Conservation Genetics (2005) 6:369–384
DOI 10.1007/s10592-005-4972-4

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

E.M. Rubidge* & E.B. Taylor
Department of Zoology and Native Fishes Research Group, University of British Columbia, 6270 University Blvd, V6T 1Z4, Vancouver, BC, Canada
*(*Corresponding author: Present address: Department of Environmental Science, Policy & Management University of California, 137 Mulford Hall #3114, Berkeley CA, 94720-3114, Fax: 604–822–2416; E-mail: erubidge@nature.berkeley.com)*

Received 6 September 2004; accepted 11 January 2005

Key words: conservation, hybridization, introduced species, spatial analysis, westslope cutthroat trout

Abstract

Westslope cutthroat trout (Oncorhynchus clarki lewisi, WCT) and introduced rainbow trout (O. mykiss, RBT) readily hybridize and introgression has occurred in many drainages across the historic native range of WCT. In British Columbia (Canada), the upper Kootenay River drainage is the heart of the WCT distribution and is thought to harbour native gene pools, but many population are thought to be under threat from hybridization with introduced rainbow trout (RBT, *O. mykiss*). In this study, we assess the extent and distribution of WCT × RBT hybridization in the upper Kootenay River drainage. We used four diagnostic nuclear loci to determine the extent of hybridization in 981 fish collected from 23 sample localities across 12 different streams in the upper Kootenay River drainage. About 14% (142/981) of individuals were identified as hybrids (an individual with both RBT and WCT alleles), 3.4% (33/981) were identified as pure RBT, and the remaining individuals were identified as pure WCT. Although pure RBT were absent from the majority of locales (20/23), we found evidence of hybridization at 78% (18/23) of the localities and the percentage of heterospecific alleles (% *I*) ranged from 0.7% to 97.1%. Only 22% (5/23) of the localities showed no evidence of hybridization. Spatial analysis showed clustering among hybridized locations and decreasing hybridization with increasing distance from Koocanusa Reservoir, suggesting that the reservoir acts as a RBT source. We found no evidence that stream order, stream magnitude, or stream elevation influenced the extent of hybridization among localities. We compared our results to an analysis conducted in 1986, which indicated that hybridization is relatively recent in the upper Kootenay River drainage and that it is increasing in magnitude and distribution. In the absence of timely management intervention, the genetic integrity of WCT populations in the heart of their Canadian range may be lost. Our results indicate the dynamic nature of hybridization in fluvial systems and that for closely related taxa such as WCT and RBT, hybridization appears to be largely influenced by physical barriers to dispersal and contact between species.

Introduction

Compared to the attention paid to extinctions in terrestrial habitats, relatively little focus has been given to species loss in freshwater ecosystems despite several studies that demonstrate a growing number of freshwater extinctions (e.g., Miller et al. 1989; Williams and Miller 1990; Ricciardi and Rasmussen 1999). In North America, freshwater fish are likely the most threatened group of vertebrates after amphibians (Bruton 1995; Ricciardi and Rasmussen 1999). One of the principal threats

to native aquatic biodiversity is the introduction of exotic species (Rhymer and Simberloff 1996). Introduced species may harm native faunas through predation, competition, disease and parasite introduction, and hybridization. Hybridization and introgression between native and non-native species impacts the native gene pool through the interaction between genetic factors that influence reproduction and gene exchange and ecological factors that influence dispersal and establishment of non-native species. Consequently, a key uncertainty in our understanding of the factors that influence the extent of hybridization is the role of the environment in promoting (or hindering) gene exchange between species.

British Columbia (BC) in western Canada is home to a diverse and unique freshwater fish fauna (McPhail and Carveth 1992, Unpublished report), but approximately 43% of the 67 recognized native freshwater fish species in BC are either red (critically imperilled) or blue (special concern) listed provincially (BC Conservation Data Centre). The introduction of non-native fish species is one of the most serious threats to BC native fishes (Taylor 2004a).

Westlope cutthroat trout (WCT, *Oncorhynchus clarkii lewisi*) is one of two subspecies of cutthroat trout native to BC. The WCT subspecies is distinguished from other subspecies of *O. clarki* by substantial differences in morphology, karyotype, and various measures of genomic divergence (see Allendorf and Leary 1988; Utter and Allendorf 1994) and is native both east and west of the Rocky Mountains in southeastern BC, southwestern Alberta, throughout Montana, and northern Idaho (Figure 1). There are also disjunct populations in Washington State, Oregon and in the South Thompson River (Fraser River drainage), Columbia River and Kettle River in BC. There have been significant declines in WCT populations throughout their historic distribution due to habitat loss and degradation, overexploitation, competition and predation by non-native salmonids, and introgressive hybridization with introduced rainbow trout (RBT, *O. mykiss*) and Yellowstone cutthroat trout (YCT, *O. clarki bouvieri*; Allendorf and Leary 1988; Liknes and Graham 1988; Shepard et al. 1997). WCT are currently blue-listed in BC (i.e., species of special concern) and under review for a federal listing under the Canadian Species at Risk Act (SARA). In the United States (US) scientists have recommended protecting only non-hybridized WCT populations under the Endangered Species Act (ESA) in order to preserve the genetic legacy of this native trout (Allendorf et al. 2004). This recommendation makes locating "genetically pure" populations vital in the conservation of this fish (i.e., those with no traces of hybridization with other species induced by human activities).

The upper Kootenay River drainage (upstream of the Canada–US border crossing near Creston, BC; Figure 2) is the heart of WCT distribution in BC and is thought to be one of the few remaining areas with genetically pure populations. Although RBT are native to many of BC's drainages, they are non-native to the upper Kootenay River drainage, but over 3,000,000 fish have been introduced repeatedly over the last 85 years (BC Ministry of Water, Land and Air Protection (MWLAP), stocking records unpublished data, Figure 2). RBT have been introduced into lower elevation tributaries of the upper Kootenay River and Koocanusa Reservoir (formed by the dam on the Kootenai River at Libby, Montana), and to high-elevation lakes including many naturally fishless, mountain lakes (BC MWLAP stocking records, unpublished data). Introductions of RBT, therefore, stem both from a main downstream source of RBT in the Koocanusa Reservoir, or dispersal from upstream sources in multiple headwater lakes.

In order to ensure the future persistence of native freshwater fish gene pools, it is important to understand the current distribution and extent of hybridization with non-native fishes, and to determine the environmental factors that may influence hybridization. Locating pure populations and recognising these as important areas for implementing protection is also an important aspect of future management of fishes threatened by hybridization with non-native species. Leary et al. (1987a) used six allozyme markers and determined that three sample sites within the White River system "unquestionably came from hybrid swarms," but the other nine rivers sampled showed no evidence of hybridization. Since 1986, RBT introductions have continued and expanded in the region (i.e. the stocking program 1986–1998 in Koocanusa Reservoir) and preliminary results indicated that hybridization has spread since its original documentation (Rubidge et al. 2001). Further documentation of spatial and temporal trends in hybridization in the upper Kootenay

