

BEHAVIORAL, ECOLOGICAL, AND FITNESS CONSEQUENCES OF HYBRIDIZATION BETWEEN
NATIVE WESTSLOPE CUTTHROAT TROUT (*Oncorhynchus clarkii lewisi*)
AND NONNATIVE RAINBOW TROUT (*O. mykiss*)

by

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ABSTRACT

Anthropogenic hybridization is one of the greatest threats to global biodiversity. Hybridization and introgression may lead to a loss of locally adapted gene complexes and ecological adaptations in native populations, yet these potential consequences have not been fully evaluated in nature. I investigated factors influencing the spread of hybridization between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and nonnative rainbow trout (*O. mykiss*) in the upper Flathead River system, Montana (USA) and British Columbia (Canada). The fundamental questions of my dissertation were: what are the behavioral, ecological, and fitness consequences of hybridization and what factors influence successful invasion of hybrids? First, I assessed the patterns of spawning between parental species and their hybrids and found that hybridization alters the spawning behavior of migratory westslope cutthroat trout, and is spreading via long distance dispersal of hybrids from downstream sources and some temporal overlap during spawning. Second, I describe for the first time how a wide range of levels of nonnative admixture affect fitness of cutthroat trout in the wild by estimating reproductive success in a recently invaded stream using parentage analysis with multilocus microsatellite markers. Small amounts of hybridization markedly reduced reproductive success, with fitness exponentially declining by ~50% with 20% nonnative genetic admixture. Finally, I evaluated the association of local-habitat features, landscape characteristics, and biotic factors with the spread of hybridization in the system, and found that hybridization increases in streams with warmer water temperatures, high land use disturbance and close proximity to the source of hybridization; however, none of these factors appeared sufficient to prevent further spread. These combined results suggest that hybrids are not only genetically different than westslope cutthroat trout but also have reduced fitness and are ecologically different, and that hybridization is likely to continue to spread if hybrid populations with high amounts of rainbow trout admixture are not reduced or eliminated. I conclude that extant aboriginal cutthroat trout are at greater conservation risk due to hybridization than previously thought and policies that protect hybridized populations need reconsideration.

CHAPTER 1

INTRODUCTION TO DISSERTATION

IntroductionSpecies Introductions

Introductions of exotic species threaten the biodiversity of native faunas throughout the world. The genomic extinction of many rare and endangered plant and animal taxa are often a direct result of anthropogenic introductions of exotic species into novel environments (Allan and Flecker 1993). Introduced species may negatively impact native biota through competition, predation, spread of disease and parasites, introgressive hybridization, and extirpation of populations (Mack et al. 2000).

Freshwater fish are likely the most threatened group of vertebrates other than amphibians in North America (Ricciardi and Rasmussen 1999). Miller et al. (1989) estimated that exotic species introductions were a factor in 68% of fish extinctions in North America. Introduced fishes have impacted nearly every major watershed in the United States (Courtenay et al. 1984), and the rate of introductions has increased dramatically in the last 50 years (Fuller et al. 1999). In many waters, fisheries management programs have transplanted nonnative sportfish to provide recreational opportunities. Additionally, many other aquatic organisms have been transported via ballast water in ships (e.g., zebra mussel, *Dreissena polymorpha*), aquarium releases,

and illegal translocations (Fuller et al. 1999). Regardless of the cause of species introductions, the establishment and proliferation of invasive species often results in the decline of native taxa and the modification of aquatic communities.

Hybridization between Native and Nonnative Species

Hybridization between native and nonnative taxa is a major consequence of species introductions. Hybridization and introgression often lead to the loss of locally adapted gene complexes and ecological adaptations in native populations, which threaten the persistence of many rare and endangered species (Rhymer and Simberloff 1996). The increasing rate of hybridization has further been exacerbated by habitat modification, suggesting that this problem will become more serious with increasing land-use practices and global climate change. Thus, an understanding of the genetic, behavioral, and ecological mechanisms that influence hybridization with nonnative taxa is critical for conservation and recovery of native species.

Although natural hybridization may lead to evolutionary novelty and speciation (Arnold 1997; Grant and Grant 1992), anthropogenic hybridization can compromise the genetic integrity of native species to the point of causing widespread introgression and extinction (Allendorf et al. 2001). Hybridization has been a major factor in 38% of fish extinctions in North America (Miller et al. 1989). For example, all subspecies of cutthroat trout, *Onchorynchus clarkii*, have been affected by intra and interspecific hybridization with introduced fishes (Gresswell 1988; Trotter 2008), resulting in most

non-hybridized populations being confined to headwater areas above barriers to migration.

The impact that hybridization will have on an ecosystem and its native organisms will primarily be related to the fitness of the hybrid offspring and the particular situation. If hybrid offspring are fertile, genetic introgression may occur when hybrids mate with first generation (F_1) progeny and/or backcrosses with either parental type. This situation often produces hybrid swarms or populations in which nearly all individuals possess a random assortment of alleles from the parental taxa. Alternatively, hybrids may not be fertile. In these cases, hybridization does not result in genetic introgression but there may still be the negative cost of wasted reproductive effort due to the production of unviable or infertile offspring. For example, bull trout *Salvelinus confluentus* were nearly replaced by nonnative brook trout *Salvelinus fontinalis* in a Montana stream due to the wastage of reproductive effort (Leary et al. 1993). Conversely, hybrids may be more fit than the parental taxa due to increases in genetic variation or formation of novel genotypes allowing exploitation of unoccupied niches (Grant and Grant 1992; Rhymer and Simberloff 1996; Reiseberg et al. 2003; Fitzpatrick and Shaffer 2007). Finally, some have suggested that hybrids may be selected against only during periodic episodes of extreme environmental conditions (Leary et al. 1995; Allendorf et al. 2004).

Local adaptation is defined as the selection for traits within a population that enhance survival and reproductive success in response to a particular environment

(Dobzhansky 1970). Hybridization may lead to the loss of local adaptations in native populations due to outbreeding depression (Allendorf et al. 2001), which is the reduction in fitness in progeny from hybrid crosses, compared to progeny from crosses between individuals from the same population (Templeton 1986). Gilk et al. (2004) found that outbreeding depression in hybrids from geographically isolated populations of pink salmon *O. gorbuscha* resulted in the erosion of fitness-related traits, including homing ability and family size.

Outbreeding depression may be either intrinsic or extrinsic. Intrinsic outbreeding depression results from genetic or chromosomal incompatibilities between the two hybridizing taxa and leads to the disruption or loss of co-adapted gene complexes (Dobzhansky 1970). An example is the data of Allendorf and Leary (1988) that demonstrated F₁ hybrids between westslope cutthroat trout (*O. c. lewisi*) and rainbow trout (*O. mykiss*) displayed slower growth and survival to 112 days post-fertilization under laboratory conditions. Extrinsic outbreeding depression is a reduction in fitness in hybrids due to environmental and ecological factors (Templeton 1986). A possible example of this may be the data of Philipp and Whitt (1991) that demonstrated hybrids between northern largemouth bass *Micropterus salmoides salmoides* and Florida largemouth bass *M. s. floridanus* had reduced survival and growth when raised in Illinois ponds.

Hybridization and Westslope Cutthroat Trout

Compared to other factors, introductions of nonnative fishes may have had the greatest effect on the decline in the distribution, abundance, and genetic diversity of native salmonid species and populations (Behnke 1992). Hybridization with nonnative taxa has been shown to reduce reproductive potential (Kitano et al. 1994) or lead to widespread introgression (Allendorf et al. 2001). Consequently, hybrid trout populations are common in many drainages in the western United States (e.g., Missouri River drainage; Liknes and Graham 1988).

The cutthroat trout *O. c. spp.* is a polytypic species native to waters in western North America exhibiting a high degree of genetic differentiation between subspecies and populations (Allendorf and Leary 1988). There are 14 recognized subspecies of cutthroat trout (Behnke 1992) and all of these except the coastal cutthroat trout *O. c. clarkii* are collectively referred to as interior cutthroat trout. Although habitat degradation and fragmentation have contributed to declines in most of the subspecies, widespread competition and genetic introgression with introduced salmonids pose significant threats to the persistence of native cutthroat trout (Allendorf and Leary 1988; Gresswell 1988; Trotter 2008). Introgression poses a serious threat to all subspecies of cutthroat trout, with two subspecies now extinct, five listed as threatened under the U. S. Endangered Species Act (ESA), and seven petitioned for listing (Trotter 2008).

Widespread transplanting of rainbow trout has had a greater impact on native cutthroat trout than any other fish introduction in North America. Since 1877, the range

of rainbow trout has been artificially expanded from western North America to include waters on all continents except Antarctica (MacCrimmon 1971; Behnke 1992).

Introductions of rainbow trout into inland drainages of the western United States has had significant impacts on indigenous cutthroat trout resulting from competition, exposure to whirling disease and widespread genetic introgression (Allendorf and Leary 1988; Fuller et al. 1999). MacCrimmon (1971) concluded that cutthroat trout have experienced local extirpations, extinctions, or reductions in abundance in nearly all drainages where rainbow trout have been introduced.

Westslope cutthroat trout are a monophyletic lineage that has been evolutionarily isolated from other taxa for 1-2 million years (Allendorf and Leary 1988; Figure 1.1). The westslope cutthroat trout is one of four major subspecies of cutthroat trout, and is genetically highly divergent from the other three major subspecies: the coastal *O.c. clarki*, Yellowstone *O.c. bouvieri*, and Lahontan *O.c. henshawi* cutthroat trout (Leary et al. 1987; Allendorf and Leary 1988; Behnke 1992). The geographic range of westslope cutthroat trout is the largest of the interior subspecies, and encompasses the Columbia, Fraser, Missouri, and Hudson Bay drainages of the United States and Canada.

The introduction of rainbow trout and subsequent hybridization with native westslope cutthroat trout threatens this highly divergent subspecies with genomic extinction (Allendorf and Leary 1988; Hitt et al. 2003; Rubidge and Taylor 2004).

Shepard et al. (2005) estimated that non-hybridized populations of westslope cutthroat

trout have drastically declined and occupy less than 10% of their historical range. Many geographically isolated populations remain in headwater reaches; however, their long term persistence is uncertain due to the deleterious effects associated with small population size and restricted habitat (Hilderbrand & Kershner 2000; Wofford et al. 2005). Further, inclusion of hybridized populations as westslope cutthroat trout for ESA listing considerations (Department of Interior 2003) may threaten the long term persistence of this species by protecting hybrids and allowing further introgression (Allendorf et al. 2004).

If hybridization is human-mediated, as is usually the case in westslope cutthroat trout, how much introgression is acceptable until the subspecies is no longer a subspecies? This is the focus of the debate on whether to include hybridized westslope cutthroat trout populations under the ESA (Allendorf et al. 2001; Allendorf et al. 2004; Allendorf et al. 2005; Campton and Kaeding 2005). Further, should hybridized populations be protected by state and federal agencies? In some systems, hybrid source populations threaten the long term persistence of non-hybridized westslope cutthroat trout populations and may thus be targeted for suppression or eradication. Conversely, fisheries managers may wish to protect slightly introgressed populations if they represent the only remaining sources of westslope cutthroat trout genotypes in a particular drainage. This dissertation addresses these questions by simultaneously examining genetic, demographic, and ecological characteristics of non-hybridized and hybrid fish in the natural environment. These data may be used to identify the level of

anthropogenic introgression that is acceptable to maintain and protect the evolutionary characteristics of native westslope cutthroat trout populations.

Hybridization in the Upper Flathead River System

Although the upper Flathead River system in Montana is considered a regional stronghold for westslope cutthroat trout, the long term persistence of these populations is threatened by the continued spread of introgression with nonnative rainbow trout (Hitt et al. 2003; Boyer et al. 2008). Previous studies have examined the spatial and temporal patterns of hybridization between westslope cutthroat trout and rainbow trout in streams of the upper Flathead River system using molecular DNA techniques (Hitt et al. 2003; Boyer et al. 2008). Temporal analyses revealed that hybridization was detected at 57% of the sites sampled from 1998 to 2001, and new rainbow trout introgression was documented in 8 of 11 (73%) sites that were determined to be non-hybridized in 1988. The spatial distribution of hybrid populations, amounts of admixture, and multilocus genotypes indicated that hybridization is spreading among sites in an upstream direction following a stepping-stone model (Hitt et al. 2003; Boyer et al. 2008) and is advancing primarily by post F_1 hybrids from source streams in the lower river (Abbot Creek; Figure 2.1). Rubidge and Taylor (2004 and 2005) reported similar results in the upper Kootenay River, Canada. Both of these studies concluded that ecological and demographic factors were probably responsible for the spread of hybridization but what these factors are remain poorly understood in the natural environment.

The upper Flathead River system provides a unique opportunity to examine hybridization in the natural environment. Unlike other systems that have already experienced widespread introgression, non-hybridized westslope cutthroat trout populations are still common in the Flathead drainage but introgression appears to be spreading rapidly (Hitt et al. 2003). The objectives of my dissertation are to: (1) compare the spatial and temporal spawning dynamics of westslope cutthroat trout, rainbow trout, and their hybrids in the upper Flathead River system; (2) quantify the relative fitness of parental types and hybrids with various proportions of rainbow trout admixture; and (3) identify environmental and ecological factors influencing the spatial and distribution of hybridization in the system. Results will be used to inform conservation and management programs aimed at restoring and protecting existing westslope cutthroat trout populations and other native plant and animal taxa.

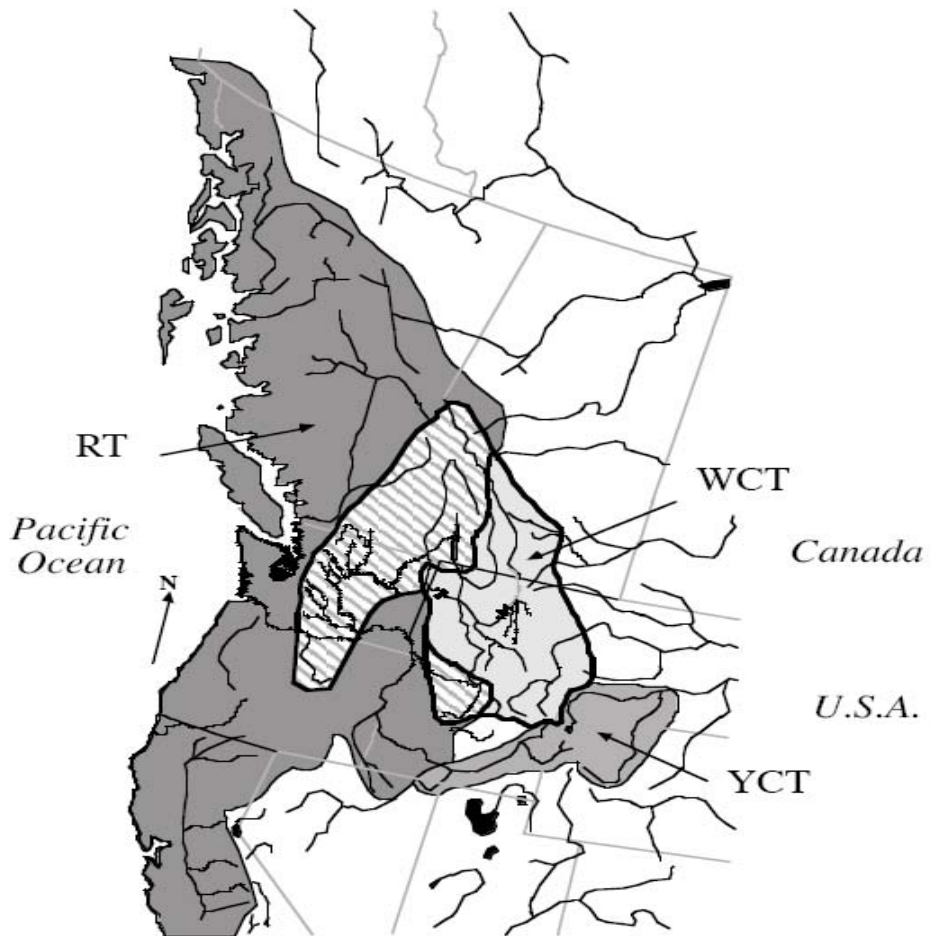


Figure 1.1. Historical distribution of *Oncorhynchus mykiss* (RT), *O. clarkii bouvieri* (YCT), and *O. c. lewisi* (WCT). Cross-hatched area represents the region of natural sympatry between WCT and RT (Adapted from Fred Allendorf).

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CHAPTER 2

SPATIAL AND TEMPORAL DYNAMICS OF SPAWNING BETWEEN NATIVE WESTSLOPE CUTTHROAT TROUT, INTRODUCED RAINBOW TROUT, AND THEIR HYBRIDS

Abstract

Populations of many native salmonids in western North America are threatened by introgression with introduced rainbow trout (*Oncorhynchus mykiss*; RBT), yet little is known about the reproductive factors influencing the spread of hybridization in the natural environment. I used radiotelemetry to assess spatial and temporal spawning distributions of native westslope cutthroat trout (*O. clarkii lewisi*; WCT; $N = 27$), introduced RBT ($N = 51$) and their hybrids ($N = 47$) in the upper Flathead River system, Montana and British Columbia, from 2000 to 2007. Radiotagged trout moved upriver towards spawning sites as flows increased during spring runoff and spawned in 29 tributaries. WCT migrated greater distances and spawned later as flows declined, whereas RBT and RBT-hybrids (backcrosses to RBT) generally spawned earlier than WCT and in low elevation streams. WCT-hybrids (backcrosses to WCT) spawned intermediately in time and space to WCT and RBT and RBT-hybrids. Both hybrid groups and RBT, however, spawned over time periods that produced temporal overlap with spawning WCT in most years. In two streams, there was spatial overlap in spawning between WCT and F_1 hybrids. In two other streams, RBT spawned in the same areas used by WCT. Abbot Creek, which is in the lower portion of the drainage, supported a

relatively high proportion (47%) of spawning by RBT and RBT-hybrids, indicating that this location is likely the main source of introgression in the area. The spatial and temporal patterns of movement indicate hybridization is spreading upstream by long distance dispersal of a few individuals with high amounts of RBT admixture. The spatial distribution of later generation backcrosses, however, suggests a stepping-stone form of invasion may also be an important mechanism for spreading nonnative genes at small scales. These observations corroborate conclusions from previous genetic studies. My data suggest that: (1) spatial and temporal overlap is occurring in the lower drainage; (2) introgression alters spawning behavior compared to migratory WCT; and (3) the spread of hybridization is likely to continue if hybrid populations, especially those with high amounts of RBT admixture, are not reduced or eliminated.

Introduction

Introductions of exotic species threaten the biodiversity of aquatic ecosystems throughout the world (Mack et al. 2000). Invasive species have altered the evolutionary pathway of native species through competitive exclusion, niche displacement, disease and parasites, hybridization, predation and ultimately extinction (Mooney and Cleland 2001). Hybridization between introduced and native taxa can be a major consequence of species introductions, especially in circumstances where nonnative species hybridize with rare or endangered species, threatening their persistence (Rhymer and Simberloff 1996).

Hybridization with introduced species has been a major factor in the decline and extinction of many native fishes throughout North America (Miller et al. 1989; Ricciardi and Rasmussen 1999). Although natural hybridization has led to evolutionary novelty and speciation in many plants and some vertebrate species (Stebbins 1950; Arnold 1997; Barton 2001), anthropogenic hybridization and introgression can lead to the extinction of native genotypes and the loss of locally adapted gene complexes and ecological adaptations (Rhymer and Simberloff 1996; Allendorf et al. 2001). Therefore, understanding genetic and ecological mechanisms that permit and constrain hybridization is important for developing effective recovery and management programs for native species at risk of hybridization with introduced taxa.

