FISHERY RESEARCH

KOOTENAI RIVER FISHERIES INVESTIGATIONS: SALMONID STUDIES

ANNUAL PROGRESS REPORT
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Project Progress Report

2007-2008 Annual Report

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CHAPTER ONE: SALMONID STUDIES

ABSTRACT

This research report addresses bull trout *Salvelinus confluentus* and redband trout *Oncorhynchus mykiss* redd surveys, population monitoring, trout distribution, and abundance surveys in the Kootenai River drainage of Idaho. The bull trout is one of several sport fish native to the Kootenai River, Idaho that no longer supports a fishery. Because bull trout are listed under the Endangered Species Act, population data will be vital to monitoring status relative to recovery goals. Thirty-three bull trout redds were found in North and South Callahan creeks and Boulder Creek in 2007. This is a decrease from 2006 and 2005 and less than the high count in 2003. However, because redd numbers have only been monitored since 2002, the data series is too short to determine bull trout population trends based on redd counts. Redband trout still provide an important Kootenai River sport fishery, but densities are low, at least partly due to limited recruitment. The redband trout proportional stock density (PSD) in 2007 increased from 2006 for a second year after a two-year decline in 2004 and 2005. This may indicate increased recruitment to or survival in the 201-305 mm length group due to the minimum 406 mm (16”) length limit initiated in 2002. We conducted 13 redd surveys and counted 44 redband trout redds from May 7 to June 3, 2007 in a 3.8 km survey reach on Twentymile Creek. We surveyed streams in the Kootenai River valley to look for barriers to trout migration. Man-made barriers, for at least part of the year, were found on Caboose, Debt, Fisher, and Twentymile creeks. Removing these barriers would increase spawning and rearing habitat for trout and help to restore trout fisheries in the Kootenai River.

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INTRODUCTION

The Kootenai River (spelled Kootenay in Canada) in Idaho no longer provides fisheries for the endangered Kootenai River white sturgeon *Acipenser transmontanus*, burbot *Lota lota*, and kokanee *Oncorhynchus nerka* because habitat changes have rendered these populations incapable of sustaining recruitment for harvest (Richards 1997). The bull trout *Salvelinus confluentus*, another sport fish native to the Kootenai River, was listed as threatened under the Endangered Species Act (ESA) in 1998.

Bull trout are distributed throughout the Kootenai River mainstem and some tributaries downstream of migration barriers in Idaho (Partridge 1983; Paragamian 1994, 1995a; Downs 1999, 2000; Walters and Downs 2001; Walters 2002). The Kootenai River is also one of 22 designated bull trout recovery units and has a recovery goal of 1,000 spawners (U.S. Fish and Wildlife Service [USFWS] 2002; USFWS 2006) (Figure 1). The bull trout is ESA listed and in two Biological Opinions (USFWS 2000); USFWS 2006) the USFWS expressed little is known of the life history of bull trout residing in the Kootenai River downstream of Kootenai Falls, MT, Idaho, and British Columbia (BC), Canada. But it is known juvenile bull trout densities ranged from 1.64/100 m^2 to 7.65/100 m^2 across four sample reaches in the Callahan Creek drainage in 2003 (Walters 2004b). Bull trout redds were first documented in Boulder Creek in 2001 and in North and South Callahan creeks in 2002 (Walters 2003, 2004a). Annual bull trout redd counts have continued on Boulder Creek since 2000 and in the Callahan Creek drainage since 2003 (Walters 2004b, 2005). The bull trout draft recovery plan states that the trend criteria for recovery will be met when the bull trout population is accepted as stable or increasing based on at least 10 years of monitoring data (USFWS 2002). The recovery plan calls for redd surveys to continue as a metric to document bull trout population trends (USFWS 2002). The continuation of bull trout redd counts in Boulder Creek and North and South Callahan creeks will provide data to help document population trends.

Redband trout *O. mykiss gairdneri* are the most popular sport fish in the Idaho reach of the Kootenai River, but densities are low, ranging from 3 fish/ha in 1993 to 11 fish/ha in 2004 (Paragamian 1995a, 1995b; Downs 2000; Walters 2005). A creel survey held in 2001 indicated the redband trout angler catch rate in the Kootenai River was about 0.18 fish/h (Walters 2002) as compared to a statewide average of about 0.94 redband/rainbow trout/h (Schill 1991). These low densities are at least partly due to limited juvenile recruitment (Walters et al. 2005). Decreased productivity in the Kootenai River downstream of Libby Dam may be another factor limiting fish populations (Woods 1982; Paragamian 1995a; Snyder and Minshall 1996). Woods (1982) reported that 63% of total phosphorus and 25% of total nitrogen in the Kootenai River system never pass through Libby Dam. A nutrient restoration experiment is currently underway to test the nutrient limitation hypothesis (IDFG and Kootenai Tribe of Idaho, unpublished data). Another possible factor limiting the Kootenai River redband trout population is angling exploitation (Walters and Downs 2001; Walters 2002). A 406 mm (16") minimum length limit and 2-fish bag limit were initiated on January 1, 2002. An annual monitoring program is necessary to determine if nutrient restoration and the more restrictive fishing regulations are benefiting the redband trout population.

