Spatial and temporal spawning dynamics of native westslope cutthroat trout, *Oncorhynchus clarkii lewisi*, introduced rainbow trout, *Oncorhynchus mykiss*, and their hybrids

Clint C. Muhlfeld, Thomas E. McMahon, Durae Belcer, and Jeffrey L. Kershner

Abstract: We used radiotelemetry to assess spatial and temporal spawning distributions of native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WCT), introduced rainbow trout (*Oncorhynchus mykiss*; RBT), and their hybrids in the upper Flathead River system, Montana (USA) and British Columbia (Canada), from 2000 to 2007. Radio-tagged trout (N = 125) moved upriver towards spawning sites as flows increased during spring runoff and spawned in 29 tributaries. WCT migrated greater distances and spawned in headwater streams during peak flows and as flows declined, whereas RBT and RBT hybrids (backcrosses to RBT) spawned earlier during increasing flows and lower in the system. WCT hybrids (backcrosses to WCT) spawned intermediately in time and space to WCT and RBT and RBT hybrids. Both hybrid groups and RBT, however, spawned over time periods that produced temporal overlap with spawning WCT in most years. Our data indicate that hybridization is spreading via long-distance movements of individuals with high amounts of RBT admixture into WCT streams and stepping-stone invasion at small scales by later generation backcrosses. This study provides evidence that hybridization increases the likelihood of reproductive overlap in time and space, promoting extinction by introgression, and that the spread of hybridization is likely to continue if hybrid source populations are not reduced or eliminated.

Résumé : La radiotélémétrie nous a servi à évaluer la répartition spatiale et temporelle de la fraie chez la truite fardée indigène du versant occidental (*Oncorhynchus clarkii lewisi*; WCT), la truite arc-en-ciel (*Oncorhynchus mykiss*; RBT) introduite et leurs hybrides dans le réseau hydrographique supérieur de la Flathead au Montana (É.-U.) et en Colombie-Britannique (Canada) de 2000 à 2007. Les truites porteuses d'une étiquette radio (N = 125) se sont déplacées vers l'amont vers les sites de fraie durant la crue printanière et ont frayé dans 29 tributaires. Les WCT ont migré sur de plus grandes distances et ont frayé dans les cours d'eau les plus en amont durant les débits maxima et durant la réduction des débits, alors que les RBT et les hybrides RBT (rétrocroisés avec RBT) ont frayé plus tôt durant l'augmentation des débits et plus en aval dans le bassin. Les hybrides WCT (rétrocroisés avec WCT) ont eu une fraie intermédiaire dans le temps et l'espace à celles des WCT, des RBT et leurs hybrides RBT. Cependant, les deux groupes d'hybrides et RBT ont frayé durant des périodes qui chevauchent la fraie des WCT la plupart des années. Nos données indiquent que l'hybridation se propage grâce aux déplacements sur de longues distances vers les cours d'eau à WCT d'individus qui possèdent un fort complément de RBT et par une invasion par degrés par les générations subséquentes rétrocroisées. Notre étude fournit des preuves que l'hybridation augmente la probabilité de chevauchement reproductif dans le temps et l'espace, ce qui favorise l'extinction par introgression; la propagation de l'hybridation va vraisemblablement continuer à moins que la source de populations d'hybrides ne soit réduite ou éliminée.

[Traduit par la Rédaction]

Introduction

Invasive species threaten the biodiversity of aquatic ecosystems worldwide (Mack et al. 2000) and are considered the second greatest threat to biodiversity loss in North America (Mooney and Cleland 2001). Hybridization (interbreeding of individuals from genetically distinct populations) between introduced and native taxa can be a major consequence of species introductions, especially when nonnative species hybridize with rare or endangered species, threatening their persistence (Rhymer and Simberloff 1996).

Received 19 November 2008. Accepted 8 May 2009. Published on the NRC Research Press Web site at cjfas.nrc.ca on 30 June 2009. J20886

Paper handled by Associate Editor Eric Taylor.

C.C. Muhlfeld.[1] US Geological Survey, Northern Rocky Mountain Science Center, Glacier Field Office, Glacier National Park, West Glacier, MT 59936, USA.
T.E. McMahon. Department of Ecology, Fish and Wildlife Program, Montana State University, Bozeman, MT 59717, USA.
D. Belcer. Montana Fish, Wildlife and Parks, 490 North Meridian Road, Kalispell, MT 59901, USA.
J.L. Kershner. US Geological Survey, Northern Rocky Mountain Science Center, P.O. Box 173492, Bozeman, MT 59717-3492, USA.

[1] Corresponding author (e-mail: cmuhlfeld@usgs.gov).

doi:10.1139/F09-073

Hybridization with introduced species has been a major factor in the decline and extinction of many native fishes throughout North America (Miller et al. 1989). Although natural hybridization can lead to evolutionary novelty and speciation in many plants and some vertebrate species (Arnold 1997; Barton 2001), human-mediated hybridization and introgression (gene flow between populations with individuals that hybridize) can lead to the extinction of native genotypes and the loss of locally adapted gene complexes and ecological adaptations (Rhymer and Simberloff 1996; Allendorf et al. 2001). Therefore, understanding the invasion patterns and mechanisms that permit and constrain hybridization is required for conservation of aquatic biodiversity and developing recovery and management programs for native species at risk of hybridization with introduced taxa.

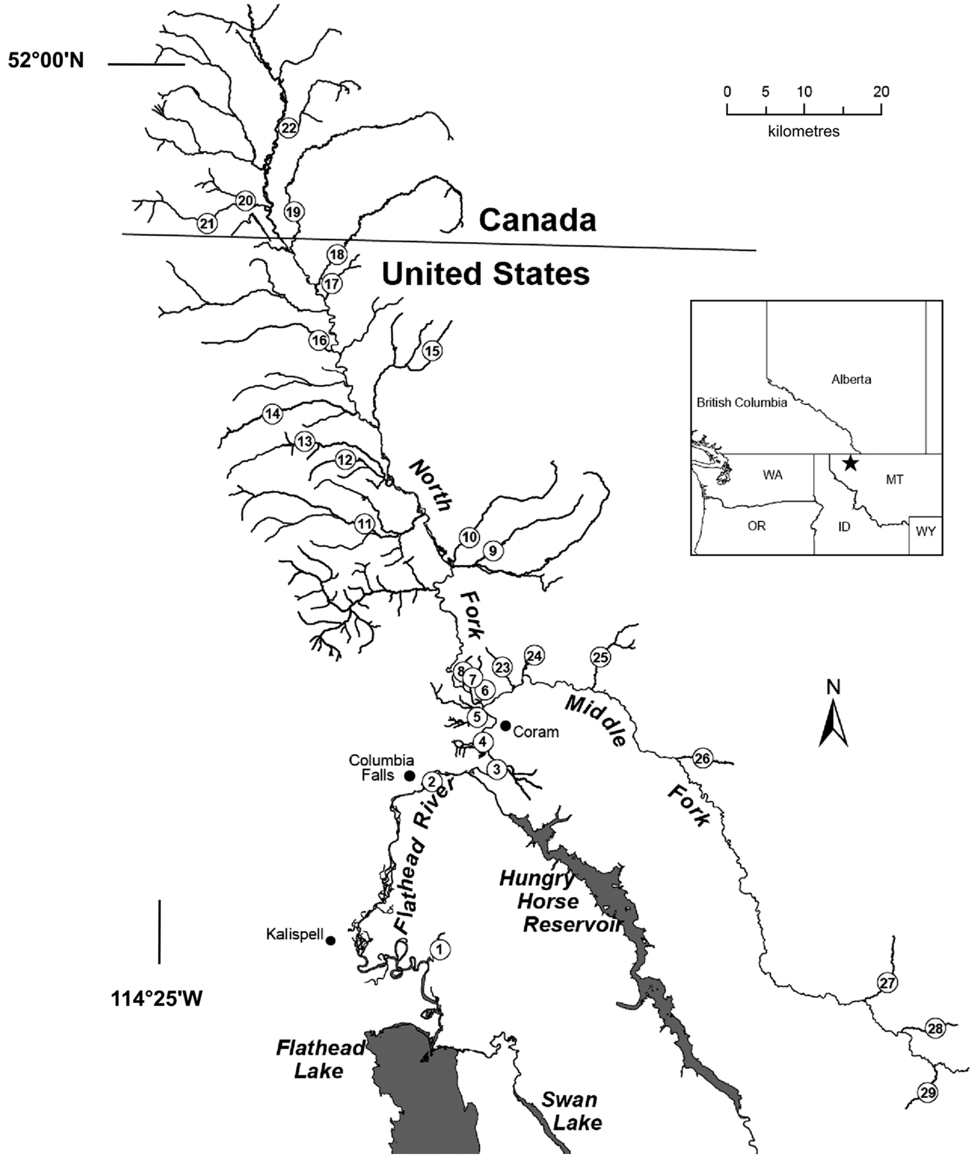
Hybridization is more common in fish than in any other vertebrate taxa (Leary et al. 1995; Allendorf et al. 2001). Many fishes have external fertilization and similar mating behaviors, facilitating interbreeding. In undisturbed ecosystems, reproductive isolation is maintained primarily by spatial and temporal reproductive isolation. However, such barriers to interbreeding are lost in the face of habitat disturbance and introduction of nonnative species (Allendorf et al. 2001). This is particularly true for salmonids in which widespread introgression among native and nonnative taxa has replaced native species over large areas throughout their native ranges, which is suspected to lead to a loss of behavioral and ecological adaptations in native populations (Allendorf and Leary 1988; Rhymer and Simberloff 1996). Reproductive behavior is intimately linked to salmonid life histories, which represent long-term adaptations to the natural environment. Thus, reproductive behavior is considered to be a critical trait influencing the persistence of native salmonid populations. The consequences of human-mediated hybridization on salmonid reproductive behavior, however, are poorly understood.

