental or human factors influence the probability and extent of admixture should improve conservation efforts for native fishes. For instance, managers could be alerted to situations where stocking should not occur, e.g., in streams with conditions that might favour non-natives and/or hybrids especially if such areas show high levels of interconnectivity with other streams) or, if stocking does occur or did occur in the past, its likely consequences to native fish gene pools (e.g., relative probability of admixture). Finally, understanding what influences admixture can help suggest potential remedial actions, e.g., streams that have marginal conditions for non-natives or that were stocked only lightly may present the best cases for recovery of native fishes.

Chapter 3

Cold tolerance limits hybridization between westslope cutthroat trout and rainbow trout

3.1 Introduction

Suitable conditions for survival play a large role in defining a species' distribution in nature. These conditions include biotic interactions that are mediated by abiotic factors that vary across a landscape (Baltz et al. 1982; Rahel 1984; Sih 1987; Dunson and Travis 1991; Warner et al. 1993; De Staso and Rahel 1994). In freshwater fishes for example, zonal patterns of species dominance are often observed in rivers and streams that flow along an elevational gradient (e.g., Paul and Post 2001). Factors that vary with elevation such as water temperature, water velocity and substrate, alter the abiotic conditions to a state that can favor one species over another up or downstream (Vannote et al. 1980; Rahel 1984; Fausch et al. 1994; Taniguchi et al. 1998). Water temperature in particular, appears to shape the distribution of salmonid fishes, where small changes of only 1 °C have been linked to the presence or absence of certain species (Fausch et al. 1994).

The temperature of the water alters metabolism and behaviour in ectothermic fishes, which in turn affects their competitive ability (Taniguchi et al. 1998; Selong et al. 2001; Bear et al. 2007). A common distribution pattern in habitats that have been stocked with non-native salmonids is a restriction of the native species to high elevation headwaters and tributaries, followed by interspecific hybrids and complete replacement by the introduced species downstream (Rahel and Hubert 1991; Fausch et al. 1994; Hitt et al. 2003; Weigel et al. 2003; Rubidge and Taylor 2005; Rasmussen et al. 2010). Cold waters at high elevations appear to provide a refuge for the native species allowing them to persist despite the absence of physical

barriers that would stop the movement of introduced trout and hybrids upstream (Paul and Post 2001; Weigel et al. 2003; Rubidge and Taylor 2005; Rasmussen et al. 2010). According to the 'elevation refuge hypothesis' first introduced by Paul and Post (2001), cold temperatures impart a competitive advantage to native trout at high elevation and the reverse is true at low elevations where introduced taxa typically dominate. Hybrids, if viable, exhibit intermediate behaviours and are found at intermediate habitats (Rasmussen et al. 2010).

Laboratory and controlled field studies have yielded mixed results in support of the elevation refuge hypothesis (see review in McHugh and Budy 2005). Some studies, such as that carried out by De Staso and Rahel (1994) on Colorado River cutthroat trout (*Oncorhynchus clarkii pleuriticus*) and brook trout (*Salvelinus fontinalis*), supported a shift in competitive ability as water temperature changed. By contrast, work by McMahon et al. (2007) failed to detect a reversal in competitive advantage favouring the native species at cold temperatures. McHugh and Budy (2005) were also unable to demonstrate that Bonneville cutthroat trout (*O. c. utah*) were more successful than exotic brown trout (*Salmo trutta*) at field sites located at high elevation. In cases where a reversal in competitive ability is not observed, McHugh and Budy (2005) argued that physiological limitations exerted a stronger influence at cold temperatures. Based on their data, the authors suggested that interspecific competition was likely driving species dominance at low elevation and that non-native fishes would displace indigenous taxa upstream to a point where their metabolic needs could no longer be met (McHugh and Budy 2005).

The elevation refuge hypothesis has been used to describe the distribution of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WSCT), which are currently threatened by hybridization with introduced rainbow trout (*O. mykiss*; RT) (Rasmussen et al. 2010). Westslope cutthroat trout inhabit approximately 20% of their historical range in southwestern Alberta (COSEWIC 2006). Fragmented populations of genetically pure WSCT are at risk due to extensive stocking of hatchery RT to enhance recreational fisheries (Allendorf and Leary 1988). Introduced RT have become naturalized in some locales and will hybridize with WSCT. The hybrids are able to reproduce and typically form a hybrid swarm at mid-elevation habitats. Although the two parental taxa have virtually identical optimal growth temperatures (WSCT: 13.6 °C; RT: 13.1 °C, Bear et al. 2007), studies by Paul and Post (2001) and Hitt et al. (2003) have found that RT tend to disperse to low elevations and warmer waters regardless of the elevation at which they were introduced. Observational differences in upper thermal tolerance

have also been established between these species, suggesting they occupy different, but overlapping thermal regimes (Bear et al. 2007). Genetically pure WSCT require cool, clear water and continue to persist only in the upper headwaters of their range, suggesting that the elevation refuge hypothesis may be applicable to their interactions with introduced salmonids (Paul and Post 2001; Rasmussen et al. 2010).

The inability of work by McMahon et al. (2007) to demonstrate a competitive advantage for the native taxa at high elevations has lead to further exploration of the theory put forth by McHugh and Budy (2005). Research by Rasmussen et al. (2010) identified a life history gradient associated with the clinal distribution of RT alleles. Fish with RT characters tended to grow more quickly and mature faster than genetically pure WSCT. Habitats at low elevations are more productive and appear to be better suited to support the energetic demands of RT. Cold-water habitats, being less productive, are able to support slow-growing WSCT. Rasmussen et al. (2010) suggested that a tradeoff exists between metabolic scope (RT) and growth efficiency (WSCT) within the system. In a more recent study (Rasmussen et al. 2012), differences in metabolism were evaluated by looking at rates of oxygen consumption, and the activity of lactate dehydrogenase and citrate synthase. The authors concluded that fish with RT alleles tended to have higher metabolic demands than fish comprised primarily of WSCT background. Hybrids generally had intermediate metabolic traits and were better able to balance the tradeoff between energetic scope and growth efficiency, allowing them to be successful further upstream than RT. That non-admixed WSCT persist in high elevation tributaries, likely reflects the physiological and metabolic limitations of RT and interspecific hybrids (Rasmussen et al. 2010, 2012).

Understanding the role of thermal limits can help pinpoint species level differences that shape the zonal distribution observed in so many studies. Despite having similar optimal growth temperatures, RT can grow over a broader range of temperatures and exhibit an upper lethal temperature 4.7 °C higher than WSCT (Bear et al. 2007). As suggested by McHugh and Budy (2005), competition is more likely to explain species dominance at low elevations that reach maximum temperatures significantly lower than physiologically defined, upper lethal limits. McHugh and Budy (2005) also highlighted a need to better understand the physiological performance of both species and their hybrids at low thermal limits.

To date, the majority of studies on freshwater fish have focused on thermal maxima, the upper threshold of temperature tolerance (see review in Beitinger et al. 2000). Typically, these studies assay the critical maximum

temperature (CTMax) defined as the maximum temperature at which a fish can maintain a normal, upright swimming position ("equilibrium"). The minimum temperature at which a fish can maintain such equilibrium is termed CTMin and has been much less commonly studied (but see Barrett et al. 2011; Darveau et al. 2012). For a comparison, upper thermal limits have been estimated in 72 studies for 103 different freshwater fish species and only fifteen studies report a lower limit across 29 species (reviewed by Beitinger et al. 2000). This disparity exists for several reasons. Maximum limits tend to be easier to measure and interspecific variability at thermal maxima is much greater. Endpoints for thermal minima can be harder to define and tend to be near 0 °C for many freshwater species. There is also a heightened interest in exploring upper thermal limits with the onset of global warming and increases in thermal stress, where fish are more likely to experience temperatures much higher than their evolutionary past (Beitinger et al. 2000).

Understanding the underlying mechanisms driving the distribution of introduced species, native taxa, and their hybrids is integral to proper recovery management. Introgressive hybridization threatens WSCT across the entirety of its historical range (Allendorf and Leary 1988; Shepard et al. 2005; COSEWIC 2006; Trotter 2008). High elevation isolates of WSCT, represent few remaining strongholds for these coldwater species, that at one time, had much more extensive distributions (Mayhood and Taylor 2011). In this chapter, I measure CTMin for WSCT and RT to test whether cold temperatures present a greater physiological constraint for RT. This may explain, in part, why rainbow trout and hybrids tend to be distributed in warmer waters at low elevations. This study presents a "first-step" in exploring the physiological limits of cold tolerance in WSCT, divergent populations of RT and their hybrids.

3.2 Materials and Methods

3.2.1 Trout populations

Westslope cutthroat trout used in this study were obtained from a source population in Connor Lake, British Columbia (Fig. 3.1). Fry were reared with the help of the Kootenay Hatchery in Cranbrook, BC, before transfer to the UBC Aquatics Facility in April 2012. Fish from this brood stock have been used in all WSCT stocking events in BC over the last three decades and represent native WSCT genotypes.

Rainbow trout from Blackwater River (BW) and Tzenzaicut Lake (TZ),

Figure 3.1: Map of source locations for westslope cutthroat trout (*Oncorhynchus clarkii lewisi*, Connor Lake) and rainbow trout (*O. mykiss*, Blackwater River (BW) and Tzenzaicut Lake (TZ)) used in critical thermal minima trials.

British Columbia, were spawned with the assistance of the Freshwater Fisheries Society of British Columbia (Fig. 3.1). These wild populations were chosen based on ease of access and divergence in specific characters. For example, BW rainbow trout are characterized by fast-growth and recommended for stocking in competitive habitats, while TZ rainbow trout are suited to colder habitats with low productivity and survival is significantly better in stocked TZ fish than BW fish (Clarke et al. 2008; Northrup and Godin 2009). Based on these characteristics, the TZ fish appear to be more similar to WSCT (i.e. slow growing, utilize habitats with low productivity), while BW fish possess attributes typical of the RT described in studies of hybridized populations (i.e. fast-growth, poor survival and highly competitive) (Rasmussen et al. 2010). The availability of sexually mature F_1 hybrids between BW and TZ, presented an opportunity to assess interpopulation and interpopulation hybrid differences. I evaluated cold tolerance in the F_2 generation and backcrosses to each parental population (Blackwater backcross, BWB; Tzenzaicut backcross, TZB) in an attempt to assess how higher-order hybrids between a WSCT-like trout (TZ) and an "average" RT (BW) perform in CTMin. Although these are not true WSCT x RT hybrids and backcrosses, they were used here to illustrate possible outcomes had WSCT x RT hybrids been obtainable for this study.

Here, I evaluated the critical thermal minima (CTMin) for 431 trout representing groups of WSCT, RT, F_2 hybrids of the RT populations and hybrid backcrosses to each parental RT population acclimated to 15 °C and 18 °C.

3.2.2 Rearing set-up

Fish were kept in an environmental chamber (manufactured by Environmental Growth Chambers) in the Biological Sciences Building at the UBC campus. Constant conditions were maintained at 12:12 L:D, and an ambient temperature of 15 °C with water supply at 10 °C. Fertilized eggs were placed into baskets constructed from 2-inch, PVC pipe and mesh netting sealed with aquarium grade silicone. The baskets were housed in a vertical incubator, with a continuous flow of freshwater. Individual baskets were labeled by family. After approximately thirty days post-fertilization, the eggs "eyed-up" and following another twenty days, alevins (larvae) began to hatch. One week, post-hatch, the alevins were transferred to plastic, containers, submerged in a fiberglass, rearing trough. One and a half-inch holes were drilled in the sides of the container, covered in mesh netting, and sealed with aquarium grade silicone to allow for water flow. The alevins remained

in these containers until their yolk sacs absorbed and they were fed crushed trout chow (approximately 3 weeks post-hatch). Fry were then transferred to wooden troughs that had been painted and sealed with fiberglass coating. The troughs were housed in a three-level shelving unit and each trough had a one-inch, PVC, water wand that supplied a continuous flow of clean freshwater. The troughs drained to a common 1.5-inch pipe which flowed to a floor drain. Trout were fed 1.2 mm Biovita trout chow.

3.2.3 Acclimation

Each test group was acclimated for a minimum of two weeks at 15 °C or 18 °C. Fish were housed in four, 195 L, fiberglass tanks that were connected to a common sump. Both the acclimation tanks and CTMin apparatus were set-up in the same environmental chamber held at 5 °C, to control water cooling and eliminate temperature fluctuations during the trials. To achieve the appropriate acclimation temperature, Odyssey Heatpro aquarium heaters were placed in the common sump, which pumped water heated to 25 °C into each tank. A nozzle controlled the flow of this water such that only a trickle of the heated water would enter the tank at any given time. Fine control of the inflow of warm water, in combination with tank water being cooled by the ambient 5 °C air, allowed me to obtain a constant acclimation temperature of 15 °C or 18 °C.

The acclimation tanks were initially set-up as a recirculating system, but were later modified to a flow-through system when a fungus killed 60% of the WSCT individuals acclimating to 18 °C. No mortalities were observed in the WSCT acclimating to 15 °C or any of the RT test groups at either acclimation temperature. These groups showed no physical signs of the fungal infection even though some were exposed to the same water as the infected WSCT in the recirculating set-up. I believe the development of fungus was brought on by heat-stress experienced by WSCT while acclimating to 18 °C (see discussion). The spread of the infection was curbed by dropping the water temperature to 10 °C and allowing fish to stabilize for one week. A header tank was added so that cool, clean water could continuously be added to the system, and the tanks were gradually brought back up to the acclimation temperatures. After the addition of the header tank and conversion to a flow-through system, WSCT acclimating to 18 °C did not show any signs of fungal infection and were able to complete the acclimation procedure. During acclimation, fish were fed 1.2 mm Biovita trout chow every day.

3.2.4 Critical thermal minima determination

CTMin experiments on trout test groups were carried out at different times over an eight month period in 2013. This was because some groups had different growth rates and I wanted to minimize the size variation amongst fish used in the study. WSCT grew much slower than RT and even at the time of trial, were smaller than the RT being tested. The two week acclimation period and number of acclimation tanks available also contributed the the duration of the study. In addition, unforeseen, technical issues, (i.e. chamber compressor failing) also caused trials to suspend for a short period of time adding to the length of the test period.

The experimental apparatus consisted of a single, large antifreeze bath in which twelve, individual containers were floated and held in place by a large Styrofoam sheet, fitted with holes to hold each container. A large, 20-gallon, Rubbermaid container was placed on a table and elevated with cement blocks above the antifreeze bath. A flap was cut into the lid of this container to facilitate the addition of dry ice to the system, allow CO_2 to escape, and to prevent uncontrolled bubbling and splatter. The container was outfitted with a brass nozzle to control the flow of cooled antifreeze entering the antifreeze bath. Below the antifreeze bath, an identical Rubbermaid container collected the antifreeze that had already flowed through the apparatus. A Little Giant, 115V-60Hz, pump in this container pumped the used antifreeze back up to the elevated Rubbermaid container to be cooled again and re-circulated through the system during the trial.

