Local Habitat, Watershed, and Biotic Factors Influencing the Spread of Hybridization between Native Westslope Cutthroat Trout and Introduced Rainbow Trout

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Abstract.—The invasion of nonnative fishes in freshwater systems is often facilitated by the interaction of biotic and abiotic factors operating at multiple spatial and temporal scales. We evaluated the association of local habitat features (width, gradient, and elevation), watershed characteristics (mean and maximum summer water temperatures, the number of road crossings, and road density), and biotic factors (the distance to the source of hybridization and trout density) with the spread of hybridization between native westslope cutthroat trout *Oncorhynchus clarkii lewisi* and introduced rainbow trout *O. mykiss* in the upper Flathead River system in Montana and British Columbia. The presence of hybridization and the proportion of rainbow trout admixture were estimated using seven diagnostic microsatellite loci. We defined logistic and linear regression models including various combinations of spatial and environmental factors and used an information-theoretic approach to evaluate the relative plausibility of these models. Models combining measures of water temperature, disturbance, and source connectivity were the best-approximating ones for the presence of hybridization. Hybridization was positively associated with mean summer water temperature and the number of upstream road crossings and negatively associated with the distance to the main source of hybridization. The best-approximating models associated with the level of introgression among hybridized sites included measures of temperature, source connectivity, and the density of trout. The proportion of rainbow trout admixture was negatively related to the distance to the source and positively related to mean summer water temperature and density. Our results suggest that hybridization is more likely to occur and spread in streams with warm water temperatures, increased land use disturbance, and proximity to the main source of hybridization. However, habitat features alone may not limit the spread of hybridization; populations with high proportions of admixture and high densities may have to be reduced or eliminated.

Exotic species are one of the greatest threats to global biodiversity and are a major concern in the conservation of freshwater ecosystems (Mack et al. 2000; Rahel 2000). Human disturbances of the landscape, such as intentional and accidental species translocations and habitat alterations, often create secondary contact between previously isolated species (Allendorf et al. 2001). In many cases, nonnative species are implicated in the decline and extinction of native biota through competition, predation, the spread of disease and parasites, and hybridization and introgression (Pimm 1989; Rahel 2000).

The invasion success of introduced species is often influenced by the interaction of abiotic and biotic factors operating at multiple spatial and temporal scales. In freshwaters, the major factors associated with the invasion and establishment of nonnative fishes include habitat conditions (local and watershed), connectivity, biotic resistance, and evolutionary history (Dunham et al. 2002; Benjamin et al. 2007; Fausch 2008). Water temperature plays a major role in determining the distribution and abundance of

stream-dwelling salmonid species and has been correlated with invasion success in freshwater systems (Paul and Post 2001; Dunham et al. 2003; McMahon et al. 2007). Human-mediated habitat disturbances that increase stream temperatures and degrade riparian and stream habitats have also been correlated with the invasions of nonnative species (Thurow et al. 1997). Furthermore, theoretical models and empirical evidence suggest that the invasion and spread of nonnative species is freshwaters is strongly related to stream connectivity and the proximity of native populations to nonnative sources. However, little information is available as to the interactive role of these factors in determining the invasion of nonnative salmonids in freshwater systems.

Hybridization can be a major consequence of species introductions, especially in circumstances in which nonnative species hybridize with rare or endangered taxa and thus threaten the persistence of those taxa. Introgressive hybridization is more common in fish than in any other vertebrate taxa. This is particularly true for salmonids, for which widespread introgression among nonnative and native taxa has often created hybrid swarms over extensive geographical areas (Allendorf and Leary 1988; Leary et al. 1995; Allendorf et al. 2001). Additionally, interspecific hybridization may cause outbreeding depression as a result of the break-up of coadapted gene complexes and the disruption of local adaptations (Templeton 1986; Barton and Hewitt 1989; Rhymer and Simberloff 1996). Thus, hybridization is considered a leading cause of the decline and extinction of many freshwater fishes throughout North America (Miller et al. 1989).

Hybridization and introgression with introduced rainbow trout *Oncorhynchus mykiss* are considered the greatest threats facing many native populations of cutthroat trout *O. clarkii* in western North America (Behnke 1992; Leary et al. 1995). Introgressive hybridization with introduced rainbow trout has been especially detrimental to native westslope cutthroat trout *O. clarkii lewisi*, threatening this highly divergent subspecies with genomic extinction (Allendorf and Leary 1988; Allendorf et al. 2001). Nonhybridized populations of westslope cutthroat trout persist in less than 10% of their historical range in the United States (Shepard et al. 2005) and less than 20% of their range in Canada (COSEWIC 2006). Consequently, many remaining populations are restricted to small, fragmented headwater habitats, where the long-term sustainability of these populations is uncertain (Hilderbrand and Kershner 2000).

The upper Flathead River system is considered a regional and rangewide stronghold for nonhybridized westslope cutthroat trout. Hybridization with introduced, nonnative rainbow trout, however, has led to a rapid spread of introgression (Hitt et al. 2003; Boyer et al. 2008), threatening the genetic and ecological characteristics of the migratory and resident populations that have persisted in the basin since the last glacial period (~14,000 years ago). This study was intended to examine the local habitat features, watershed characteristics, and biotic factors associated with the occurrence (presence or absence) and amount of hybridization (proportion of rainbow trout admixture) between native westslope cutthroat trout and nonnative rainbow trout in the upper Flathead River drainage from the headwaters of the North Fork Flathead River in Canada downstream to the mainstem Flathead River upstream of Flathead Lake. We hypothesized that hybridization would be more likely in warmer, low-elevation streams in close proximity to hybridized populations with high proportions of rainbow trout admixture. Alternatively, we predicted that westslope cutthroat trout would be more common in headwater streams characterized by colder water temperatures, less land disturbance, and greater distances from hybridized source populations. Finally, we hypothesized that the proportion of rainbow trout admixture in hybridized populations would be associated with water temperature, the density of trout *Oncorhynchus* spp., and source connectivity. Our objectives were to examine the occurrence and extent of rainbow trout introgression in relation to these abiotic and biotic factors. Understanding the factors influencing the distribution and spread of hybridization will enable fisheries managers to focus conservation and management programs for westslope cutthroat trout and other salmonids threatened with the loss of genetic integrity.

Methods

Study area.—The study area included the tributaries to the North Fork and main-stem Flathead rivers in northwestern Montana and southeastern British Columbia. The North Fork Flathead River originates in the Rocky Mountains of southeastern British Columbia and flows into northwestern Montana, where it forms the western border of Glacier National Park before joining the main-stem Flathead River, which flows into Flathead Lake (Figure 1). This interconnected drainage contains migratory and resident populations of westslope cutthroat trout, a species of special concern in Montana and a blue-listed species at risk in British Columbia. Adfluvial and fluvial populations migrate from Flathead Lake and the Flathead River, respectively, to spawn in streams within the North Fork and Middle Fork drainages (Muhlfeld et al. 2009b).

