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Timing and Location of Spawning by Nonnative Wild Rainbow Trout and Native Cutthroat Trout in the South Fork Snake River, Idaho, with Implications for Hybridization

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Abstract.—Our goal was to assess the likelihood of hybridization between introduced rainbow trout *Oncorhynchus mykiss* and native Yellowstone (YS) cutthroat trout *O. clarki bouvieri*, based upon habitat use and temporal overlap during spawning. We used radio transmitters in 1996 and 1997 to describe the spawning behavior of rainbow trout, hybrids of rainbow trout and YS cutthroat trout, and YS cutthroat trout (1997 only). Fish displayed two distinct spawning strategies, either spawning in side channels of the main stem (9 rainbow trout, 14 hybrids, and 10 YS cutthroat trout) or in tributaries (5 rainbow trout, 3 hybrids, and 7 YS cutthroat trout). Within the main stem, the majority of rainbow trout and YS cutthroat trout migrated to the same 8-km section to spawn, whereas hybrid trout spawned throughout the study site. The median spawning date for main-stem-spawning YS cutthroat trout (June 9) was significantly later than for rainbow trout (May 19) and hybrids (May 18). However, long spawning periods for rainbow trout (94 d), hybrids (113 d), and YS cutthroat trout (71 d) allowed for considerable overlap. The amount of spawning overlap varied among the four tributaries. In one tributary, complete spatial and some temporal overlap occurred (three rainbow trout, two hybrids, and two YS cutthroat trout); in another tributary no spatial or temporal overlap occurred (four rainbow trout and three YS cutthroat trout); and only YS cutthroat trout used the remaining two tributaries (one YS cutthroat trout in each). Molecular analyses verified that females of both rainbow trout and YS cutthroat trout were hybridizing and showed that the genetic composition of hybrid trout was more similar to rainbow trout than to YS cutthroat trout (mean = 64% rainbow trout markers). These results suggest that the majority of YS cutthroat trout (12 of 17) experience spatial and temporal overlap with rainbow trout and hybrids, but three tributaries may still provide some reproductive isolation for native fish.

Yellowstone (YS) cutthroat trout *Oncorhynchus clarki bouvieri* have experienced a dramatic reduction in distribution and abundance during the last century (Behnke 1992). The remaining populations are located in small headwater drainages (Young 1995 throughout Yellowstone National Park (Gresswell 1995) and in a few large rivers (Clancy 1988; Thurow et al. 1988). Major reasons for the decline include introductions of nonnative salmonids, habitat degradation, and angler exploitation (Krueger and May 1991; Leary et al. 1995; Young 1995). Most authors cite introduced salmonids, specifically rainbow trout *O. mykiss*, as having had the greatest impact on YS cutthroat trout through hybridization and competition. Hybridization is thought to have contributed to the replacement of YS cutthroat trout by rainbow trout throughout a large portion of their historical range within the upper Snake River (Thurow et al. 1988) and lower Yellowstone River drainages (Clancy 1988).

Hybridization with introduced fishes has played

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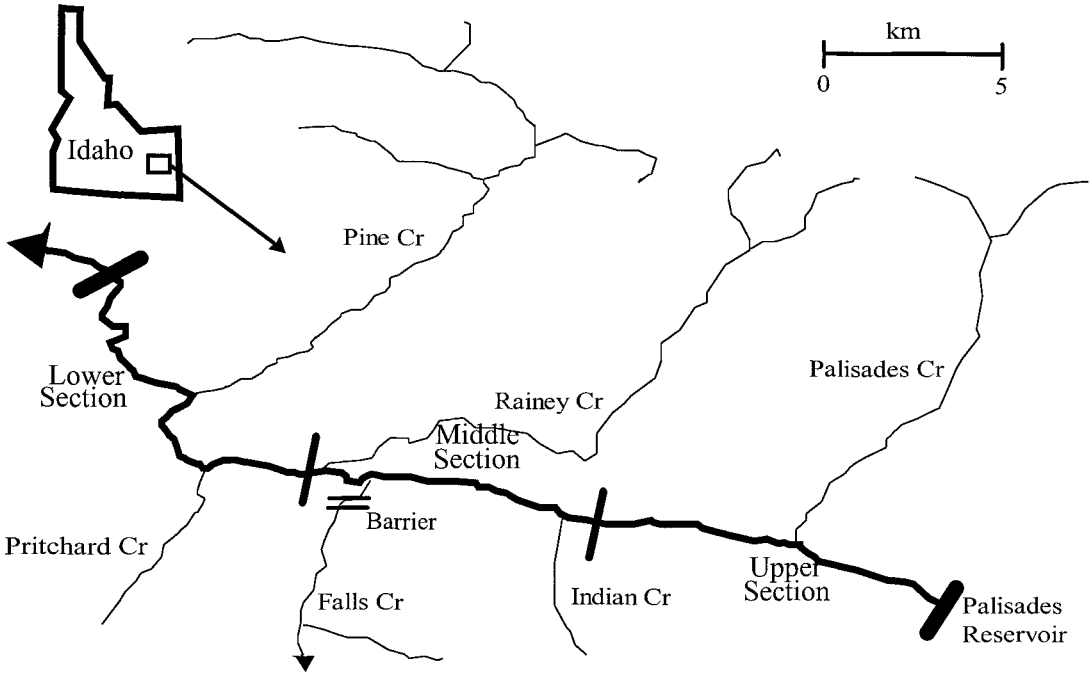

FIGURE 1.—Study site on the South Fork Snake River, Idaho.

a major role in the decline of native fish fauna throughout the western United States. Large-scale introductions placed previously allopatric species, subspecies, and stocks into sympatry, which often resulted in hybridization (Krueger and May 1991). Almost universally, the rate of hybridization between native and introduced species has been much greater than where naturally sympatric assemblages coexist. Examples include extensive hybridization between native and nonnative black bass *Micropterus* spp. (Morizot et al. 1991), pupfish (Cyprinodontidae; Echelle and Conner 1989), and most species and subspecies of salmonids (Krueger and May 1991; Leary et al. 1995).