Before the trial, 25 °C water, from the sump of the acclimation set-up, was added to the twelve containers in the antifreeze bath. Twelve fish were collected from the acclimation tanks and transferred into a bucket. A single fish was placed into each of the twelve containers once the water cooled to meet the acclimation temperature. The fish were given fifteen minutes to acclimate to the test container, during which dry ice was added to the elevated Rubbermaid container. After the fifteen minute acclimation, the nozzle of the Rubbermaid container was opened and the cooled antifreeze began to flow into the large bath. The pump in the collection container was turned on to circulate the antifreeze through the set-up. Each of the twelve containers within the antifreeze bath was connected to a digital thermometer and had an air stone, to ensure the water was saturated with oxygen and to allow for homogenous cooling. The rate of cooling was monitored using the digital thermometers and kept at a rate of -0.3 °C per minute, by the addition of dry ice to the system. The temperature at which a fish lost equilibrium was recorded as its critical thermal minima (CTMin). Once all

twelve fish lost equilibrium, the trial ended and the fish were euthanized in MS222, weighed and measured. The fish were then labeled and preserved in 95% ethanol for future potential DNA analysis. For each acclimation temperature, three CTMin trials of 12 fish were carried out for a total of 36 individuals per test group.

3.2.5 Statistical analysis

Cold tolerance data were analyzed first using simple linear regression (SLR) to test for effects of length (cm) and mass (g) on CTMin at both acclimation temperatures. Differences in means of the test groups were analyzed using ANOVA on the data collected at 15 °C acclimation (SLR yielded no effect of length or mass) and ANCOVA on the 18 °C dataset incorporating length and mass as covariates. Values corrected for differences in length and mass did not differ from actual values by more than 0.1 to 0.2 °C (see below).

3.3 Results

3.3.1 Effect of acclimation temperature

Critical thermal minima increased with acclimation temperature in all test groups (i.e., the temperature at which fish lost equilibrium increased with acclimation temperature). Simple linear regressions of CTMin on acclimation temperature were significant in both RT and WSCT test groups (RT: $r^2 = 0.059; p < 0.001$; WSCT: $r^2 = 0.256, p < 0.001$; Fig. 3.2). Significant differences in CTMin within each test group were observed in BWB, WSCT, F_2 and TZ at 15 °C and 18 °C acclimation (Fig. 3.2). Regression analysis revealed that RT experience a 0.16 °C increase in CTMin for every 1 °C increase in acclimation temperature. The rate of change was nearly double in WSCT, 0.29 °C increase in CTMin with every 1 °C increase in acclimation temperature.

3.3.2 Effect of body size

There were significant differences in fork length (cm) and mass (g) amongst test groups (ANOVA length: $p < 0.001$, mass: $p < 0.001$). Rainbow trout were generally larger than WSCT (Table. 3.1). Simple linear regression revealed no significant effect of fork length and mass on CTMin at an acclimation temperature of 15 °C ($p = 0.729$). At 18 °C acclimation, however,

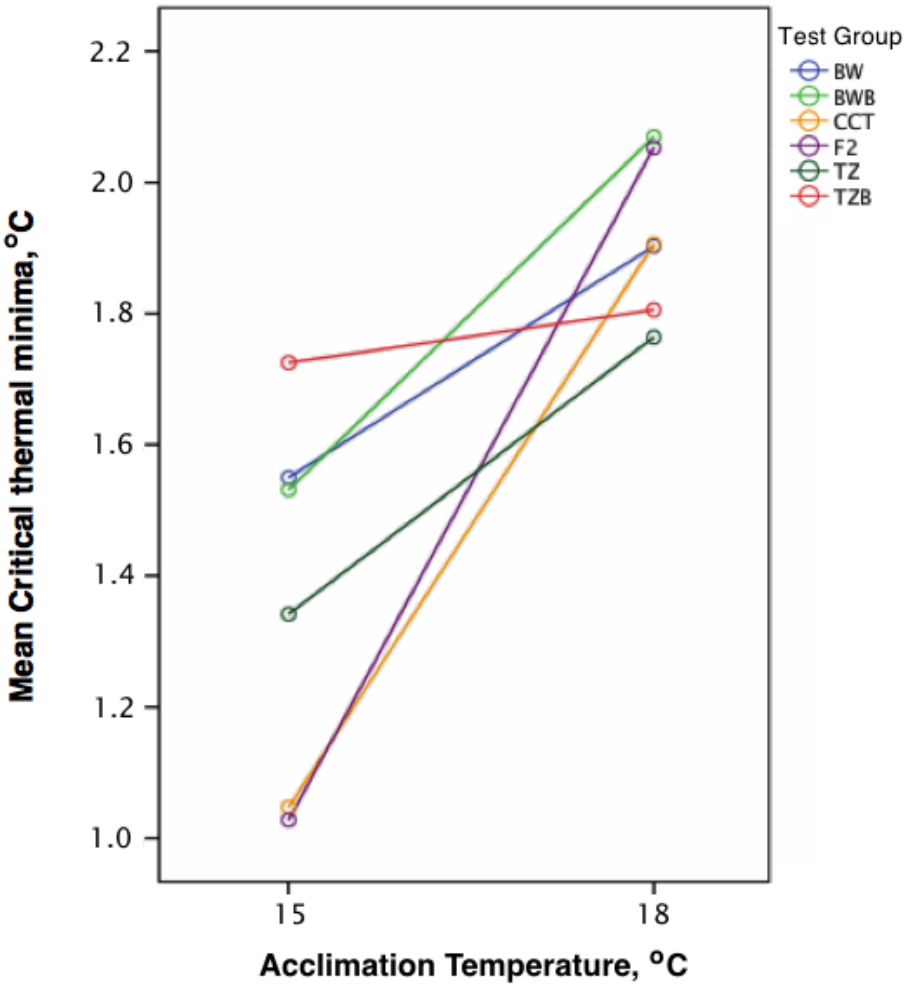

Figure 3.2: Simple linear regressions of CTMin on acclimation temperature for trout test groups. Blackwater River rainbow trout (BW; $r^2 = 0.034, p = 0.121$), Blackwater River backcross (BWB; $r^2 = 0.067, p = 0.030$), westslope cutthroat trout (WSCT; $r^2 = 0.256, p < 0.001$), Blackwater River x Tzenzaicut Lake F_1 hybrid x Blackwater River x Tzenzaicut Lake F_1 hybrid (F_2; $r^2 = 0.180, p < 0.001$), Tzenzaicut Lake rainbow trout (TZ; $r^2 = 0.053, p = 0.050$), Tzenzaicut Lake backcross (TZB; $r^2 = 0.003, p = 0.661$).

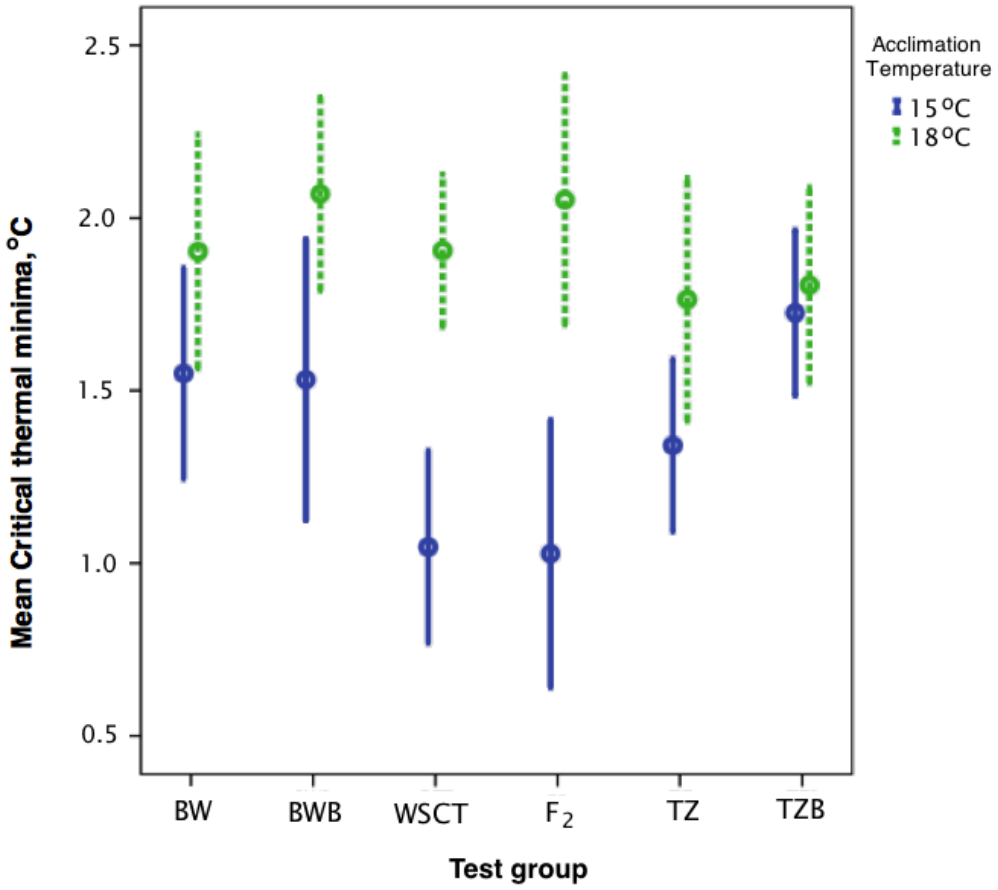

Figure 3.3: Critical thermal minima values for each trout test group at 15 °C (blue, solid line) and 18 °C (green, dashed line) acclimation with 95% confidence intervals. Blackwater River rainbow trout (BW), Blackwater River backcross (BWB), westslope cutthroat trout (WSCT), Blackwater River x Tzenzaicut Lake F_1 hybrid x Blackwater River x Tzenzaicut Lake F_1 hybrid (F_2), Tzenzaicut Lake rainbow trout (TZ), Tzenzaicut Lake backcross (TZB).

length and mass did exert a significant effect ($p = 0.028$). Analysis of covariance, controlling for the effect of fork length and mass, revealed differences in CTMin amongst test groups acclimated at 15 °C, but failed to detect any statistically significant differences for fish acclimated to 18 °C ($p = 0.006$ and $p = 0.110$, respectively; Table. 3.2). Size-corrected means generated from the ANCOVA for test fish acclimated to 15 °C did not differ from the actual values by more than 0.2 °C or 0.1 °C in 18 °C acclimated fish (Table. 3.1).

3.3.3 Interspecific differences

Rainbow trout had significantly higher CTMin than WSCT at 15 °C (1.4 °C and 1.0 °C, respectively, $p = 0.01$; Table. 3.1). At 18 °C, interspecific differences in critical thermal minima were not observed ($p = 0.929$; Table. 3.1). Here, mass seemed to be a better predictor of cold tolerance ($p = 0.022$), but only explained 3% of the variation in CTMin.

3.3.4 Intraspecific differences in rainbow trout

Average CTMin for RT test groups acclimated at 15 °C ranged from 1 °C for the F_2 fish to 1.7 °C for TZB (Table. 3.1). Differences between the test groups proved to be subtle, but statistically significant ($p = 0.006$; Table. 3.2). Levene's test of equality of error variances amongst test groups approached significance ($p = 0.085$), however, robust tests of equality of means for groups with unequal variances continued to yield significant differences between test groups (Welch $p = 0.003$; Brown-Forsythe $p = 0.007$). One-way ANOVA followed by a post-hoc test revealed that the only significant differences in CTMin amongst RT test groups were between the F_2 population and TZB (Tukey, $p = 0.02$; Fig. 3.3).

As predicted (see Materials and methods), BW fish had a higher average CTMin than TZ fish, although these differences were not statistically signficant (1.6 °C and 1.3 °C, respectively, Tukey, $p = 0.934$) and suggests that these results are not more than what would be obtained by chance.

At an acclimation temperature of 18 °C, CTMin across all test groups ranged from a low of 1.8 °C in Tzenzaicut Lake backcross (TZB) individuals, to 2.1 °C in Blackwater River backcross (BWB) (Table. 3.1). There were no statistically significant differences in means amongst the RT test groups acclimated at 18 °C when controlling for differences in fork length and mass (ANCOVA, $p = 0.647$; Table. 3.2).

Table 3.1: Critical thermal minima (CTMin), length, mass and CTMin corrected for length and mass of trout test groups acclimated at 15 °C and 18 °C. Values are mean ±s.d.; N is the number of fish; Rainbow trout (RT; all populations); westslope cutthroat trout (WSCT); RT populations: Blackwater River rainbow trout (BW); Blackwater River backcross (BWB); Blackwater River x Tzenzaicut Lake F_1 hybrid x Blackwater River x Tzenzaicut Lake F_1 hybrid (F_2); Tzenzaicut Lake rainbow trout (TZ); Tzenzaicut Lake backcross (TZB).

	15 °C Acclimation				
Test group	Fork length (cm)	Mass (g)	Mean CTMin (°C)	Size-corrected CTMin (°C)	N
RT	9.5±1.3	9.2±3.6	1.4±1.0	1.5	179
WSCT	7.3±0.8	4.0±1.7	1.0±0.8	0.8	36
BW	8.6±1.4	7.1±3.3	1.6±0.9	1.5	36
BWB	9.3±1.3	8.9±3.7	1.5±1.2	1.6	35
F2	9.9±1.2	9.7±3.3	1.0±1.2	1.1	36
TZ	10.2±1.1	10.5±3.2	1.3±0.7	1.5	36
TZB	9.6±1.0	10.0±3.4	1.7±0.7	1.8	36
	18 °C Acclimation				
RT	9.6±1.3	8.4±3.1	1.9±0.9	1.9	180
WSCT	7.4±0.8	4.7±1.9	1.9±0.7	2.0	36
BW	9.5±1.3	8.5±3.3	1.9±1.0	1.8	36
BWB	9.3±0.8	8.5±2.4	2.1±0.8	2.1	36
F2	9.8±0.9	9.5±2.8	2.0±1.0	2.0	36
TZ	10.0±1.0	9.5±3.0	1.8±0.8	1.7	36
TZB	9.6±0.8	9.6±2.2	1.9±0.9	1.9	36

Table 3.2: Summary statistics for analysis of covariance (ANCOVA) between critical thermal minima (CTMin) and body size in trout test groups at 15 °C and 18 °C acclimation using Type III sum of squares.

	15 °C Acclimation				
Source	Sum of Squares	df	Mean Square	F	Sig
Correct Model	17.667	7	2.524	2.905	0.006
Intercept	3.782	1	3.782	4.353	0.038
Length	0.408	1	0.408	0.470	0.494
Mass	0.013	1	0.013	0.015	0.903
Test group	17.079	5	3.416	3.932	0.002
Error	179.846	207	0.869		
Total	600.910	215			
Corrected Total	197.513	214			
	18 °C Acclimation				
Correct Model	9.678	7	1.383	1.701	0.110
Intercept	0.117	1	0.117	0.144	0.704
Length	3.775	1	3.775	4.644	0.032
Mass	5.757	1	5.757	7.082	0.008
Test Group	3.803	5	0.761	0.936	0.459
Error	169.082	208	0.813		
Total	972.260	216			
Corrected Total	178.760	215			

3.4 Discussion

The results of my critical thermal minima experiments reveal that there are subtle, but significant differences between species acclimated at 15 °C. Despite the variation in average CTMin amongst RT test groups, there was a statistically significant difference between average values obtained for WSCT and RT acclimated at 15 °C (1.0 °C and 1.4 °C, respectively). When acclimation temperature was increased to 18 °C, there were no significant interspecific differences observed (Table. 3.1). The loss of species level differences at high acclimation temperatures could be the result of heat stress experienced by WSCT during acclimation which may have inhibited their cold tolerance performance (see below).