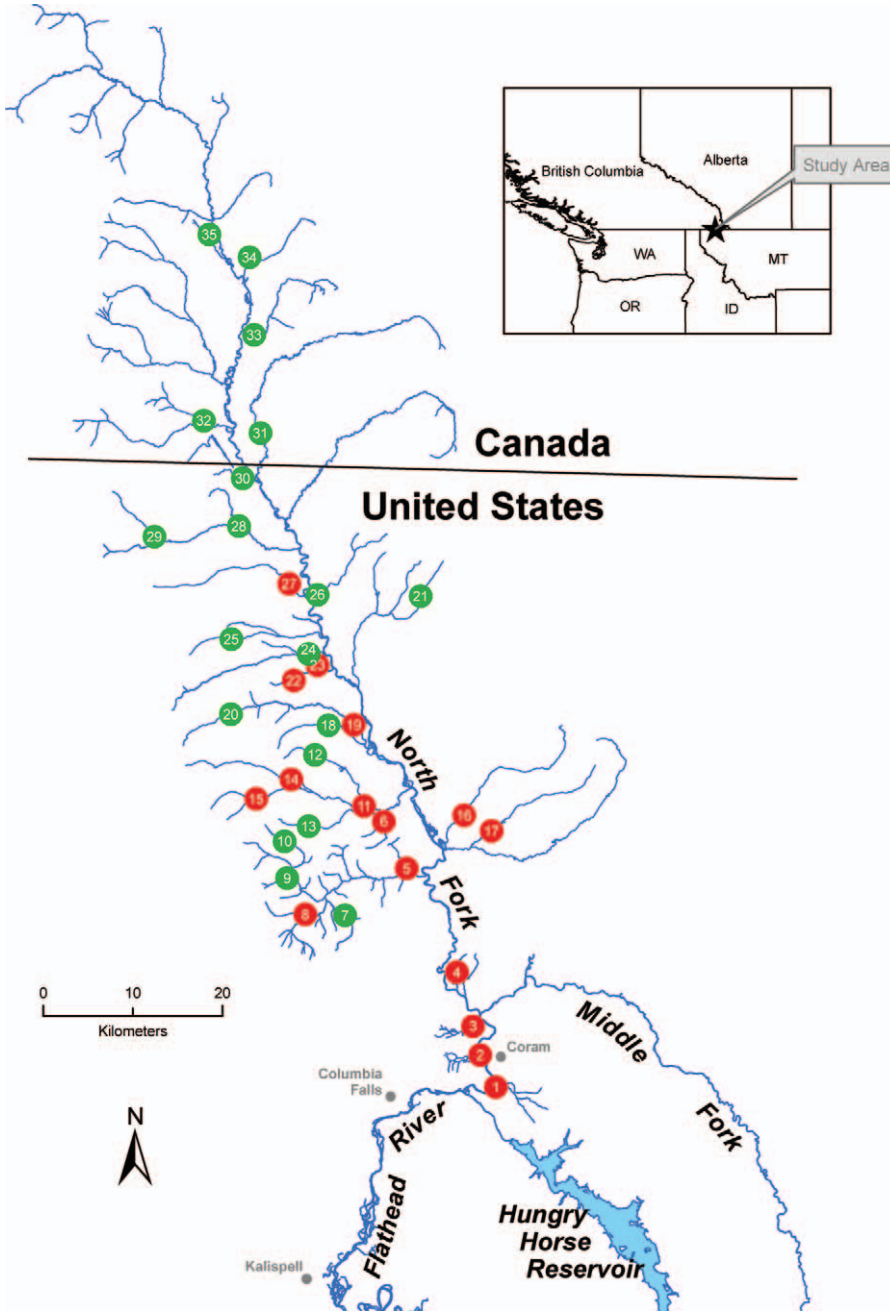Recent studies in the Flathead River drainage have

FIGURE 1.—Study area and sampling sites with hybridized (red) and nonhybridized (green) populations. The sampling site codes correspond to those in Table 1.

shown that hybridization is spreading upstream from source populations with high levels of rainbow trout ancestry. Hitt et al. (2003) found evidence of rainbow trout introgression in 7 of 11 populations that were determined to be nonhybridized in 1984, suggesting that hybridization has recently spread upstream in this system. In addition, these authors showed that the presence of hybridization was more strongly associated with neighborhood characteristics (i.e., distance and spatial attributes) than with environmental gradients.

However, their study did not assess how environmental and biotic factors influence the amount of nonnative rainbow trout introgression, nor did it include samples collected in the headwaters in Canada. Furthermore, recent genetics data (Boyer et al. 2008) and radiotelemetry studies (Muhlfeld et al., in press) indicate that the major source of hybridization in the system is Abbot Creek, a tributary to the main stem that contains a hybrid swarm with a high proportion (0.92) of rainbow trout admixture (Boyer et al. 2008). This stream is also located about 5 km downriver of a former private rainbow trout hatchery (Sekokini Springs), and anecdotal evidence suggests that approximately 70,000 rainbow trout were illegally released in 1997 when operations ceased (B. Marotz, Montana Fish, Wildlife and Parks, personal communication). Boyer et al. (2008) found that the amount of admixture tended to decrease with distance upstream from Abbot Creek, but no other abiotic or biotic factors were considered in the analysis. In this study, we expand on this research by using recent microsatellite DNA data to understand the relative importance of abiotic and biotic factors influencing both the presence/absence (occurrence) and degree of hybridization (proportion of rainbow trout admixture) throughout the interconnected river system.

Study design and data collection.—Fish population and habitat data were collected at 35 sites in the upper Flathead River system in Montana and British Columbia (Table 1; Figure 1). Streams were sampled during the low-flow period (July–September) from 2004 through 2007, and genetic samples were collected in 2003 and 2004 (Boyer et al. 2008). All sample sites were located downstream of physical barriers to fish migration. Migratory cutthroat and rainbow trout, therefore, could have theoretically accessed each site within the interconnected study area. Sampling occurred throughout the system and represented the full range of environmental and geographic variation within it (Figure 1).

Dependent variables.—We used the microsatellite DNA data reported by Boyer et al. (2008) to determine the occurrence of hybridization and the proportion of rainbow trout admixture for each site using seven diagnostic microsatellite loci. Fish were captured by electrofishing in stream reaches ranging from 250 m to 1 km in length to minimize the sampling of related individuals. Total lengths were recorded, and a portion of the fish tissue was excised and stored in a 95% solution of ethanol. The vast majority of sampled trout were less than 200 mm in length (i.e., age 1 and age 2). Population admixture was calculated as the proportion of nonnative rainbow trout alleles found among individuals within a population. Hybridization was declared present in a tributary if rainbow trout alleles were detected in the sample at one or more loci. A sample was considered to consist of nonhybridized westslope cutthroat trout if no rainbow trout alleles were detected; the power to detect rainbow trout genetic contributions as small as 1% in a hybrid swarm was at least 0.94 with our techniques (Boecklen and Howard 1997).