Reproductive isolation is important in reducing hybridization in naturally sympatric populations of salmonids. Differences in spawning locations or timing are common (Trotter 1989; Behnke 1992), although some overlap can exist without hybridization occurring (Campton and Utter 1985; Heggberget et al. 1988). However, little is known about the amount of temporal and spatial separation in spawning required to prevent hybridization between introduced and native species.

The South Fork Snake River provides a unique opportunity to address this question for populations of native YS cutthroat trout and introduced rainbow trout in a large river. Rainbow trout were stocked from the early 1900s until 1981 but represented a small proportion of the trout population (Schrader and Gamblin 1994). Since 1984, wild rainbow trout and hybrids of rainbow trout and cutthroat trout (hereafter referred to as hybrids) have increased dramatically and now constitute up to 33% of the trout abundance in some areas (Schrader and Gamblin 1996). Concern about the detrimental effects of their interbreeding with YS cutthroat trout prompted this research. The objectives were to (1) describe the location, timing, and movements associated with spawning for rainbow trout, hybrids, and YS cutthroat trout in the South Fork Snake River, and (2) describe the amount of spatial and temporal overlap between rainbow, hybrids, and YS cutthroat trout during spawning.

Study Site

This study was conducted in southeastern Idaho on a 35.9-km section of the South Fork Snake River (SFSR) from Dry Canyon to Palisades Dam (Figure 1). Within the study site, the SFSR is a sixth-order stream and ranges from 1,582 to 1,646 m in elevation. Mean monthly flows range between 42 and 709 m^3/s (1987–1996). In the upper 13.6 km of the study site, the river is confined to a single channel with low sinuosity and no side channels. From river kilometer (rkm) 22.3 to 7.0, the flood-

TABLE 1.—Watershed and stream characteristics for spawning tributaries to the South Fork Snake River within the study site; NA = not available, rkm = river kilometer. Falls Creek was not accessible due to barrier falls at mouth.

Stream	Stream order	Drainage area (km^2)	Discharge (m^3/s)		Stream length	Location of stream mouth (rkm)[a]
			Low	High		
Palisades	3	156.0	0.2	17.3	26.5	30.2
Indian	2	21.9	NA	NA	9.7	22.3
Rainey	3	145.8	0.6	10.0	23.7	15.3
Pritchard	2	36.2	NA	NA	11.4	10.9
Pine	3	163.7	0.1	22.6	26.5	6.9

[a] Distance from downstream end of study site.

plain broadens (from 0.5 to 2.0 km wide), and an extensive network of side channels exists. In the lower 8 km the river flows through a deep canyon where the floodplain narrows to 0.5 km and fewer side channels exist. Substrates throughout the study site are predominated by cobble in the main channel and cobble and gravel in side channels. Large stands of cottonwood *Populus* spp. dominate the floodplain vegetation. Five tributaries within the study area are used by YS cutthroat trout for spawning (Table 1).

A number of trout species are present within the study site. We considered the current cutthroat trout population to be YS cutthroat trout but recognized that both large-spotted and fine-spotted morphologies and their hybrids were present. Resident populations of YS cutthroat trout were also found in most of the perennial tributaries (Moore and Schill 1984). Naturally reproducing populations of rainbow trout and hybrids, as well as brown trout *Salmo trutta,* are also present throughout the main river.

Methods

Radiotelemetry.—We used radiotelemetry to follow the spawning movements of 16 rainbow trout and 14 hybrids in 1996 and 11 rainbow trout, 16 hybrids, and 28 YS cutthroat trout in 1997. Two additional rainbow trout were tagged in 1997 to help describe the timing and location of rainbow trout spawning in Pine Creek. This information was only used for Pine Creek analyses. The study site was divided into 18 segments, each 2-km. The number of fish tagged in each segment was approximately proportional to the abundance of that study group (three study groups: rainbow trout, hybrids, and YS cutthroat trout), as determined from population estimates conducted by Schrader and Gamblin (1996).

In both years fish were captured by boat electrofishing before spawning from March 6 to 15. They were anaesthetized with tricaine methanesulfonate (MS-222), and transmitters were surgically implanted according to the procedures described by Bigood (1980), as modified by Schill et al. (1994). We selected individuals greater than 325 mm total length (age 3 and older) in 1996. In 1997, we selected individuals greater than 350 mm total length in which maturing gonads could be observed through the surgical incision. Immature fish did not receive transmitters and were released after the incision was closed. All transmitters were less than 2.2% (mean, 1.3%) of fish body weight. Transmitters had an external whip antennae (8, 11, or 16 g in air) and had expected battery lives of 200, 300, and 400 d, respectively. Transmitters operated daily from 0700 to 2300 hours on a frequency of 150 MHz. Fish were held in live cages at the surgery site for 1–12 h after tagging to allow for recovery. All fish were released at their initial point of capture.

Fish were located weekly from March 15 to August 20 using a three-element directional yagi antenna. Locations were obtained using a jet boat, by vehicle, and on foot, depending on accessability. A fixed-wing aircraft was used to find fish missing for more than 2 weeks and for fish located downstream of rkm 8.0 during high flows from June 9 to July 1, 1997. Locations were recorded to the nearest 0.1 km as located on 7.5-min topographic maps and then transferred into ArcView, version 2.1, (Environmental Systems Research Institute, Inc.) for analysis.