The duration of the CTMin trials extended longer than anticipated and due to the length of time, raises the concern of differences in seasonal performance amongst test groups. Maintaining constant lab conditions during rearing and acclimation, as done in my study, should control for any effect of seasonality. In all cases, the timing of the three CTMin trials for each test group at 15 °C or 18 °C, were chosen based on the size of the individuals (to control for size variation amongst fish tested) and were never carried out in a single month, but spanned a minimum of two months. Further, significant differences in CTMin (such as that between WSCT and TZB) overlapped in the timeframe in which they were tested.

Despite sharing similar thermal optima, WSCT and RT appear to have significant differences in other aspects of their respective thermal regimes (Bear et al. 2007). Field studies have repeatedly described a gradient of species dominance that follows a gradient in elevation and water temperature when WSCT and RT exist in sympatry (Weigel et al. 2003; Hitt et al. 2003; Rubidge and Taylor 2005; Muhlfeld et al. 2009b; Rasmussen et al. 2010). In laboratory studies, interspecific differences exist when both species were tested at the upper extremes of their thermal scope (Bear et al. 2007). Rainbow trout grow over a broader range and continue to grow at temperatures beyond 20 °C, which are lethal to juvenile WSCT. When tested for upper lethal limits, RT can survive temperatures 4.7 °C above WSCT thresholds (Bear et al. 2007). My work, however, is the first to evaluate species differences between WSCT and RT at the lower limits of their thermal range.

As a whole, literature on thermal tolerance is biased towards experiments of upper thermal tolerance (UTT, Beitinger et al. 2000). This is likely because definitive endpoints are easier to observe, there is a greater degree of interspecific variation, and heat tolerance is more likely to limit species distribution in nature (discussed in Beitinger et al. 2000). I used

a non-lethal procedure that employs a gradual decline in water temperature (critical thermal methods; CTM). This method can be contrasted with incipient lethal temperature (ILT) or Fry method that evaluates thermal tolerance based on abrupt transfers to temperatures above or below acclimation until death is reported in 50% of the sample population (Fry 1947). In contrast, critical thermal methodology, utilizes a dynamic change in temperature, which is arguably more representative of conditions in the wild (see discussion in Beitinger et al. 2000). Further, CTM requires fewer acclimation temperatures and smaller sample sizes (for a detailed discussion on differences between CTM and ILT see Beitinger et al. 2000).

3.4.1 Acclimation temperature

To date, two studies have evaluated CTMin in RT acclimated to 10, 15 and 20 °C and there are no reported CTMin values for WSCT (Becker and Genoway 1979; Currie et al. 1998). The acclimation temperatures used in my study, were chosen such that a quantifiable CTMin could be obtained for both species. An acclimation temperature of 15 °C with a linear decline of 0.3 °C min^{-1} was used in my experiments so that a direct comparison could be made with previous work on RT (Becker and Genoway 1979; Currie et al. 1998). A second acclimation temperature of 18 °C with the same rate of decline was chosen in an attempt to capture species level differences that may be understated at 15 °C. An acclimation temperature above 18 °C was not employed in my study as these temperatures are likely too warm for WSCT (Bear et al. 2007), and an acclimation temperature below 15 °C was not tested as RT held at 10 °C in the previous studies, did not exhibit loss of equilibrium when the water began to freeze at 0 °C (Becker and Genoway 1979; Currie et al. 1998).

In my experiments, there was a significant effect of acclimation temperature on cold tolerance performance in WSCT and RT. Regression analysis revealed an increase of 0.29 °C in CTMin with every 1 °C increase in acclimation temperature for WSCT. This value is similar to those reported in killifish (*Fundulus heteroclitus*, Fangue et al. 2006), sheepshead minnow (*Cyprinodon variegatus*, Bennett and Beitinger 1997) and other studies of RT (Becker and Genoway 1979; Currie et al. 1998). The rainbow trout evaluated in my work, however, only exhibited a 0.16 °C increase in CTMin with every 1 °C increase in acclimation temperature. This is considerably lower than the value of 0.36 °C per 1 °C rise in acclimation reported by Becker and Genoway (1979) and Currie et al. (1998). Differences between these values could be the result of testing only two acclimation temperatures in

my study that differed by only 3 °C. Becker and Genoway (1979) and Currie et al. (1998) evaluated three acclimation temperatures at 5 °C increments. Thus, the values drawn from my study are applicable to a smaller range of temperatures and thus may not have elicited as large as response as these previous studies. Further, the RT used in my work represent different populations and crosses, which increases the potential genetic variability in cold tolerance and may translate to higher variability in cold performance. Acclimation temperature will generally explain a significant portion of the variation observed in CTMin experiments (Becker and Genoway 1979; Bennett and Beitinger 1997; Currie et al. 1998; Fangue et al. 2006). Similar work generally reported r^2 values of CTMin on acclimation temperature above 0.95. My study produced dramatically lower r^2 values likely a result of high levels of intraspecific variation (Fig. 3.2).

3.4.2 Inter- and intraspecific differences

15 °C acclimation

My results reveal a statistically significant difference in average CTMin between RT and WSCT acclimated to 15 °C (Table. 3.1). Some RT individuals were capable of performing just as well, if not better than certain WSCT, and thus, a definitve conclusion that WSCT are more cold tolerant than RT cannot be made (Appendix B Fig. B.1). Further tests of intraspecies variation in WSCT (testing other populations) would be necessary to obtain more compelling support for this hypothesis. These results do, however, agree with data that suggest the distribution of RT alleles at lower elevations may be the result of habitat preference for warmer waters (Hitt et al. 2003; Rasmussen et al. 2010, 2012). Differences in physiological response to water temperature between the two species, may be responsible for the zonal pattern observed in hybridizing populations and as a result, may be limiting the spread of RT alleles into cold headwaters (Rasmussen et al. 2010, 2012).

The F_2 crosses of RT had a mean CTMin value equivalent to WSCT at 15 °C acclimation. These results suggest that it may be possible for RT to displace WSCT at higher elevations given the appropriate combination of genes. It may simply be a matter of time and the lack of competitive pressure at lower elevations that prevent RT from seeking out and establishing populations in the upper headwaters. It is unknown whether RT individuals with high cold tolerance are competitively superior in all aspects, even at low elevations, and are therefore, never forced to seek less favourable habitats in cold extremes. Further tests are needed to determine whether RT

with high cold tolerance are competitively superior in low and high elevation habitats.

There were high levels of variation in cold performance between different populations of RT; however, statistical significance was only observed between two test groups (F_2 and TZB; Fig. 3.3). Second-generation (F_2) hybrids between "warm" (BW) and "cold" (TZ) water-adapted RT were able to perform just as well as WSCT when acclimated at 15 °C and had the highest degree of variation amongst all the groups tested. Assuming additive genetic variability for any trait, the F_2 hybrid offspring should be characterized by high levels of genetic variation as genotypic combinations of both parental genotypes as well as hybrid combinations between the two populations are represented in this group. The low CTMin obtained for this group is difficult to explain, as simple additive heritability would predict an average CTMin performance (a value intermediate to the parental populations (BW, TZ)). Unfortunately the genetic architecture of cold tolerance in salmonids is poorly understood. Quantitative trait locus (QTL) analyses would need to be carried out to identify stretches of DNA that control or are linked to cold tolerance performance in trout. This would increase our understanding of the underlying genetic controls for this trait (e.g. Xiao et al. 1995). Without this information, it is difficult to know why the backcross groups (BWB, TZB) and the F_2 hybrids performed the way they did. The patterns observed suggest that the genes controlling cold tolerance do not behave in a simple, additive manner.

Tzenzaicut fish had an average CTMin lower than BW fish (higher cold tolerance, Table. 3.1). This result is supported by reports from Clarke et al. (2008) and Northrup and Godin (2009) that TZ fish perform better in growth and survival than BW fish when stocked in colder habitats. Further, it lends support to the rationale used in my study; that wild fish populations that evolved in colder habitats perform better in cold tolerance trials. This result agrees with my predictions and suggests that the results of my study have relevance in nature.

As a whole, the CTMin values reported in my study are higher than previous reports in RT (Becker and Genoway 1979; Currie et al. 1998). At an acclimation temperature of 15 °C and a rate of temperature decline of 0.3 °C min^{-1}, Currie et al. (1998) report a CTMin of only 0.2 °C. Becker and Genoway (1979) report a similar CTMin of 0.7 °C under similar conditions. In contrast, the average CTMin of RT in my study was 1.4 °C tested under the same conditions. This discrepancy amongst individuals of the same species could be the result of minor but significant variations in methodology, experimental set-up, population, age and size of fish, etc. For

instance, RT used by Becker and Genoway (1979) were collected straight from the Columbia River, while fish used in the study by Currie et al. (1998) had been purchased from a hatchery in Missouri. My fish originated from experimental crosses using fish collected in the wild and subsequently raised under laboratory conditions. Currie et al. (1998) also used a CTMin endpoint that deviated from my methods. The temperature they reported was taken one minute after the fish exhibited an initial loss of equilibrium (LOE). I recorded my temperatures as the initial onset of LOE and did not wait one minute to record CTMin values. Becker and Genoway (1979) reported CTMin as LOE_{50} (the temperature at which 50% of a sample lost equilibrium). Further, both Becker and Genoway (1979) and Currie et al. (1998) introduced cold water directly into their system, while I used an antifreeze bath to gradually cool the test water. The fish used by Currie et al. (1998) were only four centimetres long and only 6 weeks old. My fish were considerably larger and older, and my experiments demonstrated that fork length and mass were significant predictors of cold performance in fish acclimated at 18 °C (Table. 3.2, 3.1, Appendix B Table. B.1). Analysis of variance (ANOVA) revealed that length had a positive relationship with CTMin, where longer fish tended to lose equilibrium at higher CTMin values (regression coefficient = 0.325). The opposite was true for mass. Fish that weighed more tended to have lower CTMin (regression coefficien t= -0.161). These two factors are likely to play a role in how well a fish can maintain its equilibrium.

Despite the discrepancy in the reported critical thermal minima of RT in previous studies and my own, my work does reveal that RT and WSCT exhibit marked differences in thermal minima, when tested under a common experimental procedure at least when acclimated to 15 °C.

18 °C **acclimation**

When acclimation temperature was increased to 18 °C, there were no significant intra- and interspecific differences in cold tolerance (Table. 3.2). At this temperature, body length and mass were better predictors of CTMin. This suggests that the genetically controlled, physiological differences that exist when fish are held at temperatures in the mid-range of their tolerance thresholds are no longer relevant when one or both species is beginning to experience some degree of heat-stress (Beitinger et al. 2000; Bear et al. 2007). A temperature of 18 °C represents above average summer temperatures for WSCT and approaches the upper extreme at which juvenile fish can survive (survival drops dramatically above 20 °C, Bear et al. 2007). Westslope

cutthroat trout held at this temperature may already be experiencing some level of heat stress. In my study, the spread of a fungal infection in WSCT seemed to be triggered by the high acclimation temperature. Westslope cutthroat trout acclimated to 15 °C showed no symptoms of the infection, despite exposure to the water of infected fish in the recirculating acclimation set-up. Similarly, RT showed no symptoms of the fungus at either 15 °C or 18 °C acclimation.

At 18 °C, F_2 fish loss the cold tolerance advantage observed at 15 °C acclimation (Table. 3.1). The performance of this group is similar to the results obtained for WSCT at both temperatures. It is possible that this group may have also been experiencing some degree of temperature stress at 18 °C, but the symptoms were not as conspicuous. These fish were tested in the autumn of 2012, before any experiments were done on WSCT. Because they were tested earlier, the fungus that infected the WSCT in my study may not have been in the system while the F_2 were undergoing acclimation.

3.5 Conclusions

With the exception of two test groups (F_2 and TZB), trends in cold tolerance performance met my predictions. Blackwater River (BW) fish had an average CTMin higher than Tzenzaicut Lake (TZ) fish and WSCT had a lower CTMin than pure RT genotypes and backcross individuals (Table. 3.1). While I did not resolve measurable differences at 18 °C acclimation, this may have been expected given that I was pushing the thermal limits of WSCT.

I was able to show that there are measurable differences in CTMin between WSCT and RT. This finding lends support to the theory that physiological limitations may be preventing RT from invading cooler, higher elevation habitats as suggested by work by McHugh and Budy (2005) and Rasmussen et al. (2010, 2012). This may explain, at least in part, the species gradient reported in numerous field studies that report zonal patterns of salmonid distribution (Rahel and Hubert 1991; Fausch et al. 1994; Hitt et al. 2003; Weigel et al. 2003; Rubidge and Taylor 2005; Rasmussen et al. 2010).

Some RT individuals exhibited cold tolerance comparable to WSCT which suggests that they may do just as well in cold water, however, my study still remains limited by its scope (Fig. 3.3, Appendix B Fig. B.1, B.2). Futher analysis of additional WSCT populations may reveal high levels of interspecific variation in CTMin as observed in the RT groups tested.

It is possible that RT may not survive long-term exposure to cool, often unproductive habitats. As proposed by Rasmussen et al. (2012), cold-water habitats may not meet the metabolic needs of RT and prevent their establishment in headwaters where we find genetically pure WSCT. A study evaluating the thermal requirements of RT (perhaps different populations) through all life-stages (egg to adult) would be necessary to test this idea.

The Alberta Westslope Cutthroat Trout Recovery Plan outlines efforts to restock genetically pure WSCT in habitats across their historical range (The Alberta Westslope Cutthroat Trout Recovery Team 2013). Conservation efforts for WSCT should focus on rehabilitating populations at high elevations, that provide natural refuge from RT invasion via cold water and low productivity as suggested by my study and previous work (McHugh and Budy 2005; Rasmussen et al. 2010, 2012). A better understanding of the genetics that underlie cold tolerance may also be valuable, particularly if certain populations of WSCT show a heightened tolerance to cool temperatures. If we could identify such populations, they could be useful in efforts to restock high elevation locales as their cold tolerance could provide an extra means of resistance to RT invasion and subsequent hybridization with native WSCT.

Chapter 4

Conclusion

4.1 Summary of Findings

My thesis produced three general findings:

1. Hybridization between westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WSCT) and rainbow trout (*O. mykiss*; RT) is extensive across southwestern Alberta. Over 150 sites were tested and most populations had q_{wsct} between 0.85 and 0.99. A population with q_{wsct} value below 0.99 is considered 'hybridized'.

