ASSESSMENT OF REPRODUCTIVE ISOLATION BETWEEN YELLOWSTONE

CUTTHROAT TROUT AND RAINBOW TROUT IN THE YELLOWSTONE RIVER,

MONTANA

by

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APPROVAL

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This thesis has been read by each member of the thesis committee and has been found to be satisfactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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ABSTRACT

The genomic extinction of Yellowstone cutthroat trout (*Oncorynchus clarki bouvieri*) has occurred throughout many parts of its historic range because of displacement and introgression with introduced rainbow trout (*O. mykiss*). However, fluvial cutthroat trout still retain their genetic integrity while co-existing with rainbow trout in the Yellowstone River drainage, Montana. I assessed whether spatial or temporal reproductive isolation, or both, occurs between these taxa. Time and place of spawning was determined by radio-telemetry of a total of 164 trout (98 cutthroat, 37 rainbow, and 29 cutthroat x rainbow hybrids) over three spawning seasons, from 2001 to 2003. Fish were telemetered in four areas of a 140-km segment of the mainstem Yellowstone River. Of the 164 radio-tagged fish, 73 (44 cutthroat trout, 15 rainbow trout, and 14 hybrids) were assumed to have spawned. Fifty-five (75.3%) of 73 radio-tagged fish that spawned used 16 tributaries, 17 (23.3%) used 7 river side channels, and 1 (1.4%) used the main channel of the Yellowstone River. The majority of fish that spawned (62%) used five spawning areas. These were used by 79% (N = 11) of hybrids, 61% (N = 27) of cutthroat trout, and 47% (N = 7) of rainbow trout that spawned. Spawning-area and spawning-reach overlap index values were high among all taxa. In contrast, mean migration and spawning dates of rainbow trout and hybrids were 5 to 9 weeks earlier than of cutthroat trout. Rainbow trout and hybrids began migrating and spawning in April and May when Yellowstone River discharges were lower and water temperatures were colder than discharges and temperatures during cutthroat trout migration and spawning in June and July. Spawning-period overlap index values between rainbow trout and hybrids versus cutthroat trout were typically less than half the spatial overlap index values. Therefore, difference in time of spawning is likely the predominant mechanism eliciting reproductive isolation. Management actions focused on later spawning cutthroat trout in tributaries may enhance temporal reproductive isolation from rainbow trout and hybrids.

INTRODUCTION

Hybridization of native fishes has become widespread because of the introduction of non-native fishes (Krueger and May 1991; Epifanio and Nielsen 2001) and may lead to outbreeding depression, loss of evolutionary adaptability (Leary et al. 1995), and genomic extinction of the native taxa (Allendorf et al. 2003). Hybridization may present the greatest threat to conservation of the inland subspecies of cutthroat trout *Oncorynchus clarki* (Allendorf and Leary 1988), but the limits and constraints of hybridization and introgression within salmonid populations are not well understood (Utter 2001; Hitt et al. 2003).

Yellowstone cutthroat trout *O. c. bouvieri* evolved as the only trout within the Yellowstone River drainage, as well as in the Snake River drainage above Shoshone Falls (Behnke 1992). This distribution has been reduced and the subspecies was petitioned for listing under the Endangered Species Act in 1998. Although listing was not warranted, an evaluation was made of factors that effected the decline (USFWS 2001). Among these factors were hybridization and displacement by introduced rainbow trout *O. mykiss*. Extirpation or introgression of Yellowstone cutthroat trout has occurred in over 75% of their historical stream habitat, including most mainstem rivers (May et al. 2003). However, Yellowstone cutthroat trout still co-exist with introduced rainbow trout in the mainstem of the upper Yellowstone River, Montana (Clancy 1988; Shepard 1992; Tohtz 1999). Mechanisms averting the complete displacement and hybridization of cutthroat trout by rainbow trout in the Yellowstone River are unknown. My goal was to assess

whether reproductive isolation permits co-existence between cutthroat trout and rainbow trout in the Yellowstone River.

Rainbow trout were stocked in the upper Yellowstone River, Montana, from the early 1900s until 1972 (Clancy 1988) and are the most abundant trout in the river (Tohtz 2003). Rainbow trout have hybridized with resident cutthroat trout in many tributaries of the Yellowstone River (Leary et al. 1989). In contrast, fluvial cutthroat trout spawning runs in several tributaries are composed of putatively unhybridized fish (Clancy 1988; Byorth 1990; Shepard 1992) and relatively few fluvial cutthroat x rainbow trout hybrids have been detected in the mainstem river by genetic analysis. For example, six hybrids were identified among 39 Yellowstone cutthroat trout or rainbow trout in 1983 (Leary 1983) and hybrids detected among Yellowstone cutthroat trout from three sections of the Yellowstone River were 5 of 30 (Corwin Springs), 1 of 34 (Mill Creek Bridge), and 1 of 19 (Springdale) in 1998 (Tohtz 1999). Apparently unhybridized cutthroat trout spawning populations and low numbers of hybridized fish in the Yellowstone River suggested the existence of some mechanism of reproductive separation between fluvial Yellowstone cutthroat trout and rainbow trout.

Reproductive isolation was still uncertain because hybrid individuals may be difficult to identify. Field identification of hybrids may be unreliable (Weigel et al. 2002), because phenotypic characteristics of hybrids may be similar to either parental species (Leary et al. 1984; Leary et al. 1996). Genetic tests of individual fish may be ineffective, because of the limited number of diagnostic markers available for testing (Boeklen and Howard 1997; Allendorf et al. 2001). Therefore, genetic testing of

populations is a more reliable method of determining hybridization and introgression (Leary et al. 1984). Genetic testing of putative fluvial adult cutthroat trout spawning aggregations, i.e., populations, had never been done in the Yellowstone River drainage.

Previous surveys and sampling of fluvial trout were not designed to assess reproductive isolation. Populations should first be demarcated to assess genetic composition and potential reproductive separation within sympatric populations (Olsen et al. 2002, May et al. 2003). Genetic testing and assessment of reproductive separation of fluvial trout should occur when and where genetic exchange occurs, i.e., during spawning and within spawning areas. All previous genetic samples of fluvial adult trout from the Yellowstone River drainage had been taken when and where individuals of multiple populations were present in the mainstem river. The power to detect hybridization within samples is then based on the limited number of diagnostic available to test for an individual fish rather than the sum of all individuals from a population. Fluvial fish of multiple populations may occupy the same summer and winter habitat, but spawn in different areas (Clancy 1988; Brown and Mackay 1995; Henderson et al. 2000) because habitat used for spawning is typically distinct from habitats used for feeding and protection (Northcote 1997). Migration to and aggregation within spawning habitat typically occurs in April and May by rainbow trout within Intermountain West rivers (Spoon 1985; Sandborn 1990; Henderson et al. 2000; Downing et al. 2002) and June and July by cutthroat trout (Clancy 1988; Byorth 1990; Kaeding and Boltz 2001).

Hybridization potential has been assessed by comparing the time or place of spawning of rainbow trout and cutthroat trout (Henderson et al. 2000) and could also be

used to demarcate populations and evaluate reproductive separation. Spatial or temporal segregation during spawning between coastal cutthroat *O. c. clarki* and rainbow trout within their native ranges may result in low levels of hybridization (Trotter 1989; Behnke 1992). Interbreeding may be minimized by differences in spawning times and the preference of coastal cutthroat trout for spawning in smaller tributaries, whereas rainbow trout use main river channels. Differences in timing of spawning may also minimize interbreeding of Atlantic salmon *Salmo salar* and brown trout *S. trutta* within their native ranges (Heggberget et al. 1988). Therefore, spatial or temporal reproductive isolation, or both, has been suggested as mechanisms that can limit interbreeding between native salmonid populations, and may potentially extend to nonnative populations. For example, differences in run timing and spawning areas between wild steelhead and introduced steelhead may minimize genetic and ecological interactions in Forks Creek, Washington (Mackey et al. 2001).

My hypotheses for reproductive isolation in the upper Yellowstone River drainage were based on either spatial or temporal separation. I hypothesized that spatial reproductive separation occurs because rainbow trout will spawn predominantly within side channels of the river, as occurs in other river systems (Henderson et al. 2000; Downing et al. 2002), and cutthroat trout will spawn in tributaries (Clancy 1988). Spatial separation may also occur within tributaries used for spawning if Yellowstone cutthroat trout spawn further upstream than rainbow trout (Thurow 1982; Henderson et al. 2000). I hypothesized that temporal reproductive separation may exist if rainbow trout spawn earlier than Yellowstone cutthroat trout (Henderson et al. 2000), which was partly

documented by weirs, redd counts, or both in Yellowstone River tributaries (Roberts 1986; Byorth 1990; Shepard 1992). Different aspects of the spawning behavior or habitat of either rainbow trout or cutthroat trout have been studied in the Yellowstone River drainage (Roberts 1986; Clancy 1988; Byorth 1990), but no studies have systematically compared the spawning locations and times between these taxa throughout the drainage.

My objective was to assess if, and to what degree, spatial or temporal reproductive isolation occurs among Yellowstone cutthroat trout, rainbow trout, and their hybrids in the Yellowstone River by comparing the time and place of spawning using radio-tagged individuals of these three taxa. An understanding of isolating factors could assist managers with preserving the genomic integrity of cutthroat trout through management actions that promote reproductive separation between the taxa such as selective fish barriers or spatially specific spawning habitat enhancement.

STUDY AREA

The Yellowstone River begins in northwestern Wyoming and flows north and then northeast for a total of 1,113 km to its confluence with the Missouri River in North Dakota. The Yellowstone is considered the longest "free-flowing" (not impounded) river in the continental United States. My study area encompassed 140 km of the upper Yellowstone River, from the Bear Creek confluence (elevation 1,603 m) near Gardiner, Montana, downriver to Springdale, Montana (elevation 1,286 m; Figure 1). The river gradient is 2.3 m/km and the river bed is predominantly cobble and gravel. The historical mean annual discharge was 106 m³/s and the historical mean peak discharge was 597 m³/s at the US Geological Survey (USGS) gage site at Livingston, Montana (USGS 2004). Tributaries originate primarily within publicly-owned mountainous forest lands and flow out onto the river valley bottom, which is predominantly privately-owned ranch lands. Within the river valley bottom, most tributaries are diverted for irrigation and many are dewatered and only flow to the river for a short period of time during peak run-off in late spring or early summer.

Native fishes within the study area are Yellowstone cutthroat trout, mountain whitefish *Prosopium williamsoni*, white sucker *Catostomus commersoni,* longnose sucker *C. catostomus*, mountain sucker *C. platyrhynchus*, mottled sculpin *Cottus bairdi*, and longnose dace *Rhinichthys cataractae*. Non-native fish species are rainbow trout, rainbow trout x cutthroat trout hybrids (hereafter referred to as hybrids), brown trout *Salmo trutta,* and brook trout *Salvelinus fontinalis* (rare occurrence). Angling within the

study area has been restricted to catch-and-release of Yellowstone cutthroat trout since

1984, whereas harvest of rainbow trout is allowed.

Figure 1. The upper Yellowstone River drainage showing all tributaries where spawning was observed by telemetered fish. Sections where trout were captured and implanted with radio transmitters are shown (names of sections based on Montana Department of Fish, Wildlife and Parks nomenclature).