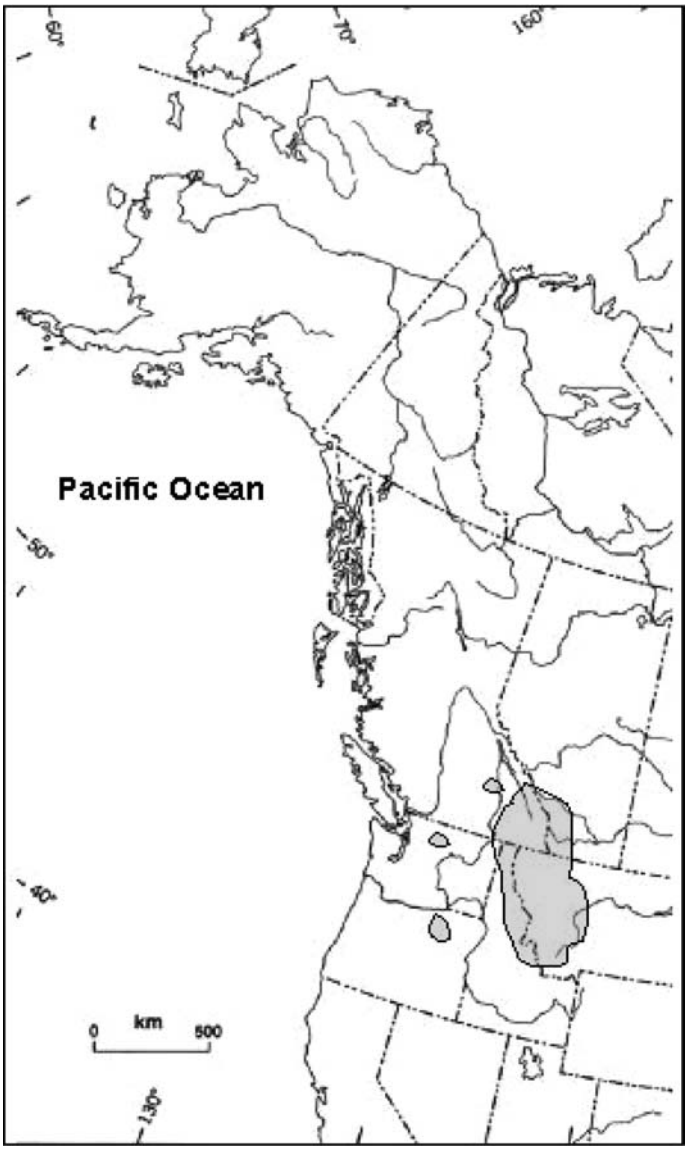

Figure 1. The native distribution of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) in western North America.

River area is, however, required to expand the baseline data.

There are many occurrences of introgressive hybridization between subspecies of native cutthroat trout and introduced RBT (e.g., Busack and Gall 1981; Leary et al. 1984; Carmichael et al. 1993; Campbell et al. 2002; Weigel et al. 2003). Consequently, it appears that there are few intrinsic (genetic) limitations to hybridization between these species. In areas of natural sympatry, RBT typically prefer larger, lower elevation, warmer streams and often spawn earlier than some subspecies of cutthroat trout (Hartman and Gill 1968; Trotter 1987; Henderson et al. 2000; Paul and Post 2001). Some evidence also suggests that lower elevation mainstem populations of WCT within the Kootenay River drainage may be at greater risk of hybridization because RBT appear to do relatively poorly in extreme headwater conditions (e.g., Bozek and Rahel 1991; Deleray et al. 1999 cited in Hitt et al. 2003; Paul and Post 2001). These observations all suggest that environmental conditions may be important factors influencing the extent of hybridization between

WCT and RBT in nature. There has, however, been little study of pre-mating isolation between native WCT and introduced RBT and previous work on WCT × RBT hybridization has produced conflicting results concerning the potential role of the environment in hybridization (Hitt et al. 2003; Weigel et al. 2003). Therefore, further study over broader environmental gradients is needed.

The objectives of our study were to determine: (i) if RBT hybridization has increased or spread in the upper Kootenay River drainage since 1986, (ii) the location of WCT populations with no evidence of hybridzation with RBT and (iii) if the incidence of hybridization is related to certain habitat characteristics (e.g., elevation, stream order and stream magnitude) to help rank populations in terms of habitat-based susceptibility to hybridization with RBT.

Materials and methods

Study location

The Kootenay River is the major tributary of the Canadian portion of the Columbia River Basin, the third largest drainage basin in BC. The headwaters of the Kootenay River occur in the Rocky Mountains in Kootenay National Park. It flows southwest through the Rocky Mountain Trench near Canal Flats, then continues south into the US before re-entering BC to join the Columbia River at Castlegar, BC. This study takes place in the upper Kootenay River drainage, which extends from its source to the first border crossing (Figure 2).

Sample collection

Caudal fin clips were collected from fish at 23 localities in 12 different river systems in the upper Kootenay River drainage (Figure 2). A total of 981 fish were included in this study; 356 were collected between June and September 1999 and 625 between June and September 2000. Fish from three localities were sampled in both years to assess temporal variation in the prevalence of hybridization. A combination of angling, electroshocking and minnow-trapping was used to sample fish. To avoid any biases in sampling, fish were clipped as they were encountered until a target sample size of $N = 30$ (see below) were reached without regard to presumed genotype. All tissue samples were stored in 95% ethanol and age class, fork length, and tentative species identification were determined for each fish. The species identification was based upon the following WCT characteristics: upper jaw extends past the posterior margin of the eye, bright red-orange slash under the base of the lower jaw, and reduced black spotting on the anterior portion of the body below the lateral line. Any individuals possessing intermediate or ambiguous phenotypes were tentatively classified as "hybrids," i.e., individuals of mixed ancestry. Age classification was based on size and retention of juvenile characteristics such as parr marks (dark oval bands on the lateral surface of subadult fish). One of four age classes was assigned: 0 + (fry or young-of-the-year, < 55 mm), 1+ (year old fish, approximately 60–130 mm), 2+ (fish larger than 130 mm that retained parr marks), and 3+ (fish larger than 180 mm that have no retention of juvenile characteristics).

Genetic analysis

The DNA was extracted from each tissue sample (10–20 mg) using the PUREGENE DNA Extraction Kit (Gentra Systems Inc.) following the manufacturer's protocol, diluted to 100 ng/μL, and stored at −20°C.

In order to identify heterospecific (RBT) alleles, fixed genetic differences between species must be identified. Markers were chosen from the literature based on the following criteria: species-specificity, repeatability, clarity (i.e. strength of banding patterns and ease of scoring), and availability. Preference was also given to co-dominant markers. We performed primer trials with 15 different potential markers and ranked them on the above criteria (detailed in Rubidge 2003). A prospective power analysis on hybrid detection found that to reliably distinguish backcross individuals from first generation hybrids (F_1) relatively few markers are needed (Boecklen and Howard 1997). For example, the probability of confusing a backcross for an F_1 using four diagnostic markers is 0.0625. Therefore, we used four markers that best fit the above criteria. Once we found markers that met these criteria on a few test individuals, we assayed individuals from both species across their distribution to confirm fixation of alleles. We tested 30 WCT