Hybridization may lead to the loss of behavioral and ecological adaptations in salmonid populations (Allendorf and Leary 1988; Rhymer and Simberloff 1996; Hedrick and Kalinowski 2000). Reproductive behavior is intimately linked to salmonid life histories which represent long term adaptations to the natural environment. Thus, reproductive behavior is considered to be a critical trait influencing the persistence of native salmonid populations. The consequences of human mediated hybridization on salmonid reproductive behavior, however, are poorly understood in the natural environment.

Introgressive hybridization with introduced rainbow trout (*Oncorhynchus mykiss*; RBT) is considered one of the greatest threats facing many salmonid populations (Behnke 1992; Leary et al. 1995). Widespread transplanting of RBT outside their native

range has caused local extirpations or reductions of native salmonids in nearly all drainages where they have been introduced (Courtenay et al. 1984). Along with habitat loss and degradation, introgressive hybridization with RBT has played a major role in the demise of all subspecies of cutthroat trout (*O. clarkii* spp.; Allendorf and Leary 1988; Gresswell 1988; Dowling and Childs 1992; Carmichael et al. 1993; Young 1995; Duff 1996; Hitt et al. 2003; Peacock and Kirchoff 2004).

Hybridization and introgression with RBT threatens the westslope cutthroat trout (*O. clarkii lewisii*; WCT) with genomic extinction (Allendorf and Leary 1988; Allendorf et al. 2004). WCT are highly divergent from the other major cutthroat trout subspecies (coastal *O. c. clarki*, Yellowstone *O. c. bouvieri*, and Lahontan *O. c. henshawi* cutthroat trout; Loudenslager and Gall 1980; Leary et al. 1987; Allendorf and Leary 1988; Behnke 1992) and their geographic range is the largest of the 13 interior subspecies. It encompasses the Columbia, Fraser, Missouri, and Hudson Bay drainages of the United States and Canada (Behnke 1992; Figure 1.1). WCT historically occupied a wide variety of aquatic habitats, from small headwater streams to large rivers and lakes. However, non-hybridized populations of WCT currently inhabit less than 10% of their historic range in the United States (Shepard et al. 2005). Many of the remaining non-hybridized populations are restricted to headwater areas above upstream dispersal barriers (Shepard et al. 2005). Consequently, hybrid forms are the dominant taxa in most drainages where WCT were native.

The timing and location of breeding are the primary isolating mechanisms between naturally sympatric salmonid taxa (Trotter 1989). The rapid increase of human mediated hybridization in many salmonid species (Busack and Gall 1981; Kitano et al. 1994; Hitt et al. 2003) suggests little reproductive segregation exists between naturally allopatric taxa. Although WCT coevolved with resident and anadromous forms of RBT in portions of the Columbia River basin (Behnke 1992), either spatial or temporal reproductive segregation has limited natural hybridization between these fishes (Campton and Utter 1985; Trotter 1989; Brown et al. 2004). In contrast, many studies have shown that introductions of nonnative RBT commonly result in widespread introgression and loss of native genotypes in WCT (Allendorf et al. 2001; Hitt et al. 2003; Rubidge and Taylor 2004; Ostberg and Rodriguez 2006) and other cutthroat trout subspecies (Gresswell 1988; Dowling and Childs 1992; Carmichael et al. 1993; Young 1995; Duff 1996; Peacock and Kirchoff 2004). The apparent lack of reproductive isolation between the introduced and native trout is likely the result of their failure to evolve isolating mechanisms in allopatry. For example, Henderson et al. (2000) and DeRito (2004) reported spatial and temporal overlap between cutthroat trout and nonnative RBT in the Snake and Yellowstone River systems, respectively. However, few studies have examined spatial and temporal dynamics of hybridization between introduced RBT and native WCT.

Although the upper Flathead River system in Montana and Canada (Figure 2.1) is considered a regional stronghold for WCT, the long term persistence of non-hybridized

populations in the drainage is threatened by a recent spread of nonnative RBT introgression (Hitt et al. 2003; Boyer et al. 2008). The drainage presents a unique opportunity to examine spawning dynamics among hybridized and non-hybridized taxa as a full range of native, nonnative and introgressed individuals are present in the system. Understanding the factors affecting reproductive isolation between introduced RBT, hybrids, and WCT in a recently invaded system will aid in the identification of the primary reproductive mechanisms in the spread of hybridization and may identify the major sources of RBT admixture in the system. To examine differences in reproductive behavior among RBT, WCT and their hybrids, I used radiotelemetry in the upper Flathead River system from 2000 to 2007. My objectives were to: (1) identify RBT and hybrid source populations in the upper Flathead River system; (2) compare the timing, location and movements of spawning by RBT, WCT, and their hybrids; and (3) describe the patterns of spatial and temporal overlap among parental taxa and hybrid types.

Methods

Study Area

The upper Flathead River drainage originates in the Rocky Mountains of British Columbia, Canada and northwestern Montana, USA, and includes Flathead Lake and the North Fork, Middle Fork, South Fork, and mainstem Flathead rivers (Figure 2.1). The drainage area is approximately 18,400 km² and is in the headwaters of the upper Columbia River basin. I implanted pre-spawning fish with radiotransmitters from the

confluence of the Middle and North Fork rivers downstream to the mainstem near Kalispell (Table 2.1; Figure 2.1). This reach supports the highest abundances of RBT x WCT fluvial hybrids in the upper river and lake system (Muhlfeld et al. 2003).

WCT exhibit both resident (remaining in natal streams throughout life) and migratory life history strategies in the Flathead Lake and River system, but migratory forms are most common (Shepard et al. 1984; Liknes and Graham 1988; Weaver and Fraley 1993; Muhlfeld et al. 2000). Migratory WCT rear in their natal stream for 1-4 years, and then migrate downstream as subadults to Flathead Lake (adfluvial) or the main stem rivers (fluvial). Adult WCT generally overwinter in the lower river or the lake, and then migrate upstream (up to 250 km, Figure 2.1) during high spring flows and spawn in tributaries. In contrast, RBT and WCT x RBT hybrids display a fluvial life history in the mainstem (this study), but little was known about their spawning behaviors prior to this study.

Timing and Location of Spawning

I used radiotelemetry to monitor the movements and spawning behavior of WCT, RBT and WCT x RBT hybrids from 2000-2007 in the upper Flathead River system, Montana (USA) and British Columbia (Canada). In 2000, 2001, 2006, and 2007, I tagged fish that visually appeared to be WCT, RBT and hybrid trout, whereas in 2002, 2003, and 2005 I exclusively targeted hybrids and RBT (Table 2.1). Morphological characters used for identification were spotting pattern, body coloration, and the presence or absence of red or orange slashes below the gill covers (Behnke 1992). Prespawning fish were

randomly captured in the mainstem Flathead River using jet boat electrofishing at night during February and early March. Captured fish that met the 2% body to transmitter:body weight ratio (Winter 1983) were anaesthetized with tricaine methane sulfonate (MS-222), surgically implanted with transmitters according to the methods described by Muhlfeld et al. (2003), held for 1-5 h after tagging, and released near their capture location. I selected individuals greater than 300 mm total length in which maturing gonads could be visually identified with an otoscope through the surgical incision. Transmitters emitted a unique code in the 148.780 MHz frequency range (with 5 second burst rates), had expected battery lives of 110 d (2000-2005) and 472 d (2006-2007), and weighed 6.7 g (air weight) and 10 g, respectively. A fin clip was collected from each fish during surgery, preserved in 95% ethanol, and analyzed at the Conservation Genetics Laboratory at the University of Montana, Missoula, to estimate degree of admixture.

Radiotagged fish were relocated three to five times per week during the spring spawning period from a jet boat, vehicle access points along the stream, and by foot using a Lotek (model SRX-400) scanning receiver equipped with an ATS 3- element Yagi antenna. Aerial surveys were conducted to locate fish that were missing for more than 1 week and to survey remote and inaccessible areas throughout the upper portions of the river system, including British Columbia (Canada), Waterton-Glacier International Peace Park, and the Bob Marshall Wilderness complex (Figure 2.1). When logistically possible, observers walked spawning streams to gain a more accurate location and to

document redd construction. Additionally, four permanent telemetry ground stations were installed near the mouths of the North Fork, Middle Fork, South Fork and mainstem Flathead River, which continuously monitored fish movements within 250 m of the antenna. Each ground station consisted of a Lotek data-logging receiver equipped with a 3-element directional Yagi antenna powered by a 12-volt deep cycle marine battery. Geo-referenced locations were obtained at each fish location using a global positioning system (GPS) unit (TSC1 Asset Surveyor, Trimble Navigation Limited, Sunnyvale, California). In a geographic information system (GIS), the point locations were overlayed on a hydrography layer for analysis of distance moved, which were calculated between consecutive locations.

I was physically unable to identify the exact timing and location of spawning for radiotagged fish due to high and turbid flows, inclement weather conditions (which precluded some aerial surveys), and the extensive size and complexity of the stream system (Figure 2.1). Therefore, I assumed that a fish had spawned if it made a pronounced (> 5 km), rapid (between consecutive relocations) movement (upriver or downriver) from its release location in the mainstem into a tributary or the mouth of a tributary or made a movement to a tributary within 5 km of its tagging location. All remaining fish were classified as nonspawners.

Spawning and movement behavior was characterized by measuring eight variables: beginning migration date, beginning migration temperature ($^{\circ}\text{C}$), beginning migration flow (m^3/s), spawning date (Julian), spawning temperature, spawning flow,

migration period (d), and migration distance (km). The beginning date of migration for each individual fish was determined as the median date between the day I first observed a fish migrating and the previous location (Swanberg 1997). Migration was defined as an upstream or downstream movement greater than 5 km from the fish's tagging location. The spawning location was defined as the farthest extent of a fish's migration, and the migration distance was defined as the fluvial distance between the location from which fish began their migration and the spawning location (Henderson et al. 2000). The migration period was defined as the time (d) from the beginning date of migration until the first relocation of the fish at or near its observed or likely spawning location. The spawning date was calculated as the midway point between migration period ending date and when it left its spawning location. The spawning period for each study group (defined below) extended from the date the first fish spawned until the last fish spawned in a particular group. Mean daily water temperature and discharge data were obtained from the U.S. Geological Survey station on the mainstem Flathead River near Columbia Falls, Montana.

Genetic Analyses

I used multiple diagnostic nuclear DNA markers to estimate the taxonomic identity of each radiotagged fish. Using this approach, non-hybridized individuals possess alleles characteristic of only one of the parental taxa at all diagnostic loci. In contrast, since half the DNA from first generation hybrids (F_1) comes from each of the parental taxa, F_1 individuals will possess alleles characteristic of both the hybridizing

taxa at all diagnostic loci. In later generation hybrids (post F_1), the amount and particular regions of DNA acquired from the parental taxa will vary among individuals in the population.

I used paired interspersed nuclear elements polymerase chain reaction (PINE PCR) techniques to assess the hybridization status of each fish tagged from 2000 through 2003 (Figure 2.2; Spruell et al. 2001; Kanda et al. 2002). Six dominant diagnostic nDNA fragments were examined for RBT and seven for WCT. For fish tagged from 2005-2007, I took advantage of a new nDNA technique that increased the ability to detect nonnative alleles in individual fish by using a combination of seven diagnostic codominant indel loci (Figure 2.2; Ostberg and Rodriguez 2004) and six diagnostic codominant microsatellite loci (R. Leary, Montana Fish, Wildlife and Parks, MFWP, unpublished data). For the PINES and Indel/microsatellite data, I calculated a hybrid index (HI) for each fish (see below). Statistical power to differentiate between parental types and early generation hybrids is high with seven diagnostic codominant markers. For example, the probability that a first generation backcross would be misclassified as an F_1 hybrid is less than 0.01 (Boecklen and Howard 1997). However, reliable discernment between parental types and later generation backcrosses requires many diagnostic loci (Floate et al. 1994). The hybrid index, therefore, likely overestimates parental types and underestimates the number of individuals of hybrid ancestry in populations containing later generation hybrids.

Fish were classified into four groups based on their HI. Individuals were classified as non-hybridized WCT if they had a HI of zero using either PINEs or indel/microsatellites. Individuals were classified as non-hybridized RBT if they had a HI of 13 (PINEs) or 26 (indel/microsatellites). F_1 hybrids were those fish with a HI of six and RBT phenotypes were detected at only the six loci where the presence of the fragment is characteristic of RBT (PINEs) and 13 (indel/microsatellite), but were heterozygous at all loci. Post F_1 hybrids with an apparently higher proportion of WCT than RBT alleles were classified as backcrosses to WCT (WCT-hybrids) and those with a higher proportion of RBT than WCT alleles were classified as backcrosses to RBT (RBT-hybrids).

Confirmation of Morphological Identification

I assessed whether morphological characteristics can be used to accurately predict the genetic status of adult fish in the field. Each fish was visually classified as either a WCT or a hybrid/RBT and then genotyped using PINEs or indel/microsats (see above) to assess the percentage of fish that were correctly classified based on their individual multilocus phenotype (PINEs) or genotype (indel/microsats). I used data collected for the years in which I simultaneously tagged WCT and hybrid/RBT spawners and nonspawners ($N = 98$ fish). Molecular analyses revealed an overall correct classification of 93%; four fish that were morphologically classified as WCT were genetically identified as WCT-hybrids and three fish visually classified to be hybrids were genetically identified as WCT.

I was unable to genotype 11 radiotagged fish. Thus, the genetic status of these fish was only visually determined. Of the fish visually classified as WCT ($N = 5$), all were estimated to have spawned in streams containing non-hybridized WCT populations (Hitt et al. 2003; Boyer et al. 2008). Similarly, of the fish visually identified as RBT ($N = 6$), all were observed spawning in Abbot Creek, a stream containing individuals with high amounts of RBT admixture (92%; Boyer et al. 2008). Thus, based on the population-level genetics data and the high accuracy of correctly classifying genetic status using morphological characteristics, I believe that the visual estimates of taxonomic identity for these 11 fish were likely reliable and unbiased.

Statistical Analyses

I used Pearson product-moment correlation analyses (two-tailed) to determine the degree of inter-correlation among the eight dependent variables (date of migration, temperature and flow at migration, migration period, spawning date, spawning temperature and flow, and migration distance). Since many of the variables were significantly ($P < 0.05$) correlated, I used multivariate analysis of variance (MANOVA) to simultaneously test for group differences on all the response variables, all of which met the assumptions of normality and homogeneity of variance (Zar 1996). First, I conducted a MANOVA to test whether there were significant effects due to genetic status, year, and sex, which were entered as fixed effects with interaction terms. Next, I conducted a separate MANOVA to assess differences among groups for the eight spawning variables. When significant differences were found ($P < 0.05$), individual

ANOVAs were used to test for differences in each spawning variable among groups, and post-hoc pairwise comparisons were conducted using Tukey's tests. ANOVA was used to test for differences in total length (mm) and weight (g) among groups followed by Tukey's tests for post-hoc comparisons. For all analyses, statistical significance was determined at the $P < 0.05$ alpha level. Statistical analyses were conducted using SPSS, Version 10.1.4 (Norusis 1990).

Results

I obtained spawning information on 27 WCT, 11 WCT-hybrids, 36 RBT-hybrids, and 51 RBT from 2000 through 2007 (Table 2.1; Table 2.2). Spawning individuals were found an average of 26 times (SD = 9) during spring (March-June). Radiotagged fish ranged from 317 to 545 mm in total length (means: WCT = 417 mm, WCT-Hybrids = 445 mm, RBT-Hybrids = 409 mm, and RBT = 404mm), and from 254 to 1,527 g in weight (means: WCT = 766 g, WCT-Hybrids = 805 g, RBT-hybrids = 674 g, and RBT = 629 g). I found significant differences in length (ANOVA, $P = 0.019$) and weight (ANOVA, $P = 0.029$) among groups. WCT-hybrids were significantly larger than RBT and RBT-hybrids ($P < 0.05$) and WCT tended to weigh more than RBT although the mean difference was nonsignificant ($P = 0.07$).

The majority of radiotagged fish made upriver migrations (98%) and spawned in a total of 29 tributaries to the mainstem, North Fork and Middle Fork Flathead River (Table 2.1; Figure 2.1). Only two fish moved downriver to access spawning tributaries to

the mainstem. There was no evidence of radiotagged fish using mainstem or side channel areas for spawning.

Behavioral Differences in Spawning and Migration

I simultaneously assessed the fixed effects of genetic group (parental and hybrid taxa), year, and sex on the eight spawning variables using MANOVA (Table 2.3; Figure 2.3). There were significant differences in the spawning and movement variables among groups (MANOVA, Wilks' $\lambda = 0.207$, $F = 7.425$, $P < 0.0001$) and years (MANOVA, Wilks' $\lambda = 0.152$, $F = 4.119$, $P < 0.0001$), but not between male and female fish (MANOVA, Wilks' $\lambda = 0.837$, $F = 2.063$, $P = 0.058$). WCT spawned at higher flows in 2006 and 2007 as compared to 2000 and 2001, but none of the other seven spawning migration variables were significantly different across years. RBT and RBT-hybrids spawned significantly later ($P < 0.001$) and at higher flows ($P < 0.001$) in 2002 likely due to a protracted and higher spring runoff. Additionally, there were no significant interactions among the fixed effects (group, year and sex) in the overall model. Therefore, I combined the data for all years and genders for subsequent analyses.

WCT spawning and migration differed significantly from RBT and the two hybrid taxa for five of the eight variables (MANOVA, Wilks' $\lambda = 0.164$, $F = 13.804$, $P < 0.0001$; Table 2.3; Figure 2.3): the flow at which they began migrating ($P = 0.003$), migration period ($P < 0.0001$), migration distance ($P < 0.0001$), spawning date ($P < 0.0001$), and spawning temperature ($P < 0.0001$). No differences were found between RBT and RBT-hybrids for any of these five variables. There were significant differences between WCT

and WCT-hybrids for spawning date, migration period, spawning temperature, and migration distance. However, WCT-hybrids only differed between RBT and RBT-hybrids for spawning temperature and migration distance.

WCT spawned during peak flows and as flows declined following peak spring runoff (May and June) and as water temperatures approached 8-9°C. The mean spawning temperature for WCT was 9.2°C. In contrast, RBT and RBT-hybrids spawned during increasing and peak spring flows (April and May) and as temperatures rose to about 6°C. Conversely, WCT-hybrids spawned throughout spring runoff (April and May) and at intermediate temperatures (mean = 7.6°C). All groups of fish began migrations on the rising limb of the hydrograph (Figure 2.4), though WCT migrated significantly farther upriver (mean = 110 km) to access tributaries (Figure 2.3*h*) as compared to WCT-hybrids (48 km), RBT-hybrids (18 km) and RBT (19 km), and the migration period for WCT was about twice (mean = 52 d) as long as RBT and hybrids (means: WCT-hybrids = 33 d, RBT-hybrids = 26 d, and RBT = 28 d; Table 2.3; Figure 2.3*g*). Mean spawning date for WCT (3 June) was significantly later than that of WCT-hybrids (13 May), RBT-hybrids (5 May) and RBT (7 May) (Figure 2.3*d* and Figure 2.4). Additionally, WCT spawned at significantly warmer temperatures (mean temperatures: WCT = 9.2°C, WCT-hybrids = 7.6°C, RBT-hybrids = 6.2°C, and RBT = 6.3°C; Table 2.3; Figure 2.3*e*).

Spatial and Temporal Overlap in Spawning

Despite the differences in spawning behavior among the groups of fish, some temporal and spatial overlap occurred (Table 2.3; Figure 2.4; Figure 2.5). The long

spawning periods produced temporal overlap between WCT and hybrid trout in 2001 and 2007 and with WCT and RBT in 2000 (Figure 2.5). No temporal overlap was found between WCT and the other groups in 2006, possibly due to small samples sizes that limit the ability to draw inferences that year ($N = 9$ fish). For all years combined, RBT had the longest spawning period (11 March – 20 June) relative to the other groups: RBT-hybrids (4 April-14 June), WCT-hybrids (19 April-3 June), and WCT (9 May-25 June) (Table 2, Figure 2.5).