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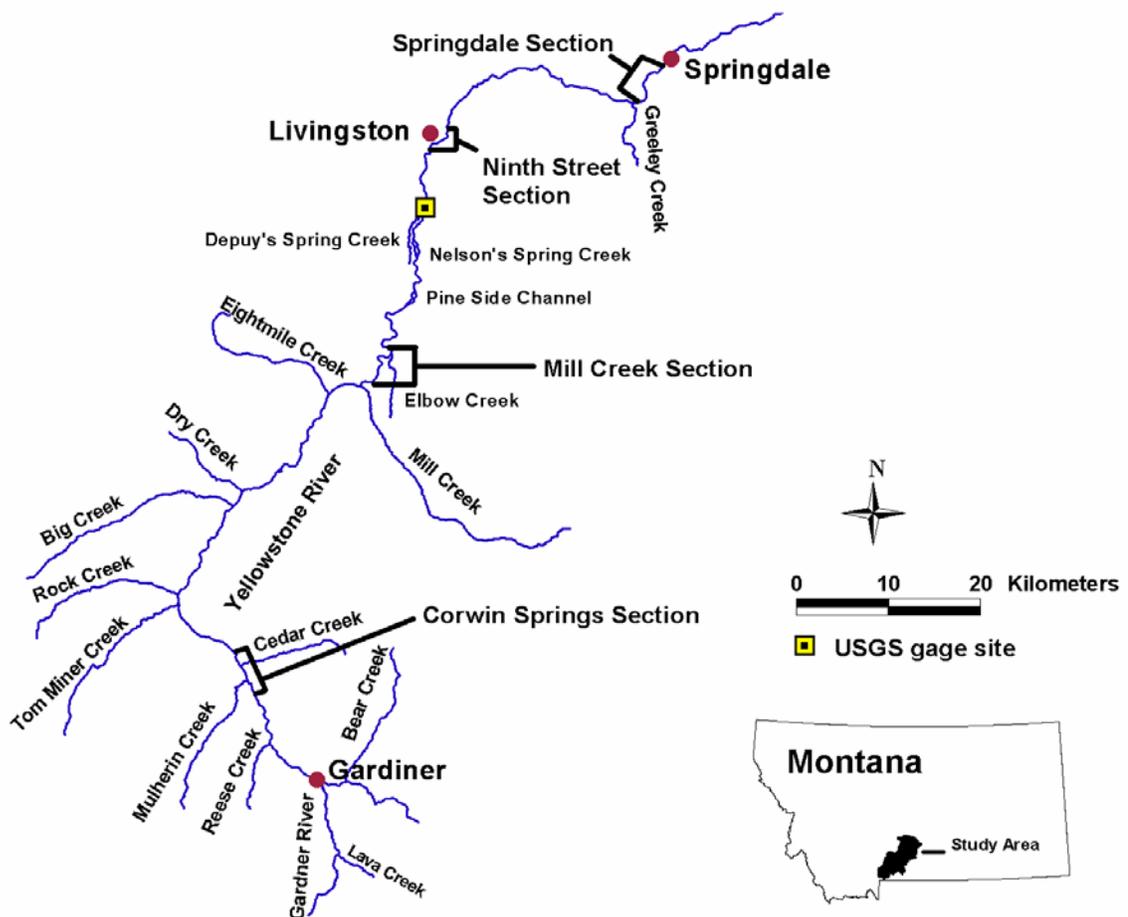
METHODS

<u>Radio telemetry</u>

I radio-tagged a total of 164 trout in the Yellowstone River during March and April of 2001, 2002, and 2003 (Table 1). Trout were captured in 5.2 to 12.9 km river reaches associated with four long-term Montana Department of Fish, Wildlife and Parks salmonid population abundance monitoring sections (Figure 1). Fish were radio-tagged in the Corwin Springs and Mill Creek Bridge sections in 2001 and 2002, and in the Ninth Street Bridge and Springdale sections in 2002 and 2003. During the first year of sampling in each section, I attempted to radio-tag 25 trout in the proportion of 15 cutthroat trout, 5 rainbow trout, and 5 hybrids. Similar proportions of each taxa were tagged in these sections during successive years. I radio-tagged a greater proportion of cutthroat trout, versus either rainbow trout or hybrids, to better ensure a representative sample of the specific spawning times and places of these putatively unhybridized trout.

Trout were captured by electrofishing (DC) with a Coffelt model VVP-15 rectifying unit from a jet boat (double-boom) in 2001 or a drift boat (single-boom) in 2002 and 2003. The drift boat was used in 2002 and 2003 because of low river discharges. Trout were anesthetized (MS-222), measured (total length, mm), weighed (g), and fin clipped (pelvic). The length at maturity of fluvial Yellowstone cutthroat trout in the Yellowstone River is about 300 mm (Clancy 1988); therefore, only fish greater than this length were radio-tagged. Lengths of radio-tagged fish ranged from 333 to 500 mm with mean lengths of 369 mm for cutthroat trout, 400 mm for hybrids, and 409 mm for rainbow trout.

Table 1. Number of radio-tagged trout (the number that subsequently spawned is within parentheses) from each river sampling section by year. Yellowstone cutthroat trout are denoted as YCT, rainbow trout are denoted as RB, and hybrids are denoted as HY. Gender is denoted as M = male and F = female.

Section	Taxa	2001 M	2001 F	2001 Total	2002 M	2002 F	2002 Total	2003 M	2003 F	2003 Total	Total M	Total F	Total Total
Corwin	YCT	10 (3)	5 (2)	15 (5)	7 (5)	5 (0)	12 (5)				17 (8)	10 (2)	27 (10)
Springs	RB	3 (1)	2 (0)	5 (1)	2 (1)	2 (1)	4 (2)				5 (2)	4 (1)	9 (3)
	HY	4 (3)	1 (0)	5 (3)	1 (1)	1 (0)	2 (1)				5 (4)	2 (0)	7 (4)
Mill	YCT	9 (4)	7 (3)	16 (7)	6 (1)	7 (6)	13 (7)				15 (5)	14 (9)	29 (14)
Creek	RB	3 (1)	2 (0)	5 (1)	2 (2)	3 (3)	5 (5)				5 (3)	5 (3)	10 (6)
Bridge	HY	3 (2)	1 (1)	4 (3)	4 (2)	0 (0)	4 (2)				7 (4)	1 (1)	8 (5)
Ninth	YCT				7 (5)	8 (2)	15 (7)	1 (1)	4 (3)	5 (4)	8 (6)	12 (5)	20 (11)
Street	RB				2 (1)	3 (1)	5 (2)	3 (0)	1 (1)	4 (1)	5 (1)	4 (2)	9 (3)
Bridge	HY				4 (2)	1 (0)	5 (2)	0 (0)	0 (0)	0 (0)	4 (2)	1 (0)	5 (2)
Springdale	YCT				6 (4)	9 (1)	15 (5)	3 (1)	4 (3)	7 (4)	9 (5)	13 (4)	22 (9)
	RB				2 (-)	3 (1)	5 (1)	2 (0)	2 (2)	4 (2)	4 (0)	5 (3)	9 (3)
	HY				2 (-)	3 (1)	5 (1)	4 (2)	0 (0)	4 (2)	6 (2)	3 (1)	9 (3)
Total	YCT	19 (7)	12 (5)	31 (12)	26 (15)	29 (9)	55 (24)	4 (2)	8 (6)	12 (8)	49 (24)	49 (20)	98 (44)
	RB	6 (2)	4 (0)	10 (2)	8 (4)	11 (6)	19 (10)	5 (0)	3 (3)	8 (3)	19 (6)	18 (9)	37 (15)
	HY	7 (5)	2 (1)	9 (6)	11 (5)	5 (1)	16 (6)	4 (2)	0 (0)	4 (2)	22 (12)	7 (2)	29 (14)
Grand Total				50 (20)			90 (40)			24 (13)			164 (73)

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A surgical incision of about 2 cm was made in the abdominal cavity and gonads were examined with the unaided eye or an otoscope to determine gender. Radio transmitters were allocated about equally between genders within taxa. Radio transmitters were implanted (Schmetterling 2001) and incisions were closed with surgical staples (Swanberg et al. 1999). Surgery duration (time on surgery table until back in the water) averaged about 3 min (range 1 to 6 min). Radio-tagged fish were held in a live cage in the river until they recovered from the anesthetic and then were released near their capture location in 2001, or at their surgery sites in 2002 and 2003. Transmitters (Lotek Wireless Inc., 8.9 g in air and 430 mm external antenna) did not exceed 3% of the body weight of radio-tagged fish (Brown et al. 1999). Twenty-five new transmitters were used during the first year of tagging in each section in both 2001 and 2002. New transmitters had a lifespan of 520 d with a monthly schedule of 37 weeks on (March 19 until December 3, 2001 and February 27 until November 13, 2002) and 12 weeks off (December 3, 2001 until February 25, 2002 and November 13, 2002 until February 5, 2003). Transmitters had a daily schedule of 13 h on (0800 – 1900) at a 3 – 3.5 s burst rate and were expected to be operable through two successive spawning seasons. Recovered transmitters were implanted in fish captured within the same river section in the subsequent year.

I attempted to relocate each radio-tagged fish from March through August once per week when fish were not in potential spawning areas and twice per week when fish were within potential spawning areas. Potential spawning areas were defined as all tributary streams or mainstem river areas with suitable spawning habitat. Suitable

spawning habitat for all taxa was based on the criteria of gravel size, water depths, and water velocities for Yellowstone cutthroat trout (Gresswell 1995) or my direct observations of spawning fish. I attempted to visually identify radio-tagged fish within potential spawning areas and observe spawning behavior (e.g., fish paired up over gravels, defense of spawning sites, and redd building) if water conditions allowed to confirm that spawning was occurring within places classified as spawning areas. Radio-tagged fish were relocated by surveys conducted by float boat, vehicle, and on foot. The observer homed in as close as possible to the position of each radio-tagged fish. Fish not initially relocated during surveys by boat, vehicle, or foot, were searched for by airplane within and outside the primary study area.

I recorded the date and field map location for each fish relocation. I used 1:24,000 aerial photos of the river (Gardiner to Springdale, Montana), 1:24,000 USGS topographic maps, and the 1:126,720 Gallatin National Forest-East Half map. Global Positioning System (GPS) coordinates were recorded for most locations. Field map locations were subsequently digitized or GPS coordinates were projected into shapefiles within a Geographic Information System: ArcView 3.2 (Environmental Systems Research Institute, Inc. 1999). Digital orthophoto quadrangles, based on 1:24,000 USGS topographic maps, were the primary layer used to plot points in ArcView. Waterway kilometers of location points were calculated from the 1:100,000 Montana hydrography layer (Montana National Resource Information System 2004). Stream distances were measured by hand using 1:24,000 digital topographic maps if a waterway route was not available for a location point within the 1:100,000 Montana hydrography layer.

Spatial and temporal spawning variables

Spatial resource variables were computed from radio-tagged fish that entered spawning areas. The largest spatial category for a spawning fish was the entire spawning area, i.e., the tributary, side channel, or mainstem location. The spawning reach of a fish was defined as the distance between the downstream-most and upstream-most relocation points within a spawning area. Spawning reaches were partitioned into 1-kilometer segments within each spawning area by waterway kilometer.

Migration and spawning times were estimated from relocation dates, movement patterns, and spawning locations. Migration was defined as a directional movement to a spawning area. Migration start date was defined as the median date between the date a migrating fish was first relocated moving to a spawning area and the previous relocation date of that fish (Swanberg 1997). Spawning start date was defined as the median date between the first date a fish was located in a spawning area and the prior relocation date of the fish when it was not in a spawning area. The spawning end date was defined as the median date between the last relocation in a spawning area and the first relocation outside of the spawning area, except for four cutthroat trout that did not leave spring creeks after spawning. Spawning end dates for these fish were defined as the dates when they had reached their maximum upstream extent within the creeks and were no longer observed spawning. The spawning period was defined as the time between the spawning start and end dates (Henderson et al. 2000). Spawning periods were partitioned into seven-day blocks, as spawning week(s).

Spatial and temporal overlap between taxa were assessed by computing Pianka's index of overlap (Pianka 1973) separately for each of the three spawning resource categories, i.e., spawning area, spawning reach, and spawning period. Overlap values ranged from 0 (no overlap) to 1 (complete overlap). Overlap values were calculated for all pairs of taxa, as well as for rainbow trout and hybrids combined versus cutthroat trout, and by individual year or aggregated over all years. Confidence intervals for all overlap values were computed using the 5th and 95th percentiles of the distribution of 2,000 bootstrap replications (Efron and Tibshirani 1993) generated using the program R 1.9.0 for Windows (R Development Core Team 2004).

Environmental variables

Discharge of the Yellowstone River and water temperatures of the river and within individual spawning areas were assessed to determine the potential influence of these variables on the timing of migration and spawning of radio-tagged fish. Yellowstone River discharge and water temperature data (measured at 15 minute intervals) were obtained from the USGS gage site at Livingston, Montana (USGS 2004; Figure 1) and used to calculate daily mean values. In addition, I installed thermographs (HOBO® and StowAway®, Onset Computer Corp.) at 18 potential spawning areas (16 tributary streams and 2 side-channels). Thermographs were located within 200 m of the mouths of tributary streams or in the middle of side-channel lengths. Thermographs were deployed from March through May and retrieved in autumn. Water temperatures were recorded every hour and daily mean temperatures were calculated.

Statistical analysis

One-way ANOVA (Neter et al. 1996) was used to test for significant differences in mean number of days and dates of migration and spawning among taxa, as well as mean daily discharges and water temperatures associated with these dates. Tukey's multiple comparison procedure was used to test for pairwise differences between mean number of days, dates, discharges, and temperatures. Two-sample t-tests were used to test for differences in mean spawn start date, spawn end date, and spawn period between genders. For each significance test and Tukey's multiple comparison family error rate, α = 0.05. All statistical analyses were conducted with Minitab 13.1 (Minitab 2000).

Genetic testing

Radio-tagged fish and putative Yellowstone cutthroat trout spawning populations were genetically tested to assess phenotypic classification, hybridization, and introgression. All fish were identified to taxon in the field using morphological features such as spotting pattern, coloration, jaw slash presence or absence, relative jaw length, and scale size. A subsample of radio-tagged fish was genetically tested (n = 30), composed of 25 fish (16 putative cutthroat trout, 5 rainbow trout, and 4 hybrids) from the Mill Creek Bridge section in 2001 and 5 fish (four cutthroat trout and one hybrid) that spawned in either Greeley or Big creeks in 2003.

