HYBRIDIZATION BETWEEN WESTSLOPE CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI LEWISI*) AND RAINBOW TROUT (*O. MYKISS*): DISTRIBUTION AND LIMITING FACTORS

by

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HYBRIDIZATION BETWEEN WESTSLOPE CUTTHROAT TROUT (*ONCORHYNCHUS CLARKI LEWISI*) AND RAINBOW TROUT (*O. MYKISS*): DISTRIBUTION AND LIMITING FACTORS

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Hybridization is perhaps the primary threat facing westslope cutthroat trout (*Oncorhynchus clarki lewisi*, WCT). Nevertheless, the distribution of hybridized populations and the limits to their expansion remain poorly understood. In this study, I used molecular genetic techniques to assess patterns of hybridization between WCT and non-native rainbow trout (*O. mykiss*, RBT) in the Flathead River system, a stronghold for native WCT. I detected WCT x RBT hybridization in 25 of 43 sample sites. Spatial analyses showed clustering among hybridized sites and decreasing percent RBT introgression with increasing upstream distance from Flathead Lake. Temporal comparisons showed new RBT introgression (7/14 sites), continued absence of RBT introgression (5/14 sites), and continued presence of RBT introgression (2/14 sites) since 1984. I found that fluvial distances to nearest hybridized and pure sites are more strongly associated with hybridization status than environmental gradients, suggesting that RBT introgression may be constrained more by demographic than environmental factors. These findings are consistent with the hypotheses that (a) RBT introgression is spreading upstream, (b) environmental gradients do not effectively limit the spread of RBT introgression, and (c) further hybridization of WCT populations is probable in the Flathead River system. This study adds to a growing literature demonstrating the importance of upstream invasive species vectors and the vulnerability of headwater communities.

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Acknowledgements

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Chapter 1.

Introduction

Non-native species have influenced nearly every biological community on earth (Mack et al. 2000). Introductions of non-native species have mirrored the dramatic expansion of transoceanic travel and commerce by humans over the last century (di Castri 1989; Elton 1958). It is estimated that at least 50,000 non-native species have been intentionally and unintentionally introduced in the United States (Pimentel et al. 2000).

Although most non-native organisms die in transport or soon upon arrival, the organisms that become established may be released from previous limiting factors (i.e., nutrients, predators, competitors, climate) and expand dramatically in numbers and distribution (Kolar and Lodge 2001). These invasive species may impose unprecedented predation, competition, and hybridization pressures on native biota and communities (Mack et al. 2000). Wilcove et al. (1998) estimated that non-native species are second only to habitat destruction as a cause of species imperilment in the United States.

Freshwater ecosystems have been dramatically impacted by introductions of non-native species (Moyle and Leidy 1992). Although most non-native fishes have been intentionally introduced for purposes of aquaculture, recreational fishing, and ecological manipulation, many of these introductions have presented unforeseen ecological and genetic consequences (Moyle et al. 1986; Krueger and May 1991). An important consequence of non-native fish introductions is hybridization between native and non-native taxa and the production of admixed populations (Allendorf et al. 2001).

The greatest threat to westslope cutthroat trout (*Oncorhynchus clarki lewisi*, WCT) is hybridization with introduced trout (Allendorf and Leary 1988). Introduced rainbow trout (*O. mykiss*, RBT) readily hybridize with WCT (Behnke 1992) and have established self-sustaining populations in the Flathead River system (FRS, Figure 1-1; Deleray et al. 1999), which is recognized as a regional stronghold for native WCT (Liknes and Graham 1988; Deeds et al. 1999). In this study, I surveyed WCT x RBT hybridization within the North and Middle Forks of the FRS and assessed potential environmental limiting factors for RBT introgression. The results of this study provide insight on spatial and temporal patterns of RBT introgression, as well as the potential for further spread.

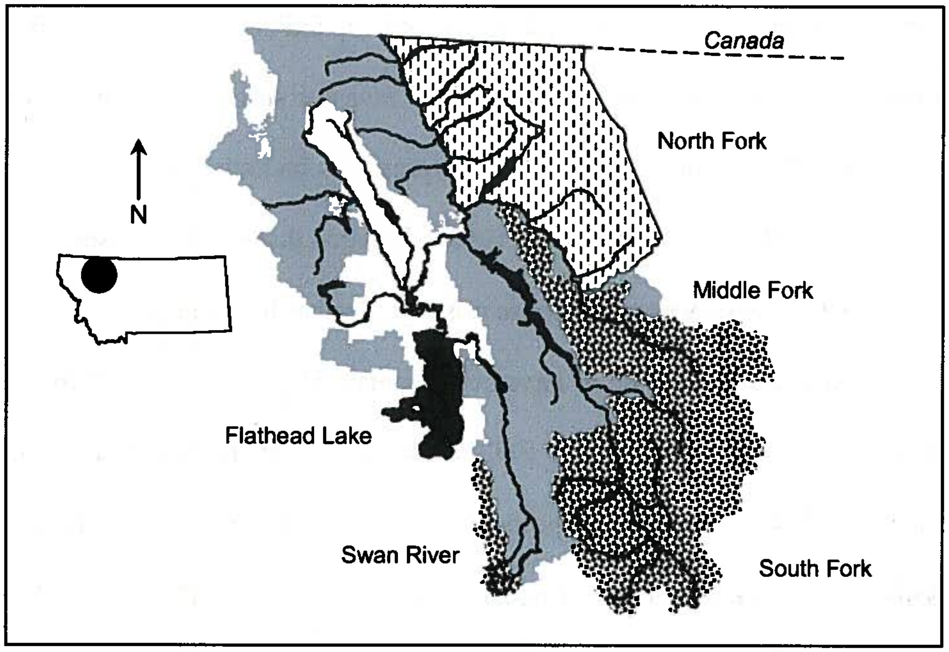

Figure 1-1. The upper Flathead River system, Montana, USA. U.S. Forest Service Wilderness Areas (dots), Flathead National Forest (gray), and Glacier National Park (vertical dashes) comprise the majority of land ownership in the study area. State Forests, Tribal lands, and private lands are not delineated.

Hybridization in freshwater fishes

Natural hybridization plays an important evolutionary role. By reinforcing species boundaries and permitting novel genetic patterns (e.g., chromosomal rearrangements and allopolyploidy), natural hybridization has influenced speciation and natural selection in many taxa (Arnold 1997). For example, the origin of tetraploidy in salmonid fishes was probably result of an interspecific hybridization event 50-100 million years ago (Utter and Allendorf 1994).

Natural hybridization is relatively common in freshwater fishes, as the result of weak ethological isolating mechanisms and r-selected reproductive strategies (Hubbs 1955). Among families of freshwater fishes, the Cyprinidae (minnows), Catostomidae (suckers), Salmonidae (whitefishes, trout, salmon, and char), and Poeciliidae (livebearers) have shown greatest levels of natural hybridization (Smith 1992). However, natural patterns of fish hybridization have been greatly altered due to anthropogenic transport of fishes (Hubbs 1995; Leary et al. 1995).

The impacts of hybridization largely depend on the fitness of the hybrid offspring. If hybrid offspring exhibit greater fitness than parental types (i.e., heterosis, Shull 1948), then selective forces may favor hybrid progeny within certain environmental parameters (i.e., bounded hybrid superiority hypothesis, Arnold 1997). For example, Ferguson et al. (1988) reported that F_1 hybrids from crosses of WCT and Yellowstone cutthroat trout (*O. c. bouvieri*, YCT) exhibited increased developmental stability (i.e., decreased fluctuating asymmetry during early development), faster rates of egg yolk absorption, and greater fry length relative to parental controls. In aquaculture applications, heterotic effects on growth and survival have been reported from intraspecific (Ayles and Baker 1983) and interspecific (Refstie and Gjedrem 1975) crosses of salmonid fishes. However, few studies have documented heterosis in fish hybrids past the F_1 generation (Cross 2000).

In contrast, if hybrid offspring exhibit decreased fitness relative to their parental types, then hybridization causes outbreeding depression (Templeton 1986). Outbreeding depression may result from genic interactions (i.e., intrinsic outbreeding depression) or between gene products and the environment (i.e., extrinsic outbreeding depression). For example, intrinsic outbreeding depression would result from the mispairing of chromosomes during meiosis or disruption of co-adapted gene complexes (*sensu* Dobzhansky 1970) and extrinsic outbreeding depression would result from the loss of local adaptations.

Evidence for intrinsic outbreeding depression in fish hybrids stems from taxonomic patterns and experimental crosses. The fact that interspecific fish hybrids are generally sterile (Hubbs 1955) suggests that intrinsic outbreeding depression may be a common mechanism for reinforcing species boundaries via meiotic incompatibilities in hybrid fishes. From a review of the aquaculture literature, Chevassus (1979) reported that experimental inter- and intrageneric crosses of salmonid fishes showed consistently poorer gametogenesis than parental controls. Poor hybrid gametogenesis has also been reported in crosses of channel catfish (*Ictalurus punctatus*) and blue catfish (*I. furcatus*) (Dunham and Argue 2000).

Extrinsic outbreeding depression has been documented in centrarchid and salmonid fishes. Aquaculture crosses between Florida largemouth bass (*Micropterus salmoides floridanus*) and northern largemouth bass (*M. s. salmoides*) have shown decreased overwinter survival rates relative to the northern subspecies (Philipp and Whitt

1991). As a result, widespread stocking of the Florida subspecies and subsequent hybridization would jeopardize local adaptations in the northern subspecies (Philipp 1991). In addition, genetic differences in fitness and performance have been shown among populations of *M. s. salmoides*, demonstrating the importance of population genetic structure and potential consequences of intraspecific hybridization in this taxon (Philipp and Claussen 1995).

Symptoms of extrinsic outbreeding depression have been extensively documented in salmonid hybrids. Leary et al. (1985a) found decreased developmental stability (i.e., increased bilateral asymmetry) in experimental hybrids between RBT, WCT, YCT, and coastal cutthroat trout (*O. c. clarki*), as well as natural hybrids between bull trout (*Salvelinus confluentus*) and brook trout (*S. fontinalis*). Increased bilateral asymmetry has also been reported from F_1 hybrids of even- and odd-year runs of pink salmon (*O. gorbuscha*) (Gharrett and Smoker 1991). The F_2 hybrids also showed reduced return rates relative to controls (Gharrett and Smoker 1991). Hawkins (1997) hypothesized that *O. c. clarki* and steelhead (i.e., anadromous RBT) hybrids would be more susceptible to predation based on her observations that hybrid alevins emerged earlier and retained larger amounts of yolk than parental controls. Brannon (1967) demonstrated the inability of intraspecific sockeye salmon (*O. nerka*) hybrids to migrate as a single population.

The deleterious impacts of hybridization may be greatest with heterosis in early-generation hybrids (i.e., F_1 and F_2 hybrids), and outbreeding depression in late-generation hybrids (i.e., post- F_2 hybrids). Under this scenario, early hybrids would maximize the spread of invading genes, ultimately resulting in decreased fitness for more individuals and populations. This scenario could result from extreme environmental conditions that do not occur every year, but nevertheless may pose severe restrictions on local populations (e.g., 100-year floods). In contrast, native salmonid populations have evolved under selective pressures of varied extremity over centuries.

Study taxa

The native range of WCT includes the headwaters of the Columbia, Missouri, Milk, and Saskatchewan Rivers in western North America (Behnke 1992). Native, disjunct populations extend from southern British Columbia to the John Day River in Oregon (Behnke 1992) (Figure 1-2). WCT populations exhibit fluvial (river-migrating), adfluvial (lake-migrating), and resident life history forms (McIntyre and Rieman 1995) and are characterized by a high degree genetic differentiation among populations (G_{st} = 0.328, Leary et al. 1985b). Hybridization with introduced trout poses the greatest threat to the persistence of WCT (Allendorf and Leary 1988).

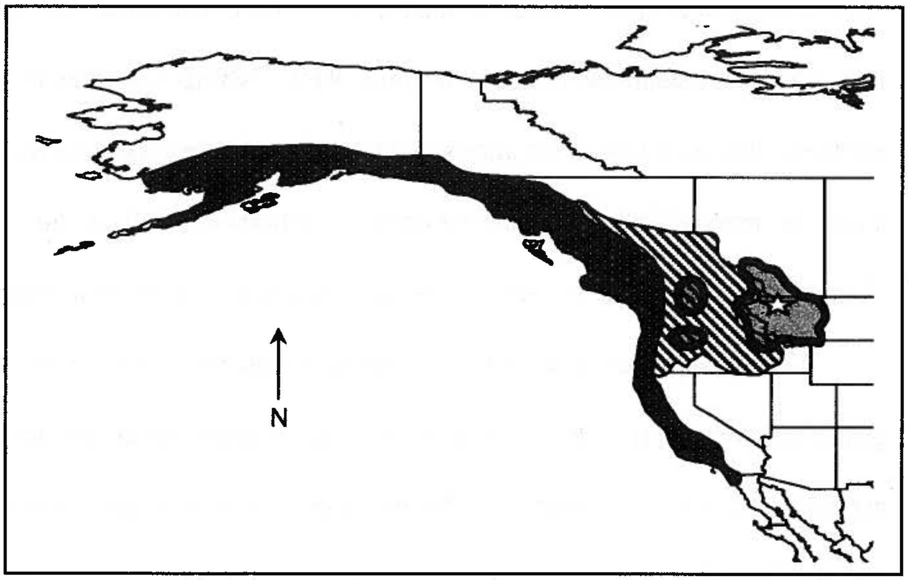

Figure 1-2. Approximate native distributions of coastal rainbow trout (dark stippled area), inland rainbow trout (cross-hatched area), and westslope cutthroat trout (dark outlined areas) in western North America (adapted from Behnke 1992). The study area is indicated with a star.

The RBT of concern in this study originate from hatchery stocks containing a mixture of coastal rainbow trout (*O. m. irideus*) and inland redband rainbow trout (*O. m. gairdneri*), although the coastal subspecies is believed to have comprised the majority of the broodstock (Needham and Behnke 1962). The native range of coastal RBT encompasses a north-south band along the Pacific Ocean from northern Baja California to central Alaska and eastern Asia. Inland RBT inhabit the interior Columbia and Fraser River basins as their native range (Figure 1-2). Both the coastal and inland subspecies exhibit anadromous life histories (steelhead), but the inland subspecies is predominantly non-migratory (Behnke 1992). Both WCT and RBT spawn during the spring with female excavation of gravel redds, deposition of eggs, and subsequent fertilization by males (Scott and Crossman 1973a, 1973b).