We were unable, because of high and turbid flow conditions during spawning, to physically determine where and when individual fish actually spawned. Therefore, we developed a set of criteria to determine the spawning status of each fish. All fish that either migrated into tributaries or moved from the main channel into a side channel were assumed to have spawned. All remaining fish were considered nonspawners. To evaluate the criteria, after the spawning season we recaptured 24% (four rainbow trout, four hybrid, and nine YS cutthroat trout) of 72 radio-tagged fish followed during spawning. They were euthanatized and their gonads inspected to determine whether they had spawned.

The location and timing of spawning for each fish were determined from its movements. The spawning location was defined by the farthest ex-

tent of a fish's migration (either upstream or downstream) and recorded to the nearest 0.1 km. We defined the migration date for each individual fish as being midway between the date we first observed the fish migrating and the date of the previous location for that fish (Swanberg 1996). This same approach was used to determine the date each individual fish entered and left its spawning location. We defined the spawning period as extending from the time an individual fish entered its spawning location until it left. To determine a spawning date for each individual fish, we used the midpoint of the fish's spawning period. Finally, the overall spawning period for each study group began when the first fish entered its spawning area and ended when the last fish left.

Genetic analyses.—We used a combination of morphological characters and molecular genetic analysis to determine the taxonomic identity of radio-tagged fish. Field techniques relied on spotting pattern, body color, mandible length, and presence or absence of coloration below the gill covers. In 1997 we used molecular genetic techniques to verify field identifications for 54 of the 57 fish. Fin tissue was collected during surgery and preserved by freezing with dry ice or by placing in 95% ethanol. Both nuclear and mitochondrial DNA markers were used to identify hybrids. Nuclear DNA is more likely to detect hybrids, especially if backcrossing has occurred with either parental species. Mitochondrial DNA, however, lends greater insight into the direction of the hybridization event.

We used 15 nuclear DNA markers based on random amplified polymorphic DNA (RAPD) analyses to detect hybrids (Williams et al. 1990; Toline et al. 1998). These molecular markers are considered fixed between rainbow trout and YS cutthroat trout, although not all known stocks of rainbow trout were examined. Therefore, we used a conservative approach to assess genetic purity. A fish was considered a hybrid if at least two loci from both species were present, and pure if at least 14 of the 15 markers were from one species. The equation used to calculate percent introgression (PI) was

$$\mathrm{PI} = (M_r/M_t) \times 100,$$

where M_r = number of markers indicating rainbow trout and M_t = the total number of markers (15). Pure YS cutthroat trout received a value of 0%, pure rainbow trout 100%; hybrid values reflected the relative contribution of each parent species to the genome.

Mitochondrial DNA were assessed using restriction fragment-length polymorphism (RFLP) analyses (Seitz 1999; Toline et al. 1999). This technique produced definitive identification of the mitochondrial genome and was used to determine whether females from both species were hybridizing.

Statistical Analyses

We conducted statistical analyses on main-stem spawners only because of small sample sizes in each tributary. We tested for differences between each study group for seven spawning migration variables: date of initial migration, migration period, date entered the spawning area, spawning period, date left the spawning area, spawning date, and distance migrated. Nonparametric tests were used for all analyses of radio-tagged fish because of large variances and small sample sizes. We used Wilcoxon's rank-sum tests to compare data between years and sexes for each spawning migration variable and Kruskal–Wallis tests to examine differences among study groups. Subsequent Bonferroni comparison tests were conducted to determine differences between each study group ($P < 0.033$). We used chi-square tests to identify differences between the number of upstream versus downstream migrants and main-stem versus tributary spawners within each study group. To examine patterns in the spawning locations of main-stem spawners, we divided the river into lower (rkm 0.0–14.0), middle (rkm 14.1–22.3) and upper (rkm 22.4–35.6; Palisades Dam) sections. We then used Fisher's exact tests to test for differences in the distribution of spawning locations used by each study group. A statistical significance value (α) of 0.10 was chosen for all tests instead of the conventional value of 0.05 because the small sample size and large variation among fish would have made it unlikely to detect differences when they actually existed. Statistical Analysis Systems (version 6.12) was used to perform all statistical computations.

Results

We obtained spawning information on 7 rainbow trout and 7 hybrids in 1996 and 7 rainbow trout, 9 hybrids, and 17 YS cutthroat trout in 1997. Within the rainbow trout and hybrids, none of the seven spawning migration variables differed significantly between years (Table 2), so data from both years were combined. Similarly, no differences in the

TABLE 2.—Medians and significance values of Wilcoxon's rank-sum tests between sampling years for the seven spawning-migration variables for rainbow trout and hybrids of rainbow trout and Yellowstone cutthroat trout in the South Fork Snake River, Idaho.

Variable	Rainbow trout			Hybrids		
	1996	1997	*P*	1996	1997	*P*
Begin migration	Apr 24	May 5	0.596	Apr 26	Apr 21	0.462
Enter spawning area	May 1	Apr 15	0.387	Apr 27	May 2	0.845
Spawning date	May 20	May 19	0.623	May 21	May 17	0.846
Leave spawning area	Jun 12	Jun 3	0.713	Jun 15	Jun 18	0.946
Migration period (weeks)	2	1	0.103	1.0	1.5	1.0
Spawning period (d)	46	34	1.0	44	56	0.847
Distance migrated (km)	6.4	5.1	0.805	7.6	5.9	0.953
Sample Size[a]	3–4	3–5		3–6	8–9	

[a] Numbers reflect the range of sample sizes used in the analyses for the seven spawning migration variables.

seven spawning migration variables were detected between sexes for rainbow trout or YS cutthroat trout (Table 3). Six of the spawning migration variables for hybrids did not differ, whereas males began spawning migrations before females ($P = 0.071$). Due to overall similarities in spawning behavior, we combined data from both sexes for all analyses.