Introgressive hybridization with introduced rainbow trout (*Oncorhynchus mykiss*; RBT) is considered one of the greatest threats facing many native salmonid populations worldwide (Behnke 1992; Leary et al. 1995). Widespread transplanting of RBT outside their native range has caused local extirpations or reductions of native salmonids in nearly all drainages in which they have been introduced, and the rate of introductions has dramatically increased over the past 50 years due to intentional and unintentional releases (Fuller et al. 1999). Along with habitat loss and degradation, introgressive hybridization with RBT has played a major role in the demise of all subspecies of cutthroat trout (*Oncorhynchus clarkii*; Allendorf and Leary 1988; Gresswell 1988; Young 1995). Hybridization and introgression with introduced RBT threatens the westslope cutthroat trout (*Oncorhynchus clarkii lewisi*; WCT) with genomic extinction, the loss of genotypes across the entire genome that are permanently lost by hybridization (Allendorf and Leary 1988; Allendorf et al. 2004). Westslope cutthroat trout are highly divergent from the other major cutthroat trout subspecies (coastal (*Oncorhynchus clarkii clarki*), Yellowstone (*Oncorhynchus clarkii bouvieri*), and Lahontan (*Oncorhynchus clarkii henshawi*) cutthroat trout; Loudenslager and Gall 1980; Leary et al. 1987; Allendorf and Leary 1988), and their geographic range is the largest of the 13 interior subspecies, encompassing the Columbia, Fraser, Missouri, and Hudson Bay drainages of the United States and Canada (Behnke 1992). Westslope cutthroat trout historically occupied a wide variety of aquatic habitats, from small headwater streams to large rivers and lakes. However, native populations have dramatically declined due to habitat degradation, fragmentation, overexploitation, and hybridization and competition with nonnative trout. Consequently, nonhybridized populations of WCT currently inhabit less than 10% of their historic range in the United States (Shepard et al. 2005) and less than 20% of their historic range in Canada (Committee on the Status of Endangered Wildlife in Canada (COSEWIC) 2006). Many of the remaining nonhybridized populations are restricted to headwater areas above upstream dispersal barriers (Shepard et al. 2005). Consequently, hybrid forms are the dominant taxa in most drainages where WCT were native.

The timing and location of breeding are the primary isolating mechanisms between naturally sympatric salmonid species. However, the rapid increase of human-mediated hybridization in many salmonid species suggests that little reproductive segregation exists between naturally allopatric taxa. Although WCT co-evolved with resident and anadromous forms of RBT in portions of the Columbia River basin (Behnke 1992), either spatial or temporal reproductive segregation has limited natural hybridization between these fishes (Brown et al. 2004; but for an exception, see Bettles et al. 2005). In contrast, many studies have shown that introductions of nonnative RBT commonly result in widespread introgression and loss of native genotypes in WCT populations (Allendorf et al. 2001; Rubidge and Taylor 2004; Ostberg and Rodriguez 2006) and other subspecies of cutthroat trout (Gresswell 1988; Young 1995). The apparent lack of reproductive isolation between the introduced and native trout is assumed to be the result of their failure to evolve isolating mechanisms in allopatry. However, few studies have tested this assumption in the natural environment.

Although the upper Flathead River system in Montana and British Columbia (Fig. 1) is considered a range-wide stronghold for WCT, the long-term persistence of nonhybridized populations in the drainage is threatened by the recent spread of nonnative RBT introgression (Hitt et al. 2003; Boyer et al. 2008; Muhlfeld 2008). The drainage presents a unique opportunity to examine spawning dynamics among hybridized and nonhybridized taxa as a full range of parentals and introgressed individuals are present in the system. Understanding the factors affecting reproductive isolation between parental and hybrids in a recently invaded system will aid in the identification of the primary reproductive mechanisms in the spread of hybridization and may identify the major sources of nonnative admixture (proportion of nonnative alleles in individuals or populations) in the system. To examine differences in reproductive behavior among RBT, WCT, and their hybrids, we used radiotelemetry in the upper Flathead River system from 2000 to 2007. We hypothesized that hybrids and RBT would spawn earlier and lower in the system compared with nonhybridized WCT, but the spawning distributions would vary spatially and temporally. Additionally, we hypothesized that hybridization is spreading by long-distance movements of individuals with high amounts of nonnative RBT admixture into nonhybridized

Fig. 1. Study area location and sample site identification in the upper Flathead River system in Montana (MT, USA) and British Columbia (Canada). Sample site codes correspond to Table 1.



WCT populations. Our objectives were (*i*) to identify RBT and hybrid source populations, (*ii*) to compare the timing, location, and movements of spawning by RBT, WCT, and their hybrids, and (*iii*) to describe the patterns of spatial and temporal overlap among parental taxa and hybrid types.

Materials and methods

Study area and populations

The upper Flathead River drainage originates in the Rocky Mountains of British Columbia, Canada, and northwestern Montana, USA, and includes Flathead Lake and the North Fork, Middle Fork, South Fork, and mainstem Flathead rivers (Fig. 1). The drainage area is approximately 18 400 km^2 and is in the headwaters of the upper Columbia River basin. We implanted prespawning fish with radio transmitters from the confluence of the Middle and North Fork rivers downstream to the mainstem near Kalispell (Fig. 1). Hydropower and flood-control operations from Hungry Horse Dam in the South Fork partially regulate the lower 69 km of the mainstem (Fig. 1), resulting in the storage of spring runoff and flow augmentation during the summer, fall, and winter months. This reach supports the highest abundances of WCT × RBT fluvial hybrids in the river and lake system (Muhlfeld et al. 2003).

WCT exhibit both resident (remaining in natal streams throughout life) and migratory life history strategies in the Flathead Lake and River system, but migratory forms are most common (Shepard et al. 1984; Liknes and Graham 1988; Muhlfeld et al. 2000). Migratory WCT rear in their natal stream for 1–4 years and then migrate downstream as subadults to Flathead Lake (adfluvial) or the mainstem rivers (fluvial). Adult WCT generally overwinter in the lower river or the lake and then migrate upstream during high spring flows and spawn in tributaries. In contrast, RBT and WCT × RBT hybrids display a fluvial life history in the mainstem (Muhlfeld et al. 2003), but little was known about their spawning behaviors prior to this study.

Table 1. The number of radio-tagged fish that spawned in each stream in the upper Flathead River system from 2000 to 2007.

		No. of radio-tagged spawners			
Code	Site name	WCT	WCT hybrid	RBT hybrid	RBT
1	Mill				1
2	Taylors		1	7	4
3	Abbot			18	28
4	Ivy			1	3
5	Rabe			4	8
6	First				1
7	Steamer			1	
8	Third		1(F_1)	2	2
9	Dutch		2	2	1
10	Anaconda		3	1	
11	Coal (NF)	1	1(F_1)		
12	Moran	1			
13	Hay	1			1
14	Red Meadow	2			
15	Akokala	2	1		
16	Tepee	3			
17	Starvation	1			
18	Kishenehn	1			
19	Sage	4			
20	Burnham	1			
21	Cauldrey	1			
22	Commerce	3			
23	Rubideau		1		
24	McDonald				1
25	Lincoln	1	1		1
26	Coal (MF)	1			
27	Granite	1			
28	Lodgepole	1			
29	Schafer	2			

Note: Sites are coded in approximate order of ascending upstream distance. Acronyms: WCT, westslope cutthroat trout; RBT, rainbow trout; WCT hybrid, WCT × RBT hybrids backcrossed to WCT; RBT hybrid, backcrosses to RBT; F_1, first-generation hybrid; NF, North Fork stream; MF, Middle Fork stream.

Timing and location of spawning

We used radiotelemetry to monitor the movements and spawning behavior of WCT, RBT, and WCT × RBT hybrids from 2000 to 2007 in the upper Flathead River system, Montana (USA) and British Columbia (Canada). In 2000, 2001, 2006, and 2007, we tagged fish that visually appeared to be WCT, RBT, and hybrid trout, whereas in 2002, 2003, and 2005, we exclusively targeted hybrids and RBT. Morphological characters used for identification were spotting pattern, body coloration, and the presence or absence of red or orange slashes below the gill covers (Behnke 1992). Prespawning fish were randomly captured in the mainstem Flathead River using jet-boat electrofishing at night during February and early March. Captured fish that met the 2% body to transmitter – body weight ratio (Winter 1983) were anaesthetized with tricaine methane sulfonate (MS-222), surgically implanted with transmitters according to the methods described by Muhlfeld et al. (2003), held for 1–5 h after tagging, and released near their capture location. We selected individuals greater than 300 mm total length in which maturing gonads could be visually identified with an otoscope through the surgical incision. Transmitters emitted a unique code in the 148.780 MHz frequency range (with 5 s burst rates), had expected battery lives of 110 days (2000–2005) and 472 days (2006–2007), and weighed 6.7 g (air weight) and 10 g, respectively. A fin clip was collected from each fish during surgery, preserved in 95% ethanol, and analyzed at the Conservation Genetics Laboratory at the University of Montana, Missoula, to estimate degree of admixture.

Radio-tagged fish were relocated three to five times per week during the spring spawning period from a jet boat, from vehicle access points along the stream, and by foot using a Lotek scanning receiver (model SRX-400, Lotek Wireless Inc., Newmarket, Ontario) equipped with an ATS three-element Yagi antenna (Advanced Telemetry Systems Inc., Isanti, Min.). Aerial surveys were conducted to locate fish that were missing for more than 1 week and to survey remote and inaccessible areas throughout the upper portions of the river system, including British Columbia (Canada), Waterton–Glacier International Peace Park, and the Bob Marshall Scapegoat Wilderness complexes (Fig. 1). When logistically possible, observers walked spawning streams to gain a more accurate location and to document redd construction. Additionally, four permanent telemetry ground stations were installed near the mouths of the North Fork, Middle Fork, South Fork, and mainstem Flathead rivers, which continuously monitored fish movements within 250 m of the antenna. Each ground station consisted of a Lotek data-logging receiver equipped with an ATS three-element directional Yagi antenna powered by a 12V deep-cycle marine battery. Georeferenced locations were obtained at each fish location using a global positioning system (GPS) unit (TSC1 Asset Surveyor, Trimble Navigation Limited, Sunnyvale, California). In a geographic information system (GIS), the point locations were overlaid on a hydrography layer for analysis of distance moved, which were calculated between consecutive locations.

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

Spawning and movement behavior was characterized by measuring eight variables including beginning migration date, beginning migration temperature (°C), beginning migration flow ($m^3 \cdot s^{-1}$), spawning date (day of year), spawning temperature, spawning flow, migration period (days), and migration distance (km). Migration was defined as an upstream or downstream movement greater than 5 km from the fish's tagging location. The beginning date of migration for each individual fish was considered to be the median date between the day when a fish migrated and the previous location. Spawning location was defined as the farthest extent of a fish's migration. Migration distance was defined as the fluvial distance between the location from which fish began their migration and the spawning location (Henderson et al. 2000). Migration period was defined as the time (days) from the beginning date of migration until the first relocation of the fish at or near its observed or likely spawning location. Spawning date was calculated as the midway point between migration period ending date and when it left its spawning location. Spawning period for each study group (defined below) extended from the date that the first fish spawned until the last fish spawned in a particular group. Mean daily water temperature and discharge data were obtained from the US Geological Survey station on the mainstem Flathead River near Columbia Falls, Montana.