Naturally sympatric populations of cutthroat trout (*O. clarki*) and RBT exhibit some spatial segregation. In southwest British Columbia, Hartman and Gill (1968) found that RBT are the dominant species in the larger rivers (drainage area >130 km^2) but that smaller streams (drainage area <13 km^2) were predominately occupied by *O. c. clarki.* Similarly, Platts (1979) observed highest densities of WCT upstream of RBT in naturally sympatric populations in Idaho. However, Maret et al. (1997) detected little environmental segregation between native YCT and RBT in the upper Snake River basin, concluding that fish introductions have drastically altered historical fish assemblage structure.

Introduced RBT have also shown distinct habitat use patterns from cutthroat trout. Although RBT were extensively stocked in high elevation waters of southern Alberta, the greatest densities of RBT are currently found in lower areas (downstream from WCT), suggesting a habitat preference for larger, warmer waters (Paul and Post 2001). Bozek and Hubert (1992) found that Colorado River cutthroat trout (*O. c. pleuriticus*) generally inhabited higher, steeper, and smaller streams than introduced RBT. However, low numbers of RBT were observed throughout the range of *O. c. pleuriticus* habitats (Bozek and Hubert 1992). Introduced RBT in the Great Smoky Mountains National Park appear to be limited to stream slopes <15% (Larson and Moore 1985). Self-sustaining populations of introduced RBT in the Flathead River system have been documented in the Flathead River mainstem, but not within tributaries to the North Fork and Middle Fork (Deleray et al. 1999).

Low-level hybridization between naturally sympatric cutthroat trout and RBT has been detected. Campton and Utter (1985) documented natural hybridization between *O. c. clarki* and anadromous RBT in 2 of 23 sample streams in Puget Sound. In experimental crosses, Hawkins (1997) confirmed that post-F_2 hybrids between these taxa were fertile, but she found some evidence of increased susceptibility to predation in hybrid alevins. In addition, hybridization between naturally sympatric WCT and inland RBT was detected in the Yaak River system of northwest Montana (Huston 1988).

In contrast, hybrid swarms commonly result from RBT stocking into historically allopatric *Oncorhynchus* populations (Behnke 1992). Previous surveys have shown introduced RBT introgression into populations of inland RBT (Williams et al. 1996), apache trout (*O. apache*, Dowling and Childs 1992; Carmichael et al. 1993), Bonneville cutthroat trout (*O. c. utah*, Hepworth et al. 1997), Colorado cutthroat trout (*O. c. pleuriticus*, Bischoff 1995; Kershner et al. 1997), Gila trout (*O. gilae*, Dowling and Childs 1992), greenback cutthroat trout, (*Oncorhynchus clarki stomias*, Allendorf and

Leary 1988), Paiute cutthroat trout (*O. c. seleniris*, Busak and Gall 1981), Lahontan cutthroat trout (*O. c. henshawi*, Gard and Flittner 1974), Yellowstone cutthroat trout (*O. c. bouveri*, Henderson et al. 2000), as well as with WCT in Montana (Leary et al. 1984), Idaho (Weigel et al. 2002), and British Columbia (Rubidge et al. 2001).

Hybridization between introduced RBT and native WCT is extensive in western Montana. Hanzel (1960:32) provided one of the first regional reports, concluding that WCT x RBT hybridization has occurred in "practically all drainages where rainbow trout were introduced." Similarly, Reinitz (1974:1) stated that "the distribution of 'pure' native cutthroat in Montana is restricted to small relict populations in the extreme headwaters of mountain streams" as the result of non-native trout stocking. Liknes and Graham (1988) estimated that unhybridized WCT populations remained only within 2.5% of their native range in Montana. However, a recent Status Review for WCT was more optimistic, determining that hybridized populations encompassed 14% and pure populations encompassed 20% of stream miles within the historic range of WCT in Montana (the remaining 66% of stream miles were untested) (Deeds et al. 1999). The Status Review also articulated the general importance of refugia and the specific importance of the Flathead River system for conservation of native WCT.

Potential limiting factors for RBT x WCT hybridization

Freshwater fish assemblages are structured by zoogeograhic, regional, and local factors (Angermeier and Winston 1998). Tonn et al. (1990) described these factors as a series of hierarchical filters through which species 'pass' for inclusion within a local species pool. Poff (1997) emphasized the interplay between autecological constraints and ecological conditions as limiting factors for species. However, RBT introductions have bypassed zoogeographic filters, leaving potential limiting factors at the regional and local scales.

Regional and local filters correspond to a nested hierarchy of habitat conditions in lotic ecosystems (*sensu* Frissell et al. 1986). Poff (1997) developed a niche model to evaluate the role of limiting factors at various habitat scales. Under this model, climate, geology, and habitat degradation present landscape-level filters; slope, stream size, thermal regime, and channel morphology present valley-level filters; and substrate sizes, groundwater-surface water interactions, sediment dynamics, and in-stream structures (e.g., large woody debris) constrain fish assemblages at the channel unit and microhabitat scales (Poff 1997).

Biotic interactions may also affect fish assemblage structure through predation and competitive exclusion (Ross 1991) and may hinder the establishment of non-native fishes (Case 1991). However, biotic interactions may be more important in species-rich systems (Elton 1958). For example, Moyle and Light (1996a) found that species interactions would not limit invasive fishes if abiotic conditions were suitable in streams of the Sierra Nevada mountains. Given the relatively low richness of fishes in Montana, RBT invasion would not be expected to be limited due to species interactions alone. However, spatial and/or temporal segregation of WCT and RBT spawning would limit the spread of RBT introgression.

Abiotic filters have apparently limited the expansion of introduced RBT populations in some areas (e.g., Larson and Moore 1985; Deleray et al. 1999) and could limit expansion of RBT hybrids. However, because WCT x RBT hybrids show phenotypic characteristics intermediate to parental types (Leary et al. 1984; Ferguson et al. 1985), RBT introgression into WCT populations may release hybrid individuals from factors that limit parental RBT. Accordingly, the presence or absence of environmental limiting factors for WCT x RBT hybridization is a crucial question for conservation of WCT.

In this study, I surveyed the distribution of WCT x RBT hybridization in North and Middle Fork tributaries of the FRS (Chapter 2) and assessed potential limiting factors for the spread of RBT introgression (Chapter 3). I examined environmental parameters for threshold effects in RBT introgression (*sensu* Moyle and Light 1996b), focusing on thermal regimes, geomorphology, and habitat degradation (*sensu* Poff 1997). I conclude in Chapter 4 by discussing the implications of this study for management and conservation of WCT. This study presents the first landscape-level assessment of potential limiting factors for RBT introgression in this recognized WCT stronghold.

Chapter 2.

Spatial and temporal patterns of hybridization

Abstract

Hybridization is perhaps the primary threat facing westslope cutthroat trout (*Oncorhynchus clarki lewisi*, WCT). Nevertheless, the dynamics of hybridization with introduced rainbow trout (*O. mykiss*, RBT) are poorly understood. I used molecular genetic techniques to assess spatial and temporal patterns of WCT x RBT hybridization within 43 sample sites in the Flathead River system, Montana. Admixed populations were detected in 58% (25/43) of the sites; the percent rainbow trout genetic contribution decreased with increasing upstream distance from Flathead Lake. Positive spatial autocorrelation was detected in North Fork tributaries (Mantel $r=0.22$, $P<0.001$), suggesting that hybridization has spread among neighboring sites. Temporal comparisons showed new rainbow trout introgression (7/14 sites), continued absence of introgression (5/14 sites), and continued presence of introgression (2/14 sites) since 1984. A minority of the admixed sites in the current study (3/25) showed significant non-random associations of alleles between loci, suggesting that rainbow trout introgression is spread primarily by post-F_1 hybrids or several generations have passed since the non-native invasions. These data indicate that RBT introgression is spreading upstream from the Flathead River mainstem.

Introduction

Non-native species have affected biological communities worldwide (Mack et al. 2000) and are a primary cause of species imperilment in the United States (Wilcove et al. 1998). Introductions of non-native fishes present an important challenge to conservation and management of aquatic ecosystems (Nico and Fuller 1999). This chapter examines spatial and temporal patterns of hybridization between non-native rainbow trout (*Oncorhynchus mykiss*, RBT) and native westslope cutthroat trout (*O. clarki lewisi*, WCT) in the Flathead River system, Montana.

Although natural hybridization plays an important evolutionary role (Arnold 1997), hybridization between native and non-native species may cause extinction of native taxa through genetic and demographic processes (Rhymer and Simberloff 1996). Ecological consequences of hybridization include intrinsic and extrinsic outbreeding depression (gene-gene interactions and gene-environment interactions) that may reduce fecundity in hybrid offspring and/or cause disruption of co-adapted gene complexes (*sensu* Dobzhansky 1970) and loss of local adaptations (e.g., Philipp and Claussen 1995). Outbreeding depression has been extensively documented from hybridization in salmonid fishes (Brannon 1967; Chevassus 1979; Leary et al. 1985; Gharrett and Smoker 1991; Gharrett et al. 1999).

Spatial patterns of natural hybridization have been explained as a balance between dispersal of hybrid offspring and selection against hybridized individuals, often resulting in a genotypic cline between two species (Barton and Hewitt 1985). In contrast, anthropogenic hybridization may result in the development of hybrid swarms if hybrid offspring are fertile. Hybrid zones, under these circumstances, are characterized by a unidirectional wave of introgression reflecting the dispersal rates in fertile hybrid offspring (e.g., stratified diffusion, Shigesada et al. 1995; telegraph model, Holmes 1993). Allendorf et al. (2001) recognized this distinction in classifying admixed (i.e., hybridized) populations of natural and anthropogenic origin.

Hybridization with introduced trout poses the greatest threat to the persistence of WCT (Allendorf and Leary 1988). The native range of WCT includes the headwaters of the Columbia, Missouri, Milk, and Saskatchewan Rivers. Native, disjunct populations occur from British Columbia to the John Day River in Oregon (Behnke 1992) (see Figure 1-1). WCT and are characterized by a high degree genetic differentiation between populations ($G_{st} = 0.328$; Leary et al. 1985b) presenting a susceptibility to extrinsic outbreeding depression.

The RBT of concern in this study originate from hatchery stocks containing a mixture of coastal rainbow trout (*O. m. irideus*) and inland redband rainbow trout (*O. m. gairdneri*), although the coastal subspecies is believed to comprise the majority of the broodstock (Needham and Behnke 1962). The native range of coastal RBT encompasses a north-south band along the Pacific Ocean from northern Baja California to central Alaska and eastern Asia. Inland RBT inhabit the interior Columbia and Fraser River basins as their native range (see Figure 1-1). Both the coastal and inland subspecies exhibit anadromous life histories (steelhead), but the inland subspecies is predominantly nonmigratory (Behnke 1992). Both WCT and RBT spawn during the spring with female excavation of gravel redds, deposition of eggs, and subsequent male fertilization (Scott and Crossman 1973a, 1973b).

Introduced RBT show distinct habitat use patterns from cutthroat trout (*O. clarki*). Although RBT were extensively stocked in high elevation waters of southern Alberta, the greatest densities of RBT are currently found in lower areas (downstream from WCT), suggesting a habitat preference for larger, warmer waters (Paul and Post 2001). Introduced RBT are also found in greatest densities downstream from WCT in Pend Oreille Lake tributaries (Hoelscher and Bjornn 1989). Bozek and Hubert (1992) found that Colorado River cutthroat trout (*O. c. pleuriticus*) generally inhabited higher, steeper, and smaller streams than introduced RBT. Self-sustaining populations of introduced RBT in the Flathead River system have been documented in the Flathead River mainstem, but not within higher-elevation tributaries to the North Fork and Middle Fork (Deleray et al. 1999).

The Flathead River system (FRS) has been recognized as an important stronghold for native WCT (Liknes and Graham 1988; Deeds et al. 1999). Although over 20 million RBT have been introduced into the FRS, the majority of RBT stocking has been limited to Flathead Lake and the Flathead River mainstem in the lower elevations within the basin. RBT stocking into rivers and lakes in this region was discontinued in 1969, but continues in ostensibly isolated ponds (May 1979).

Two possible vectors of RBT introgression have been recognized as management concerns in the FRS (C. Mulhfeld, MFWP, personal communication): a) downstream movement of RBT and RBT hybrids from historical stocking in headwater lakes and b) upstream movement of RBT or WCT x RBT hybrids from the Flathead River mainstem. In this chapter, I contrast the downstream- and upstream-source models of RBT introgression with spatial and temporal analyses. First, I assess the status of RBT introgression in the study area. Second, I examine spatial patterns of hybridization with Mantel tests and correlograms. Third, I compare these results to 1984 data to gauge the spread of RBT introgression in the study area.

Methods

Hybridization assessment

The status of RBT introgression was surveyed in 43 sample sites in the FRS (Figure 2-1; Table 2-1). Sites within the North Fork tributaries (n=31) encompassed within- and between-stream gradients. Sites within the Middle Fork tributaries (n=9) encompassed only between-stream gradients. In 41 of 43 sites, single-pass electrofishing was conducted to capture *Oncorhynchus* spp. individuals within sample reaches (>300 m). Site 43 (Whitefish River) was sampled by electrofishing from a drift boat. Site 29 (Big Creek, lower) was sampled by angling. Captured fish were anesthetized and a small portion of the anal fin was excised and stored in 95% ethanol for laboratory analyses.

Paired Interspersed Nuclear Element Polymerase Chain Reaction (PINE-PCR) techniques were used to determine the status of RBT introgression in each sample site. This methodology uses combinations of primer pairs to evaluate specific regions ("fragments") of anonymous intergenic nDNA. In this application, 6 diagnostic nDNA fragments were surveyed for RBT (Table 2-2), 7 were surveyed for WCT, and 9 were surveyed for Yellowstone cutthroat trout (*O. c. bouvieri*, YCT). These analyses were conducted at the Wild Trout and Salmon Genetics Laboratory at the University of

Montana following Spruell et al. (2001) and Kanda et al. (2002).