Radio-tagged fish displayed two distinct spawning strategies. The majority of rainbow trout (9 of 14), hybrids (14 of 16), and YS cutthroat trout (10 of 17) migrated within the SFSR and spawned in the main stem. The remaining 14 fish migrated into tributaries. Rainbow trout spawned in two, hybrid trout in one, and YS cutthroat trout in four of the tributaries. Hybrid trout used tributaries significantly less than main-stem spawning areas (chi-square, $P = 0.002$), whereas rainbow ($P = 0.285$) and YS cutthroat trout ($P = 0.467$) showed no differences in usage of main-stem and tributary spawning areas.

Main-Stem Spawning Strategy

The majority of main-stem spawners used side channels (7 of 9 rainbow trout, 12 of 14 hybrid trout, and 9 of 10 YS), whereas the remaining five individuals spawned in the main channel. Spawning locations for rainbow trout and YS cutthroat trout varied by study section. Significantly more rainbow trout (chi-square, $P = 0.013$) and YS cutthroat trout ($P = 0.007$) spawned in the middle section than in either the upper or lower sections (Figure 2). All rainbow and YS cutthroat trout initially tagged in the lower section migrated upstream, whereas five of seven fish tagged in the upper section migrated downstream. Fish in both the lower and upper sections migrated greater distances (median = 10.8 and 14.2 km, respectively) than did fish in the middle section (median = 1.7 km), although this was not statistically significant (Kruskal–Wallis, $P = 0.118$). Hybrids spawned throughout the entire study site, most spawning in the lower and middle sections. Movements of hy-

TABLE 3.—Medians and significance values of Wilcoxon's rank-sum tests for the seven spawning-migration variables for mainstem spawners (South Fork Snake River, 1996 and 1997) between male and female rainbow trout, hybrids of rainbow trout and Yellowstone cutthroat trout, and Yellowstone cutthroat trout. We did not determine the sex of one cutthroat trout and six hybrids; these fish were not included in the analyses.

Variable	Rainbow trout			Hybrids			Cutthroat trout		
	Female	Male	*P*	Female	Male	*P*	Female	Male	*P*
Begin migration	May 1	Apr 12	[a]	May 5	Apr 5	0.071	Jun 2	May 26	1.0
Enter spawning area	May 6	Apr 15	0.152	May 5	Apr 22	0.177	Jun 2	Jun 2	0.846
Spawning date	May 19	May 27	0.517	May 9	May 16	0.551	Jun 10	Jun 15	1.0
Leave spawning area	Jun 2	Jul 8	0.519	May 13	Jun 3	0.294	Jul 2	Jul 10	0.439
Migration period (weeks)	1	2	0.387	1	1	0.514	1	1.5	0.206
Spawning period (d)	30	77	0.195	13	55	0.233	15	26	0.846
Distance migrated (km)	3.5	9.2	0.515	4.0	6.5	0.178	12.0	1.7	0.245
Sample Size[a]	6	3		3–4[b]	5		5	2–3[b]	

[a] Sample size of males = 1.
[b] Numbers reflect the range of sample sizes used in the analyses for the seven spawning-migration variables.

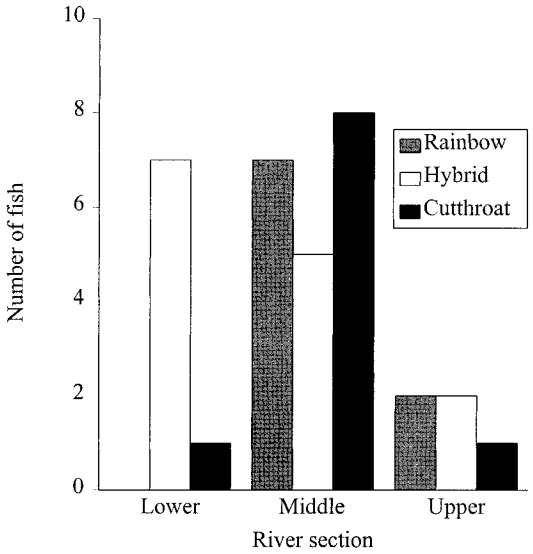

FIGURE 2.—Numbers of rainbow trout, hybrid, and Yellowstone cutthroat trout main-stem spawners by the river section, South Fork Snake River, Idaho.

brids initially tagged in the middle and upper sections were similar to rainbow and YS cutthroat trout, but most hybrids tagged in the lower section moved to spawning areas downstream. All five fish that spawned in main-channel sites used the upper section where side channels were absent.

Yellowstone cutthroat trout behaved differently than rainbow trout and hybrids for four of the seven spawning migration variables (Table 4). Significant differences were observed among the three study groups for the date they began migrating (Kruskal–Wallis, $P = 0.003$), entered spawning the areas ($P = 0.002$), spawning date ($P = 0.002$), and date they left spawning areas ($P = 0.091$). Bonferroni comparison tests for these four variables showed that YS cutthroat trout were significantly different from rainbow trout and hybrids for all but the date they left spawning areas. The median dates for each of these variables occurred 2–4 weeks earlier for rainbow trout and hybrids than for YS cutthroat trout. However, other characters showed considerable overlap between the study groups. The long spawning periods for rainbow trout (94 d), hybrids (113 d), and YS cutthroat trout (71 d) produced temporal overlap between the study groups (Figure 3). Secondly, although differences between sexes for each spawning variable were not significant, male rainbow trout and hybrids entered spawning areas earlier, left spawning areas later, and spent almost twice as long in spawning areas as did females. Finally, mitochondrial DNA analysis verified that females of both rainbow trout and YS cutthroat trout were hybridizing. Six of 13 hybrid trout had mitochondrial DNA markers for rainbow trout and seven for YS cutthroat trout.