Biotic variables.—We examined the influence of two biotic metrics, trout density and distance to the source of hybridization, on the occurrence and degree of hybridization. We considered the distance to Abbot Creek as a measure of stream connectivity to the source of hybridization in the system and trout density as a measure of the influence of demographic support in facilitating or reducing the likelihood of hybridization at each site. The stream distance from the mouth of Abbot Creek to each sample site was measured in ArcGIS 9.2 (ESRI, Redlands, California).

Trout densities were estimated in the same sections 1–2 years after the genetics sampling. Abundance estimates were conducted in 150-m sections using the multiple-pass depletion method (Zippin 1958). A hydrologic break (e.g., a riffle or vertical drop) was selected for the upper boundary, and a block net (12.7-mm mesh) was placed across the channel at the lower boundary before sampling. A minimum of three passes were completed in each section with one or two backpack electrofishing units (Smith-Root Model 15–D) working from the upstream boundary downstream to the block net. The total lengths (mm) of all captured trout were recorded. Based on length-at-age data for the upper Flathead River system (C. Muhlfeld, unpublished data), individuals less than 75 mm were considered young-of-the-year fish. These individuals were not included in the abundance estimates owing to poor sampling efficiency and variable emergence times across streams. Ten wetted widths were systematically taken every 15 m through the sample section and were used to calculate the wetted stream surface area. Population estimates were calculated using the depletion model in the MICROFISH 3.0 computer program (Van Deventer and Platts 1985), which estimates abundance from the counts and capture probabilities derived from the multi-pass sampling. Although the removal method typically produces biased and variably underestimated population density or abundance estimates, we accounted for this by maintaining similar capture probabilities across sites. Trout density (fish/ m^2) was calculated by dividing the estimate of fish population by the wetted stream surface area. At 11 of the 35 sites, abundance was estimated in more than one year. In these situations, we averaged the densities across years. Georeferenced locations were obtained at

TABLE 1.—Summary of the local habitat features, watershed characteristics, and biotic factors in each study site in the upper Flathead River drainage.

Site		Local habitat features			Watershed characteristics			
Name	Number	Gradient (%)	Elevation (m)	Stream width (m)	Maximum temperature (°C)	Mean summer temperature (°C)	Road density	Road crossings
Abbott	1	0.01	950	2.64	20.2	15.00	0.70	24
Ivy	2	0.07	977	2.03	12.6	10.60	0.50	4
Rabe	3	0.04	996	3.42	16.2	12.40	1.20	11
Third	4	0.03	962	2.18	13.3	10.70	0.07	0
Langford	5	0.02	1,130	2.56	10.8	9.40	0.43	8
Meadow	6	0.03	1,134	2.15	19.4	14.00	0.24	4
Skookoleel	7	0.08	1,200	6.10	11.8	8.70	0.49	6
Nicola	8	0.07	1,280	3.90	9.7	7.60	1.32	9
Werner	9	0.06	1,303	5.98	11.1	8.30	1.22	9
Kletomus	10	0.09	1,390	4.20	12.5	9.10	0.45	0
Cyclone, lower	11	0.02	1,260	3.78	18.6	13.10	0.83	14
Cyclone, upper	12	0.07	1,430	11.67			0.44	3
Deadhorse	13	0.04	1,260	3.60	13.7	9.80	0.42	6
North Fork Coal	14	0.03	1,259	2.30	13.9	10.00	0.71	33
South Fork Coal	15	0.05	1,340	6.50	14.1	10.20	0.45	6
Anaconda	16	0.05	1,110	5.05	16.3	12.00	0.02	1
Dutch	17	0.02	1,110	4.71	16.8	12.60	0.03	1
Moran	18	0.05	1,230	3.70	13.2	9.60	0.65	9
Hay Creek, lower	19	0.02	1,090	6.70	13.0	10.10	0.42	14
Hay Creek, upper	20	0.04	1,430	6.70	11.2	8.50	0.32	5
Akokala	21	0.03	1,340	6.30	14.8	10.90	0.03	0
South Fork Red Meadow	22	0.03	1,240	2.40	12.3	9.20	0.31	2
Red Meadow	23	0.03	1,150	7.40	15.5	11.90	0.59	31
Hawk	24	0.02	1,176	1.30	14.6	10.10	1.56	5
Moose	25	0.02	1,130	4.00	10.1	7.60	0.52	11
Ford	26	0.03	1,154	4.03	15.9	11.20	0.00	0
Tepee	27	0.03	1,210	3.80	17.7	11.80	1.05	17
Ketchikan	28	0.02	1,278	3.15	13.8	10.10	0.04	0
Tuchuck	29	0.03	1,536	5.70	12.1	9.10	0.04	1
Colts	30	0.06	1,239	3.78	12.6	9.60	0.24	0
Sage	31	0.00	1,280	13.60	12.7	10.60	0.29	29
Burnham	32	0.05	1,273	3.19	16.5	11.40	0.59	14
Commerce	33	0.02	1,334	5.92	14.3	11.20	0.29	4
Middlepass	34	0.05	1,405	5.04	11.2	9.20	0.16	8
Parker	35	0.04	1,395	4.54	8.4	6.60	0.82	1

the upstream limit of each sample section using a Global Positioning System unit (TSC1 Asset Surveyor; Trimble Navigation, Sunnyvale, California).

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

Watershed variables included measures of stream temperature and land disturbance. Thermographs were deployed at each site to record water temperatures hourly during the year in which the abundance estimates were made. The water temperature metrics used were the mean and maximum summer temperatures. The mean summer temperature was calculated as the mean of the daily averages from 1 July to 30 September. The maximum water temperature at each

site was the highest recorded temperature during the sampling period. Temperature data were unavailable for one site (upper Cyclone Creek).

Road density metrics were used as indicators of land use disturbance. Roads can alter the hydrologic and geomorphic regimes in downstream areas (Trombulak and Frissell 2000), and measures of road density and stream crossings have been correlated with the spatial extent of timber harvest activity in the Flathead River system (Hauer and Blum 1991). Therefore, we estimated road density and the number of road–stream intersections upstream of each site (Baxter et al. 1999) from the U.S. Forest Service's Flathead National Forest Infrastructure Application (INFRA) database in ArcGIS.

Data analysis.—We first tested for differences ($P <$ 0.10) between the hybridized and nonhybridized sites for each independent variable using Mann–Whitney U-tests. We used logistic and linear regression analyses to evaluate the associations between the nine independent

TABLE 1.—Extended.