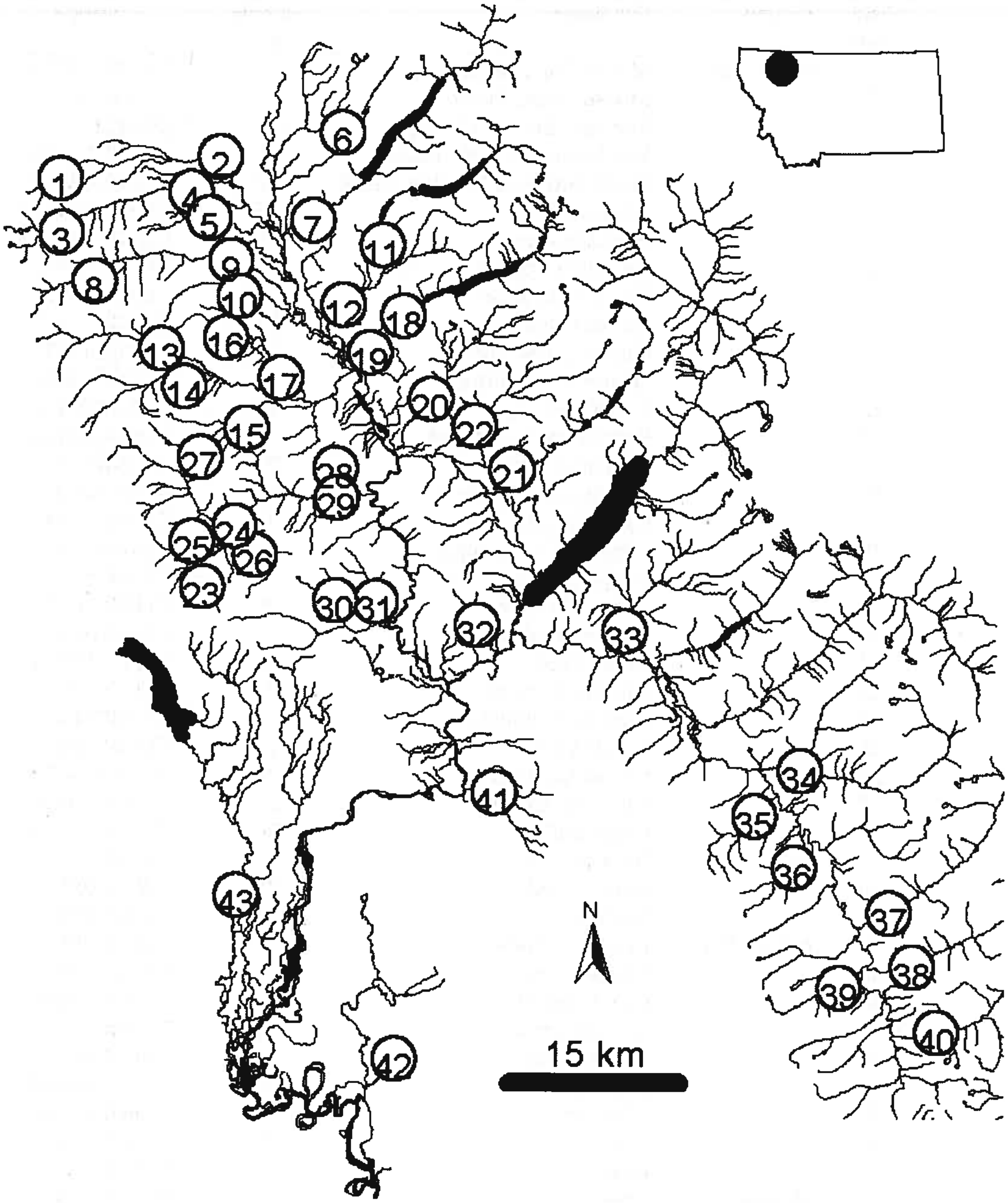

Figure 2-1. The distribution of study sites in the Flathead River system, Montana, USA. Site numbers correspond to Table 2.1.

Table 2-1. Study sites information. All sites were located within the Flathead River system, Montana, USA. Site codes correspond to Figure 2-1.

Site code	Region	Site name	Sample size	Location	Sample year(s)
1	North Fork	Moose Creek, upper	19	T35NR23WS10	2000
2		Moose Creek, lower	25	T35NR21WS8	2000
3		Red Meadow Creek, upper	25	T35NR23WS23,26	2000
4		Red Meadow Creek, lower	25	T35NR21WS8	1998, 2000
5		South Fork Red Meadow Creek	20	T35NR22WS13	2000
6		Akokala Creek	25	T35NR20WS30	2000
7		Bowman Creek	25	T35NR21WS14	2001
8		Hay Creek, upper	24	T35NR22WS32	2000
9		Hay Creek, lower	24	T35NR21WS34	2000
10		Moran Creek	21	T35NR21WS32	2000
11		Quartz Creek, upper	24	T35NR20WS21	2000
12		Quartz Creek, lower	20	T34NR20WS7	2000
13		Coal Creek (I)	15	T34NR22WS24	2000
14		South Fork Coal Creek	10	T34NR22WS26	2001
15		Deadhorse Creek	23	T33NR21WS4	2001
16		Cyclone Creek, upper	24	R43NR21WS8	2001
17		Cyclone Creek, lower	25	T34NR21WS22	1998
18		Logging Creek, upper	16	T34NR20WS2	2000
19		Logging Creek, lower	16	T34NR20WS21	2000
20		Anaconda Creek	20	T34NR20WS36	1998
21		Camas Creek	6	T33NR19WS15	1998
22		Dutch Creek	23	T33NR19WS5	1998
23		Big Creek, upper	25	T32NR22WS24	2001
24		Big Creek, middle	12	T32NR21WS7	1998, 2000
25		Nicola Creek	15	T32NR22WS12	2000
26		Skookoleel Creek	20	T32NR21WS4	2000, 2001
27		Kletomas Creek	25	T33NR21WS19	2001
28		Langford Creek	20	T33NR20WS20	1998
29		Big Creek, lower	3	T33NR20WS28	2000
30		Depuy Creek	25	T32NR20WS21	2001
31		McGinnis Creek	12	T32NR20WS27	2000
32	Middle Fork	Rubideau Creek	12	T32NR19WS33	2000
33		Lincoln Creek	22	T32NR18WS27	1998
34		Coal Creek (II)	5	T31NR17WS25	1998
35		Stanton Creek	15	T31NR17WS36	2001
36		Tunnel Creek	25	T30NR16WS18	2001
37		Park Creek	22	T30NR16WS35	1998
38		Ole Creek	20	T29NR16WS13	1998
39		Essex Creek	25	T29NR16WS15	1998
40		Bear Creek	13	T29NR15WS32	1998
41	Mainstem	Abbot Creek	18	T30NR19WS4	1998
42		Mill Creek	15	T28NR20WS15	1998
43		Whitefish River	15	T30NR21WS34	2001

PINE results were then screened for suspected WCT polymorphisms (i.e., nDNA mutations within WCT producing fragments with the same electrophoretic properties as diagnostic RBT fragments). Following Forbes and Allendorf (1991), sites with individuals showing RBT fragments at a single locus were assumed to be WCT polymorphisms. However, the presence of more than one RBT fragment size at a sampling site was assumed to indicate RBT introgression.

Admixed sites were then evaluated for linkage disequilibrium (i.e., non-random patterns of association of alleles between loci, Lewontin 1988). I used Fisher's exact test (Zar 1984) to evaluate the distribution of RBT fragments between all pair-wise comparisons. Statistical significance was determined with Bonferroni-corrected error rates based on the number of RBT markers evaluated. Sites showing linkage disequilibrium were included in binary analyses of introgression, but were not considered in evaluations of percent introgression.

Table 2-2. Paired Interspersed Nuclear Element (PINE) fragments used for the detection of RBT introgression (Spruell et al. 2001; Kanda et al. 2002).

PINE fragment number	No. base pairs	Primer pair	Primer pair type
1	70	Hpa1 5'/Hpa1 3'	SINEs[1]
2	66	Hpa1 5'/Hpa1 3'	SINEs
3	369	Fok1 5'/Tc1	SINE and transposon[2]
4	230	Fok1 5'/Tc1	SINE and transposon
5	395	Hpa1 5'/33.6+2	SINE and Jeffreys' core fragment[3]
6	266	Hpa1 5'/33.6+2	SINE and Jeffreys' core fragment

[1] Small Interspersed Nuclear Elements (see Kido et al. 1991; Tachida 1996).
[2] Transposons are base pair sequences that may move throughout the genome of an organism and that carry genes in addition to those required for transposition (Li and Graur 1991).
[3] See Jeffreys et al. (1985).

For admixed sites that lacked significant linkage disequilibrium, mean genetic contribution for RBT over all RBT fragments (GC_r) was calculated as:

$$GC_r = \frac{\sum_{i=1}^{n}\left(1-\sqrt{\frac{L_i}{N_i}}\right)}{n}$$

where the number of diagnostic RBT fragments runs from 1 to n, L_i = the number of individuals which lack fragment i, and N_i = the total number of individuals genotyped for fragment i. Parallel calculations were preformed for the percent genetic contribution of YCT (GC_y). These calculations assumed that the individuals from each site were sampled from the same population and that these populations exhibited Hardy-Weinberg proportions. The percent contribution of WCT (GC_w) was then calculated as:

$$GC_w = 1 - GC_r - GC_y$$

Sites that showed an absence of RBT (and YCT) fragments were classified as WCT sites. The power to detect introgression was defined as:

$$A^{2NX} = B$$

where A = one minus the percent introgression to be detected, B = one minus the percent chance of detecting that level of introgression, N = the number of individuals sampled, and X = the number of diagnostic non-native fragments evaluated. In this study, all sites classified as unhybridized WCT had greater than a 95% chance of detecting as little as 2% RBT introgression.

Spatial analysis

In ecology, spatial analysis concerns identification of the spatial components of environmental variability (Borchard et al. 1992). Similarities or differences among site conditions (i.e., positive and negative spatial autocorrelation) may violate assumptions of sample independence, but may indicate underlying ecological processes (Legendre 1993). For example, positive spatial autocorrelation may identify patterns of contagion in biological invasions.

I assessed patterns of spatial autocorrelation among all sites (n=43), North Fork sites (n=31), and Middle Fork sites (n=9). Mantel tests were conducted among site x site matrices for hybridization and spatial data (Mantel 1967). The hybridization matrix was coded with a binary similarity index to identify clustering among admixed sites where 1 = both sites admixed and 0 = other combinations. Spatial matrices were developed from site x site calculations of fluvial distances and Euclidean map distances.

Correlograms were developed from Mantel test results within 8 distance classes for all sites and North Fork sites (Legendre and Fortin 1989). Significance of correlations was assessed with a Bonferroni-corrected error rate, $\alpha' = 0.05/8$ (8 distance classes) = 0.0063. All Mantel tests were conducted with SMT version 1.2 (MS-DOS program by E. Bennet). Log-likelihood ratio tests (Zar 1984) were then employed to assess spatial structure among admixed and WCT sites as a function of upstream distance from Flathead Lake.

Patterns of percent RBT introgression were assessed with a trend surface analysis (Burrough 1995) in which GC_r was plotted against upstream distance from Flathead Lake for a) all admixed sites and b) admixed sites in the North and Middle Forks. Sites showing significant linkage disequibrium were not included in this analysis. Analysis of Covariance (ANCOVA) was used to compare the linear regression slopes between the Middle Fork and North Fork sites (Ott 1993).

Spatial data were used to contrast the upstream and downstream RBT source models. Evidence against the upstream source model consisted of: a) greater relative occurrence of hybridization downstream (i.e., closer fluvial distance to Flathead Lake); b) greater relative percent RBT introgression downstream; and c) greater frequency of linkage disequilibrium downstream. Evidence against the downstream source model consisted of converse results.

Temporal analysis

Rates of population growth and spread are fundamental concerns for management of non-native organisms (Lodge 1993). I assessed temporal patterns of introgression by evaluating change in hybridization status since surveys conducted in 1984. I then evaluated site-level patterns of linkage disequilibrium in PINE fragments and the distribution of F_1 hybrids.

Field collections from 1984 were coordinated by J. Huston (Montana Fish Wildlife and Parks). Allozyme surveys for these samples were conducted by the Wild Trout and Salmon Genetics Laboratory at the University of Montana. These comparisons were limited to 14 sites within the tributaries of the North Fork. Although allozymes are codominant fragments and PINEs are dominant fragments, these two types of analyses have shown concordance in hybrid detection at the population level (Smithwick 2000).

The spatial distribution of linkage disequilibrium and post-F_2 hybrid individuals also provided inferences on the spread of RBT introgression. Linkage disequilibrium results from the relatively recent introgression of relatively pure RBT into WCT populations. However, the absence of linkage disequilibrium in admixed populations may be explained by either of two scenarios: a) recent introgression of post-F_2 hybrid individuals, or b) introgression of relatively pure RBT several generations ago. As a result, the presence of linkage disequilibrium indicates recent introgression, but the absence of linkage disequilibrium in admixed sites does not exclude the possibility of recent introgression.

Temporal data contributed to the evaluation of the upstream and downstream RBT source models. Evidence against the upstream RBT source model consisted of recent introgression in the downstream areas, but not upstream areas. Evidence against the downstream RBT source model consisted of the converse relationship.

Results

Hybridization analysis

Of the 43 sample sites, 25 (58%) showed RBT introgression (Figure 2-2). Of the 18 WCT sites, 6 showed diagnostic RBT fragments that were assumed to be polymorphisms within unhybridized WCT populations (sites 2, 6, 8, 12, 27, 40; Table 2-3). Further sampling would be required to distinguish between introgression and true WCT polymorphisms in these sites. Of the 25 admixed sites, 3 showed statistically significant patterns of linkage disequilibrium (sites 4, 22, 28; Table 2-4). YCT introgression was only detected in Essex Creek (site 39).

Within the 22 admixed sites that lacked significant linkage disequilibrium, GC_r values ranged from 0.9% to 98.2% (Table 2-4). Most admixed sites (14/22) contained less than 10% genetic contribution from RBT. Sites exceeding 10% RBT introgression in the North and Middle Fork tributaries were located within approximately 25 km upstream from the North Fork – Middle Fork confluence (near Blankenship Bridge). Mainstem tributary sites (sites 41, 42, 43) showed GC_r values in excess of 89% (Figure 2-3).