Putative Yellowstone cutthroat trout spawning aggregations discerned by telemetry in four tributaries were sampled in 2003 to assess potential rainbow trout hybridization and introgression. Cedar and Mulherin creeks were selected because radio-tagged cutthroat trout had spawned in these creeks during 2001 and 2002. Greeley and

Big creeks were selected because these spawning tributaries were used by radio-tagged cutthroat in 2003. Side channels and spring creeks were not sampled because of the potential presence of fluvial fish that were not spawning. Spawning aggregations were sampled in either June (Greeley Creek) or July (all other streams). Sampling of spawning fish began near the stream mouth and progressed upstream 0.31 to 1.43 km. Fish were sampled with a backpack electrofishing unit (smooth DC) at the lowest possible voltage to minimize injury to fish and eggs. In addition, four fish were captured with hook and line from a deep pool and two spawning-mortality carcasses were sampled in Big Creek. I tried to capture at least 20 spawning trout greater than 300 mm total length, regardless of phenotype, from each stream. Captured fish were not anesthetized and were not handled beyond that needed to take a pelvic fin clip to minimize stress. Spawning aggregations of fish in each tributary were treated as populations. All fish captured from each spawning aggregation were assessed collectively to determine genetic composition and introgression of the population. The spawning aggregation samples from Greeley and Big creeks each included one radio-tagged cutthroat trout.

Fin clips were analyzed with a PCR-based method, paired interspersed nuclear DNA elements (PINE), at the University of Montana Wild Trout and Salmon Genetics Lab (Spruell et al. 2001; Kanda et al. 2002). Each fish was analyzed to determine the presence or absence of diagnostic DNA fragments, including ten for Yellowstone cutthroat trout, six for rainbow trout, and four for westslope cutthroat trout. The presence of a fragment indicates the individual is either heterozygous or homozygous for that fragment (Spruell et al. 2001). First generation hybrids will have all fragments of both

taxa because they are heterozygous at all loci. Second generation and later hybrids typically may be heterozygous for some fragments and homozygous for others.

Genotypes of the majority of radio-tagged Yellowstone cutthroat trout agreed with field-classified phenotypes, whereas the genotypes of both rainbow trout and hybrids varied in comparison to field-classified phenotypes. All individuals from the genetically tested subsample of radio-tagged Yellowstone cutthroat trout (N = 20) had diagnostic PINE fragments for Yellowstone cutthroat trout, but one also displayed a single diagnostic PINE fragment of rainbow trout. The genetically tested subsample of radio-tagged rainbow trout (N = 5) had three individuals with diagnostic PINE fragments for rainbow trout, but only one individual with no Yellowstone cutthroat trout fragments. Neither of the two putative rainbow trout with diagnostic PINE fragments for both rainbow trout and Yellowstone cutthroat trout appeared to be a first-generation hybrid. Only PINE fragments for Yellowstone cutthroat trout were detected in the remaining two putative rainbow trout. The genetically tested subsample of radio-tagged hybrids (N = 5) had two individuals with diagnostic PINE fragments for both rainbow trout and Yellowstone cutthroat trout. One of these hybrids also had a PINE fragment for westslope cutthroat trout. Of the remaining three putative hybrids, two had fragments for only rainbow trout and one had fragments only for Yellowstone cutthroat trout.

Phenotypic classifications of radio-tagged fish were not changed based on genetic results. Ninety-five percent of the subsample of radio-tagged Yellowstone cutthroat trout did not have rainbow trout PINE fragments. A high percentage of putative rainbow trout and hybrids that were radio-tagged may have been post-F_1 hybrids. Two of five

genetically tested rainbow trout were genetically identified as post-F_1 hybrids. Similarly, rainbow trout with only PINE fragments for Yellowstone cutthroat trout were also likely post-F_1 hybrids. Later generation hybrids may have an absence of some PINE fragments (Spruell et al. 2001) and given the limited number of rainbow trout diagnostic fragments (N = 6), the ability to detect rainbow trout markers may be low. Similarly, the three hybrids that had only PINE fragments for one taxon may have been later-generation hybrids. Alternatively, putative hybrids may have been misclassified as such. Fish visually classified as hybrids may have had higher levels of cutthroat trout genes than those visually identified as rainbow trout, but which were genetically identified as hybrids. Spatial and temporal reproductive overlap of cutthroat trout was compared against rainbow trout and hybrids separately, because phenotypic differences may correspond to behavioral differences, and collectively, because these fish both potentially have rainbow trout alleles.

RESULTS

Seventy-three of 164 radio-tagged trout moved into spawning areas and were assumed to have spawned there (Figure 2). These 73 spawning trout were composed of 44 cutthroat trout, 15 rainbow trout, and 14 hybrids, proportions which were similar to the proportions of taxa that were radio-tagged (Table 1). I observed spawning activity during the relocations of 33 (19 cutthroat trout, 8 rainbow trout, and 6 hybrids) of the 73 fish in spawning areas. Of the 100 fish implanted with new transmitters, only six were tracked through two spawning seasons, all during 2002 and 2003; three of these spawned during just one year, two did not spawn during either year, and one spawned in both years. It spawned in Depuy's Spring Creek during June 17 to July 26, 2002, remained in the creek until October, and again entered the creek, presumably to spawn, during April 27 to June 3, 2003. Only the first year (2002) spawning locations and times of this fish were used because of the lack of independence of the data from the successive year.

Radio-tagged spawning fish migrated from 0.1 to 51.7 km within the river to spawning areas. Mean river migration distance and range of radio-tagged taxa was 11.4 km (0.1 to 51.7 km) for cutthroat trout, 12.9 km (0.26 to 48.4 km) for rainbow trout, and 13.7 km (0.35 to 40.5 km) for hybrids. Mean river migration distance and range of radio-tagged fish by river section was 7.0 km (0.1 to 24.0 km) in Corwin Springs, 14.1 km (0.35 to 44.2 km) in Mill Creek Bridge, 9.1 km (0.26 to 51.3 km) in Ninth Street Bridge, and 18.0 km (0.98 to 51.7 km) in Springdale. Fish radio-tagged in the Corwin Springs section exclusively used spawning areas adjacent to and upriver of this tagging section. Radio-tagged fish in the three downriver sections used some of the same spawning areas.

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Figure 2. Spawning areas of radio-tagged fish in the upper Yellowstone River drainage, 2001 through 2003. Numbers of each taxon greater than one within a spawning area are denoted by the number next to the taxon symbol. Taxon symbols within tributaries are ordered according to the mean upstream distance moved by a taxon.
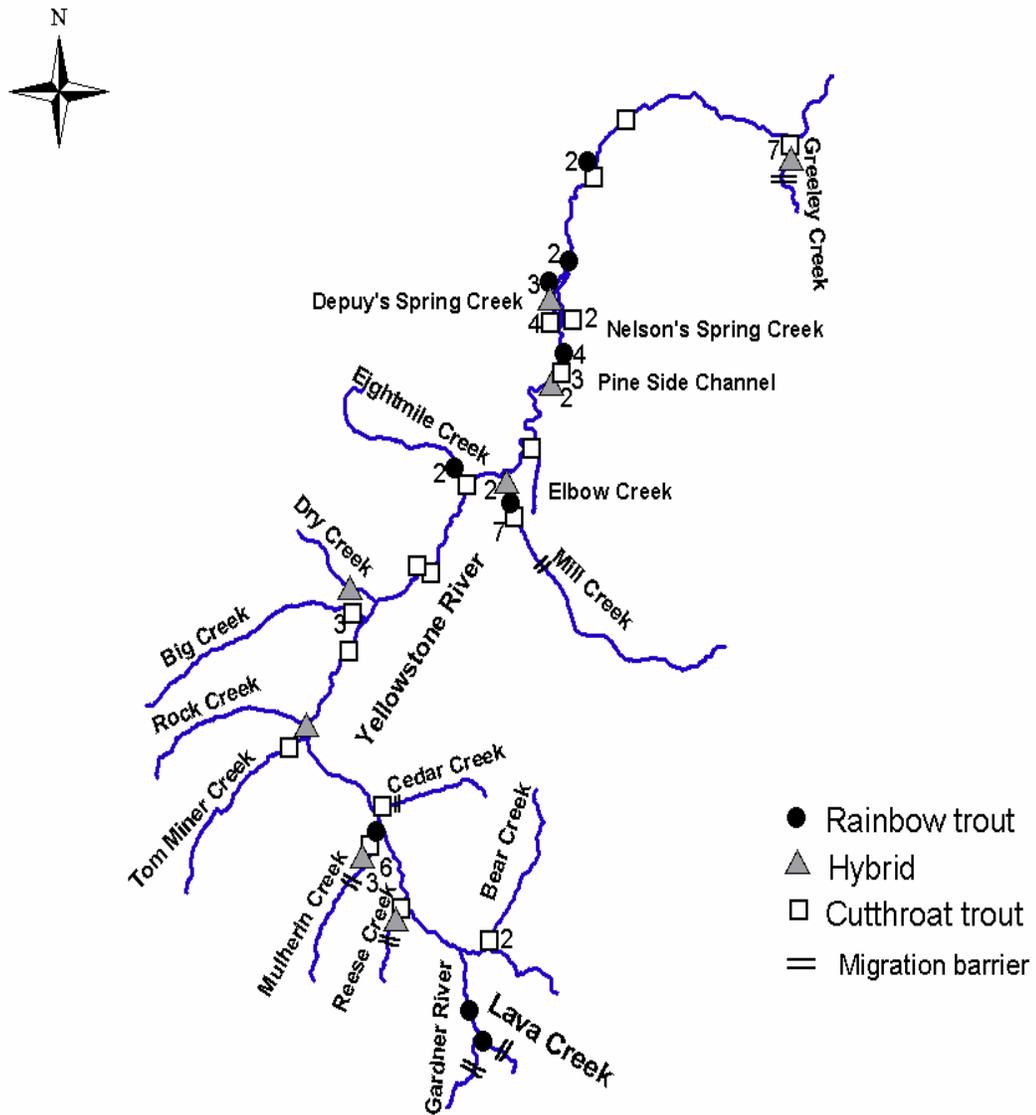
Spawning information was not collected on 91 radio-tagged fish. Twenty-nine fish did not spawn because of surgery mortality (N = 15) or pre-spawn mortality (N = 14). The percentage of surgery mortality by taxa was 7% (2 of 29) of hybrids, 8% (3 of 37) of rainbow trout, and 10% (10 of 98) of cutthroat trout. The percentage of pre-spawning mortality by taxa was 3% (1 of 37) of rainbow trout, 13% (13 of 98) of cutthroat trout, and none of the hybrids. Twenty-three fish made migrations during the spring, but were never found in spawning areas. Sixteen of these fish (3 rainbow trout, 3 hybrids, and 10 cutthroat trout) may have spawned in unidentified mainstem river spawning areas or perhaps their migration was not completed because of mortality. These fish were not found in spawning areas despite a relatively short amount of time between relocations (range 1 to 11 d, mean = 7 d). It is likely that the remaining 7 radio-tagged fish (2 cutthroat trout and 5 rainbow trout) spawned in areas that were not covered during relocation surveys because they were missing between relocations for 14 to 30 d (mean = 22 d). The five rainbow trout migrated to spawn shortly after being radio tagged and before weekly relocation surveys began of the full study area. Thirty-nine fish (10 rainbow trout, 10 hybrids, and 19 cutthroat trout) did not migrate. These fish were alive in the river through the spawning season but made no discernable movements to spawn.

Apparent mortality of spawning and nonspawning fish was high during and after the spawning season. Forty (55%) of 73 documented spawning fish died or expelled tags as a result of spawning, including 60% of rainbow trout, 59% of cutthroat trout, and 36% of hybrids. Nine fish died during the subsequent summer through winter period. Final location or status of 17 fish that spawned was unknown. These fish were tracked after

spawning until the subsequent winter but survival was uncertain based upon the last relocation. Seven fish survived either through a second year, until the end of the study period, or until their transmitter batteries expired. Some nonspawning fish either died during their applicable taxon spawning season (N = 25) or from the spawning season through the first winter (N = 17). The final locations or status of 17 fish that did not spawn was unknown and 3 survived through their second season, until the end of the study period, or until transmitter batteries expired.

The majority of radio tags were recovered after apparent fish mortality. Most of the recovered tags were no longer in the fish and tags were typically found within the river or along its banks. Predation likely occurred on 15% (N = 24) of radio-tagged fish based on recovery locations outside of waterways, i.e., below nests, perches, or in fields. Predators of radio-tagged fish were primarily birds, including eagles, ospreys, and herons (in order of the number of suspected predation events), but may have included mink and otters. Four radio-tagged fish (three cutthroat trout and one hybrid) were entrained within irrigation ditches originating from four different tributary streams. Three radio tags were recovered within or adjacent to redds, indicating potential expulsion of tags during spawning.