Site		Biotic factors			
Name	Number	Distance to source (km)	Hybridization present	Trout density (fish/m^2)	% Rainbow trout admixture
Abbott	1	0.0	Yes	0.16	91.6
Ivy	2	6.4	Yes	0.08	49.3
Rabe	3	13.9	Yes	0.22	49.1
Third	4	16.9	Yes	0.19	65.8
Langford	5	40.3	Yes	0.12	33.1
Meadow	6	58.3	Yes	0.06	3.5
Skookoleel	7	54.2	No	0.04	0.0
Nicola	8	55.1	Yes	0.07	1.8
Werner	9	56.0	No	0.08	0.0
Kletomus	10	62.7	No	0.10	0.0
Cyclone, lower	11	59.7	Yes	0.07	11.6
Cyclone, upper	12	59.7	No	0.05	0.0
Deadhorse	13	67.9	No	0.14	0.0
North Fork Coal	14	67.9	Yes	0.23	7.3
South Fork Coal	15	74.6	Yes	0.02	0.6
Anaconda	16	48.3	Yes	0.07	20.6
Dutch	17	49.3	Yes	0.04	13.0
Moran	18	64.4	No	0.06	0.0
Hay Creek, lower	19	64.7	Yes	0.05	1.4
Hay Creek, upper	20	81.0	No	0.07	0.0
Akokala	21	86.8	No	0.01	0.0
South Fork Red Meadow	22	77.2	Yes	0.07	0.3
Red Meadow	23	75.0	Yes	0.15	2.2
Hawk	24	74.1	No	0.10	0.0
Moose	25	89.6	No	0.12	0.0
Ford	26	84.7	No	0.08	0.0
Tepee	27	87.7	Yes	0.05	1.3
Ketchikan	28	103.3	No	0.22	0.0
Tuchuck	29	108.4	No	0.11	0.0
Colts	30	107.0	No	0.08	0.0
Sage	31	114.1	No	0.01	0.0
Burnham	32	116.4	No	0.03	0.0
Commerce	33	130.7	No	0.05	0.0
Middlepass	34	139.5	No	0.05	0.0
Parker	35	143.7	No	0.05	0.0

variables and the occurrence (presence/absence) of hybridization among all study sites and the proportion of rainbow trout admixture among hybridized sites, respectively. First, we attempted to reduce the number of independent variables in the final variable sets to avoid potential model selection biases caused by such large candidate model sets (Ramsey and Schafer 2002; Taper 2004; Kutner et al. 2004). Therefore, for the logistic and linear regression analyses we included all nine variables in both forward and backward stepwise regression processes and included all of the variables selected in the first model selection as the final variable set, regardless of whether or not they were retained in the final model. For the logistic regression analysis, the stepwise model included stream width, mean summer water temperature, the number of road crossings, and the distance to the source of hybridization, whereas in the linear regression analysis mean summer water temperature, stream width, the distance to the source, and trout density were included.

Next, we used all subsets of the logistic and linear regression models (a priori) representing all possible combinations of the four remaining variables in each analysis and employed an information-theoretic approach to evaluate the relative plausibility of the competing models (Burnham and Anderson 2002). A Hosmer–Lemeshow goodness-of-fit test of the global model (including all factors) indicated that the logistic model provided a good fit to the presence/absence data. Therefore, we used Akaike's information criterion (Akaike 1973) with adjustment for small sample size (AIC$_c$; Hurvich and Tsai 1989) to rank the competing models relative to the one with the lowest score. Models were considered equally plausible if their AIC$_c$ scores were within 2.0 of that of the best model (Burnham and Anderson 2002). The classification cutoff was 0.5 for each logistic model, and all models included a constant and an error term. For the linear regression analysis, it was necessary to perform a logit transformation on the proportion of rainbow trout

TABLE 2.—Model selection results for candidate logistic regression models with various combinations of local habitat features (stream width), watershed characteristics (mean summer water temperature and number of upstream road crossings), and biotic factors (distance to the source of hybridization) in relation to the occurrence of hybridization between native westslope cutthroat trout and nonnative rainbow trout at 35 sites in the upper Flathead River drainage. The number of parameters (k) includes intercept and error terms. Models were ranked according to their corrected Akaike information criterion values (AIC_c).

Model	k	AIC_c	ΔAIC_c
Mean temperature, distance to source, number of road crossings	5	28.98	0.00
Mean temperature, distance to source	4	29.02	0.04
Mean temperature, distance to source, width	5	32.02	3.04
Mean temperature, distance to source, number of road crossings, width	6	32.12	3.14
Distance to source, number of road crossings	4	32.67	3.69
Distance to source, number of road crossings, width	5	33.48	4.50
Distance to source	3	35.32	6.34
Distance to source, width	4	36.73	7.75
Mean temperature	3	42.05	13.07
Mean temperature, number of road crossings, width	5	42.46	13.48
Mean temperature, width	4	42.70	13.72
Mean temperature, number of road crossings	4	43.46	14.48
Number of road crossings, width	4	45.35	16.37
Width	3	48.69	19.71
Number of road crossings	3	49.28	20.30

admixture in order to meet the assumptions of normality and homogeneity of variance and account for the correct variation behavior of the proportional data (i.e., using multiple alleles across all fish in each sample). The final variable selection and model development followed the same procedures as for the logistic regression analysis.

Results

A total of 971 individuals were collected from 35 locations in 33 streams (mean per stream, 28; SD, 7). Nineteen of the 35 locations (54%) showed no evidence of rainbow trout introgression (Table 1; Figure 1). Streams with hybrid populations were smaller (mean width, 3.9 m; range, 2.0–7.4 m) and lower in elevation (mean, 1,137 m; range, 950–1,280 m) than streams with nonhybridized westslope cutthroat trout (mean width, 5.4 m; range, 1.3–13.6 m [Mann-Whitney $U = 97.5$, $P = 0.07$]; mean elevation,

TABLE 3.—Coefficients (B) and standard errors (SEs) for the two most plausible logistic regression models of the occurrence of hybridization between native westslope cutthroat trout and nonnative rainbow trout in the upper Flathead River drainage (see Table 2).

Variable	B	SE
Model 1		
Mean temperature	0.955	0.56
Distance to source	−0.103	0.043
Number of road crossings	0.128	0.086
Constant	−3.532	4.64
Model 2		
Mean temperature	1.104	0.584
Distance to source	−0.099	0.047
Constant	−4.131	4.518

1,304 m; range, 1,130–1,536 m [$U = 47.0$, $P < 0.01$]). The mean and maximum summer temperatures were significantly higher in streams with hybrids. The mean water temperature was 11.5°C (range, 7.6–15.0°C) in streams containing hybrid populations and 9.6°C (range, 6.6–11.4°C) in streams with nonhybridized westslope cutthroat trout populations ($U = 63.5$, $P < 0.01$); the maximum temperature averaged 20.2°C among hybridized populations, versus 16.5°C among nonhybridized populations ($U = 82.5$, $P = 0.03$). Hybrid populations occurred in streams with significantly ($U = 99.5$; $P = 0.08$) more upstream road intersections (mean, 11; range, 0–33) than those containing westslope cutthroat trout (mean, 6; range, 0–29), but no differences were detected for road density ($U = 123.5$; $P = 0.35$). No differences in gradient were found between streams occupied by hybrid trout (mean, 0.04; range, 0.01–0.07) and nonhybridized westslope cutthroat trout (mean, 0.04; range, 0.01–0.09; $U = 110.5$, $P = 0.17$). The same was true for the density of trout (nonhybridized sites: mean $= 0.076$ fish/m^2; hybridized sites: mean $= 0.103$ fish/m^2) ($U = 116$, $P = 0.24$).