Genetic analyses

We used paired interspersed nuclear elements polymerase chain reaction (PINE PCR) techniques to assess the hybridization status of each fish tagged from 2000 through 2003 (Appendix A, Table A1; Spruell et al. 2001; Kanda et al. 2002). We used combinations of primer pairs to evaluate specific regions (“fragments”) of anonymous DNA for each taxon. Six dominant nDNA fragments were examined for RBT and seven for WCT (Spruell et al. 2001). For fish tagged from 2005 to 2007, we took advantage of a new nDNA technique that increased the ability to detect nonnative alleles in individual fish by using a combination of seven diagnostic co-dominant indel loci (*Occ34*, *Occ35*, *Occ36*, *Occ37*, *Occ38*, *Occ42*, *Om55* (Ostberg and Rodriguez 2004)) and six diagnostic co-dominant microsatellite loci (*Ssa408* (Cairney et al. 2000); *Oki10* (Smith et al. 1998); *Omm1037-1*, *Omm1037-2*, *Omm1050* (Rexroad et al. 2002); *Omy0004* (Holm and Brusgaard 1999); *Omy1001* (Spies et al. 2005)). For the PINES and indel–microsatellite data, we calculated a hybrid index (HI) for each fish (Appendix A, Fig. A1). Statistical power to differentiate between parental types and early generation hybrids is high, with seven diagnostic co-dominant markers. For example, the probability that a first-generation backcross would be misclassified as a first-generation (F_1) hybrid is less than 0.01 (Boecklen and Howard 1997). However, reliable discernment between parental types and later generation backcrosses requires many diagnostic loci (Floate et al. 1994). The hybrid index, therefore, likely overestimates parental types and underestimates the number of individuals of hybrid ancestry in populations containing later generation hybrids.

Fish were classified into four groups based on their HI (Appendix A, Fig. A1). Individuals were classified as nonhybridized WCT if they had a HI of zero using either PINEs or indel–microsatellites. Individuals were classified as nonhybridized RBT if they had a HI of 13 (PINEs) or 26 (indel–microsatellites). F_1 hybrids were those fish with a HI of 6 using PINEs (RBT phenotypes were detected at only the six loci where the presence of the fragment is characteristic of RBT) and 13 using indel–microsatellites, but were heterozygous at all loci. Post-F_1 hybrids with higher proportions of WCT than RBT alleles were classified as backcrosses to WCT (WCT hybrids) and those with a higher proportion of RBT than WCT alleles were classified as backcrosses to RBT (RBT hybrids). The two F_1s monitored during the study were grouped with the WCT hybrids because their movement and spawning behavior were similar and to increase the samples size in that category. Although fish classified as RBT have diagnostic RBT alleles at all loci, recent genetic studies in the system indicate that these individuals may have originated from a private RBT hatchery (see Discussion) or from a stream (Abbot Creek) containing a hybrid swarm with 92% RBT admixture. Therefore, some of the fish classified as nonhybridized RBT may have contained a few WCT alleles. Finally, the sample size of fish with low levels of RBT admixture was lower than the samples sizes of WCT and fish with high amounts of RBT admixture. This was due, in part, to our field identification and preferential selection of fish with morphological characteristics of parental types and high proportions of RBT admixture.

Confirmation of morphological identification

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

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

Statistical analyses

Because some of the eight dependent spawning and movement variables were significantly correlated (Appendix A, Table A2), multivariate analyses were used to simultaneously test for group differences on all the response variables, all of which met the assumptions of normality and homogeneity of variance (Zar 1996). First, we used a multivariate general linear model (GLM multivariate) to test whether there were significant effects due to genetic status (group), year, and sex, which were entered as fixed effects with interaction terms. Next, we used multivariate analysis of variance (MANOVA) to assess differences among groups for the eight spawning variables. Individual analyses of variance (ANOVAs) were used to test for differences in each spawning variable among groups, and post-hoc pairwise comparisons were conducted using Tukey’s tests. For all analyses, statistical significance was determined at an alpha level of 0.05. Statistical analyses were conducted using SPSS, version 10.1.4 (Norusis 1990).

Table 2. Summary statistics for radio-tagged fish monitored in the upper Flathead River drainage from 2000 through 2007.

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

Note: For mean length and mean weight, the number in parentheses is the standard deviation. Acronyms: WCT, westslope cutthroat trout; RBT, rainbow trout; WCT hybrid, WCT × RBT hybrids backcrossed to WCT; RBT hybrid, backcrosses to RBT; SD, standard deviation.

Results

We obtained spawning information on 27 WCT, 11 WCT hybrids, 36 RBT hybrids, and 51 RBT from 2000 through 2007 (Tables 1–2). Spawning individuals were found an average of 26 times (standard deviation, SD = 9) during spring (March–June). Radio-tagged fish ranged in total length from 317 to 545 mm (means: WCT = 417 mm, WCT hybrids = 445 mm, RBT hybrids = 409 mm, and RBT = 404 mm) and in weight from 254 to 1527 g (means: WCT = 766 g, WCT hybrids = 805 g, RBT hybrids = 674 g, and RBT = 629 g).

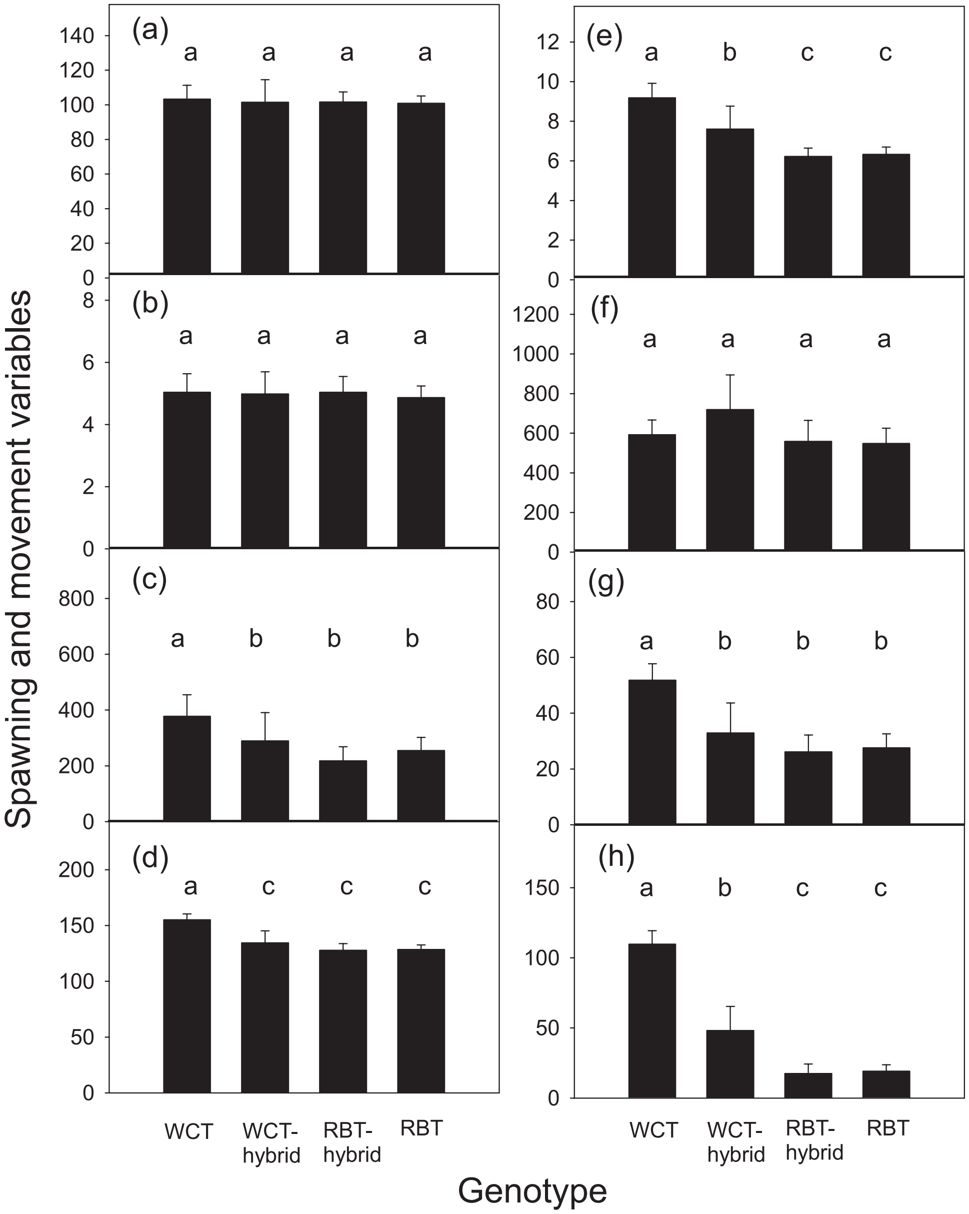
The majority of radio-tagged fish made upriver migrations (98%) and spawned in a total of 29 tributaries to the mainstem, North Fork, and Middle Fork Flathead rivers (Table 1). Only two fish moved downriver to access spawning tributaries to the mainstem. There was no evidence of radio-tagged fish using mainstem or side-channel areas for spawning.

Behavioral differences in spawning and migration

We simultaneously assessed the fixed effects of group, year, and sex on the eight spawning variables using multivariate GLM (Fig. 2). There were significant differences in the spawning and movement variables among groups (MANOVA, Wilks' $\lambda = 0.207$, $F_{[21,213]} = 7.425$, $P < 0.0001$) and years (MANOVA, Wilks' $\lambda = 0.152$, $F_{[42,350]} = 4.119$, $P < 0.0001$), but not between male and female fish (MANOVA, Wilks' $\lambda = 0.837$, $F_{[7,74]} = 2.063$, $P = 0.058$). WCT spawned at higher flows in 2006 and 2007 as compared with 2000 and 2001 (ANOVA, $P < 0.001$), but none of the other seven spawning migration variables was significantly different across years (ANOVA, $P > 0.05$). RBT and RBT hybrids spawned significantly later (ANOVA, $P < 0.001$) and at higher flows (ANOVA, $P < 0.001$) in 2002, likely due to a protracted and higher spring runoff. Additionally, there were no significant interactions among the fixed effects (group, year, and sex) in the overall model. Therefore, we combined the data for all years and genders for subsequent analyses.

