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[Article]

Influences of Wildfire, Habitat Size, and Connectivity on Trout in Headwater Streams Revealed by Patterns of Genetic Diversity

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Abstract.—Wildfire is an important natural process in many stream ecosystems, but the ability of fish to respond to wildfire-related disturbances is increasingly constrained by human activities that fragment and degrade stream habitats. In this study, we used molecular genetic markers (nuclear microsatellites) to examine the effects of wildfire and related disturbances along with habitat fragmentation on native rainbow trout in the Boise and Payette River basins, Idaho. We surveyed the genetic diversity of fish in 55 tributary streams to compare the level of diversity in samples without the recent influence of wildfire with that of those influenced by stand-replacing wildfire and those influenced by both wildfire and a severe channel-reorganizing disturbance. Stream habitats also varied substantially in size (catchment basin area) and isolation caused by road culverts. Based on prior work in our study streams, we expected that both wildfire and channel reorganization would reduce local population sizes significantly. Accordingly, we expected that wildfire-related disturbances would reduce genetic diversity via founder effects or population bottlenecks. Our results, however, showed little evidence of these influences. In contrast, the level of genetic diversity was lower in fish collected upstream of culvert barriers, probably because of restricted gene flow. We also observed the expected positive correlation between habitat size and genetic diversity, which suggested the importance of larger local population sizes and habitat diversity in maintaining genetic diversity. An unexpected finding was that 15 of the 55 samples showed genetic evidence of hybridization between rainbow trout *Oncorhynchus mykiss* and nonnative cutthroat trout *O. clarkii*. The results of this study suggest that human influences such as barriers to dispersal and introductions of nonnative fish may pose greater threats to populations of native trout than wildfire itself.

In western North America, wildfire can be a major driver of disturbance in headwater streams (Reeves et al. 1995; Rieman and Clayton 1997; Gresswell 1999). Though these disturbances can be dramatic and in some cases devastating to stream habitats and species in the short term, the prevailing view is that wildfire-related disturbances are an important contributor to the natural functioning of stream ecosystems over longer time frames (Bisson et al. 2003; Minshall 2003). The loss of vegetation and decreased infiltration capacity of the soil that accompany wildfire can render small, steep headwater streams particularly vulnerable to extreme flooding and debris flows, leading to channel-reorganizing disturbances (Benda et al. 2003; Miller et al. 2003; Wondzell and King 2003). In some instances, these events have led to the local extirpation of fish (Rieman and Clayton 1997; Brown et al. 2001; Minshall 2003; Burton 2005; Sestrich 2005). Over time, populations may recover as habitat conditions

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improve, sometimes because the disturbance event itself contributes to key habitat-forming processes (Reeves et al. 1995; Minshall 2003).

Wildfire has emerged as a major management issue for native trout *Oncorhynchus* spp. because the natural ability of fish populations to respond to wildfire-related disturbances is becoming increasingly constrained (Rieman et al. 2003). The fragmentation and degradation of habitats by humans is believed to increase the vulnerability of local populations of native trout to extirpation by wildfire (Dunham et al. 2003). In larger, interconnected stream networks, local trout populations appear to be resilient to wildfire-related disturbances, perhaps because of migration or dispersal among local habitats (Rieman et al. 1997; Gresswell 1999; Burton 2005; Dunham et al. 2007). By contrast, the natural resilience of fish populations may be seriously compromised when habitats have become fragmented and degraded to the point that fish movement is highly restricted and the availability of potential immigrants is reduced (Brown et al. 2001). The influences of habitat fragmentation in terms of both reduced habitat or population size and increased isolation have been inferred from a limited number of case studies documenting the patterns of fish population responses to fire (e.g., Dunham et al. 2003).

Understanding the responses of trout populations to wildfire is complicated by logistical difficulties in tracking populations over long time frames as well as collecting pre- and postdisturbance information. Consequently, most of the evidence is from single populations or habitats and the studies are highly opportunistic by nature. To our knowledge, there are no studies in which multiple populations of headwater fish have been studied systematically to contrast the influences of habitat fragmentation and wildfire-related disturbances (Dunham et al. 2003). In this study, we conducted a broad-scale retrospective comparison of genetic diversity in trout sampled from multiple habitats in two major river networks that have experienced wildfire over large portions of their catchments within the past 20 years (Burton 2005). Previous work has examined the distribution and abundance of rainbow trout *O. mykiss* in response to a host of relatively recent and extensive wildfires across these basins (Rieman et al. 1997; Burton 2005; Dunham et al. 2007). Here, we extend this work to examine a large number of streams ($n = 55$), which allowed us to examine the effects of wildfire in comparison with those of habitat size and fragmentation. We focused on the most abundant and widespread native species present in the headwater streams in our system—rainbow trout—contrasting the genetic diversity among fish in headwater streams (1) without recent wildfire, (2) with a stand-replacing fire within the past 20 years, and (3) with a recent stand-replacing wildfire and a severe fire-related channel-reorganizing disturbance assumed to have reduced population sizes significantly. In addition to contrasting disturbance histories, we examined the effects of variation in habitat size and isolation caused by culverts where roads cross streams (Clarkin et al. 2005; Wofford et al. 2005).

To measure (indirectly) the response of rainbow trout to disturbance history, habitat size, and isolation, we used molecular genetic markers (nuclear microsatellites; Sunnucks 2000). We reasoned that given a sufficient number of genetic markers, even short-term and recent population bottlenecks or founder events associated with disturbances or habitat fragmentation would be reliably indicated by the patterns of within-population genetic diversity. This is supported theoretically (Hedrick 1999) as well as by evidence from studies of experimental bottlenecks in fish (Leberg 1992; Richards and Leberg 1996; Spencer et al. 2000), landscape genetic studies of trout evaluating known recent bottlenecks or founder events (Heath et al. 2002; Ostergaard et al. 2003; Wofford et al. 2005; Neville et al. 2006b), and the responses of other species to relatively recent disturbances (Curtis and Taylor 2004; Baucom et al. 2005; Wahbe et al. 2005). Accordingly, the examination of molecular markers can be an efficient alternative to the traditional ecological approach of tracking population sizes and abundance over time (Neville et al. 2006a). Whereas the distribution and abundance of a population can rebound quickly after a disturbance, the patterns of genetic diversity caused by bottleneck and founder events should persist and remain observable over many years (Garza and Williamson 2001).