The best-approximating logistic regression model contained the watershed variables mean summer water temperature and number of upstream road crossings in combination with the biotic variable distance to the source of hybridization (Table 2). However, one other model (with the variables mean summer water temperature and distance to the source of hybridization) was equally plausible. Both models had overall classification accuracies greater than 85%. The occurrence of hybridized trout was positively associated with mean summer water temperature and the number of
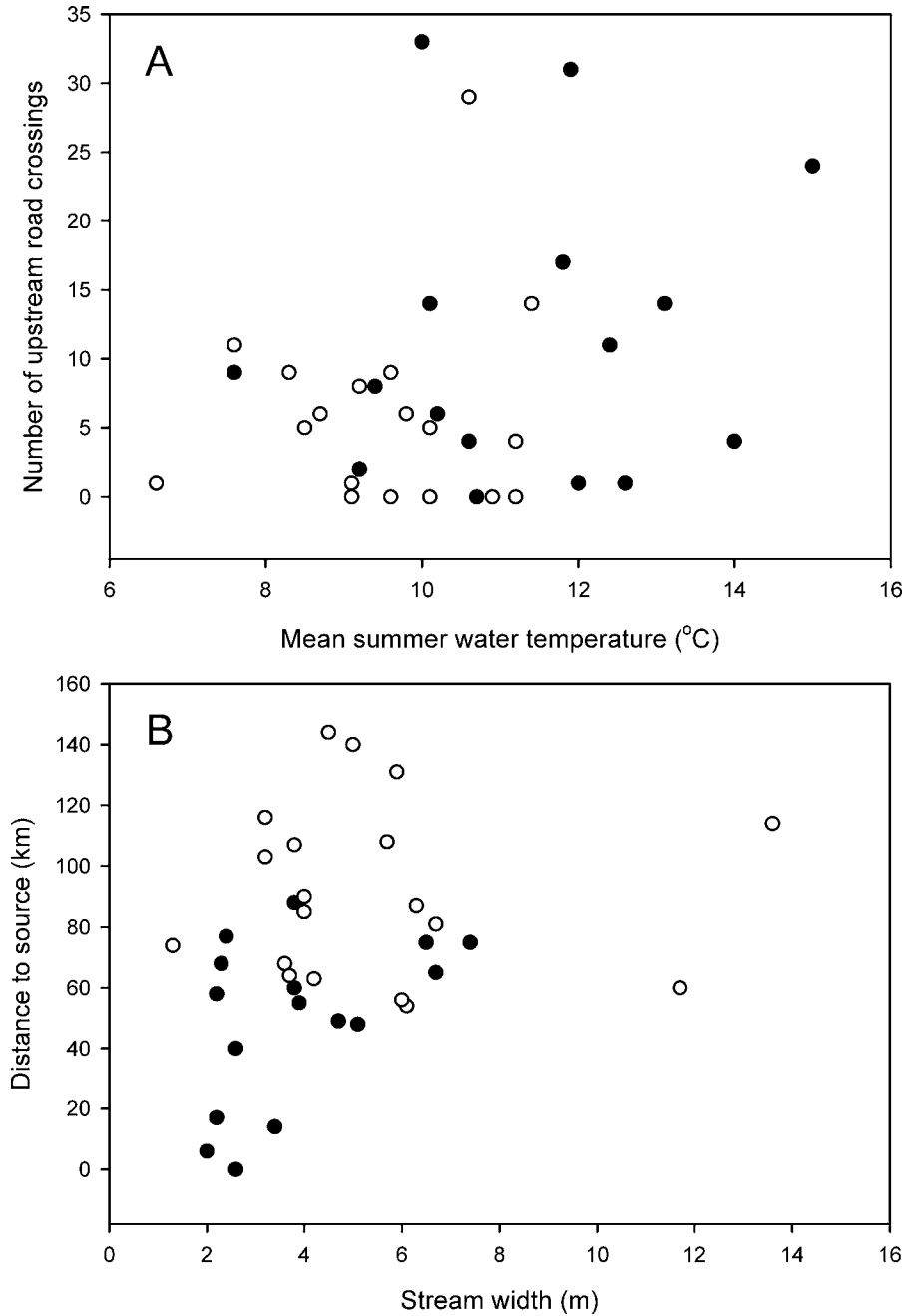
FIGURE 2.—Presence (closed circles) and absence (open circles) of hybridization between native westslope cutthroat trout and nonnative rainbow trout in relation to **(A)** mean summer water temperature and the number of upstream road crossings and **(B)** stream width and the distance from the source of hybridized individuals.

upstream road crossings and negatively associated with the distance to the source of hybridization (Table 3; Figure 2). The best-approximating linear regression models associated with the level of introgression among hybridized sites included summer water temperature, distance to the source, and trout density (Table 4). The proportion of rainbow trout admixture (logit transformed) was negatively related to the

TABLE 4.—Model selection results for candidate linear regression models of the proportion of nonnative genetic admixture between native westslope cutthroat trout and nonnative rainbow trout in 16 hybridized streams in the upper Flathead River drainage. Density refers to the density of both trout species at the sampling site; other variables are explained in Table 2.

Model	k	AIC_c	ΔAIC_c
Mean temperature, distance to source	4	48.871	0
Mean temperature, distance to source, density	5	49.169	0.298
Distance to source	3	49.518	0.647
Distance to source, density	4	50.494	1.623
Mean temperature, distance to source, width	5	52.958	4.087
Distance to source, width	4	53.093	4.222
Mean temperature, distance to source, density, width	6	54.455	5.584
Distance to source, density, width	5	54.856	5.985
Mean temperature, density	4	72.573	23.702
Density	3	73.205	24.334
Mean temperature, density, width	5	74.563	25.692
Mean temperature, width	4	74.619	25.748
Density, width	4	74.965	26.094
Width	3	75.264	26.393
Mean temperature	3	75.284	26.413

distance to the source and positively related to mean temperature and density (Table 5). The occurrence and amount of introgression was negatively related to stream width in both regression analyses (Figure 3), although it was not included in the top models.

Discussion

Conservation of aquatic biodiversity requires an understanding of the invasion patterns and factors promoting extinction by hybridization. We evaluated the influence of local habitat features, large-scale watershed characteristics, and biotic factors associated with the spread of hybridization between an introduced, nonnative species and a native species of conservation concern. Our results provide evidence

TABLE 5.—Model coefficients (B) and standard errors (SEs) for the four most plausible linear regression models of the proportion of rainbow trout admixture in the upper Flathead River drainage (see Table 4).

Variable	B	SE
Model 1		
Mean temperature	0.242	0.121
Distance to source	−0.072	0.009
Constant	−1.39	1.559
Model 2		
Mean temperature	0.251	0.111
Distance to source	−0.065	0.009
Density	6.376	3.422
Constant	−2.512	1.551
Model 3		
Distance to source	−0.077	0.009
Constant	1.586	0.506
Model 4		
Distance to source	−0.071	0.01
Density	6.017	3.923
Constant	0.639	0.784

supporting the hypothesis that hybridization is more likely to occur and spread in streams with warm water temperatures, increased land use disturbance, and proximity to the main source of hybridization. Our findings provide fisheries managers with a better understanding of the factors that promote the success of invasions and the loss of biodiversity through extinction by hybridization.