In November of 2007, the US Army Corps of Engineers (USACE) and Bonneville Power Administration (BPA) resumed load following from the Libby Dam. The process of load following is ramping the discharge from Libby Dam in accordance with power demands. Hourly ramping rates range higher in the winter than summer and can range from 56.6 to 198.2 m^3/s while ramp down rates can range from 14.2 to 99.1 m^3/s (USFWS 2006). Typically, discharge from Libby Dam is higher during the weekdays than weekends or holidays. Ramping rates can rapidly change velocities in fish holding areas and dewater or flood the river margins. Ramping rates and river velocities have been the subject of study regarding white sturgeon spawning and burbot migration (Paragamian 2000; Paragamian and Wakkinen 2008), but the effects on redband trout behavior or movement have not been addressed. Resident redband trout are common from Libby Dam downstream to Bonners Ferry and are likely affected by power peaking during winter months when they are not active and productivity is low. Increased water volume causes changes in redband trout habitat by altering prey drift, increasing velocity, and depth. The effects of such operations on stream biota and habitat are in need of evaluation in the Kootenai River. When juvenile trout begin to establish their home range in their second year of life, their movements become less dramatic (Behnke 1992). Small trout in tailwater fisheries are influenced more by dam operations than large trout (McKinney et al. 2001). We studied the effects of load following operations on redband trout movement and habitat use during episodes of rapid flow manipulations as well as low flows.

We surveyed streams in the Kootenai Valley to locate barriers to trout migration as part of our Statement of Work to the Bonneville Power Administration. Tributaries to the Kootenai River are important spawning and rearing areas for redband trout, bull trout, cutthroat trout, and kokanee (Walters et al. 2005; Walters 2007). Once barriers were found, we estimated the amount of fish habitat that could be gained upstream of the barriers. The potential increase in fish habitat would help to prioritize the most important barriers for future removal efforts. Locating stream barriers was considered the first step for future work that would remove barriers as funding permitted.

OBJECTIVES

1. To have a recovered and fishable bull trout population of 1,000 adults or more that qualifies for delisting status within the decade.

2. To have an improved redband trout population that increases angler catch per hour from the recent 0.18 trout/h to 0.94 trout/h.

STUDY AREA

The Kootenai River flows south from British Columbia into Montana, northwest into Idaho, then north back into British Columbia and Kootenay Lake (Figure 1). It flows out of the west arm of Kootenay Lake and enters the Columbia River at Castlegar, British Columbia. In the U.S., the Kootenai River is regulated by Libby Dam in Montana (Figure 1). In Idaho, the Kootenai River has the following three reaches: 1) the Canyon Reach (22 km) from the Montana border to the Moyie River, 2) the Braided Reach (10 km) from the Moyie River to Bonners Ferry, and 3) the Meandering Reach (73 km) from Bonners Ferry to the Canadian border (Fredericks and Hendricks 1997). The Meandering Reach has a relatively slow velocity and substrates consisting mainly of sand, silt, and clays (Partridge 1983). Dikes on either side of the river in this reach reduce flooding of the adjacent agricultural lands.

The study area for the redband trout winter telemetry was much more restricted than the general study area. The upper location of redband trout winter telemetry study reach was at Leonia (rkm 270, location of a gauging station), Idaho at universal transverse Mercator (UTM) 11U 570083E 53856454N and while the lower end of the study reach was to the Search and Rescue boat ramp (rkm 246) located in Bonners Ferry UTM 11U 549384E 5393710N. This reach included the canyon and braided reach of the Kootenai River. The reach includes the Moyie River UTM 11 U 559845 5394984. The canyon reach of the Kootenai River is characterized by long 1-3 meter deep runs ending in large pools 3-20 m deep. Substrate in the canyon reach is dominated by boulder and cobble. Downstream of the canyon reach is the braided reach of the Kootenai. This reach starts as the Kootenai River exits the steep canyon 1.5 km downstream of the Moyie River and continues for 13 km to Bonners Ferry Idaho. The braided reach has many islands and side channels and is dominated by cottonwood (*Populus* sp.) forests along the banks. This reach contains more riffle and less deep pool habitat than the canyon reach. Substrate in the braded reach is dominated by gravel and cobble.