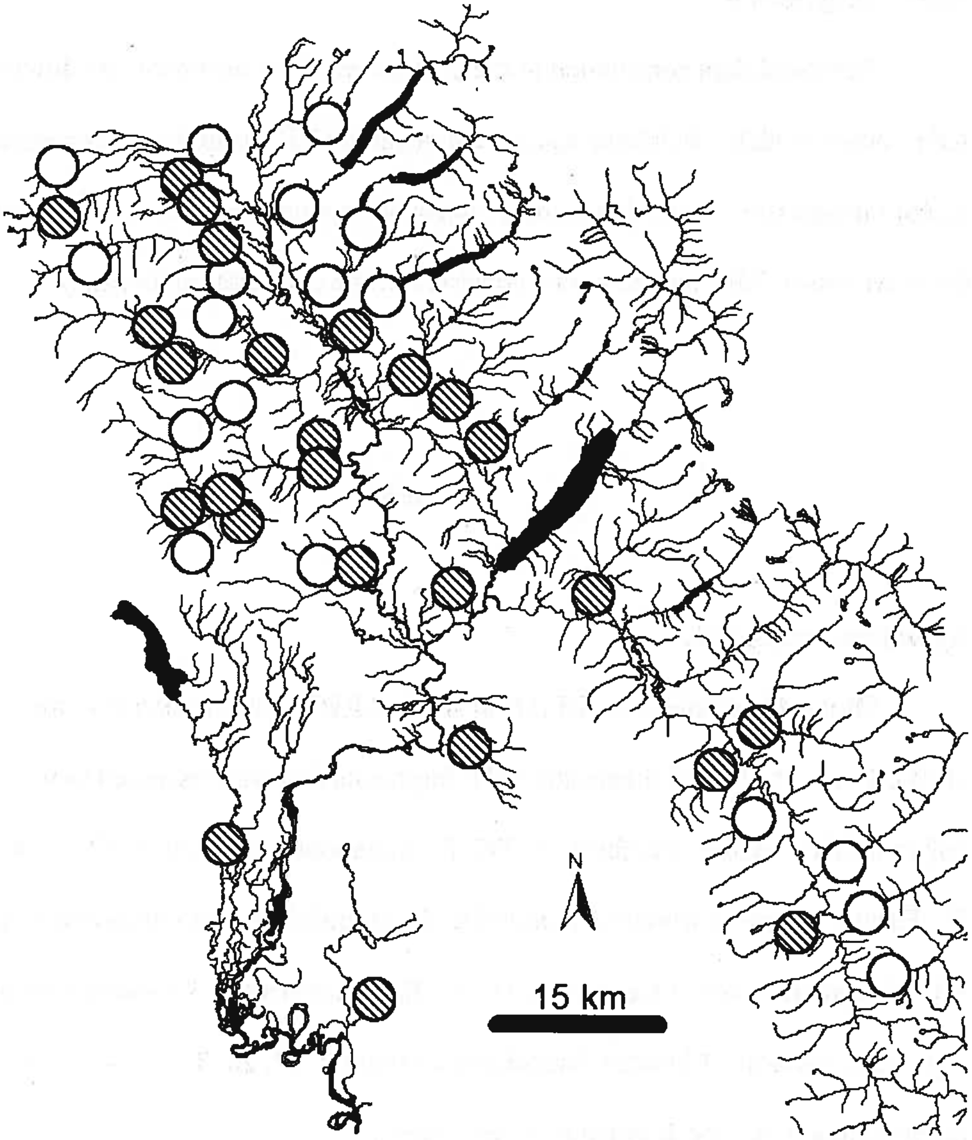

Figure 2-2. The distribution of WCT (open circles) and admixed populations of WCT x RBT (hatch-marked circles) in the study area.

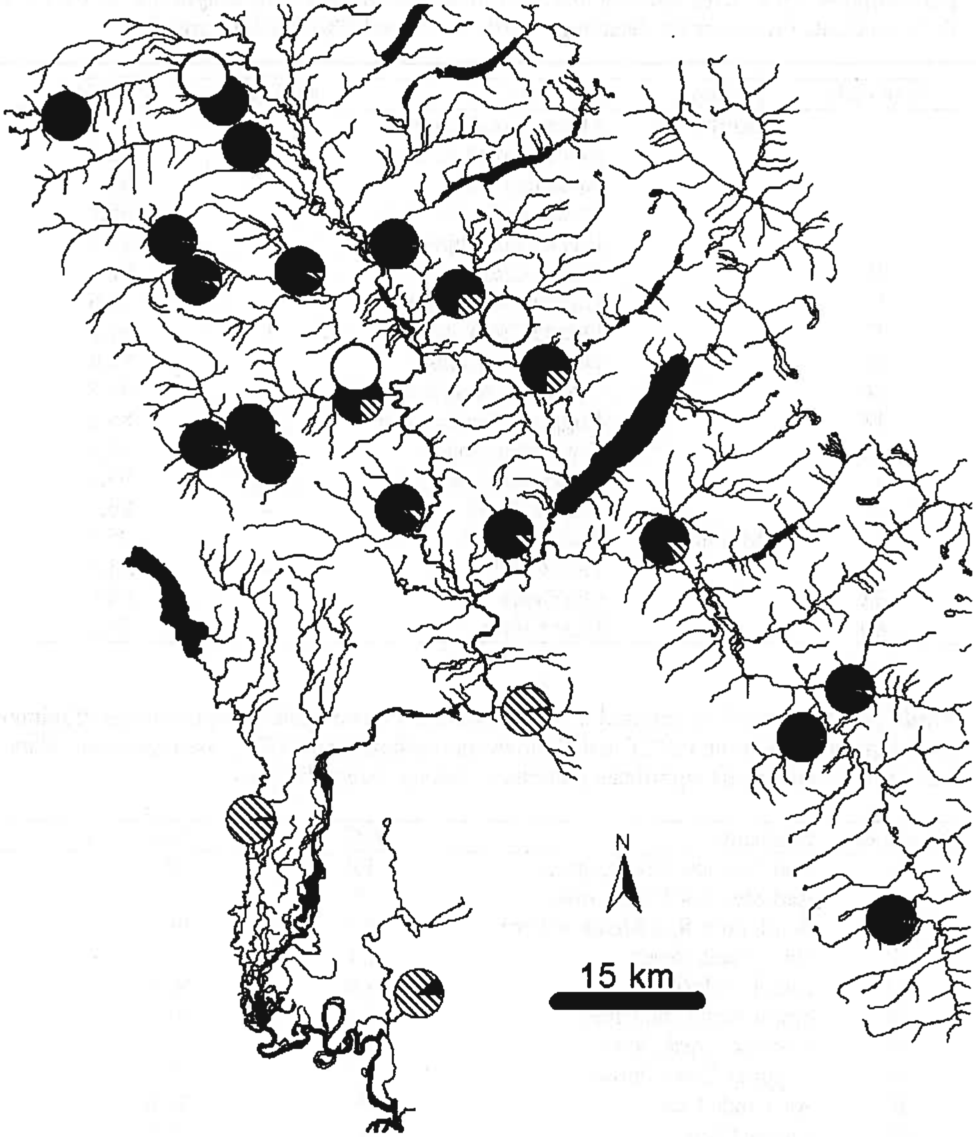

Figure 2-3. Relative contributions of RBT, WCT, and YCT in admixed sample sites. In each pie chart, the black area corresponds to the percent WCT contribution and diagonal hatched area corresponds to the percent RBT contribution. In Essex Creek (site 39), the YCT contribution is indicated with vertical hatch marks. Open circles indicate sites with RBT introgression, but where percent calculations were inappropriate (see text).

Table 2-3. Non-hybridized WCT sites detected in the study area. Plus signs indicate probable WCT polymorphisms (i.e., sites showing one PINE fragment size normally diagnostic for RBT). P(1%) and P(2%) indicate the power for detecting as little as 1% and 2% RBT introgression.

Site code	Region	Site name	WCT poly.	P (1%)	P (2%)
1	North Fork	Moose Creek, upper	-	89.9	99.0
2		Moose Creek, lower	+	95.1	99.8
6		Akokala Creek	+	95.1	99.8
7		Bowman Creek	-	95.1	99.8
8		Hay Creek, upper	+	94.5	99.7
10		Moran Creek	-	92.1	99.4
11		Quartz Creek, upper	-	94.5	99.7
12		Quartz Creek, lower	+	91.0	99.2
15		Deadhorse Creek	-	93.8	99.6
16		Cyclone Creek, upper	-	94.5	99.7
18		Logging Creek, upper	-	85.5	97.9
23		Big Creek, upper	-	95.1	99.8
27		Kletomas Creek	+	95.1	99.8
30		Depuy Creek	-	95.1	99.8
36	Middle Fork	Tunnel Creek	-	95.1	99.8
37		Park Creek	-	93.0	99.5
38		Ole Creek	-	80.0	99.2
40		Bear Creek	+	79.2	95.7

Table 2-4. Admixed sites detected in the study area. Percent genetic contributions of rainbow trout (GC_r), westslope cutthroat trout (GC_w), and Yellowstone cutthroat trout (GC_y) are presented. Stars indicate sites that showed statistically significant patterns of linkage disequilibrium.

Site code	Site name	GC_r	GC_w	GC_y
3	Red Meadow Creek, upper	1.4	98.6	0
4	Red Meadow Creek, lower	*	*	0
5	South Fork Red Meadow Creek	0.9	99.1	0
9	Hay Creek, lower	1.1	98.9	0
13	Coal Creek (I)	3.6	96.4	0
14	South Fork Coal Creek	6.1	93.9	0
17	Cyclone Creek, lower	7.4	92.6	0
19	Logging Creek, lower	2.1	97.9	0
20	Anaconda Creek	27.4	72.6	0
21	Camas Creek	24.5	75.5	0
22	Dutch Creek	*	*	0
24	Big Creek, middle	1.4	98.6	0
25	Nicola Creek	4.0	96.0	0
26	Skookoleel Creek	1.7	98.3	0
28	Langford Creek	*	*	0
29	Big Creek, lower	24.2	75.8	0
31	McGinnis Creek	9.8	90.2	0
32	Rubideau Creek	11.1	88.9	0
33	Lincoln Creek	18.5	81.5	0
34	Coal Creek (II)	5.3	94.7	0
35	Stanton Creek	2.3	97.7	0
39	Essex Creek	1.4	95.2	3.5
41	Abbot Creek	97.5	2.5	0
42	Mill Creek	89.4	10.6	0
43	Whitefish River	98.2	1.8	0

Spatial analysis

Based on fluvial distances, Mantel tests indicated a zone of positive spatial autocorrelation within 19 km from locations within all sites (Mantel r=0.12, P<0.003; Figure 2-4a) and North Fork sites (Mantel r=0.22, P<0.001; Figure 2-4c). However, Euclidean distances for these areas showed no statistically significant patterns of positive spatial autocorrelation (Figures 2-4b and 2-4d). Negative spatial autocorrelation was detected within North Fork sites at 2 of 4 fluvial distance classes greater than 34 km (34-39 km: Mantel r=-0.12, P<0.002; 44-49 km: Mantel r=-0.15; P<0.002; Figure 2-4c). Pooled data showed no statistically significant patterns of spatial autocorrelation within all sites, North Fork sites, or Middle Fork sites for either fluvial or Euclidean distances.

Within the North Fork basin, more unhybridized sites were in tributaries entering the mainstem above 30 km upstream from the Middle Fork confluence than below 30 km (11 and 3 sites, respectively), but this difference was only marginally significant (*G*=1.89, 0.10<P<0.25). In contrast, the distribution of admixed sites in the North Fork showed greater proportionality among these areas (*G*=1.42, 0.25<P<0.50). All Middle Fork sites were admixed within 40 km upstream from the North Fork confluence, above which 4 of 5 sites were unhybridized WCT.

The magnitude of RBT introgression generally decreased with increasing upstream distance from Flathead Lake (Figure 2-5). North Fork and Middle Fork sites showed similar distance effects with mainstem sites (ANCOVA interaction term, P>0.45) and excluding mainstem sites (ANCOVA interaction term, P>0.45). Although the mainstem sites (sites 41, 42, 43) were influential points, their absence did not change the general pattern of decreasing RBT introgression with increasing distance from Flathead Lake for either the North Fork or Middle Fork sites (Figure 2-5).

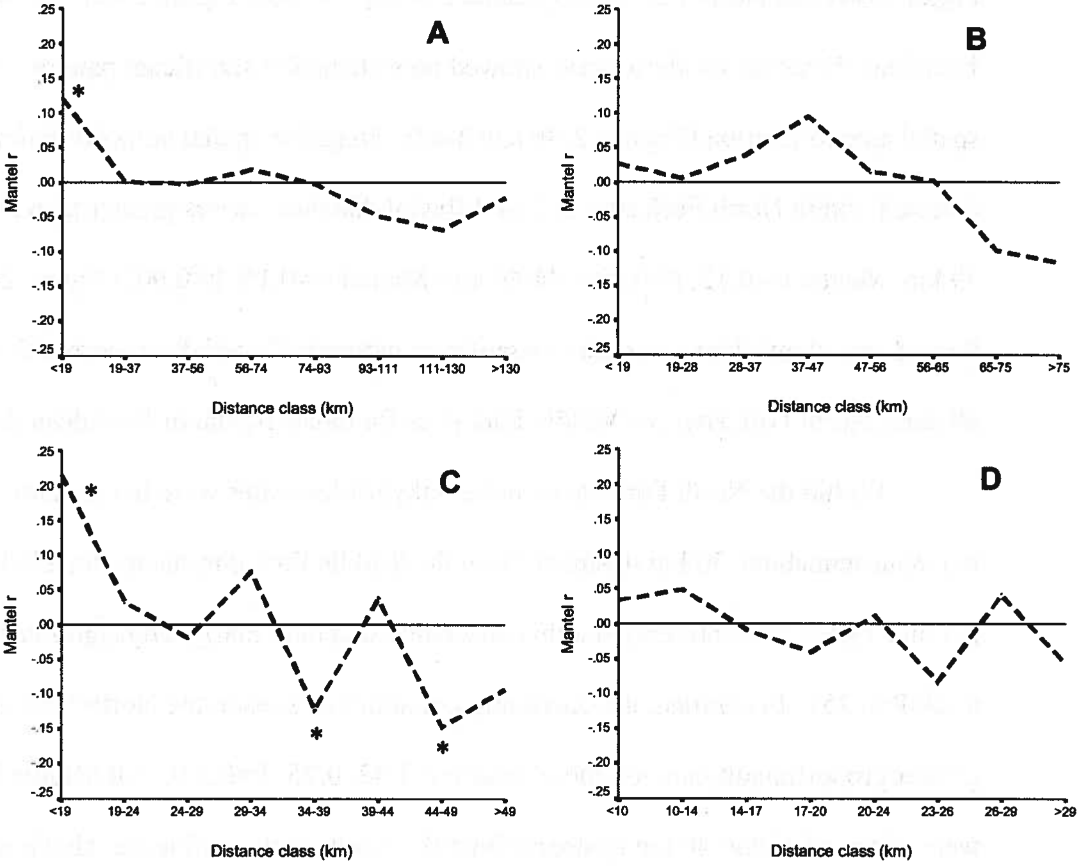

Figure 2-4. Mantel correlograms for RBT introgression. Spatial matrices were calculated from A) fluvial distances in all sites (n=43), B) Euclidean distances in all sites (n=43), C) fluvial distances in North Fork sites (n=31), and D) Euclidean distances in North Fork sites (n=31). Stars indicate significant correlations, using a Bonferroni-corrected error rate, α′ = 0.05/8 (8 distance classes) = 0.0063.