Distribution of spawning locations varied geographically among the four groups of fish. WCT spawned in headwater tributaries, RBT and RBT-hybrids in the lower drainage, and WCT-hybrids generally spawned in the middle portion of the drainage, overlapping with WCT spawning locations (Table 2.1; Figure 2.1). Furthermore, I found evidence of spatial overlap (fish spawning in the same stream) among WCT and RBT in Hay and Lincoln creeks and found F_1 hybrids spawning in Coal and Third creeks representing previous matings between parental species.

I found evidence that hybridization is spreading upstream via long upstream movements of a few individuals with a high proportion of RBT admixture. Two RBT males made long distance migrations and spawned in tributaries used by radiotagged WCT during the study. One RBT migrated 73 km upriver and spawned in the lower section of Hay Creek in 2002, the same general location that a WCT used for spawning in 2000. Similarly, in 2002 a RBT made an upriver movement of 67 km and spawned in Lincoln Creek, the same stream that was used by a WCT and a WCT-hybrid for spawning

in 2007. Also, I tracked two first-generation (F_1) hybrids during the study. One F_1 hybrid moved 67 km upriver and spawned in Coal Creek in 2003, which is the same stream a WCT used for spawning in 2006, and the remaining F_1 hybrid spawned near the confluence of the North Fork and mainstem Flathead River in 2002, along with several other RBT and RBT-hybrids during the study. Finally, individuals with a high proportion of RBT admixture made long distance movements to spawning areas that WCT-hybrids used for spawning; four fish migrated a mean distance of 55 km and spawned in Dutch and Anaconda creeks.

The majority of RBT and RBT-hybrids spawned in tributaries in the lower portions of the drainage, however. These streams included (in order of use): Abbot, Taylors, Rabe, Ivy, Third, and Mill creeks and unnamed tributaries in the Coram area. Abbot Creek supported a high proportion of spawning by RBT and RBT-hybrids (52%), indicating that this location is the main source of introgression in the study area (Table 2.1). The spawning behaviors of several radiotagged RBT and RBT-hybrids suggest that a former private RBT hatchery (Sekokini Springs) that ceased operations in 1996 (see discussion), located about 2 km downstream of the confluence of the Middle and North Forks near Abbot Creek, is likely the original source of the recent proliferation of hybridization in the upper Flathead River system. Five RBT/RBT-hybrids made pronounced upstream migrations to the upper mainstem Flathead River and then displayed short upriver and downriver movements in close proximity to the hatchery site, suggesting they originated from the hatchery. Moreover, the majority of RBT and

RBT-hybrids spawned in streams in close proximity (± 5 km) to the hatchery during the study.

Discussion

Many native salmonids in western North America are threatened by introgressive hybridization with introduced RBT, yet prior to this work little was known about the reproductive factors influencing the spread of hybridization in recently invaded systems. My findings show that RBT, WCT and their hybrids can vary spatially and temporally in spawning producing zones of contact (e.g., hybrids zones: Barton and Hewitt 1985) between parental species leading to introgression in native populations. I found some evidence of overlap in spawning locations in space and time between parental and hybrid groups mostly in the lower and middle portions of the drainage. Similarly, using radiotelemetry Henderson et al. (2000) found that the majority of Yellowstone cutthroat trout demonstrated spatial and temporal overlap with RBT and hybrids in the mainstem and lower tributaries to the South Fork Snake River in Idaho. My study also provides evidence that individuals with low and high levels of RBT admixture differ in spawning behavior compared to non-hybridized WCT, and that WCT backcrossed hybrids display intermediate spawning behavior between that of non-hybridized WCT and RBT.

Spatial and Temporal Distribution of Spawning

Based on the spatial distribution of spawning, I found that most RBT and RBT-hybrids spawned in tributaries in lower elevations, non-hybridized WCT in the headwater reaches, and WCT-hybrids distributed intermediately in the drainage. These results are consistent with many genetic studies that have been conducted in situations where RBT are introduced into waters containing allopatric populations of WCT (Rubidge and Taylor 2004; Hitt et al. 2003; Weigel et al. 2003; Robinson 2007). In this study, the observed spatial distribution of hybrids and parental species appears attributable to upriver invasion of individuals with high amounts of RBT admixture into non-hybridized WCT populations (R. Leary, unpublished data; Hitt et al. 2003; Boyer et al. 2008; this study). The purported illegal release of an estimated 70,000 RBT individuals in 1997 from a private hatchery in the lower portion of the drainage appears to have played a significant role in the recent proliferation of introgression in the system (Boyer et al. 2008). I found that the vast majority of radiotagged RBT and RBT-hybrids spawned in a relatively few streams located near the probable release point and some of these individuals appeared to be returning to the hatchery to spawn in the early spring. The observed gradient of hybridization, however, may not simply be the result of the initial release location as RBT typically prefer to spawn in lower elevation streams in most systems in which they are introduced (Fausch et al. 2001). For instance, in the east slopes of the Rocky Mountains where RBT have been stocked extensively over a wide range of elevations, Paul and Post (2001) found that RBT are most common at

lower elevations where they often outcompete and hybridize with native WCT populations.

Although non-hybridized WCT populations still exist, my data indicate that many of these populations are at high risk of genetic introgression. The spatial and temporal differences in spawning between WCT and RBT and hybrids are not absolute and thus, are not strong enough to preclude further hybridization. Moreover, when reproductive overlap occurs and hybrid progeny are produced hybridization may spread and genomic extinction of parental taxa can occur even when hybrid offspring experience reduced fitness due to the 'ratchet effect' wherein all progeny of hybrids will be hybrids (Epifanio and Philipp 2001; Allendorf et al. 2004). Finally, previous studies have suggested that environmental factors may not be strong enough to constrain invasion and subsequent introgression (Hitt et al. 2003; Rubidge and Taylor 2005). Additional research is needed to test the hypotheses that hybridization between WCT and introduced RBT leads to a reduction in fitness (Arnold and Hodges 1995) from a loss of local adaptation (extrinsic outbreeding depression) or disruption of co-adapted gene complexes (intrinsic outbreeding depression).

I did not find any evidence of radiotagged fish using mainstems or side-channel habitats for spawning. Spatial and temporal overlap of the parental species and hybrid types during spawning occurred exclusively in tributaries to the mainstem, North Fork and Middle Fork Flathead Rivers. In contrast, Henderson et al. (2000) found complete spatial and temporal overlap between introduced RBT and native Yellowstone cutthroat

trout within mainstem locations of the South Fork Snake River in Idaho. Also, DeRito (2004) reported that RBT spawned in side channels to a greater extent than Yellowstone cutthroat trout in the Yellowstone River, Montana, but spatial overlap occurred in one side channel used by the parental taxa and hybrids. However, DeRito (2004) did not find any evidence of temporal overlap in tributaries.

Cutthroat trout exhibit a wide variety of life history variation (Liknes and Graham 1988; Gresswell et al. 1994). In the Flathead system, migratory WCT spawn in tributaries where juveniles rear for one to four years prior to emigrating to large rivers or lakes where they grow to maturity (Shepard et al. 1984; Liknes and Graham 1988) and some of these movements can be extensive (up to 155 km). High water velocities and sporadic discharges during spring runoff in the unregulated and partially regulated mainstems may reduce emergence success in redds (Weaver and Fraley 1993; Schmetterling 2000), provide inadequate substrate size and intragravel flow (Weaver and Fraley 1993), wash away fry (Fausch et al. 2001) and provide sub-optimal nursery habitat during the first year of growth (Shepard et al. 1984). Thus, WCT may be adapted to tributary spawning in the Flathead system to optimize survival and persistence.

Radiotelemetry was useful to identify streams where hybridization is occurring and to understand how hybridization is spreading in the river system. I found that most RBT and RBT-hybrids spawned in streams in the lower drainage and that Abbot Creek which contains a hybrid swarm with a 92% RBT admixture likely serves as the major source of RBT introgression in the upper Flathead River system. These results are

consistent with recent molecular genetic studies (Hitt et al. 2003; Boyer et al. 2008) that found a significant negative correlation in RBT admixture with upstream distance from Abbot Creek in the system. The spatial spawning distribution and movement patterns of RBT, RBT-hybrids, and F_1 hybrids indicates hybridization is being promulgated upstream via long distance movements of a few individuals with high amounts of RBT admixture (the continent-island invasion model; Arnold 1997). Additionally, the spatial distribution of later generation backcrosses suggests that hybridization is also spreading by dispersal between neighboring populations (the stepping-stone invasion model; Kimura and Weiss 1964).

In general RBT and RBT-hybrids spawned in low elevation streams with relatively stable flows that were fed by springs or headwater lakes, whereas WCT spawned in mountain streams dominated by snow melt runoff. The upper Flathead River system is a relatively cold and unproductive system, so RBT and RBT-hybrid populations have become established (through extensive propagule pressure) in low elevations and “spring creeks” that are relatively higher in productivity and that contain stable flows that are more conducive to spawning earlier in the spring. Similarly, other studies have found that RBT outcompete cutthroat trout in productive, low elevation streams (Paul and Post 2001; Robinson 2007) probably because of their higher thermal tolerances (Bear et al. 2007), greater metabolic and growth requirements (Robinson 2007), better swimming ability (Seiler and Keeley 2007), and high variation in habitat use (Raleigh et

al. 1984). Undoubtedly, propagule pressure has played a major role in this spatial pattern.

Spawning Behavior

I found that WCT displayed significantly different spawning behavior than any other group. First generation hybrids and individuals backcrossed to WCT displayed spawning and movement characteristics that were generally intermediate to those of WCT and RBT and RBT-hybrids. RBT-hybrids behaved similarly to RBT. The spawning behaviors of WCT represent adaptations to the environment (Northcote 1997), which have been selected for at least since the Wisconsin glacial period (~11,000-14,000 years ago). WCT likely adapted to migrate during high spring flows to access the full extent of available habitat and to spawn as flows decline to minimize sediment deposition on redds (Weaver and Fraley 1993), substrate scour (Fausch et al. 2001) and to avoid high water velocities that wash away trout fry (Fausch et al. 2001). My data suggest that hybridization may produce earlier spawning times, leading to earlier emergence times, and possibly reduced recruitment success. Loss of local adaptations may be especially detrimental to indigenous species during periods of extreme environmental conditions, such as fire, drought, and winter flooding (Leary et al. 1995; Allendorf et al. 2004). Regardless of whether or not hybridization results in a reduction in fitness, my results clearly indicate it alters spawning behavior compared to native WCT.

Differences in spawning characteristics of cutthroat trout and hybrids may be attributed to spatial and temporal aspects of the environment (Gresswell et al. 1997). In

my study, WCT spawned in a variety of streams that differed in elevation, aspect and drainage area (Chapter 4), yet I did not detect significant variation in the timing and location of breeding among WCT individuals or populations. Instead, I found that WCT consistently spawned in streams as flows declined following peak runoff, which parallels that observed in other studies (Bjornn and Mallet 1964; Shepard et al. 1984; Schmetterling 2000; Schmetterling 2001).

Native WCT populations exhibit a high degree of genetic divergence over short geographical distances (Allendorf and Leary 1988; Taylor et al. 2003; Boyer et al. 2008) which indicates low straying rates among populations. Conversely, the rapid spread of hybridization indicates a high rate of dispersal and gene flow from hybrid and RBT sources (Boyer et al. 2008). I found that hybridization is spreading upriver, in part, by long distance movements of individuals with high amounts of RBT admixture that likely strayed into streams containing non-hybridized WCT. Furthermore, I hypothesize that male RBT and hybrids may be the primary vectors in the spread of hybridization in the system which has been observed in other salmonid species (Hard and Hoard 1999; Kitano et al. 1994).

Hybridization and reproductive segregation may be dynamic events that vary temporally due to abiotic factors, such as river discharge and temperature, and in many systems hybridization has rapidly increased over short time frames (Hitt et al. 2003; Rubidge and Taylor 2004). In 2002, a high flow year, I found that RBT and RBT-hybrids spawned significantly later than all other years and, coincidentally or not, I observed

long distance spawning movements by two RBT males into streams containing non-hybridized WCT. Similarly, DeRito (2004) found evidence of temporal variation in spawning among years in the Yellowstone River system; RBT and hybrids spawned earlier with earlier peak spring runoff, increasing the likelihood of overlap with later spawning Yellowstone cutthroat trout.

The purpose of the hybrid index classification was to describe the multilocus phenotype or genotype of individuals. Due to the random reshuffling of alleles during sexual reproduction, the power to discriminate between parental types and later generation hybrids is a function of the number of marker loci used (Boecklen and Howard 1997). With 13 diagnostic nuclear PINE or indel/microsatellite loci, there is much less than a one percent chance a first generation backcross would be misidentified as a F_1 . The ability to distinguish between non-hybridized parental species and later generation backcrosses, however, is more problematic. For example, if an individual fish has 10% of its genes from RBT there is a six percent chance it would possess no RBT alleles at the 13 diagnostic indel/microsatellite loci. In contrast, with the six PINE diagnostic loci in which the presence of the fragment is characteristic of rainbow trout there is a 28% chance no RBT alleles would be detected in such an individual. Consequently, due to this sampling error I probably overestimated the number of parental types and underestimated the number of hybrids. In particular, I likely overestimated the number of RBT in the study. Boyer et al. (2008) found that none of the streams used by what I classified as RBT in this study contained non-hybridized RBT

populations. Rather these streams contained hybrid swarms with a high degree of RBT admixture (e.g., Abbot Creek). This may explain the fact that the RBT and RBT-hybrids differed little in spawning characteristics.

Management Implications and Conclusions

Currently there are no official policy guidelines for treating hybrids under the Endangered Species Act (ESA; Allendorf et al. 2004; Allendorf et al. 2005). Recent debate has focused on establishing a clear definition on what constitutes a 'pure' species and what level of anthropogenic introgression is acceptable (Allendorf et al. 2005; Campton and Kaeding 2005). Furthermore, the USFWS (Department of the Interior 2003) established criteria for determining whether fish populations should be considered under the unit for listing. These criteria included morphological characteristics and "reproductive discreteness between individuals that conform morphologically to the WCT subspecies." This study provides insight into this debate and listing assumptions by linking genetic status with behavioral characteristics of non-hybridized WCT and hybrid fish during spawning. My data indicate that individuals with only a low proportion of RBT alleles differed in spawning behavior compared to non-hybridized WCT, suggesting that the listing criteria may need to be reconsidered.

Inclusion of hybridized populations as WCT may threaten the long term persistence of this subspecies by protecting hybrids and allowing further introgression (Allendorf et al. 2004). Eradication or suppression of hybrid populations, therefore, may be required as an effective management strategy for reducing the spread of

hybridization. I found that Abbot Creek is the major source of hybridization in the system. Currently, MFWP is actively suppressing this population through the removal of adults during spawning and installing barriers to preclude access to spawning habitats. I believe that pro-active conservation measures like these should be considered for slowing or stopping the spread of hybridization in the Flathead and other similar systems. Alternatively, in situations where there are still parental individuals present a selective removal program may be an effective option to constrain or prevent introgression. However, the ability to accurately visually determine the identity of non-hybridized and hybrid fish is necessary for this approach to be successful. I compared the genetic status of individual adult fish with visual classifications in the field and found that morphological characteristics accurately differentiated WCT and hybrids 93% of the time. These results suggest that phenotypic characteristics may provide a reasonably accurate and cost effective means of identifying adult hybrids in the field. Prior to using field identification I recommend that individuals first compare the accuracy of the characteristics they have chosen to genetic identification (e.g. Leary et al. 1996; Meyer et al. 2006; Weigel et al. 2002). This is especially critical for detecting hybrids with low levels of RBT admixture.

Understanding the spatial and temporal dynamics of reproduction between introduced and native fishes is important for estimating the probability of hybridization and for identifying the mechanisms responsible for human mediated hybridization in the natural environment. Prior to this work the spawning dynamics of introduced RBT,

native WCT, and their hybrids were largely unknown. I assessed the temporal and spatial dynamics of spawning between introduced RBT and native WCT in a large interconnected river system. My study demonstrates that introductions of RBT into previously allopatric populations of WCT may result in genetically admixed populations because of a lack of complete reproductive segregation during spawning. The data also suggest that hybrids do not have the same spawning characteristics as non-hybridized WCT. Telemetry data revealed that hybridization is spreading via long range movements of RBT and RBT-hybrids into streams containing non-hybridized WCT and possibly by dispersal of later generation hybrids from neighboring streams. This suggests both continent-island and stepping-stone models of RBT invasion. Finally, the data suggest that Abbot Creek is a main source of hybridization in the system. Although some streams in the upper portions of the system may still provide some reproductive isolation, hybridization and introgression is likely to continue to spread unless populations with high amounts of RBT admixture are reduced or eliminated.

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Table 2.1. The number of radiotagged fish that spawned in each stream in the upper Flathead River system from 2000 to 2007. Sites are coded in approximate order of ascending upstream distance. Acronyms: WCT = westslope cutthroat trout, RBT = rainbow trout, WCT-hybrid = WCT x RBT hybrids backcrossed to WCT, RBT-hybrid = backcrosses to RBT, F_1 = first-generation hybrid, NF = North Fork stream, and MF = Middle Fork stream.

Code	Site name	Number of Radiotagged Spawners			
		WCT	WCT-Hybrid	RBT-Hybrid	RBT
1	Mill				1
2	Taylors		1	7	4
3	Abbot			18	28
4	Ivy			1	3
5	Rabe			2	5
6	First				1
7	Steamer			1	
8	Third		1(F_1)	2	2
9	Dutch		2	2	1
10	Anaconda		3	1	
11	Coal (NF)	1	1(F_1)		
12	Moran	1			
13	Hay (upper)				
13	Hay (lower)	1			1
14	Red Meadow	2			
15	Akokala	2	1		
16	Tepee	3			
17	Starvation	1			
18	Kishenehn	1			
19	Sage	4			
20	Burnham	1			
21	Cauldrey	1			
22	Commerce	3			
23	Rubideau		1		
24	McDonald				1
25	Lincoln	1	1		1
26	Coal (MF)	1			
27	Granite	1			
28	Lodgepole	1			
29	Schafer	2			

Table 2.2. Summary statistics for radiotagged fish monitored in the upper Flathead River drainage from 2000 through 2007. Acronyms: WCT = westslope cutthroat trout, RBT = rainbow trout, WCT-hybrids = WCT x RBT hybrids backcrossed to WCT, RBT-hybrids = backcrosses to RBT, and SD = standard deviation.

Group	N	Mean Length (mm)	Mean Weight (g)	Mean spawning date	SD (d)	Mean distance (km)	SD
<u>2000</u>							
WCT	7	410 (51)	720 (342)	6/7	15	118	32
RBT	9	392 (24)	573 (120)	5/3	7	20	14
<u>2001</u>							
WCT	5	392 (39)	615 (205)	6/9	9	105	7
WCT-hybrids	1	464	933	5/6		77	
RBT-hybrids	7	380 (22)	527 (101)	5/11	16	19	20
RBT	9	410 (31)	666 (160)	5/6	7	13	11
<u>2002</u>							
WCT-hybrids	1	442	920	6/3		50	
RBT-hybrids	9	417 (55)	735 (313)	5/23	16	15	7
RBT	11	390 (27)	579 (140)	5/22	18	27	25
<u>2003</u>							
WCT-hybrids	1	435	781	5/25		67	
RBT-hybrids	8	410 (37)	632 (154)	5/3	14	22	33
RBT	12	410 (59)	628 (347)	5/5	15	18	17
<u>2005</u>							
RBT-hybrids	7	413 (26)	690 (170)	4/23	10	10	4
RBT	6	423 (36)	778 (177)	4/29	13	17	9
<u>2006</u>							
WCT	3	474 (53)	1082 (374)	6/3	20	99	35
WCT-hybrids	1	465	901	5/7		1	
RBT-hybrids	3	447 (48)	974 (357)	4/17	7	5	5
RBT	2	420 (14)	624 (85)	4/26	14	18	4
<u>2007</u>							
WCT	12	419 (37)	776 (222)	5/29	11	109	23
WCT-hybrids	7	441 (31)	760 (145)	5/11	18	48	23
RBT-hybrids	2	395 (28)	588 (153)	5/12	4	50	4
RBT	2	402 (22)	541 (85)	4/30	8	16	10

Table 2.3. Means for the eight spawning variables for spawning radiotagged westslope cutthroat trout, rainbow trout and hybrids in the upper Flathead River system, Montana (USA) and British Columbia (Canada). Acronyms: WCT = westslope cutthroat trout; WCT-Hybrid = westslope cutthroat trout backcross; RBT-Hybrid = rainbow trout backcross; RBT = rainbow trout.