We predicted that rainbow trout from habitats experiencing recent wildfire, especially those that also underwent channel reorganization, should have lower levels of genetic diversity as a result of recent bottlenecks or founder events. We also predicted that rainbow trout sampled from larger habitats would exhibit greater genetic diversity owing to greater resident population sizes or habitat diversity, whereas those from habitats isolated by culverts would have lower diversity (Neville et al. 2006b). The results of this work were assessed to contrast the relative influences of wildfire, habitat size, and human-associated isolation on native rainbow trout. To our knowledge, this is the first attempt to evaluate these collective influences across a broad network of local habitats, and it thus provides a critical empirical evaluation of current thinking about wildfire and the

management of human influences to conserve native fishes.

Methods

Selection of sampling sites.—This study took place in the Boise and Payette River basins in central Idaho, which is an ideal region for studying wildfire-related disturbances because significant portions of these basins burned between 1989 and 2003 (Rieman et al. 1997; Burton 2005; Dunham et al. 2007). We confined our selection of streams to those with perennial flow along a range of elevations likely to support rainbow trout (973–2,096 m), selecting smaller tributary streams for sampling because they are most likely to show the long-term effects of fire and fire-related disturbances (Dunham et al. 2007). In addition, because nonnative trout were introduced into the Boise and Payette rivers throughout the last century, we chose streams that were far from reservoirs and lakes (likely sources of introduced trout that could hybridize with native trout, thereby affecting genetic patterns; see the section on hybridization assessment below).

The 55 streams selected for sampling were distributed widely across the river basins we sampled (Figure 1) and had the range of conditions needed to test our hypotheses, including size (catchment areas 193–8,622 ha), disturbance histories (unburned, burned, and burned and reorganized; Dunham et al. 2007), and isolation (upstream of barriers to fish movement versus freely connected habitats). The classification of wildfires within the sampled catchments utilized a database derived from remotely sensed satellite imagery (http://fsgeodata.fs.fed.us/mtbs/index.html). Streams without a recent (<100 years) history of wildfire were classified as "unburned." Streams that had experienced stand-replacing wildfire within most of their catchments within the last 20 years were classified as "burned." Burned streams that also experienced channel-reorganizing events after being subjected to wildfire were classified as "reorganized" (Figure 1). Owing to the high topographic relief of the stream catchments, severe floods and debris flows commonly follow wildfires and cause severe alteration to the stream channels (Benda et al. 2003; Miller et al. 2003). The channel reorganizations in our study area probably caused a significant reduction in fish numbers and, in many cases, local extirpation (Rieman et al. 1997; Burton 2005). The occurrence of channel reorganization was determined by examination of aerial photos from 1969, 1979, 1988, and 1996 (C. Luce, U.S. Forest Service, Rocky Mountain Research Station, personal communication) and on-the-ground verification. Additionally, we included two populations from sites that had not been burned but that had experienced massive reorganizing events similar to those stemming from wildfire. These samples were characterized as reorganized for our analyses.

For all three levels of disturbance (unburned, burned, and reorganized), we sampled areas with two levels of isolation: "connected" or "isolated." As indicated earlier, isolation was caused by culverts placed under road crossings that prevented the upstream movement of fish. We followed the national inventory and assessment protocol for culverts (Clarkin et al. 2005) to verify that the habitats we classified as isolated were upstream of culverts that were clear barriers to the movement of rainbow trout.

Habitat size was measured in terms of watershed area (ha) upstream of the sampled locations near tributary confluences or upstream of culvert barriers by means of geographical information systems software (ArcGIS; www.esri.com) and 30-m digital elevation data. To the fullest extent possible, we selected streams with comparable catchment areas within each isolation and disturbance history category.

Ultimately, 21 streams were classified as unburned, 21 as burned, and 13 as reorganized (including the two without recent wildfire; Table 1). The percentage of wildfires of moderate to high severity averaged 33% (range, 6–66%) in the burned catchments and 42% (0–75%) in the reorganized catchments. Analysis of variance (ANOVA) among the three stream types indicated that the differences in catchment area (a potentially confounding covariate) were not statistically significant ($F = 1.59$, df = 54, $P = 0.21$). Of the 55 streams sampled, 23 represented habitats isolated by a culvert that posed a barrier to fish movement (11 unburned, 9 burned, and 3 reorganized; Table 1). Of the 32 stream catchments that had been burned, wildfires occurred in 1988 ($n = 1$), 1989 ($n = 5$), 1992 ($n = 6$), 1994 ($n = 15$), 2000 ($n = 3$), and 2003 ($n = 2$).

Fish sampling and tissue collections.—All sampling occurred in the summer of 2004. Within each stream, rainbow trout were sampled via electrofishing (Model 12B electrofisher; Smith Root, Vancouver, Washington). Sampling consisted of a single upstream electrofishing pass beginning either above a culvert barrier or at least 300 m above the confluence of the tributary with the Boise or Payette River. Sampling continued upstream until a sufficient number of rainbow trout were collected for analysis (whenever possible, we sampled at least 30 individuals). Field crews took care not to sample family groups by avoiding young-of-the-year fish as well as by spreading the shocking effort over a large length of the stream (see Hansen et al. 1997). After capture, the rainbow trout were anesthetized with tricaine methanesulfonate (MS-222) and