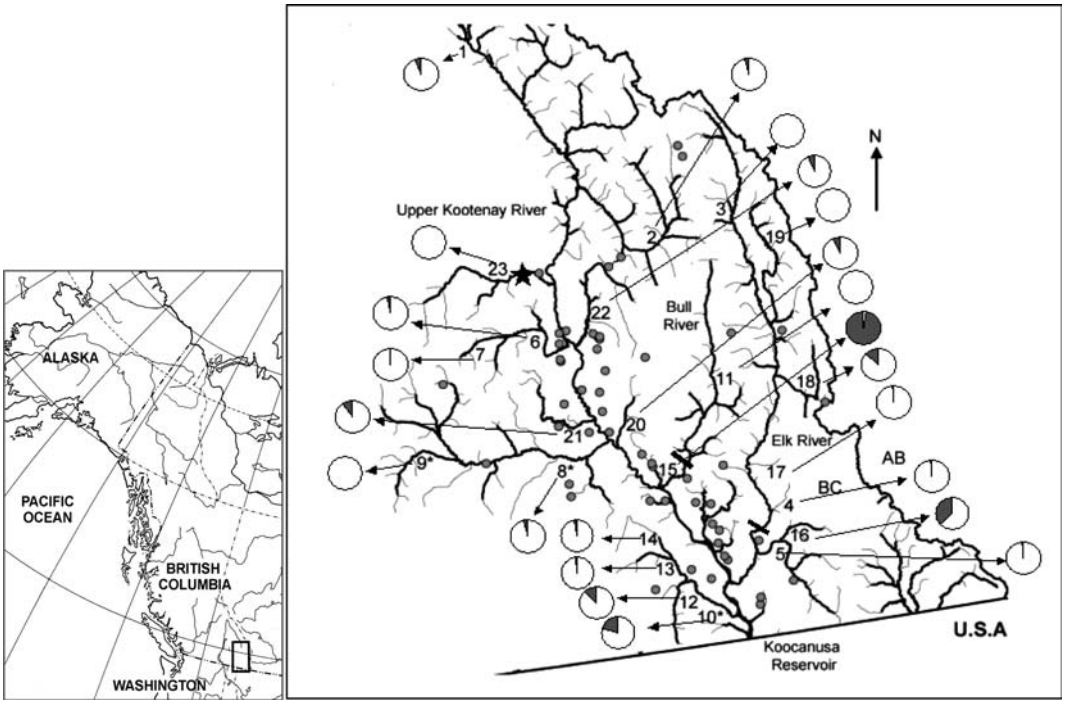

Figure 2. Sample localities examined for the presence of westslope cutthroat trout, rainbow trout, and their hybrids in the upper Kootenay River drainage. (1) Upper Kootenay River mainstem; (2) White River; (3) upper Elk River; (4) Morrissey Creek; (5) Wigwam River; (6) lower Skookumchuk Creek; (7) upper Skookumchuk Creek; (8) lower St. Mary River; (9) upper St. Mary River; (10) lower Gold Creek; (11) upper Bull River; (12) Bloom Creek at Gold Creek; (13) Teepee Creek at Gold Creek; (14) upper Gold Creek; (15) lower Bull River; (16) Lodgepole Creek; (17) Coal Creek; (18) Michel Creek; (19) Fording River; (20) Wild Horse River; (21) Mather Creek; (22) Lussier River; (23) Findlay Creek. Localities (1)–(11) were sampled in 1999, localities (12)–(23) were sampled in 2000. *Note*: Three systems were sampled in both years; upper and lower St. Mary River and lower Gold Creek and are indicated by the asterisk (*). Each grey dot represents a locality where rainbow trout were stocked between 1915 and 1998 (data from BC MWLAP stocking records); one locality may have been stocked numerous times. Pie charts represent the proportion of species alleles at each site; shaded area indicates % RBT alleles, white area indicates % WCT alleles. Black bars represent hydro dams and the star represents a canyon, both barriers to upstream fish migration. Inset shows study area in western North America. BC–British Columbia, AB–Alberta, USA–United States of America.

individuals from three populations that were believed to be pure (Findlay Creek, upper Bull River, and Connor Lakes) and 20 RBT individuals from populations in California to Russia and several BC populations that are used for hatchery production (i.e. Lardeau River and Pennask Lake populations). In addition to these tests, the authors that developed two of the chosen markers verified their status as species specific for the same alleles that we observed on 118 RBT from six different populations and 57 WCT from two populations in Idaho (Ostberg and Rodriguez 2002).

All four markers chosen are co-dominant markers with species diagnostic differences to identify ''hybrid'' individuals (Table 1). We define ''hybrids'' as any individual bearing a mixture of alleles from both species (see below). Ikaros and Heatshock cognate are coding genes, but the primers amplify intron regions of these genes. Species-specific variants of these introns enabled identification of individuals when cut with the appropriate restriction enzymes (RFLPs) (Baker et al. 2002). The other two markers used to identify WCT, RBT and their hybrids (Occ 16 and Om 13) are species diagnostic simple sequence repeats (SSR) designed by Ostberg and Rodriguez (2002). The Occ 16 and Om 13 are diagnostic based on fixed differences in allele frequencies of SSR (Ostberg and Rodriguez 2002), a type of microsatellite that is widespread throughout eukaryotic genomes.

Table 1. Primer sequences, PCR conditions (annealing temperature, degree centigrade/number of cycles), and species-specific diagnostic allele sizes for molecular markers used in DNA analyses of westslope cutthroat trout (WCT), rainbow trout (RBT) and their hybrids

Primer	Sequence 5′–3′	Annealing temperature per number of cycles	Enzyme	Diagnostic allele sizes (base pairs)
Hsc 71F	ctg cgt atc atc aat gag cc	60,56/8,32	Taq I	WCT: 568, 367, 249*
Hsc 71R	gat cag gac ggt cat gac			RBT: 616, 352, 216
IK F	ctt cga gtg caa cct ctg	48/45	Hinf I	WCT: 519, 294
IK R	att ttc ttt gcc acc gag g			RBT: 813
Occ 16F	gac aga cac att aag agt agt	50/30	N/A	WCT: 380
Occ 16R	cag taa tac agg tac agt atg			RBT: 280
Om 13F	gct gtt agg cta tat ttg ata t	56/30	N/A	WCT: 190
Om 13R	gaa aga tga gta aaa cta ttc			RBT: 175

*Diagnostic band for all cutthroat trout subspecies, other two bands may vary within cutthroat subspecies, all fish in this study were fixed for all three bands.N/A: non-applicable because no restriction enzymes were used.

DNA amplification

PCR reactions were run with varying conditions for each marker (Table 1). A typical PCR reaction consisted of a total volume of 20 μL with 10 ng template DNA, 0.8 μM each primer, 0.2 mM each dNTP, 1.5 mM $MgCl_2$, 1 × Invitrogen *Taq* DNA Polymerase buffer (20 mM Tris–HCl, pH 8.4, 50 mM KCl), and one unit of *Taq* DNA polymerase. All PCR reactions were run using a PTC–100 thermal cycler (MJ Research). Restriction digests were performed as per manufacturer's instructions (New England Biolabs), overnight, using 6 μL of PCR product in a total volume of 15 μL. The results of the PCR and the restriction fragment length polymorphisms were visualized using 2–3% agarose gels stained with ethidium bromide.

Hybrid identification

Individual fish were identified by their genotype at the four loci. If they were homozygous at all loci for the WCT alleles or the RBT alleles they were classified as pure WCT or pure RBT, respectively. If a heterozygote was observed at one or more of the four loci then that individual was classified as a "hybrid." We use the term "hybrid" to include everything from a first generation hybrid (heterozygous at all loci) to a backcrossed individual (heterozygous at one or more loci and homozygous for one of the parental species at the remaining loci) to an *n*th (post F_1) generation hybrid (homozygous for alternating parent species at two or more loci). The error associated with distinguishing between a parental genotype and a second (BC-2) or third generation (BC-3) backcross is quite high. For example, with four markers, an approximately 25% chance exists that a BC-2 will be classified as a parental individual, with BC-3 and BC-4 there is a greater chance (~51% and ~72%, respectively) of misclassification (Boecklen and Howard 1997). Therefore, our analyses may underestimate the number of hybrid individuals and overestimate the number of parental individuals in each population.

RBT hybridization

We assessed the degree of hybridization at each locality using the equation

$$I(\%)(\#\ \text{RBT alleles}/8) \times 100. \quad (1)$$

The presence of RBT or heterospecific alleles (% heterospecific alleles, *I*) at each site was quantified by dividing the number of RBT alleles out of total possible alleles (8) for each individual then this value was multiplied by 100. The mean was calculated for each sample locality. This analysis provided a comparative measure of the presence of RBT alleles in WCT populations across localities.

Statistical power to detect the presence of RBT alleles at each locality was calculated using equation (2) from Kanda et al. (2002):

$$a = (1 - q)^{2nx}, \quad (2)$$

where q is the desired frequency of non-native alleles to detect, n the number of fish sampled, x the number of diagnostic markers and α is equal to 1 minus the probability of detection. For example,

all localities (except one) have at least 30 individuals; therefore, with four diagnostic markers we had a 91% chance of detecting as little as a 1% genetic contribution from RBT in each population. The main objective of this regional study was not to determine the precise ancestry of each individual (e.g. a fourth-generation backcross), but to detect RBT introgression in each population.