Spawning behavior of hybrids was more similar to rainbow trout than YS cutthroat trout. We observed no differences between hybrids and rainbow trout for the seven spawning migration variables (Table 4). Surprisingly, the median dates that hybrids began migrating, entered spawning the areas, spawned, and the length of spawning periods were further from YS cutthroat trout than from rainbow trout. Hybrids were intermediate between the parental species for the date they left the spawning areas, whereas the migration period, distance migrated, and direction of migration were similar among study groups. We used molecular analyses to determine the genetic composition of the 10 hybrids that spawned. These fish were more introgressed with rainbow trout than YS cutthroat trout (mean = 64% rainbow markers; Figure 4).

Tributary Spawning Strategy

Varied amounts of habitat use and overlap were observed in the four tributaries used for spawning.

TABLE 4.—Results from statistical tests conducted on main-stem spawners (South Fork Snake River) for the seven spawning-migration variables. Sample size, range, median, significance values of Kruskal–Wallis tests, and Bonferroni comparison tests are displayed. Significant differences between rainbow trout and cutthroat trout for Bonferroni comparison tests are indicated by R–C and between hybrids (rainbow trout and Yellowstone cutthroat trout) and cutthroat trout by H–C.

Variable	Rainbow trout			Hybrids		
	N	Range	Median	*N*	Range	Median
Begin migration	7	Apr 9–May 6	Apr 28	13	Apr 2–May 19	Apr 21
Enter spawning area	9	Apr 9–May 12	Apr 26	14	Apr 2–May 19	Apr 29
Spawning date	9	Apr 22–Jun 4	May 19	14	Apr 22–Jun 10	May 18
Leave spawning area	9	May 5–Jul 12	Jun 3	14	May 12–Jul 24	Jun 15
Migration period (weeks)	7	1–2	1	13	1–3	1
Spawning period (d)	9	12–84	34	14	8–92	51
Distance migrated (km)	9	2.6–16.4	5.1	15	0.4–27.0	6.5

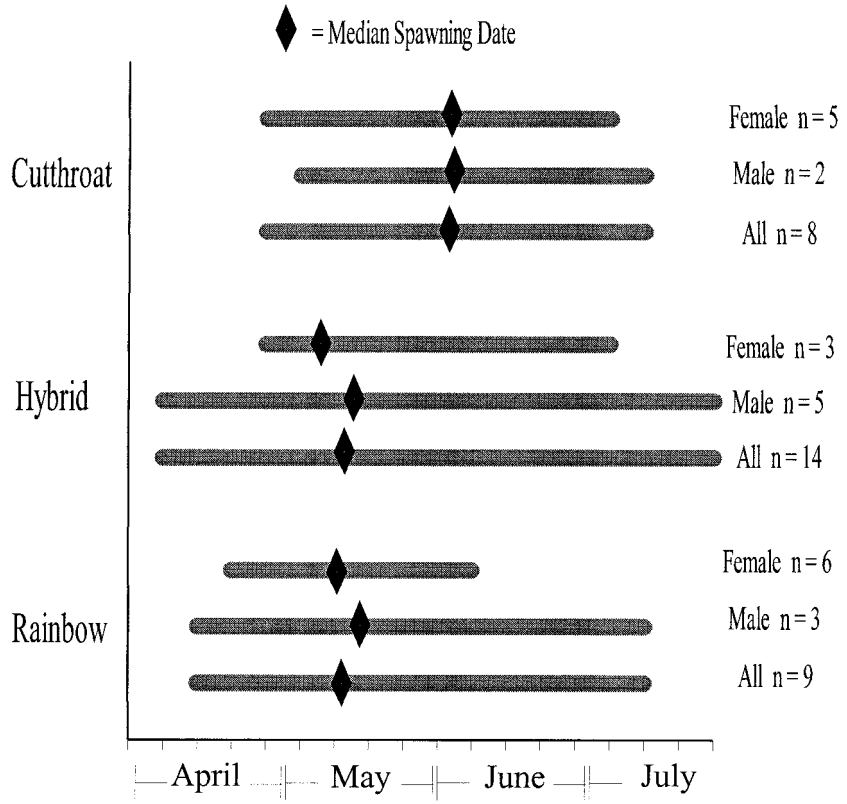

FIGURE 3.—Spawning periods for all radio-tagged rainbow trout, hybrids, and Yellowstone cutthroat trout spawning in the main stem of the South Fork Snake River, Idaho. Spawning periods for males, females, and both sexes combined were designated as beginning on the date the first fish entered its spawning area and ending when the last fish left.

In Pine Creek, YS cutthroat trout migrated further upstream to reach spawning areas (ranged 15.4–24.5 km, $N = 3$) than rainbow trout (range 2.0–9.0 km, $N = 4$; Figure 5). Similarly, the spawning periods differed between study groups. Rainbow trout spawned from March 20 to June 9 and YS cutthroat trout from June 13 to July 28 (Figure 6). Hybrids did not spawn in Pine Creek. In Palisades Creek, the spawning locations and spawning periods of all three study groups coincided. All fish spawned in the lower 2.0 km of the creek. The spawning periods were April 22 to June 16 ($N = 3$) for rainbow trout, May 24 to June 21 ($N = 2$) for hybrids, and June14 to July 3 ($N = 2$) for YS cutthroat trout. Only YS cutthroat trout spawned in Rainey and Indian creeks (one fish in each creek).

TABLE 4.—Extended.