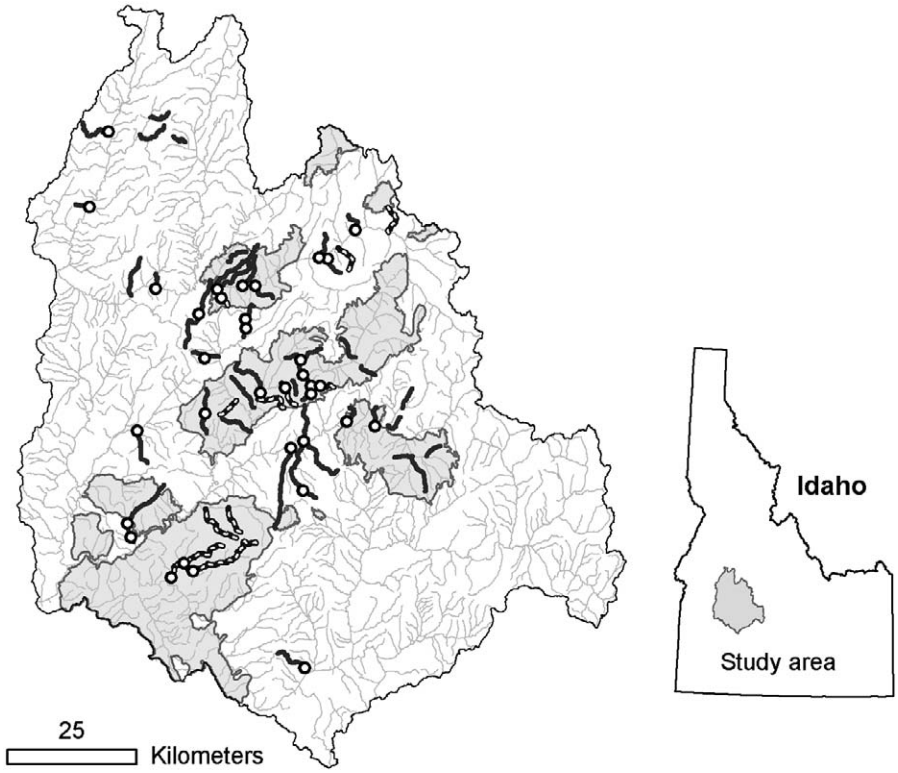

FIGURE 1.—Map of the Boise and Payette River basins showing the locations of the headwater tributary streams where rainbow trout tissue samples were collected in 2004. Streams without a history of channel-reorganizing events are represented by solid lines, those that experienced such an event within 15 years of sample collection by dashed lines. Circles indicate the presence of culverts. Areas with recent wildfires (1989–2003) are shaded.

small fin clips were taken from the caudal fin and immediately stored in solutions of 95% ethanol for later analysis. Because the roads in this mountainous region often follow river channels, most of the culverts were located close to the confluence of a tributary with its river. For this reason, we did not collect samples below barriers.

Molecular genetic protocols.—Total genomic DNA was extracted using DNeasy extraction kits (Qiagen, Valencia, California) and diluted to 5 ng/μL after quantification with fluorometry. Polymerase chain reaction (PCR) and fragment sizing with an Applied Biosystems (Foster City, California) Prism 3730 DNA Analyzer were performed by the Nevada Genomics Center (Reno). We used fourteen fluorescently labeled tri- and tetranucleotide microsatellite loci (Table 2) isolated from rainbow trout (Rexroad and Palti 2003) and Lahontan cutthroat trout (*Oncorhynchus clarkii henshawi*; Peacock et al. 2004). The PCRs were performed in 15-μL reactions using 20 ng of DNA and the reagent concentrations and thermal protocols listed in Table 2. Individuals were genotyped manually with Genemapper version 3.0 (Applied Biosystems).

Hybridization assessment.—Throughout the last century, both hatchery-origin rainbow trout and two subspecies of cutthroat trout were introduced into the Boise River basin (pre-1980s stocking used Yellowstone cutthroat trout, *O. c. bouvieri*; later stocking used westslope cutthroat trout, *O. c. lewisii*; M. Campbell, Idaho Department of Fish and Game, personal communication). Unfortunately, no genetic markers were available to distinguish hatchery-origin from native rainbow trout, and because we could not determine which individuals were affected by rainbow trout stocking, we designed our sampling protocol to avoid areas close to lakes or reservoirs, which are common sources of invasion by nonnatives (Adams et al. 2001). However, markers were available to distinguish the various subspecies of cutthroat trout from rainbow trout (Ostberg and Rodriguez 2004). Each individual was therefore assessed for hybridization with Yellowstone or westslope cutthroat trout using a single PCR multiplex of seven biparental,

TABLE 1.—Rainbow trout populations sampled in the Boise and Payette River basins. Shown are the GPS coordinates in UTMs (east, north), disturbance treatment (B = burned, UN = unburned, R = reorganized), the presence of culverts (Y = yes, N = no), catchment basin size, whether or not cutthroat trout hybrids were detected based on genetic data (Y = yes, N = no), and the number of tissue samples collected (*N*).