2. Amongst the locales tested, water temperature, elevation, distance to nearest stocking site and distance to nearest railway were significant predictors of levels of admixture. 'Genetically pure' populations of WSCT are more common in cool, high elevation habitats that are far from stocking sites. These results are consistent with previous work done over smaller spatial scales. The complex interaction with distance to railway is difficult to interpret; however, railways were historically used to stock RT in southwestern Alberta.

3. Rainbow trout and WSCT exhibit subtle, but significant differences in average CTMin when acclimated to 15 °C. At this acclimation temperature, WSCT were able to withstand an average water temperature cooler than RT. This result provides additional evidence that RT and hybrids may prefer warmer water and are limited in their upstream spread by physiological demands that cannot be met in cold temperature habitats. These constraints may be preventing RT and hybrids from overtaking the final WSCT "strongholds" at high elevations when physical barriers are not present.

My analyses provide evidence that hybridization is indeed widespread in southwestern Alberta and efforts to mitigate the loss of WSCT to genomic extinction are urgently needed. The identification of landscape variables that appear to influence levels of hybridization may assist recovery plans.

For instance, low elevation sites near former RT stocking locales are likely to show a high degree of hybridization and may contain genetically pure RT. The likelihood of recovering pure WSCT in these types of habitats may be very low. Efforts to restock WSCT in these sites without the elimination of existing hybridized individuals and RT may be futile, if conditions in these higher stream order habitats indeed favor RT and hybrids over WSCT. Work by Henderson et al. (2000) and Hitt et al. (2003) suggest that hybrids disperse more than the parental taxa and are the agent of spread. Target sites for recovery will require elements that suppress RT movement, such as thermal or physical barriers.

4.2 Defining 'Genetic Purity'

The standards set to classify a population as 'hybridized' or 'genetically pure' for conservation purposes have been widely debated (Allendorf et al. 2005; Campton and Kaeding 2005). Allendorf et al. (2001) recommend stringent guidelines, setting a 1% threshold of allowable admixture, such that 'pure' populations are those with $q_{wsct} \geq 0.99$. They argued that because hybrids with low levels of RT admixture experience a significant reduction in fitness in laboratory studies, any level of admixture in WSCT populations would be undesirable. Further, protecting WSCT under such a conservative limit would act to preserve the evolutionary legacy of the WSCT gene pool, while acknowledging that polymorphisms may still be shared between the two species or that historical, natural hybridization between them may have occurred (resulting in admixture of no more than 1%). These guidelines would also reduce the likelihood of protection of populations with higher levels of admixture that may serve as a means for introgression into pure populations. Campton and Kaeding (2005) contested the $\leq$1% admixture threshold, and stated that admixture is present in naturally sympatric populations of WSCT and RT. These authors also added that in the long term, failing to protect populations with as low as 10% introgression would dramatically reduce our capacity to recover WSCT. For instance, if only populations with $\leq$1% admixture qualified for protection and the remaining populations were subject to eradication, and if eradication was not 100%, as is often the case, the remaining 'pure' populations could continue to mix with residual admixed individuals and show evidence of introgression after several generations and may no longer be subject to protection under strict conservation guidelines. This could continue on until we have depleted our reserve of WSCT populations and no longer have any populations to pro-

tect that meet the ≤1% admixture standard. Muhlfeld et al. (2009a) showed that fitness of WSCT in nature may decline by up to 50% with as little as 20% admixture with RT, although the mechanisms of such fitness declines are unknown. More such studies under different environmental contexts are required before we can make broad generalizations about the fitness consequences of introgression given a starting level of admixture. Many who work in this field agree that until the fitness consequences of introgression in nature are better understood, case-by-case assessments concerning conservation of individual populations is the best policy (see discussion in The Alberta Westslope Cutthroat Trout Recovery Team 2013).

Many of the sites measured in my study were found to have admixture levels (q_{wsct}) significantly lower than 0.99 (the amount of hybridizaton is significant). The recovery plan outlined for WSCT in Alberta, adopt the standards set by Allendorf et al. (2001) to identify the populations that are top priorities for conservation, citing the same rationale; that slight levels of admixture significantly reduces the fitness of female and male trout as demonstrated by Muhlfeld et al. (2009a), and that 1% admixture accounts for historical hybridization between species. The recovery team also recognize that "populations with low levels of hybridization ($q_{wsct} \geq 0.95$ but < 0.99) may be important for species conservation and recovery", as these populations may contain migratory or adfluvial life history forms, may be adapted to unique environments, or be the least introgressed population within a geogrphic area. They may also have unique behaviours or phenotypes that researchers and experts consider important to conserve (The Alberta Westslope Cutthroat Trout Recovery Team 2013).

4.3 Anthropogenic Hybridization

It is important not to ignore the role that hybridization plays in evolution. Hybridization introduces genetic variation that can act as a source for beneficial adaptations (Verspoor and Hammart 1991). Hybridization with a closely related taxon may also rescue small populations experiencing inbreeding depression (Willi et al. 2007). Hybridization may even play a role in speciation; examples of allopolyploid speciation exist in plants, where parental species that differ in chromosome number hybridize and produce an offspring that is unable to reproduce with either parental species because it carries double the number of chromosomes of the parental species (Widmer and Baltisberger 1999). Some species of fish have also arisen as a result of introgressive hybridization (Demarais et al. 1992; reviewed in Taylor 2004).

By contrast, hybrids that arise from anthropogenic activity generally negatively impact an ecosystem by threatening native species (Allendorf and Leary 1988; Rhymer and Simberloff 1996). 'Anthropogenic hybridization' as described by Allendorf et al. (2001) needs to be reduced and "hybrids that originate as a result of human activity should only be protected if the hybrids contain the only remaining genetic information from a species that has otherwise been lost by genetic mixing or when their origin is unclear."

4.4 Concluding Thoughts and Future Directions

My research builds upon previous work on hybridization between WSCT and RT in North America. I employed a larger suite of genetic markers to better estimate levels of admixture and extend my analysis of landscape variables across a larger spatial scale. The congruence between environmental factors highlighted in my model and that of previous studies brings generality to the findings. Our ability to detect hybrids has increased with growth in genetic technology. Increasing sample sizes and developing genome-wide methods to identify specific genes that differentiate WSCT, RT and their hybrids will provide more precise estimates of admixture and population structure (Hohenlohe et al. 2011). Fine-scale, geographic mapping and measurement of abiotic and anthropogenic variables that exist in WSCT habitats are also needed to obtain a better understanding of the complex interactions which lead to spatial variability in hybridization levels across the range of WSCT. Temporal sampling and genetic analysis will give us a better understanding of how admixture values change over time and an evaluation of the life history requirements of WSCT will also be vital to develop effective conservation strategies.

My data revealed a measurable difference in average CTMin between WSCT and RT at 15 °C acclimation (1.0 °C and 1.4 °C, respectively). This supports previous laboratory work, which suggests that RT are better adapted to warmer temperatures, being able to tolerate temperatures significantly higher than WSCT (Bear et al. 2007). My results also agree with field research that propose a habitat preference, supported by species level differences in metabolism and life history strategy (Rasmussen et al. 2010, 2012). Together, these studies provide a foundation for the hypothesis put forth by McHugh and Budy (2005), that physiological constraints to cold water at high elevations prevent the spread of RT into the headwaters, and that temperature-mediated competition with RT is likely excluding WSCT from low elevations.

Carrying forward, molecular research into the genetic basis and architecture of thermal tolerance will provide further insight into the evolutionary history of physiological adaptation in each taxon. If lower thermal limits are physiologically constraining the movement and establishment of RT upstream, determining the genes responsible for thermal tolerance may also help us identify RT populations that are unlikely to overtake remaining WSCT populations if stocking continues (i.e., one management solution to continued demands for RT could be to stock genotypes that exhibit poor cold tolerance). In conclusion, the results of my thesis agree with and extend previous research that identify water temperature as a major element influencing levels of hybridization in WSCT, and contributes to the understanding of how cold water in high elevation habitats may provide refuge for indigenous taxa threatened by hybridization with non-native species.

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Appendix A

Chapter 2

Table A.1: Summary of genetic data for sampling locations in southwestern Alberta and southeastern British Columbia, Canada. Abbreviations: N, number of individuals sampled; Mean q_{wsct}: average admixture value; F_{IS}: inbreeding coefficient; 95% CI: 95% confidence interval of F_{IS}; H_O: observed heterozygosity; H_E: expected heterozygosity; N_A: number of alleles.

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Bow	Altrude	Altrude (middle)	AM	32	28	51.2335	-116.0421	2007, 2008	0.988	0.003	(-0.178-0.142)	0.22	0.22	2.0
Bow	Altrude	Arnica Lake	AR	33	28	51.2219	-115.9984	2008	0.993	-0.025	(-0.209-0.123)	0.20	0.20	2.1
Bow	Altrude	Boom Cr, lower	PC-LBC	31	30	51.2494	-116.0213	2006	0.929	0.253	(0.043-0.393)	0.14	0.18	2.6
Bow	Altrude	Boom Cr, upper	PC-UBC	29	21	51.2597	-116.0718	2006	0.909	0.094	(-0.109-0.224)	0.20	0.22	3.2
Bow	Altrude	Lower Twin Lake	LTwin	35	31	51.2018	-115.9813	2008	0.991	0.094	(-0.062-0.221)	0.19	0.21	3.1
Bow	Altrude	Smith Lake	SML	30	52	51.2493	-115.9253	2007	0.991	0.133	(0.032-0.217)	0.24	0.28	2.6

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Bow	Altrude	Upper Twin Lake	UT	34	30	51.2125	-115.9843	2008	0.985	-0.062	(-0.206-0.065)	0.20	0.19	2.2
Bow	Bow mainstem	Bow River upper	BR	4	30	51.6509	-116.3839	2006	0.988	0.361	(0.210-0.477)	0.22	0.30	3.3
Bow	Cascade	Cuthead Creek	CC	13	30	51.4404	-115.6958	2008	0.993	0.026	(-0.112-0.114)	0.23	0.24	2.4
Bow	Cascade	Elk Lake	PC-ElL	25	24	51.2883	-115.6509	2006	0.989	-0.020	(-0.155-0.078)	0.37	0.36	2.6
Bow	Cascade	Sawback Lake	SBL	18	31	51.3498	-115.7675	2008	0.993	0.960	(0.842-1.000)	0.01	0.08	1.3
Bow	Cascade River	Block Lake	BLK	17	29	51.3724	-115.8339	2008	0.995	-0.003	(-0.168-0.151)	0.21	0.21	2.2
Bow	Corral	Corral Creek	CC	11	56	51.4487	-116.1162	2009	0.977	0.101	(0.032-0.162)	0.23	0.24	2.4
Bow	Corral	Corral Creek	PC-CC	12	25	51.4487	-116.1162	2006	0.946	0.135	(-0.029-0.265)	0.36	0.42	3.3
Bow	Elbow	Canyon Creek	AFW-CC	57	30	50.8952	-114.7772	2006	0.974	0.004	(-0.132-0.097)	0.38	0.39	3.7
Bow	Elbow	Canyon Creek	J-E15	58	33	50.8955	-114.7753	2008	0.941	-0.052	(-0.159-0.023)	0.40	0.38	4.2
Bow	Elbow	Prairie Creek	AFW-PC	60	27	50.8799	-114.8794	2006	0.985	-0.068	(-0.385-0.044)	0.25	0.23	1.5
Bow	Elbow	Quirk Creek	AFW-QC	71	28	50.8073	-114.7544	2006	0.892	0.269	(-0.003-0.402)	0.24	0.32	2.8
Bow	Elbow	Ranger Creek (higher up)	J-E10a	55	26	50.9106	-114.7070	2007	0.650	-0.072	(-0.196-0.002)	0.68	0.64	4.7
Bow	Elbow	Silvester Creek	AFW-SiC	62	25	50.8648	-114.7238	2006	0.992	0.216	(0.082 -0.303)	0.24	0.30	2.1
Bow	Elbow	Tributary to Canyon Creek	J-E15b	56	37	50.9139	-114.9164	2008	0.975	-0.010	(-0.133-0.085)	0.36	0.36	3.4
Bow	Fish	Fish Creek	AFW-FC	64	21	50.8509	-114.6192	2006	0.912	0.070	(-0.100-0.166)	0.43	0.46	4.5

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Bow	Forty Mile Creek	Mystic Lake	OCMY	28	29	51.2783	-115.7498	1999	0.992	-0.136	(-0.318- -0.001)	0.32	0.28	2.2
Bow	Ghost	Johnson Cr	AFW-JC	15	17	51.3904	-115.0910	2006	0.992	0.194	(-0.189- 0.338)	0.27	0.32	2.6
Bow	Ghost	Margaret Creek	AFW-MaC	14	30	51.4026	-115.0711	2006	0.970	0.151	(-0.117- 0.290)	0.26	0.30	2.8
Bow	Ghost	Meadow Creek	AFW-MeC	19	27	51.3388	-115.0836	2006	0.970	0.173	(-0.074- 0.310)	0.22	0.26	3.0
Bow	Ghost	Waiparous Creek	AFW-WC	23	29	51.2869	-114.8395	2006	0.985	0.069	(-0.053- 0.147)	0.29	0.31	3.1
Bow	Healy	Healy Lake #1	HL1	44	21	51.0829	-115.8555	2008	0.981	0.002	(-0.194- 0.107)	0.31	0.31	2.4
Bow	Healy	Healy Lake #2	HL2	45	30	51.0853	-115.8592	2008	0.992	-0.092	(-0.251- 0.035)	0.20	0.19	2.2
Bow	Healy	Healy Lake #3	HL3	42	25	51.0911	-115.8627	2008	0.982	-0.028	(-0.208- 0.093)	0.28	0.28	2.5
Bow	Helen	Katherine Lake	PC-KL	1	26	51.6857	-116.3914	2006	0.075	0.252	(0.082- 0.341)	0.28	0.37	3.2
Bow	Highwood	Bear Creek	J-P16	104	30	50.3483	-114.4595	2007	0.117	0.033	(-0.039- 0.065)	0.74	0.77	8.5
Bow	Highwood	Bear Creek	J-P16a	106	27	50.3393	-114.4926	2008	0.787	0.149	(-0.031- 0.247)	0.39	0.46	5.2
Bow	Highwood	Cutthroat Creek	AFW-CuC	92	26	50.4763	-114.4912	2006	0.990	0.081	(-0.117- 0.215)	0.34	0.36	2.9
Bow	Highwood	Deep Creek	J-H11	120	29	50.4275	-114.4829	2007	0.993	0.017	(-0.131- 0.121)	0.31	0.38	3.3
Bow	Highwood	Etherington Creek above barrier	J-H24b	107	30	50.3364	-114.6246	2007	0.980	-0.105	(-0.254- 0.044)	0.22	0.20	2.0
Bow	Highwood	Etherington Creek below barrier	J-H24a	108	30	50.3393	-114.6247	2007	0.988	-0.116	(-0.313- 0.073)	0.22	0.19	1.9
Bow	Highwood	Flat Creek	AFW-FlC	93	30	50.4654	-114.5587	2006	0.975	0.214	(0.065- 0.320)	0.30	0.38	3.3