MANOVA found overall differences in spawning and migration behaviors among the study groups (Wilks' $\lambda = 0.164$, $F_{[21,330]} = 13.804$, $P < 0.0001$; Table 2; Fig. 2) for five of the eight spawning and migration variables: the flow

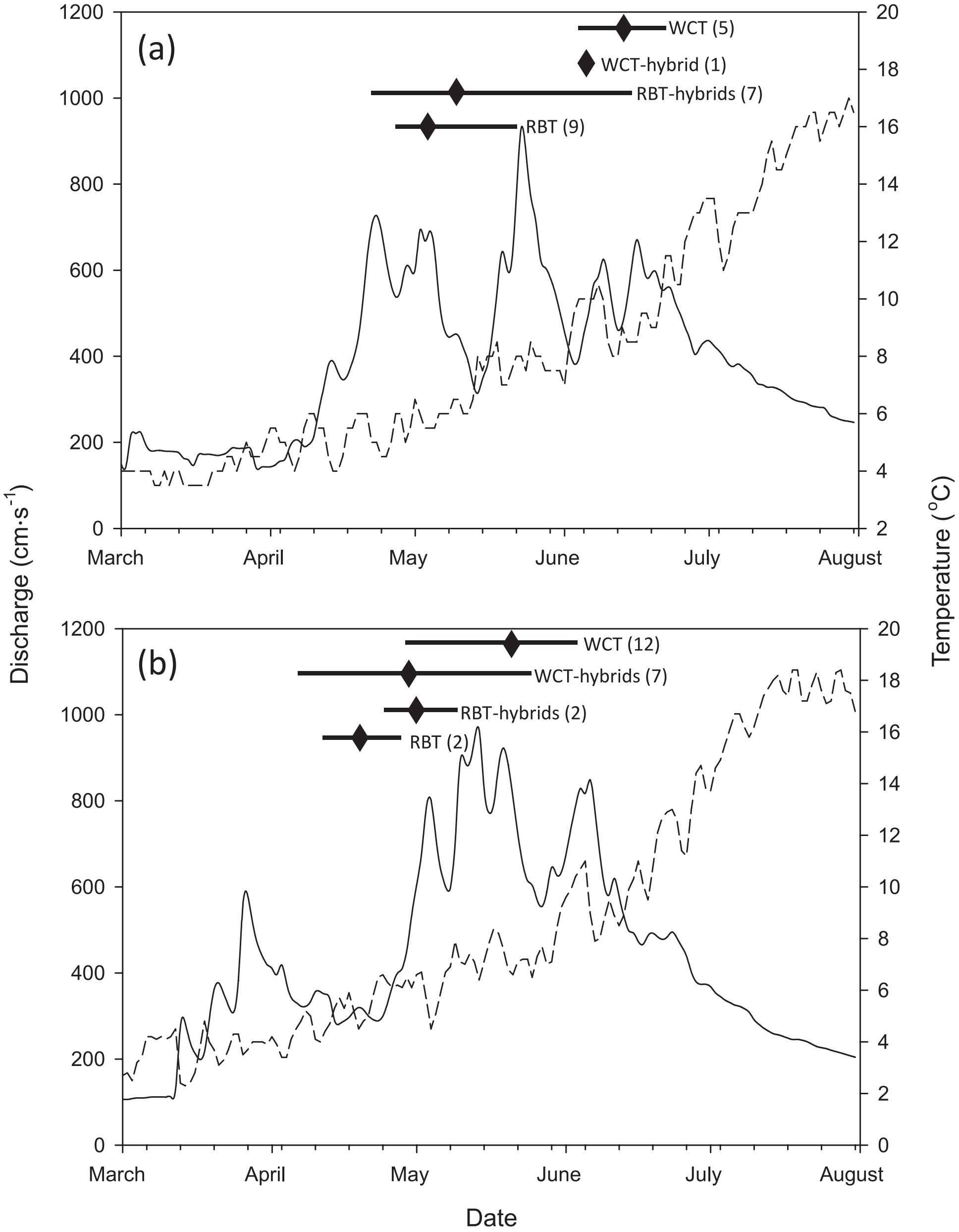
Fig. 2. Comparisons of movement and spawning characteristics (±95% confidence interval, CI) for eight variables: (*a*) beginning migration date (day of year), (*b*) beginning migration temperature (°C), (*c*) beginning migration flow ($m^3 \cdot s^{-1}$), (*d*) spawning date (day of year), (*e*) spawning temperature (°C), (*f*) spawning flow ($m^3 \cdot s^{-1}$), (*g*) migration period (days), and (*h*) migration distance (km). For each measurement, means that do not share letters are significantly different (Tukey's test, $P < 0.05$). Acronyms: WCT, westslope cutthroat trout; RBT, rainbow trout; WCT hybrid, WCT × RBT hybrid trout backcrossed to WCT; RBT hybrid, WCT × RBT hybrid trout backcrossed to RBT.



at which they began migrating (ANOVA, $F_{[3,121]} = 5.016$, $P = 0.003$), migration period (ANOVA, $F_{[3,121]} = 14.738$, $P < 0.0001$), migration distance (ANOVA, $F_{[3,121]} = 140.329$, $P < 0.0001$), spawning date (ANOVA, $F_{[3,121]} = 21.106$, $P < 0.0001$), and spawning temperature (ANOVA, $F_{[3,121]} = 27.338$, $P < 0.0001$). Tukey's post-hoc comparisons revealed that WCT differed from RBT and RBT hybrids in the flow at which they began migrating ($P < 0.01$), migration period ($P < 0.0001$), migration distance ($P < 0.0001$), spawning date ($P < 0.0001$), and spawning temperature ($P < 0.0001$). Conversely, no differences were found between RBT and RBT hybrids for any of these five variables ($P > 0.05$). Furthermore, there were significant differences between WCT and WCT hybrids for spawning date ($P = 0.001$), migration period ($P = 0.012$), spawning temperature ($P = 0.017$), and migration distance ($P < 0.001$). However, WCT hybrids only differed between RBT and RBT hybrids for migration distance ($P < 0.001$) and RBT hybrids for spawning temperature ($P = 0.036$).

WCT spawned during peak flows and as flows declined following peak spring runoff (May and June) and a mean water temperature of 9 °C (Fig. 3). In contrast, RBT and RBT hybrids spawned during increasing and peak spring flows (April and May) and as temperatures rose to about 6 °C. WCT hybrids spawned throughout spring runoff (April and May) and at intermediate temperatures (mean = 7.6 °C). All groups of fish began migrations on the rising limb of the hydrograph at similar flows and temperatures (Figs. 2*a*–2*c*).

Fig. 3. The spawning periods and mean spawning dates (diamonds) for each taxa as related to the hydrograph and thermograph at Columbia Falls in the mainstem Flathead River in (*a*) 2001 and (*b*) 2007. Acronyms: WCT, westslope cutthroat trout; RBT, rainbow trout; WCT hybrids, WCT × RBT hybrid trout backcrossed to WCT; RBT hybrids, WCT × RBT hybrid trout backcrossed to RBT. Solid lines represent flow and dotted lines represent temperature. Sample sizes (number of fish) are in parentheses.

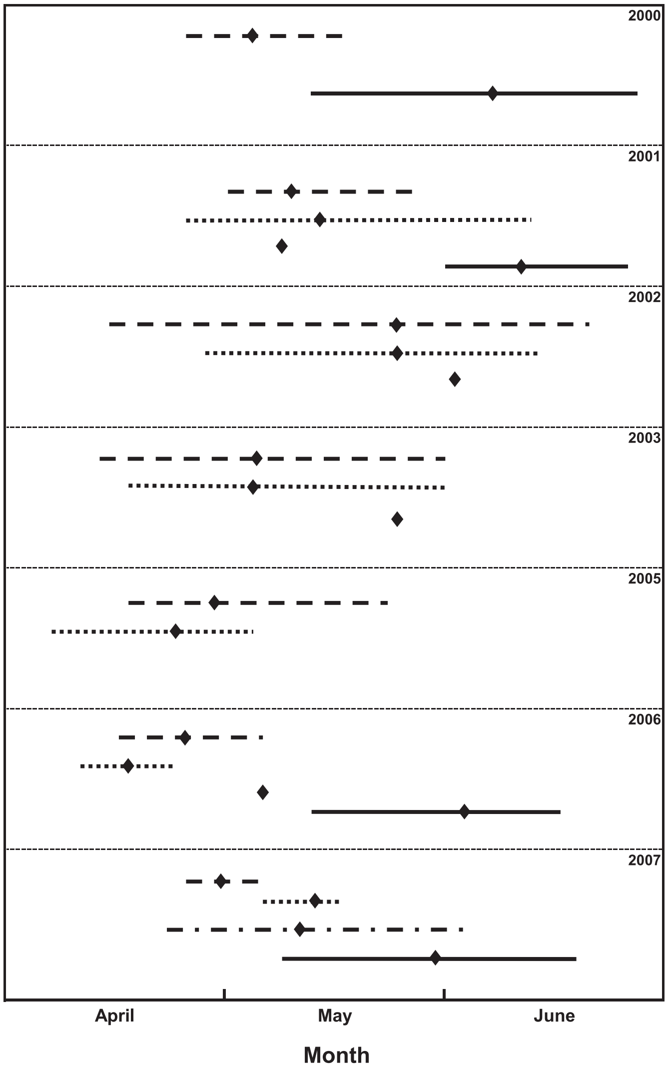


Mean spawning date for WCT (3 June) was significantly later that those of WCT hybrids (13 May), RBT hybrids (5 May), and RBT (7 May) (Figs. 2*d* and 3). Additionally, WCT spawned at significantly warmer temperatures (means: WCT = 9.2 °C, WCT hybrids = 7.6 °C, RBT hybrids = 6.2 °C, and RBT = 6.3 °C; Fig. 2*e*). Migration period for WCT was about twice (mean = 52 days) as long as for RBT and hybrids (means: WCT hybrids = 33 days, RBT hybrids = 26 days, and RBT = 28 days; Fig. 2*g*), likely because WCT migrated significantly farther upriver (mean = 110 km) to access spawning tributaries (Fig. 2*h*) as compared with WCT hybrids (48 km), RBT hybrids (18 km), and RBT (19 km).

Spatial and temporal overlap in spawning

Despite the differences in spawning behavior among the groups of fish, some temporal and spatial overlap occurred (Figs. 3–4). The long spawning periods produced temporal overlap between WCT and hybrid trout in 2001 and 2007 and between WCT and RBT in 2000 (Fig. 4). No temporal overlap was found between WCT and the other groups in 2006, possibly due to small samples sizes that limit the ability to draw inferences that year (*N* = 9 fish); the observed spawning periods increased with the more fish we tagged. For all years combined, RBT had the longest spawning period (11 March – 20 June) relative to the other groups: RBT hybrids (4 April – 14 June), WCT hybrids (19 April – 3 June), and WCT (9 May – 25 June) (Fig. 4).