Population	Coordinates	Disturbance	Culvert?	Size (ha)	Hybrids?	*N*
Boise River basin						
Lost	617363, 4859399	B	Y	1,544	N	30
Lost Man	627779, 4841402	UN	Y	922	N	36
Little Rattle	605553, 4827193	R	N	2,395	Y	32
Big Owl	619797, 4860070	B	N	1,817	Y	31
Wren	621025, 4858553	R	N	1,022	N	36
Cottonwood	599930, 4840616	B	N	5,358	Y	35
South Fork Sheep	609473, 4835723	R	N	3,178	N	36
Trail	628933, 4862554	B	N	1,954	Y	32
North Fork Beaver	619795, 4860070	UN	N	1,451	N	36
Trapper	625185, 4858858	R	Y	897	Y	31
Trail	649467, 4846505	B	N	888	N	36
Steppe	636706, 4854452	B	Y	482	N	36
Flint	652471, 4848270	B	N	436	N	30
Buck	628932, 4850502	UN	N	3,009	N	36
Evans	628494, 4806396	UN	N	1,150	N	28
Devils	613648, 4836822	R	N	1,246	Y	29
King	647451, 4857531	UN	N	372	Y	35
Bow	637796, 4866928	B	N	639	N	36
Robin	639479, 4864677	B	N	818	N	36
German	609279, 4852700	B	N	2,305	N	36
Eagle	642207, 4853567	B	Y	712	Y	30
Hunter	627685, 4866326	B	Y	640	N	36
Pine	596033, 4851729	UN	N	1,910	N	36
Wood	614961, 4858221	R	N	767	N	30
China Fork	644769, 4853590	UN	N	626	Y	22
Steamboat	625068, 4866712	B	N	300	N	36
Banner	617013, 4872064	UN	N	2,285	N	36
Granite	628436, 4851752	UN	Y	1,720	N	37
Lamar	611323, 4867076	UN	N	613	N	36
Robert Lee	626123, 4862338	B	N	560	Y	22
Camp Gulch	649000, 4847583	B	N	675	N	36
Horse Heaven	630312, 4861323	R	N	641	N	36
Roaring River	623701, 4841192	UN	Y	6,061	N	36
McDonald	637179, 4866553	R	N	287	N	35
Rattlesnake	604053, 4826843	R	N	4,635	N	45
Hungarian	615908, 4854092	B	Y	750	Y	31
Payette River basin						
Danskin	594523, 4879223	UN	Y	2,587	N	36
Rattlesnake	589627, 4902101	R	N	8,622	Y	33
Big Pine	599530, 4880328	UN	Y	4,587	Y	34
Smokey	611416, 4880589	R	Y	193	N	35
Tie	586447, 4896174	UN	Y	400	N	36
Kirkham	616511, 4880948	B	Y	1,040	Y	28
Chapman	635198, 4887885	R	N	1,526	N	36
Peace	596588, 4909763	UN	N	3,505	N	36
Lick	612624, 4881134	B	Y	882	N	31
Archie	618714, 4880515	B	Y	1,212	N	36
Ucon	598522, 4913552	UN	N	421	Y	35
Rock	609710, 4880247	UN	Y	3,402	N	36
Fox	638177, 4891788	UN	Y	628	N	42
MacDonald	632101, 4886628	UN	Y	1,126	N	36
South Fork Bear	644624, 4891677	R	Y	1,477	N	36
Wet Foot	590233, 4910781	UN	Y	2,453	N	36
Casner	631241, 4886252	UN	Y	1,018	N	36
Miller	611675, 4881683	B	Y	1,303	N	36
Park	613905, 4885555	B	N	537	N	41

codominant markers developed for hybrid detection (see Table 3). Each of the primers that we used amplifies an allele specific to either rainbow trout or cutthroat trout in "pure" individuals but can yield a heterozygous genotype in hybrids (Ostberg and Rodriguez 2004). After the initial hybridization, which would lead to heterozygous genotypes at all loci (see below), backcrossing with individuals of either parental

TABLE 2.—Polymerase chain reaction (PCR) laboratory protocols for microsatellite loci used to genotype rainbow trout in the Boise and Payette River basins. In some cases, loci were combined in multiplex PCRs.

PCR type	Locus (-i)	Reference	Primer (μM)	PCR mix[a]	Thermal protocol
Multiplex 1	*Omm1286*	Rexroad and Palti (2003)	0.15	Qiagen MP	95°C for 15 min; 34 cycles of 95°C (30 s), 56°C (1.5 min), and 72°C (30 s); 30 min at 62°C
	Omm1295		0.05		
	Omm1178		0.1		
Multiplex 2	*Och20*	Peacock et al. unpublished	0.1	Qiagen MP	95°C for 15 min; 34 cycles of 95°C (30 s), 62°C (1.5 min), and 72°C (30 s); 30 min at 62°C
	Omm1220	Rexroad and Palti (2003)	0.04		
	Omm1235		0.1		
	Omm1236		0.2		
	Omm1231		0.1		
Multiplex 3	*Och6*	Peacock et al. (2004)	0.18	Qiagen MP	95°C for 15 min; 25 cycles of 95°C (30 s), 67°C–52°C touchdown (1.5 min), and 72°C (30 s); 10 cycles of 95°C (30 s), 54°C (1.5 min), and 72°C (30 s); 30 min at 62°C
	Och9		0.06		
	Och10		0.12		
Single locus	*Omm1234*	Rexroad and Palti (2003)	0.2	Single	95°C for 5 min; 36 cycles of 95°C (30 s), 66°C (30 s), and 72°C (30 s); 30 min at 72°C
Single locus	*Omm1177*		0.2		95°C for 5 min; 36 cycles of 95°C (30 s), 58°C (30 s), and 72°C (30 s); 30 min at 72°C
Single locus	*Omm1173*		0.2		95°C for 5 min; 36 cycles of 95°C (30 s), 67°C (30 s), and 72°C (30 s); 30 min at 72°C
Single locus	*Omm1272*		0.2		95°C for 5 min; 36 cycles of 95°C (30 s), 67°C (30 s), and 72°C (30 s); 30 min at 72°C

[a] Qiagen MP = Qiagen multiplex mix (commercial) with 1 unit HotStart DNA polymerase and 3 mM $MgCl_2$ at pH 8.7; single-locus PCR with 1× buffer with 3.5 mM $MgCl_2$, 0.83 mM deoxynucleotide triphosphates, and 1 unit Titanium *Taq* polymerase.

species will cause "pure" genotypes to reemerge at some loci, masking the signal of hybridization. However, the combination of the seven loci used here should confer high power to detect hybridization with cutthroat trout in our system. For instance, a power analysis based on seven codominant markers suggests that the probability of mistakenly categorizing a first-generation back-crossed individual as a pure parental type is 0.0078 (see Boecklen and Howard 1997). Individuals that were heterozygous at any of the seven loci were identified as hybrids and dropped from the analyses of genetic diversity and the hypothesized influences of habitat size, isolation, and disturbance history. However, to clarify the patterns of introgression, hybrid individuals were further classified based on their specific genotypes as follows (see also Rubidge and Taylor 2004): individuals that were heterozygous at all loci were classified as F_1 hybrids (the product of a pure rainbow trout and a pure cutthroat trout); those that were homozygous at one or more loci for only one parental species were classified as backcrosses (the product of a hybrid and a pure parental type); and those that had at least one locus that was homozygous for each parent species were classified as post-F_1 generation hybrids (the product of a hybrid and a hybrid).