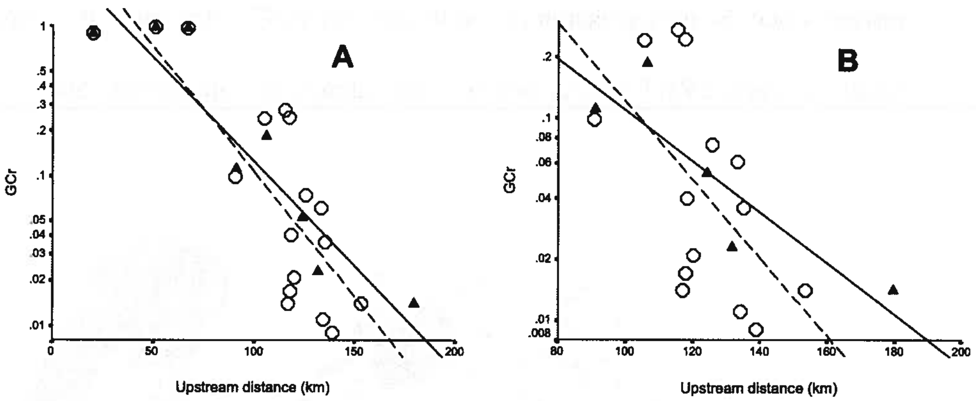

Figure 2-5. Relationship between the percent genetic contribution of RBT (*GC_r*) and upstream distance from Flathead Lake for A) all admixed sites and B) admixed sites within the North Fork and Middle Fork. Circles indicate North Fork sites (n=14); triangles indicate Middle Fork sites (n=5); and triangles within circles indicate mainstem sites (n=3).

Temporal analysis

Genetic surveys from 1984 showed RBT introgression in 2 of 14 sites within the North Fork of the Flathead River (sites 28 and 29). These two sites showed RBT introgression in the current survey, although the magnitude of RBT introgression increased in site 29 (and was not calculated in site 28 because this site showed significant linkage disequilibrium). New RBT introgression was detected in 7 of 14 sites. Five of the 14 sites continued to remain free from RBT introgression (Table 2-5; Figure 2-6). None of these sites are separated from the North Fork mainstem by known barriers to fish migration.

Only 3 sites showed statistically significant patterns of linkage disequilibrium. Two of these sites were within the southern portion of the North Fork (sites 22, 28) and one site was located near the northern extent of the study area (site 4). Each of these sites showed at least one apparent F_1 hybrid. The rarity of linkage disequilibrium (and F_1 hybrids) suggests that hybridization is spread primarily by post-F1 hybrids and/or several generations have passed since the introgression of relatively pure RBT. However, this result also indicates relatively recent RBT introgression in the linkage disequilibrium sites.

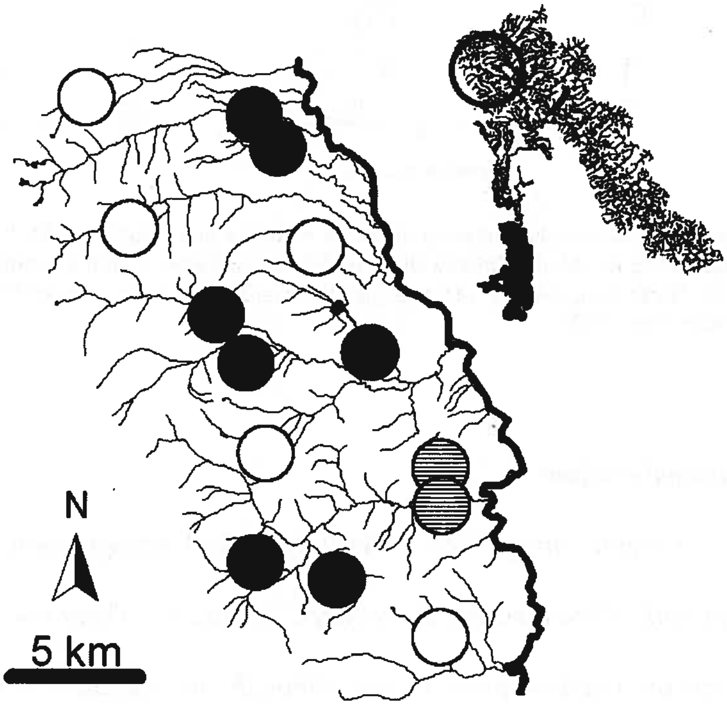

Figure 2-6. Temporal change in RBT introgression from 1984 - 2001 in the North Fork of the Flathead River system. Black circles indicate sites where new RBT introgression was detected (7/14 sites). Open circles indicate sites that have remained free from RBT introgression (5/14 sites). Horizontal hatch marks indicate sites where RBT introgression was detected in 1984 and current surveys.

Table 2-5. A comparison of RBT introgression (*GCr*) between 1984 and current study. Field collections for 1984 data were coordinated by J. Huston (Montana Fish, Wildlife, and Parks). GC_r could not be calculated for sites 4 and 28 due to evidence against Hardy-Weinberg proportions.

Site code	Site name	N - 1984	GC_r – 1984	GC_r – this study
1	Moose Creek, upper	27	0	0
4	Red Meadow Creek, lower	22	0	Introgression present
5	South Fork, Red Meadow Creek	24	0	0.01
8	Hay Creek	25	0	0
10	Moran Creek	29	0	0
13	Coal Creek	26	0	0.04
14	South Fork, Coal Creek	25	0	0.06
17	Cyclone Creek	23	0	0.07
25	Nicola Creek	25	0	0.04
26	Skookoleel Creek	11	0	0.02
27	Kletomas Creek	25	0	0
28	Langford Creek	15	0.02	Introgression present
29	Big Creek, lower	19	0.01	0.24
30	Depuy Creek	27	0	0

Discussion

The extent of RBT introgression in the FRS is greater than previously documented. The North Fork sites showed evidence of new RBT introgression since 1984. Patterns of positive spatial autocorrelation suggest that the spread of RBT introgression is facilitated by hybrid individuals straying among populations in the study area. Moreover, the rarity of F_1 hybrids and linkage disequilibrium suggests that the observed patterns of RBT introgression are not the result of multiple RBT stocking events.

These data are consistent with the hypothesis that RBT introgression is spreading upstream from Flathead Lake and the mainstem of the Flathead River. Spatial data show decreasing numbers of admixed sites and decreasing percent RBT contribution with increasing fluvial distance from Flathead Lake. Although temporal comparisons against 1984 data did not conclusively distinguish between upstream and downstream source models, sites showing linkage disequilibrium are not found within headwater areas, suggesting that relatively recent introgression of relatively pure RBT occurs more frequently at downstream sites than upstream sites.

However, the presence of a F_1 hybrid (and consequent linkage disequilibrium) in lower Red Meadow Creek (site 4) presents an interesting complication. Although this site is located in the lower section of the Red Meadow Creek drainage, this site is over 100 km upstream from Flathead Lake. Although headwater lakes feed this stream (and these lakes are popular for recreational fishing), the downstream movement of illegally stocked RBT is unlikely because the upstream site (site 3) did not show recent RBT introgression.

Extensive upstream migrations of RBT and RBT hybrids have been documented in the FRS and elsewhere. McMichael and Pearsons (2001) documented upstream migrations of hatchery steelhead >12 km in Washington. Recent telemetry studies in the FRS (C. Muhlfeld, MFWP, unpublished data) have shown upstream spawning migrations of WCT x RBT hybrids over 50 km. These fish were captured in the mainstem of the Flathead River and spawned in Camas Creek (near site 21). The closest site neighbor to Camas Creek is Dutch Creek, which showed evidence of linkage disequilbrium in the current study. As a result, the telemetry data are concordant with the genetics results of this study, although migrations from the mainstem to lower Red Meadow Creek have not been observed.

The spatial analyses in this study demonstrated the importance of fluvial measurements in assessing contagious processes among sites. Although Euclidean (i.e., straight-line) distances lacked significant Mantel correlations with hybridization status, fluvial distances showed significant positive and negative autocorrelation. By extension, Mantel correlations in aquatic ecology that rely exclusively on Euclidean distances may underestimate spatial autocorrelation by overlooking processes driven by fluvial connections (e.g., Olden et al. 2001; Wilkinson and Edds 2001; *cf.* Magnan et al. 1994). This finding also underscores the importance of fluvial processes in the spread of RBT introgression.

The spatial patterns of hybridization in this study support previous assessments of the population genetic structure within WCT. Leary et al. (1985b) determined that WCT maintain relatively high levels of genetic differentiation among populations (i.e., $G_{st} = 0.328$). Their survey included 9 sites within different streams of the FRS, but did not assess within-stream variation (Leary et al. 1985b). However, within-stream population genetic structure of WCT has also been detected (Leary et al. 1997), but has not been comprehensively surveyed. The current study provides anecdotal evidence for fine-scale population segregation based on the proximities between admixed and pure WCT sites (site comparisons 9-10; 18-19; 23-24; 30-31; 35-36) as well as the distribution of suspected WCT polymorphisms (sites 2, 6, 8, 12, 27, 40). It is therefore probable that the current survey underestimates the number of local WCT populations lost to RBT introgression in the study area.

Chapter 3.

Limiting factors for hybridization

Abstract

Conservation of westslope cutthroat trout (*Oncorhynchus clarki lewisi*, WCT) depends on environmental limitations to rainbow trout (*O. mykiss*, RBT) introgression. I compared environmental metrics and spatial statistics with introgression status using matrix correlations, categorical tests, and classification analyses to assess potential limiting factors in the Flathead River system (FRS). Neighborhood data (i.e., fluvial distances to nearest hybridized and pure sites) were more strongly associated with introgression status than environmental metrics, suggesting that RBT introgression may be constrained more by demographic than environmental factors. Although classification analysis provided weak evidence for geomorphic limiting factors at elevations >1450 m, these headwater areas are probably not large enough to support isolated WCT populations (i.e., 95% segments <8 km). I conclude that the spread of RBT introgression is not restricted by low-elevation environmental factors in the FRS and that further spread of RBT introgression is likely within the study area.

Introduction

A fundamental issue in ecology concerns the relationship between the intrinsic capacity for species dispersal and extrinsic constraints to that dispersal. For non-native species, this interplay determines the limits to range expansion and, consequently, the extent of impacts to native biota (Stauffer 1984). Hybridization between non-native and native species presents a unique problem in this regard because hybridized individuals may not be constrained by the factors that limit parental forms of the invading taxon.

Hybridization presents an important challenge for management and conservation of westslope cutthroat trout (*Oncorhynchus clarki lewisi*, WCT), a subspecies threatened by hybridization with introduced rainbow trout (*O. mykiss*, RBT) (Allendorf and Leary 1988). WCT are native within the headwaters of the Columbia and Missouri river basins and commonly occupy the highest stream elevations occupied by fishes (McIntyre and Rieman 1995). In contrast, RBT typically do not utilize the headwater reaches of streams in their native habitat (western North America and eastern Asia, see Figure 1-1) (Hartman and Gill 1968). In naturally sympatric RBT and cutthroat trout (*O. clarki*) populations, hybridization is rare (Campton and Utter 1985; Huston 1988), but is commonly observed where RBT have been stocked into native WCT waters (Behnke 1992).

As one of the most extensively transplanted fishes on earth, RBT have demonstrated the ability to survive and reproduce under a wide range of environmental conditions (Nico and Fuller 1999). However, self-sustaining populations of introduced RBT reflect the general habitat associations of native populations (MacCrimmon 1971). In native and introduced populations, RBT have shown associations with variation in patterns of water temperature, channel geomorphology, and habitat degradation.

In an analysis of the global distribution of introduced RBT, MacCrimmon (1971:696) concluded that "water temperature and precipitation are the primary environmental factors affecting the survival of the species" and that "naturalized populations do not become established if water temperatures do not fall below 13 °C." Laboratory experiments have shown RBT thermal preferences at 19.2- 19.8 °C (Cherry et al. 1977); 13.0 °C (Garside and Tait 1958); 18-19 °C (McCauley and Pond 1971). Based on experience in hatchery cultivation, Piper (1982) documented that eggs will not develop properly in RBT broodfish in water temperatures exceeding 13.3 °C (in WDE 1996). Lethal temperatures for RBT have been recorded between 25 – 26.5 °C (Alabaster and Welcomme 1962, Bidgood and Berst 1969, Charlton 1970, Hokanson et al. 1977 in Jobling 1981). Preferred water temperatures for RBT are greater than those for WCT (McIntyre and Rieman 1995).

Geomorphic parameters (e.g., stream slope, substrate composition, valley form, elevation, sinuosity) may also constrain the distribution of RBT. These abiotic parameters influence fish assemblage structure by altering the spatial and temporal microhabitat availability and by limiting rates of reproduction and survival as a function of stream discharge (Schlosser 1995) and groundwater-surface water interactions (Sowden and Power 1985). For example, Fausch et al. (2001) hypothesized that RBT invasion success was limited by egg scouring during spring peak flows (i.e., decreased RBT recruitment).

Anthropogenic degradation of stream habitat has been associated with increased invasions of non-native fishes (Moyle and Light 1996b) and increased competitive abilities of introduced fish (e.g., *Salvelinus fontinalis* invasion into WCT habitat, Shepard et al. 1998). Sedimentation, increased water temperatures, and microhabitat homogenization (e.g., streambank armoring) are commonly associated with human land use practices that affect fish assemblage structure (Schlosser 1991). Warmer stream temperatures may create opportunities for RBT introgression in the study area.

The Flathead River system (FRS) in Montana has been recognized as the largest stronghold for native WCT (Liknes and Graham 1988; Deeds et al. 1999). Although introduced RBT have formed self-sustaining populations in Flathead River mainstem (Deleray et al. 1999), no previous study has investigated the role of environmental limiting factors for RBT x WCT hybridization in the FRS. In this Chapter, I examine potential coarse-scale limiting factors for RBT hybridization, focusing on thermal regimes, geomorphological patterns, and habitat degradation.