Fig. 4. Spawning periods and mean spawning dates (diamonds) of 125 radio-tagged trout during 2000–2003 and 2005–2007: 27 westslope cutthroat trout (solid lines), 11 WCT × RBT hybrids backcrossed to WCT (dashed–dotted lines), 36 WCT × RBT hybrids backcrossed to RBT (dotted lines), and 51 rainbow trout (broken lines). Sample sizes are provided in Table 2. The diamonds with no lines represent the spawning date of individual fish.



Distribution of spawning locations varied geographically among the four groups of fish. WCT spawned in headwater tributaries, RBT and RBT hybrids spawned in the lower drainage, and WCT hybrids generally spawned in the middle portion of the drainage, overlapping with WCT spawning locations (Table 1; Fig. 1). Furthermore, we found evidence that hybridization is spreading upstream via long upstream movements of a few individuals with a high proportion of RBT admixture. Two RBT males made long-distance migrations and spawned in tributaries used by radio-tagged WCT during the study. One RBT migrated 73 km upriver and spawned in the lower section of Hay Creek in 2002, the same general location that a WCT used for spawning in 2000. Similarly, in 2002, a RBT made an upriver movement of 67 km and spawned in Lincoln Creek, the same stream that was used by a WCT and a WCT hybrid for spawning in 2007. Also during the study, we tracked two F_1 hybrids representing previous matings of parental taxa. One F_1 hybrid moved 67 km upriver and spawned in Coal Creek (North Fork) in 2003, which is the same stream that a WCT used for spawning in 2006, and the remaining F_1 hybrid spawned in a tributary near the confluence of the North Fork and mainstem Flathead rivers in 2002, along with several other RBT and RBT hybrids during the study. Finally, individuals with a high proportion of RBT admixture made long-distance movements to spawning areas that WCT hybrids used for spawning; four fish migrated a mean distance of 55 km and spawned in Dutch and Anaconda creeks.

However, the majority of RBT and RBT hybrids spawned in tributaries in the lower portions of the drainage (Table 1; Fig. 1). These streams included (in order of use) Abbot, Rabe, Taylors, Ivy, Third, Dutch, Anaconda, and Mill creeks. Abbot Creek supported a high proportion of spawning by RBT and RBT hybrids (52%), indicating that this location is the main source of introgression in the study area (Table 1). The spawning behaviors of several radio-tagged RBT and RBT hybrids suggest that a former private RBT hatchery (Sekokini Springs) that ceased operations in 1997 (see Discussion), located about 2 km downstream of the confluence of the Middle and North Forks near Abbot Creek, is likely the original source of the recent proliferation of hybridization in the upper Flathead River system. Three RBT and two RBT hybrids made pronounced upstream migrations to the upper mainstem Flathead River and then displayed short upriver and downriver movements in close proximity to the hatchery site, suggesting that they originated from the hatchery, before apparently spawning in Rabe Creek. Moreover, the majority of RBT and RBT hybrids spawned in streams in close proximity (±5 km) to the hatchery during the study.

Discussion

Conservation of native biota requires understanding the invasion patterns and the mechanisms that promote extinction by hybridization. In North America, many populations of native cutthroat trout are threatened by introgressive hybridization with nonnative salmonids, yet prior to our study, little was known about the reproductive factors influencing the spread of hybridization in the natural environment. Our data illustrate that contact between previously allopatric species results in hybridization and introgression due to incompletely evolved reproductive isolation mechanisms. The timing and location of spawning varied spatially and temporally producing zones of contact (e.g., hybrids zones; Barton and Hewitt 1985) between parental species, which results in loss of integrity of the native cutthroat trout gene pool. The spawning distributions and movement patterns indicate that hybridization is being promulgated upstream via long-distance movements of individuals with high amounts of RBT admixture (the continent–island invasion model; Arnold 1997). Additionally, the spatial distribution of later generation backcrosses suggests that hybridization is also spreading by dispersal between neighboring populations (the stepping-stone invasion model; Kimura and Weiss 1964). Combined, these data suggest that hybrid source populations are contributing to the spread of hybridization in the system and provide evidence that hybridization increases the likelihood of reproductive overlap in time and space, promoting extinction by introgression (Rhymer and Simberloff 1996).

Spatial and temporal distribution of spawning

Based on the spatial distribution of spawning, we found that most RBT and RBT hybrids spawned in tributaries in lower elevations, nonhybridized WCT spawned in the headwater reaches, and WCT hybrids were distributed intermediately in the drainage. However, the spawning distributions also revealed that WCT experience spatial and temporal overlap in spawning with RBT and individuals with various RBT ancestries, mostly in the lower and middle portions of the drainage. These results are consistent with many genetic studies that have been conducted in situations in which RBT are introduced into waters containing native cutthroat trout (Gunnell et al. 2008; Metcalf et al. 2008) and WCT (Weigel et al. 2003; Rubidge and Taylor 2004; Ostberg and Rodriguez 2006) populations. Our telemetry results also corroborate recent genetic studies in the system that found gametic disequilibrium at some of the spawning sites and a decrease in the proportion of RBT admixture with increasing distance from lower elevation tributaries (Hitt et al. 2003; Boyer et al. 2008). Similarly, Henderson et al. (2000) found that the majority of spawning Yellowstone cutthroat trout demonstrated spatial and temporal overlap with RBT and hybrids in lower portions of the South Fork Snake River in Idaho.

The observed spatial distribution of hybrids and parental species appears attributable to upriver invasion of individuals with high amounts of RBT admixture into nonhybridized WCT populations. The purported illegal release of an estimated 70 000 RBT individuals in 1997 from a private hatchery in the lower portion of the drainage likely played a significant role in the recent proliferation of introgression in the system. The vast majority of radio-tagged RBT and RBT hybrids spawned in a relatively few streams located near the probable release point, and some of these individuals appeared to be returning to the hatchery to spawn in the early spring. The observed gradient of hybridization and spawning distributions, however, may not simply be the result of the initial release location, as RBT typically use low-elevation areas of systems in which they are introduced (Fausch et al. 2001), possibly because RBT have higher thermal tolerances (Bear et al. 2007), greater metabolic and growth requirements (Robinson 2007), better swimming abilities (Seiler and Keeley 2007), and high variation in habitat use (Raleigh et al. 1984). For instance, in the east slopes of the Rocky Mountains where RBT have been stocked extensively over a wide range of elevations, Paul and Post (2001) found that RBT are most common at lower elevations where they often compete for food resources and hybridize with native salmonid populations.

We did not find any evidence of radio-tagged fish using mainstems or side-channel habitats for spawning. It is possible that these habitats are not suitable for spawning in this system due to high and sporadic water velocities that wash away fry (Fausch et al. 2001), reduce emergence success (Weaver and Fraley 1993), and provide suboptimal nursery habitat during the first year of growth (Shepard et al. 1984). In contrast, in the South Fork Snake River in Idaho, Henderson et al. (2000) found complete spatial and temporal overlap between introduced RBT and native Yellowstone cutthroat trout within side channels in the mainstem. Also, DeRito (2004) reported that RBT spawned in side channels to a greater extent than Yellowstone cutthroat trout in the Yellowstone River, Montana, but that spatial overlap occurred in one side channel used by the parental taxa and hybrids. However, unlike our findings, DeRito (2004) found minimal evidence of temporal overlap in tributaries used for spawning.

Radiotelemetry was useful for identifying streams in which hybridization is occurring and for understanding how hybridization is spreading in this large, interconnected system. We found that most RBT and RBT hybrids spawned in streams in the lower drainage and that Abbot Creek, which contains a hybrid swarm with a 92% RBT admixture, likely serves as a major source of RBT introgression in the upper Flathead River system. These results are consistent with recent genetic studies in this system (Boyer et al. 2008) and nearby Kootenay River system (Rubidge and Taylor 2005) that found a significant negative correlation in the proportion of RBT admixture with upstream distance from hybrid sources.

Spawning behavior

Our study also provides evidence that individuals with low and high levels of RBT admixture differ in spawning behavior compared with nonhybridized WCT. Our findings indicate that (*i*) WCT display significantly different spawning behaviors than RBT and hybrids, (*ii*) F_1 hybrids and individuals backcrossed to WCT display spawning and movement characteristics generally intermediate to those of WCT and RBT and RBT hybrids, and (*iii*) hybrids backcrossed to RBT behave similarly to RBT. The spawning behaviors of native WCT probably represent adaptations to the environment, which have been selected for at least since the Wisconsin glacial period (~14 000 years ago). WCT may possibly migrate during high spring flows to access the full extent of available habitat and spawn as flows decline to minimize sediment deposition (Weaver and Fraley 1993) and substrate scour (Fausch et al. 2001) and to avoid high water velocities that wash away trout fry (Fausch et al. 2001). Our data suggest that hybridization may produce earlier spawning times, leading to earlier emergence times, and possibly reduced recruitment success. Loss of local adaptations may be especially detrimental to indigenous species during periods of extreme environmental conditions, such as fire, drought, and winter flooding (Leary et al. 1995; Allendorf et al. 2004).

Increased straying rates in the invasive taxon can contribute to extinction of native species by hybridization. Native WCT populations exhibit a high degree of genetic divergence over short geographical distances (Allendorf and Leary 1988; Boyer et al. 2008), indicating low straying rates among populations. Conversely, the rapid spread of hybridization indicates a high rate of dispersal and gene flow from hybrid and RBT sources. Our data indicate that hybridization is spreading upriver, in part, by long-distance movements of individuals with high amounts of RBT admixture that likely strayed into streams containing nonhybridized WCT, which corroborates conclusions from a recent genetics study in the system (Boyer et al. 2008).

Hybridization and reproductive segregation may be dynamic events that vary temporally due to abiotic factors, such as river discharge and temperature, and in many systems, hybridization has rapidly increased over short time frames (Hitt et al. 2003; Rubidge and Taylor 2004). For example, temporal genetic analyses in the Flathead system revealed that hybridization was detected at 57% of the sites sampled from 1998 to 2001, and new RBT introgression was documented in 7 of 11 (73%) sites that were determined to be nonhybridized in 1984. In 2002, a high flow year, we found that RBT and RBT hybrids spawned significantly later than in all other years, and coincidentally or not, we observed long-distance spawning movements by two RBT males into streams containing nonhybridized WCT, facilitating interbreeding among parental types (Muhlfeld et al. 2009*a*). Similarly, DeRito (2004) found evidence of temporal variation in spawning among years in the Yellowstone River system; RBT and hybrids spawned earlier with earlier peak spring runoff, increasing the likelihood of overlap with later-spawning Yellowstone cutthroat trout.