Marker evaluation and general population structure.—We treated the collections of fish in the tributary streams as if they represented actual populations, conducting standard tests of allele frequency data to look for evidence of pooling across these samples. We assessed each population for Hardy–Weinberg equilibrium at each locus using FSTAT (Goudet 2001), adjusting the critical significance levels to account for simultaneous tests. We evaluated the general population structure based on pairwise F_{ST} values calculated in FSTAT. We also performed an analysis of molecular variance using GenAlEx version 6.2 (Peakall and Smouse 2006; Beck et al. 2008; Smouse et al. 2008) to determine the degree of variation attributable to the genetic divergence among samples within and between the Boise and Payette River basins. This analysis

TABLE 3.—Polymerase chain reaction laboratory protocols for multiplexed loci used to identify rainbow trout–cutthroat trout hybrids in the Boise and Payette River basins. Qiagen multiplex mix was used (see Table 2); the thermal protocol was 95°C for 15 min; 34 cycles of 95°C (30 s), 57°C (1.5 min), and 72°C (30 s); then 30 min at 62°C (Ostberg and Rodriguez 2004).

Loci	Primer (μM)
Omm55	0.2
Occ38	0.2
Occ37	0.2
Occ34	0.2
Occ42	0.2
Occ35	0.1
Occ36	0.4

involved 999 permutations and was based on the genetic distance PhiPT, which can be compared among codominant and haploid–binary data (Peakall and Smouse 2006).

Measures of genetic diversity.—Using FSTAT, we calculated Nei's (1987) unbiased measure of gene diversity (H_E) and allelic richness (R_S), a rarified estimate of the number of alleles that is independent of the sample size (El Mousadik and Petit 1996; Petit et al. 1998; Leberg 2002). Though we assessed both metrics for comparison, allelic richness has been shown to be much more sensitive than gene diversity to population bottlenecks (see Spencer et al. 2000; Gapare et al. 2008 and the references therein) and thus was expected to be more effective at capturing the disturbance-related dynamics in our study. We also calculated Garza and Williamson's (2001) *M*-ratio, an indicator of genetic bottlenecks. The *M*-ratio characterizes the changes that occur after a bottleneck in the distribution of allele sizes relative to the number of alleles in a population. Empirical data from populations with documented demographic histories suggest that those that have historically been stable had *M*-ratios above 0.82, while those with known bottlenecks had *M*-ratios less than 0.70 (Garza and Williamson 2001). In addition to being a continuous metric (ranging from 0 to 1) that can be statistically correlated to our study factors, this metric has performed well in capturing known founder events and likely bottlenecks in other trout populations that were not well characterized by other approaches to testing for genetic bottlenecks (see Neville et al. 2006b). We also considered the linkage disequilibrium among loci as an indicator of within-population processes (Bartley et al. 1992), but recent work has demonstrated that a very large sample size is needed for accurate estimation (England et al. 2005), and we judged this measure to be infeasible with the limited resources we had available.

Analysis of factors influencing genetic diversity.—We used nonparametric Spearman rank correlation to determine the degree of correlation between habitat size and genetic variability, performing separate analyses for each genetic metric (R_S, H_E, and *M*). Similarly, we used nonparametric Kruskal–Wallis tests to determine the relationship between our categorical variables (connectivity [subject to a barrier or connected] and disturbance [unburned, burned, or reorganized]) and each measure of genetic diversity.

Results

Hybrid Assessment

Of the 1,974 fish included in the original data set, 86 cutthroat trout–rainbow trout hybrids were identified and removed from further analysis. These hybrids were spread across 15 of the 55 populations (Table 1). In these 15 populations, the percentage of the original sample that was made up of hybrids ranged from 2.5 to 40, with 5 populations having fewer than 10% hybridized individuals and 4 having more than 30%. Five individuals were classified as F_1 hybrids (found in only two populations), 78 were rainbow trout backcrosses, and 1 was deemed a post-F_1 hybrid. Two individuals had cutthroat trout alleles and were removed from further analysis but could not be categorized with confidence owing to poor amplification. No pure cutthroat trout or cutthroat-backcrossed individuals were identified. Although only one of our hybrid loci (*Omm55*; see Ostberg and Rodriguez 2004) had the ability to distinguish Yellowstone from westslope cutthroat trout, no Yellowstone cutthroat trout alleles were identified in any individuals.

Marker Evaluation and General Population Structure

After removal of cutthroat trout hybrids, our final data set comprised 1,888 rainbow trout dispersed across 55 populations. The final number of successfully analyzed nonhybrid fish per population averaged 34 individuals and ranged from 22 to 44. One of our microsatellite loci (*Och6*) exhibited consistent heterozygosity deficits across populations and was thus dropped from the study, leaving a final set of 13 loci. Of the 715 F_{IS} values evaluated (13 loci $\times$ 55 populations), one would expect 36 deviations from 0 in either direction. Randomization tests indicated that 52 of the 715 F_{IS} values were significantly greater than 0 ($P < 0.05$), indicating a heterozygosity deficit, and 31 were significantly smaller than 0, indicating a heterozygosity excess (see Table A.1 in the appendix in the online version of this article). In both instances, the deviations were spread across loci and populations and did not show consistent patterns suggesting either amplification issues (i.e., null alleles) or the pooling of fish from different populations (i.e., a Wahlund effect). When adjusted for tablewide significance (adjusted nominal level of 5% = 0.00007; see Goudet 2001), none of the F_{IS} values deviated significantly from zero in either direction.