	WCT			WCT-Hybrid			RBT-Hybrid			RBT		
	<i>N</i>	Range	Mean	<i>N</i>	Range	Mean	<i>N</i>	Range	Mean	<i>N</i>	Range	Mean
Begin migration	27	9 March-29 May	12-April	11	3 March-23 May	10-April	36	6 March-14 May	10-April	51	10 March-16 May	9-April
Begin temperature (°C)	27	2-7.5	5	11	3.9-6.6	5	36	1.0-8.0	5	51	1.5-8.5	4.9
Begin flow (m ³ /s)	27	100-801	377	11	100-603	290	36	95-580	218	51	90-693	255
Migration period (d)	27	27-87	52	11	7-67	33	36	2-77	26	51	2-74	28
Spawning date	27	9 May-25 June	3-June	11	19 April-3 June	13-May	36	4 April-14 June	6-May	51	11 March-20 June	7-May
Spawning temperature (°C)	27	6.5-13	9.2	11	5.0-11	7.6	36	4.0-8.5	6.2	51	3.0-10.0	6.3
Spawning flow (m ³ /s)	27	251-892	592	11	300-1152	719	36	113-1177	559	51	182-1228	548
Migration distance (km)	27	59.7-155	109.7	11	1.3-82.4	48.1	36	1.9-102	17.5	51	0.5-73.3	19.1

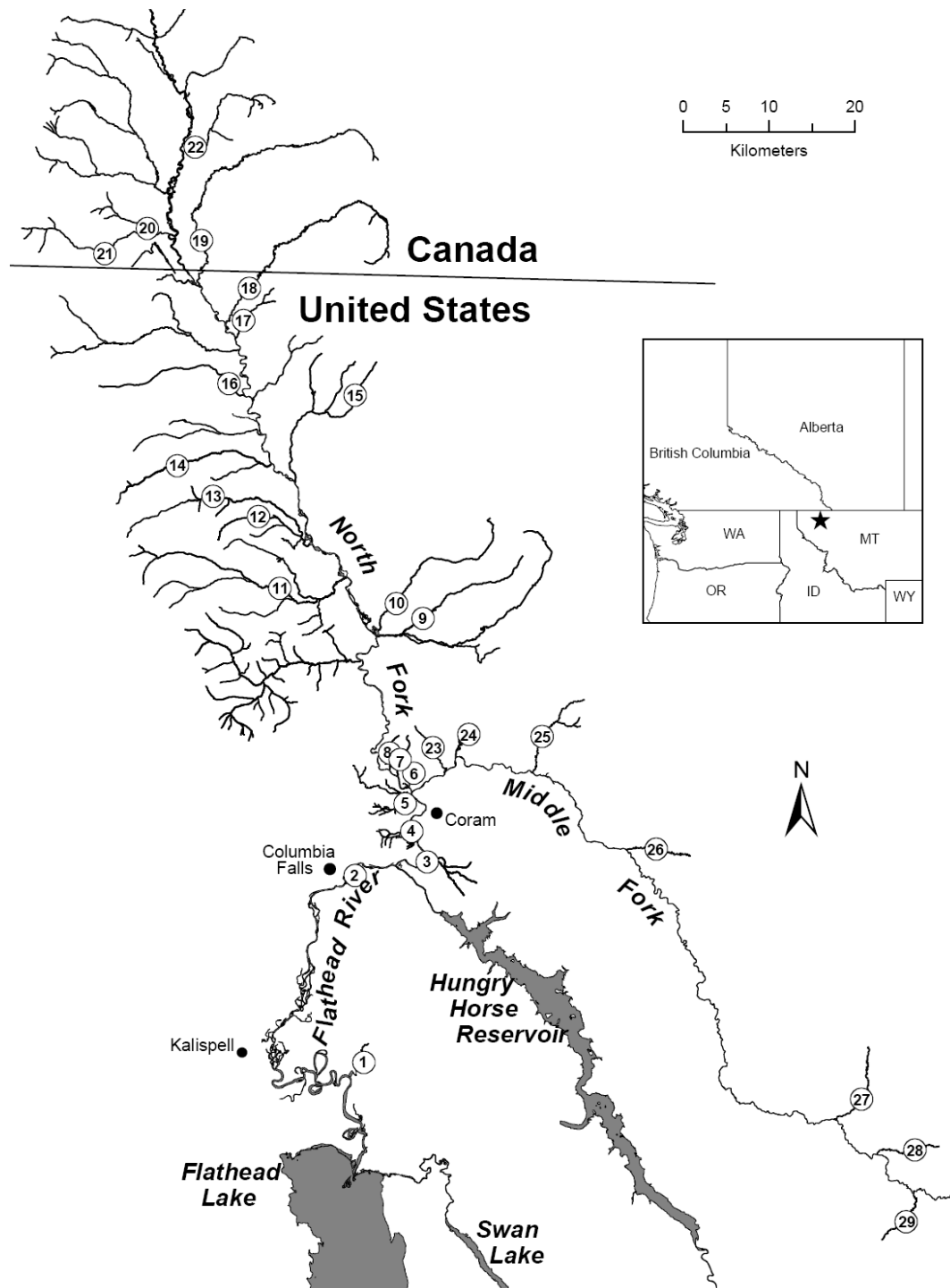


Figure 2.1. Study area location and sample site identification in the upper Flathead River system in Montana (USA) and British Columbia (Canada). Sample site codes correspond to Table 2.1.

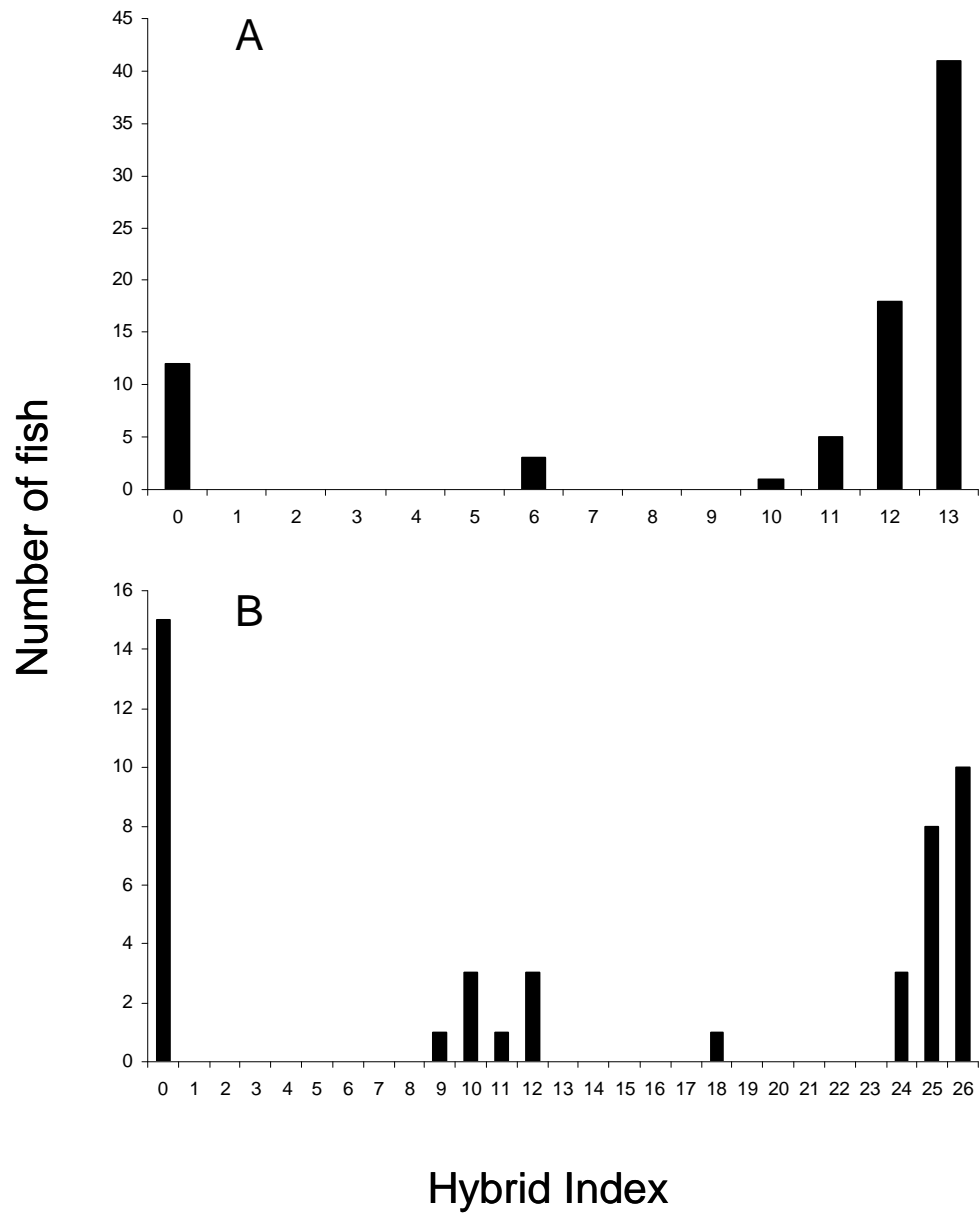


Figure 2.2. Hybrid index scores of 125 radio-tagged westslope cutthroat trout, rainbow trout and hybrids based on (A) paired interspersed nuclear element polymerase chain reaction (PINE PCR) of 13 dominant loci ($N = 80$) and (B) a combination of six co-dominant indel loci and seven co-dominant microsatellite loci ($N = 45$).

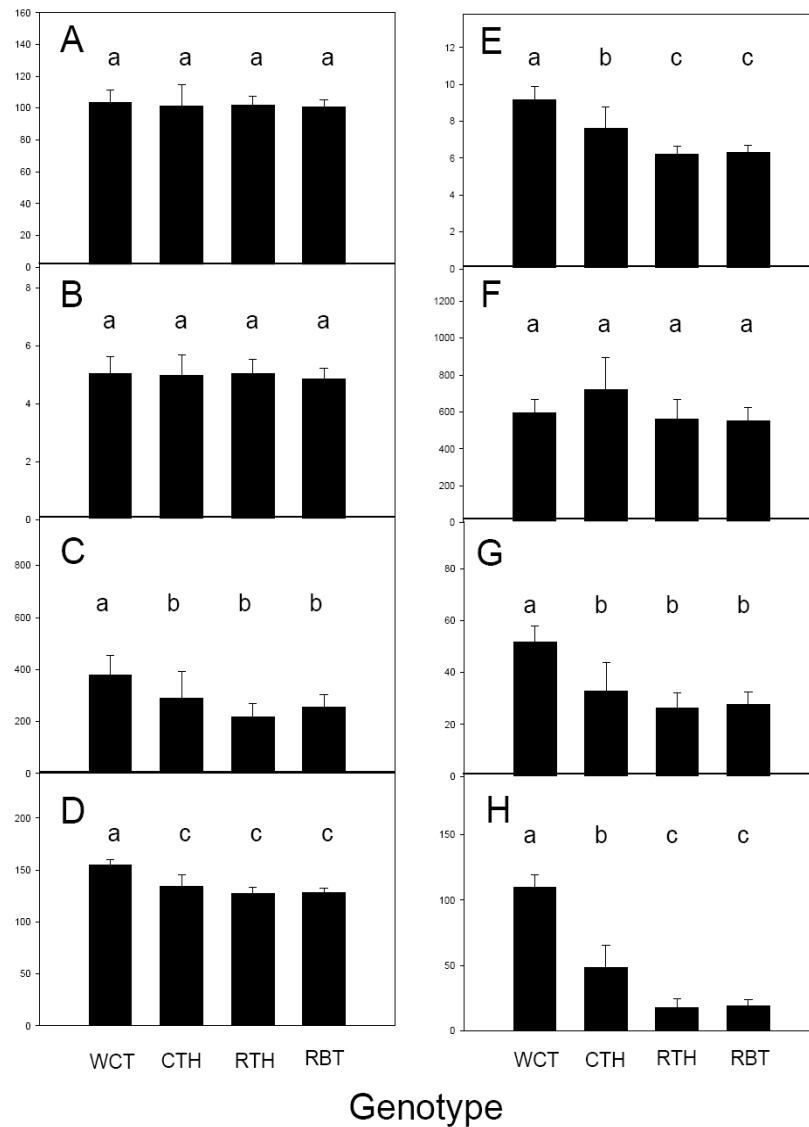


Figure 2.3. Comparisons of mean movement and spawning characteristics (± 95 CI) for eight spawning variables: (A) beginning migration date, (B) beginning migration temperature, (C) beginning migration flow, (D) spawning date, (E) spawning temperature (F), spawning flow, (G) migration period, and (H) migration distance. For each measurement, means that do not share letters are significantly different (Tukey's test, $P < 0.05$). Acronyms: WCT, westslope cutthroat trout; RBT, rainbow trout; CTH, WCT x RBT hybrid trout backcrossed to WCT; RTH, WCT x RBT hybrid trout backcrossed to RBT.

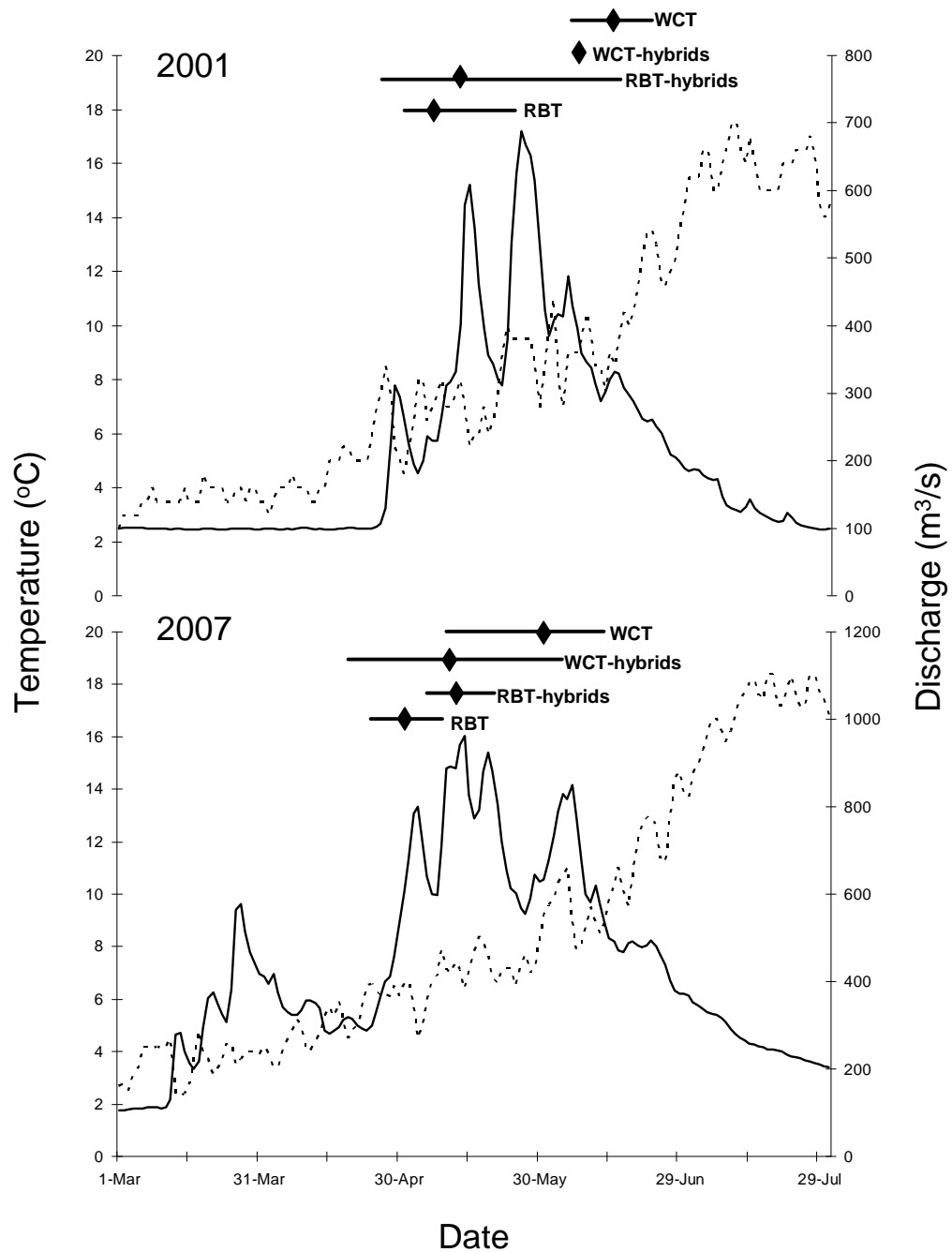


Figure 2.4. The spawning periods and mean spawning dates (diamonds) for each taxa as related to the hydrograph and thermograph at Columbia Falls in the mainstem Flathead River in 2001 and 2007. Acronyms: WCT, westslope cutthroat trout; RBT, rainbow trout; WCT-hybrids, WCT x RBT hybrid trout backcrossed to WCT; RBT-hybrids, WCT x RBT hybrid trout backcrossed to RBT. Solid lines are flow and dotted lines are temperature.

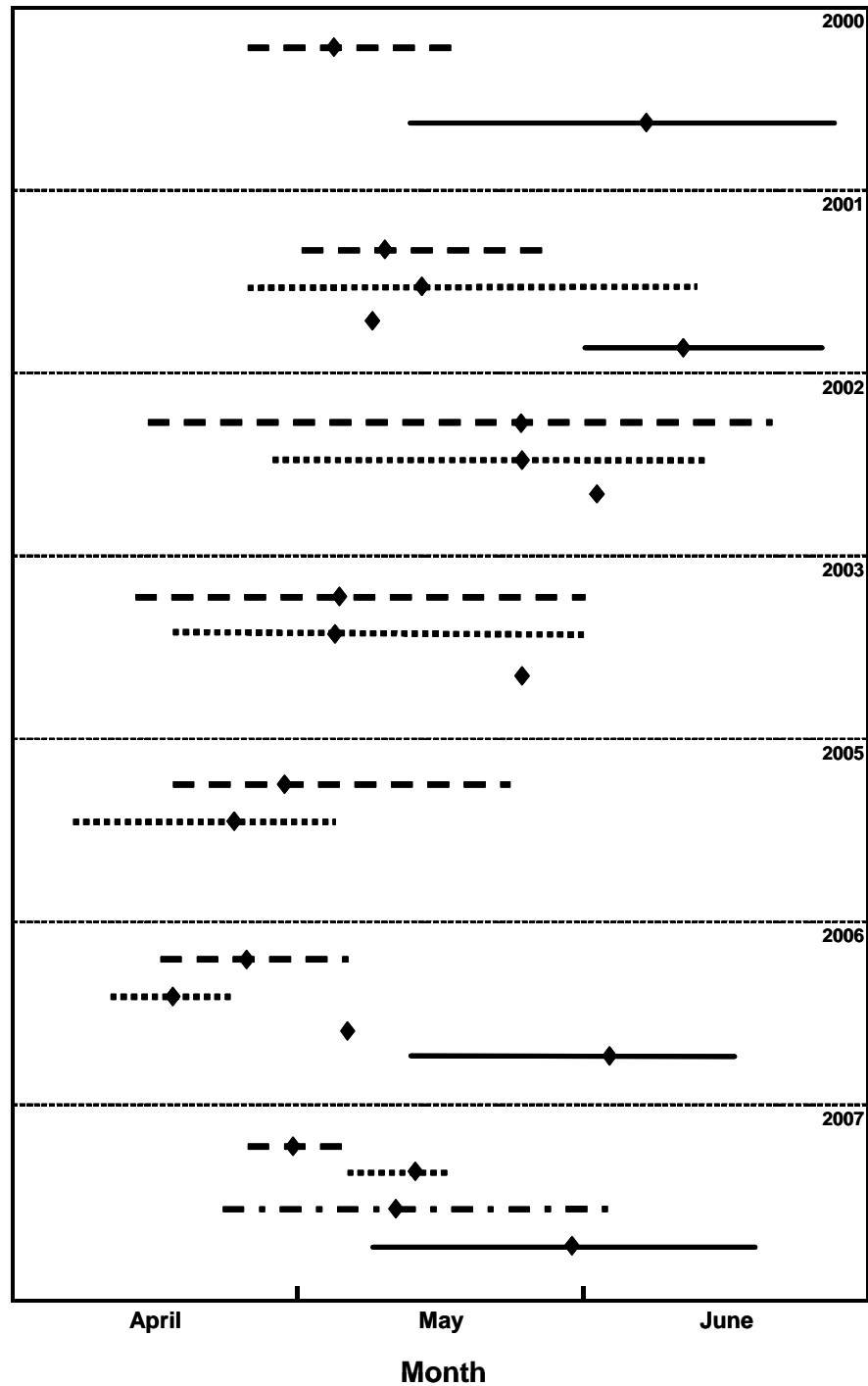


Figure 2.5. Spawning periods and mean spawning dates (diamonds) of 125 radiotagged trout during 2000-2003 and 2005-2007: 27 westslope cutthroat trout (solid lines), 11 WCT x RBT hybrids backcrossed to WCT (dash-dot lines), 36 WCT x RBT hybrids backcrossed to RBT (short dotted lines), and 51 rainbow trout (long dotted lines).