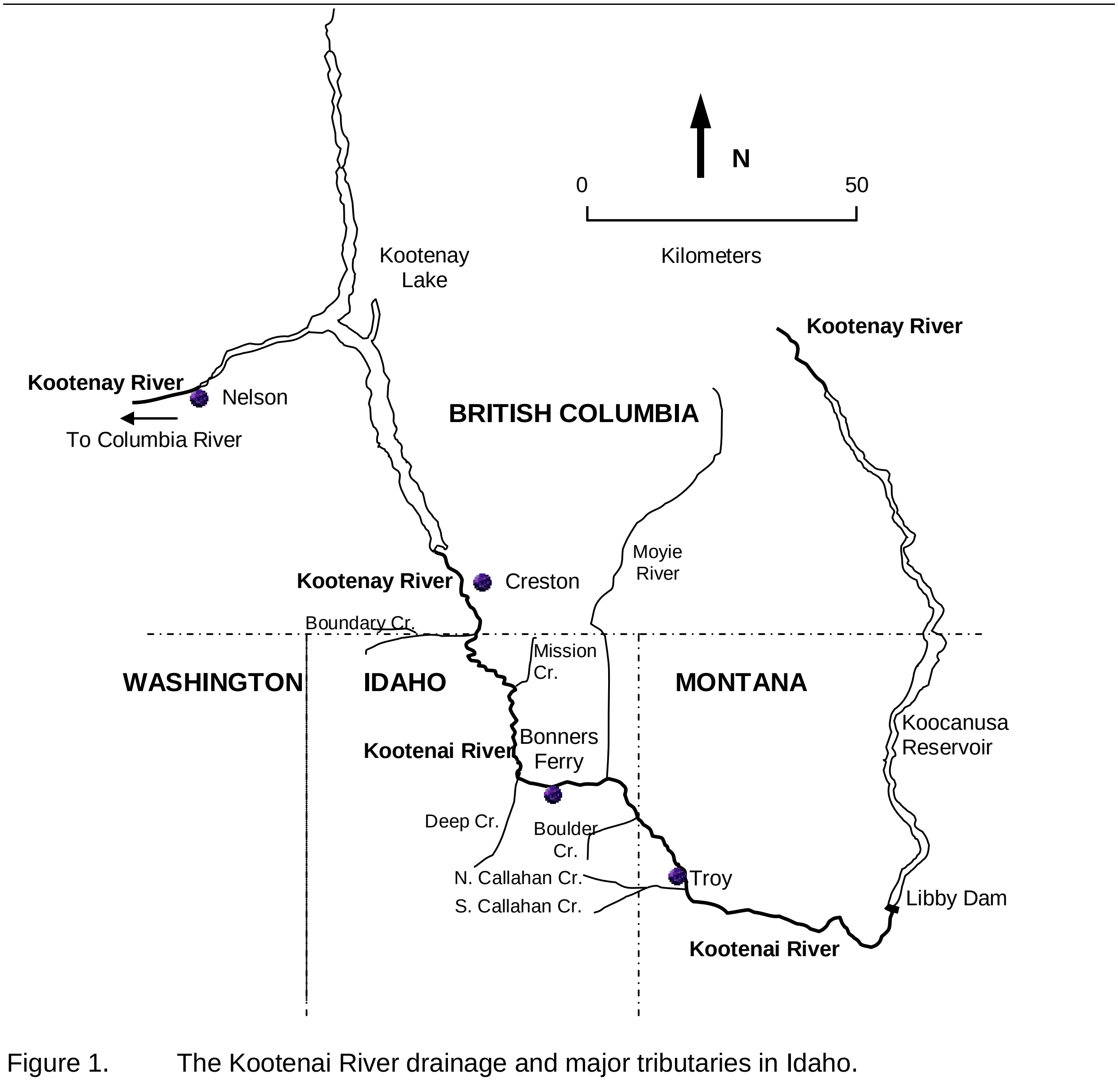

Figure 1. The Kootenai River drainage and major tributaries in Idaho.

METHODS

Bull Trout Redd Surveys

Bull trout redd surveys were conducted along index transects on Boulder Creek and North and South Callahan creeks (Walters 2004b). Each index transect was hiked once during midday. Disturbed and cleaned gravel or cobble areas showing a pit and tailspill were identified as bull trout redds (Shepard and Graham 1983; Dunham et al. 2001). Lengths of observed bull trout were also estimated to the nearest cm total length (TL).

Redband Trout Stock Status

In fall 2007, redband trout were sampled while electrofishing several sections of the Kootenai River from rkm 250 (Cow Creek) to rkm 275.5 (near Boulder Creek). Redband trout were measured in total length (TL), weighed (g), and released. Redband trout catch-per-unit-effort (CPUE), relative weights (W_r), proportional stock density (PSD), and quality stock density (QSD) were then calculated (Anderson 1976; Wege and Anderson 1978; Anderson and Neumann 1996). These variables are measured annually to monitor the redband trout population size structure. Relative weights were calculated for redband trout length groups of 201-305 mm TL, 306-406 mm TL, and >406 mm TL using the standard weight (W_s) equation for lotic rainbow trout populations proposed by Simpkins and Hubert (1996). Proportional and quality stock densities were calculated for redband trout >305 mm TL and >406 mm TL, respectively, using 200 mm TL as stock length (Schill 1991). Confidence intervals (95%) were estimated for the PSD and QSD using the table provided by Gustafson (1988). The 2007 redband trout population statistics were compared to those from previous years to help evaluate the 2002 regulations change.

Redband Trout Winter Telemetry

River Flows and Temperature

The Kootenai River flow volume was monitored at the Leonia Gauging station located on the Idaho Montana Border (rkm 270). River flows were checked from the internet using the USGS website after tracking was completed to get an exact volume at the start of each tracking run. Temperatures were taken in the field using handheld biological grade thermometers at the start of each telemetry run.

Radio Tagging Redband Trout

We used Advanced Telemetry Systems (ATS) models F1540, F1560, F1580, and F1835 radio transmitters to monitor redband trout winter movements. The expected life of each transmitter model was 35, 42, 94, and 251 days, respectively. Transmitter frequencies ranged from 30-31 Mhz, operated at pulse widths of 22 ms and pulse rates of 55 pulses/min. Transmitter weights were 2 g for 1540, 2.9 g for 1560, 3.9 g for 1580, and 14 g for the 1835 model. Some of the radio transmitters expired in the middle of the study period; thus, we collected five additional redband trout in order to maintain the same sample size through the duration of the study. Transmitters did not exceed 2% of the weight of the fish as recommended by Winter (1996). Transmitters were implanted in redband trout following the shielded needle technique described by Downs (2000). Redband trout collected in the canyon reach were captured with electrofishing equipment as described by Downs (2000) and released at the location of surgery within 1 km of the capture location. Redband trout were also captured in the braided reach using hook and line, transmitters were implanted following the same methods as above, and they were released at the location of capture.

Redband Trout Winter Telemetry

Redband trout telemetry was scheduled 2-4 times weekly and before and after operational flow changes were made at Libby Dam. The study reach on the Kootenai River was divided into three sections with random telemetry observations (Snedecor and Cochran 1989. Telemetry timing was also done randomly in morning and afternoon sessions to eliminate bias of daily feeding movements made by fish. Fish were located using two-point triangulation from a jet boat using an ATS R4500S receiver and directional antennae. Care was taken not to disturb fish until the fish's exact location was determined. Using triangulation and at a distance of 25 m the actual location of the fish could be determined within a 2 m circle; this circle was defined as the fish location, or FL. Kootenai River flow was obtained from a US Geological Survey gauging station at Leonia, Montana.

ATS fixed receivers were placed at the top and bottom of the reach to detect any redband trout that traveled outside the study reach. If redband trout could not be located by boat, a fixed wing aircraft was used to locate redband trout and the boat could then be used to verify fish locations.