Spatial assessment

Fifty-five of 73 (76%) radio-tagged fish spawned in 16 tributaries, 17 (23%) in 7 river side channels, and 1 (1%) in the main channel of the Yellowstone River (Figure 2). Spawning activity was noted during relocations of the 73 radio-tagged fish in 10 tributaries, 3 side channels, and the one main channel location. Some spawning areas

were used exclusively by one radio-tagged taxon, including 11 by cutthroat trout, 4 by rainbow trout, and 2 by hybrids. However, the majority of fish that spawned (N = 45 or 62%) used five spawning areas. These were used by 79% (N = 11) of hybrids, 61% (N = 27) of cutthroat trout, and 47% (N = 7) of rainbow trout (Figure 3). All three taxa spawned in Mulherin (N = 10), Mill (N = 10), and Depuy's Spring (N = 8) creeks and the Pine Creek side channel (N = 9), and cutthroat trout and a hybrid spawned in Greeley Creek (N = 8). Rainbow trout spawned in side channels (8 of 15 or 53%) more than cutthroat trout (7 of 44 or 16%) or hybrids (2 of 14 or 14%). However, of the fish that spawned in side channels, 100% (2 of 2) of hybrids, 50% (4 of 8) of rainbow trout, and 43% (3 of 7) of cutthroat trout used the Pine Creek side channel. Use of the same spawning areas by high proportions of all three taxa resulted in high spawning-area overlap index values among taxa (Table 2). Spawning-area overlap between rainbow trout and hybrids combined versus cutthroat trout ranged from 0.34 to 0.84 annually and was 0.67 over all years in aggregate. Spawning-area overlap between hybrids and cutthroat trout was greater (0.78) than between hybrids and rainbow trout (0.51) or between rainbow trout and cutthroat trout (0.40).

All three taxa consistently spawned within the same reaches of spawning areas. Most tributary spawning reaches used by all taxa (45 of 55 or 82 %) were within 4 km of the tributary mouths (Figures 4 through 6). Seven of the ten fish that spawned more than 4 km from a tributary mouth were in Depuy's Spring Creek. The spawning reaches of

these seven fish (4 cutthroat trout and 3 hybrids) were a maximum of six kilometers from the mouth. The same spawning reaches were also used by all three taxa in the Pine Creek side channel.

Figure 3. Distribution of spawning by radio-tagged trout among the five most heavily used spawning areas in the Yellowstone River system, 2001 to 2003. Percentages were based on the total number of spawning fish of each taxon in all spawning areas: 15 rainbow trout, 14 hybrids, and 44 cutthroat trout.
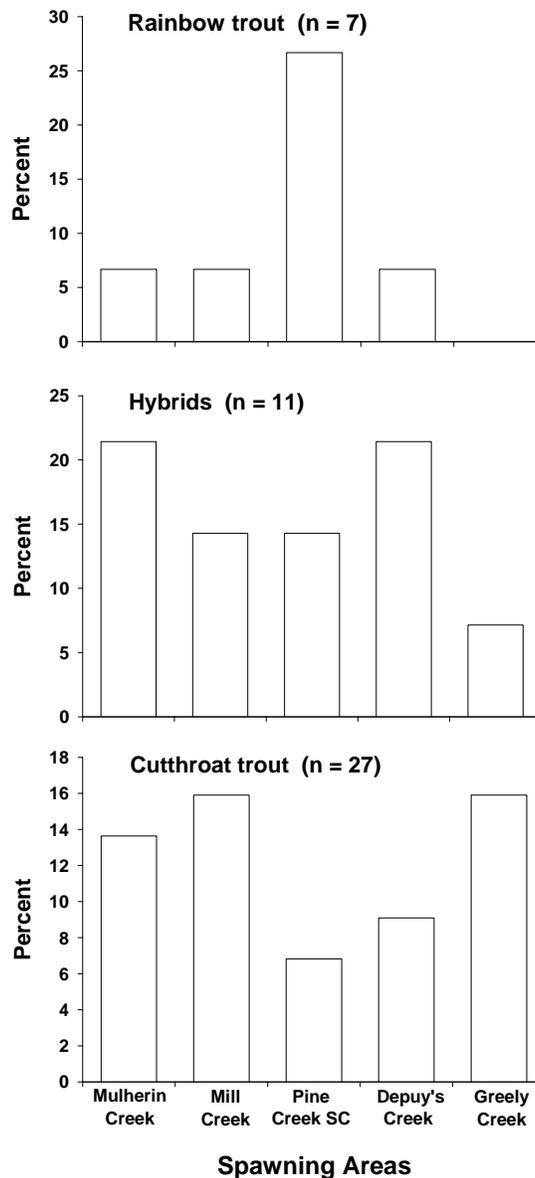
Table 2. Pianka overlap index values of spawning-area and spawning-reach overlap between taxa by year and for all years combined. The overlap value and 95% confidence interval (in parentheses) are followed by the numbers of fish of each taxon or taxa. Overlap values of zero do not have a bootstrapped confidence interval (denoted by a dash).

Comparison	Taxa	2001	2002	2003		All years combined
Spawning area	Rainbow trout vs. hybrid	0.95 (0.00-1.00) 2, 6	0.43 (0.00-0.74) 10, 6	0.00	(-) 3, 2	0.51 (0.08-0.72) 15, 14
	Rainbow trout vs. cutthroat trout	0.87 (0.15-0.94) 2, 12	0.25 (0.00-0.45) 10, 24	0.00	(-) 3, 8	0.40 (0.10-0.56) 15, 44
	Hybrid vs. cutthroat trout	0.82 (0.19-0.92) 6, 12	0.38 (0.00-0.57) 6, 24	0.75 (0.00-0.97) 2, 8		0.78 (0.33-0.84) 14, 44
	Rainbow trout and hybrid vs. cutthroat trout	0.84 (0.26-0.94) 8, 12	0.34 (0.05-0.54) 16, 24	0.40 (0.00-0.81) 5, 8		0.67 (0.31-0.77) 29, 44
Spawning reach	Rainbow trout vs. hybrid	0.67 (0.00-0.92) 2, 6	0.27 (0.00-0.49) 10, 6	0.00	(-) 3, 2	0.35 (0.03-0.50) 15, 14
	Rainbow trout vs. cutthroat trout	0.70 (0.00-0.79) 2, 12	0.24 (0.00-0.38) 10, 24	0.00	(-) 3, 8	0.36 (0.07-0.47) 15, 44
	Hybrid vs. cutthroat trout	0.54 (0.04-0.70) 6, 12	0.58 (0.00-0.72) 6, 24	0.71 (0.00-0.90) 2, 8		0.70 (0.23-0.76) 14, 44
	Rainbow trout and hybrid vs. cutthroat trout	0.65 (0.11-0.75) 8, 12	0.48 (0.03-0.61) 16, 24	0.48 (0.00-0.78) 5, 8		0.66 (0.24-0.70) 29, 44

Figure 4. Spawning reaches of individual fish within the Yellowstone River drainage, 2001. Each line connecting symbols represents a spawning reach of an individual fish (N = 19, does not include 1 fish that spawned in the main river channel): 11 cutthroat trout (solid lines, squares), 6 hybrids (dash-dot lines, triangles), and 2 rainbow trout (dotted lines, circles). Symbols within each line represent relocation points. Relocation points within 80 m of each other are shown as a single symbol with the number of superimposed relocation points listed above the symbol. The total number of relocation points for a fish is shown at the end of the line within parentheses. Spawning observations were made at relocation points denoted by solid symbols. Spawning areas (SP = spring creek and SC = side channel) on the y-axis of each graph are ordered by elevation at waterway kilometer 0 (at tributary mouth or side channel confluence) from highest to lowest.
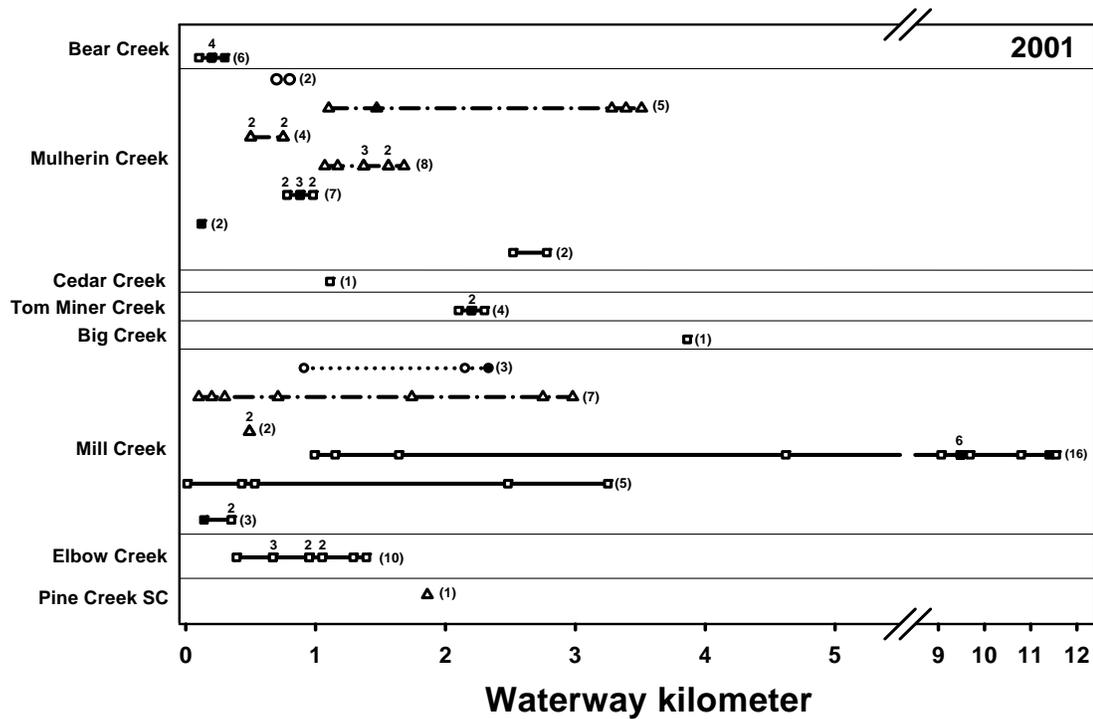
Figure 5. Spawning reaches of individual fish within the Yellowstone River drainage, 2002. Each line connecting symbols represents a spawning reach of an individual fish (N = 40): 24 cutthroat trout (solid lines, squares), 10 rainbow trout (dotted lines, circles) and 6 hybrids (dash-dot lines, triangles). See Figure 4 legend, for graph format details.
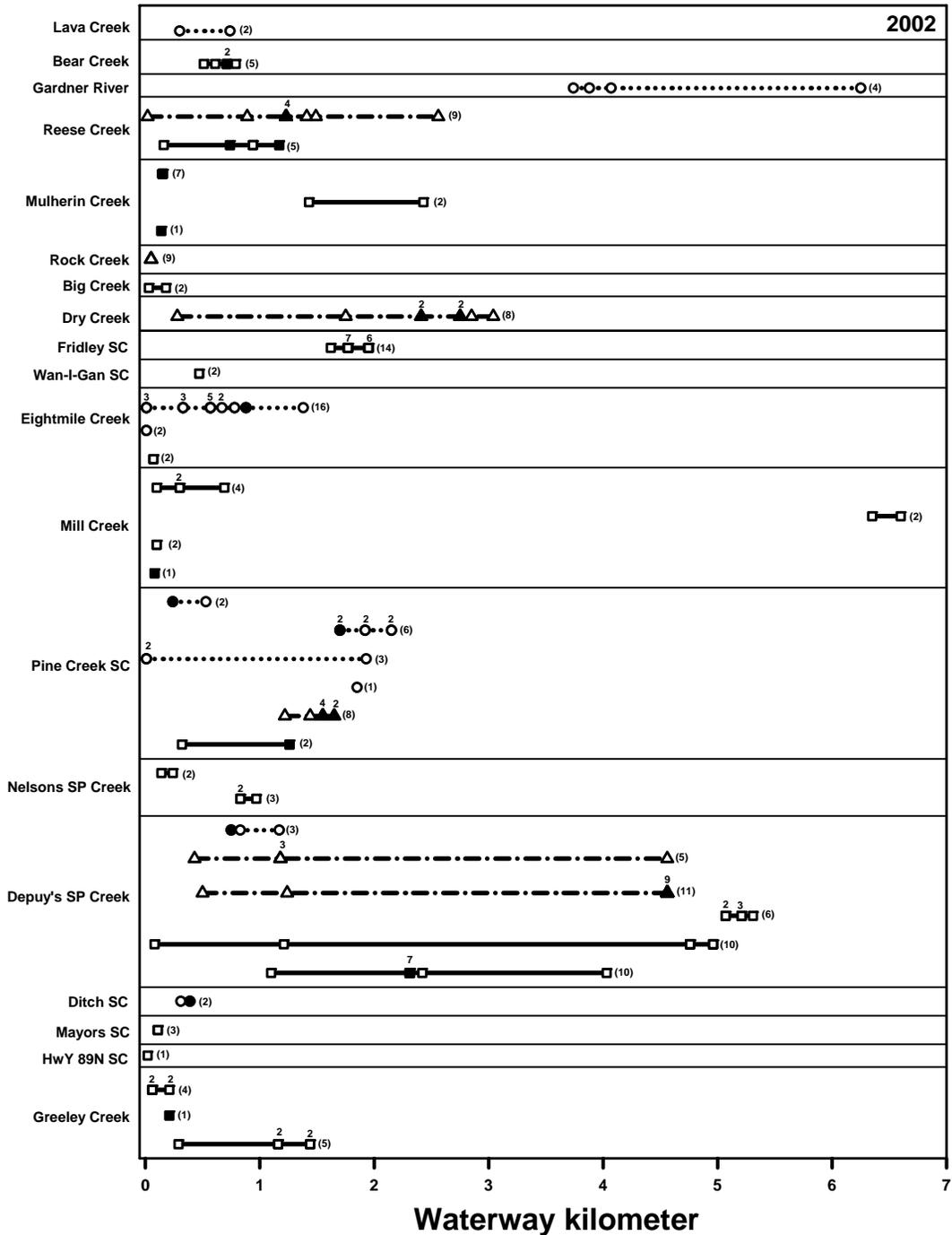
Figure 6. Spawning reaches of individual fish within the Yellowstone River drainage, 2003. Each line connecting symbols represents a spawning reach of an individual fish (N = 13): 8 cutthroat trout (solid lines, squares), 3 rainbow trout (dotted lines, circles) and 2 hybrids (dash-dot lines, triangles). See Figure 4 legend, for graph format details.
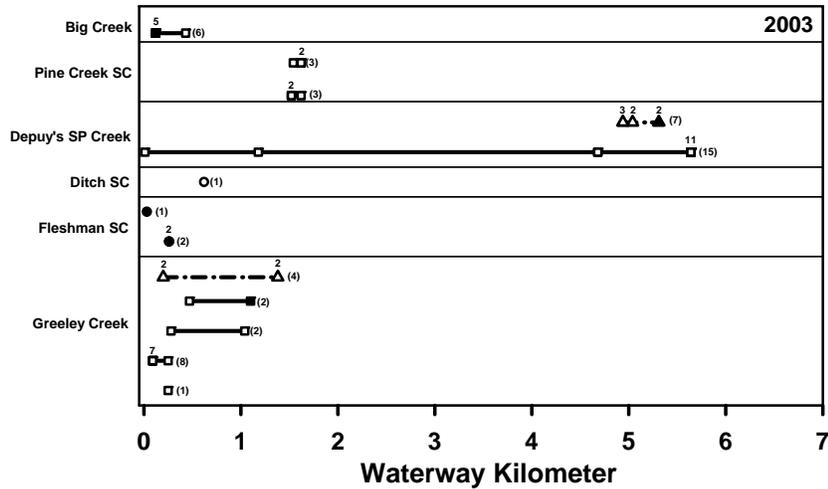
Spawning-reach overlap between rainbow trout and hybrids versus cutthroat trout was 0.66 considering all years in aggregate (Table 2). Spawning-reach overlap between hybrids and cutthroat trout (0.70) was greater than the overlap between hybrids and rainbow trout (0.35) or between rainbow trout and cutthroat trout (0.36).