Methods

Environmental limiting factors would exhibit repeated patterns at the landscape scale and between transitions from hybridized to pure sites. Under the presence of limiting factors, admixed and WCT sites would show non-random associations with the distribution of introgression. Conversely, if limiting factors were not acting, hybridization would be distributed throughout measured environmental gradients and environmental data would not distinguish between hybridized and pure sites. I tested the null hypothesis of "no environmental limiting factors" with matrix correlations, categorical tests, and classification analysis.

Environmental data

Collection of environmental data was organized around three categories: water temperature regime, channel geomorphology, and degradation status. These divisions represent coarse categories of environmental conditions that have been demonstrated to influence assemblage structure in stream fishes (Schlosser 1995). I contrasted these categories with nearest-neighbor data (i.e., fluvial distances to nearest hybridized and pure site) to assess the relative importance of environmental limiting factors and contagious spatial processes (Clark and Evans 1954).

Water temperature metrics included measures of magnitude and variability (Moyle and Light 1996b), including a) thermal range, b) mean daily maximum temperature, c) standard deviation of daily mean temperatures, d) percent mean temperature days <10 °C, and e) percent mean temperature days >15 °C. Thermographs were deployed at each site to record water temperatures at bi-hourly measurements. The sampling interval extended from 27 June to 20 September (85 days) between 1998 and 2001. All thermographs were calibrated to ± 0.2 °C before and after use.

Channel geomorphology was assessed with metrics derived from U.S. Geological Survey 7.5 minute maps. Site gradient and elevation were determined from contour intervals and stream order (Strahler method) was calculated from mapped perennial streams. Upstream basin size and channel sinuosity was calculated from ArcView® Spatial Analyst.

I used upstream road density and number of road – stream intersections as a surrogate for degradation status (Baxter et al. 1999). Forest roads affect the physical, biotic, and chemical conditions in downstream areas by altering geomorphic and hydrologic regimes (Trombulak and Frissell 2000). Road densities are strongly associated with timber management intensity in the FRS (Hauer and Blum 1991). Road data were obtained from U.S. Census Bureau 2000 TIGER files and mapped with ArcView® Spatial Analyst.

Principal Components Analysis (PCA) was used to reduce covariation within data categories. Before this analysis, percent data were arcsin-square root transformed and all other data were log (x+1) transformed to reduce outliers and normalize data (Wilkinson and Edds 2001). All categories were extracted into one principal component that explained the majority of variance (Table 3-1) and was readily interpretable. PCA scores were used for subsequent analyses.

Limiting factor analysis

Associations between RBT introgression and environmental gradients were assessed with Mantel tests (Mantel 1967), log-likelihood ratio tests (Zar 1984), and discriminant function analysis (Jongman et al. 1995). I used Mantel tests to screen environmental data for spatial autocorrelation and to assess patterns of non-random associations between environmental conditions and hybridization status. Site x site matrices of environmental data were constructed from Euclidean distances of PCA scores for a) all sites, b) North Fork sites, and c) Middle Fork sites. Hybridization matrices were constructed from a binary similarity index where 1 = both sites hybridized and 0 = other combinations (see Chapter 2). Each of the environmental matrices was tested against a spatial matrix (Euclidean map distances) to assess their spatial component and the hybridization matrix to assess their importance for introgression. Significance was

assessed at a Bonferroni-corrected error rate, $\alpha' = 0.10/15$ (15 possible comparisons) = 0.007.

I used log-likelihood ratio tests (G-tests) to assess associations between environmental conditions and introgression status (Zar 1984). PCA gradients were divided into 3 score classes. The observed number of hybridized and pure sites within each data class was contrasted against the expected number of sites within each class, based on the per-site hybridization probability. Zero scores were x+1 transformed and outliers (sites 42, 43) were excluded. G-tests are similar to Chi-squared tests but are more robust to small sample sizes (Williams 1976).

Discriminant function analysis (DFA) was employed to assess the predictive capacity of environmental data to explain the presence or absence of hybridization in the study area (Jongman et al. 1995). DFA was employed with PCA results at three scales: a) all sites, b) North Fork sites, and c) Middle Fork sites. The percent correct classification was calculated for each combination of data categories.

Table 3-1. Raw data summary and Principal Components Analysis results. All metric categories were extracted with one Principal Component for all $\lambda > 1$.

Category	Metric	Mean ± 1 standard deviation (Range)	% variance explained in PC I	Metric loadings into PC I
Thermal			80.8%	
	Water temperature range (°C)	9.85 ± 2.77 (10.25)		0.826
	Maximum daily average water temperature (°C)	13.87 ± 4.02 (13.81)		0.984
	Standard deviation: daily average water temperature (°C)	1.50 ± 0.60 (2.65)		0.908
	% summer days with average water temperature <10°C	0.45 ± 0.40 (1.00)		-0.920
	% summer days with average water temperature >15°C	0.17 ± 0.29 (1.00)		0.849
Geomorphological			55.6%	
	Site gradient	0.04 ± 0.03 (0.10)		-0.602
	Upstream basin size (km^2)	71.36 ± 65.57 (298.20)		0.884
	Channel sinuosity	1.14 ± 0.10 (0.50)		0.706
	Stream order (Strahler)	2.56 ± 0.80 (3.00)		0.739
	Site elevation (m)	1185 ± 175 (870)		-0.770
Degradation			84.1%	
	Road density (km/km^2)	0.38 ± 0.40 (1.58)		0.917
	# stream-road intersections	10 ± 20 (112)		0.917
Neighbors			58.2%	
	Fluvial distance to nearest WCT site (km)	17.66 ± 15.07 (80.20)		0.763
	Fluvial distance to nearest admixed site (km)	12.29 ± 8.14 (35.90)		0.763

Results

Admixed sites showed extensive variability in thermal regime, geomorphology, and degradation status. Admixed sites were detected throughout the majority of the range of variability within each PCA gradient (Figure 3-1). The only significant association with RBT introgression was detected from neighbors data at the all-sites scale (Mantel r = 0.24, P<0.007; Table 3-2). At the North Fork scale, neighbors data showed the greatest correlation with RBT introgression status, but this relationship was not statistically significant. Mantel tests detected no spatial autocorrelation in environmental or neighbors data (Table 3-2).

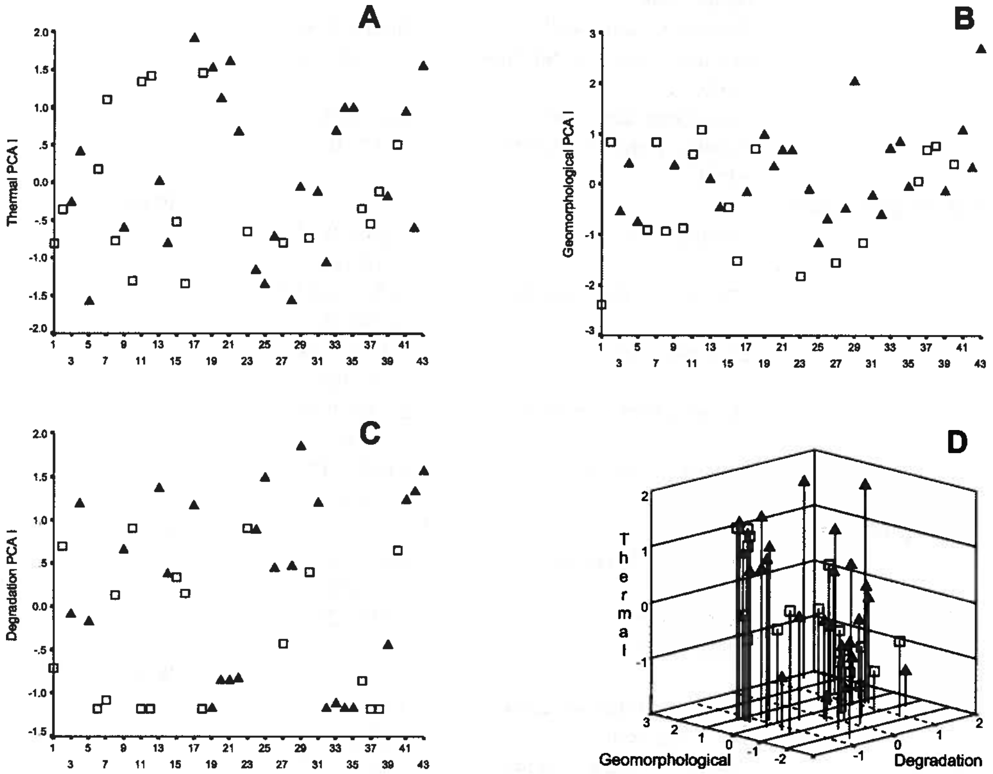

Figure 3-1. Environmental variability among admixed and WCT sites for A) thermal, B) geomorphological, C) degradation, and D) combined PCA gradients. PCA metric loadings are given in Table 3-1. Triangles indicate admixed sites; squares indicate WCT sites.

Table 3-2. Mantel correlations for comparisons between geographic distances (Euclidean), RBT introgression, environmental factors, and nearest fluvial neighbors. Statistical significance was assessed using a Bonferroni-corrected error rate, $\alpha' = 0.10/15$ (15 possible comparisons for each site group) = 0.007. Probability is based on 10000 permutations, *$P<0.007$, **$P<0.0007$.

Site group	Matrix	1. RBT	2. Thermal	3. Degradation	4. Geomorph.	5. Neighbors
All (n=43)	Euclidean	-0.10	0.03	0.07	0.09	-0.04[1]
	1.	—	-0.008	0.05	-0.12	0.24*
	2.		—	0.08	0.38**	0.07
	3.			—	0.16*	0.14
	4.				—	0.10
North Fork (n=31)	Euclidean	-0.06	0.13	0.09	0.12	0.11[1]
	1.	—	-0.008	0.008	-0.14	0.19
	2.		—	0.19*	0.44**	0.06
	3.			—	0.22	0.28
	4.				—	0.14
Middle Fork (n=9)	Euclidean	0.10	0.20	-0.10	0.10	0.39[1]
	1	—	0.34	0.12	0.14	0.32
	2.		—	-0.27	0.13	-0.16
	3.			—	0.06	0.49
	4.				—	-0.08

[1] A lack of spatial autocorrelation was also observed among fluvial distances.

Log-likelihood ratio tests showed significant disproportionality between WCT sites and geomorphological data ($P<0.05$) as well as neighbors data among PCA categories ($P<0.05$, Table 3-3). However, the disproportionality of admixed sites for neighbors data exceeded geomorphology, although neither was significant at the 0.05 level ($0.05<P<0.10$ and $0.10<P<0.25$, respectively). Bootstrap sampling showed that the mean deviation from random in neighbors data exceeded the 90% confidence interval for geomorphological data (Figure 3-2). Thermal data showed the least divergence from expected values for admixed and WCT sites ($P>0.75$, Figure 3-3).

DFA results varied among North Fork, Middle Fork, and all-site scales (Table 3-4). Neighbors data exerted the greatest effects on the predictive capacity of DFA models for 3 variables at the all-sites and North Fork scales. With 2 variables, degradation and geomorphological data were the best predictors of introgression status at the all-sites scale, while degradation and neighbors data were the best predictors at the North Fork scale. However, although neighbors data were the best single variable predictor in North Fork sites, they were not for the all-sites scale. In contrast, introgression status of Middle Fork sites was more influenced by thermal data, although thermal data was the least important variable at the all-sites and North Fork scales (Table 3-4).

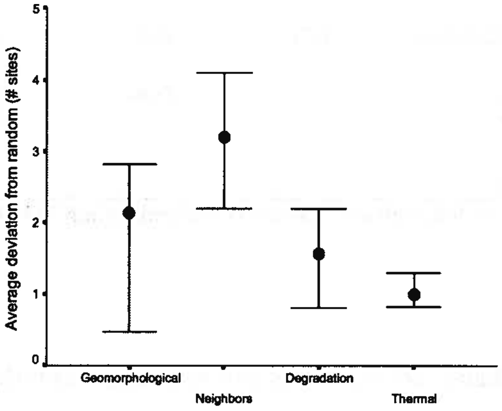

Figure 3-2. A comparison of departure from randomness in the distribution of hybridized and pure sites in the study area. Circles indicate mean values for the absolute difference between observed and expected site counts within three data classes per category (G-test results). Whiskers indicate 90% confidence intervals derived from bootstrap sampling (5000 replicates).

Table 3-3. Log-likelihood ratio test results for WCT and admixed sites comparing observed and expected numbers of sites within 3 environmental PCA gradients in 3 classes.

Category	Hybridization status	*G*	P
Thermal	WCT	0.51	P>0.75
	WCT x RBT	0.41	P>0.75
Geomorphological	WCT	6.60	P<0.05
	WCT x RBT	3.24	0.10<P<0.25
Degradation	WCT	1.82	0.25<P<0.50
	WCT x RBT	1.44	0.25<P<0.50
Neighbors	WCT	6.25	P<0.05
	WCT x RBT	4.73	0.05<P<0.10

Table 3-4. Discriminant function analysis results. N = neighbors PCA scores, T = thermal PCA scores, D = degradation PCA scores, G = geomorphic PCA scores.