CHAPTER 3

HYBRIDIZATION REDUCES FITNESS OF CUTTHROAT TROUT IN THE WILD

Abstract

Hybridization with introduced species threatens many native fishes worldwide, yet the effects of hybridization on fitness remain poorly understood. I estimated the reproductive success (number of offspring per adult) of native westslope cutthroat trout, nonnative rainbow trout, and their hybrids in a natural stream using parentage analysis with multilocus microsatellite markers. Small amounts of hybridization markedly reduced the reproductive success of hybrids compared to cutthroat trout in the wild with fitness declining by ~50% with only 20% nonnative genetic admixture. This outbreeding depression suggests that extant aboriginal cutthroat trout populations are at greater conservation risk than previously thought and policies that protect hybridized populations may need reconsideration.

Introduction

Invasive species are one of the greatest threats to global biodiversity (1, 2). One of the most pernicious threats to native species is hybridization and genetic introgression with exotic taxa (3-5). These irreversible changes have contributed to the demise and extinction of many populations and species of plants and animals.

Furthermore, hybridization is exacerbated by habitat modification (3) so this problem will likely become more serious with increasing land use development and global climate change. Consequently, conservation of native biota requires an understanding of the ecological and evolutionary consequences of anthropogenic hybridization, but such data for endangered and rare species are limited in nature.

The legal status of hybrids is one of the most controversial questions in endangered species law (3, 6-9). In the United States, there currently is no official policy for how to classify hybrids under the Endangered Species Act (ESA). The most contentious issue has been establishing a threshold for deciding how much hybridization is permissible for hybrids to be considered part of the native taxon (3, 10). Ideally, these decisions should be informed by assessing the fitness consequences of different levels of hybridization, especially at low levels of hybridization. Unfortunately, this information is unavailable for any vertebrate species. To date, federal agencies have addressed this issue on a case-by-case basis, and have relied heavily on morphological data (11-13). This ruling is reasonable when no other data are available, but assumes that morphological similarity is indicative of genetic and ecological similarity. This assumption, however, has not been tested.

Hybridization is more common in fish than any other vertebrate taxa (3, 14). Many fish species have external fertilization and similar mating behaviors, facilitating interbreeding. In undisturbed ecosystems, reproductive isolation is maintained primarily by spatial and temporal reproductive isolation rather than well developed pre-

and post-mating isolating mechanisms (15). However, such barriers to interbreeding are lost in the face of habitat disturbance and introduction of nonnative species (3). This is particularly true for salmonids where widespread introgression among native and nonnative taxa has replaced native species over large areas throughout their native ranges (14, 16). Rainbow trout (*Oncorhynchus mykiss*), the most widely introduced fish species in the world (17), produce fertile offspring when crossed with cutthroat trout (*O. clarkii*), and introgression often continues until a hybrid swarm is formed and all native cutthroat genomes are lost (14). A major consequence of such interspecific hybridization may be the break up co-adapted gene complexes and disruption of local adaptations (18, 19). Introgression poses a serious threat to all 14 subspecies of cutthroat trout in western North America (20, 21) due to widespread stocking and invasion of nonnative rainbow trout into historical cutthroat trout habitats. Two subspecies are now extinct, five listed as threatened under the ESA, and seven petitioned for listing (22).

Interbreeding between westslope cutthroat trout (*O. c. lewisi*) with nonnative rainbow trout (16) exemplifies the conservation challenges of interspecific hybridization. Westslope cutthroat trout historically occupied an estimated 90,800 km of aquatic habitats throughout the Columbia, Fraser, Missouri, and Hudson Bay drainages of the United States and Canada (23). Non-hybridized populations now persist in less than 10% of their historic range and many are restricted to small, fragmented headwater habitats where their long term sustainability is uncertain (23). Distinguishing between

hybrid and non-hybridized cutthroat trout based on morphology is difficult especially when individual fish contain low levels of rainbow trout admixture (24). Because of this difficulty, westslope cutthroat trout populations with less than 20% rainbow trout admixture were included as non-hybridized westslope cutthroat trout in a recent ESA status review by the U.S. Fish and Wildlife Service (USFWS, 13). The decision to use the 20% threshold continues to be controversial because the potential fitness effects of this amount of admixture are unknown and the use of this arbitrary threshold will profoundly influence the decision to list by determining the number of populations classified as part of the native taxon (25-27). Moreover, protection of fish with even low levels of admixture may facilitate continued expansion of hybridization. Consequently, there is an urgent need to better understand how introgression affects fitness in the wild.

Methods

In this study, I describe for the first time how a wide range of levels of admixture affect fitness in the wild. Genetic parentage analysis (28) was used to compare the reproductive success of native westslope cutthroat trout, nonnative rainbow trout, and westslope cutthroat/rainbow trout hybrids in a natural stream (Langford Creek, Flathead River drainage, Montana, United States, 28). Hybridization between westslope cutthroat trout and rainbow trout in Langford Creek has been ongoing for several generations and the breeding population is currently composed of non-hybridized

westslope cutthroat trout and rainbow trout, first generation hybrids, and later generation backcrosses having a wide range of rainbow trout admixture. Westslope cutthroat trout and rainbow trout in Langford Creek both exhibit migratory life histories. After emergence, juveniles rear in the stream for 1-3 years before emigrating to the Flathead River or Flathead Lake where they grow to maturity and return as adults to spawn in the spring. During the springs (April-June) and summers (July-September) of 2003-2007, I captured 185 adults ($N = 61$ females, $N = 124$ males) migrating upstream in the creek to spawn and 648 emigrating juveniles. All fish were genotyped at 16 microsatellite loci for parentage analyses. Reproductive success was measured as the number of offspring per parent for each spawning year. Based on the most conservative parentage analysis (28), I assigned 397 emigrating juveniles to a mother, 389 to a father, and 149 of these juveniles to both parents. The proportion of rainbow trout admixture of each adult and juvenile was estimated using eight microsatellite loci that are diagnostic between these taxa (28). The effects of the proportion rainbow trout admixture, fish length, and spawning year on reproductive success were evaluated using generalized linear mixed models (GLMM).

Results and Discussion

The GLMM showed that the proportion of rainbow trout admixture among spawning females and males had a strong negative effect on reproductive success (Table 3.1). Fitness declined sharply with an increase in the proportion of nonnative

rainbow trout admixture (Figure 3.1A; Figure 3.2A). These data closely fit an exponential decay curve indicating that even small amounts of nonnative rainbow trout introgression rapidly reduce fitness in later generation hybrids (Figure 3.1B; Figure 3.2B). Overall, I observed about a 25% decline in reproductive success with 10% rainbow trout admixture and ~50% decline with 20% admixture (28). Length had no detectable effect on female reproductive fitness and only a small, but statistically significant effect, on male fitness (Table 3.1). Furthermore, the small standard deviation of the year random effect indicates that annual fluctuation in reproductive success is modest (generally < 25%).

Although there was an exponential decline in fitness with increasing levels of introgression, paternity analysis showed that two hybrid males had an inordinately high reproductive success, comprising a total of 30% of the total offspring (Figure 3.2A). One of these males, a hybrid with 75% rainbow trout admixture, mated with four known female westslope cutthroat trout (based on the parentage analysis for parent-pairs) and produced the highest number of juvenile offspring in my dataset. Together, these data indicate that a few, relatively fit males with a high proportion of rainbow trout genes are likely promulgating the spread of hybridization in the stream.

The fitness of first generation (F_1) hybrids was relatively high for both sexes and similar to that of non-hybridized westslope cutthroat trout (Figure 3.1A, Figure 3.2A). This result may be explained by the fact that F_1 hybrids have a maximum amount of genetic variation and have intact haploid genomes from each parental species. The

fitness of F_1 westslope cutthroat trout/rainbow trout hybrids, however, did not persist after backcrossing presumably due to disruption of co-adapted gene complexes (15, 17).

My results present a paradox—genetic introgression reduces fitness but progresses rapidly. My data present a partial explanation for this conundrum. First, I observed that F_1 hybrids can have relatively high fitness. This facilitates subsequent generations of backcrossing. Second, I observed that a few post F_1 hybrid males had reproductive success as high as or substantially higher than non-hybridized westslope cutthroat trout. This decreases the efficiency of selection against hybrid genotypes and may be a conduit through which genetic introgression proceeds. Third, in this river system, hybridization is spreading upstream from hybrid source populations following continent and stepping-stone models of invasion (29, 30). Lastly, westslope cutthroat trout/rainbow trout hybrids have higher straying rates than non-hybridized westslope cutthroat trout (30) further promoting the spread of nonnative rainbow trout genes among populations. Therefore, the combined information supports a “hybrid ratchet effect” which predicts that the genomic extinction of parental taxa can occur despite heavy fitness costs for the hybrid offspring due to dispersal of parental types and the continued production of hybrids by hybrids (31).

Several important questions remain unanswered. First, I do not know the life stage(s) at which outbreeding depression occurred. My estimate of fitness includes spawning date, mating success, gamete production, egg-to-fry emergence, and juvenile survival, and selection could have acted at any of these times. Second, I do not know

the genetic mechanism reducing fitness because outbreeding depression was not observed until later generation backcrossing, but I suspect that intrinsic outbreeding selection from the breakup of co-adapted gene complexes is a significant factor. Third, the extent to which outbreeding depression may be affected by the environment (extrinsic selection) is unknown. Lastly, I do not know the evolutionary fate of hybrid populations. If selection is strong enough, it may be able to purge deleterious rainbow trout genes from hybridized populations.

How much hybridization is acceptable to maintain the evolutionary legacy of a species, subspecies or evolutionary significant unit? I provide the first direct quantitative estimates of the fitness consequences of anthropogenic hybridization employing the relatively new genetic tool of paternity analysis to explore this question in detail. This approach could be readily adapted to other species where level of hybridization has been contentious and intractable, such as with the red wolf (*Canis rufus*; 7) and spotted owl (*Strix occidentalis*; 9).

Given the widespread replacement of native cutthroat trout with hybrids, I suspect that non-hybridized populations will continue to be lost if hybrid source populations with high proportions of rainbow trout admixture are not suppressed or eliminated (30). My data illustrate how quickly fitness declines when salmonid species interbreed because of human mediated secondary contact in nature. My results also suggest that small amounts of admixture that may not be morphologically detectable may have negative effects on the fitness of trout in the wild and that conservation and

policy programs protecting hybridized populations may need reconsideration in systems containing extant populations of native trout.

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Table 3.1. Generalized linear mixed model (GLMM) analysis of male and female reproductive success. The response variable, reproductive success, was estimated both using a conservative parentage analysis which deleted individuals with tied juvenile-parent assignments and using fractional allocation (28). First generation hybrid adults (Figure 3.1A, Figure 3.2A) were not included as parents in the analysis ($N = 14$ females and 15 males) nor were the three male outliers (Figure 3.2A). Coefficients describe the effect of covariates on the natural log of mean fitness. The covariates are proportion of rainbow trout admixture, sex, and total fish length (mm). Year is a random effect. Because there are only two classes in the category sex, the intercept coefficient can be perceived as the intercept for females while the intercept for males is that plus the sex-M coefficient. Similarly, the length coefficient can be thought of as the effect for females, while the effect of length for males is that plus the sexM:length coefficient.

Factor	Coefficient	Std. Error	DF	t-value	p-value
<i>Using fractional allocation</i>					
Female intercept	3.8540	0.5730	143	6.7100	0.0000
Proportion rainbow trout admixture	-3.3530	0.4500	143	-7.4500	0.0000
sex-M	-3.0300	0.7600	143	-3.9900	0.0001
length	0.0028	0.0018	143	-1.5010	0.1354
sex-M:length	0.0065	0.0026	143	2.5410	0.0121
Random effect std. deviation: Year = 0.1091; Individual (residual) = 2.1090.					
<i>Deleting tied assignments</i>					
Female intercept	2.7100	0.6600	143	4.1000	0.0001
Proportion rainbow trout admixture	-3.0400	0.4600	143	-6.5600	0.0000
sex-M	-2.7500	0.8500	143	-3.2200	0.0016
length	-0.0004	0.0020	143	-0.1980	0.8432
sex-M:length	0.0053	0.0028	143	1.9100	0.0576
Random effect std. deviation: Year = 0.1863; Individual (residual) = 1.8695.					

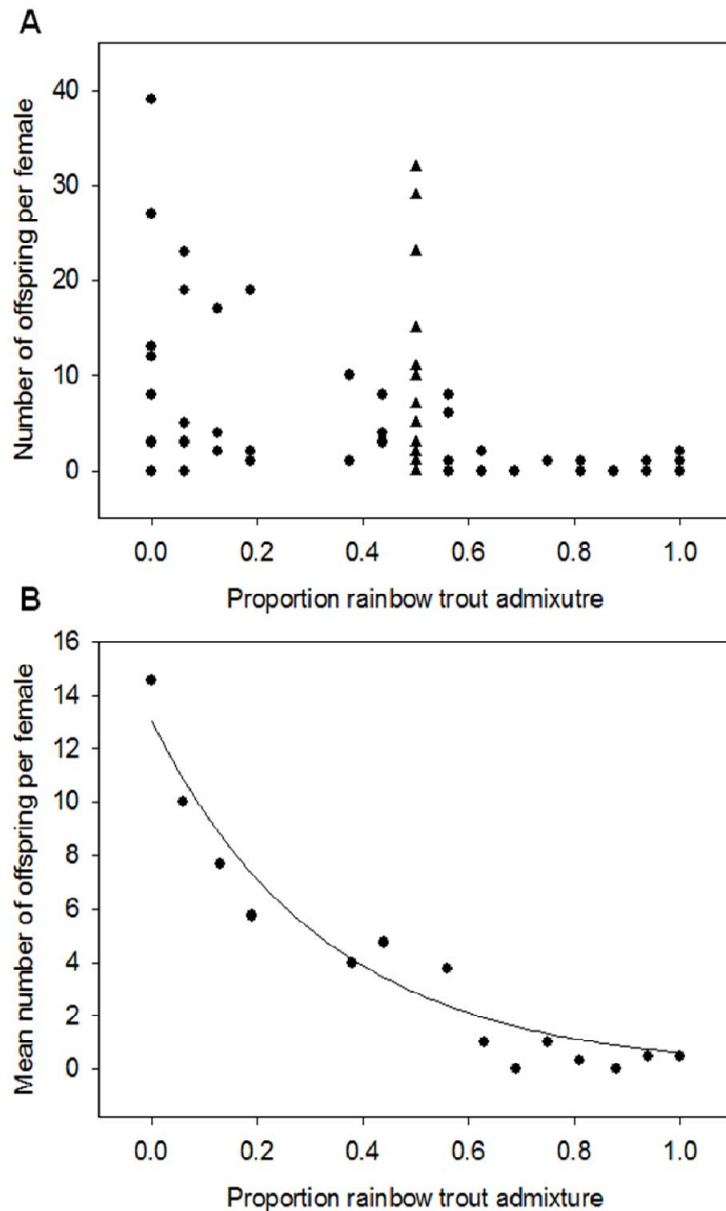


Figure 3.1. (A) Number of offspring per female versus the proportion of nonnative rainbow trout admixture. The plot includes 61 mothers and 397 juvenile assignments using parentage analysis (28). Each point represents an estimate for an individual fish from a spawn-year. Circles represent westslope cutthroat trout, rainbow trout and later-generation hybrids, and triangles are first-generation hybrids. (B) Mean number of offspring per female (data from Figure 3.1A) plotted against the proportion of rainbow trout admixture. A declining exponential regression fitted to the data ($y = 13.039 e^{-3.040x}$) suggests that fitness declines by ~50% with 20% nonnative rainbow trout admixture in the wild. First-generation hybrids ($N = 14$) were not included in the regression.

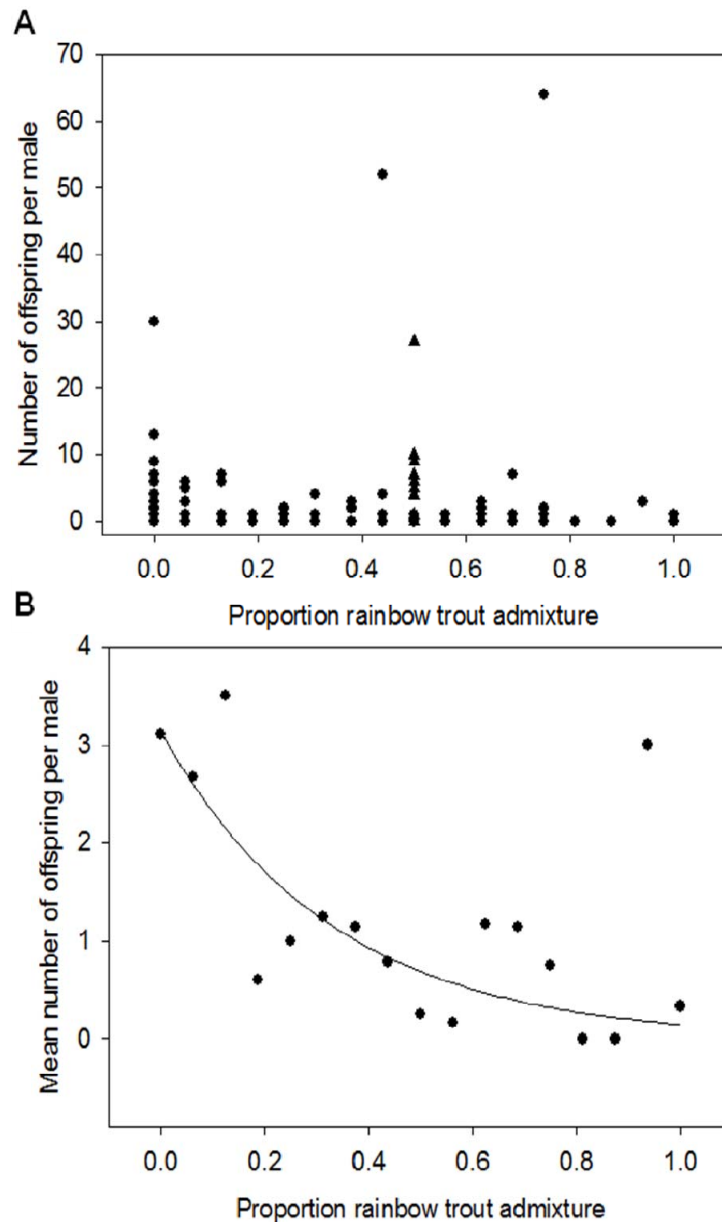


Figure 3.2. (A) Number of offspring per male versus the proportion of nonnative rainbow trout admixture. The plot includes 124 fathers and 389 juvenile assignments using parentage analysis (28). Each point represents an estimate for an individual fish from a spawn-year. Circles represent westslope cutthroat trout, rainbow trout and later-generation hybrids, and triangles are F₁ hybrids. (B) Mean number of offspring per male (data from Figure 3.2A) plotted against the proportion of rainbow trout admixture. A declining exponential regression fitted to the data ($y = 3.140 e^{-3.040x}$) suggests that fitness declines by ~50% with 20% nonnative rainbow trout admixture in the wild. The intercept is adjusted for the mean of the sex-M:length interaction term. First generation hybrids ($N = 16$) were not included in the regression.