Temporal assessment

Rainbow trout and hybrids migrated before cutthroat trout. Mean dates of migration start were all significantly different among taxa ($P < 0.001$; Table 3), and all pairwise comparisons were significantly different. Mean dates of migration start of hybrids and rainbow trout were about six to nine weeks earlier than the corresponding date of cutthroat trout. Mean durations of migration were not different among taxa ($P = 0.867$).

Table 3. Mean migration and spawning time variables of rainbow trout (N = 15), hybrids (N = 14), and cutthroat trout (N = 44) in the Yellowstone River drainage, Montana, 2001 through 2003. Dates are presented as Julian day with calendar dates in parentheses. Significance values of one-way ANOVA are displayed.

Variable	Rainbow trout		Hybrid		Cutthroat trout		P
	Mean	Range	Mean	Range	Mean	Range	
Migration start	101 (Apr 11)	86-118 (Mar 27-Apr 28)	117 (Apr 27)	91-187 (Apr 1-Jul 6)	161 (Jun 10)	110-187 (Apr 20-Jul 6)	< 0.001
Migration period (d)	10	1-36	8	1-52	9	1-34	0.867
Spawning start	108 (Apr 18)	94-156 (Apr 4-Jun 5)	126 (May 6)	91-187 (Apr 1-Jul 6)	169 (Jun 18)	132-194 (May 12-Jul 13)	< 0.001
Spawning end	122 (May 2)	104-163 (Apr 14-Jun 12)	150 (May 30)	122-198 (May 2-Jul 17)	187 (Jul 6)	152-226 (Jun 1-Aug 14)	< 0.001
Spawning period (d)	14	4-55	24	7-40	17	2-75	0.170

Rainbow trout and hybrids spawned before cutthroat trout. Rainbow trout and hybrids began spawning in April, whereas most cutthroat trout began spawning in June or later (Figure 7). Most rainbow trout and hybrids spawned between mid April and late May, whereas most cutthroat trout spawned from late June through mid July. Spawning by rainbow trout and hybrids extended into June and July, respectively, whereas one cutthroat trout spawned in August. Mean dates of spawn start and spawn end were all significantly different among taxa (all $P < 0.001$; Table 3), and all pairwise comparisons were significantly different. Mean dates of spawn start and spawn end of hybrids and rainbow trout were about five to nine weeks earlier than the corresponding dates of cutthroat trout. Mean spawn end dates of rainbow trout and hybrids were significantly different from the mean spawn start date of cutthroat trout ($P < 0.001$; Figure 8). Radio-tagged rainbow trout and cutthroat trout did not spawn at the same time within the same spawning areas (Figure 9). Similarly, hybrids did not spawn at the same time as cutthroat trout during the same year within four areas that were used by all three taxa (Mulherin and Mill creeks in 2001, Pine side channel and Depuy's Spring Creek in 2002). Hybrids were present during cutthroat trout spawning during the same year within three areas (Reese Creek in 2002, Depuy's Spring and Greeley creeks in 2003). However, hybrids left these spawning areas earlier than cutthroat trout, which were still spawning in June and July. Mean durations of spawning periods were not different among taxa ($P = 0.170$). The mean number of days between relocations used to calculate migration start, spawn start, and spawning end dates did not differ among taxa ($P = 0.977$, $P = 0.300$, and $P = 0.968$, respectively).

Figure 7. Distributions of weeks that radio-tagged trout were present in spawning areas in the Yellowstone River drainage, 2001 through 2003.
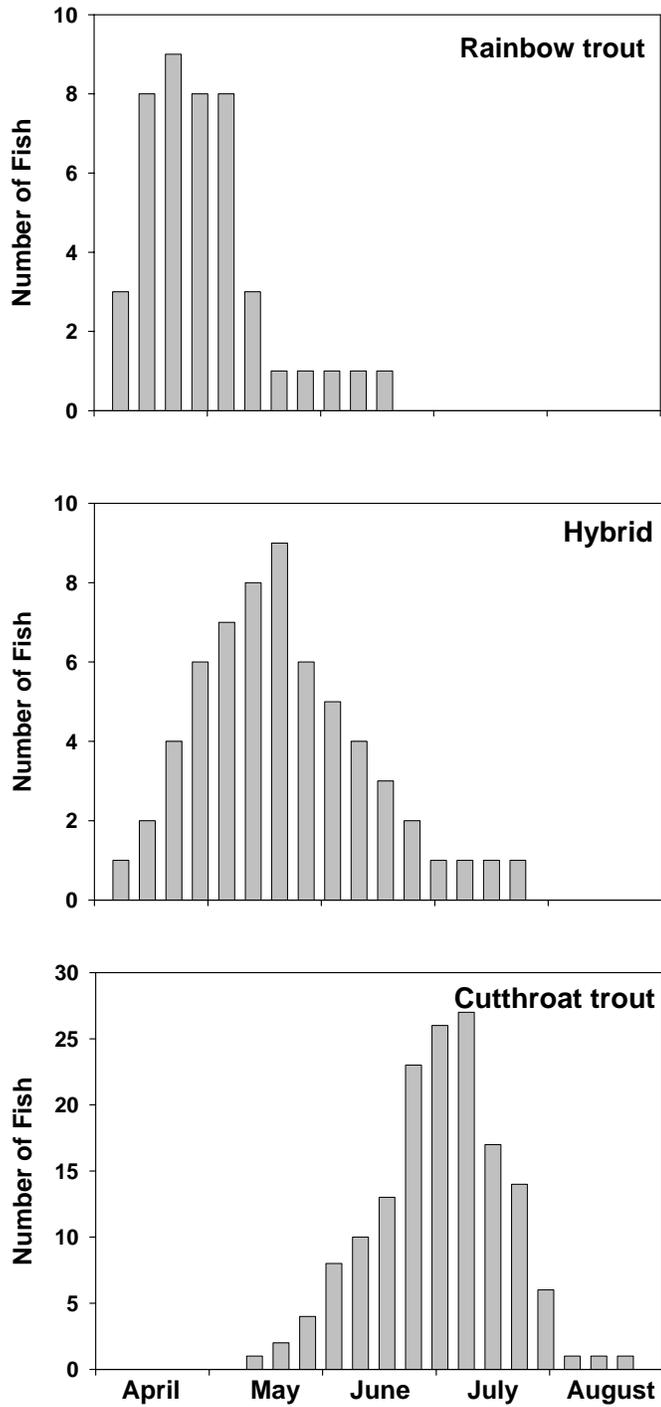
Figure 8. Comparison of rainbow trout and hybrid spawning end dates versus cutthroat trout spawning start dates. Boxes represent the 25th to 75th percentile and the line within the box marks the mean. Lines extending from the box indicate the 10th and 90th percentiles and horizontal dashes represent outlying points.
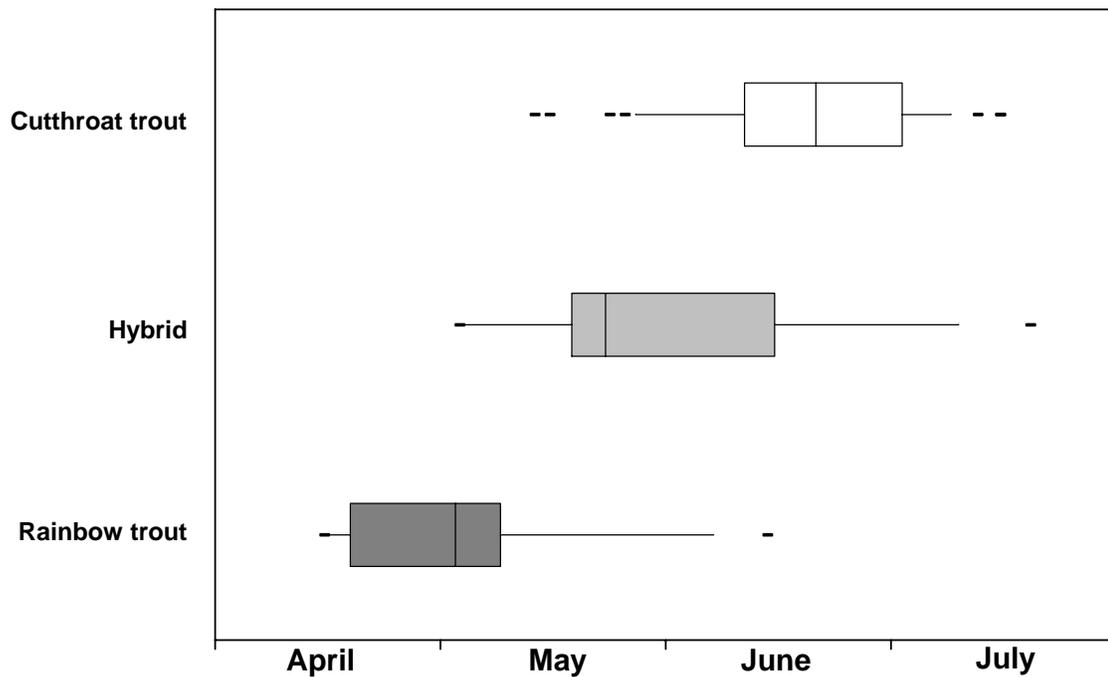
Little overlap of spawning periods occurred between cutthroat trout and the two other taxa. Spawning-period overlap index values between rainbow trout and hybrids versus cutthroat trout were 0.09 to 0.19 for individual years and 0.20 considering all years in aggregate. Spawning-period overlap index values between rainbow trout or hybrids versus cutthroat trout were 0.04 and 0.30, respectively (Table 4). The highest spawning-period overlap index value (0.66) was between hybrids and rainbow trout.