Our results are concordant with those of other studies in Europe and North America that have found that invasion success is often facilitated by a complex interaction of many abiotic and biotic factors, such as local habitat conditions, temperature, connectivity, human influences, and biotic resistance (Paul and Post 2001; Dunham et al. 2002; Rich et al. 2003; Kitano 2004; Carveth et al. 2006; Jeschke and Strayer 2006). However, the relative influences of these factors vary among geographical areas and hybrid zones of native cutthroat trout and nonnative rainbow trout. For example, Rubidge and Taylor (2005) showed that the level of hybridization decreased with increasing distance from Koocanusa Reservoir in British Columbia (indicating that the reservoir acts as a source of rainbow trout) but found no evidence that stream order, magnitude, or elevation influenced the extent of hybridization among localities. Conversely, Weigel et al. (2003) found evidence of ecological barriers (e.g., water temperature) restricting the spread of hybridization between westslope cutthroat trout and nonnative rainbow trout in the Clearwater River system of Idaho. These authors found that many tributaries located close to the original stocking locations did not contain hybridized populations and that the degree of hybridization showed negative associations with site elevation and positive associations with stream width. In contrast, Gunnell et al. (2008) found that the primary

factor influencing the geographic distribution of rainbow trout introgression with native Yellowstone cutthroat trout (*O. clarkii bouvieri*) was fluvial distance from the stocking locations and, to a lesser extent, stream elevation. In our study, the independent variables site elevation, distance to the source, and water temperature were correlated, making it difficult to ascertain the relative effects of each variable on the geographic distribution of hybridization in the system.

Source proximity strongly influenced the occurrence and proportion of rainbow trout admixture across the large, interconnected drainage. Other studies have also shown that invasion success and hybridization are largely governed by the spatial arrangement of nonnative source populations. For instance, in the upper Kootenay River drainage in British Columbia, Rubidge and Taylor (2005) showed clustering among hybridized locations and decreasing hybridization with increasing distance from Koocanusa Reservoir. Likewise, Gunnell et al. (2008) found that rainbow trout introgression with native Yellowstone cutthroat trout declined with distance from a known rainbow trout stocking source. We found that the distance to the source of hybridization appears to be the leading factor associated with the presence of hybridization and the amount of rainbow trout admixture among hybridized sites. However, the spread of hybridization was also influenced by the additive effects of temperature and a measure of land use degradation, suggesting that hybridization is facilitated by a complex combination of spatial and environmental factors in this westslope cutthroat trout–rainbow trout hybrid zone.

The observed genotypic gradient of decreasing hybrid occurrence and decreasing levels of introgression from main-stem sites to upper-elevation tributaries is consistent with the results of several studies in other rainbow trout–cutthroat trout hybrid zones (Kruse et al. 2000; Weigel et al. 2003; Rubidge and Taylor 2005; Gunnell et al. 2008). Elevation was significantly related to the occurrence and level of hybridization, but we expected this to be the case since site elevation increased with increasing distance from the main source of hybridization (Abbot Creek) and elevation is strongly correlated with water temperature. Similarly, Weigel et al. (2003) found that the level of introgression between introduced rainbow trout and native westslope cutthroat trout was negatively related to elevation. In contrast, Rubidge and Taylor (2005) found no evidence that stream size and elevation influence the extent of hybridization among sites in the Kootenay River drainage, although their study streams did not include the wide range of site elevations included in ours.

Hybridization was more likely at warmer sites and

the level of rainbow trout introgression was positively related to mean summer water temperature, suggesting that temperature is also a noteworthy factor promoting invasion by nonnative rainbow trout and hybridization with native cutthroat trout. Water temperature plays an important role in determining the distribution of many stream-dwelling salmonid species owing to its direct effects on physiology, behavior, and ecological interactions (Paul and Post 2001; Dunham et al. 2003; Carveth et al. 2006; McMahon et al. 2007). Indeed, water temperature has an important influence on the distribution and abundance of westslope cutthroat trout throughout their current range (Shepard et al. 2005). For example, Sloat et al. (2005) found that westslope cutthroat trout resided in streams with cool water temperatures (maximum daily temperature, ≤16.5°C) in the Madison River drainage in Montana, which is nearly identical to our findings in the Flathead River system (mean maximum temperature, 16.5°C). Although westslope cutthroat trout and rainbow trout have nearly identical optimum growth temperatures, rainbow trout have higher upper tolerance limits and grow over a wider range of temperatures than westslope cutthroat trout, according to a laboratory study (Bear et al. 2007). Also, rainbow trout are native to lower-elevation systems along the Pacific coast (MacCrimmon 1971). These temperature relationships, therefore, may account for the displacement of native cutthroat trout by nonnative rainbow trout in montane systems.