Spatial analysis of hybridization

If hybridization is spreading from a downstream RBT source (i.e. Koocanusa Reservoir) to surrounding tributaries, then one would expect the highest percentage of heterospecific alleles (*I*%) to be in close proximity to Koocanusa Reservoir and that % *I* would decrease at localities further upstream. If the opposite is true, and % *I* is higher further upstream, then an upstream RBT source is more likely. To test if the RBT source is Koocanusa Reservoir, we examined the relationship between % *I* and riverine distance to Koocanusa Reservoir. The values of % *I* across all 23 sites could not be normalized with the appropriate transformations; therefore, we used a non-parametric Spearman Rank Correlation (Zar 1999). We conducted the correlation twice, once with all localities ($n = 23$) and again without localities that were located above upstream migration barriers ($n = 16$) to determine if these barriers were associated with reduced hybridization upstream.

We used two methods to test for patterns in the distribution of hybridization across localities. To determine if localities containing hybrid individuals were found closer together than those that did not contain hybrids (positive spatial autocorrelation) we used a Mantel test (Mantel 1967). We constructed two distance matrices, one based on geographic distance between pairs of localities and the other based on the hybrid state of the pairs of localities. More specifically, the hybrid state matrix was a binary-coded matrix compiled of zeros and ones where 0 = both localities hybridized and 1 = any other combination. We compared the hybrid matrix with a straight-line distance (Euclidian distance) matrix and a fluvial distance matrix. Both fluvial and straight-line distances between all 23 pairs of localities were calculated from ArcView GIS 3.2. We also tested the effects of upstream migration barriers using a partial Mantel test. The partial Mantel test compared the fluvial distance matrix and the hybrid matrix and controlled for a third matrix representing the presence (1) or absence (0) of migration barriers between certain pairs of localities. All tests were carried out using the statistical software R package (Casgrain and Legendre 2001) and 9,999 permutations were conducted for each test.

The second analysis involved a Principal Components Analysis (PCA). We used the PCA to determine if a pattern existed between the physical site characteristics and the presence or absence of RBT hybridization. In addition, because most of the variables were intercorrelated, PCA was used to quantify the independent patterns of variation. If a pattern is revealed it may provide insight into certain locality characteristics that promote or hinder interspecific matings. We collected data on the physical characteristics of each sample stream to determine if any of these were associated with the presence or absence of hybridization. The locality characteristics used in the analysis were stream order, stream magnitude, elevation, stream gradient, and distance to nearest hybridized population. These variables are useful in giving a coarse-grained analysis of environmental influences on hybridization rates, and provide a good starting point for future research on the role of the environment in hybridization between these two species. Stream order and stream magnitude were obtained from the BC government FishWizard website (http://pisces.env.gov.bc.ca). Locality elevations were recorded from topographical maps (1:50,000 scale) and estimated to the nearest 25 m. We calculated the average stream gradient from the change in elevation from the mouth of the stream to the sampling site, and divided by the distance between these two points. The last variable included was the distance to the nearest hybridized neighbouring (NHN) site. This distance was measured using ArcView GIS 3.2. All 23 localities were included in this analysis and the stream magnitude and NHN variables were both square root transformed to remove skewness.

Results

Hybrid detection

Five hundred and sixty-three adults (age 3+), 304 juveniles (age 2+), 96 fingerling (age 1+) and 18

fry (age 0+) were sampled in total. Most fish sampled were between 16 and 35 cm in length (Rubidge 2003).

One hundred and forty-two hybrids (14%) and 33 RBT (3.4%) were identified from the 981 samples collected across sites in both years. The remaining 806 samples were identified as WCT. The percentage of hybrids differed significantly between age class: 8.9% of adults, 23.0% of juveniles, 20.8% of fingerling and 11.1% of fry were identified as hybrids ($\chi^2 = 34.8$, df = 3, $P < 0.0001$).

Field identification of hybrids significantly underestimated the occurrence of hybrid individuals identified genetically ($\chi^2 = 58.5$, df = 1, $P < 0.0001$). Only 28 of the 142 hybrids identified genetically were correctly identified as hybrids in the field. Fifteen fish genetically identified as cutthroat were misidentified in the field as hybrids. Another 14 were recorded as RBT upon capture, and only six of these were confirmed genetically, the other eight were genetically identified as hybrids. These results suggest that the field-based assessment of hybrids used in this study was not very accurate.

The majority of the hybrid individuals were observed in the year 2000 samples (114/625), where 18% of the individuals sampled were identified to be of mixed ancestry compared to only 8% in 1999 (28/356). The majority of the RBT were also found in 2000 (31 in 2000 and only 2 in 1999).

RBT introgression in WCT populations

Eighteen of the 23 localities showed the presence of RBT alleles, leaving only five that showed no evidence of hybridization (Table 2, Figure 2). There was evidence of backcrossing at all 18 localities containing hybrid individuals, indicating that varying levels of introgression has occurred. There was a large range in the percentage of heterospecific alleles present (% *I*) across localities, from less than 1% in upper Skookumchuk Creek to 97.1% in the lower Bull River. A one-way analysis of variance revealed that % *I* differed significantly across localities ($F = 51.04$, $P < 0.001$). The majority of localities containing hybrid individuals (13/18) had less than 10% heterospecific alleles.

Three localities that were sampled both in 1999 and 2000 (lower Gold Creek, lower St. Mary River and upper St. Mary River) showed no significant differences in % *I* between years ($P = 0.53$, $P = 0.71$, respectively, upper St. Mary was 0% *I* in both years), consequently temporal samples were pooled when calculating % *I* for these rivers (Table 2). We found evidence that a naturalized population of RBT exists in the lower Bull River. Twenty-five of the 30 individuals collected from lower Bull River were classified as RBT, and five were hybrids (97.1 % *I*). The next highest value of % *I* was found at Lodgepole Creek (37.5%), a tributary of the Wigwam River, then lower Gold Creek (20.6%), and then Michel Creek (13.1%) on the Elk River system (Figure 2). The only other locality to show more than 10% heterospecific alleles was Bloom Creek (12.2%) a tributary of lower Gold Creek. In fact, in the Gold Creek system, we sampled four areas and there was a striking decrease in hybridization with increasing distance from Koocanusa Reservoir (20.6% in the lower reaches of Gold Creek near the mouth to 2.5% at the site furthest away from the reservoir $r = -0.957$, $P = 0.043$.

No evidence of hybridization was found at 5/23 localities (Findlay Creek; upper St. Mary River, Fording River, upper Elk River, and the upper Bull River; Figure 2). The upper St. Mary River has been sampled extensively (131 fish total) and not one hybrid has been detected. A power analysis revealed virtually 100% confidence in detecting as little as 1% introgression in the upper St. Mary River (Table 2). The lower St. Mary River (below St. Mary Lake), however, has experienced significantly more RBT hybridization ($t = 3.814$, df = 134, $P < 0.0001$).

Spread of hybridization

There was a significant negative correlation between distance to Koocanusa Reservoir and the presence of RBT alleles ($r_s = -0.486$, $P = 0.019$). Further, this relationship remained significant when localities upstream from migration barriers were removed ($r_s = -0.568$, $P = 0.023$).