Habitat Measurements

Habitat measurements were taken in the FL after redband trout were located to within 2 m of their exact location. Depth was measured with a handheld Polar Vision digital depth sounder from the side of the boat. Velocities were taken from the side of the boat using a Flow Mate™ model 2000 portable flow meter with staff. Distance from each bank was recorded using a Bushnell™ Yardage Pro Sport laser range finder. Substrate types were classified as sand (<2 mm), gravel (2-64 mm), cobble (64-256 mm), boulder (512-4,096 mm) (Harrelson et al 1994). Cover was defined as anything that could conceal fish from above from avian predators. Dominant substrate and cover type were measured visually within the FL at each fish location. When water depth and clarity did not allow visual identification of substrate a fishing grade television camera and monitor were used. A Garmin™ 130 Rhino Global Positioning System (GPS) unit was used to store a waypoint and UTM coordinates at each fish location. Euclidian distance (straight-line distance between two points) that fish moved was then determined using the Pythagorean Theorem and the UTMs from the previous fish location.

Twentymile Creek

Twentymile Creek Habitat Measurements

Measurement of habitat transects started at a random distance upstream of the bottom of the stream reach. We divided the stream into logical sections using property lines, landmarks, and stream barriers. We measured one transect for each 100 m section of stream within our reaches. We used a random number generator for numbers from 1-100 and counted this distance in m from the bottom of each reach to our first transect. This was repeated for each 100 m section of stream to prevent bias from natural patterns. At each transect we characterized seven habitat variables including wetted width, depth, velocity, substrate, substrate embeddedness, distance to cover, and cover type. Stream widths at each transect were measured perpendicular to the current and recorded to the nearest 0.1 m. Each transect was broken down into 0.25 m sections, and habitat measurements were taken at the midpoint of each section (Schuett-Hames et al. 1999). Depth was measured to the nearest 0.01 m and velocity was measured to the nearest 0.01 m/s at 0.6 x depth when depth was less than or equal to 0.75 m and at 0.8 x depth and 0.2 x depth and averaged when depth was >0.75 m.

In order to classify substrate we collected a sample at each section midpoint. The substrate sample was measured on the intermediate axis to the nearest 0.01 mm and classified as silt (<.60 mm), sand (0.06-2 mm), very fine gravel (2-4 mm), fine gravel (4-8 mm), medium gravel (8-16 mm), course gravel (16-32 mm), very course gravel (32-64 mm), small cobble (64-128 mm), large cobble (128-256 mm), boulder (>356 mm), bedrock, or embedded wood. Substrate embeddedness was defined as the percentage of the surface area of larger particles (gravel, cobble, boulder) that was covered by fine sediment. Distance to cover was measured from the section midpoint to the edge of the nearest cover. Cover types included undercut bank, overhanging vegetation, woody debris, pool or deep water, whitewater, and boulders. We installed a staff gage just upstream of the Highway 95 culvert to monitor stream flow.

Redd Surveys and Redd Measurements

We conducted 13 redd surveys on Twentymile Creek between May 7 and June 19, 2007 during midday hours and on sunny days when practical. The survey reach for Twentymile Creek was from above the Highway 95 culvert upstream 3.8 km to the first fish passage barrier. The reach was hiked in an upstream manner and redds were identified as areas of relatively clean gravel with a visual upstream pit and downstream tailspill (Grost et al. 1991; Holecek and Walters 2007; Walters et al. in progress). Redds were marked with flagging tape and numbered in the order in which they were found.

Redd area was determined by multiplying the total length of the redd, from the upstream edge of the pit to the downstream edge of the tailspill, by the average width, measured at 25%, 50% and 75% of length. Depths and velocities were measured at the pit head, mid-pit, and mid-tailspill portions of the redds. Velocity was measured at 60% of depth with a Marsh-McBirney electronic flow meter. Primary and secondary tailspill substrate were visually estimated for each redd, based on the Wentworth Scale (Harrelson et al. 1994). We also measured distance to cover from the center of the pit to the edge of the cover.

Barrier Surveys

We walked tributaries to the Kootenai River in Idaho to locate fish passage barriers. The surveyed streams included Curley, Caboose, Debt, Trout, Fisher, Parker, Long Canyon and Twentymile creeks (Figure 2). Streams were walked from the mouth upstream until natural barriers to fish migration were encountered.