We detected a general pattern of nonhybridized populations persisting in colder, headwater streams at higher elevations than those occupied by hybridized populations. These results are consistent with those of other studies that have examined the genetic distribution of hybridization in situations in which previously allopatric populations of nonnative rainbow trout and native westslope cutthroat trout have come into artificial secondary contact (Weigel et al. 2003; Rubidge and Taylor 2005). At first glance, the data appear to support the elevation refugia hypothesis. That is, cold temperatures in headwater streams impart a competitive advantage to native salmonids and thus account for the greater resistance to invasion and the displacement of nonnatives (Paul and Post 2001; McMahon et al. 2007). However, the overlap in temperature regimes and local habitat conditions among sites with and without hybrids and the significant association between the amount of admixture and the distance to the source of hybridization suggest that headwater streams will not provide a refuge from hybridization if the sources of hybridization persist and spread in the system. Furthermore, because westslope cutthroat trout and rainbow trout
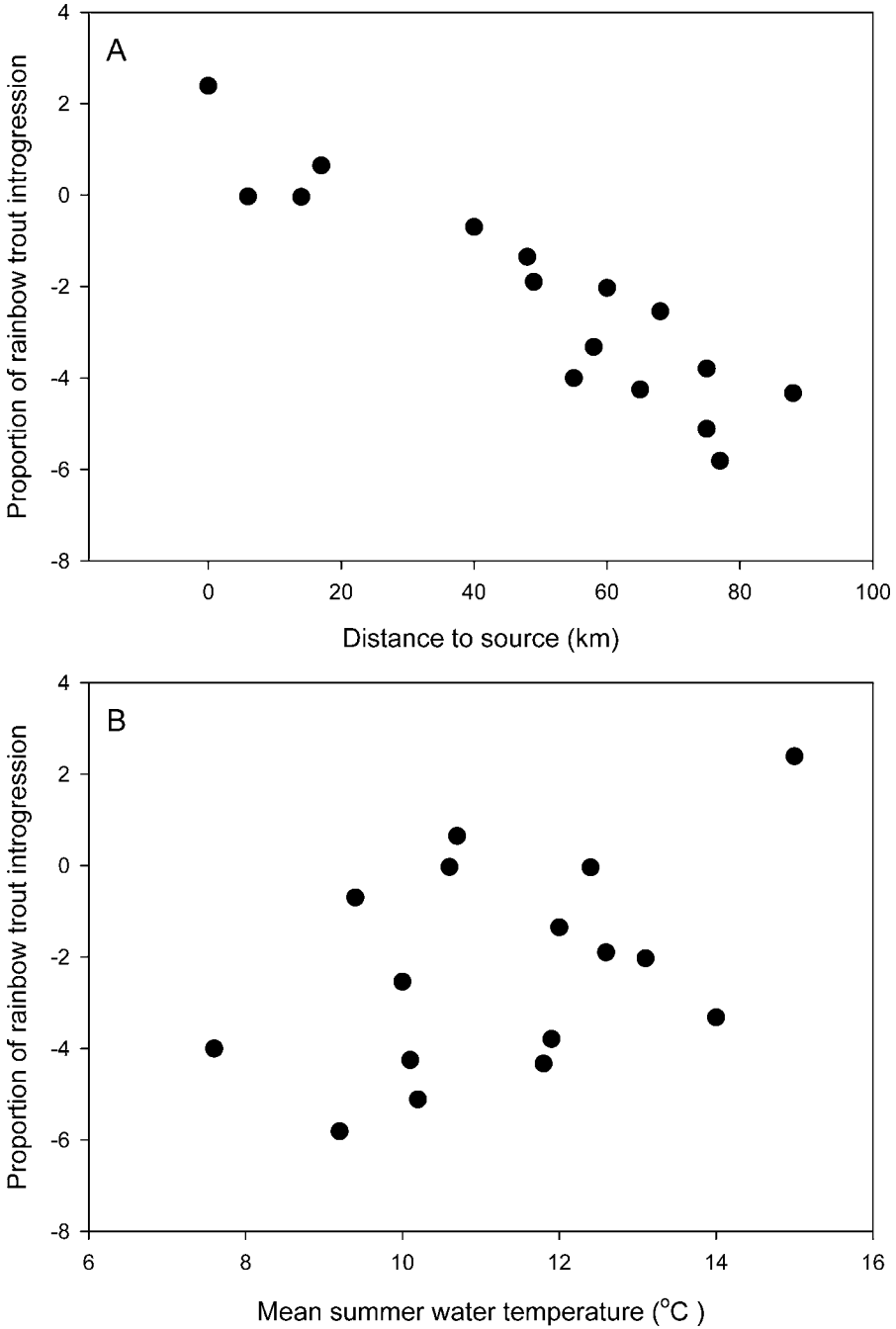
FIGURE 3.—Proportion of rainbow trout introgression (logit transformed) versus **(A)** the distance from the source of hybridized individuals, **(B)** mean summer water temperature, **(C)** trout density, and **(D)** stream width.

have virtually identical optimum growth temperatures in the laboratory (13.68°C and 13.18°C, respectively; Bear et al. 2007), temperature alone may not prevent or slow the spread of hybridization. Additional research is

needed to compare the thermal preferences of hybrids with those of both parental species in natural environments.

The association between the presence of hybridiza-
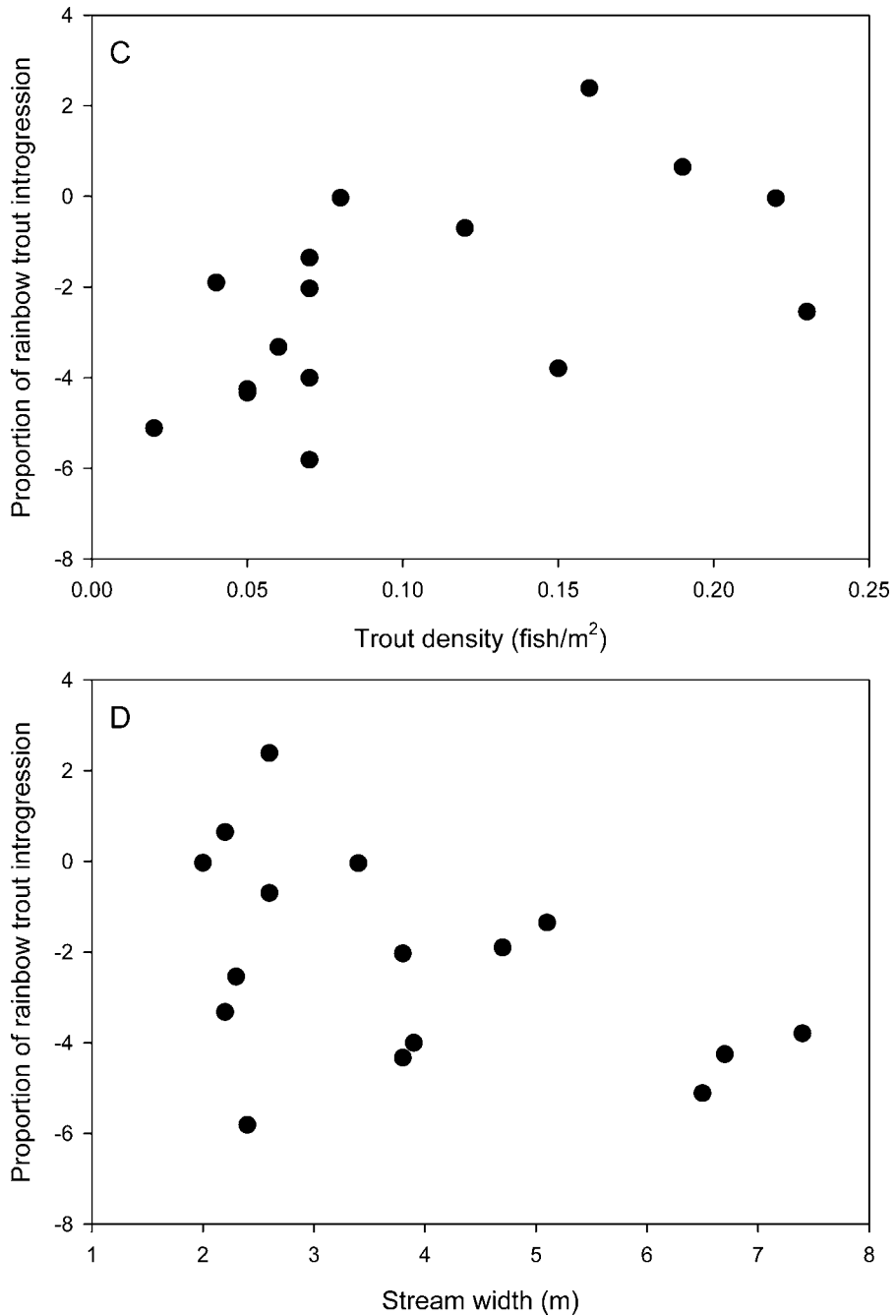
FIGURE 3.—Continued.