Limitations

Differences in spawning characteristics of cutthroat trout and hybrids may also be attributed to the current spatial distribution of hybridization in the system. Prior to the introduction of RBT in the system, WCT likely used spawning tributaries in lower elevations presently dominated by RBT and hybrids. Therefore, it is plausible that some of the observed differences in spawning behavior (e.g., migration distance and migration period) may be due, in part, to the fact that WCT now persist predominately in higher-elevation tributaries. Additionally, we were unable to measure water temperature and flow within spawning tributaries, and these environmental cues may have differed from the measured variables in the mainstem. Nonetheless, the major differences in spawning behavior appear to be related to temperature and flow, as WCT consistently spawned in streams as flows declined following peak runoff and as stream temperatures approached 9 °C, which is consistent with other studies (Bjornn and Mallet 1964; Shepard et al. 1984; Schmetterling 2001). Photoperiod may also be an important factor linked to spawning time, as recent research has identified a gene in RBT that is linked to a spawning time quantitative trait locus (see Leder et al. (2006) and references therein). This could explain, in part, why spawning date is significantly different between WCT and individuals with some amount of RBT admixture.

The purpose of the hybrid index classification was to describe the multilocus phenotype or genotype of individuals. Because of the random reshuffling of alleles during sexual reproduction, the power to discriminate between parental types and later generation hybrids is a function of the number of marker loci used (Boecklen and Howard 1997). With 13 diagnostic nuclear PINE or indel–microsatellite loci, there is much less than a 1% chance that a first-generation backcross would be misidentified as an F_1. The ability to distinguish between nonhybridized parental species and later-generation backcrosses, however, is more problematic. For example, if an individual fish has 10% of its genes from RBT, there is a 6% chance that it would possess no RBT alleles at the 13 diagnostic indel–microsatellite loci. In contrast, with the six PINE diagnostic loci, the presence of the fragment being characteristic of RBT, there is a 28% chance that no RBT alleles would be detected in such an individual. Consequently, because of this sampling error, we probably overestimated the number of pure parental types and underestimated the number of hybrids. This may explain the fact that the RBT and RBT hybrids differed little in spawning characteristics.

Conservation implications

Although nonhybridized WCT populations still exist, our data indicate that many of these populations are at high risk of genetic introgression. The spatial and temporal differences in spawning between WCT and RBT and hybrids are not absolute and, thus, are likely not strong enough to preclude further hybridization. Environmental factors may also not be strong enough to constrain invasion and subsequent introgression in this system and others (Hitt et al. 2003; Rubidge and Taylor 2005; Muhlfeld et al. 2009*b*). Moreover, when reproductive overlap occurs and hybrid progeny are produced, hybridization may spread and genomic extinction of parental taxa can occur, even when hybrid offspring experience reduced fitness due to the "ratchet effect" whereby all progeny of hybrids will be hybrids (Epifanio and Philipp 2001; Allendorf et al. 2004). Muhlfeld et al. (2009*a*) found that small amounts of hybridization markedly reduced the reproductive success of native WCT in a recently invaded stream (Langford Creek) in the Flathead system. Hybridization appears to be spreading, however, due to relatively high reproductive success of F_1 hybrids and high reproductive success of a few males with high levels of admixture, which corroborates our telemetry data.

Currently, there are no official policy guidelines for treating hybrids under the Endangered Species Act (ESA; Allendorf et al. 2004, 2005). Recent debate has focused on establishing a clear definition on what constitutes a "pure" species and what level of anthropogenic introgression is acceptable to maintain the evolutionary legacy of this subspecies (Allendorf et al. 2005; Campton and Kaeding 2005). This study provides insight into this ongoing debate by linking genetic status with behavioral characteristics of nonhybridized WCT and hybrid fish during spawning. Our data revealed that hybrids backcrossed to WCT and RBT differed in spawning behavior compared with nonhybridized WCT, suggesting that hybridization may change local adaptations in native WCT populations by producing earlier spawning times.

Currently, hybridizing taxa are not protected under the ESA and their consideration has been determined on a case-by-case basis. Populations with up to 20% nonnative genetic admixture were classified as WCT under the unit considered for listing in a recent status review (US Fish and Wildlife Service (USFWS) 2003). A primary concern with protecting hybrid populations is that inclusion of these populations as WCT may threaten the persistence of this subspecies by protecting hybrid sources and allowing further introgression and loss of ecological adaptations (Allendorf et al. 2004). Our results suggest that introgressive hybridization erodes discrete reproductive behavior of the native taxon and that hybridization is spreading from source hybrid populations in lower portions of the system. Eradication or suppression of hybrid populations, therefore, may be required as an effective management strategy for reducing the spread of hybridization. Proactive conservation measures, such as removing adults during spawning and installing barriers to migration (Fausch et al. 2006), are being implemented in the Flathead and other systems to slow or stop the spread of hybridization. Alternatively, in situations where there are still parental individuals present, a selective removal program may be an effective option to constrain or prevent introgression. The ability to accurately visually determine the identity of nonhybridized and hybrid fish is necessary for this approach to be successful. Our results suggest that morphological phenotypic characteristics may provide a reasonably accurate and cost-effective means of identifying adult hybrids with high proportions of RBT admixture in the field; however, morphological identification of individuals with low to moderate amounts of admixture can be unreliable (Leary et al. 1984; Behnke 1992; Weigel et al. 2002). Prior to using field identification, we recommend that biologists first compare the accuracy of the characteristics that they have chosen with genetic identification of individuals with varying levels of admixture (e.g., Weigel et al. 2002).

Genetic studies in this system (Hitt et al. 2003; Boyer et al. 2008) and others (Weigel et al. 2003; Rubidge and Taylor 2005) have concluded that in the absence of physical migration barriers, continued introgression with introduced trout will likely lead to the genomic extinction of WCT. Our telemetry results provide additional support for this prediction. Our study demonstrates that introductions of RBT into previously allopatric populations of WCT may result in genetically admixed populations because of a breakdown in reproductive segregation in time and space, promoting extinction by introgression. We conclude that introgression is likely to continue to spread unless populations with high amounts of nonnative genetic admixture are reduced or eliminated.

Acknowledgements

Bonneville Power Administration and Montana Fish, Wildlife and Parks provided funding for this work. Genetic analysis was conducted at the Conservation Genetics Laboratory, Missoula, Montana. We thank Robb Leary, Sally Painter, Paul Spruell, and Kathy Knudsen for conducting the genetic analyses. We thank Bob Gresswell, Steven Kalinowski, Matthew Boyer, Brian Marotz, and three anonymous reviewers for helpful suggestions and comments. Field data were collected by Steve Glutting, Rick Hunt, John Wachsmuth, and Matthew Boyer. Brian Marotz and Joel Tohtz provided logistical and administrative support during this project. We thank Steve Glutting for providing Fig. 1.

References

Allendorf, F.W., and Leary, R.F. 1988. Conservation and distribution of genetic variation in a polytypic species: the cutthroat trout. Conserv. Biol. **2**(2): 170–184. doi:10.1111/j.1523-1739.1988.tb00168.x.

Allendorf, F.W., Leary, R.F., Spruell, P., and Wenburg, J.K. 2001. The problems with hybrids: setting conservation guidelines. Trends Ecol. Evol. **16**(11): 613–622. doi:10.1016/S0169-5347(01)02290-X.

Allendorf, F.W., Leary, R.F., Hitt, N.P., Knudsen, K.L., Lundquist, L.L., and Spruell, P. 2004. Intercrosses and the U.S. Endangered Species Act: should hybridized populations be included as westslope cutthroat trout. Conserv. Biol. **18**(5): 1203–1213. doi:10.1111/j.1523-1739.2004.00305.x.

Allendorf, F.W., Leary, R.F., Hitt, N.P., Knudsen, K.L., Boyer, M.C., and Spruell, P. 2005. Cutthroat trout hybridization and the U.S. Endangered Species Act: one species, two policies. Conserv. Biol. **19**(4): 1326–1328. doi:10.1111/j.1523-1739.2005.00223.x.

Arnold, M.L. 1997. Natural hybridization and evolution. Oxford University Press, New York.

Barton, N.H. 2001. The role of hybridization in evolution. Mol. Ecol. **10**(3): 551–568. doi:10.1046/j.1365-294x.2001.01216.x. PMID:11298968.

Barton, N.H., and Hewitt, G.M. 1985. Analysis of hybrid zones. Annu. Rev. Ecol. Evol. Syst. **16**: 113–148.

Bear, E.A., McMahon, T.E., and Zale, A.V. 2007. Comparative thermal requirements of westslope cutthroat trout and rainbow trout: implications for species interactions and development of thermal protection standards. Trans. Am. Fish. Soc. **136**(4): 1113–1121. doi:10.1577/T06-072.1.

Behnke, R.J. 1992. Native trout of western North America. American Fisheries Society, Bethesda, Maryland. AFS Monograph No. 6.

Bettles, C.M., Docker, M.F., Dufour, B., and Heath, D.D. 2005. Hybridization dynamics between sympatric species of trout: loss of reproductive isolation. J. Evol. Biol. **18**(5): 1220–1233. doi:10.1111/j.1420-9101.2005.00935.x. PMID:16135118.

Bjornn, T.C., and Mallet, J. 1964. Movements of planted and wild trout in an Idaho river system. Trans. Am. Fish. Soc. **93**(1): 70–76. doi:10.1577/1548-8659(1964)93[70:MOPAWT]2.0.CO;2.

Boecklen, W.J., and Howard, D.J. 1997. Genetic analysis of hybrid zones: number of markers and power of resolution. Ecology, **78**: 2611–2616.

Boyer, M.C., Muhlfeld, C.C., and Allendorf, F.W. 2008. Rainbow trout (*Oncorhynchus mykiss*) invasion and the spread of hybridization with native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). Can. J. Fish. Aquat. Sci. **65**: 658–669. doi:10.1139/F08-001.

Brown, K.H., Patton, S.J., Martin, K.E., Nichols, K.M., Armstrong, R., and Thorgaard, G.H. 2004. Genetic analysis of interior Pacific Northwest *Oncorhynchus mykiss* reveals apparent ancient hybridization with westslope cutthroat trout. Trans. Am. Fish. Soc. **133**(5): 1078–1088. doi:10.1577/T03-217.1.

Cairney, M., Taggart, J.B., and Høyheim, B. 2000. Characterization of microsatellite and minisatellite loci in Atlantic salmon (*Salmo salar* L.) and cross-species amplification in other salmonids. Mol. Ecol. **9**(12): 2175–2178. doi:10.1046/j.1365-294X.2000.105312.x. PMID:11123640.

Campton, D.E., and Kaeding, L.R. 2005. Westslope cutthroat trout, hybridization, and the U.S. Endangered Species Act. Conserv. Biol. **19**(4): 1323–1325. doi:10.1111/j.1523-1739.2005.00222.x.