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Bow	Highwood	Greenfeed Creek (south fork)	J-P17a	109	29	50.3229	-114.4718	2007	0.952	0.058	(-0.085-0.175)	0.25	0.27	2.7
Bow	Highwood	Hay Creek	J-St14f	112	30	50.3048	-114.3374	2007	0.454	0.004	(-0.086-0.058)	0.70	0.71	6.3
Bow	Highwood	J-H11a Deep Creek tributary	J-H11a	96	30	50.4362	-114.5061	2007	0.992	-0.002	(-0.123-0.080)	0.25	0.25	2.0
Bow	Highwood	J-H4h Sullivan Creek	J-H4h	86	29	50.5504	-114.5708	2007	0.582	-0.007	(-0.097-0.048)	0.70	0.69	7.9
Bow	Highwood	J-H4i Sullivan Creek	J-H4i	87	30	50.5375	-114.5913	2007	0.720	0.061	(-0.054-0.145)	0.58	0.61	7.0
Bow	Highwood	J-H7a Unnamed trib to Flat Creek	J-H7a	95	30	50.4483	-114.5012	2007	0.994	-0.168	(-0.305--0.071)	0.32	0.28	1.6
Bow	Highwood	Loomis Creek (lower)	J-H33	94	22	50.4632	-114.8128	2008	0.968	0.040	(-0.076-0.107)	0.40	0.42	3.9
Bow	Highwood	Marston Creek	J-H12	100	30	50.4124	-114.5202	2007	0.677	0.083	(-0.048-0.182)	0.52	0.57	4.2
Bow	Highwood	McPhail Creek (lower)	J-H30	101	30	50.4162	-114.7490	2007	0.429	0.011	(-0.096-0.085)	0.73	0.74	6.6
Bow	Highwood	McPhail Creek (upper)	J-H30a	98	30	50.4298	-114.8044	2007	0.706	0.041	(-0.070-0.106)	0.57	0.61	6.1
Bow	Highwood	Muir Creek	J-H30b	99	25	50.4235	-114.7891	2007	0.897	-0.131	(-0.240--0.05)	0.55	0.60	3.1
Bow	Highwood	North Sullivan Creek (1.5 km up)	J-H4c	88	28	50.5266	-114.4345	2007	0.041	0.156	(0.061-0.207)	0.62	0.73	7.4

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Bow	Highwood	Pekisko Creek (headwaters)	J-P21	114	30	50.2856	-114.4657	2007	0.962	0.041	(-0.123-0.167)	0.24	0.25	2.6
Bow	Highwood	Pekisko Creek above falls	AFW-PeC	105	27	50.3440	-114.4551	2006	0.947	-0.072	(-0.204-0.052)	0.29	0.28	2.0
Bow	Highwood	Picklejar #4	AFW-PjL #4	90	29	50.5188	-114.7831	2006	0.991	0.101	(-0.138-0.264)	0.20	0.23	1.6
Bow	Highwood	Picklejar L #2	AFW-PjL #2	90	26	50.5182	-114.7759	2006	0.990	0.144	(-0.170-0.403)	0.11	0.12	1.4
Bow	Highwood	Salt Creek	J-P20	113	30	50.3041	-114.4611	2007	0.959	0.005	(-0.160-0.140)	0.22	0.22	2.6
Bow	Highwood	Sheppard Creek (south fork)	J-St14d	110	30	50.3206	-114.3967	2008	0.932	-0.071	(-0.183-0.004)	0.39	0.37	3.3
Bow	Highwood	South Sullivan Creek (6 km up)	J-H4f	91	30	50.5004	-114.5535	2007	0.731	0.038	(-0.094-0.114)	0.53	0.55	6.2
Bow	Highwood	South Sullivan Creek trib (5 km up)	J-H4g	89	30	50.5162	-114.5659	2007	0.769	-0.048	(-0.157-0.033)	0.61	0.58	5.2
Bow	Highwood	Unnamed to Pekisko Cr	J-P15	103	21	50.3570	-114.4592	2007	0.080	0.073	(-0.016-0.119)	0.63	0.68	7.0
Bow	Highwood	Unnamed to upper Pekisko	J-P19	111	28	50.3098	-114.4468	2007	0.949	0.021	(-0.119-0.121)	0.23	0.23	2.4
Bow	Highwood	Zephyr Creek	J-H18	102	30	50.3851	-114.5757	2007	0.986	0.050	(-0.072-0.154)	0.33	0.35	2.4
Bow	Johnston	Johnston Creek	JC	27	17	51.2827	-115.8212	2006	0.293	0.192	(-0.011-0.347)	0.22	0.27	2.9

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Bow	Johnston	Luellen Lake U Laval Clips	OCLL	20	17	51.3402	-115.9169	1999	0.436	-0.010	(-0.245-0.066)	0.46	0.46	3.8
Bow	Jumpingpound	Coxhill Cr below falls	J-J11	50	35	51.0072	-114.8186	2007	0.900	-0.025	(-0.113-0.034)	0.44	0.43	4.8
Bow	Jumpingpound	Jumpingpound Creek	AFW-JuC	54	27	50.9666	-114.9578	2006	0.973	-0.101	(-0.217--0.029)	0.38	0.34	2.9
Bow	Jumpingpound	Pine Cr. middle	J-J7	46	17	51.0184	-114.7674	2007	0.962	0.058	(-0.114-0.171)	0.28	0.30	3.6
Bow	Jumpingpound	Unnamed to Coxhill Cr	J-J11c	49	33	51.0085	-114.8257	2007	0.901	0.024	(-0.101-0.114)	0.38	0.39	3.3
Bow	Jumpingpound	Unnamed to Up Jumping-pound	J-J19	51	30	50.9890	-114.9547	2007	0.965	-0.086	(-0.212-0.020)	0.32	0.29	3.1
Bow	Jumpingpound	Unnamed to Up Jumping-pound	J-J20	52	21	50.9757	-114.9537	2007	0.941	-0.046	(-0.180 -0.028)	0.40	0.38	2.8
Bow	Kananaskis	Evan-Thomas Creek	J-EVTH	61	34	50.8820	-115.1219	2009	0.993	-0.092	(-0.243-0.028)	0.20	0.18	2.0
Bow	Kananaskis	Spotted Wolf Creek	AFW-SWC	81	25	50.8424	-115.3445	2006	0.889	0.188	(0.047-0.296)	0.22	0.27	3.1
Bow	Kananaskis	Spotted Wolf Creek	B-K1	83	15	50.6611	-115.0973	2008	0.939	-0.089	(-0.260-0.012)	0.30	0.28	2.7
Bow	Moraine	Babel Creek-Consolation Lake	BCC	21	27	51.3099	-116.1491	2008	0.989	0.043	(-0.143-0.172)	0.31	0.33	2.8
Bow	Mosquito	Mosquito Cr	PC-MC	3	30	51.6592	-116.3176	2006	0.981	0.204	(0.006-0.318)	0.25	0.31	3.0
Bow	Outlet	Outlet Creek	OC	16	29	51.4001	-116.1225	2008	0.994	0.001	(-0.240-0.239)	0.07	0.07	1.3
Bow	Pipestone	Big Fish Lake	BFL	5	62	51.6423	-116.1985	2009	0.993	0.487	(0.407-0.551)	0.19	0.36	3.0

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Bow	Pipestone	Deer Lake	DL	6	47	51.6271	-116.1625	2009	0.994	0.043	(-0.131-0.187)	0.11	0.12	1.6
Bow	Pipestone	Little Fish Lake	LFL	9	47	51.6436	-116.1809	2009	0.994	0.068	(-0.094-0.225)	0.12	0.13	1.7
Bow	Pipestone	Moose Lake	ML	2	49	51.6634	-116.2010	2009	0.994	0.097	(-0.043-0.181)	0.11	0.13	1.6
Bow	Pipestone	Pipestone R	PC-PR	8	20	51.6103	-116.1321	2006	0.959	0.302	(0.115-0.411)	0.23	0.31	2.3
Bow	Redearth	Black Rock L	PC-BRL	36	27	51.1210	-115.9152	2006	0.959	0.260	(0.043-0.415)	0.18	0.22	2.3
Bow	Redearth	Egypt L	PC-EgL	41	27	51.0989	-115.9166	2006	0.956	0.128	(-0.030-0.252)	0.26	0.30	2.9
Bow	Redearth	Haiduk L	PC-HL	37	25	51.1201	-115.9431	2006	0.432	0.168	(0.039-0.232)	0.47	0.56	4.4
Bow	Redearth	Mummy L	PC-ML	43	21	51.0892	-115.9139	2006	0.017	0.088	(-0.107-0.249)	0.38	0.42	2.9
Bow	Redearth	Pharaoh Cr	PC-PC	38	30	51.1196	-115.8982	2006	0.969	-0.022	(-0.174-0.100)	0.28	0.28	2.4
Bow	Redearth	Pharaoh L	PC-PL	39	27	51.1143	-115.9110	2006	0.985	-0.004	(-0.215-0.170)	0.25	0.25	2.1
Bow	Redearth	Scarab L	PC-SL	40	28	51.1005	-115.9039	2006	0.590	0.137	(0.015-0.210)	0.44	0.50	3.8
Bow	Sheep	Coal Creek (upper reach)	J-S12a	85	30	50.5682	-114.5825	2007	0.977	0.013	(-0.153-0.130)	0.24	0.24	2.7
Bow	Sheep	Death Valley Creek	J-T11d	79	29	50.7111	-114.5236	2007	0.010	0.099	(-0.013-0.173)	0.62	0.69	7.6
Bow	Sheep	Fisher Creek	AFW-FeC	69	25	50.8081	-114.6199	2006	0.775	0.228	(0.099-0.316)	0.40	0.51	4.8
Bow	Sheep	Gorge Creek (10 km above falls)	J-S17b	82	29	50.6595	-114.7462	2007	0.991	-0.088	(-0.254-0.057)	0.20	0.18	1.7

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Bow	Sheep	Gorge Creek (3 km above falls)	J-S17a	80	30	50.6646	-114.6860	2007	0.954	0.009	(-0.165-0.184)	0.19	0.19	2.2
Bow	Sheep	Muskeg Creek (upper)	J-T20a	73	25	50.7762	-114.6859	2007	0.068	0.074	(-0.014-0.128)	0.69	0.74	7.4
Bow	Sheep	North Coal Creek	J-S12b	84	31	50.6125	-114.5835	2008	0.929	0.197	(0.081-0.274)	0.37	0.45	4.8
Bow	Sheep	Ware Creek (headwaters)	J-T11g	78	30	50.7138	-114.6471	2007	0.715	0.032	(-0.080-0.109)	0.60	0.62	6.4
Bow	Spray	Gloria L	PC-GL	66	28	50.8645	-115.6049	2006	0.989	0.638	(0.181-0.914)	0.02	0.04	1.3
Bow	Spray	Marvel Lake U Laval Clips	MLULaval, OCM	63	34	50.8777	-115.5569	1999	0.993	0.088	(-0.107-0.249)	0.12	0.12	1.9
Bow	Spray	Smuts Cr	AFW-SC	68	25	50.8424	-115.3445	2006	0.897	0.201	(0.027-0.308)	0.23	0.29	2.9
Bow	Spray	Terrapin L	PC-TeL	65	30	50.8648	-115.5918	2006	0.983	0.505	(-0.129-0.778)	0.03	0.07	1.5
Bow	Spray	Upper Spray River	PC-USP	75	31	50.7412	-115.3934	2006	0.992	-0.104	(-0.252-0.013)	0.19	0.17	2.0
Bow	Spray	Watridge Creek	AFW-WatC	67	17	50.8486	-115.4201	2006	0.966	0.194	(-0.167-0.376)	0.15	0.19	2.3
Bow	Taylor	O'Brien L	PC-O'B	26	28	51.2866	-116.0829	2006	0.937	0.204	(0.091-0.276)	0.34	0.42	3.9
Bow	Taylor	Taylor Creek	TC	22	26	51.3076	-116.0250	2008	0.910	0.222	(0.080-0.312)	0.31	0.39	4.2
Bow	Taylor	Taylor L	PC-TaL	24	20	51.2958	-116.0957	2006	0.018	0.032	(-0.130-0.103)	0.38	0.39	3.5
Columbia	Kootenay	2nd Honeymoon Creek	2HC	47	29	51.0315	-115.9903	2008	0.981	-0.079	(-0.216-0.025)	0.34	0.31	2.7
Columbia	Kootenay	Daer Creek	DC	72	29	50.8129	-115.9603	2008	0.943	-0.054	(-0.159-0.009)	0.40	0.38	3.7

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Columbia	Kootenay	Dolly Varden Creek	DVC	70	30	50.8272	-116.0146	2008	0.916	0.044	(-0.098-0.138)	0.40	0.42	4.2
Columbia	Kootenay	Honeymoon Creek	HC	48	29	51.0275	-115.9856	2008	0.981	-0.147	(-0.283--0.048)	0.33	0.29	2.3
Columbia	Kootenay	Lost Creek	LC	59	29	50.8975	-116.0710	2008	0.964	-0.114	(-0.239--0.027)	0.41	0.37	3.2
Columbia	Kootenay	Meadow Creek	MCA	74	35	50.7638	-115.9528	2008	0.963	-0.057	(-0.146-0.004)	0.39	0.37	3.9
Columbia	Kootenay	Nixon Creek	NC	76	27	50.7423	-115.9211	2008	0.896	-0.037	(-0.165-0.046)	0.47	0.45	4.0
Columbia	Kootenay	Pitts Creek	PC	77	37	50.7378	-115.9008	2008	0.964	-0.049	(-0.145-0.021)	0.41	0.39	3.7
Columbia	Kootenay	Vermillion Creek	VR	53	33	50.9823	-115.9505	2008	0.965	0.129	(0.030-0.195)	0.34	0.39	3.1
Oldman	Castle	Carbondale (middle)	ACA-152	133	18	49.4521	-114.4091	2009	0.911	0.031	(-0.093-0.090)	0.38	0.39	4.0
Oldman	Castle	Carbondale (middle)	ACA-59	136	21	49.4372	-114.4313	2009	0.957	-0.040	(-0.147-0.023)	0.37	0.35	3.9
Oldman	Castle	Carbondale (upper)	ACA-61	139	16	49.4059	-114.4981	2009	0.993	0.035	(-0.136-0.133)	0.31	0.32	2.6
Oldman	Castle	Carbondale River	AFW-CaR	138	22	49.4218	-114.4671	2006	0.988	-0.126	(-0.276--0.016)	0.38	0.34	2.9
Oldman	Castle	Gardiner Creek	D-C3	142	29	49.3983	-114.4592	2007	0.993	0.028	(-0.102-0.122)	0.33	0.34	3.0
Oldman	Castle	Gladstone (middle)	ACA-52	144	15	49.3664	-114.2158	2009	0.945	-0.158	(-0.295--0.097)	0.50	0.43	4.1
Oldman	Castle	Goat Creek	ACA-57	129	29	49.4954	-114.5397	2009	0.992	0.518	(0.262-0.735)	0.48	0.99	1.5
Oldman	Castle	Gorge Creek	ACA-78	131	29	49.4702	-114.4274	2009	0.939	0.134	(-0.135-0.326)	0.16	0.18	2.8
Oldman	Castle	Lost Creek	AFW-LoC	134	27	49.4492	-114.4870	2006	0.982	0.160	(0.011-0.256)	0.31	0.37	3.5