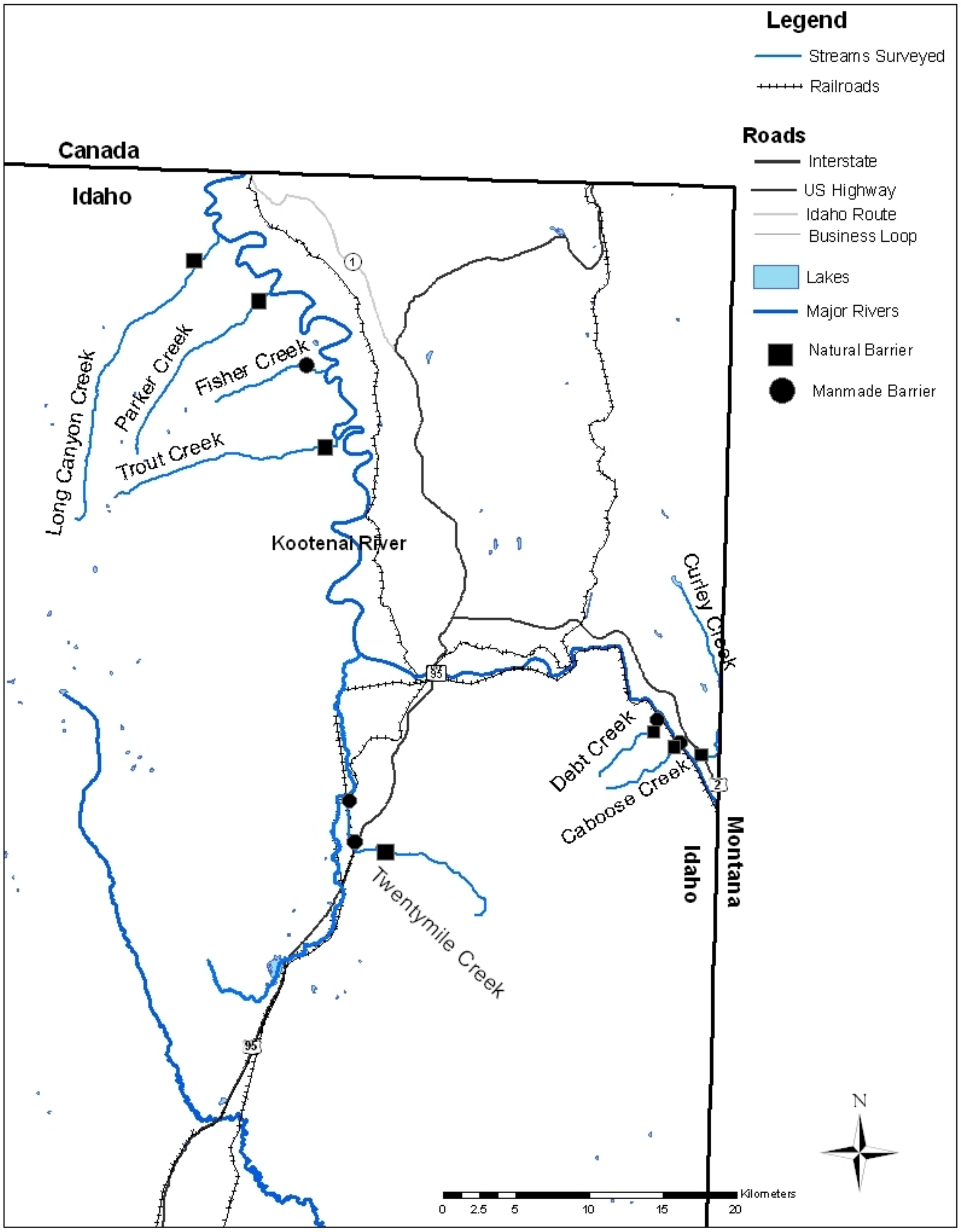

Figure 2. Location of stream barriers surveyed in 2008.

Once potential barriers were located, stream gradients, flow velocity, height of jumps, and the depth and size of plunge pools were measured to determine if the obstruction was a barrier to fish migration. We measured stream gradients with a hand-held Suunto inclinometer. Flow velocity was measured with a Marsh-McBirney flow meter. Data on potential fish barriers were entered in the software Fish Xing 3.0 to determine if it would be a barrier to fish migration.

RESULTS

Bull Trout Redd Surveys

Three bull trout redds were observed in the Callahan Creek drainage; all were observed in North Callahan Creek. A summary of bull trout redd counts for the Kootenai River drainage in Idaho since 2000 is presented in Table 1.

Redband Trout Population Monitoring

In fall 2007, 111 redband trout were collected during 9,836 s (2.73 h) of electrofishing effort on several standard sections of the Kootenai River from rkm 250 to rkm 275.5, for a total catch per unit effort of 41.4 fish/h. A summary of electrofishing CPUE data collected for redband trout since 2000 is provided in Appendix A. The redband trout proportional stock density (PSD) was 42 (95% CI = 10), while the quality stock density (QSD) was 4. Average relative weight values were 83 for the 201-305 mm length group, 81 for the 306-406 mm length group, and 84 for fish >406 mm. A summary of redband trout population metrics collected since 1993 is given in Appendix B.

Redband Trout Winter Telemetry

River Flows and Temperature

River flows fluctuated from 40 to 195 m^3/s during the redband trout telemetry investigation. Flow fluctuations during load following operations changed river volumes as much as 392 m^3/s from one telemetry interval to the next (Figure 3). The flows peaked and decreased nine times during the study period.

Temperature was influenced by flow volume and air temperature. During load following operations, the temperatures of Lake Koocanusa had a greater influence on river temperature in the study reach. At base flows from Libby Dam of about 113 m^3/s, water temperatures were greatly influenced by air temperature and during prolonged periods of cold weather such as the week of Jan 22 when air temperature dropped to -0.5°C (Figure 3).

Table 1. Numbers of bull trout redds in the Kootenai River drainage of Idaho, 2000 through 2007.