Model version	All sites (n=43) % correct classification	North Fork sites (n=31) % correct classification	Middle Fork sites (n=9) % correct classification
N, T, D, G	0.674	0.742	1.00
N, D, G	0.674	0.742	0.778
N, T, D	0.651	0.710	0.889
T, D, G	0.605	0.677	1.00
N, T, G	0.651	0.710	0.889
N, T	0.605	0.677	0.667
N, D	0.558	0.710	0.778
N, G	0.535	0.677	0.556
T, G	0.535	0.613	0.889
T, D	0.581	0.613	0.667
D, G	0.651	0.677	0.667
N	0.512	0.677	0.667
G	0.512	0.645	0.667
T	0.558	0.548	0.667
D	0.558	0.581	0.556

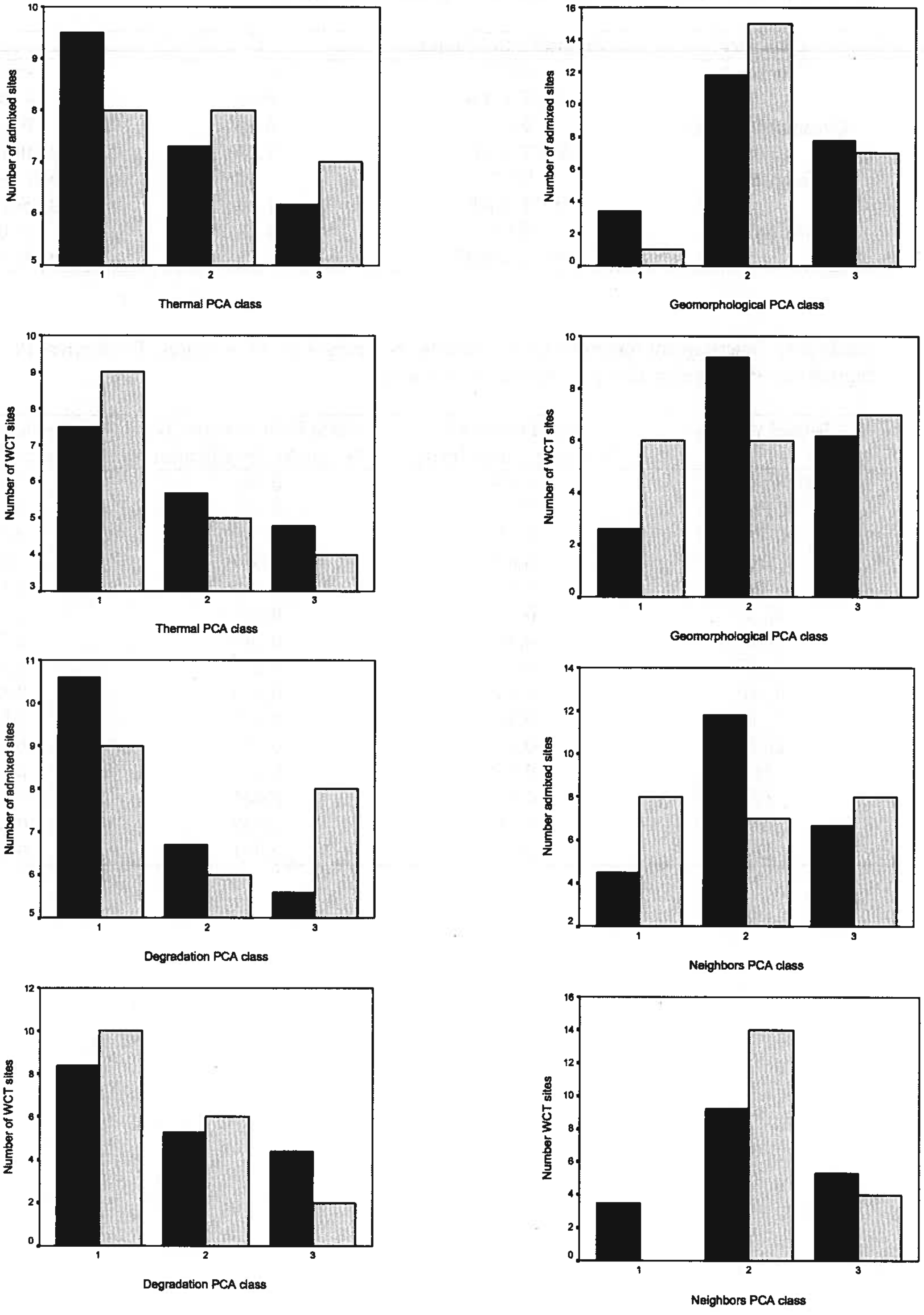

Figure 3-3. Log-likelihood ratio comparisons for admixed and WCT sites in 3 PCA categories. Solid bars indicate expected numbers of sites; hatch-marked bars indicate observed numbers of sites. P-values are reported in Table 3-3.

Discussion

Thermal regime

Populations of self-sustaining RBT have been documented in the mainstem of the Flathead River (Deleray et al. 1999), which supports warmer waters than headwater streams. However, Mantel tests, categorical tests, and classification analyses showed that hybridization is distributed randomly with respect to water temperature range, maximum summer temperature, standard deviation of daily maximum temperatures, and percent days below 10°C and above 15°C. Although introduced populations of RBT may be limited by thermal gradients, it appears that RBT x WCT hybrids are not restricted by thermal regimes in the study area.

Geomorphology

I found some evidence for geomorphological limits to RBT introgression. Log-likelihood ratio tests and classification analyses showed that geomorphological data were non-randomly associated with the distribution of admixed and pure sites. A transition from hybridized to unhybridized sites occurred above elevations of 1450 m and greater than 9% slopes. However, because only two unhybridized sites were found above these conditions, these results are suggestive, but not conclusive, about the role of geomorphic limiting factors.

However, even if these environmental conditions restrict RBT introgression, the headwater areas would probably be too small to maintain isolated WCT populations. Within the North and Middle Fork basins, over 400 stream segments exist above this elevation gradient. Of these, >95% are less than 8 km in length, a distance unlikely to support a census population of 2,500 individuals (corresponding to N_e=500) (Hilderbrand and Kershner 2000). As a result, it is unlikely that geomorphic limiting factors will be important in limiting RBT introgression within the study area.

Degradation

Admixed sites were associated with increased road densities and increased numbers of road-stream intersections, relative to unhybridized sites. Conversely, the maximum road density of unhybridized sites was 0.88 km/km^2 (site 23) and hybridized sites exceeded this value (sites 25, 31, 42). Although these differences were not statistically significant in categorical tests or Mantel tests, these results are not inconsistent with Moyle and Light's (1996b) hypothesis that degraded sites are more susceptible to invasion by stream fishes.

Neighbors

Neighbors data showed the strongest association with patterns of RBT introgression. As demonstrated in matrix correlations, categorical tests, and classification analyses, sites closer to hybridized neighbors tended to be hybridized and vice versa. This result is consistent with the hypothesis that WCT x RBT hybrids are straying among populations, thereby spreading RBT introgression. This model of RBT invasion would exhibit stratified diffusion (*sensu* Shigesada et al. 1995) as populations are sequentially invaded by RBT x WCT hybrids and subsequent offspring stray to neighboring populations.

Chapter 4.

Synthesis

This study brings new insight to the subject of hybridization between native westslope cutthroat trout (*Oncorhynchus clarki lewisi*, WCT) and introduced rainbow trout (*O. mykiss*, RBT) in the Flathead River system (FRS). First, this study redefines the distribution of WCT and admixed populations in the study area. RBT introgression was detected in 55% (17/31) of the North Fork sample sites and 56% (5/9) of the Middle Fork sample sites. Temporal comparisons showed that hybridization has spread within North Fork tributaries since 1984.

Second, this study highlights the susceptibility of native WCT to introgression by RBT in the study area. Although pure RBT may be excluded from headwater areas in the FRS, WCT x RBT hybrids are not. Instead, neighborhood statistics (i.e., distances to nearest hybridized and pure sites) showed stronger associations with introgression than environmental gradients. My findings also support the hypotheses that a) RBT introgression is spreading upstream from Flathead Lake and the Flathead River mainstem and b) RBT introgression is spreading among sites. Although I found weak evidence for geomorphological limiting factors above elevations of 1450 m, remnant stream reaches would probably be too small to support isolated WCT populations.

The U.S. Fish and Wildlife Service should consider these findings to determine the distribution and threats to WCT. Using stream reaches delineated by Montana Fish, Wildlife, and Parks, I estimate that the extent of RBT x WCT hybridization in the North and Middle Forks of the FRS is more than triple that considered in the Status Review (Deeds et al. 1999). Additionally, the environmental variability among hybridized sites suggests that the Status Review's assumption that headwater streams "are relatively secure from colonization by nonnative fishes..." (Deeds et al. 1999:158) is inaccurate for WCT x RBT hybrids. Although admixed populations may have an important role in for restoration, they should not be confused with WCT for identifying distributions.

Management responses to this problem may improve the prognosis for native WCT. Because it appears that RBT introgression is spreading upstream, control of source populations in the Flathead River mainstem may decrease hybridization at the landscape scale. This finding lends support for on-going efforts to reduce RBT spawning in Abbot Creek (C. Muhlfeld, MFWP, personal communication). Increasing fishing pressure on RBT and WCT x RBT populations may also decrease the spread of RBT introgression. However, this would require fishing regulations within entire streams, due to the impracticality of angler identification of hybrid individuals. In contrast, all members of hybrid swarms should be considered hybridized, albeit at low levels.

In many cases, management of headwater cutthroat trout populations involves the construction of upstream passage barriers to protect a stream from pioneering nonnative fishes. For example, nearly all remnant WCT populations in the headwaters of the Missouri River are found above migration barriers (B. Shepard, personal communication). However, barrier construction could jeopardize migratory populations of federally-threatened bull trout (*Salvelinus confluentus*). In these cases, the conservation trade-offs of barrier construction are probably unwarranted.

Two caveats should accompany interpretations from this study. First, the percent genetic contribution of RBT (*GCr*) depends on whether or not the sampled individuals consist of one population that exhibits Hardy-Weinberg (H-W) proportions. Second, I assume that individuals sampled from a site are representative of spawning populations near that area. I examine the potential bias from violating these assumptions below.

Calculations of *GCr* depend on assumed proportions of homozygous and heterozygous individuals in the sample population. Because Paired Interspersed Nuclear Elements (PINEs) are dominant, one cannot distinguish between individuals that are heterozygous and homozyous for a present RBT fragment. Consequently, *GCr* is derived from the frequency of absence of the RBT marker, not its presence. In a population exhibiting H-W proportions, the frequency of absence would equal the squared proportion of WCT markers (i.e., q^2). In turn, one minus the square root of q^2 would equal the percent RBT contribution from that locus.

However, conditions for H-W proportions may be violated. First, the sample may consist of individuals from more than one population. Effectively, this would reduce the sample sizes and increase the Type II error probabilities (i.e., increased failure to detect admixed populations when they exist). Second, H-W assumes that gene flow is not occurring, but the presence of RBT introgression demonstrates that it is. Third, H-W assumes that natural selection is not acting, but hybridization may confer fitness costs. Although these violations of H-W proportions may bias *GCr* values, in no case would these conditions result in the misdiagnosis of a site as "admixed" when in fact it was pure WCT.

The second caveat is that sampled individuals are representative of a breeding population near that area. However, some migratory individuals may have been included in the sampled individuals. Although migratory fish may spawn in the lower reaches of streams used by resident WCT (Johnson 1963), there is some potential for non-representative individuals in all sample sites.

I found subadult WCT individuals (<100 mm total length) in 40 of 43 sample sites, suggesting that most sites include locally-reproducing populations. However, within Big Creek (site 29), Whitefish River (site 43), and Camas Creek (site 21), only individuals >100 mm were found. Given the low sample size (n=3), lack of juvenile fish, and close proximity to the North Fork mainstem, it is possible that the Big Creek site consists of fluvial or adfluvial migrants from another drainage. Similarly, the Whitefish River sample may consist of adfluvial Whitefish Lake fish. However, radio telemetry studies have shown fluvial WCT x RBT hybrids spawning in Camas Creek (C. Muhlfeld, MFWP, unpublished data), providing additional evidence that admixed populations reproduce in this tributary.

The results of this study present new research questions for the FRS. First, this study highlights the importance of genetic surveys to assess the distribution of WCT. Additional sampling should be conducted to fully assess the distribution of WCT and admixed populations. Second, this study demonstrates that relatively large influxes of RBT introgression are possible within 3-5 generations. To better understand temporal trends, additional surveys should resample sites with 1984 data (see Table 2-5). Third, the role of Camas, Dutch, and Anaconda Creeks (Glacier National Park) as WCT x RBT source populations should be investigated.

Fundamental questions about the effects of RBT introgression remain unanswered. First, the fitness consequences of RBT introgression should be investigated. Although laboratory crosses have demonstrated intermediate traits in WCT x RBT hybrids (Leary et al. 1984a; Ferguson et al. 1985), no studies have compared vital rates among WCT and admixed populations. Understanding the effects of natural selection on WCT x RBT hybrids would provide new insight on the long-term impacts of RBT introgression.

Second, the effects of RBT introgression on straying rates should be assessed. Because WCT are characterized by genetic diversity among populations (and, by inference, relatively low straying rates; Leary et al. 1985b) and homing in salmonids has a genetic basis (Smith 1985), RBT introgression could impair the ability of hybrid trout to home. Additionally, RBT introgression could introduce a genetic predisposition to stray. Under either scenario, hybridization would facilitate further RBT introgression (*sensu* Simberloff and Von Holle 1999). To test this hypothesis, one would first have to determine the extent of fine-scale population genetic structure within the study area.

A third emerging issue concerns the role of habitat degradation and restoration in the spread of RBT introgression. Degradation may decrease the availability of microhabitats for WCT (via homogenization), potentially inducing straying, emigration, and increased spread of RBT introgression. Conversely, restoration activities to increase habitat complexity may reduce straying and emigration rates, thus slowing the spread of RBT introgression.

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Appendix. Paired Interspersed Nuclear Element (PINE)-PCR (Spruell et al. 2001) raw data for admixed sites. Fragment presence = 1 and absence = 0. Blank cells indicate results that were not scorable.

Site 3. Red Meadow Creek, upper.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
2	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
3	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
4	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
6	0	1	1	0	1	1	0	1	0	0	0	1	0	1	0	0	1
7	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
8	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
9	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
10	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
11	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0	0	1
12	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
13	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
14	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
15	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
16	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
17	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
18	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
19	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0	0	1
20	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
21	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
22	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0	0	1
23	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
24	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
25	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1

Site 4. Red Meadow Creek, lower.

Primer Pair	Hpal 5'/Hpal 3'						Fokl 5'/Tc1						Hpal 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
2	0	1	1	0	1	0	0	1	0	0	0	1					1
3	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
4	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
6	0	0	1	1	0	1	0	1	0	0	0	1					
7	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
8	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
9	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
10	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
11	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
12	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1
13	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
14	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
15	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
16	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
17	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
18	0	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1
19	0	1	1	1	1	1	0	1	0	0	0	1					
20	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
21	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
22	0	1	1	1	1	1	1	1	0	0	0	1	0	1	1	0	1
23	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
24	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
25	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1

Site 5. South Fork of Red Meadow Creek.