Spatial analysis

There was positive spatial autocorrelation between the binary hybrid matrix and straight line distance ($r = 0.192$, $P = 0.05$) suggesting that localities with RBT alleles present are clustered geographi-

Table 2. Mean percent heterospecific alleles (% *I*) in westslope cutthroat trout populations throughout the upper Kootenay River drainage

Locality (see Figure 2 for location)	Year (*n*)	Mean % RBT alleles (% *I*)	Power to detect 1% introgression (1–α)
1. Upper Kootenay River mainstem	1999 (15)	5.8	0.70
2. White River	1999 (33)	3.8	0.93
3. Upper Elk River	1999 (38)	0.0	0.95
4. Morrissey Creek	1999 (30)	1.3	0.91
5. Wigwam River	1999 (34)	1.5	0.94
6. Lower Skookumchuk Creek	1999 (33)	3.4	0.93
7. Upper Skookumchuk Creek	1999 (40)	0.7	0.96
8. *Lower St. Mary River	1999 (31)	4.4, 3.8 (pooled mean)	0.92
*Lower St. Mary River	2000 (104)	3.6	>0.99
9. *Upper St. Mary River	1999 (31)	0.0	0.92
*Upper St. Mary River	2000(100)	0.0	>0.99
10. *Lower Gold Creek	1999 (36)	18.4, 20.6 (pooled mean)	0.94
*Lower Gold Creek	2000 (30)	23.3	0.91
11. Bloom Creek	2000 (30)	12.2	0.91
12. Teepee Creek	2000 (30)	2.5	0.91
13. Upper Gold Creek	2000 (30)	2.5	0.91
14. Upper Bull River	1999 (36)	0.0	0.94
15. Lower Bull River	2000 (30)	97.1	0.91
16. Lodgepole Creek	2000 (30)	37.5	0.91
17. Coal Creek	2000 (40)	1.4	0.96
18. Michel Creek	2000 (30)	13.2	0.91
19. Fording River	2000 (30)	0.0	0.91
20. Wild Horse River	2000 (45)	7.5	0.97
21. Mather Creek	2000 (30)	9.7	0.91
22. Lussier River	2000 (30)	6.7	0.91
23. Findlay Creek	2000 (32)	0.0	0.92

*Localities sampled in both years that showed no significant differences in % *I* between years were pooled by locality for calculating % *I*.

cally. Not only distance, however, but also the complexity of stream networks connecting localities appeared to be important in influencing hybridization because a higher correlation co-efficient was observed when comparing fluvial distance and presence of RBT alleles (r = 0.230,

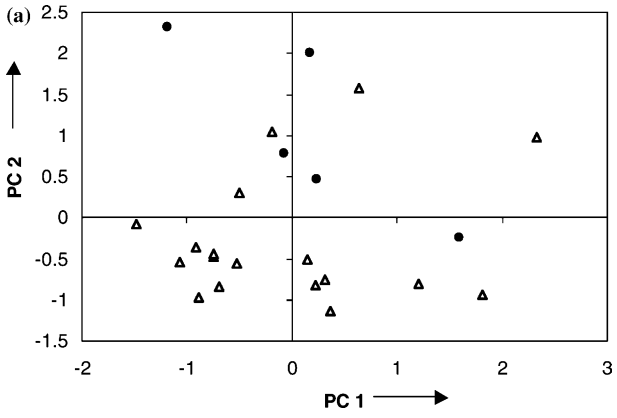

Figure 3. Plot of all 23 localities against values of the two first principal components extracted from the correlation matrix of the locality environmental data. Black circles represent locations with no rainbow trout hybridization; open triangles represent locations with rainbow trout hybridization. PC 1 increases with increasing stream size; PC 2 increases with increasing elevation and nearest hybridized neighbour site (NHN).

$P = 0.032$) than when using straight-line distance. The partial Mantel test that controlled for the presence of upstream migration barriers revealed a slightly stronger positive correlation between fluvial distance and the presence of RBT alleles ($r = 0.259$, $P = 0.023$).

Three principal components that together explained 88.2% of the variation in the environmental data were extracted from the correlation matrix of the five variables (Table 3). Stream order and magnitude loaded heavily on principal component 1 (PC 1) suggesting it was a general "stream size" component. Principal component 2 (PC 2) represents the "isolation" component of the variation among localities because both elevation and NHN loaded heavily on PC 2. Average stream gradient loaded heavily on the third component (PC 3). Genetically pure westslope cutthroat trout ("non-hybridized") localities and those with some RBT alleles ("hybridized") were both characterized by a broad range of stream gradients and sizes, but there was detectable separation between non-hybridized and hybridized localities along PC 2 (Figure 3). Higher values of PC 2 indicate sites that are located at higher elevations and at greater distances from hybridized sites (i.e. more isolated). Four of the five pure WCT localities are located on the positive side of PC 2 axis indicating that more pure populations were sampled at high elevations and further from localities containing hybrid individuals. The one non-hybridized locality with a negative PC 2 score is the upper Bull River, which is located above a migration barrier. Fourteen of the hybridized localities are found in lower elevation areas and closer to neighbouring hybridized localities. Consequently, hybridization appears to be more prevalent at, but not exclusive to, lower elevation streams and rivers because we found a significant difference between the mean values of PC 2 scores for non-hybridized and non-hybridized WCT localities ($t = -3.2$, df = 21, $P = 0.004$).

Table 3. Principal components analysis results of environmental variables for 23 localities showing the loadings of these variables for the first three components after Varimax rotation.

Variable (units)	Loading on		
	PC 1	PC 2	PC 3
Stream order	0.903	−0.017	0.235
Elevation (m)	−0.215	0.843	0.342
Stream gradient (m/km)	0.065	0.034	0.967
NHN (km)	0.293	0.853	−0.243
Stream magnitude	0.906	0.080	0.155
% Cumulative variance	36.6	65.1	88.2

Variables related to stream size loaded on PC 1, elevation and distance to nearest hybridized neighbour site (NHN) loaded heavily on PC 2, and stream gradient loaded heavily on PC 3.

Discussion

Geographic and temporal trends in hybridization

An important component of analyses of hybridization are an understanding of temporal trends in interaction between species. Temporal analysis is important not only to understand the stability of hybrid zones and their potential for spreading, but also because it can differentiate between models that structure hybrid zones as well as identify cases of historical versus contemporary hybridization (Taylor 2004b). We analysed 23 localities in the upper Kootenay River drainage for hybridization between WCT and RBT; 18 of these showed evidence of hybridization and five appeared to be pure westslope cutthroat trout populations. It is important to note that our power to detect 1% heterospecific alleles (Table 2) was similar or greater to that of Leary et al. (1987a), who previously surveyed seven of our localities; their power estimates ranged from 62% in the upper St. Mary River to 99% at Skookumchuk Creek whereas ours ranged from 70% in the upper Kootenay River locality to over 99.9% in the upper St. Mary River locality. Leary et al. (1987a) used two more diagnostic markers than we did (6 versus 4); however, our sample sizes were larger in most cases. A comparison of all available genetic data on WCT × RBT hybridization indicates an increase in the number of hybridized populations in the upper Kootenay River drainage from 1986 to 1999 (Table 4). A detailed population genetic analysis of the hybridizing populations in the upper Kootenay River drainage also supports these findings that hybridization in this drainage is relatively recent (Rubidge and Taylor 2004).

More hybrid fish were detected in the year 2000 than in 1999. This observed pattern does not necessarily mean that the presence of RBT and hybridization has increased over one year, but rather that the expansion of the sampling regime

included more hybridized populations. In rivers that were sampled in both years there was no difference in the percent of heterospecific alleles detected suggesting that the rate of hybridization had not increased over one year (assuming there is no age-specific selection acting against hybrids). In contrast, when examining all samples across populations, there was a detectable difference in proportion of hybrids between age classes; juvenile and fingerling fish had more than twice the proportion of hybrids than the adult age class suggesting that hybridization has increased in recent years (assuming minimal environmental interaction). Field identification of trout using morphology greatly underestimated the number of hybrids in this study. A more rigorous analysis of morphology may have resulted in a higher accuracy of identifying hybrids in the field. For example, Weigel et al. (2003) showed that the most reliable characters in identifying WCT × RBT hybrids are intensity of the lower jaw red slash, basibranchial teeth, spot shape, and ratio of head length to total length (HL:TL). Weigel et al. (2002), however, concluded that a hybridized population had to contain at least 50% RBT admixture to be consistently identified based on these phenotypic characters which reinforces the importance of genetic analysis in less extreme situations.