The F_{ST} values indicated substantial and significant differentiation among populations, with a systemwide F_{ST} estimate of 0.094 (95% confidence interval, 0.087–0.102). Pairwise F_{ST} values ranged from 0.004 to 0.354, and all but two comparisons were statistically significant. The two insignificant comparisons (Buck and Granite creeks; Trail and Robert Lee creeks) were between pairs of neighboring populations in the Boise River basin with varying characteristics. Though Granite Creek was isolated, both Buck and Granite creeks were unburned while both Trail and Robert Lee

TABLE 4.—Results of nonparametric analyses assessing the relationships between three indicators of genetic diversity (allelic richness [R_S], gene diversity [H_E], and M-ratio [see text]) and study factors in the Boise and Payette rivers. Spearman rank correlation (r_s; df = 2, $\alpha = 0.05$) was used to evaluate the relationships between habitat size and each genetic metric, while the Kruskal–Wallis test (df = 1, $\alpha = 0.05$) was used to assess the relationships between the categorical variables and each genetic metric.

Response	Hypothesis	Variable	Median	25th quartile	75th quartile	Test results
R_S	Habitat size	Catchment area	7.71	6.95	8.82	$r_s = 0.49$, df = 53, $P = 0.0001$
	Disturbance	Unburned	7.86			
		Burned	7.62			
		Reorganized	8.43			
	Connectivity	Connected	8.30	7.52	9.20	
		Barrier	7.22	5.37	8.49	$\chi^2 = 5.95$, df = 1, $P = 0.015$
H_E	Habitat size	Catchment area	0.74	0.72	0.78	$r_s = 0.36$, df = 53, $P = 0.007$
	Disturbance	Unburned	0.75			
		Burned	0.75			
		Reorganized	0.77			
	Connectivity	Connected	0.77	0.75	0.78	
		Barrier	0.75	0.67	0.78	$\chi^2 = 3.56$, df = 1, $P = 0.06$
M-ratio	Habitat size	Catchment area	0.72	0.69	0.76	
	Disturbance	Unburned	0.74			
		Burned	0.72			
		Reorganized	0.71			
	Connectivity	Connected	0.73			
		Barrier	0.70			

creeks were connected and had been burned. The largest pairwise F_{ST} value was between Evans Creek, the most peripheral sample from the southeast Boise River basin, and Kirkham Creek in the Payette River basin. Evans Creek was unburned and connected, while Kirkham Creek had been burned and its population was isolated by a culvert below a paved road. Analysis of molecular variance showed that 2% of the genetic variance was partitioned between the major river systems while 16% was partitioned among populations within these river basins (both were significant at $P = 0.001$).

Patterns of Genetic Diversity

Gene diversity (H_E) averaged across loci ranged from 0.45 to 0.84 and allelic richness (R_S) from 3.4 to 10.71. The M-ratio ranged from 0.51 to 0.83, 17 out of the 55 populations having a ratio of 0.69 or less, which suggests a genetic bottleneck. Only one population had a ratio as high as 0.82, which, based on empirical data, suggests demographic stability (Garza and Williamson 2001).

A nonparametric Spearman rank correlation showed that R_S and H_E were significantly related to habitat size (Table 4; Figures 2, 3) but that the M-ratio was not. A nonparametric Kruskal–Wallis test indicated that allelic richness was significantly impacted by isolation but not by disturbance history. Neither H_E nor the M-ratio were significantly affected by either categorical variable, although isolated populations had marginally significant ($P = 0.06$) reductions in H_E.

Discussion

Disturbances are often associated with reduced genetic diversity in natural populations (e.g., Curtis and Taylor 2004; Haag et al. 2005; Jensen et al. 2005). Here, however, rainbow trout from headwater streams with drastically different disturbance histories (e.g., wildfire and channel reorganization versus unburned streams) were not detectably different based on any of our metrics of genetic diversity. We expected that fire-related disturbance would reduce the genetic diversity of local populations via founder effects or population bottlenecks, but our results showed little evidence of these influences. In contrast, allelic richness was reduced in populations upstream of fish movement barriers, which we expected owing to the restricted gene flow and presumably smaller sizes of populations upstream of barriers (Wofford et al. 2005; Neville et al. 2006b). We also observed the expected increase in genetic diversity (R_S and H_E) as habitat size increased, presumably because of larger population sizes in the larger habitats or greater population stability in larger catchments with greater habitat diversity (e.g., Benda et al. 2004). Additionally, our results confirm the results of other empirical studies in finding that R_S may be a more sensitive metric than H_E when one is evaluating influences on genetic diversity (Spencer et al. 2000; Gapare et al. 2008). The differences among R_S, H_E, and the M-ratio underscore the importance of considering multiple measures of genetic diversity and the value of relatively simple measures such as R_S.

The lack of a detectable influence of disturbance history on the genetic diversity of rainbow trout could