Committee on the Status of Endangered Wildlife in Canada. 2006. COSEWIC assessment and update status report on the westslope cuttthroat trout *Oncorhynchus clarkii lewisi* (British Columbia population and Alberta population) in Canada. Committee on the Status of Endangered Wildlife in Canada, Ottawa, Ontario. Available at www.sararegistry.gc.ca/status/status_e.cfm.

DeRito, J.N. 2004. Assessment of reproductive isolation between Yellowstone cutthroat trout and rainbow trout in the Yellowstone River, Montana. M.Sc. thesis, Department of Ecology, Montana State University, Bozeman, Montana.

Epifanio, J., and Philipp, D. 2001. The extinction of parental lineages from introgressive hybridization: the effects of fitness, initial proportions of parental taxa, and mate choice. Rev. Fish Biol. Fish. **10**(3): 339–354. doi:10.1023/A:1016673331459.

Fausch, K.D., Taniguchi, Y., Nakano, S., Grossman, G.D., and Townsend, C.R. 2001. Flood disturbance regimes influence rainbow trout invasion success among five holarctic regions. Ecol. Appl. **11**(5): 1438–1455. doi:10.1890/1051-0761(2001)011[1438:FDRIRT]2.0.CO;2.

Fausch, K.D., Rieman, B.E., Young, M.K., and Dunham, J.B. 2006. Strategies for conserving native salmonid populations at risk from nonnative fish invasions: tradeoffs in using barriers to upstream movement. USDA Forest Service General Technical Report No. RMRS-GTR-174.

Floate, K.D., Whitham, T.G., and Keim, P. 1994. Morphological versus genetic markers in classifying hybrid plants. Evolution, **48**(3): 929–930. doi:10.2307/2410500.

Fuller, P.L., Nico, L.G., and Williams, J.D. 1999. Nonindigenous fishes introduced into inland waters of the United States. American Fisheries Society, Bethesda, Maryland. AFS Special Publication No. 27.

Gresswell, R.E. 1988. Status and management of interior stocks of cutthroat trout. American Fisheries Society, Bethesda, Maryland. AFS Symposium No. 4.

Gunnell, K., Tada, M.K., Hawthorne, F.A., Keeley, E.R., and Ptacek, M.B. 2008. Geographic patterns of introgressive hybridization between native Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*) and introduced rainbow trout (*O. mykiss*) in the South Fork of the Snake River watershed, Idaho. Conserv. Genet. **9**(1): 49–64. doi:10.1007/s10592-007-9302-6.

Henderson, R., Kershner, J.L., and Toline, C.A. 2000. Timing and location of spawning by nonnative wild rainbow trout and native cutthroat trout in the South Fork Snake River, Idaho, with implications for hybridization. N. Am. J. Fish. Manage. **20**(3): 584–596. doi:10.1577/1548-8675(2000)020<0584:TALOSB>2.3.CO;2.

Hitt, N.P., Frissell, C.A., Muhlfeld, C.C., and Allendorf, F.W. 2003. Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki lewisi*, and nonnative rainbow trout, *Oncorhynchus mykiss*. Can. J. Fish. Aquat. Sci. **60**(12): 1440–1451. doi:10.1139/f03-125.

Holm, L.E., and Brusgaard, K. 1999. Two polymorphic dinucleotide repeats in rainbow trout (*Oncorhynchus mykiss*). Anim. Genet. **30**(2): 162–163. doi:10.1046/j.1365-2052.1999.00382-2.x.

Kanda, N., Leary, R.F., Spruell, P., and Allendorf, F.W. 2002. Molecular genetic markers identifying hybridization between the Colorado River – greenback cutthroat trout complex and Yellowstone cutthroat trout or rainbow trout. Trans. Am. Fish. Soc. **131**(2): 312–319. doi:10.1577/1548-8659(2002)131<0312: MGMIHB>2.0.CO;2.

Kimura, M., and Weiss, G.H. 1964. The stepping stone model of population structure and the decrease of genetic correlation with distance. Genetics, **49**(4): 561–576. PMID:17248204.

Leary, R.F., Allendorf, F.W., Phelps, S.R., and Knudsen, K.L. 1984. Introgression between westslope cutthroat trout and rainbow trout in the Clark Fork River drainage, Montana. Proc. Mont. Acad. Sci. **43**: 1–18.

Leary, R.F., Allendorf, F.W., Phelps, S.R., and Knudsen, K.L. 1987. Genetic divergence and identification of seven cutthroat trout subspecies and rainbow trout. Trans. Am. Fish. Soc. **116**(4): 580–587. doi:10.1577/1548-8659(1987)116<580:GDAIOS>2.0.CO;2.

Leary, R.F., Allendorf, F.W., and Sage, G.K. 1995. Hybridization and introgression between introduced and native fish. Am. Fish. Soc. Symp. **15**: 91–101.

Leder, E.H., Danzmann, R.G., and Ferguson, M.M. 2006. The candidate gene, Clock, localizes to a strong spawning time quantitative trait locus region in rainbow trout. J. Hered. **97**(1): 74–80. doi:10.1093/jhered/esj004. PMID:16407529.

Liknes, G.A., and Graham, P.J. 1988. Westslope cutthroat trout in Montana: life history, status, and management. Am. Fish. Soc. Symp. **4**: 53–60.

Loudenslager, E.J., and Gall, G.A.E. 1980. Geographic patterns of protein variation and subspeciation in the cutthroat trout. Syst. Zool. **29**(1): 27–42. doi:10.2307/2412624.

Mack, R.N., Simberloff, D., Lonsdale, W.M., Evans, H., Clout, M., and Bazzaz, F.A. 2000. Biotic invasions, epidemiology, global consequences and control. Ecol. Appl. **10**(3): 689–710. doi:10.1890/1051-0761(2000)010[0689:BICEGC]2.0.CO;2.

Metcalf, J.L., Siegle, M.R., and Martin, A.P. 2008. Hybridization dynamics between Colorado's native cutthroat trout and introduced rainbow trout. J. Hered. **99**(2): 149–156. doi:10.1093/jhered/esm118. PMID:18238784.

Miller, R.R., Williams, J.D., and Williams, J.E. 1989. Extinction of North American fishes during the past century. Fisheries, **14**(6): 22–38. doi:10.1577/1548-8446(1989)014<0022:EONAFD>2.0.CO;2.

Mooney, H.A., and Cleland, E.E. 2001. The evolutionary impact of invasive species. Proc. Natl. Acad. Sci. U.S.A. **98**(10): 5446–5451. doi:10.1073/pnas.091093398. PMID:11344292.

Muhlfeld, C.C. 2008. Behavioral, ecological, and fitness consequences of hybridization between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and nonnative rainbow trout (*Oncorhynchus mykiss*). Ph.D. dissertation, Department of Ecology, Montana State University, Bozeman, Montana.

Muhlfeld, C.C., Glutting, S., Hunt, R., and Marotz, B. 2000. Seasonal distribution and movements of native and nonnative fishes in the upper Flathead River drainage, Montana. Montana Fish, Wildlife and Parks. Final report to Bonneville Power Administration, Portland, Oregon.

Muhlfeld, C.C., Glutting, S., Hunt, R., Daniels, D., Boyer, M., Wachsmuth, J., and Marotz, B. 2003. Hungry Horse Mitigation Program, 2002 Annual Progress Report: investigations of the Flathead River Native Species Project. BPA Project No. 199101903, Kalispell, Montana.

Muhlfeld, C.C., Kalinowski, S.T., McMahon, T.E., Taper, M.L., Painter, S., Leary, R.F., and Allendorf, F.W. 2009*a*. Hybridization rapidly reduces fitness of a native trout in the wild. Biol. Lett. **5**(3): 328–331. doi:10.1098/rsbl.2009.0033. PMID:19324629.

Muhlfeld, C.C., McMahon, T.E., Boyer, M.C., and Gresswell, R.E. 2009*b*. Local habitat, watershed, and biotic factors influencing the spread of hybridization between native westslope cutthroat trout and introduced rainbow trout. Trans. Am. Fish. Soc. In press.

Norusis, M.J. 1990. SPSS/PC+ 4.0 base manual for the IBM PC/XT/AT and PS/2. SPSS Inc., Chicago, Illinois.

Ostberg, C.O., and Rodriguez, R.J. 2004. Bi-parentally inherited species-specific markers identify hybridization between rainbow trout and cutthroat trout subspecies. Mol. Ecol. **4**(1): 26–29. doi:10.1046/j.1471-8286.2003.00554.x.

Ostberg, C.O., and Rodriguez, R.J. 2006. Hybridization and cytonuclear associations among native westslope cutthroat trout, introduced rainbow trout, and their hybrids within the Stehekin River drainage, North Cascades National Park. Trans. Am. Fish. Soc. **135**(4): 924–942. doi:10.1577/T05-209.1.

Paul, A.J., and Post, J.R. 2001. Spatial distribution of native and nonnative salmonids in streams of the eastern slopes of the Canadian Rocky Mountains. Trans. Am. Fish. Soc. **130**(3): 417–430. doi:10.1577/1548-8659(2001)130<0417:SDONAN>2.0.CO;2.

Raleigh, R.F., Hickman, T., Solomon, R.C., and Nelson, P.C. 1984. Habitat suitability information: rainbow trout. US Fish and Wildlife Service Report No. FWS/OBS-82/10.60.

Rexroad, E., III, Coleman, R.L., Gustafson, A.L., Hershberger, W.K., and Killefer, J. 2002. Development of rainbow trout microsatellite markers from repeat enriched libraries. Mar. Biotechnol. **3**(1): 12–16. doi:10.1007/s10126-001-0058-6.

Rhymer, J.M., and Simberloff, D. 1996. Extinction by hybridization and introgression. Annu. Rev. Ecol. Syst. **27**(1): 83–109. doi:10.1146/annurev.ecolsys.27.1.83.

Robinson, M.D. 2007. The ecological consequences of hybridization between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*O. mykiss*) in southwestern Alberta. M.Sc. thesis, University of Lethbridge, Lethbridge, Alberta, Canada.

Rubidge, E.M., and Taylor, E.B. 2004. Hybrid zone structure and the potential role of selection in hybridizing populations of native westslope cutthroat trout (*Oncorhynchus clarki lewisi*) and introduced rainbow trout (*O. mykiss*). Mol. Ecol. **13**(12): 3735–3749. doi:10.1111/j.1365-294X.2004.02355.x. PMID:15548287.

Rubidge, E., and Taylor, E.B. 2005. An analysis of spatial and environmental factors influencing hybridization between native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*O. mykiss*) in the upper Kootenay River drainage, British Columbia. Conserv. Genet. **6**(3): 369–384. doi:10.1007/s10592-005-4972-4.