Stream	Year	Section start point description	Start point UTM coordinates[a]		Section end point description	End point UTM coordinates[a]		*Number of bull trout redds*
			Eastings	Northings		Eastings	Northings	
Boulder Cr.	2000	mouth	569849	5386164	waterfalls 1.9 km upstr.	568641	5385028	*0*
Boulder Cr.	2001	mouth	569849	5386164	waterfalls 1.9 km upstr.	568641	5385028	*2*
Boulder Cr.	2002	mouth	569849	5386164	waterfalls 1.9 km upstr.	568641	5385028	*2*
Boulder Cr.	2003	mouth	569849	5386164	waterfalls 1.9 km upstr.	568641	5385028	*0*
Boulder Cr.	2004	mouth	569849	5386164	waterfalls 1.9 km upstr.	568641	5385028	*0*
Boulder Cr.	2005	mouth	569849	5386164	waterfalls 1.9 km upstr.	568641	5385028	*1*
Boulder Cr.	2006	mouth	569849	5386164	waterfalls 1.9 km upstr.	568641	5385028	*0*
Boulder Cr.	2007	mouth	569849	5386164	waterfalls 1.9 km upstr.	568641	5385028	*0*
N. Callahan Cr.	2002	100 m downstr. of Smith Cr.	569501	5365990	Waterfalls barrier	568218	5366538	*13*
N. Callahan Cr.	2003	Jill Cr., Montana[b]	570786[c]	5365340[c]	Waterfalls barrier	568218	5366538	*32*
N. Callahan Cr.	2004	Jill Cr., Montana	570786[c]	5365340[c]	Waterfalls barrier	568218	5366538	*17*
N. Callahan Cr.	2005	Jill Cr., Montana	570786[c]	5365340[c]	Waterfalls barrier	568218	5366538	*10*
N. Callahan Cr.	2006	Jill Cr., Montana	570786[c]	5365340[c]	Waterfalls barrier	568218	5366538	*29*
N. Callahan Cr.	2007	Jill Cr., Montana	570786[c]	5365340[c]	Waterfalls barrier	568218	5366538	*3*
S. Callahan Cr.	2002	bridge on forest rd. 4554	570596	5362719	Forest Rd. 414 bridge (trailhead #154)	566519	5361191	*3*[e]
S. Callahan Cr.	2003	bridge on forest rd. 4554	570596	5362719	Forest Rd. 414 bridge (trailhead #154)	567347	5360822	*10*
S. Callahan Cr.	2004	bridge on forest rd. 4554	570596	5362719	Forest Rd. 414 bridge (trailhead #154)[d]	567347	5360822	*8*
S. Callahan Cr.	2005	bridge on forest rd. 4554	570596	5362719	Forest Rd. 414 bridge (trailhead #154)	567347	5360822	*5*
S. Callahan Cr.	2006	bridge on forest rd. 4554	570596	5362719	Forest Rd. 414 bridge (trailhead #154)	567347	5360822	*4*
S. Callahan Cr.	*2007*	*bridge on forest rd. 4554*	*570596*	*5362719*	*Forest Rd. 414 bridge (trailhead #154)*	*567347*	*5360822*	*0*

[a] UTM Zone 11; WGS84 datum.
[b] On 9/24/2003 the section from approximately 500 m downstream of Jill Creek upstream to Jill Creek was also surveyed, but no redds were seen.
[c] Estimated from electronic version of topographic map.
[d] S. Callahan was also surveyed from the Forest Road 414 bridge upstream approximately 500 m, but no redds were seen.
[e] One additional redd was found within 0.9 km upstream of Forest Road 414 bridge.

Radio Tagging Redband Trout

From September 27, 2007 through December 11, 2007, we captured 27 redband trout and implanted radio and ultrasonic transmitters in each. Twenty-one redband trout were captured in the canyon reach of the Kootenai River using electrofishing gear and six redband trout were captured by angling in the braided reach. Mean length for redband trout implanted with radio transmitters was 311 mm TL (range = 219–482 mm TL; SD = 64.7) and mean weight was 320 g (range = 107-921 g; SD = 212.8). Mean length for fish implanted with ultrasonic transmitters was 213 mm TL (range = 212-215 mm TL; SD = 0.7) and mean weight was 82 g (range = 74-87; SD = 5.7). Our radio transmitters had an expected life range of 70 to 502 d; however, most transmitters that expired during the study period lasted at least one month longer than the expected life.

Redband Trout Winter Telemetry

We successfully monitored 22 redband trout from 10/10/07 to 4/15/08 for a total of 1,191 contacts. Individual contacts through telemetry for redband trout ranged from 33 to 73 (Table 2). In most cases, the distance fish moved was not associated with changes in flow volume. Most fish did not move far or frequently throughout the winter and tended to stay in one location for weeks at a time. R^2 values for fish movement plotted with flow volume changes were not above 0.2 for any of the fish, but the fish with the highest r^2 value did make obvious movements when flows changed.

The proportion of fish using pool habitats relative to runs, riffles, and eddies increased as temperatures dropped in October and November (Figures 4 and 5). River temperatures stayed below 6°C for the remainder of the study period. Temperatures began to steadily increase in March but remained below 6°C until mid-April. Fish found in pools and runs moved to slightly faster water and could be seen feeding on mature insects on the water surface in mid-March.

In fall and early spring redband trout showed a more uniform distribution between habitat types; during winter, redband trout moved to slow runs and pool habitats (Figure 5). At the start of the study in October, fish were found in runs, riffles, and eddies. Most fish made gradual movements into large deep pools or slow sections of runs and stayed there for most of the winter making small movements within the pool or run.

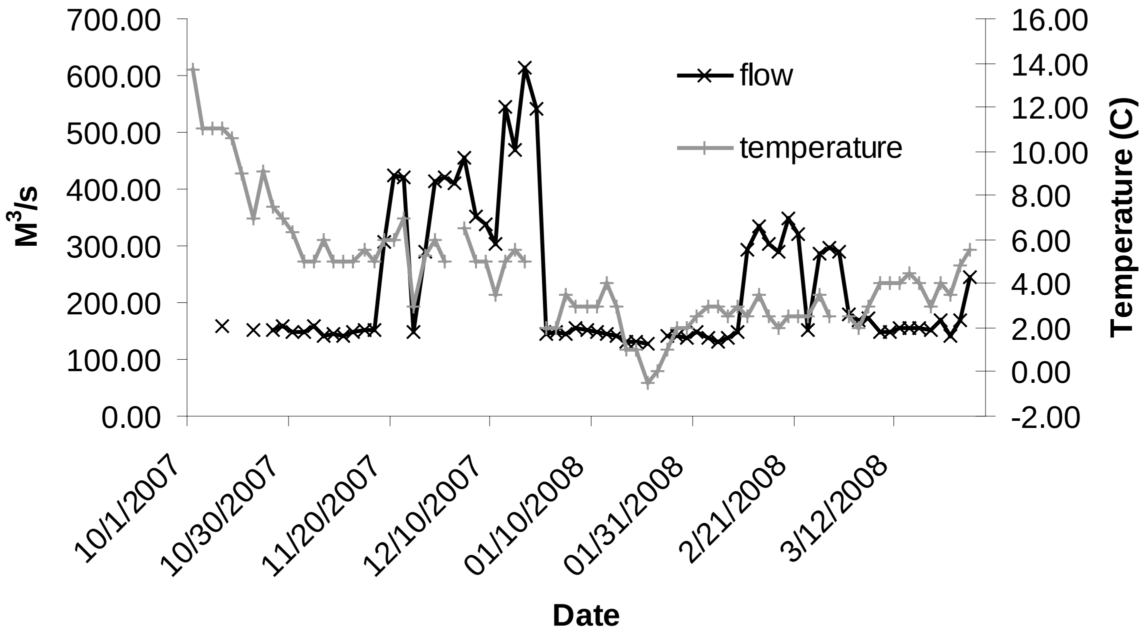

Figure 3. Mean daily temperature (°C) and flow (m^3/s) for the Kootenai River at the Leonia gauging station.