We found a negative correlation between the degree of hybridization and geographic distance from Koocanusa Reservoir, implying that hybridization is spreading upstream. It thus appears that RBT alleles could spread throughout the drainage unless RBT or hybrids are restricted by physical barriers, or removed via natural selection. Exogenous selection against hybrids at upstream localities (i.e., a reduction in RBT alleles at greater distances from Koocanusa Reservoir, perhaps due to an environmental gradient) would also be consistent with the observed correlation. If selection were causing the pattern, however, we would expect the frequency of RBT alleles to decrease over time in locations upstream of the reservoir, whereas our results show that the frequency of RBT alleles has in fact increased in three upstream sample localities since the previous analysis of Leary et al. (1987a). Therefore, it appears that the RBT introductions into Koocanusa Reservoir from 1986 to 1998 have provided a source population of RBT and subsequent hybrids that are spreading from the reservoir to surrounding areas. Natural hybridization has probably occurred historically between RBT and WCT in other portions of the range of WCT (Leary et al. 1987b; Brown et al. 2004), but our documentation of hybridization at localities where it was previously undetected (Leary et al. 1987a) clearly indicates recent, anthropogenically induced hybridization in the upper Kootenay River. Our results also suggest that migration barriers (hydro dams on the Bull River and the Elk River and a natural impassable canyon on Findlay Creek) limiting RBT or hybrids from moving upstream. Evidence of hybridization in tributaries above the hydro dam on the Elk River; however, is indicative of other RBT sources in the upper Kootenay River system.

Many of the remaining non-hybridized populations throughout the range of WCT are restricted to isolated headwaters (Shepard et al. 1997; Mayhood 1999; Hilderbrand and Kershner 2000), and non-hybridized populations in the upper Kootenay River drainage seem to be no exception. All five localities with no evidence of

Table 4. Summary table for all existing data on percent heterospecific alleles between native westslope cutthroat trout and introduced rainbow trout in seven river systems in the upper Kootenay River drainage in British Columbia

River system	1986 (Leary et al. 1987a) (%)	1999 (%)	2000 (%)
Skookumchuk Cr.	0	3.4	Not sampled
White R.	5.3	3.8	Not sampled
Wigwam R.	0	1.5 (main stem)	37.5 (tributary)
Upper St. Mary R.	0	0	0
Lower St. Mary R.	Not sampled	4.4	3.6
Upper. Elk R.	0	0	Not sampled
Lower Elk R.	Not sampled	1.2	1.3
Upper Bull R.	0	0	Not sampled
Lower Gold Cr.	0	18.4	23.3

introgression with RBT are located on tributaries further upstream from the mainstem upper Kootenay River than their hybridized counterparts. If hybridization continues to spread without any physical or environmental impediment, RBT alleles will likely extend into these upstream areas via RBT dispersal or hybrid trout straying from localities where hybridization has occurred. The upper Bull River and Findlay Creek westslope cutthroat trout, however, are located above impassable physical barriers that prevent access by downstream fish, and are thus are at much lower risk of hybridization as long as rainbow introductions do not occur above the barriers. The other non-hybridized populations (upper St. Mary River, Fording River, and upper Elk River) are not separated by physical barriers from hybridized areas and may be vulnerable to hybridization. Our results, therefore, highlight the likely non-equilibrium dynamics of hybrid zones in fluvial habitats where sources of non-native taxa are spreading both from upstream and downstream sources. In such situations, studies of hybridization conducted over short time periods are unlikely to give a complete picture of the threats to native faunas and what becomes increasingly important is the ability to understand and predict the rate of spread of non-native species.

Environmental correlates of hybridization

We found no evidence of some environmental limitation of hybridization based on stream order, stream magnitude, and stream gradient. The only measured factor that appears to be constraining hybridization in this system is the degree of isolation from other hybridized populations or from Koocanusa Reservoir. Hitt et al. (2003) conducted an analysis of hybridization between WCT and RBT in the Flathead River (Montana) and among several potentially limiting factors (thermal regime, habitat degradation, geomorphology, and location of neighbouring and hybridized populations statistics), only nearest–neighbour data was significantly associated with extent of hybridization. These data are consistent with our study and both, consequently, suggest that the spread of RBT hybridization is facilitated via hybrids straying to neighbouring populations. By contrast, stream width and stream elevation showed positive and negative associations, respectively, with RBT × WCT hybridization in the Clearwater River, Idaho (Weigel et al. 2003). At least some hybridization occurred, however, over a wide range of stream habitat conditions in the Weigel et al. (2003) analysis. This latter observation, coupled with the lack of environmental correlates of hybridization in our study and that of Hitt et al. (2003) all suggest that, other than the presence of migration barriers, little environmental resistance to WCT hybridization with introduced RBT exists.

Although there is little evidence of environmental factors limiting the spread of hybridization, other environmental parameters that we did not measure such as habitat availability and stream flow, may be important in regulating the establishment or spread of naturalized RBT populations. Recently, flow regime was identified as a factor influencing the invasion success of RBT; in particular, a match between timing of fry emergence and months of low flow appears to be associated with successful invasion (Fausch et al. 2001). Evidence also exists that suggests that the availability of winter habitat limits RBT recruitment in the Snake River (Idaho), and that age−0 trout survived only where complex bank habitat was present (Mitro and Zale 2002). Consequently, an analysis of flow regimes and habitat types within the upper Kootenay River drainage may aid in predicting which tributaries are at greatest risk of RBT invasion, a pre-requisite to hybridization with native WCT.

The behaviour of hybrids may also influence the extent of hybridization. For instance, the apparent lack of RBT in most streams and the presence of RBT alleles at 78% of localities may result from hybrids straying from areas where both parental species exist [cf. Henderson et al. 2000 for Yellowstone cutthroat trout (YCT, *O. clarkii bouveri*) × RBT hybrids]. The positive results of the Mantel test, indicating that hybridized localities are found in closer proximity to each other than they are to pure localities, also supports the idea of hybrid straying. The correlation was strengthened using fluvial distance and controlling for the presence of upstream migration barriers. This result strongly supports the importance of connectivity between localities and suggests that hybridization may be facilitated via hybrid straying in the upper Kootenay River drainage (cf. Hitt et al. 2003).

The widespread hybridization between sympatric WCT and non-native RBT in our study and

others (e.g., Hitt et al. 2003; Weigel et al. 2003) contrasts with the situation in coastal areas of western North America where coastal cutthroat trout (CCT, *O. clarkii clarki*) and RBT are more commonly found in natural sympatry. Although hybridization between CCT and RBT is not uncommon there are many instances where sympatric populations show little to no evidence of hybridization (Campton and Utter 1985; Johnson et al. 1999; Young et al. 2001; Docker et al. 2003). Differences between the subspecies of cutthroat trout in their susceptibility to hybridization with RBT may stem from the greater opportunity for evolution of reproductive isolating barriers in sympatric CCT and RBT (Taylor 2004b). In addition, RBT introduced into the range of WCT are of hatchery origin and artificial propagation of RBT may alter any natural patterns of spawning timing or habitat choice that could promote hybridization (Docker et al. 2003). The interaction between native WCT and hatchery-produced, non-native RBT, therefore, illustrate how natural (evolution in allopatry) and human-induced (hatchery production and introductions) can influence hybridization under natural conditions.

Upstream sources of hybridization

Although the majority of hybridized localities were observed in lower elevation areas, Michel Creek, where we found 13.1% heterospecific alleles, is at a higher elevation in the Elk River system that is isolated from the mainstem upper Kootenay River by an impassible upstream barrier. Only two other localities on the Elk River system above the barrier (out of five total) showed evidence of RBT hybridization (Morrissey Creek and Coal Creek), and levels at these localities were much lower (1.5% and 1.2 %, respectively). Both of these localities are found downstream from the Michel Creek, suggesting an upstream RBT source in the Elk River system.