Cutthroat trout				Bonferroni comparison tests
N	Range	Median	*P*	
8	Apr 30–Jun 15	May 26	0.003	R-C, H-C
8	Apr 30–Jun 23	May 26	0.002	R-C, H-C
8	May 29–Jul 2	Jun 9	0.002	R-C, H-C
9	Jun 15–Jul 10	Jul 2	0.091	
8	1–2	1	0.580	
8	10–63	18	0.240	
10	0.0–17.4	6.8	0.886	

Status of Radio-Tagged Fish

We did not obtain spawning information for 38 of the 85 fish that were implanted with transmitters. Seven fish died within the first 4 weeks (surgery mortality), 12 fish died from 5 to 10 weeks after surgery but before spawning migrations (prespawning mortality), and three transmitters failed. Of the remaining 16 nonspawners, we concluded that 11 fish did not spawn based on their movement patterns. The five remaining fish were recaptured after the spawning season and confirmed to have not spawned. Radio-tagged fish

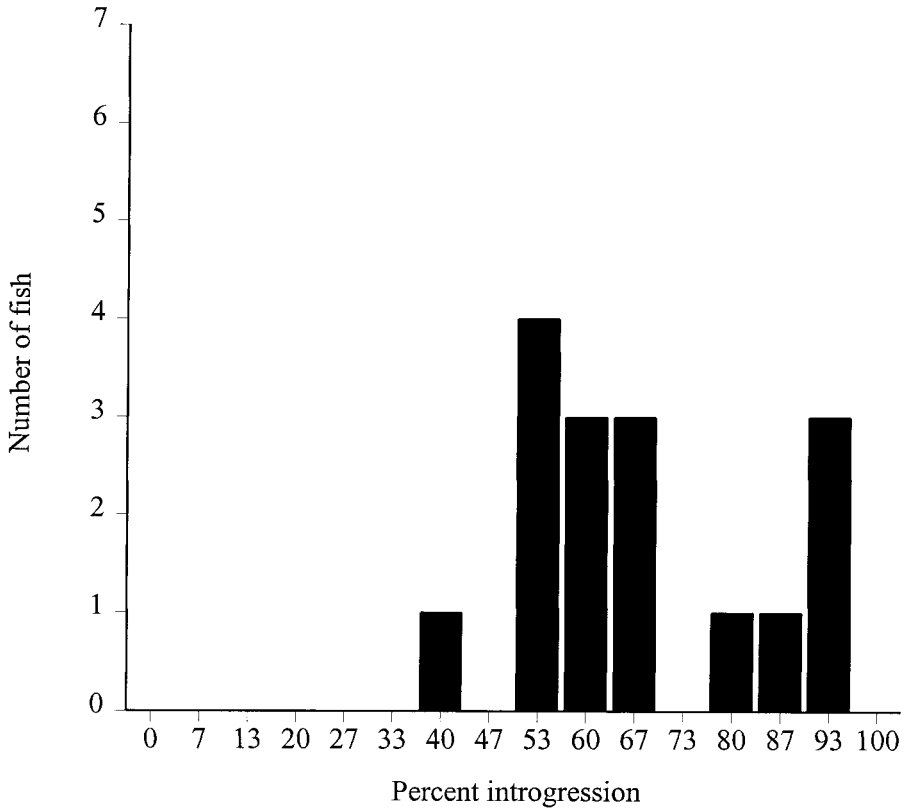

FIGURE 4.—Genetic composition of 16 radio-tagged hybrids of rainbow trout and Yellowstone cutthroat trout based on 15 diagnostic markers produced from random amplified polymorphic DNA analyses.

ranged in total length from 342 to 521 mm (medians: rainbow trout = 448 mm, hybrids = 447 mm, YS cutthroat trout = 420 mm). Yellowstone cutthroat trout were significantly smaller than rainbow trout and hybrids (Kruskal–Wallis, $P = 0.047$). There was no significant difference between the lengths of spawners versus nonspawners for rainbow trout (Wilcoxon's rank-sum, $P = 0.577$), hybrids ($P = 0.572$), or YS cutthroat trout ($P = 0.451$).

Validation of Methodologies

To determine if the assessments of whether fish spawned were accurate, we recaptured, euthanatized, and examined the gonads of 17 radio-tagged fish after the spawning seasons. All four tributary spawners and all seven main-stem spawners had spawned. Five of six fish that were classified as nonspawners did not spawn; the one fish that spawned stayed in and apparently spawned in the main channel.

The molecular analyses indicated that we accurately identified in the field 51 of the 54 fish that we radio-tagged. All rainbow trout and YS cutthroat trout were correctly identified, whereas three hybrids were misclassified, two as rainbow trout and one as a YS cutthroat trout. These results suggest that approximately 2 (6%) of the 34 radio-tagged fish that were not genetically verified might have been misidentified.

Discussion

Reproductive isolation appears to be an important factor in preventing hybridization between related fish species (Hubbs 1955; Leary et al. 1995). Complete spatial separation (Clancy 1988) or temporal separation (Thurow 1982; Huston et al. 1984; Likenes and Graham 1988) during spawning may prevent hybridization in the few streams where introduced rainbow trout and native cutthroat trout coexist. Similarly, in systems where these species coevolved, nearly complete temporal and spatial segregation during spawning has been reported (Hartman and Gill 1968; Trotter 1989). Rainbow trout generally spawn lower in the drainages and before cutthroat trout.