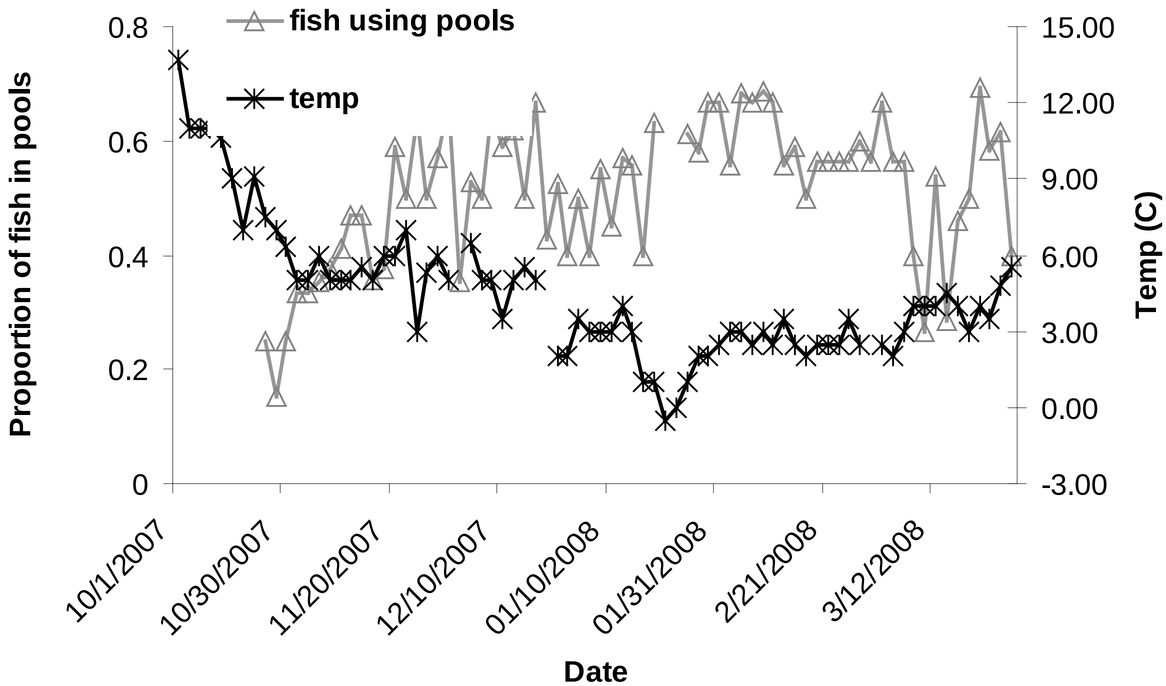

Figure 4. Mean daily temperature (°C) and the proportion of radio tagged redband trout in pool habitat.

Table 2. Redband trout movements and their relation to changes in volume.

Radio tag frequency	r^2 value	Movements during flow ramping	Total movements	Total observations
30.010	0.00006	3	9	*35*
30.030	0.05	1	3	*33*
30.050	0.005	0	2	*46*
30.060	0.005	3	12	*47*
30.110	0.009	5	11	*35*
30.120[a]	0.17	1	2	*65*
30.130	0.037	2	10	*67*
30.140	0.023	4	12	*43*
30.150	0.035	4	10	*42*
30.160	0.002	0	6	*43*
30.180	0.014	2	7	*41*
30.260	0.022	4	9	*60*
30.270	0.023	3	14	*61*
30.471	0.013	2	8	*72*
30.542	0.013	5	13	*71*
30.562	0.086	5	13	*73*
30.592[a]	0.193	6	13	*67*
30.621	0.001	2	13	*68*
30.682	8.00E-06	6	8	*34*
30.692	0.019	3	12	*65*
30.732	0.001	3	13	*45*
30.701	*0.001*	*3*	*6*	*73*

[a] This redband trout showed obvious movements associated with flow ramping operations.