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Oldman	Castle	Lost Creek trib	ACA-62	135	26	49.4484	-114.4966	2009	0.989	0.027	(-0.097-0.119)	0.31	0.32	2.7
Oldman	Castle	Lynx Creek	AFW-LyC	130	30	49.4741	-114.4713	2006	0.990	0.215	(-0.112-0.453)	0.1	0.13	1.7
Oldman	Castle	Lynx Creek (lower)	ACA-83	132	30	49.4630	-114.4468	2009	0.992	0.010	(-0.115-0.100)	0.07	0.08	1.5
Oldman	Castle	Lynx Creek trib	ACA-121	128	29	49.5235	-114.5229	2009	0.993	0.150	(-0.150-0.472)	0.05	0.05	1.5
Oldman	Castle	MacDonald Creek	D-C1	140	30	49.3997	-114.5227	2007	0.986	-0.018	(-0.156-0.105)	0.38	0.37	3.2
Oldman	Castle	OHagen Creek	D-C4	137	30	49.4252	-114.3911	2007	0.992	0.068	(-0.061-0.167)	0.17	0.18	2.0
Oldman	Castle	Scarpe Creek	D-C6	149	34	49.2339	-114.2560	2008	0.984	0.070	(-0.071-0.164)	0.31	0.34	3.5
Oldman	Castle	South Castle River (lower)	ACA-65	141	19	49.3801	-114.3332	2009	0.874	-0.052	(-0.181-0.015)	0.49	0.47	4.1
Oldman	Castle	South Castle River (upper)	ACA-71	150	18	49.2225	-114.2285	2009	0.985	-0.015	(-0.175-0.081)	0.30	0.30	2.4
Oldman	Castle	West Castle River (lower)	ACA-159	143	20	49.3748	-114.3720	2009	0.850	0.098	(-0.127-0.227)	0.44	0.48	5.2
Oldman	Castle	West Castle River (lower)	ACA-84	147	28	49.3445	-114.4098	2009	0.947	0.078	(-0.079-0.208)	0.35	0.38	4.2
Oldman	Castle	West Castle River (upper)	ACA-68	148	27	49.2375	-114.3496	2009	0.994	0.064	(-0.111 -0.205)	0.12	0.13	1.5
Oldman	Castle	Whitney Creek	AFW-WhC	146	25	49.3599	-114.9252	2006	0.939	0.129	(-0.011-0.237)	0.38	0.43	3.9
Oldman	Castle	Whitney Creek (lower)	ACA-60	145	29	49.3587	-114.1525	2009	0.925	-0.067	(-0.162--0.006)	0.12	0.13	1.5
Oldman	Crowsnest	Blairmore Creek	AFW-BC	125	26	49.6682	-114.4377	2006	0.931	0.218	(0.026-0.329)	0.31	0.39	4.0

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Oldman	Crowsnest	Crowsnest D-Cr1 nr tourist office	D-Cr1	126	30	49.6300	-114.6090	2007	0.994	0.128	(-0.064-0.281)	0.15	0.17	1.6
Oldman	Crowsnest	Island Creek	AFW-IC	127	23	49.6284	-114.7013	2006	0.968	0.253	(0.122-0.354)	0.38	0.39	3.3
Oldman	Livingstone	Deep Creek	AFW-DC	120	30	49.9761	-114.5565	2006	0.978	0.305	(0.107-0.458)	0.19	0.27	2.9
Oldman	Livingstone	Livingstone River above falls	AFW-LR	117	24	50.1664	-114.4610	2006	0.989	0.149	(-0.069-0.296)	0.29	0.34	3.1
Oldman	Livingstone	Livingstone River below falls	D-O2	118	31	50.0881	-114.4271	2008	0.864	0.113	(-0.005-0.194)	0.39	0.44	4.8
Oldman	Oldman mainstem	Beaver Creek	D-O1	123	30	49.8055	-113.9663	2008	0.969	-0.001	(-0.117-0.079)	0.41	0.41	4.5
Oldman	Oldman mainstem	Hidden Creek	AFW-HC	119	25	49.9762	-114.5566	2006	0.991	-0.020	(-0.155-0.084)	0.34	0.33	2.4
Oldman	Oldman mainstem	Sharples Creek	D-O3	121	29	49.8809	-114.0692	2008	0.990	0.013	(-0.107-0.103)	0.38	0.38	3.2
Oldman	Racehorse	North Racehorse Cr above falls	AFW-NRC	122	27	49.8434	-114.5745	2006	0.990	0.169	(0.025-0.247)	0.32	0.38	3.2
Oldman	Racehorse	Vicary Creek	AFW-VC	124	21	49.7538	-114.4885	2006	0.990	0.093	(-0.075-0.216)	0.38	0.41	3.4
Oldman	Waterton	Carthew Lake	CA	152	30	49.0286	-113.9912	2008	0.904	0.045	(-0.087-0.140)	0.34	0.35	3.4
Oldman	Waterton	Crypt Lake	CLR	154	27	49.0012	-113.8407	2008	0.764	0.035	(-0.091-0.130)	0.37	0.39	3.3
Oldman	Waterton	Goat Lake	GO	10	27	51.4471	-115.8592	2008	0.982	-0.042	(-0.201-0.076)	0.25	0.24	2.2
Oldman	Waterton	Lineham Lake	LIN	153	28	49.0258	-114.0675	2008	0.770	0.081	(-0.040-0.159)	0.40	0.43	3.7
Oldman	Waterton	Lone Lake	LOL	151	35	49.0887	-114.1313	2008	0.843	0.066	(-0.053-0.146)	0.33	0.35	3.1

Major Drainage	Sub-basin	Stream/Lake	Sample site	# on Fig. 2.1	N	Latitude	Longitude	Sample Year	Mean q_{wsct}	F_{IS}	(95% CI)	H_O	H_E	N_A
Oldman	Willow	Corral Creek	DW4	115	30	50.2548	-114.4225	2007	0.992	0.026	(-0.112-0.114)	0.34	0.34	2.8
Oldman	Willow	Johnson Creek	DW2	116	28	50.2158	-114.4047	2007	0.827	-0.031	(-0.160-0.006)	0.36	0.44	4.5

Table A.2: Variable data of streams used in RANDOMFOREST to generate admixture model. Site code: sampling location (see Table. A.1 for location details); q_{wsct}: average admixture; meand: mean depth at sample site (m); maxd: maximum depth at sample site (m); temp: water temperature at time of sampling (°C); barrier: distance to nearest barrier (m); elev: elevation (m), streamo: Strahler stream order; road: euclidean distance to nearest road (m); pipeline: euclidean distance to nearest gas/oil pipeline (m); powerline: euclidean distance to nearest power line (m); railline: euclideaen distance to nearest rail line (m); stockint: total number of stocking events at the stocking site; snumber: number of fish stocked at the stocking site; year2: total years between 2010 and year of last stocking at stocking site; diststock: fluvial distance to nearest stocking site (m).

Site Code	q_{wsct}	meand	maxd	temp	barrier	elev	streamo	road	pipeline	powerline	railline	stockint	snumber	year2	diststock
ACA-121	0.993	0.48	0.80	11.1	8905	1605	3	1094	10388	11707	10819	10	87500	59	8929
ACA-152	0.911	0.80	1.50	10.7	833	1355	6	126	3305	13437	12037	3	26500	59	409
ACA-159	0.850	0.54	1.00	9.1	8538	1372	5	330	8082	20709	19776	3	26500	59	5506
ACA-57	0.992	0.20	0.30	4.0	7914	1581	4	139	11970	14956	14164	6	23400	71	8027
ACA-59	0.957	0.81	1.30	10.3	2472	1393	6	71	5371	15602	14204	4	21710	66	1228
ACA-60	0.925	0.27	0.35	10.0	15555	1436	3	193	347	10993	12596	1	6000	74	4084
ACA-61	0.993	0.32	0.40	9.0	7503	1519	4	138	11291	21021	19641	3	12700	41	2474
ACA-62	0.989	0.18	0.25	11.0	4224	1493	4	134	9620	17182	15826	6	23400	71	1933

Site Code	q_{wsct}	meand	maxd	temp	barrier	elev	streamo	road	pipeline	powerline	railline	stockint	snumber	year2	diststock
ACA-65	0.874	1.06	2.00	9.2	7186	1356	5	211	6632	18648	19080	64	3101151	0	2797
ACA-68	0.994	0.54	1.00	9.1	5124	1563	3	1193	16592	25806	26392	1	6300	45	1880
ACA-71	0.985	0.34	0.60	11.6	12670	1521	5	153	10442	18419	18809	2	5960	41	5564
ACA-83	0.992	0.61	1.40	14.2	296	1419	4	99	5924	13672	12296	3	26500	59	3084
ACA-84	0.947	0.90	1.60	9.3	7594	1397	4	431	12305	24939	23537	1	5000	69	7032
D-C3	0.993	0.20	0.50	8.0	8958	1529	4	2525	10931	22067	20668	3	12700	41	1726
D-C4	0.992	0.40	1.00	10.0	3980	1435	3	12	4099	15885	14483	3	26500	59	3498
D-C6	0.984	0.50	1.40	8.0	10301	1512	3	1312	11810	19596	20082	2	5960	41	3504
D-Cr1	0.994	0.20	0.40	6.0	12656	1377	4	70	984	1211	483	15	121720	45	2028
D-O3	0.990	0.20	1.50	11.0	19783	1386	3	9	2961	7586	32936	22	20935	18	3976
D-W2	0.992	0.10	0.30	8.0	7972	1590	4	372	230	12258	47344	3	33300	59	6417
DW4	0.992	0.20	0.40	6.0	4557	1586	3	3290	3259	13303	46204	2	8400	45	776
GO	0.988	0.35	1.10	10.0	6477	1619	4	38	14444	19654	56283	7	117880	41	4926
J-E10a	0.650	0.20	0.50	15.0	5241	1425	3	1052	3390	20423	26774	51	112568	0	1653
J-H11	0.993	0.20	0.70	13.0	1000	1437	3	843	16479	23247	43767	1	5000	66	6074
J-H11a	0.992	0.10	0.50	10.0	929	1514	2	2690	17979	25152	45174	1	5000	66	4817
J-H12	0.677	0.15	0.30	12.0	1413	1482	3	89	19418	24481	46741	1	5000	66	3338
J-H18	0.986	0.20	0.75	8.0	332	1485	4	682	17614	23505	51359	22	237767	25	575
J-H24a	0.988	0.35	1.10	10.0	6450	1619	4	38	14444	19654	56283	7	117880	41	4926
J-H30	0.429	0.30	1.00	9.0	2999	1599	4	1423	26643	11966	62449	1	2340	31	10
J-H30a	0.706	0.20	0.80	8.0	4100	1712	4	3944	30374	8857	64836	1	2340	31	4173
J-H30b	0.897	0.15	0.75	8.0	4882	1703	3	3617	29118	9636	64302	1	2340	31	2916
J-H33	0.968	0.40	1.00	7.0	858	1760	4	2058	33536	9167	63442	42	478769	0	4985
J-H4c	0.041	0.20	1.00	15.0	6607	1350	4	1457	10624	17120	37196	15	312340	31	3639
J-H4f	0.731	0.15	1.00	6.0	5172	1563	3	2840	19502	25567	45668	15	312340	31	5294

Site Code	q_{wsct}	meand	maxd	temp	barrier	elev	streamo	road	pipeline	powerline	railline	stockint	snumber	year2	diststock
J-H4g	0.769	0.15	0.50	7.0	6568	1593	3	2886	18982	25371	45396	15	312340	31	5853
J-H4h	0.582	0.30	0.50	6.0	2753	1577	4	210	17152	24019	43730	15	312340	31	7245
J-H4i	0.720	0.20	0.40	9.0	4338	1634	4	60	19121	25914	45700	15	312340	31	8001
J-H7a	0.994	0.10	0.40	9.0	1980	1482	3	3453	17525	25643	44582	1	5000	66	5828
J-J11	0.900	0.30	2.00	8.5	312	1469	4	161	184	15355	16340	8	14980	24	3289
J-J11c	0.901	0.20	0.50	9.0	630	1469	3	686	707	15271	16030	8	14980	24	3258
J-J19	0.965	0.30	1.00	9.0	7747	1644	3	1	4714	7970	13752	3	1150	11	5623
J-J20	0.941	0.15	0.30	8.5	8799	1675	3	1	4468	9046	15063	3	1150	11	6841
J-P15	0.080	0.10	0.30	12.0	1355	1463	3	1084	14090	17765	44553	6	31000	40	2024
J-P16	0.117	0.30	0.50	14.0	463	1477	3	1962	13353	17431	44813	3	30500	66	2380
J-P16a	0.787	0.40	1.10	12.0	1860	1593	3	3438	11945	19390	47352	3	30500	66	941
J-P17a	0.952	0.20	0.50	9.0	2229	1580	3	4900	10396	17518	46456	3	30500	66	1423
J-P19	0.949	0.15	0.40	13.0	3936	1551	2	6007	9461	15449	45255	1	10000	71	2820
J-P20	0.959	0.20	0.40	8.0	4316	1591	3	6724	8635	16300	46432	1	10000	71	1647
J-P21	0.962	0.15	0.30	10.0	2991	1631	3	5371	6620	16324	47499	1	10000	71	2040
J-S12a	0.977	0.20	1.00	5.0	948	1590	4	1861	17070	24148	43543	2	9200	60	7329
J-S12b	0.929	0.20	1.00	12.5	1484	1483	4	4013	13176	23285	41662	2	9200	60	2605
J-S12e	0.182	0.60	1.80	12.0	1375	1395	5	1532	10782	20873	38979	2	9200	60	1443
J-S17	0.698	1.00	3.00	15.0	0	1460	5	320	13791	28087	45716	3	30000	60	3395
J-S17a	0.954	0.40	1.20	16.0	163	1565	4	359	13312	27592	47119	2	50000	69	3717
J-S17b	0.990	0.30	0.50	10.0	4147	1687	4	4652	16341	23884	51406	2	50000	68	1400
J-St14d	0.932	0.20	0.40	11.0	4929	1458	3	5538	8127	12253	41488	3	30500	66	6333
J-St14f	0.454	0.30	0.60	15.0	2684	1359	3	2080	4408	7739	38279	1	2125	42	3468
J-T11d	0.010	0.30	0.70	18.0	8071	1320	4	104	2549	19270	35001	3	32000	70	2275
J-T11g	0.715	0.30	0.70	13.0	5324	1497	4	3	7316	27931	43660	1	10000	73	5400
J-T20a	0.068	0.20	0.50	14.0	6493	1566	3	2439	4022	31901	40911	1	10000	73	2476

Appendix B

Chapter 3

Table B.1: Critical thermal minima, length and mass data for each trout tested. Rainbow trout (RT); Blackwater River rainbow trout (BW); Blackwater River backcross (BWB); westslope cutthroat trout (WSCT); Blackwater River x Tzenzaicut Lake F_1 hybrid x Blackwater River x Tzenzaicut Lake F_1 hybrid (F_2); Tzenzaicut Lake rainbow trout (TZ); Tzenzaicut Lake backcross (TZB).