tion and the number of upstream road crossings suggests that hybridization is also more likely in streams with increased disturbance. Similarly, Shepard (2004) found that invasive brook trout *Salvelinus fontinalis* displaced native westslope cutthroat trout in a southwestern Montana stream with higher water temperatures, lower frequencies of debris and pools, and greater erosion and deposition of fine sediments than two adjacent, undisturbed streams. Land use disturbances can make systems more prone to the successful invasion of nonnative competitors by changing the availability and quality of habitats, which

may result in the displacement or complete replacement of native taxa (Allendorf et al. 2001; Jeschke and Strayer 2006). This has been observed for a variety of salmonid species (Taylor et al. 1984; Fausch et al. 2001; Paul and Post 2001; Shepard 2004) as well as other vertebrate species (Haig et al. 2004; Schwartz et al. 2004) and many plant species (Arnold 1997). Road metrics are often used as a surrogate for habitat disturbance because they may negatively impact salmonid populations by increasing stream sediment loads; obstructing fish movements; degrading spawning, rearing, and reproductive habitats; and providing vectors for fishing pressure and the stocking of nonnative species (Meehan 1991; Trombulak and Frissell 2000; McCaffery et al. 2007). Other disturbances unaccounted for in this study, such as drought, wildfire, and flooding, may also affect the invasion success of rainbow trout in novel environments. Fausch et al. (2001) concluded that flood disturbance regimes strongly influenced the invasion success of rainbow trout in five Holarctic regions with similar temperature regimes. These authors showed that invasive rainbow trout are more successful when fry emerge in areas and periods of low flood probability as opposed to areas with summer and fall floods that wash them away.

Hybrid zones are areas of contact between genetically distinct populations where hybridization occurs and are formed and maintained by selection and dispersal (Barton and Hewitt 1989). Our results suggest that the dispersal of hybridized individuals from hybrid source populations is a significant factor in the spread of hybridization in the upper Flathead River system; these results are corroborated by a recent telemetry study (Muhlfeld et al. 2009b) and fine-scale genetic analyses (Boyer et al. 2008). However, Muhlfeld et al. (2009a) found that hybridization rapidly reduces fitness in later-generation westslope cutthroat trout–rainbow trout hybrids in a stream in the North Fork Flathead River (Langford Creek; site 5 in this study). Despite the apparent occurrence of outbreeding depression, the authors concluded that hybridization may still spread because of (1) the relatively high reproductive success of first-generation hybrids and a few males with high levels of admixture; (2) higher straying rates in rainbow trout (Boyer et al. 2008); and (3) and the fact that all of the progeny of hybrids are hybrids (Epifanio and Philipp 2001). Thus, source connectivity and dispersal barriers probably explain the distribution patterns of hybridization in this system.

We found that the proportion of rainbow trout admixture among hybridized sites was positively related to the abundance of trout. Although biotic resistance from native species may limit the extent to which competitors become established (Pimm 1989),

we hypothesized that sites with higher densities would be more susceptible to hybridization because rainbow trout and westslope cutthroat trout have similar mating behaviors that would facilitate interbreeding and the formation of hybrid swarms. Alternatively, our results suggest that introgressed populations have a fitness advantage. However, we do not believe that this is the case because fish densities were not significantly different among hybridized and pure sites and our recent work indicates that hybridization rapidly reduces fitness in later-generation hybrids (Muhlfeld et al. 2009a). Additional research is needed to understand the demographic and ecological consequences of hybridization in old and new hybrid swarms in a variety of stream environments.

Covariation among spatial and environmental variables and stocking history precludes us from making definitive conclusions regarding the relative influences of factors that limit or promote the spread of hybridization. This is a problem in many invasive species studies owing to the increase in the prevalence of unauthorized introductions of nonnative species (Rahel 2000). The sites where disturbance was more common and temperatures were warmer were also closer to the source of hybridization than the colder, less disturbed sites in the headwaters. Additionally, the purported illegal release of an estimated 70,000 rainbow trout in 1997 from a private hatchery in the lower portion of the drainage probably played a significant role in the recent proliferation and current distribution of hybridized trout in the system, and many studies have shown that propagule pressure (the number and frequency of introduced individuals) plays an important role in the establishment and spread of exotic species (see Lockwood et al. 2006 for a review). The observed spatial distribution of hybridization may not be entirely the result of stocking history, however, as rainbow trout tend to establish population strongholds at low-elevation sites in the drainages into which they are introduced. For example, Weigel et al. (2003) found a pattern of elevational separation between native westslope cutthroat trout and nonnative rainbow trout despite the fact that the rainbow trout had access to all of the sampling locations in their study area. Similarly, Paul and Post (2001) showed that rainbow trout stocked extensively over a wide range of elevations on the eastern slopes of the Rocky Mountains generally moved downstream and hybridized with and displaced native cutthroat trout populations.

Management Implications and Conclusions

Our data suggest that westslope cutthroat trout are particularly susceptible to hybridization with nonnative rainbow trout in situations in which anthropogenic

habitat disturbances increase water temperature and degrade stream habitats. Habitat degradation and fragmentation have been identified as leading factors in the decline and extirpation of westslope cutthroat trout populations throughout their range (Shepard et al. 2005). Currently, the headwaters of the North Fork Flathead River in British Columbia have been targeted for coal bed methane development and open-pit coal mining. Our research shows that this area supports the majority of the remaining nonhybridized westslope cutthroat trout populations in the transboundary system (Boyer et al. 2008; Muhlfeld et al., in press; this study). Thus, protection of pure migratory populations and their critical spawning and rearing habitats in the headwater portion of the drainage are critical to the long-term persistence of nonhybridized westslope cutthroat trout populations and migratory life history forms in the Flathead River and similar freshwater systems.

The petition to list the westslope cutthroat trout as a threatened species under the Endangered Species Act was recently denied because the subspecies is widely distributed, numerous nonintrogressed westslope cutthroat trout populations are distributed in secure habitats throughout the subspecies' historic range, and numerous westslope cutthroat trout are non-introgressed or nearly so. (USFWS 2003).

Although headwater streams currently contain nonhybridized westslope cutthroat trout populations, our data suggest that habitat conditions alone are not sufficient to maintain "secure" habitats in open systems and that headwater streams will not provide refuge from hybridization if the sources of hybridization persist in this open system. Indeed, many studies have found that pure cutthroat trout populations only persist above upstream migration barriers in situations in which nonnative rainbow trout have been introduced (Sloat et al. 2005; Ostberg and Rodriguez 2006; Metcalf et al. 2008).

Our results indicate (1) that hybridization is likely to spread further, causing additional westslope cutthroat trout populations to be lost, unless populations with high amounts of rainbow trout admixture are suppressed or eliminated and (2) that the protection of hybridized populations facilitates the expansion of hybridization. To preserve nonhybridized westslope cutthroat trout populations, managers should consider eradicating hybridized populations with high levels of rainbow trout admixture and restoring streams characterized by warm temperatures and high levels of disturbance.

Acknowledgments

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