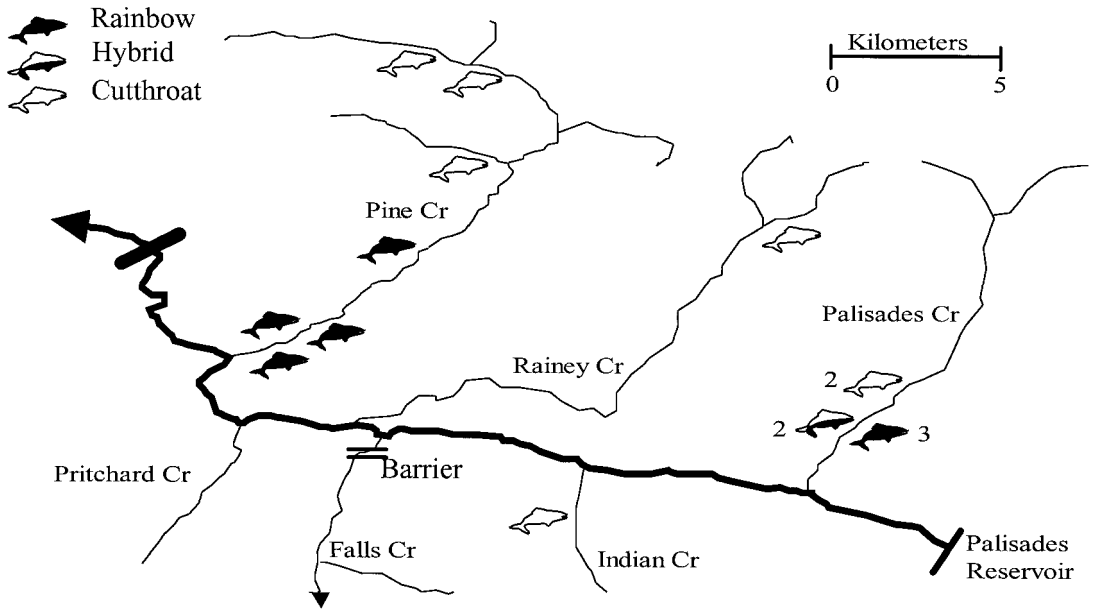

FIGURE 5.—Spawning locations for each rainbow trout, hybrid, and cutthroat trout that pawned in tributaries of the South Fork Snake River, Idaho. In Palisades Creek (Cr), numbers reflect the number of individuals for each taxon.

Main-stem spawners in the SFSR did not display either the spatial or temporal separation observed in streams where these species have historically coexisted. In the main stem, YS cutthroat trout spawning areas completely overlapped those used by rainbow trout and hybrids, suggesting an absence of spatial segregation. Although median spawning dates for rainbow and hybrids were significantly different than for YS cutthroat trout, long spawning periods within each study group allowed for some temporal overlap. These results suggest that main-stem-spawning YS cutthroat trout lack the spatial and possibly temporal separation necessary to prevent hybridization.

The spawning overlap and subsequent potential for hybridization varied among tributaries. In Pine Creek, rainbow trout spawned earlier and lower in the tributary than YS cutthroat trout. This pattern is similar to that observed in the Blackfoot River, Idaho (Thurow 1982), where the two species coexist, and in coastal streams where they coevolved (Hartman and Gill 1968; Trotter 1989). In addition, no radio-tagged hybrids spawned in this tributary, suggesting that hybridization may not be occurring. In Palisades Creek, fish from all study groups spawned in the lower 2 km, even though high-quality spawning areas were abundant further upstream (Miller and Roby 1957; Moore 1980). Some overlap in spawning periods was also observed. An irrigation diversion at tributary kilometer 1.3 limits recruitment from upstream spawning areas (Miller and Roby 1957; Moore and Schill 1984). Researchers have found that reductions in spawning habitat encourage hybridization in many systems by decreasing the number of potential spawning areas (Hubbs 1955; Leary et al. 1995). This may explain the limited use of upstream spawning areas and the presence of hybrid trout in Palisades Creek. Although sample sizes were low, only YS cutthroat trout spawned in Rainey and Indian creeks. Thus YS cutthroat trout in these tributaries appeared to experience the smallest threat from hybridization, although they probably represent a minor component of the spawning population.

Results from molecular analyses confirmed that rainbow trout, hybrids, and YS cutthroat trout are readily interbreeding and provided additional information on the composition and direction of genetic introgression. Initially, it was predicted that most hybrid trout would be produced from crosses between male YS cutthroat trout and female rainbow trout because many male YS cutthroat trout in the SFSR were ready to spawn before the females (Idaho Department of Fish and Game, unpublished data). However, mitochondrial DNA analysis did not support this prediction; i.e., the maternal parents of radio-tagged hybrid trout were