Fish Number	Test Group	Species	Acc. Temp °C	CTMin, °C	Fork Length (cm)	Mass (g)
1	BW	RT	15	2.5	7.5	4.73
2	BW	RT	15	1.1	8.1	5.27
3	BW	RT	15	1.4	8.6	6.58
4	BW	RT	15	1.4	7.5	4.43
5	BW	RT	15	2.5	9.9	10.20
6	BW	RT	15	1.8	7.7	4.83
7	BW	RT	15	1.1	10.1	10.53
8	BW	RT	15	3.3	8.5	6.43
9	BW	RT	15	2.6	7.1	3.81
10	BW	RT	15	1.8	7.4	3.68
11	BW	RT	15	2.3	6.0	2.56
12	BW	RT	15	1.7	7.0	3.77
13	BW	RT	15	1.1	7.6	4.62
14	BW	RT	15	0.5	9.5	9.29
15	BW	RT	15	2.0	7.4	4.51
16	BW	RT	15	1.5	8.9	7.89
17	BW	RT	15	1.2	9.1	7.88
18	BW	RT	15	2.1	7.7	5.09
19	BW	RT	15	2.8	7.6	5.11
20	BW	RT	15	1.8	9.5	8.96

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
21	BW	RT	15	2.4	7.2	4.48
22	BW	RT	15	3.6	6.9	3.82
23	BW	RT	15	2.7	8.8	7.76
24	BW	RT	15	1.8	7.2	4.31
25	TZ	RT	15	1.2	10.3	10.20
26	TZ	RT	15	0.7	10.3	10.67
27	TZ	RT	15	0.6	8.3	5.73
28	TZ	RT	15	0.5	12.0	17.75
29	TZ	RT	15	0.9	9.7	8.91
30	TZ	RT	15	0.1	11.6	14.90
31	TZ	RT	15	0.8	10.7	11.38
32	TZ	RT	15	2.5	10.8	11.64
33	TZ	RT	15	1.0	9.1	6.72
34	TZ	RT	15	2.1	8.7	6.30
35	TZ	RT	15	0.3	9.8	8.67
36	TZ	RT	15	0.3	12.4	18.42
37	F2	RT	15	1.1	9.8	9.33
38	F2	RT	15	0.6	10.8	11.35
39	F2	RT	15	0.2	11.6	15.52
40	F2	RT	15	0.4	8.9	7.94
41	F2	RT	15	1.0	8.4	6.53
42	F2	RT	15	1.6	12.5	18.63
43	F2	RT	15	2.0	9.5	8.05
44	F2	RT	15	1.8	10.7	10.95
45	F2	RT	15	1.6	9.1	7.95
46	F2	RT	15	0.3	9.7	9.76
47	F2	RT	15	0.3	9.8	9.61
48	F2	RT	15	2	11.2	14.51
49	TZ	RT	18	1.1	12.2	16.68
50	TZ	RT	18	1.2	10.6	10.66
51	TZ	RT	18	1.7	11.0	12.60
52	TZ	RT	18	0.8	9.7	8.51
53	TZ	RT	18	0.5	10.4	10.60
54	TZ	RT	18	1.1	10.8	11.72
55	TZ	RT	18	3.1	9.5	7.99
56	TZ	RT	18	1.9	10.9	13.00
57	TZ	RT	18	1.5	10.3	10.42

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
58	TZ	RT	18	1.6	12.1	15.85
59	TZ	RT	18	0.8	12.0	15.24
60	TZ	RT	18	1.9	10.0	9.58
61	TZ	RT	15	1.0	9.3	7.26
62	TZ	RT	15	0.2	11.4	13.96
63	TZ	RT	15	2.0	10.3	9.71
64	TZ	RT	15	1.8	10.2	9.56
65	TZ	RT	15	0.5	10.0	9.24
66	TZ	RT	15	1.0	6.7	3.03
67	TZ	RT	15	3.3	9.9	8.98
68	TZ	RT	15	2.1	11.2	13.80
69	TZ	RT	15	1.2	9.7	8.74
70	TZ	RT	15	1.3	9.1	6.70
71	TZ	RT	15	0.8	10.2	9.71
72	TZ	RT	15	1.3	10.2	9.66
73	F2	RT	18	3.7	10.1	11.11
74	F2	RT	18	1.8	9.9	8.08
75	F2	RT	18	2.1	11.2	13.00
76	F2	RT	18	3.2	9.6	8.52
77	F2	RT	18	2.2	10.6	11.98
78	F2	RT	18	3.7	10.8	12.49
79	F2	RT	18	5.1	10.3	9.62
80	F2	RT	18	4.3	10.5	11.54
81	F2	RT	18	1.6	10.5	10.87
82	F2	RT	18	2.6	10.3	11.90
83	F2	RT	18	1.7	11.2	14.45
84	F2	RT	18	2.5	8.9	7.41
85	F2	RT	15	0.8	10.9	12.17
86	F2	RT	15	1.8	10.5	10.02
87	F2	RT	15	0.7	9.1	6.76
88	F2	RT	15	0.2	8.2	5.15
89	F2	RT	15	1.6	9.1	5.60
90	F2	RT	15	0.9	11.0	12.53
91	F2	RT	15	2.0	7.6	4.05
92	F2	RT	15	2.6	9.7	8.52
93	F2	RT	15	0.3	8.2	5.47
94	F2	RT	15	2.1	9.9	8.85

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
95	F2	RT	15	0.3	10.5	11.34
96	F2	RT	15	5.1	11.5	13.36
97	F2	RT	18	0.5	10.2	10.66
98	F2	RT	18	1.9	10.8	14.51
99	F2	RT	18	0.9	10.3	10.26
100	F2	RT	18	1.3	9.1	6.37
101	F2	RT	18	1.9	9.1	7.69
102	F2	RT	18	0.4	9.1	8.56
103	F2	RT	18	2.5	11.3	14.38
104	F2	RT	18	2.6	9.4	8.18
105	F2	RT	18	0.7	7.6	4.90
106	F2	RT	18	0.7	9.6	8.40
107	F2	RT	18	2.4	9.1	7.86
108	F2	RT	18	1.8	10.5	11.80
109	BW	RT	15	1.2	9.0	7.34
110	BW	RT	15	0.4	11.1	15.80
111	BW	RT	15	1.6	10.5	11.18
112	BW	RT	15	0.4	10.1	9.70
113	BW	RT	15	0.5	9.5	8.24
114	BW	RT	15	0.1	11.9	16.13
115	BW	RT	15	1.3	10.7	13.06
116	BW	RT	15	0.5	8.5	5.86
117	BW	RT	15	-0.1	8.3	5.43
118	BW	RT	15	1.1	9.1	7.44
119	BW	RT	15	0.3	8.1	5.32
120	BW	RT	15	1.5	9.8	8.47
121	TZ	RT	18	0.6	9.1	7.45
122	TZ	RT	18	0.6	10.6	11.52
123	TZ	RT	18	2.6	9.6	7.69
124	TZ	RT	18	1.7	9.2	7.26
125	TZ	RT	18	1.1	10.9	11.77
126	TZ	RT	18	0.0	8.8	6.13
127	TZ	RT	18	2.9	7.9	4.70
128	TZ	RT	18	3.0	9.8	8.03
129	TZ	RT	18	2.7	8.5	5.28
130	TZ	RT	18	0.8	10.9	11.92
131	TZ	RT	18	1.4	9.6	8.06

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
132	TZ	RT	18	1.2	10.4	9.74
133	BW	RT	18	0.0	9.7	9.86
134	BW	RT	18	1.5	8.7	5.88
135	BW	RT	18	1.9	8.0	5.22
136	BW	RT	18	0.7	10.1	10.64
137	BW	RT	18	1.7	9.2	8.24
138	BW	RT	18	2.7	11.4	15.11
139	BW	RT	18	1.6	7.4	4.72
140	BW	RT	18	0.1	8.0	3.65
141	BW	RT	18	0.7	9.4	8.35
142	BW	RT	18	1.3	8.9	7.45
143	BW	RT	18	2.1	7.9	5.21
144	BW	RT	18	0.6	10.8	13.27
145	F2	RT	15	-0.1	9.8	10.42
146	F2	RT	15	0.1	10.6	12.03
147	F2	RT	15	0.9	9.8	9.51
148	F2	RT	15	0.0	10.8	12.01
149	F2	RT	15	3.2	6.8	3.61
150	F2	RT	15	-0.2	9.9	10.03
151	F2	RT	15	-0.2	11.7	14.88
152	F2	RT	15	1.3	10.4	9.69
153	F2	RT	15	1.1	9.8	10.28
154	F2	RT	15	-1.1	10.1	10.90
155	F2	RT	15	0.5	9.1	5.76
156	F2	RT	15	0.2	8.3	6.84
157	BWB	RT	15	1.8	10.0	9.20
158	BWB	RT	15	0.1	9.0	7.56
159	BWB	RT	15	1.4	7.8	4.70
160	BWB	RT	15	0.1	8.7	6.64
161	BWB	RT	15	0.8	7.0	3.45
162	BWB	RT	15	1.2	7.3	3.84
163	BWB	RT	15	2.4	8.6	6.47
164	BWB	RT	15	2.3	8.3	5.40
165	BWB	RT	15	0.3	7.2	4.06
166	BWB	RT	15	0.6	10.1	10.68
167	BWB	RT	15	1.3	10.5	10.7
168	F2	RT	18	1.7	9.1	7.33

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
169	F2	RT	18	1.0	9.9	9.47
170	F2	RT	18	2.4	8.5	6.07
171	F2	RT	18	1.6	9.2	7.29
172	F2	RT	18	3.0	8.2	5.53
173	F2	RT	18	3.3	9.2	7.09
174	F2	RT	18	1.4	10.1	9.63
175	F2	RT	18	1.8	8.8	6.37
176	F2	RT	18	1.4	9.9	9.23
177	F2	RT	18	1	11.1	13.31
178	F2	RT	18	1.4	8.1	5.29
179	F2	RT	18	1.8	10.7	11.73
180	BWB	RT	15	3.7	10.4	9.85
181	BWB	RT	15	1.2	8.4	5.47
182	BWB	RT	15	1.6	11.7	15.83
183	BWB	RT	15	1.0	9.3	7.67
184	BWB	RT	15	2.4	11.9	15.39
185	BWB	RT	15	2.6	9.5	7.43
186	BWB	RT	15	5.1	8.5	5.97
187	BWB	RT	15	3.6	7.4	3.82
188	BWB	RT	15	1.3	9.0	7.25
189	BWB	RT	15	1.3	8.1	5.31
190	BWB	RT	15	1.7	9.3	7.82
191	BWB	RT	15	2.5	7.2	4.15
192	BWB	RT	18	1.8	10.5	9.47
193	BWB	RT	18	2.4	8.0	4.81
194	BWB	RT	18	2.2	8.8	6.08
195	BWB	RT	18	1.2	9.0	6.99
196	BWB	RT	18	0.9	9.2	6.70
197	BWB	RT	18	2.6	7.9	4.36
198	BWB	RT	18	3.4	9.1	6.57
199	BWB	RT	18	4.5	9.1	6.79
200	BWB	RT	18	3.0	9.3	6.20
201	BWB	RT	18	3.1	8.3	4.98
202	BWB	RT	18	1.9	7.8	4.60
203	BWB	RT	18	1.5	10.0	9.37
204	TZB	RT	15	1.0	9.7	8.03
205	TZB	RT	15	1.2	12.5	18.10

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
206	TZB	RT	15	1.3	10.3	10.67
207	TZB	RT	15	0.2	8.4	5.63
208	TZB	RT	15	0.8	9.2	7.10
209	TZB	RT	15	1.0	9.3	7.06
210	TZB	RT	15	2.1	7.9	4.50
211	TZB	RT	15	2.7	9.5	8.01
212	TZB	RT	15	2.3	7.9	4.40
213	TZB	RT	15	0.3	8.0	4.30
214	TZB	RT	15	1.0	9.9	8.71
215	TZB	RT	15	1.1	8.9	6.41
216	BW	RT	18	1.1	8.5	5.50
217	BW	RT	18	2.0	6.9	3.53
218	BW	RT	18	1.4	10.5	12.34
219	BW	RT	18	4.0	10.1	7.68
220	BW	RT	18	2.6	9.0	6.21
221	BW	RT	18	0.9	8.5	6.41
222	BW	RT	18	3.1	7.5	4.01
223	BW	RT	18	3.2	7.9	5.07
224	BW	RT	18	2.9	8.6	6.56
225	BW	RT	18	2.8	11.5	14.51
226	BW	RT	18	1.3	10.9	13.14
227	BW	RT	18	2.1	11.5	14.88
228	BW	RT	18	1.6	10.1	9.76
229	BW	RT	18	1.7	11.4	12.02
230	BW	RT	18	1.9	11.3	11.32
231	BW	RT	18	4.4	9.4	6.96
232	BW	RT	18	1.7	9.9	9.20
233	BW	RT	18	2.2	9.3	7.33
234	BW	RT	18	1.6	9.6	7.82
235	BW	RT	18	3.5	10.2	9.56
236	BW	RT	18	1.4	9.4	8.05
237	BW	RT	18	2.7	10.2	9.54
238	BW	RT	18	1.8	8.9	6.56
239	BW	RT	18	1.7	10.3	10.63
240	CCT	WSCT	15	0.6	7.8	4.35
241	CCT	WSCT	15	0.8	6.9	3.30
242	CCT	WSCT	15	1.4	6.6	2.60

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
243	CCT	WSCT	15	0.5	6.8	3.06
244	CCT	WSCT	15	1.7	7.0	3.37
245	CCT	WSCT	15	1.5	7.0	2.96
246	CCT	WSCT	15	1.2	6.6	3.12
247	CCT	WSCT	15	2.9	7.7	4.07
248	CCT	WSCT	15	0.5	6.8	2.96
249	CCT	WSCT	15	1.2	7.5	3.80
250	CCT	WSCT	15	2.2	6.1	2.31
251	CCT	WSCT	15	1.2	7.3	3.82
252	CCT	WSCT	15	2.1	6.6	3.02
253	CCT	WSCT	15	0.8	6.8	2.92
254	CCT	WSCT	15	0.3	7.6	4.40
255	CCT	WSCT	15	1.3	7.8	4.8 0
256	CCT	WSCT	15	1.6	6.5	2.32
257	CCT	WSCT	15	-0.1	8.0	4.99
258	CCT	WSCT	15	2.0	7.5	4.10
259	CCT	WSCT	15	2.3	10.0	10.69
260	CCT	WSCT	15	2.0	7.5	4.14
261	CCT	WSCT	15	0.0	7.8	4.49
262	CCT	WSCT	15	0.9	8.1	7.19
263	CCT	WSCT	15	0.4	8.3	5.85
264	CCT	WSCT	15	0.5	7.4	4.08
265	CCT	WSCT	15	0.1	9.0	7.66
266	CCT	WSCT	15	1.2	6.6	2.58
267	CCT	WSCT	15	1.1	7.2	3.96
268	CCT	WSCT	15	0.0	7.1	4.29
269	CCT	WSCT	15	0.2	6.8	3.33
270	CCT	WSCT	15	1.6	7.0	3.86
271	CCT	WSCT	15	2.0	6.4	2.40
272	CCT	WSCT	15	0.2	7.0	3.06
273	CCT	WSCT	15	-0.5	7.5	4.72
274	CCT	WSCT	15	1.9	7.0	3.57
275	CCT	WSCT	15	0.1	6.7	2.37
276	TZ	RT	18	3.1	9.4	6.36
277	TZ	RT	18	1.6	9.5	7.83
278	TZ	RT	18	1.4	8.7	6.48
279	TZ	RT	18	1.2	8.6	6.00