Recently, the BC government ceased the RBT stocking program in Koocanusa Reservoir out of concern for hybridization. Stocking, however, continues in many "landlocked" high-elevation lakes throughout the region (B. Westover, BC MWLAP, Cranbrook, BC, pers. comm. 2003). These high-elevation lakes are often isolated and naturally fishless and are considered to have a low risk of introduced fish dispersing from them to other areas. For instance, BC RBT stocking records show that the closest stocking site to Michel Creek is Summit Lake, a small lake 5 km upstream from the site sampled on Michel Creek. Between 1961 and 1995 nearly 50,000 RBT were released into this lake (BC MWLAP stocking records, unpublished data). Although no pure RBT were found at this locality, the presence of a hybrid individual classified as a RBT backcross suggests that RBT are present (Rubidge 2003). It is likely, however, that some rainbow have spilled out of the lake at some point (possibly during snowmelts when flows are high) and have been swept downstream into Michel Creek. This evidence of hybridization above the hydro dam on the Elk River indicates that ceasing lower elevation RBT introductions will not stop the spread of hybridization in this river system. The most obvious factor preventing upstream migration of non-native fish is the presence of impassable barriers such as waterfalls or high-gradient streams. Stocking above these barriers, however, does not prevent downstream movement of non-native fish into previously inaccessible habitat. Non-native brook trout (*Salvelinus fontinalis*) stocked into headwater lakes have been shown to disperse both downstream and upstream to colonize new habitats (Adams et al. 2001). Physical or velocity barriers to upstream movements could allow non-native fishes access to an entire stream network from even a small number of initial headwater introductions (Adams et al. 2001).

Conservation implications and conclusions

Hybridization between introduced RBT and westslope cutthroat trout appears to have increased and spread since its original documentation in southeastern BC. This increase is most likely a result of the continued and expanded introductions of RBT into the Koocanusa Reservoir and adjacent tributaries. Given the high levels of introgression documented in other drainages (eg., Leary et al. 1984; Hitt et al. 2003; Weigel et al. 2003), and the increase of hybridization documented here, the most obvious step to minimize impacts on native *O. clarkii lewisi* populations would be to cease all RBT introductions into the geographic range of westslope cutthroat trout. Although ceasing exotic RBT introductions may

reduce hybridization, it would not necessarily solve the hybridization issue. In the absence of selection against hybrid genotypes, introgressed westslope cutthroat trout populations will persist indefinitely. Therefore, locating and protecting non-hybridized populations should be the highest priority for resource managers.

Evidence from our study suggests that the environment factors measured do not play a significant role in limiting the spread of hybridization, indicating that populations unaffected by hybridization have most likely avoided it simply because they are more isolated from RBT stocking localities and hybridized populations. Therefore, if RBT introductions continue, all WCT populations are likely vulnerable to hybridization unless they are protected by upstream migration barriers (and this is only effective if there is no upstream source of RBT). For hybridized populations, possible restoration strategies include moving westslope cutthroat trout to isolated headwater reaches, chemical treatment to remove introduced species, and constructing barriers to prevent invasion from downstream non-native trout (e.g., Tews et al. 2000). Although each of these strategies is not without problems (see Leary et al. 1995; Tews et al. 2000), in BC, because hybridization is relatively recent, and introgression levels remain relatively low (13 of 18 populations contained less than 10% heterospecific alleles), they may have some utility.

Hybridization with non-native RBT has been listed as the greatest threat to remaining WCT populations in US and a growing threat in Canada (Leary and Allendorf 1988; Costello and Rubidge 2004). The upper Kootenay River drainage in BC was one of the last areas in the WCT range where populations were thought to be free from RBT hybridization, but our results clearly indicate that this is not the case. Hybridization and introgression have increased and spread to at least nine tributaries of the upper Kootenay River drainage from over recent years (1986–2000). The evolution of both species largely in allopatry appears to have resulted in few intrinsic behavioural or genetic barriers to gene exchange. This situation, coupled with hatchery production of RBT and generally similar environmental preferences between the species have probably promoted hybridization upon artificial secondary contact following widespread introductions of RBT into the native range of WCT. Given the apparent ease of hybridization and introgression between these species, if conservation of native westslope cutthroat trout gene pools is to be a priority, further RBT introductions should not be permitted. In addition, identification of further populations of WCT free from introgression should be undertaken and they should be given high priority for conservation. Our results emphasize the temporally dynamic nature of hybridization especially when it involves introduction of a non-native species. In such cases, it is very important to incorporate appropriate temporal sampling to gain a more realistic understanding of the state of interaction between species.

Acknowledgements

We would like to thank P. Corbett, S. Bennett, J. Addison (Mirkwood Ecological Consulting Ltd.) and J. Baxter (Baxter Environmental) among others, for assisting in the collection of the tissue samples used in this study. Conversations with B. Westover (Ministry of Land, Water and Air Protection, Cranbrook, BC) provided useful information about past and current RBT stocking practices in the upper Kootenay River drainage. Early versions of this manuscript were improved by the helpful suggestions of A. B. Costello, S. Otto, D. Schluter, R. Waples, and three anonymous reviewers. This study was funded in part by the Columbia Kootenay Fisheries Renewal Partnership and Global Forest (GF−18–2001–170) as well as by the Natural Sciences and Engineering Research Council of Canada.

References

Adams SB, Frissell CA, Rieman BE (2001) Geography of invasion in mountain streams: Consequences of headwater lake fish introductions. *Ecosystems*, **4**, 296–307.

Allendorf FW, Leary RF (1988) Conservation and distribution of genetic variation in a polytypic species, the cutthroat trout. *Conserv. Biol.*, **2**, 170–184.

Allendorf FW, Leary RF, Hitt NP, Knudsen KL, Lindquist LL, 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.

Baker J, Bentzen P, Moran P (2002) Molecular markers distinguish coastal cutthroat trout from coastal rainbow trout/steelhead and their hybrids. *Trans. Am. Fish. Soc.*, **131**, 404–417.

Boecklen WJ, Howard DJ (1997) Genetic analysis of hybrid zones: numbers of markers and power of resolution. *Ecology*, **78**, 2611–2616.

Bozek MA, Rahel FJ (1991) Assessing habitat requirements of young Colorado River cutthroat trout by use of macrohabitat and microhabitat analyses. *Trans. Am. Fish. Soc.*, **120**, 571–581.

Brown KH, Patton SJ, Martin KE, Nichols KM, Armstrong R, Thorgaard GH (2004) Genetic analysis of interior Pacific Northwest *Oncorhynchus mykiss* reveals apparent ancient hybridization with westslope cutthroat trout. *Trans. Am. Fish. Soc.*, **133**, 1078–1088.

Bruton MN (1995) Have fishes had their chips? The dilemma of threatened fishes *Environ. Biol. Fishes*, **43**, 1–27.

Busack CA, Gall GAE (1981) Introgressive hybridization in populations of Paiute cutthroat trout (*Salmo clarki seleniris*). *Can. J. Fish. Aquat. Sci.*, **38**, 939–951.

Campbell MR, Dillon J, Powell MS (2002) Hybridization and introgression in a managed, native population of Yellowstone cutthroat trout: Genetic detection and management implications. *Trans. Am. Fish. Soc.*, **131**, 364–375.

Campton DE, Utter FM (1985) Natural hybridization between steelhead trout (*Salmo gairdneri*) and coastal cutthroat trout (*S.clarki*) in two Puget Sound streams. *Can. J. Fish. Aquat. Sci.*, **42**, 110–119.

Carmichael GJ, Hanson JN, Schmidt ME, Morizot DC (1993) Introgression among Apache, cutthroat and rainbow trout in Arizona. *Trans. Am. Fish. Soc.*, **122**, 121–130.