Figure 9. Spawning periods of 73 individual fish: 44 cutthroat trout (solid lines), 15 rainbow trout (dotted lines), and 14 hybrids (dash-dot lines) during 2001 to 2003. Spawning areas (SP = spring creek and SC = side channel) on the y-axis of each graph are ordered by elevation at waterway kilometer 0, from upriver (top of graph) to downriver.
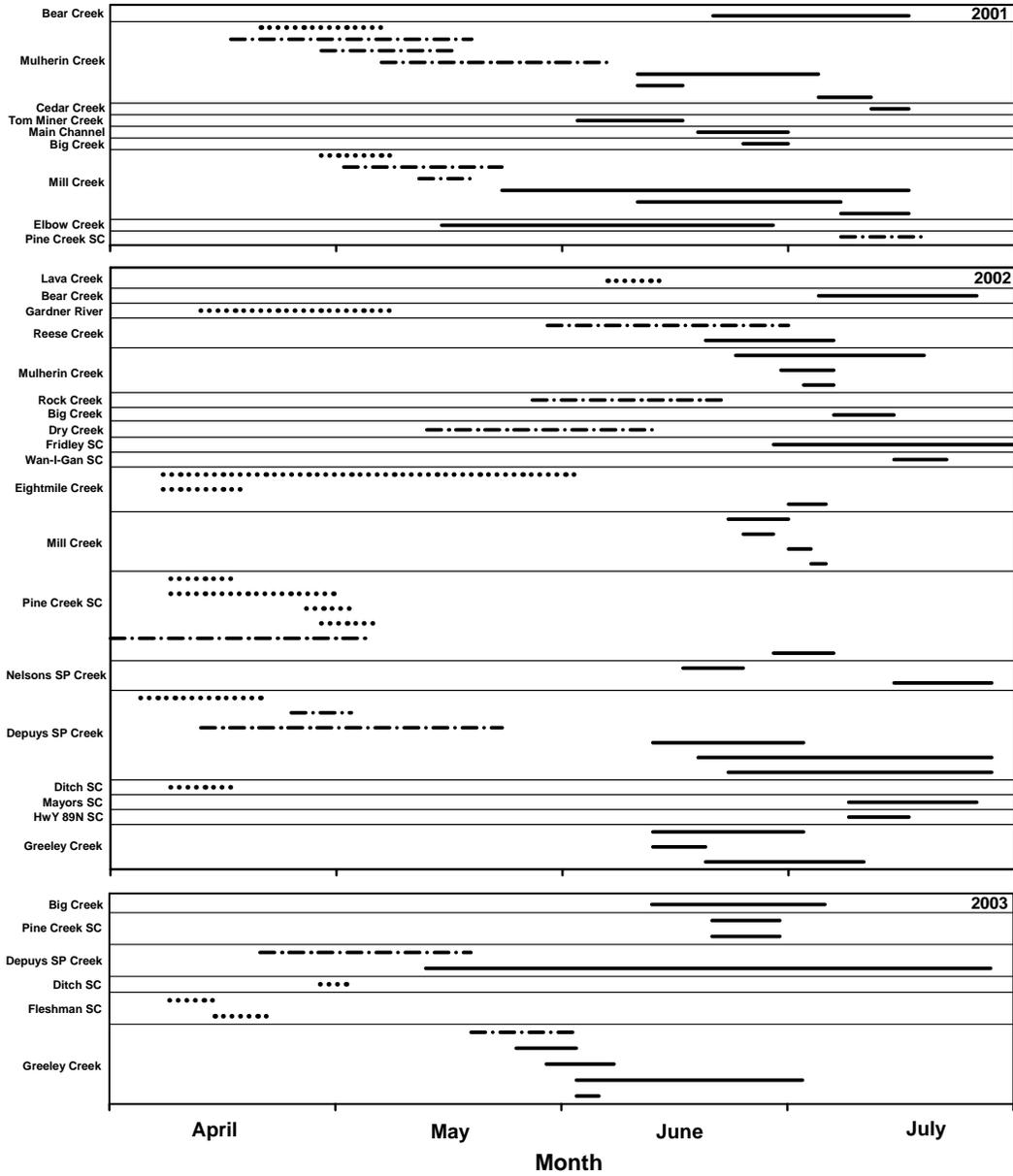
Table 4. Pianka overlap index values of spawning-period overlap between taxa by year and for all years combined. The overlap values and 95% confidence intervals (in parentheses) are followed by the numbers of fish of each taxon or taxa. Overlap values of zero do not have a bootstrapped confidence interval (denoted by a dash).

Comparison	Taxa	2001	2002	2003	All years combined
Spawning period	Rainbow trout vs. hybrid	0.68 (0.32-0.82) 2, 6	0.70 (0.17-0.94) 10, 6	0.36 (0.00-0.71) 3, 2	0.66 (0.30-0.85) 15, 14
	Rainbow trout vs. cutthroat trout	0.00 (-) 2, 12	0.01 (0.00-0.05) 10, 24	0.00 (-) 3, 8	0.04 (0.00-0.12) 15, 44
	Hybrid vs. cutthroat trout	0.24 (0.01-0.50) 6, 12	0.18 (0.01-0.38) 6, 24	0.29 (0.00-0.56) 2, 8	0.30 (0.10-0.54) 14, 44
	Rainbow trout and hybrid vs. cutthroat trout	0.18 (0.01-0.41) 8, 12	0.09 (0.01-0.23) 16, 24	0.19 (0.00-0.46) 5, 8	0.20 (0.06-0.35) 29, 44

Mean spawning start dates and mean spawning end dates were not significantly different between genders for rainbow trout and cutthroat trout. The sample size of hybrid females (N = 2) was insufficient to calculate a representative mean to compare to males. The mean spawning period of rainbow trout did not differ between genders (N = 9 females and 6 males). The mean spawning period of cutthroat trout males (22 d, N = 24) was significantly longer ($P = 0.013$) than cutthroat trout females (11 d, N = 20).

Environmental variables

Rainbow trout and hybrids began migrating and spawning at lower discharges and colder temperatures than cutthroat trout (Figure 10 and Table 5). The annual peak mean daily discharge at the Livingston gage station occurred on May 15, 2001, June 2, 2002, and June 1, 2003. All 15 rainbow trout and 13 of 14 hybrids migrated prior to the these dates, whereas 37 of 44 cutthroat trout migrated on or after the peak dates. Mean discharges of the Yellowstone River at the start of migration and spawning of rainbow trout (44 m³/s and 78 m³/s, respectively) and hybrids (76 m³/s and 110 m³/s, respectively) were significantly lower ($P < 0.001$) than the mean river discharges (310 m³/s and 293 m³/s, respectively) during the migration and spawning start of cutthroat trout (Table 5). Similarly, mean river temperatures during the migration and spawning start of rainbow trout (8.1 °C and 8.7 °C, respectively) were significantly colder ($P < 0.001$) than the mean river temperatures (11.3 °C and 13.1 °C) during the migration and spawning start of cutthroat trout (Table 5). Mean river temperature during the mean migration start date of hybrids was not significantly different from cutthroat trout, but mean river temperature

during the spawning start of hybrids (9.5 ºC) was significantly colder (*P* < 0.001) than the mean river temperature during the spawning start of cutthroat trout (Table 5).   No significant differences existed between the mean river discharges or temperatures during the migration or spawning start dates of rainbow trout and hybrids. The mean spawning area temperature during cutthroat trout spawning start (N = 33) was 11.3 ºC.   Sample sizes of water temperatures collected within spawning areas during the spawning start of rainbow trout (N = 2) and hybrids (N = 6) were insufficient to calculate a representative mean for these taxa. These taxa spawned primarily before thermographs were installed or in spawning areas where thermographs were not installed.

Figure 10. Annual migration start date ranges of taxa compared to the Yellowstone River hydrograph and thermograph at Livingston, 2001 through 2003.
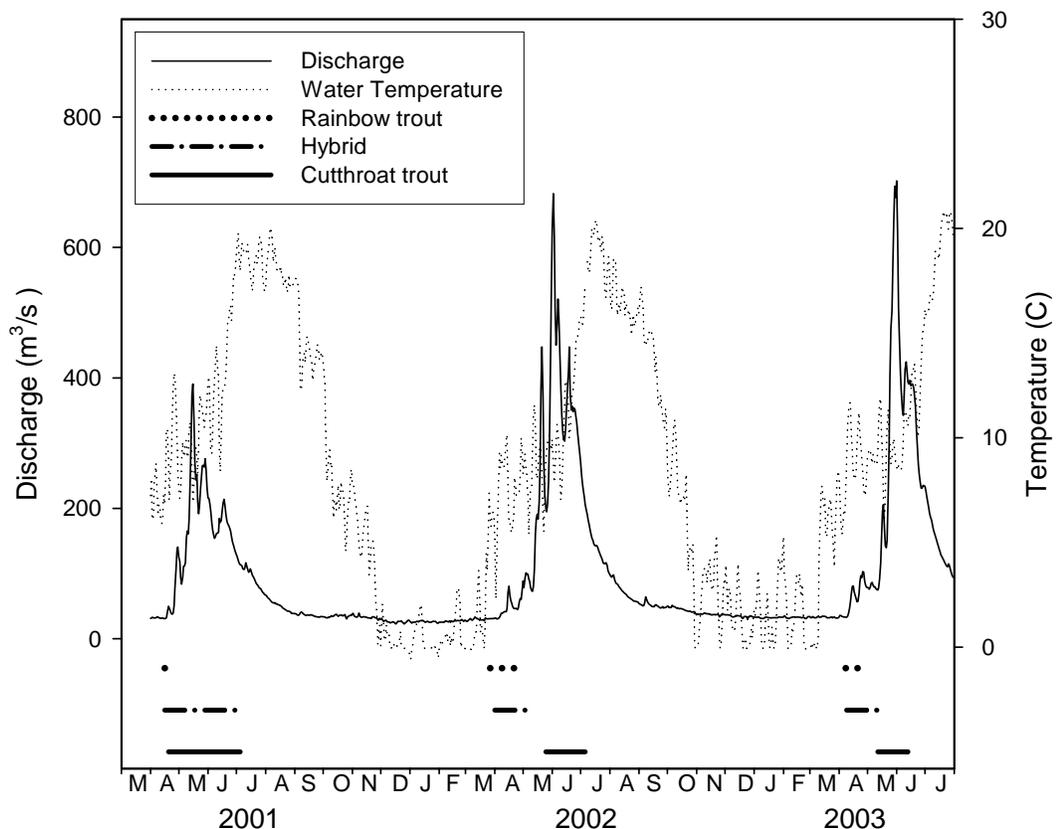
Table 5. Mean daily discharge and temperature of the Yellowstone River at Livingston during migration and spawn start dates of rainbow trout (N = 15), hybrids (N = 14), and cutthroat trout (N = 44), 2001 through 2003.

Variable	Rainbow trout		Hybrids		Cutthroat trout		P
	Mean	Range	Mean	Range	Mean	Range	
River discharge (m³/s) during migration start	44	31-106	76	31-206	310	50-702	< 0.001
River temperature (ºC) during migration start	8.1	6.1-11.7	9.7	5.5-18.7	11.3	7.3-19.8	< 0.001
River discharge (m³/s) during spawn start	78	31-450	110	31-242	293	75-702	< 0.001
River temperature (ºC) during spawn start	8.7	6.1-11.7	9.5	5.5-18.7	13.1	7.5-20.0	< 0.001

Spatial versus temporal assessment

Spatial distributions of spawning of rainbow trout and hybrids versus cutthroat trout within the five most-used spawning areas were more similar than temporal distributions. All three taxa primarily used the same spawning reaches (Figure 11), but most rainbow trout and hybrids spawned in April and May and had left spawning areas before most cutthroat trout began spawning in June. Similarly, annual or aggregated spawning-period overlap index values between rainbow trout and hybrids individually or combined versus cutthroat trout were less than spawning-reach overlap index values (Figure 12). In contrast, spawning-period overlap values were greater than spawning-reach overlap values for all years between rainbow trout and hybrids.

Genetic testing

Rainbow trout introgression was not detected in three of four spawning aggregations of putative Yellowstone cutthroat (Table 6). Rainbow trout introgression (2%) was detected in the Cedar Creek spawning aggregation, where one fish was identified in the field as a hybrid and two fish had nonnative PINE markers. DNA fragments representative of westslope cutthroat trout were detected in three of four spawning aggregations. Genetic compositions of spawning aggregations were 97.5% to 100% Yellowstone cutthroat trout.