Supplemental Material

Study Population

My study was conducted in Langford Creek, a second-order tributary of the North Fork Flathead River in northwestern Montana, USA. Prior to the 1980s, the stream supported a migratory population of westslope cutthroat trout (WCT), but recent genetics data indicate significant nonrandom association of nonnative rainbow trout (RBT) alleles among individuals (gametic disequilibrium), indicating that the population was recently hybridized but had not developed into a hybrid swarm (30). The populations are migratory (see main text), and the age distribution estimated from empirical data in Langford Creek suggest that hybrid and WCT juveniles emigrate from the stream to the river at the ages of 1-3 (mean, 2). The stream has a drainage area of 12.4 km²; elevations range from 1045 m to 1143 m; channel gradients average 2-3 percent; average wetted stream width is 2.5 m; and the streambed is dominated by gravels.

I operated a fish weir near the mouth of Langford Creek to sample adult trout (ripe individuals) during their upstream spawning migration from 2003 to 2007 and out-migrating juveniles from 2004 to 2007 (Table 3.2). Juveniles (>75 mm, total length) and adults were unable to pass up or downstream without being sampled except during extreme spring floods. The weir was continuously operated the first year of the study (March 2003-February 2004) and no trout were captured during the winter months

(December-March). Therefore, the weir was operated from March through November each subsequent year (2004-2007). Because age-0 individuals (<75 mm) were too small to be captured in the weir, fry traps were deployed immediately downstream of the weir in 2007 (June-November) that sampled ~75% of the total cross sectional area of the channel. I did not detect an outmigration of age-0 fish which corroborates previous studies in the Flathead system (S1) and is concordant with studies demonstrating poor survival associated with age-0 emigration in salmonid species (e.g., S2). Length, date, and sex (of adults) were recorded for each captured fish. A portion of fin tissue was excised and stored in 95% ethanol for genetic analysis.

Genetic Analyses

Genetic analyses were conducted at the University of Montana Conservation Genetics Laboratory at the University of Montana, Missoula, USA. DNA was extracted using a detergent-based cell lysis buffer and ammonium acetate protein precipitation followed by isopropyl alcohol DNA precipitation. DNA was resuspended in 100ul TE buffer. DNA was diluted 1:10 and polymerase chain reaction (PCR) amplified in a PTC-200 thermocycler (MJ Research Inc., Waltham, MA) using the QIAGEN Multiplex PCR Kit (QIAGEN, Valencia, CA). All multiplex reactions used a total volume of 10 ul and follow the QIAGEN Microsatellite protocol. Two different touchdown PCR profiles were used, one with an initial annealing temperature of 58°C stepping down to 48°C, and the other started at 70°C stepping down to 55°C. *Ssa407* was PCR amplified separately with Fisher Taq DNA Polymerase (Fisher Scientific, Pittsburgh PA) using a final concentration of

1.75mM MgCl₂. PCR products were visualized on an ABI3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) in the Murdoch DNA Sequencing Facility at the University of Montana, Missoula, USA. Allele sizes were determined using the ABI GS600LIZ ladder, (Applied Biosystems Inc., Foster City, CA). Chromatogram output was viewed and analyzed using GeneMapper version 3.7 (Applied Biosystems Inc., Foster City, CA).

All sampled fish were genotyped using sixteen highly polymorphic microsatellite loci (Table 3.3, S3- S12). Eight of these loci have non-overlapping (diagnostic) sets of alleles in WCT and RBT and, therefore, are useful for estimating the ancestry of individuals (Table 3.3). To estimate individual RBT admixture, I calculated a hybrid index based on genotypes at the eight diagnostic loci (30). This index ranges from 0 for WCT (no RBT alleles) to 1 for RBT (2 RBT alleles at each locus) and is calculated by dividing the total number of diagnostic RBT alleles in an individual by $2X$ (where X is the number of diagnostic loci). First generation hybrids (F_1 s) between WCT and RBT have a hybrid index of 0.5 and are heterozygous for alleles from the parental taxa at all loci. Although fish classified as RBT have diagnostic RBT alleles at all loci, recent genetic studies in the system indicate that these individuals likely originated from a source stream (Abbot Creek) containing a hybrid swarm with 97% RBT admixture (30). Statistical power to discriminate parental types from F_1 hybrids is high with eight diagnostic markers ($1-\beta = 0.9961$). However, discerning between non-hybridized parental types and later generation backcrosses requires more diagnostic loci (S13). Consequently the hybrid

index likely overestimates parental types and underestimates the number of individuals with low levels of admixture.

Parentage Analyses

Mendelian exclusion was used for parentage identification. Maximum likelihood methods (e.g., *S14*) were not used because genotypes in this population are not in Hardy-Weinberg equilibrium. In order to account for genotyping error and mutation, I permitted parents and offspring to have “mismatched” genotypes (i.e. not sharing alleles) at one out of the 16 loci (*S15*). If I could not find a parent for a juvenile that satisfied this criterion, I assumed the parent was not sampled and dropped the juvenile from subsequent analyses. Parentage analysis was first performed using mother-father-offspring triplets and this assigned 149 juveniles to parents. Next, parentage was assigned for each sex separately. In some cases, more than one adult shared the same number of mismatching loci to an offspring and a parent could not be identified based on the juvenile’s age estimated from empirical length-at-age data ($N = 43$ juveniles in the female analysis and 41 juveniles in the male analysis). These ties were treated by either removing them from the dataset (previously reported) or using fractional allocation in which each juvenile was partially allocated based on the number of tied parents (*S16*). I only included juvenile fish less than 110 mm (~age-1 fish) from the 2004 juvenile cohort because adults were sampled for the first time in 2003 and likely produced these individuals. All sampled juveniles from 2005 through 2007 were used in the parentage analysis. As a conservative measure, all five years of adults were included

as potential parents of all juveniles because of repeat spawning and the possibility of incomplete adult sampling (S17).

Statistical Analyses

Not all emigrating juveniles can be ascribed unambiguously to parents but were equally likely to have been spawned by two or even several potential parents. This was true for 31% of the juveniles. I repeated the analysis with two distinct data sets: one in which only offspring which could be unambiguously assigned were included and one where offspring were fractionally assigned to all equally likely parents. Reproductive fitness is the number of offspring each male and female produced. Three males (with 0%, 44% and 75% RBT admixture) produced disproportionately high numbers of juvenile offspring and were eliminated from the statistical analysis as outliers. It is not that these data points are not real, but that they are clearly different from the rest of the data, and should be considered separately.

I investigated the effects of the covariates proportion rainbow trout admixture, sex, length, and year using generalized linear models and generalized linear mixed models (S18). The response variable was either the number of uniquely assigned offspring or the number of offspring including fractional assignments. A natural log link function with a quasi-Poisson error distribution was used. This error distribution is appropriate for both discrete and continuous data and will handle extra-Poisson variation (over-dispersion). Analyses were conducted in R version 2.5.1 and primarily used the functions `glm` (Package 'stats' version 2.5.1) and `glmmPQL` (Package 'MASS'

version 7.2-34). Model selection was done in glm treating all covariates as fixed effects. All combinations of proportion rainbow trout admixture, sex, length, and their interactions with and without a year effect were considered. Model selection was based on minimum jackknifed relative prediction error. Satisfyingly, the fixed model selected by this criterion also had the smallest estimated over-dispersion parameter. The final model was estimated using glmmPQL treating year as a random effect.

The estimated mixed effects model (see Table 3.1) has a strongly significant intercept and terms for proportion rainbow trout admixture and sex. There is also a significant sex by length interaction. The large negative coefficient for proportion rainbow trout admixture indicates that fitness drops very rapidly with hybridization with rainbow trout. In fact, only a small proportion of rainbow trout genetic admixture is required to reduce fitness to 50% of that of non-hybridized cutthroat trout: 0.21 (95% CI = 0.16 to 0.28) in the fractional allocation analysis and 0.23 (0.95% CI = 0.18 to 0.33) in the deleted ties analysis. However, although I showed a dramatic fitness decline, I do not have the statistical resolution to determine the exact form of the decline at very low levels of hybridization.

My estimates of the impact of hybridization on reproductive success are conservative for several reasons. I consider reproductive success with the ties deleted to be the primary data set because it requires less data manipulation and the biases introduced by deleting ties, which are primarily among fish with low rainbow trout admixture, will lead to conservative estimates of the effect of introgression on

reproductive fitness. However, the results from the analysis with the ties fractionally allocated as the response variable mirrors all qualitative results from the analysis with the tied assignments removed (Figure 3.3). Thus, my conclusions are robust to the handling of tied assignments. I believe that the fractional allocation analysis estimate of the proportion rainbow trout admixture is more likely to be accurate than that of the deleted ties analysis. Further, there is an unknown amount of measurement error associated with the assignment of individuals to hybrid class. This error stems from both laboratory error and from the random sampling of a limited number of marker loci from the trout genome. As this error is in a predictor variable, it will introduce a bias reducing the magnitude of the estimated effects.

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Table 3.2. Number of trout *Oncorhynchus spp.* males (N_{males}) and females (N_{females}), total number of adult spawners (N_T), male length size range (M_{SR}), average male length (Avg_M), female length size range (F_{SR}), average female length (Avg_F), male mean enter date (M_{DATE}), female mean enter date (F_{DATE}), by spawn year (2003-2007).

Spawn Year	N males	N females	N_T	M_{SR}	Avg_M	F_{SR}	Avg_F	M_{DATE}	F_{DATE}
2003	18	5	23	189-496	292	359-610	463	30-Apr	9-May
2004	22	18	40	150-510	305	320-478	396	7-May	7-May
2005	31	13	44	169-447	229	284-430	379	10-May	10-May
2006	37	21	58	125-410	237	180-475	345	11-May	17-May
2007	16	4	20	164-289	226	264-422	336	6-May	15-May
Total	124	61	185						

Table 3.3. Microsatellite loci used to genotype adult and juvenile trout in Langford Creek. * Loci are diagnostic between westslope cutthroat trout and rainbow trout.

Locus	Final [Primer] uM	Initial annealing temperature (°C)	Reference
Multiplex 1			
<i>Ogo8*</i>	0.12	58	Olsen et al., 1998 (S3)
<i>Omm1019*</i>	0.20	58	Rexroad et al., 2002 (S4)
<i>Omm1050*</i>	0.20	58	Rexroad et al., 2002 (S4)
<i>Omm1060*</i>	0.12	58	Rexroad et al., 2002 (S4)
<i>Omm1037-1</i>	0.20	58	Rexroad et al., 2002 (S4)
<i>Omy0004*</i>	0.20	58	Holm et al., 1998 (S5)
Multiplex 2			
<i>Omy1001</i>	0.30	70	Spies et al., 2005 (S6)
<i>Ogo4*</i>	0.20	70	Olsen et al., 1998 (S3)
<i>Ssa456*</i>	0.10	70	Angers et al., 1995 (S7)
<i>Sfo8</i>	0.30	70	Small et al., 1998 (S8)
<i>Ots 101</i>	0.20	70	Slettan et al., 1995 (S9)
Multiplex 3			
<i>Ogo3</i>	0.20	58	Olsen et al., 1998 (S3)
<i>Oki10</i>	0.15	58	Smith et al., 1998 (S10)
<i>Ots 107</i>	0.10	58	Nelson and Beacham, 1999 (S11)
<i>Ssa408*</i>	0.20	58	Cairney et al., 2000 (S12)
<i>Ssa407</i>	0.40	60	Cairney et al., 2000 (S12)

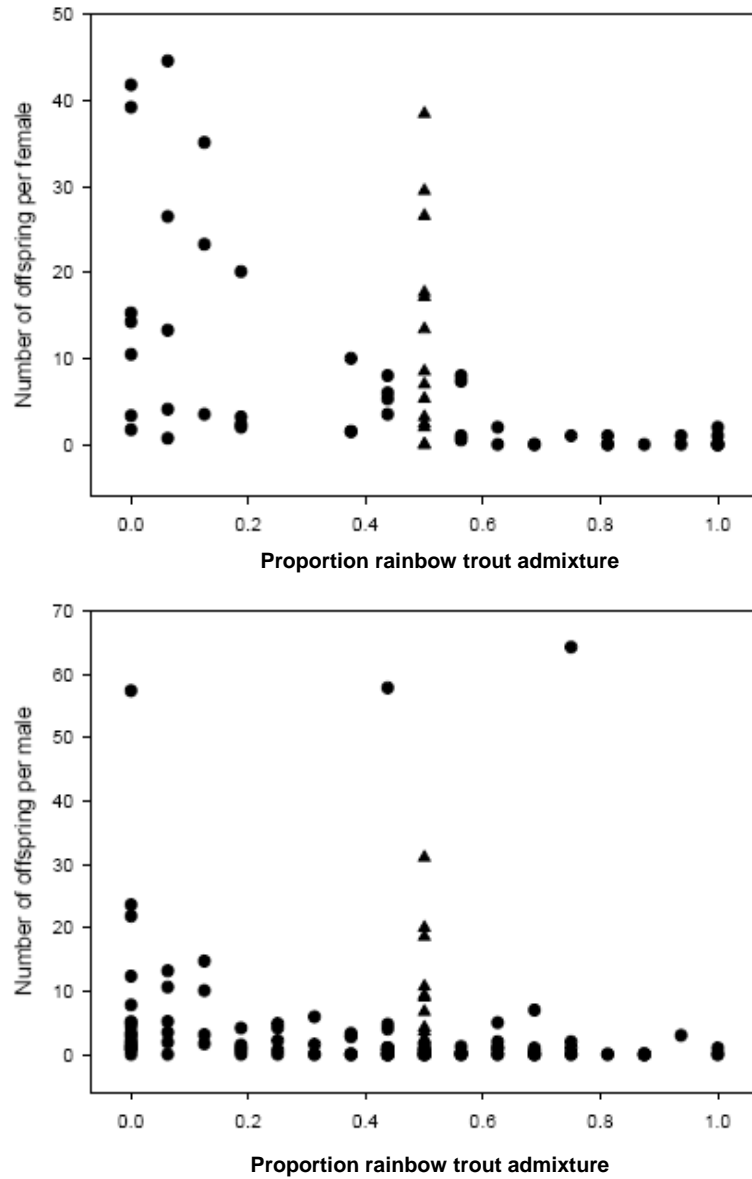


Figure 3.3. Number of offspring per female versus the proportion of nonnative rainbow trout admixture. The female plot (upper) includes 61 mothers and 536 juvenile assignments using parentage analysis with fractional allocation (28). The male plot (bottom) includes 124 fathers and 544 juvenile assignments using parentage analysis with fractional allocation (28). Each point represents an estimate for an individual fish from a spawn-year. Circles represent WCT, RBT, and later-generation hybrids, and triangles are first generation hybrids.

CHAPTER 4

LOCAL HABITAT, LANDSCAPE, AND BIOTIC FACTORS ASSOCIATED WITH THE
DISTRIBUTION AND SPREAD OF HYBRIDIZATION BETWEEN NATIVE WESTSLOPE
CUTTHROAT TROUT AND INTRODUCED RAINBOW TROUTAbstract

The invasion of nonnative fishes in freshwater systems is often facilitated through the interaction of environmental and biotic factors operating at multiple spatial and temporal scales. I evaluated the association of local habitat features, large scale landscape characteristics, and biotic factors with the spread of hybridization between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WCT) and nonnative rainbow trout (*O. mykiss*; RBT) at sample sites in 35 streams of the upper Flathead River system in Montana, U.S.A., and British Columbia, Canada. The presence or absence of hybridization and the proportion RBT admixture for each sampled population were estimated using seven diagnostic microsatellite loci. Local habitat features were measures of stream size, gradient, and elevation. Landscape variables were measures of mean and maximum summer water temperature and of land disturbance (upstream road density and the number of upstream road crossings). Biotic factors were measures of neighborhood effects (distance to source of hybridization) and trout abundance. First, I defined nine candidate logistic regression models that represented various combinations of these factors and used an information-theoretic approach to evaluate

the relative plausibility of competing models. Three models combining stream and landscape characteristics of mean summer temperature, stream width, and number of road crossings in combination with the biotic variable distance to the source of hybridization were the most plausible. Overall classification accuracies were greater than 85%. The presence of hybridization was positively associated with mean summer water temperature and number of upstream road crossings and negatively correlated with distance to the source of hybridization and stream width. Next, linear regression analyses were used to assess the relationship between the proportion of RBT admixture with these factors. Distance to the source of hybridization was the only variable significantly related to the proportion RBT admixture among sites with hybridized trout; RBT admixture decreased with increasing stream distance from the likely source of hybridization. My results suggest that WCT may be particularly susceptible to hybridization with nonnative RBT in streams where anthropogenic habitat disturbance increases water temperatures and degrades stream habitat. However, habitat features alone are unlikely to protect presently non-hybridized populations of WCT from hybridization in the future unless nearby hybridized populations are reduced or eliminated.

Introduction

Invasions of nonindigenous species threaten the biodiversity of aquatic and terrestrial ecosystems worldwide (Mack et al. 2000). Hybridization arising from anthropogenic disturbances on the landscape such as intentional and accidental species relocations and habitat alterations often create secondary contact between reproductively isolated species (Allendorf et al. 2001). Hybridization associated with invasions of nonindigenous fishes may lead to a loss of unique genetic, behavioral and ecological adaptations in native populations (Templeton 1986; Rhymer and Simberloff 1996). In some cases, native taxa are replaced by hybrid swarms resulting in genomic extinction of the native population (Allendorf and Leary 1988; Allendorf et al. 2001). Thus, hybridization is considered a leading cause in the decline and extinction of many fish species throughout North America (Miller et al. 1989).

The invasion of nonnative fishes in freshwater systems is often influenced by the interaction of environmental and biotic factors operating at multiple spatial and temporal scales. The primary factors associated with invasion and establishment of nonnative species are habitat conditions (local and landscape), connectivity, proximity to source populations, and biotic resistance (Benjamin et al. 2007). Water temperature is an important habitat characteristic influencing the distribution and abundance of many stream dwelling salmonid species and has been associated with nonnative species invasions in many freshwater systems (Paul and Post 2001; Dunham et al. 2003;

McMahon et al. 2007). For example, Bear et al. (2007) found that rainbow trout *Oncorhynchus mykiss* (RBT) grew over a wider range and at higher temperatures than westslope cutthroat trout *O. clarkii lewisi* (WCT) in a laboratory study. This relationship may account for displacement of native salmonids by rainbow trout at lower elevation sites (Paul and Post 2001). Additionally, anthropogenic habitat disturbances that increase stream temperatures and degrade riparian and stream habitats have also been correlated with invasion success (Thurow et al. 1997). Shepard (2004) found that that invasive brook trout *Salvelinus fontinalis* displaced native WCT in a southwestern Montana stream with relatively higher water temperatures, decreased debris and pool frequencies, and increased erosion and deposition of fine sediments compared to two adjacent, undisturbed streams. Finally, non-hybridized populations that are in close proximity to nonnative sources may be especially vulnerable to hybridization and the production of hybrid swarms (Mayr 1970; Hitt et al. 2003; Benjamin et al. 2007). However, little information exists regarding the interactive role of these factors in determining the spread and degree of hybridization between nonnative RBT and native salmonids.