Casgrain P, Legendre P (2001) The R Package for multivariate and spatial analysis, version 4.03 — User's Manual. Departement des sciences biologiques, Universite de Montreal. www.fas.umontreal.ca/BIOL/legendre.

Costello AB, Rubidge E (2004) Status review of cutthroat trout (Oncorhynchus clarki spp.) in Canada. Report for the Committee on the Status of Endangered Wildlife In Canada. Ottawa, Ontario.

Docker MF, Dale A, Heath DD (2003) Erosion of interspecific reproductive barriers resulting from hatchery supplementation of rainbow trout sympatric with cutthroat trout. *Mol. Ecol.*, **12**, 3515–3521.

Fausch KD, Taniguchi Y, Nakano S, Grossman GD, Townsend CR (2001) Flood disturbance regimes influence rainbow trout invasions success among five holarctic regions. *Ecol. Appl.*, **11**, 1438–1455.

Hartman GF, Gill CA (1968) Distributions of juvenile steelhead and cutthroat trout, *Salmo gairdneri* and *S.clarkii clarki* within streams in Southwestern. *B.C. J. Fish. Res. Bd. Can.*, **25**, 33–48.

Henderson R, Kershner JL, Toline CL (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. *North Am. J. Fish. Manage.*, **20**, 584–596.

Hilderbrand RH, Kershner JL (2000) Conserving inland cutthroat trout in small streams: How much stream is enough? *North Am. J. Fish. Manage.*, **20**, 513–520.

Hitt NP, Frissell CA, Muhlfeld CC, Allendorf FM (2003) Spread of hybridization between native westslope cutthroat trout, *Oncorhynchus clarki clarki*, and nonnative rainbow trout, *Oncorhynchus mykiss*. *Can. J. Fish. Aquat. Sci.*, **60**, 1440–1451.

Johnson OW, Ruckelshaus MH, Grant WS, Waknitz FW, Gharrett AM, Bryant GJ, Neely K, Hard JJ (1999) Status review of coastal cutthroat trout from Washington, Oregon and California. NOAA Tech. Mem. NMFS-NWFSC−37.

Kanda N, Leary RF, Spruell P, Allendorf FW (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**, 312–319.

Leary RF, Allendorf FW, Phelps SR, Knudsen KL (1984) Introgression between westslope cutthroat and rainbow trout in the Clark Fork River drainage. *Proc. Montana Acad. Sci.*, **43**, 1–18.

Leary RF, Allendorf FW, Knudsen KL (1987a) Genetic divergence among populations of westslope cutthroat trout in the upper Kootenay River drainage, British Columbia. *Populations Genetics Laboratory Report Univ. of Montana*, **87**, 1–17.

Leary RF, Allendorf FW, Phelps SR, Knudsen KL (1987b) Genetic divergence among seven species of cutthroat trout and rainbow trout. *Trans. Am. Fish. Soc.*, **116**, 580–587.

Leary RF, Allendorf FW, Sage GK (1995) Hybridization and introgression between introduced and native fish. *Am. Fish. Soc. Symp.*, **15**, 91–101.

Liknes GA, Graham PJ (1988) Westlope cutthroat trout in Montana: Life history, status and management. *Am. Fish. Soc. Symp.*, **4**, 53–60.

Mantel N (1967) The detection of disease clustering and a generalized regression approach *Cancer Res.*, **27**, 209–220.

Mayhood DW, (1999) Provisional evaluation of the status of westslope cutthroat trout in Canada. In: Proceedings of the Biology and Management of Species and Habitats at Risk (ed. Darling LM), http://www.fwresearch.ab.ca/library, Kamloops, B.C.

Miller RR, Williams JD, Williams JE (1989) Extinctions of North American fishes during the past century. *Fisheries*, **14**, 22–38.

Mitro MG, Zale AV (2002) Seasonal survival, movement and habitat use of age−0 rainbow trout in the Henrys Fork of the Snake River, Idaho. *Trans. Am. Fish. Soc.*, **131**, 271–286.

Ostberg C, Rodriguez J (2002) Novel molecular markers differentiate between *Oncorhynchus mykiss* (rainbow trout and steelhead) and the *O. clarki* (cutthroat trout) subspecies. *Mol. Ecol. Notes*, **2**, 197–202.

Paul AJ, Post JR (2001) Spatial distribution of native and nonnative salmonids in streams of the eastern slopes of the Canadian Rocky Mountains. *Trans. Am. Fish. Soc.*, **130**, 417–430.

Rhymer JM, Simberloff D (1996) Extinction by hybridization and introgression. *Ann. Rev. Ecol. Syst*., **27**, 83–109.

Ricciardi A, Rasmussen JB (1999) Extinction rates of North American freshwater fauna. *Conserv. Biol*., **13**, 1220–1222.

Rubidge E ,(2003) Molecular Analysis of Hybridization between Native Westslope Cutthroat Trout (*Oncorhynchus clarkii lewisi*) and Introduced Rainbow Trout (*O. mykiss*) in Southeastern British Columbia. MSc Thesis, University of British Columbia.

Rubidge E, Taylor EB (2004) Hybrid zone structure and the potential role of selection in hybridizing populations of native westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) and introduced rainbow trout (*O. mykiss*). Mol. Ecol., 13, 3735–3749.

Rubidge E, Corbett P, Taylor EB (2001) A molecular analysis of hybridization between native westslope cutthroat trout and introduced rainbow trout in southeastern British Columbia, Canada. *J. Fish Biol*., **59**, 42–54.

Shepard BB, Sanborn B, Ulmer L, Lee DD (1997) Status and risk of extinction for westslope cutthroat trout in the Upper Missouri River Basin, Montana. *North Am. J. Fish. Manage*., **17**, 1158–1172.

Shepard BB, May BE, Urie W (2002) Status of westslope cutthroat trout (*Oncorhynchus clarkii lewisi*) in the United States: 2002. Montana Fish, Wildlife and Parks, Bozeman, MT.

Taylor EB (2004a) An analysis of homogenization and differentiation of Canadian freshwater fish faunas with an emphasis on British Columbia. Can. J. Fish. Aquat. Sci., 61: 68–79.

Taylor EB (2004b) Evolution in mixed company In: Evolution Illuminated. Salmon and their Relatives (eds. AP Hendry SC Stearns), pp. 232–263. Oxford University Press, Oxford.

Tews A, Enk M, Hill W, Dalbey S, Liknes GA (2000) Westslope cutthroat trout (*Oncorhynchus clarkii lewisi*). In: North central Montana Status and Restoration Strategies. Special Report by Montana, Fish Wildlife and Parks in collaboration with Lewis and Clark National Forest.

Trotter PC1987Cutthroat Native Trout of the WestColorado Associated University PressBoulder, Colorado.

Utter FM, Allendorf FW (1994) Phylogenetic relationships among species of *Oncorhynchus*: a consensus view. *Conserv. Biol*., **8**, 864–867.

Weigel DE, Peterson JT, Spruell P (2002) A model using phenotypic characters to detect introgressive hybridization in wild westslope cutthroat trout and rainbow trout. *Trans. Am. Fish. Soc*., **131**, 389–403.

Weigel DE, Peterson JT, Spruell P (2003) Introgressive hybridization between native cutthroat trout and introduced rainbow trout. *Ecol. Appl*., **13**, 38–50.

Williams JE, Miller RR (1990) Conservation status of the North American fish fauna in fresh water. *J. Fish Biol*., **37**, 79–85.

Young WP, Ostberg CO, Keim P, Thorgaard GH (2001) Genetic characterization of hybridization and introgression between anadromous rainbow trout (*Oncorhynchus mykiss irideus*) and coastal cutthroat trout (*O. clarkii clarki*). *Mol. Ecol*., **10**, 921–930.

Zar, J.H. (ed.) (1999) *Biostatistical Analysis*, 4th ednth edn. Prentice-Hall Inc, Upper Saddle River, New Jersey, USA.