Figure 11. Comparison of spawning reaches versus spawning periods of radio-tagged fish during within the five most-used spawning areas of the Yellowstone River drainage, 2001 to 2003. Each line connecting symbols represents a spawning reach of an individual fish. Symbols within each line represent relocation points. Pine Creek Side Channel fish were assumed to have entered the side channel from either upriver or downriver, depending on their previous relocation positions in the main channel.
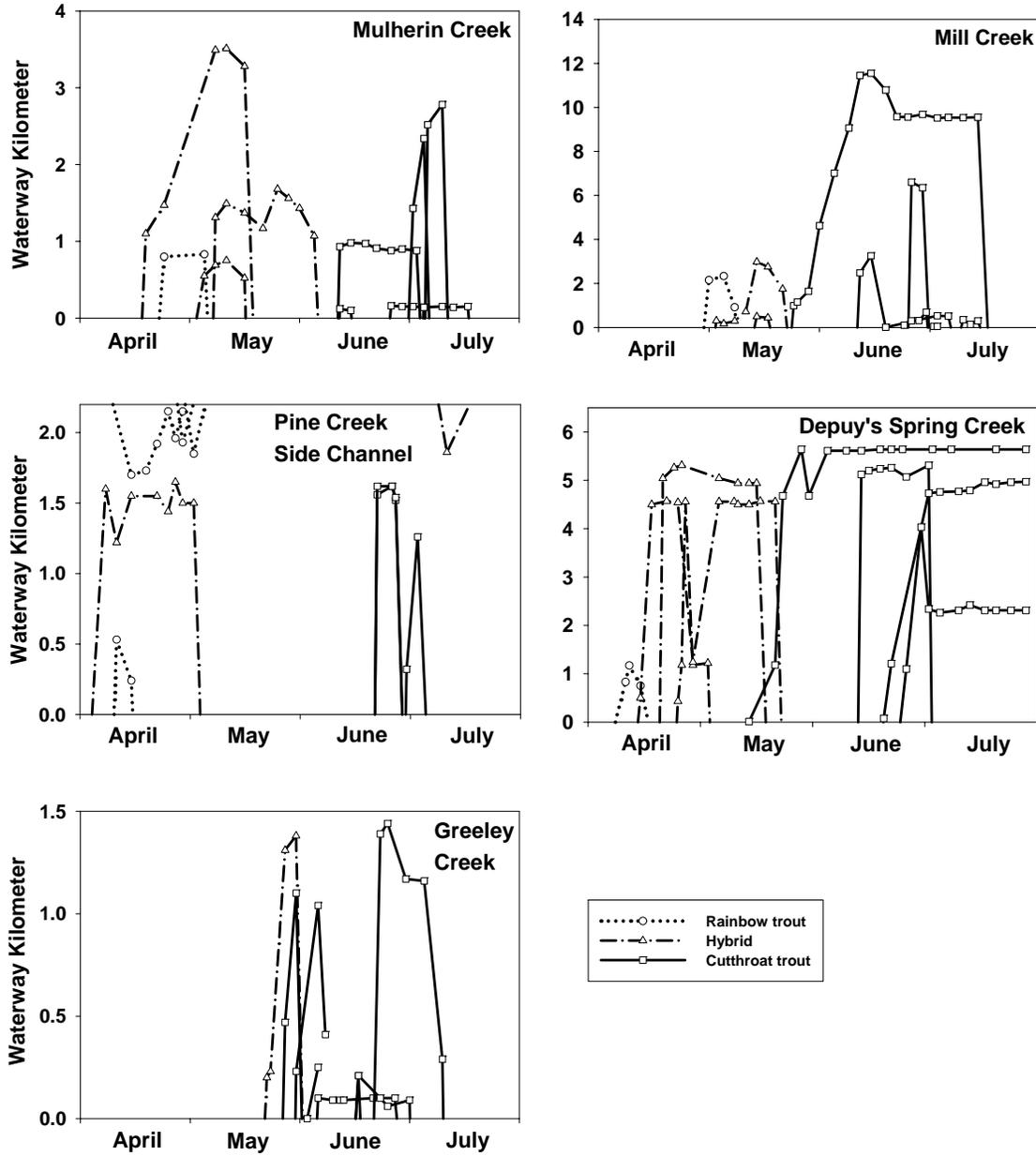
Figure 12. Spawning-reach overlap index values versus spawning-period overlap index values by year and for all years combined. Taxa pair symbols increase in size with year from 2001 through 2003 and solid symbols represent all years combined. The dashed diagonal line indicates equal temporal and spatial overlap. Points below the line indicate greater temporal overlap than spatial overlap, whereas points above the line indicate greater temporal than spatial overlap between a taxa combination. The vertical distance of a point from the diagonal line indicates the magnitude of difference between the two overlap values.
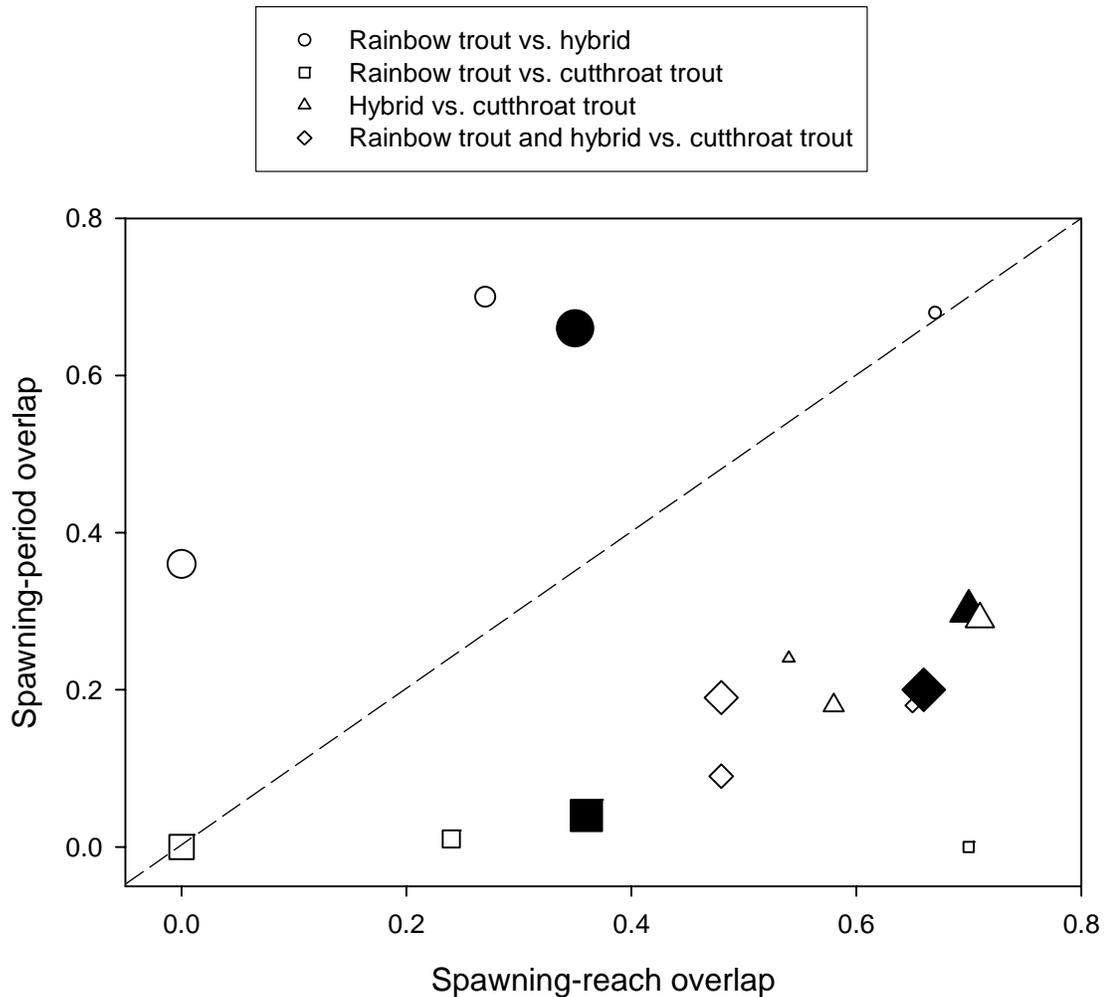
Table 6. Genetic compositions of cutthroat trout spawning aggregations, as determined by PINE, from four tributaries in 2003. The number of individuals in a sample is N. The genetic composition of the samples is indicated as a percentage of cutthroat trout, rainbow trout, or other (either a false-positive or a true-positive DNA fragment for westslope cutthroat trout).

| Stream | N | Genetic Percentage | | | Individuals with non-native markers | |
		Cutthroat trout	Rainbow trout	Other	Rainbow trout	Other
Mulherin	20	99.2	0.0	0.8	0	1
Cedar	24	97.5	2.0	0.5	1	1
Big	18	100	0.0	0.0	0	0
Greeley	16	99.2	0.0	0.8	0	1

DISCUSSION

Temporal separation and spatial overlap

Differences in time of spawning among salmonids may prevent genetic and ecological interactions (Heggberget et al. 1988; Quinn et al. 2000; Mackey et al. 2001). Migration and spawning dates of rainbow trout and hybrids were 5 to 9 weeks earlier than cutthroat trout in the Yellowstone River and 2 to 4 weeks earlier in the South Fork Snake River (Henderson et al. 2000). Introgressive hybridization from earlier spawning rainbow trout or hybrids may be low because spawning-period overlap was low between rainbow trout and hybrids versus cutthroat trout in the Yellowstone River. For example, no fluvial rainbow trout and only two hybrids were collected during sampling of putative cutthroat trout spawning aggregations during June and July. In South Fork Snake River tributaries, Yellowstone cutthroat trout migrated four to six weeks after fish with rainbow trout alleles had stopped migrating (Host 2003).

Yellowstone cutthroat trout spawn from late April through early August, depending on latitude, altitude, water temperature, and runoff conditions (Gresswell and Varley 1988). Fluvial and adfluvial fish appear to have similar timing throughout the Yellowstone River drainage. I observed cutthroat trout spawning from April 27 to August 14, a range that others have observed spawning in this area. Cutthroat trout spawning peaked from late June or early July in a mountain stream (Byorth 1990) and spawning occurred from early June until late July in a spring creek (Roberts 1986). Upriver of my study area, cutthroat trout began spawning in the Yellowstone River outlet of Yellowstone Lake from May 23 to June 16 (mean = June 4) with durations of 30 to 45

days (mean = 37 d) (Kaeding and Boltz 2001). Mean date of peak spawning in 27 tributaries of Yellowstone Lake ranged from May 23 to July 3 with a mean of June 10 (Gresswell et al. 1997). Cutthroat trout spawned from April 30 to July 10 (median spawning date of June 9) in the South Fork Snake River (Henderson et al. 2000).

The spawning time distributions of introduced rainbow trout in Intermountain West rivers have earlier peak and end dates than Yellowstone cutthroat trout. I observed rainbow trout spawning from April 4 through June 12. Fluvial rainbow trout spawned from early April through mid June in the Bighorn River (Sanborn 1990), from March through May in the Missouri River (Spoon 1985), from late March through early June (median spawn dates of April 30 and May 1) in the Madison River (Downing et al. 2002), and from early April through mid July (median spawn date of May 19th) in the South Fork Snake River (Henderson et al. 2002). Rainbow trout typically spawn during spring along the Pacific Coast of northern California, where many of the hatchery stocks for the species originated (Fausch et al. 2001).

Differences in timing of spawning could be related to different responses to environmental conditions for migration and spawning (Quinn and Adams 1996). Rainbow trout spawn during stable flow periods within their native range (Fausch et al. 2001) and introduced rainbow trout may spawn during similar conditions, prior to the spring peak in the hydrograph (Downing et al. 2002). Accordingly, all rainbow trout and 93% of hybrids migrated prior to the annual peak of runoff in the Yellowstone River when river temperatures had yet to warm considerably. In contrast, I observed 84% of cutthroat trout migrating during or after the annual peak in the hydrograph, at higher

discharges and warmer temperatures than rainbow trout and hybrids. Spawning cutthroat trout generally move to spawning areas as water temperature rises and discharge decreases after the spring runoff peak (Thurow 1982; Byorth 1990). Ninety-two percent of cutthroat trout spawning migrations into Clear Creek, a tributary of Yellowstone Lake, occurred after the peak discharge of the creek during ten years between 1979 and 1991 (Gresswell et al. 1997). Cutthroat trout migration during the descending limb of the hydrograph may be an adaptation to migrate when the greatest amount of spawning habitat is accessible (Gresswell 1995; Schmetterling 2001).

Species that segregate by breeding in response to different environmental conditions may continue to do so if those conditions are maintained, whereas greater overlap, interaction, and hybridization may occur when conditions are modified (Hubbs 1955; Carlson et al. 1985). Dams regulate the timing and magnitude of the flow regimes of most mainstem rivers where Yellowstone cutthroat trout no longer co-exist with rainbow trout (May et al. 2003), except in the regulated South Fork Snake River (Henderson et al. 2000). If rainbow trout, hybrids, and cutthroat trout respond to different river discharges and temperatures to begin migration and spawning, then the unregulated flows of the Yellowstone River may provide a hydrologic regime that maintains temporal reproductive separation. The degree of temporal separation between rainbow trout and hybrids and cutthroat trout may vary annually depending on the river hydrograph and thermograph. Spawning occurred 1-2 weeks earlier in Cedar Creek in 1988 than in 1989, probably in response to water temperature warming faster in 1988 (Byorth 1990). Water temperatures likely warmed sooner in the creek because discharge

had peaked earlier, as potentially indicated by the peak mean daily discharge for the Yellowstone River occurring 11 days earlier in 1988 than 1989. Earlier discharge peaks may indicate that cutthroat trout will migrate and spawn earlier (Thurow 1982), increasing the likelihood of overlap with earlier spawning rainbow trout and hybrids. However, spawning-period overlap between rainbow trout and hybrids in the 2001, the year with the earliest annual river peak, was similar or greater than the overlap observed in 2002 and 2003 when the annual peaks in the river hydrograph occurred 17 to 18 days later.