All subspecies of cutthroat trout in western North America are threatened by introgressive hybridization with introduced RBT (Allendorf and Leary 1988; Behnke 1992). RBT introductions have been especially detrimental to native WCT populations due to widespread introgression (Allendorf et al. 2001) which threatens this highly divergent subspecies of cutthroat trout with genomic extinction (Allendorf & Leary

1988; Behnke 1992). Shepard et al. (2005) estimated that non-hybridized populations of WCT persist in less than 10% of their historical range. Many of the remaining populations are restricted to small, fragmented headwater habitats where the long-term sustainability of populations is uncertain (Hilderbrand and Kershner 2000).

The upper Flathead River system is considered a regional stronghold for WCT. Recent invasion of RBT, however, has led to a rapid spread of introgression threatening the genetic and ecological characteristics of migratory and resident populations (Hitt et al. 2003; Boyer et al. 2008; Chapter 2). This study was intended to examine abiotic and biotic factors associated with the occurrence and degree of hybridization throughout the upper Flathead River drainage from the headwaters in Canada downstream to the mainstem Flathead River upstream of Flathead Lake. I hypothesized that hybridization would be more likely in small, low elevation streams with increased land disturbance in close proximity to hybridized populations that contain high trout densities. Furthermore, I predicted WCT would be more common in headwater streams that have colder water temperatures, less land disturbance, and are further from hybridized populations. Finally, I hypothesized that the proportion of rainbow trout admixture would be positively related to density, temperature, and land disturbance.

My objectives were to examine the occurrence and extent of RBT introgression in relation to these factors. Understanding the importance of environmental and biotic factors influencing the spread of hybridization will provide guidance for prioritization

and implementation of effective conservation and recovery programs for WCT and other salmonids threatened with loss of genetic integrity.

Methods

Study Area

The study area included tributaries to the North Fork Flathead River, a fifth order river, that drains an area roughly 4,000 km² and flows approximately 160 km south to its confluence with the Middle Fork. The North Fork Flathead River originates in the Rocky Mountains of British Columbia, Canada and flows across the international boundary into northwest Montana, where it forms the western border of Glacier National Park before joining the mainstem Flathead River that flows into Flathead Lake (Figure 4.1). This drainage contains migratory populations of bull trout *S. confluentus* which is a threatened species in the United States and westslope cutthroat trout which is a species of special concern in Montana (USA) and a blue-listed species at risk in British Columbia. Both species migrate from Flathead Lake and the lower Flathead River in Montana to spawn in U.S. and Canadian tributaries.

Previous genetic studies in the Flathead River drainage (Hitt et al. 2003; Boyer et al. 2008) documented sources of RBT introgression in lower elevation tributaries and a decline in the proportion of admixture with increasing upstream distance from these sites. Furthermore, Hitt et al. (2003) found evidence of new RBT introgression in seven of 11 populations that were determined to be non-hybridized in 1984 suggesting that

hybridization has recently spread in an upstream direction in the system. Montana Fish, Wildlife & Parks (MFWP) stocking records indicate that over 20 million RBT were stocked in the lower elevations of the Flathead River drainage (Flathead Lake and mainstem Flathead River) beginning in the late 1800s and continuing until 1969. Additionally, unintentional introductions of RBT likely occurred from Sekokini Springs, a privately owned RBT hatchery in the lower valley. Anecdotal evidence suggests that approximately 70,000 RBT were illegally released in the lower section of the North Fork Flathead River in 1997 when Sekokini Springs ceased operations (Boyer et al. 2008).

Study Design and Data Collection

I evaluated the association of local habitat features, large scale landscape characteristics, and biotic factors with patterns of occurrence, density, and degree of hybridization between native WCT and nonnative RBT in 35 locations in 33 streams of the upper Flathead River system in Montana and British Columbia (Table 4.1; Figure 4.1). The study occurred during the low flow period (July-September) from 2004 through 2007. All sample sites were located downstream of physical barriers to fish migration. WCT and RBT, therefore, could have theoretically accessed each site within the interconnected study area. Sampling occurred throughout the system and represented the full range of environmental and geographic variation from the headwaters in Canada downstream to the lower valley bottom in the U.S.

The presence or absence of hybridization and the proportion RBT admixture for each sampled population was estimated using seven diagnostic microsatellite loci. I

calculated population admixture as the proportion of RBT alleles found among individuals within a population (Boyer et al. 2008). These methods and data are presented by Boyer et al. (2008). A total of 971 individuals were collected from 35 locations (2003-2007; mean per stream, 28; SD, 7). To minimize sampling of related individuals, fish were captured by electrofishing or angling in stream sections ranging from 250 m to 1 km long. Hybridization was declared present in a tributary if RBT alleles were detected in the sample at one or more loci. A sample was considered to be non-hybridized WCT if no RBT alleles were detected; the power to detect as little as a 1% RBT genetic contribution in a hybrid swarm with our techniques was at least 0.94 (Boecklen and Howard 1997).

Biotic Variables

I examined the influence of fish density and distance to the source of hybridization on the presence or absence of hybridization. These biotic metrics were used to assess the role of demographic support in facilitating or preventing the spread of hybridization. Previous genetic surveys (Hitt et al. 2003; Boyer et al. 2008) have shown that Abbot Creek contained a hybrid swarm between WCT and RBT with a high proportion (0.98) of RBT admixture. Telemetry studies (Chapter 2) also indicated the stream supports a relatively high proportion of spawning by migratory hybrids. Finally, Hitt et al. (2003) and Boyer et al. (2008) found that the amount of admixture tended to decrease with distance upstream from Abbot Creek. Thus, I calculated the stream distance from the mouth of Abbot Creek to each sample site in ArcGIS 9.2 (ESRI,

Redlands, Calif.), as a metric of a neighborhood effect (subsequently referred to as distance to source of hybridization).

I conducted fish abundance estimates in the same section in which the genetic data were collected using the multiple pass depletion method (Zippin 1958).

Abundance estimates were conducted between July and September within 1-2 years following the genetics sampling. Population estimates were conducted in stream sections 150 m in length. A hydrologic break (e.g., riffle or vertical drop) was selected for the upper boundary and a block net (12.7 mm mesh) was placed across the channel at the lower boundary prior to sampling. A minimum of three passes were completed in each section with one or two backpack electrofishing units (Smith-Root Model 15-D) working from the upstream boundary downstream to the lower block net. If capture probability (P) exceeded 0.60 on the third pass, electrofishing was terminated. If not, electrofishing continued until P exceeded 0.60. Lengths (to the nearest mm) of all captured trout were recorded. Based on length-at-age data for the upper Flathead system (C. Muhlfeld, unpublished data), individuals less than 75 mm were considered to be young-of-the-year fish. These individuals were not included in the abundance estimates due to poor sampling efficiency and variable emergence times across streams.

Geo-referenced locations were obtained in each sample section using a global positioning system (GPS) unit. Ten wetted widths were systematically taken every 15 m through the sample section. Population estimates were calculated using the MICROFISH 3.0 computer program (Van Deventer and Platts 1985). Fish density (fish/m²) was

calculated by dividing the fish population estimate by the wetted stream surface area. For 11 of the 35 sites, abundance was estimated in more than one year. In these situations, I averaged the densities across years.

Local Habitat and Landscape Variables

Local habitat features included measures of stream size, gradient, and elevation. Site gradient and elevation were derived from 1:25,000 U.S. Geological Survey maps using ArcGIS. Mean stream width was calculated as the average of the ten wetted width measurements collected during the population estimate.

Landscape variables included measures of temperature and land disturbance. Thermographs were deployed at each site to record hourly water temperatures during the year in which abundance estimates were completed. Water temperature metrics used were mean summer temperature and summer maximum temperature. The mean summer temperature was calculated as the mean of all the daily averages from 1 July–30 September. The maximum water temperature at each site was the highest recorded temperature during the sampling period. Temperature data were unavailable for upper Cyclone Creek.

Road density metrics were used as indicators of land use disturbance because roads often alter hydrologic and geomorphic regimes in downstream areas (Trombulak and Frissell 2000). Furthermore, road density metrics have been correlated to the spatial extent of timber harvest activity in the Flathead system (Hauer and Blum 1991). The upstream road density and the number of upstream road stream intersections were

calculated for each site (Baxter et al. 1999) from the U.S. Forest Service's Flathead National Forest INFRA database in ArcGIS (Hitt et al. 2003).

Data Analysis

I used two separate regression approaches to analyze the data. Logistic regression analyses were used to evaluate the relationship between the nine independent variables and the presence or absence of hybridization. Linear regression analysis was used to assess the relationship of these variables with the level of RBT admixture among hybrid populations. For logistic regression analyses, I first tested for differences between hybridized and non-hybridized sites for each independent variable using Mann-Whitney U tests. Non-significant ($P > 0.10$) variables were excluded from subsequent analyses (gradient, road density, fish density). Next, I conducted a Pearson rank correlation analysis to identify interactions between the remaining variables. The local habitat variables elevation and width were significantly correlated ($P < 0.05$), as were mean temperature and maximum temperature ($P < 0.001$). Thus, I selected variables from this group that had the most functional significance or that had the strongest univariate relationship with the occurrence of hybridization. Based on these criteria, the final variables for the logistic regression analyses were stream width, mean summer water temperature, number of road crossings, and distance to source of hybridization.

I defined nine candidate logistic regression models that represented various combinations of these four factors and used an information-theoretic approach to

evaluate the relative plausibility of competing models (Burnham and Anderson 1998; Thompson and Lee 2000; Rich et al. 2003). I first used a global model relating the occurrence of hybridization to all local habitat, landscape, and biotic factors and then conducted a Hosmer-Lemeshow goodness-of-fit test to see whether the model met the assumptions of logistic regression. If the model met these assumptions ($P < 0.05$), I used logistic regression analysis to assess the relative plausibility of each model. I used a small-sample size adjustment (AICc; Hurvich and Tsai 1989) to Akaike's information criterion (AIC; Akaike 1973) and relative Akaike weights (w_i) to rank competing models. Models with lower Akaike information criterion (AICc) scores and larger Akaike weights were considered more plausible models. Models were ranked in terms of the difference between their AICc score and the lowest score (20.77). Models were considered equally plausible unless their Akaike weights were less than one-eighth the value of the model with the highest Akaike weight (Thompson and Lee 2000; Rich et al. 2003). Among streams where hybridization was detected, I examined how the proportion of RBT admixture varied among the abiotic (gradient, elevation, stream width, maximum and mean temperature, road density, road crossings) and biotic (distance to source of hybridization and fish density) variables using simple linear regression (Norusis 1990).

Results

Habitat and Landscape Characteristics

Nineteen of 35 sites (54%) showed no evidence of RBT introgression (Table 4.1; Figure 4.1). Streams with hybrid populations were smaller (mean width, 3.9 m; range, 2.0-7.4 m) and lower in elevation (mean, 1137 m, range, 950-1280 m) compared to streams with WCT (mean width, 5.4 m; range, 1.3-13.6 m, $U = 97.5$, $P = 0.07$; mean elevation, 1304 m, range, 1130 m to 1536 m; Mann-Whitney $U = 47.0$, $P < 0.01$). Mean and maximum summer temperatures were significantly higher in streams with hybrids. The mean summer water temperature was 11.5°C (range, 7.6 - 15.0°C) for streams with hybrids and 9.6°C (range, 6.6 - 11.4°C) for streams with WCT ($U = 63.5$, $P < 0.01$), and maximum temperatures averaged 20.2°C versus 16.5°C , ($U = 82.5$, $P = 0.03$), respectively. Hybrid populations occurred in streams with more upstream road intersections (mean, 11; range, 0-33) than those with WCT (mean, 6; range, 0-29), but no differences were detected for road density ($U = 123.5$; $P = 0.35$). Similarly, I found no differences in gradient between streams occupied by hybrid trout (mean, 0.04; range, 0.01-0.07) and non-hybridized WCT (mean, 0.04; range, 0.01-0.09; $U = 110.5$, $P = 0.17$), nor among densities of trout between WCT and hybrid sites ($U = 116$, $P = 0.24$) (WCT: mean, 0.076 fish/m^2 ; hybrids: mean, 0.103 fish/m^2).

Occurrence and Degree of Hybridization

The best approximating logistic regression model contained the watershed variables of mean temperature and number of upstream road crossings in combination with the biotic variable distance to source of hybridization (Table 4.2). However, two other models, one comprising mean temperature and distance to source and the other comprising all of the variables (the global model) were equally plausible (w_i greater than one-eighth of highest w_j). All three models had overall classification accuracies greater than 85%. The occurrence of hybridized trout was positively associated with mean summer water temperature and the number of upstream road crossings and negatively correlated with distance to the source of hybridization and stream width. Linear regression analysis showed that the distance to the source of hybridization was significantly related to the proportion RBT admixture among hybridized populations ($r^2 = 0.845$; $df = 34$; $P < 0.001$). In contrast, none of the other eight variables that I examined were significantly associated with proportion of RBT admixture ($P > 0.05$).

Discussion

I examined the relative importance of local habitat features, large scale landscape characteristics, and biotic factors associated with the spread of hybridization between native WCT and nonnative RBT in a recently invaded, large interconnected river system. Results support my hypothesis that hybridization is more likely to occur in

streams with warm water temperatures, increased land-use disturbance, and in sites located in close proximity to the major source of hybridization. Similarly, other studies in Europe and North America (Paul and Post 2001; Rich et al. 2003; Shepard 2004; Carveth et al. 2006; Jeschke and Strayer 2006; Benjamin et al. 2007) have found that the synergistic effects of habitat conditions and connectivity influence nonnative species invasions and the displacement of native fishes.

Hybridization was more likely at warmer sites suggesting increased water temperatures may promote invasion by nonnative RBT and hybridization with native WCT. Water temperature plays an important role in determining the distribution of many stream dwelling salmonid species due to its synergistic effects on physiology, behavior, and ecological interactions (Paul and Post 2001; Dunham et al. 2003; McMahon et al. 2007). Temperature is an important factor influencing the distribution and abundance of WCT populations (Sloat et al. 2005; Shepard et al. 2005). This subspecies has the lowest thermal optima and lowest upper tolerance limits of North American salmonids (Bear et al. 2007). Sloat et al. (2005) found that WCT occurred in streams with cool water temperatures (maximum daily temperature, $\leq 16.5^{\circ}\text{C}$) in Montana, which is very similar to my findings (mean maximum temperature, 16.5°C).

I detected a general pattern of WCT persisting in colder streams at higher elevations than hybrids. These results are consistent with many studies that have examined the genetic distribution of hybridization in situations where RBT are introduced into waters containing allopatric populations of WCT (Rubidge and Taylor

2004; Weigel et al. 2003; Robinson 2007). At first glance, my data appear to support the elevation refugia hypothesis. That is, cold temperatures in headwater streams impart a competitive advantage to native salmonids and thus account for increased resistance to invasion and displacement of nonnatives (Paul and Post 2001; McMahon et al. 2007). The overlap in temperature regimes and local habitat conditions, however, among sites with and without hybrids and the significant association between amount of admixture and distance to source of hybridization suggest that headwater streams may not provide refuge from hybridization if sources of hybridization continue to persist in the system.

The correlation between the presence of hybridization and the number of upstream road crossings suggests that hybridization is also more likely in streams with increased disturbance. Land use disturbances can make systems more prone to successful invasion of nonnative competitors by changing the availability and quality of habitats which eventually may lead to the displacement of native taxa (Allendorf et al. 2001; Jeschke and Strayer 2006). This has been observed for a variety of salmonids (Taylor et al. 1984; Fausch et al. 2001; Post and Paul 2001; Shepard 2004), as well as many plant (Arnold 1997) and other vertebrate species (Haig et al. 2004; Schwartz et al. 2004). In montane river systems, forest roads affect the physical, biological, and chemical characteristics of downstream areas by altering hydrologic and geomorphic processes (Trombulak and Frissell 2000), and thus in part determine physical and biological characteristics of stream habitats (Montgomery and Buffington 1998). Roads

may negatively impact salmonid stream habitat by increasing stream sediment loads, obstructing fish movements, and degrading spawning, rearing and reproductive habitats (Furniss et al. 1991; Meehan 1991; Ripley et al. 2005). In the Flathead system, McCaffrey et al. (2007) found that various measures of road effects (i.e., road density, number of roads in use, and number of road crossings) were positively correlated with increased sedimentation in the stream substrate. High levels of fine sediment are known to decrease emergence success of incubating salmonid embryos (Chapman 1988), including WCT (Weaver and Fraley 1993) and RBT (Fudge et al. 2008).

The presence of hybrid populations with high proportions of RBT admixture in the lower drainage may also simply reflect stocking history, which may have confounded my results. The purported illegal release of an estimated 70,000 RBT in 1997 from a private hatchery in the lower portion of the drainage likely played a significant role in the recent proliferation and distribution of hybridized trout in the system (Boyer et al. 2008). This coincides with a dramatic increase in the spread of hybridization in the system during the late 1990s to the present (Hitt et al. 2003; Boyer et al. 2008). Many studies have shown that propagule pressure, the number and frequency of introduced individuals, plays an important role in the establishment and spread of exotic species (see Lockwood et al. 2006 for a review). The spatial distribution of hybridization in the Flathead system, however, may not entirely be the result of stocking history, as RBT tend to outcompete cutthroat trout in productive, low elevation streams (Fausch et al. 2001; Paul and Post 2001; Robinson 2007). RBT have higher thermal tolerances (Bear et

al. 2007), greater metabolic and growth requirements (Robinson 2007), better swimming ability (Seiler and Keeley 2007), and broad niche requirements (MacCrimmon 1971) than WCT. For instance, in the east slopes of the Rocky Mountains where RBT have been stocked extensively over a wide range of elevations RBT have moved downstream often hybridizing or displacing native cutthroat trout populations (Paul and Post 2001).

Source proximity strongly influenced the occurrence and proportion of nonnative RBT admixture across this large drainage. Previous genetic studies have demonstrated that Abbot Creek is likely the main source of hybridization in the system and that the spread of hybridization was largely explained by the proximity of sites to this locality (Hitt et al. 2003; Boyer et al. 2008). Likewise, in the upper Kootenay River drainage in British Columbia, Rubidge and Taylor (2005) examined the spatial and environmental factors influencing hybridization between native WCT and introduced RBT. They found clustering among hybridized sites and that the likelihood of finding hybrids decreased with distance from Koocanusa Reservoir suggesting that the reservoir serves as a main source of hybridization. Other research on stream dwelling salmonids has shown that source connectivity and local habitat characteristics influence invasion and displacement or replacement of native taxa (Rich et al. 2003; Benjamin et al. 2007).

Surprisingly, fish density did not influence the occurrence or degree of hybridization. Although biotic resistance from native species may limit competitors from becoming established (Pimm 1989), I hypothesized that sites with higher densities

would be more susceptible to hybridization because RBT and WCT have similar mating behaviors which facilitates interbreeding. My results, however, suggest that the probability of hybridization may be more closely related to neighborhood rather than density effects, as distance to source of hybridization was a significant factor in all the models.

Elevation was also significantly related to the degree of RBT introgression. I expected this to be the case since site elevation increased with increasing distance from Abbot Creek and elevation is strongly correlated with water temperature. Similarly, Weigel et al. (2003) examined the distribution of introgressive hybridization between native WCT and nonnative RBT in two subbasins of the Clearwater River, Idaho. They found that the level of introgression was negatively related to elevation and positively related to stream size. In contrast, Rubidge and Taylor (2005) found no evidence of stream size or elevation influencing the extent of hybridization among sites in the Kootenay River drainage, in British Columbia.