Schmetterling, D.A. 2001. Seasonal movements of fluvial westslope cutthroat trout in the Blackfoot River drainage, Montana. N. Am. J. Fish. Manage. **21**(3): 507–520. doi:10.1577/1548-8675(2001)021<0507:SMOFWC>2.0.CO;2.

Seiler, S.M., and Keeley, E.R. 2007. Morphological and swimming stamina differences between Yellowstone cutthroat trout (*Oncorhynchus clarkii bouvieri*), rainbow trout (*Oncorhynchus mykiss*), and their hybrids. Can. J. Fish. Aquat. Sci. **64**(1): 127–135. doi:10.1139/F06-175.

Shepard, B.B., Pratt, K.L., and Graham, P.J. 1984. Life histories of westslope cutthroat trout and bull trout in the upper Flathead River Basin, Montana. Montana Department of Fish, Wildlife and Parks, Helena, Montana.

Shepard, B.B., May, B.E., and Urie, W. 2005. Status and conservation of westslope cutthroat trout within the western United States. N. Am. J. Fish. Manage. **25**(4): 1426–1440. doi:10.1577/M05-004.1.

Smith, C.T., Koop, B.F., and Nelson, R.J. 1998. Isolation and characterization of coho salmon (*Oncorhynchus kisutch*) microsatellites and their use in other salmonids. Mol. Ecol. **7**(11): 1614–1616. PMID:9819912.

Spies, B., Brasier, D.J., O'Reilly, P.T.L., Seamons, T.R., and Bentzen, P. 2005. Development and characterization of novel tetra-, tri- and dinucleotide microsatellite markers in rainbow trout (*Oncorhynchus mykiss*). Mol. Ecol. Notes, **5**(2): 278–281. doi:10.1111/j.1471-8286.2005.00900.x.

Spruell, P., Bartron, M.L., Kanda, N., Allendorf, F.W., and McEachran, J.D. 2001. Detection of hybrids between bull trout (*Salvelinus confluentus*) and brook trout (*Salvelinus fontinalis*) using PCR primers complementary to interspersed nuclear elements. Copeia, **2001**(4): 1093–1099. doi:10.1643/0045-8511(2001)001[1093:DOHBBT]2.0.CO;2.

US Fish and Wildlife Service. 2003. Endangered and threatened wildlife and plants: 12 month finding for a petition to list the westslope cutthroat trout as threatened through its range. Fed. Regist. **68**: 46989–47009.

Weaver, T.M., and Fraley, J.J. 1993. A method to measure emergence success of westslope cutthroat trout fry from varying substrate compositions in a natural stream channel. N. Am. J. Fish. Manage. **13**(4): 817–822. doi:10.1577/1548-8675(1993)013<0817:AMTMES>2.3.CO;2.

Weigel, D.E., Peterson, J.T., and Spruell, P. 2002. A model using phenotypic characteristics to detect introgressive hybridization in wild westslope cutthroat trout and rainbow trout. Trans. Am. Fish. Soc. **131**(3): 389–403. doi:10.1577/1548-8659(2002)131<0389:AMUPCT>2.0.CO;2.

Weigel, D.E., Peterson, J.T., and Spruell, P. 2003. Introgressive hybridization between native cutthroat and introduced rainbow trout. Ecol. Appl. **13**(1): 38–50. doi:10.1890/1051-0761(2003)013[0038:IHBNCT]2.0.CO;2.

Winter, J.D. 1983. Underwater biotelemetry. *In* Fisheries techniques. *Edited by* L.A. Nielsen and D.L. Johnson. American Fisheries Society, Bethesda, Maryland. pp. 371–395.

Young, M.K. (*Editor*). 1995. Conservation assessment for inland cutthroat trout. USDA Forest Service, Rocky Mountain Forest and Range Experimental Station, Fort Collins, Colorado. General Technical Report No. RM-256.

Zar, J.H. 1996. Biostatistical analysis. 3rd ed. Prentice-Hall, Englewood Cliffs, New Jersey.

Appendix A

Table A1. Paired interspersed nuclear element polymerase chain reaction (PINE PCR) fragments used for the detection of *Oncorhynchus mykiss* introgression.[a,b]

Taxon	PINE fragment number	No. of base pairs	Primer pair	Primer pair type
O. mykiss	1	70	Hpa1 5′/Hpa1 3′	SINEs
	2	66	Hpa1 5′/Hpa1 3′	SINEs
	3	369	Fok1 5′/Tc1	SINE and transposon
	4	230	Fok1 5′/Tc1	SINE and transposon
	5	395	Hpa1 5′/33.6+2	SINE and Jeffreys' core fragment
	6	266	Hpa1 5′/33.6+2	SINE and Jeffreys' core fragment
O. clarkii lewisi	1	153	Hpa1 5′/Hpa1 3′	SINEs
	2	72	Hpa1 5′/Hpa1 3′	SINEs
	3	69	Hpa1 5′/Hpa1 3′	SINEs

Table A1 (*concluded*).

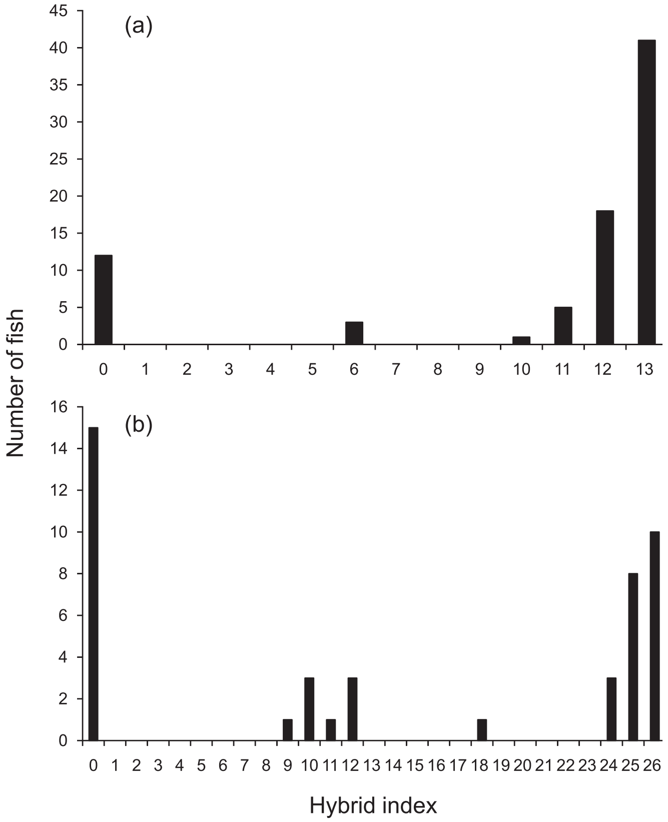
Taxon	PINE fragment number	No. of base pairs	Primer pair	Primer pair type
	4	366	Fok1 5′/Tc1	SINE and transposon
	5	110	Fok1 5′/Tc1	SINE and transposon
	6	388	Hpa1 5′/33.6+2	SINE and Jeffreys' core fragment
	7	148	Hpa1 5′/33.6+2	SINE and Jeffreys' core fragment

Note: SINE, small interspersed nuclear elements.

[a] Kanda et al. 2002. Trans. Am. Fish. Soc. **131**(2): 312–319. doi:10.1577/1548-8659(2002)131<0312: MGMIHB>2.0.CO;2.

[b] Spruell et al. 2001. Copeia, 2001(4): 1093–1099. doi:10.1643/0045-8511(2001)001[1093:DOHBBT]2.0.CO;2.

Fig. A1. Hybrid index scores of 125 radio-tagged westslope cutthroat trout, rainbow trout, and hybrids based on paired interspersed nuclear element polymerase chain reaction (PINE PCR) of (*a*) 13 dominant nDNA fragments (*N* = 80 fish) and (*b*) a combination of six co-dominant indel loci and seven co-dominant microsatellite loci (*N* = 45 fish). The hybrid index score is calculated as the number of nonnative rainbow trout alleles in each fish.



(Table A2 appears on the next page.)

Table A2. Pearson's product moment correlations for eight spawning habitat variables.

		Spawning variables							
Correlations		Beginning migration date	Beginning temperature	Beginning flow	Spawning date	Migration period	Spawning temperature	Spawning flow	Migration distance
Beginning migration date	Pearson's correlation	1	0.7280	0.5625	0.4027	–0.4818	0.3008	0.2092	0.0837
	Significance (two-tailed)		0.0000	0.0000	0.0000	0.0000	0.0007	0.0192	0.3531
	N	125	125	125	125	125	125	125	125
Beginning temperature	Pearson's correlation	0.7280	1	0.2167	0.1666	–0.4719	0.2173	–0.0237	0.0720
	Significance (two-tailed)	0.0000		0.0152	0.0633	0.0000	0.0149	0.7930	0.4248
	N	125	125	125	125	125	125	125	125
Beginning flow	Pearson's correlation	0.5625	0.2167	1	0.3535	–0.1495	0.2536	0.3339	0.2967
	Significance (two-tailed)	0.0000	0.0152		0.0001	0.0960	0.0043	0.0001	0.0008
	N	125	125	125	125	125	125	125	125
Spawning date	Pearson's correlation	0.4027	0.1666	0.3535	1	0.6080	0.7242	0.4730	0.6006
	Significance (two-tailed)	0.0000	0.0633	0.0001		0.0000	0.0000	0.0000	0.0000
	N	125	125	125	125	125	125	125	125
Migration period	Pearson's correlation	–0.4818	–0.4719	–0.1495	0.6080	1	0.4323	0.2713	0.5023
	Significance (two-tailed)	0.0000	0.0000	0.0960	0.0000		0.0000	0.0022	0.0000
	N	125	125	125	125	125	125	125	125
Spawning temperature	Pearson's correlation	0.3008	0.2173	0.2536	0.7242	0.4323	1	0.0189	0.6346
	Significance (two-tailed)	0.0007	0.0149	0.0043	0.0000	0.0000		0.8345	0.0000
	N	125	125	125	125	125	125	125	125
Spawning flow	Pearson's correlation	0.2092	–0.0237	0.3339	0.4730	0.2713	0.0189	1	0.1086
	Significance (two-tailed)	0.0192	0.7930	0.0001	0.0000	0.0022	0.8345		0.2278
	N	125	125	125	125	125	125	125	125
Migration distance	Pearson's correlation	0.0837	0.0720	0.2967	0.6006	0.5023	0.6346	0.1086	1
	Significance (two-tailed)	0.3531	0.4248	0.0008	0.0000	0.0000	0.0000	0.2278	
	N	125	125	125	125	125	125	125	125