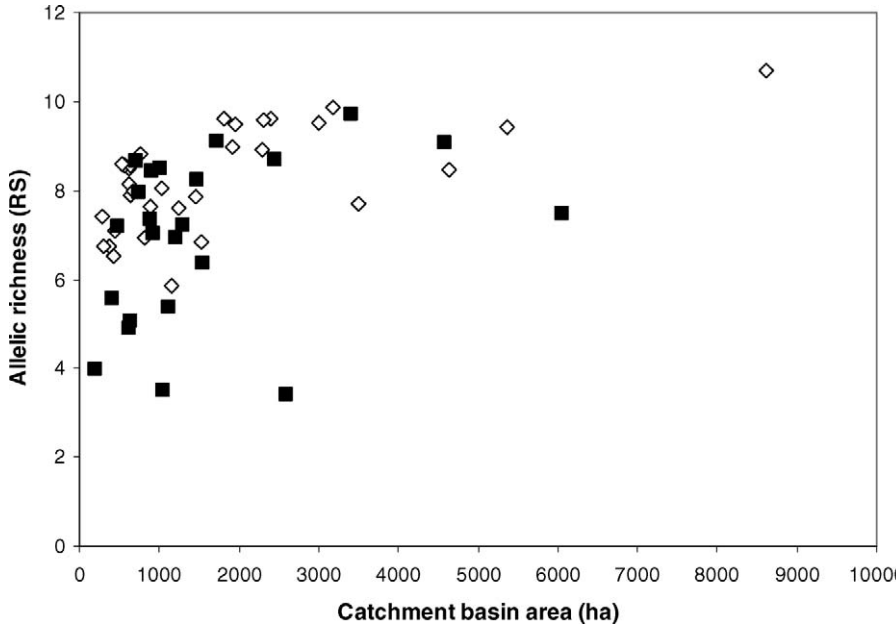

FIGURE 2.—Allelic richness detected in populations of rainbow trout versus catchment area for sites in the Boise and Payette River basins unimpeded by culverts (diamonds) and those isolated by culverts (squares).

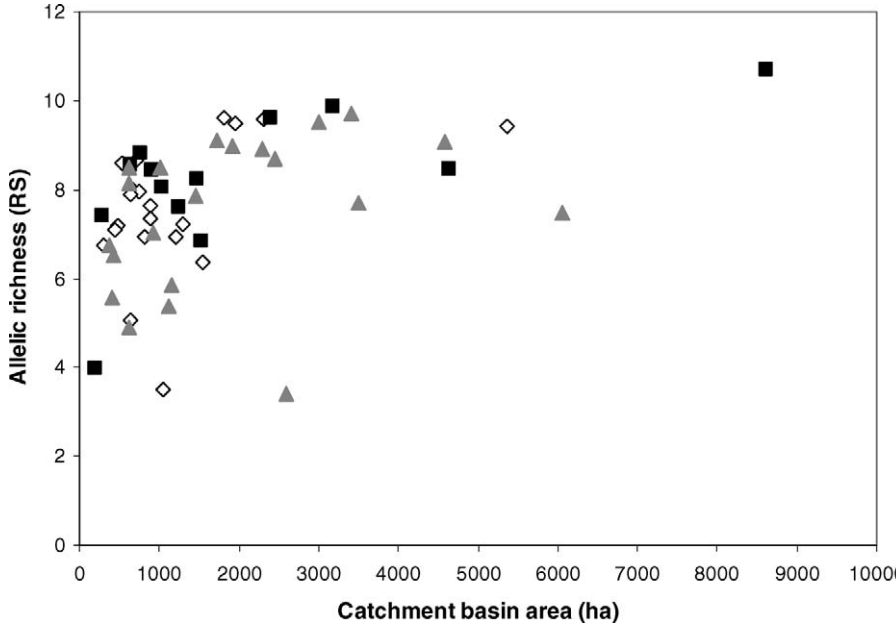

FIGURE 3.—Allelic richness detected in populations of rainbow trout versus catchment area for sites in the Boise and Payette River basins with different disturbance histories. Triangles represent sites without recent wildfires, diamonds sites with recent stand-replacing wildfires, and squares sites with channel-reorganizing events within the past 15 years.

be attributable to several factors, including the lack of the assumed founder or bottleneck effects. However, trout populations have been extirpated by fires in several instances (Propst et al. 1992; Dunham et al. 2003), and it seems unlikely that the populations were not severely reduced after major fire-related disturbances in our study area, particularly those resulting in channel-reorganizing events. Additionally, some of the streams that we studied were sampled by Burton (2005) immediately after the wildfires and debris flows, and fish were not detected. The sampling conducted by Burton (2005) was not spatially continuous, however, and it may be that fish were present between sampling sites. Alternatively, incomplete detectability of fish due to low sampling efficiency within sampled locations (Rosenberger and Dunham 2005) may have been an important factor in that study. Even with these sampling issues, it is noteworthy that Burton (2005) did not detect fish in these streams immediately after the disturbance. A longer-term evaluation of these and other streams in the Boise River basin detected rainbow trout at every site sampled (Dunham et al. 2007), so if the numbers of fish immediately after channel-reorganizing events were comparable to those in later years, at least some fish should have been detected.

In cases in which the expected losses of diversity due to known disturbances are not observed, genetic recovery by rapid dispersal into disturbed sites is often implicated (Lytle and Poff 2004; Fauvelot et al. 2006; Werth et al. 2006). The ability of salmonids to move through and use multiple habitats within river networks is believed to increase the resilience of populations in the face of wildfire and other types of disturbance (Dunham et al. 2003; Consuegra et al. 2005; Neville et al. 2006a). Here, the rapid increases in the number of fish detected in the years after the disturbance (Burton 2005) could have resulted from immigrants from other habitats, migratory fish returning to the stream that were not present at the time of the disturbance (Rieman et al. 1997), or fish colonizing from localized refuges within streams. It is likely that a combination of all these factors is important. In one sense, the relatively large degree of genetic differentiation indicated by many pairwise F_{ST} values suggests that recovery often results from the latter two processes. Both immigration by migratory fish moving back into their natal sites and fish that had taken refuge within an affected stream would maintain population segregation and potentially within-population diversity (Consuegra et al. 2005; see Neville et al. 2006b for comparison). Direct studies of movements by fish in this system are lacking, and we do not have samples from the main-stem Payette or Boise rivers with which to assign potentially migratory fish to their tributaries of origin, so we cannot speculate on the relative roles of the possible dispersal and recovery processes. However, all depend critically on habitat connectivity, and the fact that the populations isolated upstream of movement barriers showed substantially lower levels of genetic diversity suggests that habitat connectivity and movement are important.