Management Implications and Conclusions

Sites where disturbance was more common and temperatures were warmer were closer to Abbot Creek than colder, less disturbed sites. The results, therefore, are confounded by interrelated environmental and human-mediated factors that synergistically influence invasion success and the extent of hybridization in the system. In this system, the distance from Abbot Creek likely represents the main factor that affects the probability that a site will be encountered and invaded by a hybrid or RBT.

Under this scenario, sites with higher water temperature increase the likelihood the invasion will be successful. In the upper Flathead River system, many sites close to Abbot Creek tend to be relatively warm and more disturbed resulting in a high probability of successful invasion by hybrids. Sites that are colder, less disturbed, and more distant from Abbot Creek, however, are probably not completely safe from future introgression due to the ratchet effect (Epifanio and Philipp 2001) of hybridization, long-distance dispersal of individuals with high proportions of nonnative RBT admixture, and the unknown correlation between an individual's thermal tolerance and its proportion of admixture.

My data suggest that WCT may be particularly susceptible to hybridization with nonnative RBT in situations where anthropogenic habitat disturbance increases water temperatures and degrades stream habitat. Indeed, habitat degradation and fragmentation are also leading causes of the decline and extirpation of WCT throughout their range (Shepard et al. 2005). Increased stream temperatures due to dewatering of the stream channel, alteration of stream hydrology and riparian vegetation, and global climate change may be especially detrimental to non-hybridized WCT populations. Additionally, my data suggest that road building and increased sedimentation in spawning and rearing habitats may promote hybridization. Currently, the headwaters of the North Fork Flathead River in British Columbia have been targeted for coalbed methane development and open-pit coal mining. This area supports the majority of the remaining non-hybridized WCT populations in the drainage. Protection of these

headwater migratory populations and associated spawning and rearing habitats is probably critical for long term persistence of WCT populations and the migratory life history in the Flathead system.

The petition to list the WCT as a threatened species under the Endangered Species Act (ESA) was recently denied because the subspecies is “widely distributed, numerous non-introgressed WCT populations are distributed in secure habitats throughout the subspecies’ historic range, and numerous WCT are non-introgressed or nearly so” (U.S Court of Appeals, Case 07-5179, May 2007). My results and previous work (Hitt et al. 2003; Boyer et al. 2008) do not support the assumption that non-hybridized populations are “secure” from introgression with nonnative RBT. The focus of this study was to identify factors that affect the occurrence and degree of hybridization. Although headwater streams currently contain non-hybridized WCT populations, my data suggest that environmental and biotic factors alone may not be sufficient to maintain ‘secure’ habitats in open systems. Indeed, Sloat et al. (2005) found that where RBT are present the only non-hybridized WCT populations occurred upstream of barriers to fish migration. Furthermore, neighborhood effects largely explained the distribution and degree of RBT admixture in the Flathead system. Thus, these data suggest that hybridization and introgression is likely to continue to spread and the loss of additional WCT populations is imminent unless populations with high amounts of RBT admixture are reduced or eliminated (Boyer et al. 2008). Management strategies for preserving non-hybridized WCT populations may consider attempting to

eradicate hybridized populations with high levels of RBT admixture by targeting streams with warmer temperatures and high land use disturbance as my results suggest that these are the likely source populations for hybridization to spread.

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Table 4.1. Summary of local-habitat features, landscape characteristics, and biotic factors in each study site in the upper Flathead River drainage, Montana and British Columbia, Canada.

Site name	Site #	Local-habitat features			Watershed characteristics				Biotic factors			
		Gradient (%)	Elevation (m)	Stream width (m)	Maximum temperature (°C)	Mean summer temperature (°C)	Road density	Road crossings	Distance to source (km)	Present/absent	Trout density (fish/m ²)	% RBT admixture
Abbott	1	0.01	950	2.64	20.2	15.00	0.70	24	0.0	yes	0.16	91.6
Ivy	2	0.07	977	2.03	12.6	10.60	0.50	4	6.4	yes	0.08	49.3
Rabe	3	0.04	996	3.42	16.2	12.40	1.20	11	13.9	yes	0.22	49.1
Third	4	0.03	962	2.18	13.3	10.70	0.07	0	16.9	yes	0.19	65.8
Langford	5	0.02	1130	2.56	10.8	9.40	0.43	8	40.3	yes	0.12	33.1
Meadow	6	0.03	1134	2.15	19.4	14.00	0.24	4	58.3	yes	0.06	3.5
Skookoleel	7	0.08	1200	6.10	11.8	8.70	0.49	6	54.2	no	0.04	0.0
Nicola	8	0.07	1280	3.90	9.7	7.60	1.32	9	55.1	yes	0.07	1.8
Werner	9	0.06	1303	5.98	11.1	8.30	1.22	9	56.0	no	0.08	0.0
Kletomus Cyclone, lower	10	0.09	1390	4.20	12.5	9.10	0.45	0	62.7	no	0.10	0.0
Cyclone, upper	11	0.02	1260	3.78	18.6	13.10	0.83	14	59.7	yes	0.07	11.6
Deadhorse	12	0.07	1430	11.67	na	na	0.44	3	59.7	no	0.05	0.0
North Coal	13	0.04	1260	3.60	13.7	9.80	0.42	6	67.9	no	0.14	0.0
South Fork Coal	14	0.03	1259	2.30	13.9	10.00	0.71	33	67.9	yes	0.23	7.3
Anaconda	15	0.05	1340	6.50	14.1	10.20	0.45	6	74.6	yes	0.02	0.6
Dutch	16	0.05	1110	5.05	16.3	12.00	0.02	1	48.3	yes	0.07	20.6
Moran	17	0.02	1110	4.71	16.8	12.60	0.03	1	49.3	yes	0.04	13.0
Hay Creek, lower	18	0.05	1230	3.70	13.2	9.60	0.65	9	64.4	no	0.06	0.0
	19	0.02	1090	6.70	13.0	10.10	0.42	14	64.7	yes	0.05	1.4

Table 4.1 Continued

Site name	Site #	Local-habitat features			Watershed characteristics				Biotic factors			
		Gradient	Elevation (m)	Stream width (m)	Maximum temperature (°C)	Mean summer temperature (°C)	Road density	Road crossings	Distance to source (km)	Present/absent	Trout density (fish/m ²)	% RBT admixture
Hay Creek, upper	20	0.04	1430	6.70	11.2	8.50	0.32	5	81.0	no	0.07	0.0
Akokala	21	0.03	1340	6.30	14.8	10.90	0.03	0	86.8	no	0.01	0.0
South Fork Red												
Meadow	22	0.03	1240	2.40	12.3	9.20	0.31	2	77.2	yes	0.07	0.3
Red Meadow	23	0.03	1150	7.40	15.5	11.90	0.59	31	75.0	yes	0.15	2.2
Hawk	24	0.02	1176	1.30	14.6	10.10	1.56	5	74.1	no	0.10	0.0
Moose	25	0.02	1130	4.00	10.1	7.60	0.52	11	89.6	no	0.12	0.0
Ford	26	0.03	1154	4.03	15.9	11.20	0.00	0	84.7	no	0.08	0.0
Tepee	27	0.03	1210	3.80	17.7	11.80	1.05	17	87.7	yes	0.05	1.3
Ketchikan	28	0.02	1278	3.15	13.8	10.10	0.04	0	103.3	no	0.22	0.0
Tuchuck	29	0.03	1536	5.70	12.1	9.10	0.04	1	108.4	no	0.11	0.0
Colts	30	0.06	1239	3.78	12.6	9.60	0.24	0	107.0	no	0.08	0.0
Sage	31	0.00	1280	13.60	12.7	10.60	0.29	29	114.1	no	0.01	0.0
Burnham	32	0.05	1273	3.19	16.5	11.40	0.59	14	116.4	no	0.03	0.0
Commerce	33	0.02	1334	5.92	14.3	11.20	0.29	4	130.7	no	0.05	0.0
Middlepass	34	0.05	1405	5.04	11.2	9.20	0.16	8	139.5	no	0.05	0.0
Parker	35	0.04	1395	4.54	8.4	6.60	0.82	1	143.7	no	0.05	0.0

Table 4.2. Model selection results for a candidate set of logistic regression models containing various combinations of local habitat features (stream width), landscape characteristics (mean summer water temperature, number of upstream road crossings), and biotic factors (distance to source of hybridization) in relation to the occurrence of hybridization between native westslope cutthroat trout and nonnative rainbow trout in 35 locations from 33 streams in the upper Flathead River drainage, Montana (USA) and British Columbia (Canada).

Model	Number of parameters	AICc	Akaike weight	Percent correct
Mean temperature, number of crossings, distance to source	3	0.00	0.4543	88.2
Mean temperature, distance to source	2	0.72	0.3175	88.2
Width, mean temperature, number of crossings, distance to source (global)	4	2.06	0.1619	85.3
Width, mean temperature, road crossings	3	4.51	0.0477	82.4
Distance to source	1	7.41	0.0112	71.4
Width, distance	2	8.43	0.0067	68.6
Mean temperature	1	14.14	0.0004	67.6
Mean temperature, road crossings	2	15.16	0.0002	70.6
Width	1	20.77	0.0000	68.6

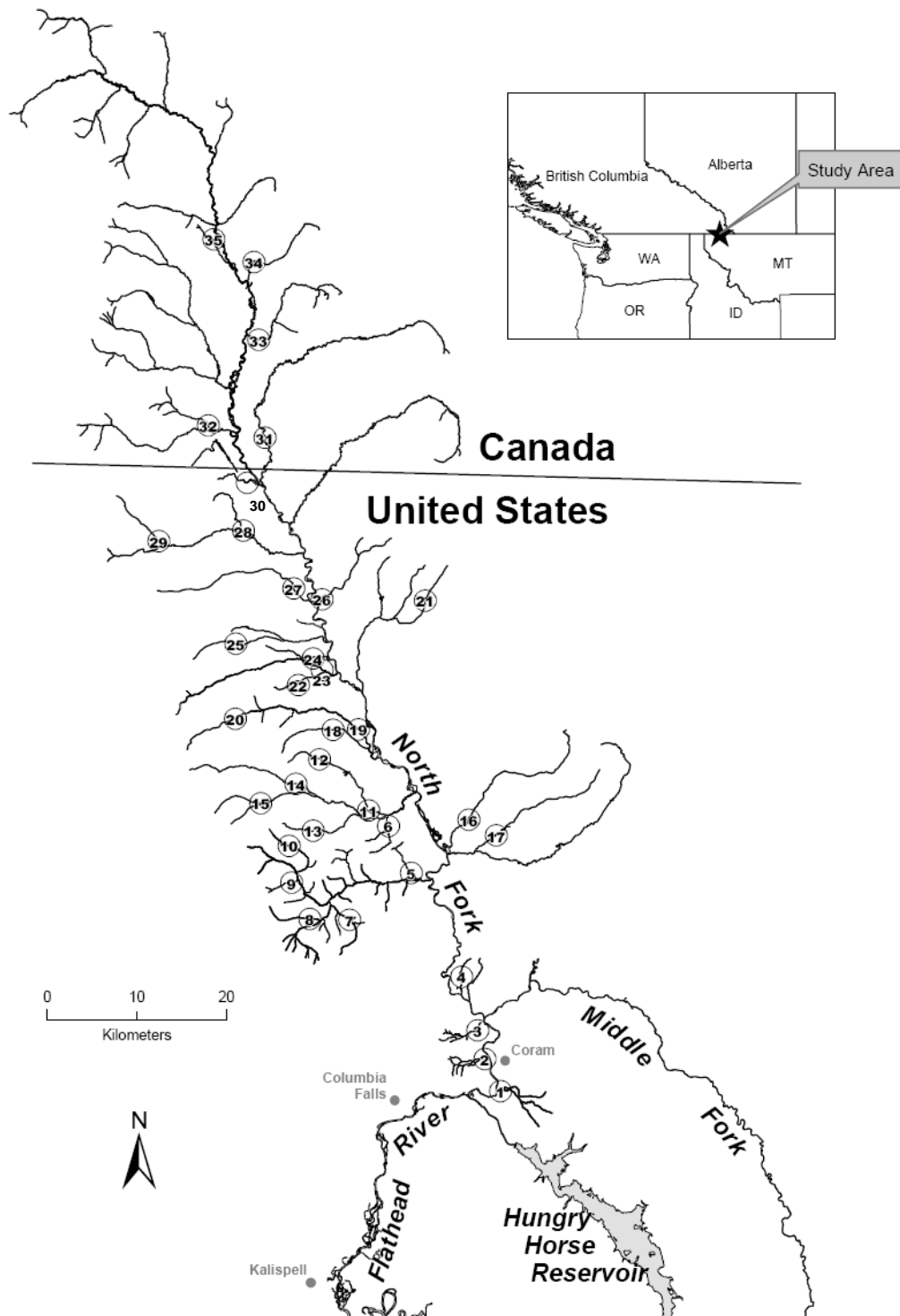
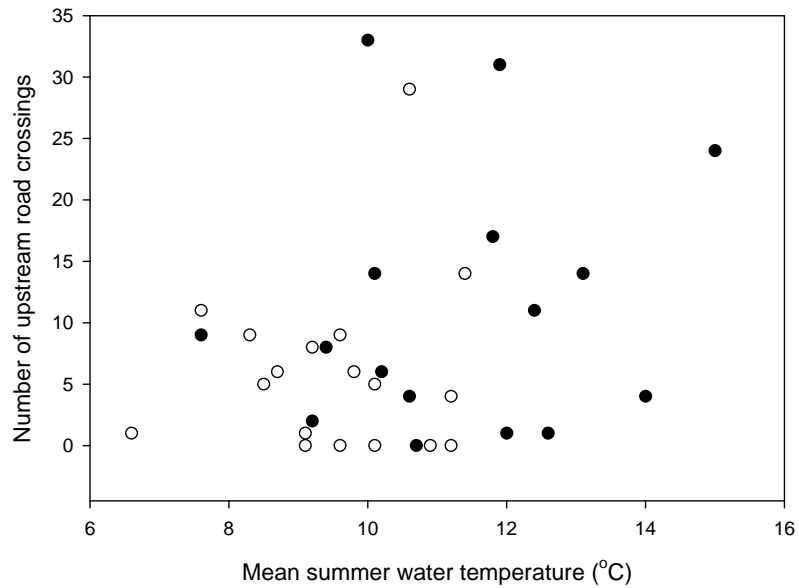
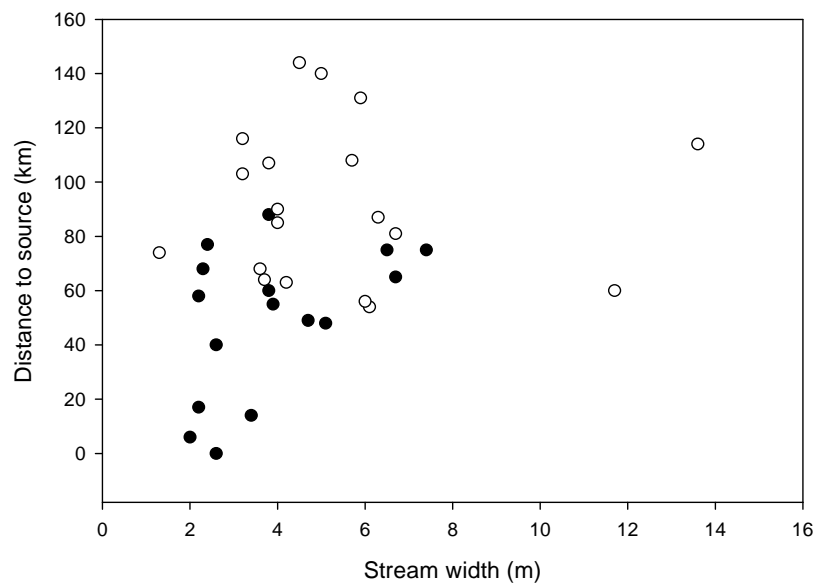


Figure 4.1. Study area and sample site identification. Sample site codes correspond to Table 4.1.

A**B**

4.2. The presence (closed circles) and absence (open circles) of hybridization between native WCT and nonnative RBT in relation to: (A) mean summer water temperature (°C) and number of upstream road crossings, and (B) stream width (m) and distance from hybrid source (km).

CHAPTER 5

CONCLUSIONS AND MANAGEMENT RECOMMENDATIONS

The fundamental question of my dissertation was to determine what are the behavioral, ecological and fitness consequences of hybridization between introduced rainbow trout and native westslope cutthroat trout? I found that hybridization in the upper Flathead River system is spreading rapidly due to a combination of two mechanisms: (1) long distance movements and reproduction in upper drainage tributaries by a few highly introgressed rainbow trout males with high reproductive success; and (2) stepping-stone dispersal of hybrids colonizing and successfully reproducing in streams emanating from the original hybridization source of Abbot Creek, resulting in near local extirpation of non-hybridized westslope cutthroat trout in lower drainage tributaries. My results also showed that spawning behavior has a strong genetic component and that introgression significantly altered the spawning behavior of westslope cutthroat trout relative to hybrids and rainbow trout. Most importantly, my results clearly demonstrated that even small amounts of hybridization markedly reduce reproductive success in the wild. Finally, I found that the presence and degree of hybridization was most closely governed by the proximity to Abbot Creek, the likely source of rainbow trout in the drainage, but also was greater in streams with warmer temperatures and higher levels of land use disturbance.

Despite heavy fitness costs, I found that the spread of hybridization and introgression through the system appears to be due to a variety of factors. First, it seems to be spreading via the hybrid ratchet effect. That is, genomic extinction of parental taxa can occur despite heavy fitness costs for the hybrid offspring due to dispersal of parental types and the continued production of hybrids by hybrids. I observed that a few F_1 hybrids have relatively high fitness which facilitates subsequent backcrossing. I also observed that a few post F_1 hybrid males had reproductive success as high as or substantially higher than non-hybridized westslope cutthroat trout. This decreases the efficiency of selection against hybrid genotypes and may be a conduit through which genetic introgression proceeds.

My findings have significant implications to the protection and restoration of genetically pure westslope cutthroat trout populations. The proposed listing of westslope cutthroat trout under the ESA has been controversial. This controversy arises from two main factors. First, is the heavy reliance on the use of morphology to identify individuals and populations under the unit considered for listing. Second, is the inclusion of populations with up to 20% nonnative introgression as considered to be behaviorally and ecologically equivalent to westslope cutthroat trout. My results clearly indicate the latter assumption is incorrect. For example, with 20% introgression my data showed that fitness declined by ~50%. Instead of representing a conservation strategy, therefore, my results indicate that protection of populations at this level of introgression will likely serve as a means of promoting the continued loss of westslope

cutthroat trout populations by further hybridization and introgression. My data suggest that the level of anthropogenic introgression needed to maintain fitness and protect the evolutionary characteristics of westslope cutthroat trout populations is likely between zero and ten percent. However, future studies are needed to assess the fitness and life history impacts of very low levels (<10%) of nonnative introgression.

I conclude that westslope cutthroat trout are at greater conservation risk than previously thought. Given the continued replacement of westslope cutthroat trout with hybrids, I suspect that non-hybridized populations will continue to be lost if hybrid source populations with high proportions of rainbow trout admixture are not suppressed or eliminated, like the active suppression efforts currently underway in Abbot Creek. Other management schemes that may prove useful in stopping or slowing the spread of hybridization include: (1) selectively removing hybrid spawners in recently invaded streams; (2) installing barriers to preclude access to spawning areas used by hybrids or to isolate non-hybridized populations; and (3) modification of flow or habitat conditions that favor native populations. My data illustrate how quickly fitness declines and reproductive and migratory behaviors change when rainbow and cutthroat trout interbreed. My results also suggest that small amounts of admixture that may not be morphologically detectable may have negative effects on the fitness of westslope cutthroat trout. Future research should focus on examining fitness in older, established hybrid swarms to determine if fitness remains depressed after several generations, and assessing how very low levels (<10%) of nonnative admixture affect fitness using

laboratory and field studies. Conservation and policy programs protecting hybridized populations need reconsideration in systems containing non-hybridized westslope cutthroat trout populations.