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
280	TZ	RT	18	1.4	11.0	11.19
281	TZ	RT	18	1.8	9.2	7.25
282	TZ	RT	18	4.8	10.2	9.02
283	TZ	RT	18	4.2	9.1	6.70
284	TZ	RT	18	2.0	9.4	7.24
285	TZ	RT	18	1.2	10.3	10.15
286	TZ	RT	18	2.8	9.6	8.55
287	TZ	RT	18	2.2	11.1	12.68
288	TZB	RT	18	2.2	10.3	10.70
289	TZB	RT	18	0.6	9.6	9.29
290	TZB	RT	18	0.9	11.3	13.58
291	TZB	RT	18	1.7	9.0	7.36
292	TZB	RT	18	1.3	10.0	10.10
293	TZB	RT	18	1.0	10.1	10.98
294	TZB	RT	18	2.6	9.1	6.91
295	TZB	RT	18	3.3	10.5	9.66
296	TZB	RT	18	2.2	10.5	11.92
297	TZB	RT	18	3.0	10.1	10.56
298	TZB	RT	18	3.0	8.7	6.17
299	TZB	RT	18	1.1	9.9	9.16
300	CCT	WSCT	18	2.5	7.7	4.53
301	CCT	WSCT	18	1.5	6.9	3.43
302	CCT	WSCT	18	1.7	7.1	3.68
303	CCT	WSCT	18	2.3	7.0	3.53
304	CCT	WSCT	18	1.9	8.9	7.13
305	CCT	WSCT	18	1.6	7.8	4.67
306	CCT	WSCT	18	1.8	7.0	3.66
307	CCT	WSCT	18	2.8	6.8	3.39
308	CCT	WSCT	18	1.7	8.3	5.92
309	CCT	WSCT	18	1.6	7.4	4.23
310	CCT	WSCT	18	3.6	6.8	3.37
311	CCT	WSCT	18	1.8	7.4	4.27
312	TZ	RT	15	1.5	11.2	11.66
313	TZ	RT	15	1.9	10.5	12.20
314	TZ	RT	15	1.0	10.9	13.37
315	TZ	RT	15	1.1	11.3	13.02
316	TZ	RT	15	2.1	10.1	9.45

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
317	TZ	RT	15	2.1	10.4	11.33
318	TZ	RT	15	1.7	9.3	8.01
319	TZ	RT	15	1.5	9.5	8.66
320	TZ	RT	15	2.1	10.1	10.31
321	TZ	RT	15	1.8	10.4	9.97
322	TZ	RT	15	2.2	11.3	14.30
323	TZ	RT	15	1.8	11.2	13.12
324	CCT	WSCT	18	1.8	6.2	2.18
325	CCT	WSCT	18	1.9	7.2	4.25
326	CCT	WSCT	18	1.6	8.0	5.63
327	CCT	WSCT	18	0.6	7.9	4.74
328	CCT	WSCT	18	1.1	7.4	4.47
329	CCT	WSCT	18	2.5	7.1	3.94
330	CCT	WSCT	18	3.0	7.4	4.94
331	CCT	WSCT	18	1.8	9.6	10.75
332	CCT	WSCT	18	0.8	7.2	4.18
333	CCT	WSCT	18	2.5	6.4	3.20
334	CCT	WSCT	18	2.8	6.9	4.15
335	CCT	WSCT	18	1.4	8.0	5.92
336	CCT	WSCT	18	2.1	7.6	4.01
337	CCT	WSCT	18	1.2	8.5	6.68
338	CCT	WSCT	18	1.5	9.1	10.03
339	CCT	WSCT	18	1.7	7.2	4.02
340	CCT	WSCT	18	2.3	6.5	3.01
341	CCT	WSCT	18	1.5	6.6	2.72
342	CCT	WSCT	18	2.5	7.9	5.16
343	CCT	WSCT	18	1.4	7.2	5.36
344	CCT	WSCT	18	2.4	6.3	2.41
345	CCT	WSCT	18	0.9	7.4	4.66
346	CCT	WSCT	18	1.8	8.9	7.84
347	CCT	WSCT	18	2.7	6.2	2.99
348	BWB	RT	15	0.1	11.3	16.87
349	BWB	RT	15	2.0	10.4	11.93
350	BWB	RT	15	1.6	10.3	12.36
351	BWB	RT	15	-0.1	10.0	11.45
352	BWB	RT	15	1.1	9.5	9.10
353	BWB	RT	15	-0.1	10.3	11.91

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
354	BWB	RT	15	0.1	9.7	10.64
355	BWB	RT	15	0.3	9.8	11.20
356	BWB	RT	15	1.8	11.5	16.14
357	BWB	RT	15	2.8	10.1	11.60
358	BWB	RT	15	2.5	9.5	9.17
359	BWB	RT	15	1.2	9.6	9.35
360	BWB	RT	18	2.2	9.3	8.43
361	BWB	RT	18	1.7	9.6	9.78
362	BWB	RT	18	1.4	9.1	8.63
363	BWB	RT	18	1.1	9.7	10.27
364	BWB	RT	18	1.9	10.0	10.42
365	BWB	RT	18	2.2	10.0	10.88
366	BWB	RT	18	3.3	9.4	9.19
367	BWB	RT	18	3.1	8.2	6.24
368	BWB	RT	18	2.0	10.8	12.57
369	BWB	RT	18	0.9	9.5	9.47
370	BWB	RT	18	1.8	8.2	5.82
371	BWB	RT	18	1.0	9.1	7.71
372	BWB	RT	18	2.9	10.8	13.33
373	BWB	RT	18	1.7	9.4	8.41
374	BWB	RT	18	2.1	9.8	10.37
375	BWB	RT	18	1.4	10.0	10.64
376	BWB	RT	18	1.4	9.9	10.90
377	BWB	RT	18	1.2	10.4	12.28
378	BWB	RT	18	2.4	9.3	9.32
379	BWB	RT	18	2.5	10.2	11.31
380	BWB	RT	18	2.0	8.8	8.10
381	BWB	RT	18	1.2	9.8	10.57
382	BWB	RT	18	1.6	8.4	7.07
383	BWB	RT	18	3.0	7.9	5.71
384	TZB	RT	18	2.5	9.0	8.22
385	TZB	RT	18	1.2	8.9	7.99
386	TZB	RT	18	1.5	9.0	7.69
387	TZB	RT	18	3.1	11.1	14.60
388	TZB	RT	18	1.2	9.1	8.05
389	TZB	RT	18	1.5	10.4	12.76
390	TZB	RT	18	1.6	9.0	7.76

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
391	TZB	RT	18	2.7	9.3	9.02
392	TZB	RT	18	2.5	9.6	9.88
393	TZB	RT	18	2.4	8.9	7.76
394	TZB	RT	18	2.1	9.8	10.46
395	TZB	RT	18	1.2	10.8	14.14
396	TZB	RT	18	0.6	9.9	10.86
397	TZB	RT	18	1.4	8.9	8.64
398	TZB	RT	18	0.5	8.4	7.71
399	TZB	RT	18	1.4	10.5	12.23
400	TZB	RT	18	1.7	7.7	5.49
401	TZB	RT	18	1.6	10.0	12.04
402	TZB	RT	18	3.0	9.1	7.74
403	TZB	RT	18	1.1	9.6	10.08
404	TZB	RT	18	0.7	9.3	9.30
405	TZB	RT	18	3.4	9.9	10.56
406	TZB	RT	18	1.3	8.4	6.47
407	TZB	RT	18	1.9	9.4	8.73
408	TZB	RT	15	1.6	9.9	10.70
409	TZB	RT	15	1.8	9.8	11.06
410	TZB	RT	15	1.6	10.0	13.25
411	TZB	RT	15	2.0	11.5	17.32
412	TZB	RT	15	1.8	9.8	10.80
413	TZB	RT	15	2.1	11.4	16.40
414	TZB	RT	15	2.8	9.6	10.48
415	TZB	RT	15	1.6	9.9	10.92
416	TZB	RT	15	1.6	11.4	16.43
417	TZB	RT	15	1.4	9.0	8.96
418	TZB	RT	15	1.9	10.4	13.05
419	TZB	RT	15	2.8	9.5	10.21
420	TZB	RT	15	1.3	8.7	8.81
421	TZB	RT	15	2.5	10.2	12.44
422	TZB	RT	15	1.6	10.0	10.53
423	TZB	RT	15	1.1	9.8	10.88
424	TZB	RT	15	1.6	9.5	9.70
425	TZB	RT	15	1.2	9.0	9.12
426	TZB	RT	15	2.9	9.4	10.40
427	TZB	RT	15	2.7	10.2	12.23

Fish Number	Test Group	Species	Acc. Temp °C	Ctmin °C	Fork Length (cm)	Mass (g)
428	TZB	RT	15	2.3	9.0	9.64
429	TZB	RT	15	1.6	8.7	8.18
430	TZB	RT	15	2.7	8.6	7.47
431	TZB	RT	15	2.6	9.8	11.48

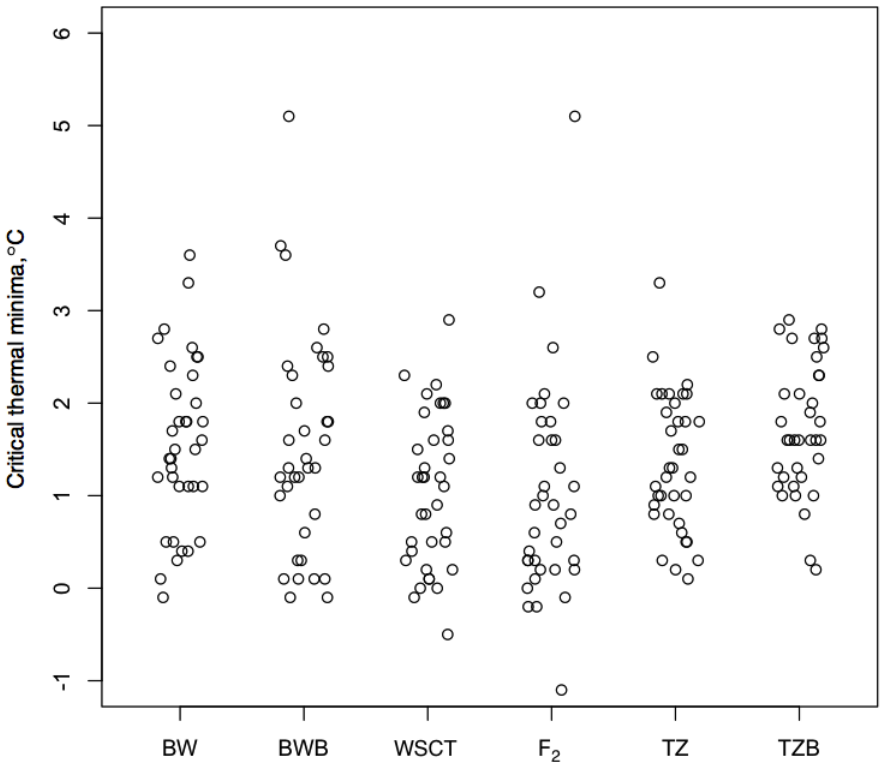

Figure B.1: Spread of CTMin data of trout test groups acclimated at 15 °C. Blackwater River rainbow trout (BW); Blackwater River backcross (BWB); westslope cutthroat trout (WSCT); Blackwater River x Tzenzaicut Lake F_1 hybrid x Blackwater River x Tzenzaicut Lake F_1 hybrid (F_2); Tzenzaicut Lake rainbow trout (TZ); Tzenzaicut Lake backcross (TZB).

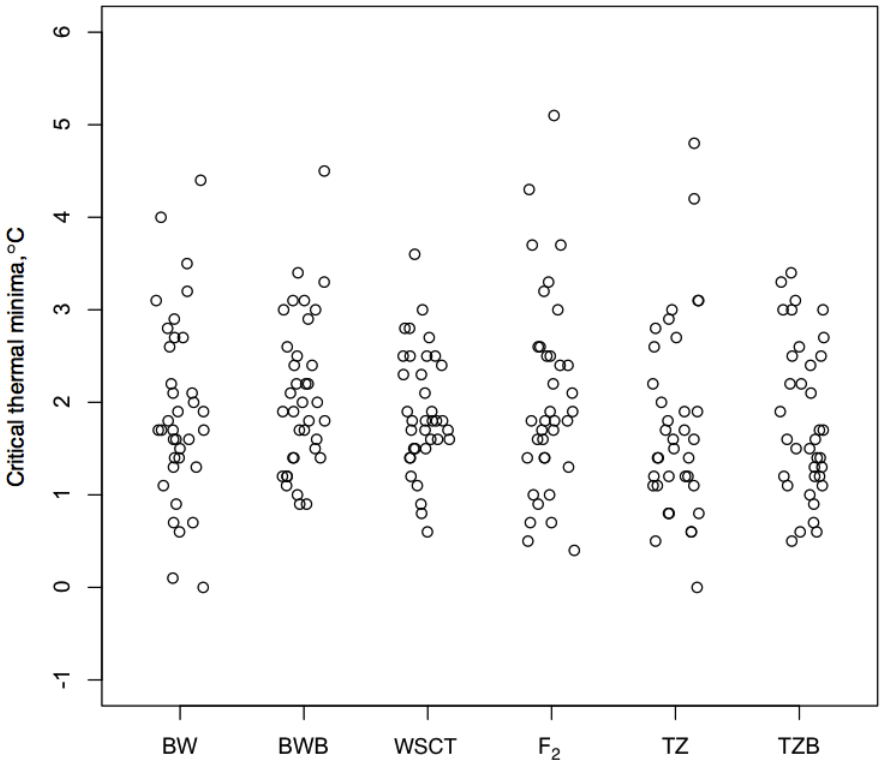

Figure B.2: Spread of CTMin data of trout test groups acclimated at 18 °C. Blackwater River rainbow trout (BW); Blackwater River backcross (BWB); westslope cutthroat trout (WSCT); Blackwater River x Tzenzaicut Lake F_1 hybrid x Blackwater River x Tzenzaicut Lake F_1 hybrid (F_2); Tzenzaicut Lake rainbow trout (TZ); Tzenzaicut Lake backcross (TZB).