Although the maintenance of connectivity has obvious importance for the conservation of fish in river networks, it can also increase the threat of invasion by nonnative fish (Fausch et al. 2009). Even after purposefully sampling habitats remote from likely sources of nonnative fish, we found fish with cutthroat trout characteristics and genetic evidence of hybridization with native rainbow trout in some populations. Many studies have emphasized the conservation problem of hybridization between these two species, though typically in terms of nonnative rainbow trout hybridizing with native cutthroat trout (e.g., Hitt et al. 2003; Weigel et al. 2003; Rubidge and Taylor 2004, 2005; but see Kozfkay et al. 2007). Cutthroat trout–rainbow trout hybrids were detected in more than 25% of the sampled locations, yet only 4% of individuals were identified as hybrids overall. None of our samples were characterized as hybrid swarms, and no pure cutthroat trout or cutthroat trout backcrosses were observed. Except for the two sites where F_1 hybrids were found, hybridization with cutthroat trout does not seem to be of recent origin, and thus it is probably still possible to keep the genomes of native rainbow trout intact. Ongoing monitoring of the patterns of hybridization may be warranted if protection of the native genomes of the rainbow trout in this system is a management priority.

Another potential genetic threat to the native rainbow trout in this study system is the historical stocking of hatchery rainbow trout to provide angling opportunities. We were unable to quantify the influences of hatchery-origin rainbow trout in this work. Fertile nonnative rainbow trout were stocked through 2002, and stocking since then has been with sterile triploid hatchery trout. Nonnative rainbow trout are stocked primarily in larger river and reservoir habitats in this system (J. Dillon, Idaho Department of Fish and Game, personal communication), but it is possible that individuals from the earlier stocking have moved into our study streams and hybridized with wild fish. It is thus possible that gene flow from nonnative rainbow trout has confounded the patterns that we observed. For example, the greater genetic diversity among rainbow trout in habitats without culvert barriers may be due to the presence of hatchery-origin trout or hybrids and an influx of novel alleles that is not observed in isolated populations. However, many of the sites in which we observed cutthroat trout hybrids were above barriers, suggesting that the influence of connectivity is not

confounded by hybridization. Additionally, if naturalized fish of 100% hatchery origin were present in our samples, we would expect to see a Wahlund effect (an excess of homozygous genotypes) from the combination of two distinct gene pools (hatchery and native); however, tests of allele frequencies against those expected under Hardy–Weinberg equilibrium did not show this. Unfortunately, we could not use this test to determine whether hybrids of native and hatchery-origin rainbow trout were present, because one generation of random mating would erase this signal. Future work could address this issue in more detail, such as by tracking the movements of hatchery fish to see whether they survive to spawn and attempt to reproduce in tributaries or by developing genetic markers to distinguish hatchery from native rainbow trout. Other work based on molecular markers similar to those applied here has found only a limited influence of nonnative rainbow trout on native populations (Araki et al. 2007; Matala et al. 2008).

Although this study represents one of the most spatially extensive studies of the responses of fish to wildfire (e.g., Dunham et al. 2003), there were several factors that we were unable to address statistically. First, owing to the limited sizes of our treatment groups we did not examine the potential for interactions among the three major influences on genetic diversity. It might be expected, for example, that isolation would increase the vulnerability of local populations to disturbances such as debris flows after a wildfire (Dunham et al. 2003). However, in the case of culverts, major flooding and erosion commonly cause fish movements to be restored by breaching the road crossing, destroying the culvert, or transporting it downstream (Howell 2006). Thus, depending on the time it takes to replace a culvert and whether or not the new culvert permits fish movement, there is often at least a temporary opportunity for fish movement and gene flow. We could not test the influence of culvert replacements because they were not documented at most of the sites that we studied. This would be a fruitful area for further study, with parallels in other work examining the influence of time since isolation on the persistence of populations upstream of barriers (e.g., Morita and Yamamoto 2002) and the direct influence of barriers on within-population genetic diversity (Yamamoto et al. 2004, 2006; Wofford et al. 2005; Small et al. 2007). In any case, isolation caused by culverts was strong enough to be readily apparent in terms of reduced allelic richness overall, in spite of the fact that many culverts may not have been in place for long time periods (>20 years).

Time since disturbance is another important factor that we did not explicitly consider. We did not have enough samples from within 1–3 years of a disturbance for comparison with those with older disturbance histories. However, direct observations in this system suggest that the abundance of rainbow trout recovers within a few years (Rieman et al. 1997; Burton 2005). Thus, it is possible that reductions in local population sizes and genetic variation are very short-lived. Furthermore, the very dynamic nature of the habitats in the system that we studied could promote dispersal and gene flow by forcing fish to emigrate during disturbance events or by favoring selection for increased dispersal ability (Hanski et al. 2004; Lytle and Poff 2004; Whiteley et al. 2006). The effects of disturbances such as those studied here may be more evident in systems with less physical connectivity or dispersal ability among the species studied (Dunham et al. 2003).

In a management context, the results of this work highlight the negative influences of habitat fragmentation and parallel the results of a large and growing body of research on trout and salmon facing similar challenges. Wildfire does represent a major source of disturbance to the streams we studied, but in interconnected habitats fish populations appear to be naturally resilient. In contrast to these large events, artificial barriers that restrict movement can have profound influences by disrupting connectivity (Park et al. 2008). Introductions of nonnative cutthroat and rainbow trout pose additional threats to the native rainbow trout we studied, as they do in many other cases (Dunham et al. 2003; Fausch et al. 2009). Collectively, these influences may over time reduce the resilience of the native rainbow trout in our system to wildfire or drive local populations to extinction even in the absence of disturbances.

The traditional approach to managing wildfire has tended to treat the effects of the fire itself as the most important threat, yet it is widely accepted that wildfire is an important natural process in stream ecosystems (Bisson et al. 2003). Many of the examples of fish responses to wildfire involve cases in which local populations were permanently extirpated because their habitats were degraded and fragmented to the point that they could not withstand further disturbance (Brown et al. 2001). The results of this study suggest that extirpation need not be the case. Proactive management of human-related threats to fish populations and the maintenance of natural movement processes would make it more likely that fish would persist in the face of natural disturbances.

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