In contrast to the low level of temporal reproductive overlap, all three taxa spawned in the same places in the Yellowstone River drainage. Five spawning areas in the study area were used by 62% of all radio-tagged fish that spawned. Spatial reproductive overlap of the three taxa occurred primarily in tributaries in the Yellowstone River, whereas the three taxa overlapped within mainstem locations of the South Fork Snake River (Henderson et al. 2000). Some spawning areas were used exclusively by a low number of individuals of the same taxon in the Yellowstone River drainage. However, the majority of spawning areas used by a single radio-tagged taxon were used by cutthroat trout, which may be attributable to the greater number of this taxon that was radio-tagged and subsequently spawned versus either rainbow trout or hybrids. As initially hypothesized, rainbow trout spawned in side channels to a greater extent than cutthroat trout, but spatial overlap occurred because one side channel (Pine Creek) was used by all three taxa.

Habitat constriction may increase hybridization (Leary et al. 1995). No separation was observed among taxa within Yellowstone River tributaries, whereas separation of the same taxa was documented within one tributary of the South Fork Snake River (Henderson et al. 2000). Limited availability of spawning habitat among and within Yellowstone River tributaries may cause high spawning-area and -reach overlap. Irrigation water diversion occurs in almost all Yellowstone River tributaries and many are inaccessible to spawning fluvial trout because of dewatering of their lower reaches (Clancy 1988). Inaccessibility of most potential spawning tributaries forces spawning adults to concentrate in the few remaining accessible tributaries. Dewatering of Yellowstone River tributaries led Clancy (1988) to classify only a few tributaries as high-quality spawning areas for cutthroat trout in the 1980s. These were the same tributaries that were used for spawning by radio-tagged fish during my study. Spawning habitat may also be limited within the accessible tributaries. For example, radio-tagged fish moved upstream to nearly the maximum extent possible in Greeley, Depuy's Spring (temporary barrier from beaver dam), Mill, Cedar, Mulherin, Reese, and Lava creeks (the only tributary of a tributary used for spawning). In these, radio-tagged fish migrated to about the full extent upstream to natural barriers, i.e., waterfalls and cascades, except in Mill and Reese creeks. In these two creeks, barriers constructed to prevent rainbow trout introgression of resident headwater populations limit the accessible extent for migratory fish.

Several study design factors may have affected estimates of spatial and temporal reproductive overlap. Spatial overlap may have been underestimated because of the

unequal numbers of taxa that were radio-tagged. Higher spatial overlap would be expected if all three taxa had been radio-tagged in equal numbers, given that high spawning-area and spawning-reach overlap was exhibited among taxa with unequal numbers. However, my conclusion of high spatial overlap would be strengthened by this possible bias. Conversely, spatial overlap could have been overestimated because I could not precisely estimate the actual length of stream used for spawning by all individual fish. However, I believe this potential bias did not affect my conclusions because spawning activity was noted throughout individual spawning reaches, spawning areas were used to a limited extent, and salmonids can spawn in multiple locations with multiple partners (Brown and Mackay 1995; Schmetterling 2001; Taggart et al. 2001). Temporal overlap could have been overestimated between rainbow trout with either hybrids or cutthroat trout, because early spawning rainbow trout may not have been adequately represented by radio-tagged fish. There was evidence that some rainbow trout may have begun migrating to spawning areas prior to initiation of radio-tagging. Fluvial rainbow trout migrated into Mill Creek in mid March (Tohtz 2003) and prior to this time during all years only 14% of rainbow trout were radio-tagged. In addition, Nelson and Depuy's spring creeks may support spawning rainbow trout in late winter (Roberts 1986; Shepard 1992) and this time period was not included in this study. These fish may be descendants of a past stocking of the Arlee hatchery strain of rainbow trout that was cultured for fall spawning (B. Shepard, Montana Department of Fish, Wildlife and Parks, personal communication).

Hybrid spawning times overlapped more with rainbow trout than cutthroat trout, whereas hybrid spawning areas and reaches overlapped more with cutthroat trout. Similarly, spawning times of hybrids are nearer to rainbow trout than to cutthroat trout in the South Fork Snake River (Henderson et al. 2000). Therefore, hybrids may be more likely to interbreed with rainbow trout, potentially averting a potential source of rainbow trout alleles from cutthroat trout. For example, genetically tested putative rainbow trout and hybrids were likely post-F_1 hybrids, indicating repeated interbreeding of hybrids with rainbow trout. However, rainbow trout x cutthroat trout hybrids may be vectors of introgression of cutthroat trout populations (Hitt et al. 2003). Hybrids, more so than rainbow trout, overlapped cutthroat trout spawning locations and times in the Yellowstone River drainage. For example, radio-tagged hybrids were observed spawning within the same tributaries at the same time as radio-tagged cutthroat trout. Mitochondrial DNA testing of hybrids indicates that females of both rainbow trout and cutthroat trout may hybridize (Henderson et al. 2000) and that backcross hybrids are produced from male hybrids mating with pure females (Young et al. 2001). Longer mean spawn periods of cutthroat trout males versus females may indicate that male cutthroat trout may have a greater probability of mating with hybrid females, rather than the reciprocal combination. However, low numbers of hybrid females spawned in my study and therefore prevented a meaningful sample to test for gender differences of spawning variables within this taxon.

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Genetic testing

Genetic confirmation of hybridization, or lack thereof, for an individual organism is limited by the number of available diagnostic markers (Boeklen and Howard 1997). Some hybridized individuals that are genetically tested will only show diagnostic markers for a single taxon, because of the random reshuffling of alleles during sexual reproduction (Allendorf et al. 2001). The relatively low incidence of DNA fragments diagnostic for rainbow trout in fish I identified as Yellowstone cutthroat trout could be related to my ability to correctly identify Yellowstone cutthroat trout or the relatively limited power of genetic tests, or both. Ninety-five percent of the subsample of radio-tagged Yellowstone cutthroat trout had no detectable rainbow trout markers, which was comparable to other samples of putative cutthroat trout from the Yellowstone River (Leary 1983; Tohtz 1999). Rainbow trout markers were not detected in 76 (99%) of 77 individual putative cutthroat trout in spawning aggregations. The presence of two radio-tagged cutthroat trout in spawning aggregations with no rainbow trout introgression provides further evidence that the radio-tagged cutthroat trout might not have been hybridized.

Hybridization between taxa likely includes a wide range of recombinants (Barton and Hewitt 1985). If most putative rainbow trout and hybrids were later-generation hybrids, then these two taxa could be considered a single group. Spatial and temporal reproductive overlap of cutthroat trout was assessed versus rainbow trout and hybrids together, because these fish both have potential rainbow trout alleles. However, phenotypic identification may have allowed for a more refined distinction in degree of

hybridization because hybrids had different spawning times and places than rainbow trout.

Genetic testing of Yellowstone cutthroat trout spawning aggregations sampled during the latter part of the cutthroat trout spawning period showed little or no evidence of rainbow trout introgression. However, rainbow trout introgression of cutthroat trout spawning aggregations may be higher during the earlier periods of cutthroat trout spawning because of late-spawning hybrids. Putative rainbow trout spawning aggregations should also be sampled to assess hybridization and introgression. Cutthroat trout introgression of rainbow trout populations may be high because of the high temporal overlap of hybrids with rainbow trout.

PINE fragments resembling those for westslope cutthroat trout were found in three of four spawning aggregations of Yellowstone cutthroat trout and one radio-tagged hybrid. These fragments may indicate that westslope cutthroat trout from a historical stocking introgressed with Yellowstone cutthroat trout, or these fragments may actually be non-diagnostic for westslope cutthroat trout. The future threat of westslope cutthroat trout introgressive hybridization with Yellowstone cutthroat trout is low because parental types of westslope cutthroat trout are not known to exist within the Yellowstone River.

<u>Status of radio-tagged fish</u>

Surgery mortality percentages were about the same among taxa, whereas cutthroat trout had higher pre-spawn mortality than the other two taxa. Cutthroat trout had the longest pre-spawning period for a taxon and this likely accounts for the higher pre-spawning mortality of cutthroat trout versus the other two taxa. Despite high pre-

spawning mortality, there were greater numbers of cutthroat trout alive during the start of the spawning period versus the other taxa because a greater number of cutthroat trout were radio-tagged.

High apparent mortality of radio-tagged fish after surgeries and spawning was similar to that observed in other studies. The combined percentage of surgery and pre-spawn mortality (18%) was comparable to that among the same taxa in the South Fork Snake River (22%; Henderson et al. 2000). About one-third of the surgery and pre-spawn mortality occurred within the Corwin Springs section of the Yellowstone River in 2001. An irregularity during surgeries, e.g., ineffective procedures or contaminated equipment is suspected. I found spawning mortality of cutthroat trout (59%) that was higher than the 13-48% reported by Gresswell (1995), but my observed rainbow trout spawning mortality (60%) was similar to estimates of 43 to 84% by Hartman et al. (1962). My estimates of spawning mortality may be high, because transmitter expulsion can occur without subsequent mortality or morbidity (Lucas 1989; Chisholm and Hubert 1985). Three transmitters that were in cutthroat trout were recovered in or near redds, potentially indicating expulsion during spawning.

Radio transmitters may increase the predation risk of a fish (Adams et al. 1998). Predation was indicated for 15% of the radio tagged fish in this study, mostly from birds. Antennas were about 1.1 times the average length of radio-tagged fish and trailed an average of 27 cm past the caudal fin. The plastic coated antenna may have allowed radio-tagged fish to be more easily seen or captured by predators.

Migratory trout may spawn in alternate years (Gresswell 1995). This may account for the twenty-four percent of radio-tagged fish that did not migrate to spawn during this study. Alternate year spawning was suggested for cutthroat trout in the Yellowstone River because the average growth of cutthroat trout is slower for fish that spawn in consecutive years versus the cutthroat trout population as a whole (Clancy 1987).

<u>Management implications</u>

Temporal reproductive separation may be facilitated by management actions that maintain and extend tributary conditions for cutthroat trout to spawn later than either rainbow trout or hybrids. These management actions may include water leases, diversion screening, selective barriers, and stream closure regulations.

Water rights leases on Mulherin, Cedar, Big, Mill, and Locke creeks are held by the Montana Department of Fish, Wildlife, and Parks. Leases are intended to maintain instream flows levels for fluvial cutthroat trout spawning and the emergence and outmigration of their offspring (Hennessey 1998). Water is leased from April or May through October. The critical time for the leases occurs when peak irrigation demands are greatest, typically June through August when cutthroat trout are spawning or eggs are incubating. Continuation of water leases on these streams, or the addition of water leases on other streams, would continue or expand the opportunities for later spawning and incubating cutthroat trout.

Preventing fish entrainment into tributary irrigation diversions would allow more cutthroat trout adults and fry to successfully emigrate from tributaries. Entrainment of

radio-tagged trout, in addition to untagged fluvial fish, was observed in four different tributary diversions. All fish were likely entrained when they attempted to migrate back to the Yellowstone River after spawning. Entrainment rates ostensibly increase as the amount of water diverted increases and stream runoff decreases during the summer. Diversion screening would provide increased downstream passage of fluvial fish, especially during the summer when cutthroat trout are spawning and their fry are outmigrating.

Installation of fish barriers and selective passage of only cutthroat trout above the barriers have shown promise of reducing introgression between rainbow trout and Yellowstone cutthroat trout within tributaries of the South Fork Snake River (Host 2003). Similarly, weirs could be used on Yellowstone River tributaries to prevent upstream passage of earlier spawning rainbow trout and hybrids and then pass later spawning cutthroat trout. Issues that need to be addressed when considering the feasibility of such actions include weir construction and effectiveness, artificial selection of genotypes based on phenotypic criteria, and genetic composition of resident trout upstream of the barrier site (Host 2003).

Private lands and the waterways that run through them are critical to spawning habitat for migratory fishes. Eighty-nine percent (65 of 73) of radio-tagged fish spawned in waterways that were within privately-owned lands. Cooperative partnerships with private land owners will be required for water leasing, diversion screening, and selective barrier sites.

Yellowstone River tributaries in the study area are closed to fishing from November 30 until the third Saturday in May. This closure covers the majority of time when rainbow trout and hybrids are spawning but allows catch-and-release fishing when cutthroat trout are spawning in tributary streams. Although tributary streams receive relatively low angling pressure compared to the mainstem river, angling may disrupt spawning and illegal take of cutthroat trout may occur by anglers unable to differentiate between species (Schmetterling 2001). However, longer stream closures may need to be balanced against the possibility of opening streams earlier in the year to promote harvest of rainbow trout and hybrids.

Resistance of a native taxon to hybridization does not preclude its displacement (Utter 2001) and hybridization may increase if introduced fish increase and the native taxon continues to decline (Hubbs 1955; Krueger and May 1991). Rainbow trout are currently the most abundant trout in the Yellowstone River. If rainbow trout continue to increase and cutthroat trout further decline then hybridization may increase. Therefore, hybridization and introgression between rainbow trout and cutthroat trout within spawning aggregations should be monitored to determine the effectiveness of management actions and long-term trends. Systematic sampling of key spawning aggregations every 5 to 10 years may detect changes in hybridization and introgression.

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