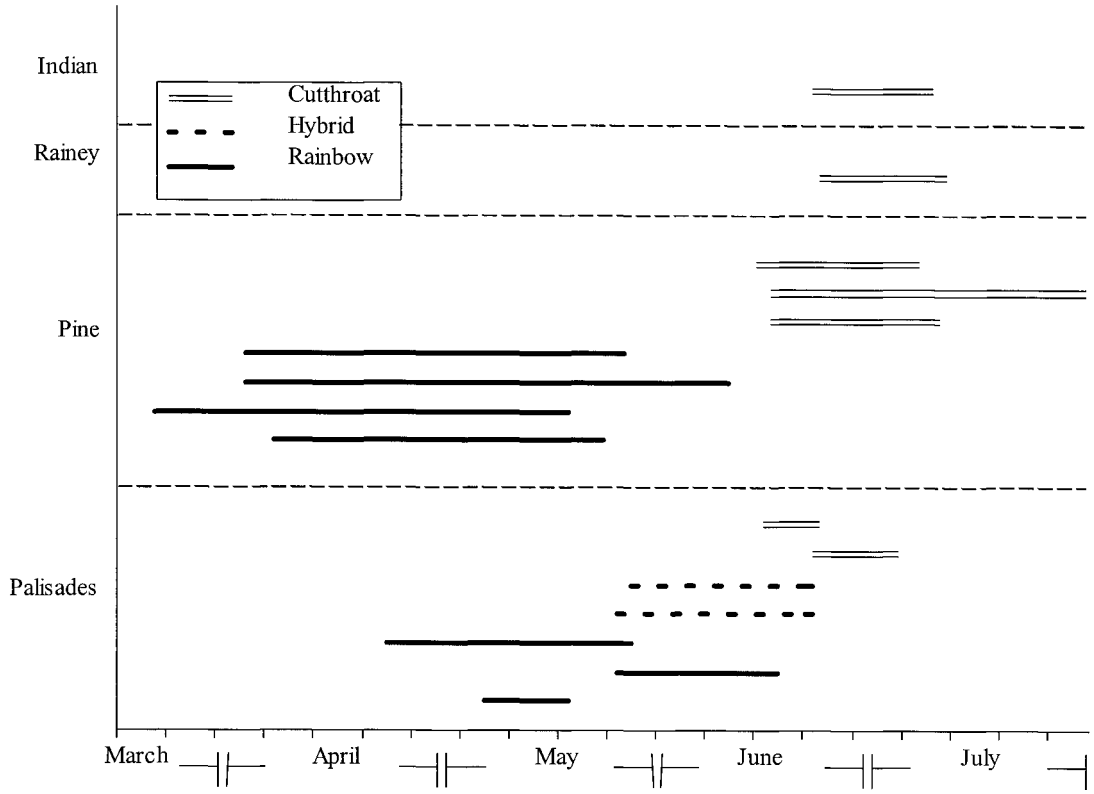

FIGURE 6.—The spawning period for each rainbow trout, hybrid, and cutthroat trout by tributary (Indian, Rainey, Pine, and Palisades creeks) in which they spawned.

evenly distributed between rainbow trout and YS cutthroat trout. This result again indicates that some overlap exists between spawning periods. It also suggests an absence of mate-pairing behaviors that would discourage interspecific breeding.

Interestingly, nuclear DNA analyses showed that most hybrids either spawned with other hybrids or backcrossed with rainbow trout, but they rarely backcrossed with YS cutthroat trout (Figure 3). We recognize that our radio-tagged fish may not have represented a random sample of the hybrid population, thereby biasing these results. However, additional sampling ($N = 21$) in side-channel spawning areas verified these findings (unpublished data). This may explain why the spawning characteristics for hybrids were more similar to rainbow trout than YS cutthroat trout. Another explanation may include differential survival of offspring between the reciprocal male $\times$ female crosses, as Herke et al. (1990) observed between northern pike *Esox lucius* and chain pickerel *E. niger*. However, no differences were found in survival or growth of hybrids produced by reciprocal crosses of rainbow trout and golden trout *O. aguabonita* (Halliburton et al. 1983).

The results of this study show substantial overlap in the spawning locations used by rainbow trout, hybrids, and YS cutthroat trout and some overlap in spawning periods. This indicates that a large portion of the YS cutthroat trout population in the SFSR may currently be at risk from hybridization. Comparisons our results with those of those of a previous study (Moore and Schill 1984) indicate that rainbow trout have expanded their range within the last decade to include spawning areas in Pine Creek. Continued expansion of rainbow trout and hybrids into tributaries would result in further encroachment on spawning areas currently used by only YS cutthroat trout.

Two aspects of the sampling may have affected the degree of overlap observed. Not finding any information describing when fish spawn once they reach their spawning area, we used the midpoint of the spawning period to define when a fish spawned. Therefore, spawning dates we reported may be imprecise, as well as vary among individ-

uals, sexes, spawning strategies, and study groups. Secondly, the period from 1980 to 1994, when rainbow trout and hybrid populations expanded, were mostly drought years. Conversely, 1995, 1996, and 1997 were characterized by normal to extremely high flows. Many authors have observed a later spawning period for salmonids during high-flow years (Kiefling 1978; Gresswell and Varley 1988; Likenes and Graham 1988), which may have affected the extent of temporal overlap we observed.

Management Implications

The Idaho Department of Fish & Game manages the SFSR as a native YS cutthroat trout fishery. This study was designed to provide baseline information necessary to develop management strategies to protect the genetic integrity and population viability of the YS cutthroat trout. The distribution of rainbow trout and hybrid spawning sites throughout the SFSR and two tributaries suggests that any management activity to control rainbow trout and hybrids will need to be intensive and widespread. Understanding the timing of spawning, locations of major spawning areas, and migration patterns of each species should increase the success of management activities.

Conservation strategies to protect native fishes have focused on the removal of nonnative species (Larson et al. 1986; Leary et al. 1995). The ability to accurately and quickly distinguish hybrid trout from the parental species is necessary for successful removal efforts. Past studies have shown that hybrid trout produced from crosses between rainbow trout and most cutthroat trout subspecies are often difficult to visually differentiate from their parents (Leary et al. 1984; Behnke 1992). For example, in a small stream in California, efforts to remove hybrids of Paiute cutthroat *O. clarki seleniris* and rainbow trout were unsuccessful because of similarities in coloration and spotting patterns (Busack and Gall 1981). Our finding that YS cutthroat trout in the SFSR can be visually separated from rainbow trout and hybrids greatly increases the likelihood of success for removal efforts. However, continued molecular verification will be necessary because the genetic structure of hybrids may change and affect the accuracy of current identification techniques.

Few removal efforts have been conducted in large rivers. Recently, electrofishing was used in the Colorado River system with marginal success (P. Badame, Utah State University, personal communication). Results of electrofishing from side channels during the rainbow trout and hybrid spawning periods suggest that capture rates will need to be increased to be effective and economically feasible (our unpublished data). Promoting angler harvest to control unwanted species is another option, although there are few examples where this technique has worked. To be effective, these techniques must reduce the rainbow trout and hybrid populations to levels that minimize threat to YS cutthroat trout. Past examples of this approach in large systems do not exist.

Protecting the genetic integrity of tributary spawners may prove more effective and simpler than for main-stem spawners. Instream weirs have been successfully used to trap upstream-migrating YS cutthroat trout in several systems (Moore and Schill 1984; Clancy 1988). This technique should be an effective means of capturing and removing tributary-spawning rainbow trout and hybrids, thereby minimizing the threat of hybridization for this portion of the population.

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