Primer Pair	Hpal 5'/Hpal 3'						Fokl 5'/Tc1						Hpal 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
2	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
3	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
4	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
5	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
6	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
7	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
8	0	1	1	0	1	0	0	0	1	0	1	0	0	1	0	1	1
9	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
10	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
11	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
12	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
13	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
14	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
15	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
16	0	1	0	0	1	0	0	0	1						0	1	0
17	0	1	0	0	1	0	0	0	1						0	1	0
18	0	1	0	0	1	0	0	0	1	0	1	1	0	1	0	1	0
19	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
20	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0

Site 9. Hay Creek, lower.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
2	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
3	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
4	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
5	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
6	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
7	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
8	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
9	1	1	0	0	1	0	0	0	1	0	1	0	0	1	1	1	0
10	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
11	0	1	1	0	1	0	0	0	1	0	1	0	0	1	0	1	1
12	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
13	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
14	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
15	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
16	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
17	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
18	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
19				0	1	0	0	0	1								
20	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
21	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
22	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
23	0	1	0	0	1	0	0	0	1	0	1	0	0	1	0	1	0
24	0	1	1	0	1	0	0	0	1	0	1	0	0	1	0	1	1

Site 13. Coal Creek (North Fork Flathead River)

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
2	0	1	1	1	1	1	0	1	0	0	0	1					
3	0	1	1	0	1	0	0	1	0	0	0	1	0	1	1	0	1
4	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
6	0	1	1	0	1	0	0	1	0	0	0	1					
7	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
8	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
9	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
10	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
11	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
12	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
13	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
14	0	1	1	0	1	0	1	1	0	0	0	1	0	1	0	0	1
15	0	1	1	0	1	0	0	1	1	0	0	1	1	1	0	0	1

Site 14. South Fork of Coal Creek

Primer Pair	Hpal 5'/Hpal 3'						Fokl 5'/Tc1						Hpal 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	0	1	0	1	1	1	1	0	0	0	0	1	0	0	0
2	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
3	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
4	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
6	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
7	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
8	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
9	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	0
10	0	1	1	0	1	0	1	1	1	0	0	1	1	1	0	0	0

Site 17. Cyclone Creek, lower.

Primer Pair	Hpal 5'/Hpal 3'						Fokl 5'/Tc1						Hpal 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1			0	0	1	0				0	0	1					
2			1	0	1	0	1	0	0	0	0	1					
3	0	1	1	0	1	0	0	1	0	0	0	1					
4	0	1	1	0	1	1	1	1	1	0	0	1					
5	0	1	1	0	1	0	0	1	0	0	0	1					
6	0	1	1	0	1	0	0	1	0	0	0	1					
7			1	0	1	0			1	0	0	1					
8	0	1	1	0	1	0	0	1	0	0	0	1					
9	0	1	1	0	1	0	0	1	0	0	0	1					
10	0	1	1	0	1	0	0	1	0	0	0	1					
11	0	1	1	0	1	0	0	1	0	0	0	1					
12	0	1	1	0	1	0	0	1	0	0	0	1					
13	0	1	1	1	1	0	0	1	0	0	0	1					
14	0	1	1	0	1	0	0	1	1	0	0	1					
15		1	0	0	1	0	0	1	0	0	0	1					
16			1	0	1	0			1	0	0	1					
17			1	0	1	0			1	0	0	1					
18	0	1	1	0	1	0	0	1	0	0	0	1					
19			1	0	1	0			1	0	0	1					
20	0	1	1	0	1	0				0	0	1					
21	0	1	1	0	1	0				0	0	1					
22	0	1	1	0	1	1				0	0	1					
23	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	
24	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	
25	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0	0	

Site 19. Logging Creek, lower.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
2	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
3	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
4	0	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1
5	0	1	0	1	1	0	0	1	0	0	0	0	0	1	0	0	1
6	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
7	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
8	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
9	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
10	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
11	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
12	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
13	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
14	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
15	0	1	0	0	1	0	0	1	0	0	0	1	0	1	0	0	1
16	0	1	0	0	1	1	0	1	0	0	0	1	1	1	1	0	1

Site 20. Anaconda Creek.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1		1	1	0	1	0				0	0	1					
2		1	1	1	1	0				0	0	1					
3			1	1	1	1				0	0	1					
4		1	1	1	1	1				0	0	1					
5		1	1	1	1	1											
6		1	1	0	1	0											
7		1	1	1	1	0											
8			1	1	1	0											
9		1	1	0	1	1											
10			1	0	1	0											
11		1	1	1	1	1											
12		1	1	0	1	0				0	0	1					
13			1	1	1	0	1	0	1	0	0	1					
14		1	1	1	0	1	0	1	0	0	0	1					
15							1	1	0	0	0	1					
16		1	1	1	1	1	0	1		0	0	1					
17		1	1	1	1	0	1	1	0	0	0	1					
18		1	1	1	0	1	0	1	1	0	0	1					
19		1	1	0	1	0	0	1	0	0	0	1					
20		1	1	0	1	1				0	0	1					

Site 21. Camas Creek.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0	0	1
2	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
3	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1
4	0	0	0	1	0	1	1	1	1	0	0	1	1	1	1	0	1
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1		0	1
6	0	1	0	0	1	0	0	1	1	0	0	1	1	1	0	0	1

Site 22. Dutch Creek.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	1	0	1	0	0	0	1	1	1	1	0	
2	0	1	1	1	1	1	0	1	1	0	0	1	1	1	1	0	
3	0	1	0	1	1	1	1	1	0	0	0	1	0	1	1	0	
4	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	
5	0	1	1	0	1	0	1	1	1	0	0	1	1	1	0	0	
6	0	1	1	0	1	0	1	1	0	0	0	1	0	1	0	0	
7	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	
8	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	
9	0	1	1	1	1	1	0	1	0	0	0	0	1	1	1	0	
10	0	1	1	1	1	1	1	1	1	0	0	1	1	1	1	0	
11	0	1	1	0	1	1	1	1	0	0	0	1	1	0	1	0	
12	0	1	1	0	1	0	1	1	0	0	0	1	0	1	0	0	
13	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	
14	0	1	1	0	1	1	0	1	0	0	0	1	0	1	1	0	
15	0	1	1	0	0	1	1	1	1	0	0	1	1	0	1	0	
16	0	1	0	1	1	1	1	1	1	0	0	1	1	1	1	0	
17	0	1	1	0	1	0	1	1	1	0	0	1	1	1	0	0	
18	0	0	1	1	1	0	1	1	0	0	0	0			0	0	
19	0	1	1	1	1	1	1	1	0	0	0	1	1	1	1		1
20	0	1	1	0	1	0	0	1	1	0	0	1					1
21	0	1	1	0	1	1	1	1	1	0	0	1					1
22		1	1	1	1	0											1
23		1	1	1	1	1											1

Site 24. Big Creek, middle.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
2	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
3	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
4	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
6	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
7	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
8	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
9	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
10	0	1	1	1	1	0	0	1	0	0	0	1	1	1	0	0	1
11	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
12	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1

Site 25. Nicola Creek.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	1	0	1	0	0	0	1	0	1	1	0	1
2	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0	0	1
3	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0	0	1
4	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
6	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
7	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
8	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
9	0	1	1	0	1	0	1	1	1	0	0	1	0	1	1	0	1
10	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
11	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
12	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
13	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
14	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
15	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1

Site 26. Skookoleel Creek.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
2	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
3	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
4	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
6	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
7	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
8	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
9	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
10	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
11	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
12	0	1	1	0	1	1	1	1	1	0	0	1	1	1	0	0	1
13	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
14	0	1	1	0	1	0	0	1	0	0	0	1					
15	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
16	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
17	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
18	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
19	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
20	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1

Site 28. Langford Creek.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0			0	0	0	1					
2	0	1	1	1	1	1			0	0	0	1					
3	0	0	0	1	1	1			1	0	0	1					
4	0	1	1	0	1	1			1	0	0	1					
5	0	1	1	0	1	0			0	0	0	1					
6	0	1	1	1	1	1	0	1	1	0	0	1					
7	0	1	1	0	1	0	0	1	0	0	0	1					
8	0	1	1	0	1	0			0	0	0	1					
9	0	1	1	1	1	1			1	0	0	1					
10	0	1	0	1	1	1	1	1	1	0	0	0					
11	0	1	1	0	1	1	0	1	1	0	0	1					
12	0	1	1	0	1	0	0	1	0	0	0	1					
13	0	1	1	0	1	0	1	1	0	0	0	1					
14	0	0	1	1	0	1	1	1	1	0	0	1					
15	0	1	1	0	1	0			1	0	0	1					
16	0	1	1	0	1	0	1	1	0	0	0	1					
17	0	1	1	1	1	0	1	1	1	0	0	1					
18	0	0	1	1	0	1	0	1	1	0	0	1					
19	0	1	1	1	1	1	1	1	0	0	0	1					
20	0	1	1	1	1	1	0	1	0	0	0	1					

Site 29. Big Creek, lower.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1
2	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
3	0	1	1	1	1	1	0	1	0	0	0	1	0	1	1	0	1

Site 31. McGinnis Creek.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
2	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
3	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
4	0	1	1	1	1	1	0	1	0	0	0	1	0	1	1	0	1
5	0	1	1	0	0	1	0	1	0	0	0	1	0	1	0	0	1
6	0	1	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1
7	0	1	1	0	0	1	0	1	0	0	0	1	0	1	0	0	1
8	0	1	1	0	0	0	0	1	0	0	0	1	0	1	0	0	1
9	0	1	1	0	0	0	0	1	1	0	0	1	0	1	1	0	1
10	0	1	1	1	0	1	0	1	0	0	0	1	0	1	0	0	1
11	0	1	1	0	0	0	0	1	0	0	0	1	0	1	0	0	1
12	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1

Site 32. Rubideau Creek.

Primer Pair	Hpal 5'/Hpal 3'						Fokl 5'/Tcl						Hpal 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	0	1	1	1	1	0	1	1	0	0	1	1	1	1	0	1
2	0	0	1	1	1	1	1	1	1	0	0	1	1	1	1	0	1
3	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
4	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
5	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
6	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
7	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
8	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
9	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
10	0	0	1	1	1	1	0	1	0	0	0	1	1	1	1	0	1
11	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
12	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1

Site 33. Lincoln Creek.

Primer Pair	Hpal 5'/Hpal 3'						Fokl 5'/Tcl						Hpal 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	1	1	0			0	0	0	1					
2	0	1	1	1	1	0			0	0	0	1					
3	0	1	0	1	0	1			0	0	0	1					
4	0	1	1	0	1	0			0	0	0	1					
5	0	1	1	0	1	0			0	0	0	1					
6	0	1	1	0	1	1			1	0	0	1					
7	0	0	1	1	1	0			1	0	0	1					
8	0	1	1	0	1	1			1	0	0	1					
9	0	1	1	0	1	1			0	0	0	1					
10	0	1	1	0	1	0			0	0	0	1					
11	0	1	1	0	1	0			0	0	0	1					
12	0	0	1	1	1	0			0	0	0	1					
13	0	1	1	0	0	1			0	0	0	0					
14	0	1	1	0	1	1			1	0	0	1					
15	0	1	1	1	1	1			1	0	0	1					
16	0	1	1	0	1	0			0	0	0	1					
17	0	1	1	1	1	0			0	0	0	0					
18	0	1	1	1	1	0			0	0	0	1					
19	0	1	1	0	1	0			0	0	0	1					
20	0	1	1	1	1	0			0	0	0	1					
21	0	1	1	0	1	0			0	0	0	1					
22	0	1	1	0	1	1			0	0	0	1					

Site 34. Coal Creek (Middle Fork Flathead River).

Primer Pair	Hpal 5'/Hpal 3'						Fokl 5'/Tcl						Hpal 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	
2	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	
3	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	
4	0	1	1	1	1	0	1	1	0	0	0	1	1	1	0	0	
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	

Site 35. Stanton Creek.

Primer Pair	Hpal 5'/Hpal 3'						Fok1 5'/Tc1						Hpal 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
2	0	0	1	0	1	0	1	1	0	0	0	1	0	1	0	0	1
3	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
4	0	1	1	0	1	0	1	1	0	0	0	1	0	1	0	0	1
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
6	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
7	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
8	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
9	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
10	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
11	0	1	1	0	1	0	1	1	0	0	0	1	0	1	0	0	1
12	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
13	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
14	0	1	1	0	1	0	0	1	0	0	0	1	1	1	0	0	1
15	0	0	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1

Site 39. Essex Creek.

Primer Pair	Hpal 5'/Hpal 3'						Fok1 5'/Tc1						Hpal 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
2	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
3	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
4	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
5	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
6	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
7	0	1	1	0	1	0	0	1	0	0	1	1	0	1	0	0	1
8	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
9	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
10	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
11	0	1	1	0	1	0	1	1	0	0	1	1	0	1	0	0	1
12	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
13	0	1	1	0	1	0	0	1	0	1	0	1	0	1	0	0	1
14	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
15	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0	0	1
16	0	1	1	0	1	0	0	1	0	0	1	1	0	1	0		1
17	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0		1
18	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0		1
19	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0		1
20	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0		1
21	0	1	1	0	1	0	0	1	0	0	0	1	0	1	0		1
22	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0		1
23	0	1	1	0	1	0	0	1	0	0	1	1	0	1	0		1
24	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0		1
25	0	1	1	1	1	0	0	1	0	0	0	1	0	1	0		1

Site 41. Abbot Creek.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	0	0	1	0	1	1	0	1	0	0	0			1	0	0
2	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
3	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
4	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
5	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	0
6	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	0
7	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
8	0	1	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
9	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	0
10	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
11	0	1	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
12	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
13	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
14	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
15	0	0	0	1	0	1	1	0	1	0	0	0					
16	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	1
17	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0
18	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	0

Site 42. Mill Creek

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	
2	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	
3	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	
4	0	0	0	1	0	1	1	0	1	0	0	1					
5	0	0	0	1	0	1	1	0	1	0	0	1					
6	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	
7	0	0	0	1	0	1	1	0	0	0	0	0	1	0	1	0	
8	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	
9	0	0	0	1	0	1	1	0	0	0	0	0	1	0	1	0	
10	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	
11	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	
12	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	
13	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	
14	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	
15	0	0	0	1	0	1	1	0	0	0	0	1	1	0	1	0	

Site 43. Whitefish River.

Primer Pair	Hpa1 5'/Hpa1 3'						Fok1 5'/Tc1						Hpa1 5'/33.6+2				
No. base pairs	232	153	72	70	69	66	369	366	230 (235)	159	138	110	395	388	266	248	148
Diagnostics	YCT	YCT WCT	YCT WCT	RBT	YCT WCT	RBT	RBT	YCT WCT	RBT	YCT	YCT	WCT	RBT	YCT WCT	RBT	YCT	YCT WCT
1	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
2	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
3	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
4	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
5	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
6	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
7	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	
8	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	
9	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
10	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
11	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
12	0	0	0	1	0	1	1	0	1	0	0	1	1	0	1	0	
13	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
14	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	
15	0	0	0	1	0	1	1	0	1	0	0	0	1	0	1	0	