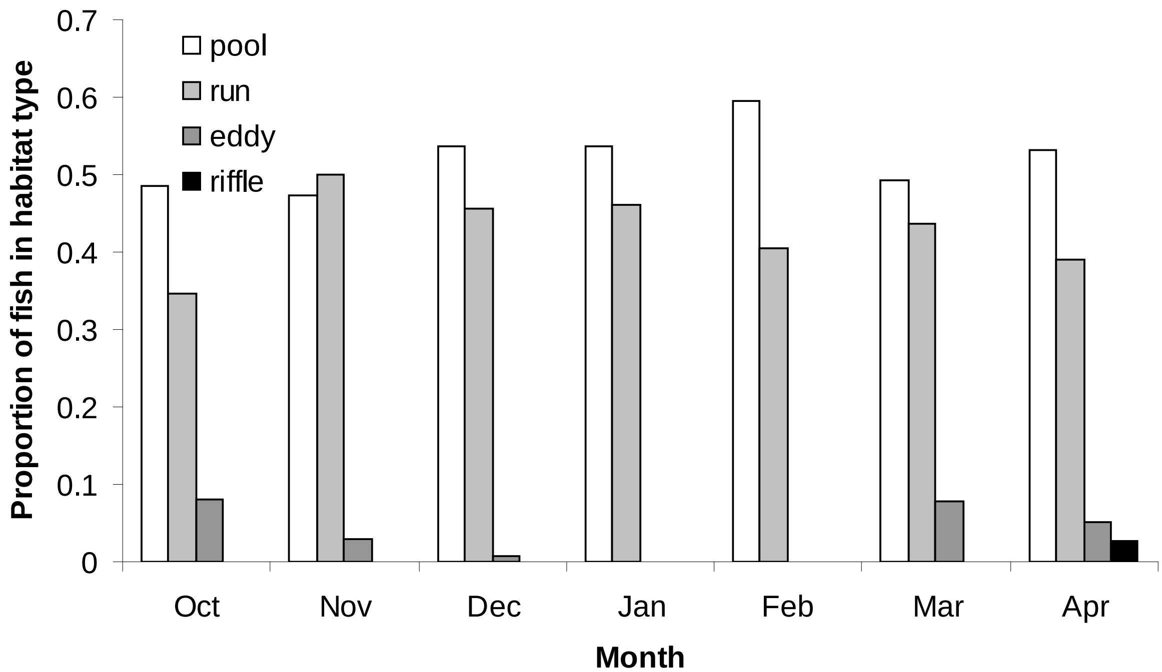

Figure 5. Monthly proportion of redband trout found in pool, run, eddy, and riffle habitat.

Redband trout did not move far during load-following operations even though in most cases flow fluctuated rapidly. Base flows were defined as volumes less than 168 m^3/s. Flows fluctuated mildly due to precipitation and temperature, but when flow volumes exceeded 168 m^3/s it occurred during load-following operations (Figure 6). Typically fish only moved as far as the nearest cover to find lower velocities and suitable habitat during flow load following. Preliminary results showed two of the 22 fish made frequent movements over 20 meters when flows changed. These two fish occupied areas where the habitat changed from pool to fast run when volumes increased. When this occurred, they moved to refuge areas as far as 1 km away, with lower velocities and woody cover. As flows decreased these refuge sites were dewatered and these two fish moved back to their previous locations. RBT living in pools were able to easily find lower velocities even when volumes where high. Preliminary results showed that velocities within the FL in pool habitats remained low even when flow volumes increased by 300% (Figures 7 and 8). Fish living in runs showed some increase in velocity with higher volumes in early spring but fish in run habitats were also able to find refuge as volumes increased.

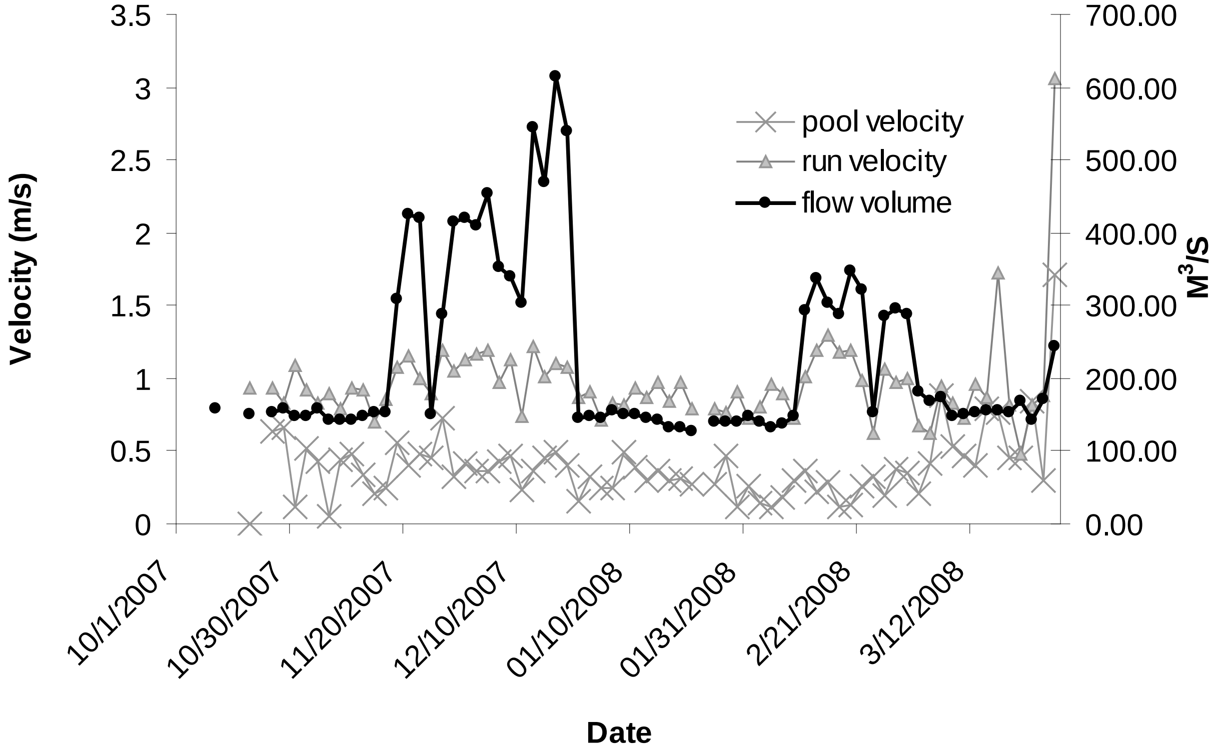

Figure 6. Measured average velocities as related to flow volume for redband trout locations in pools and runs.

The most common substrates observed within the FL were cobble, boulder, gravel, and sand, respectively (Figure 7). Substrate was classified as unknown in some deep pools and when substrate was not visible due to turbidity. With higher flows it became more difficult to visually measure substrate and the proportion of unknown substrate increased.

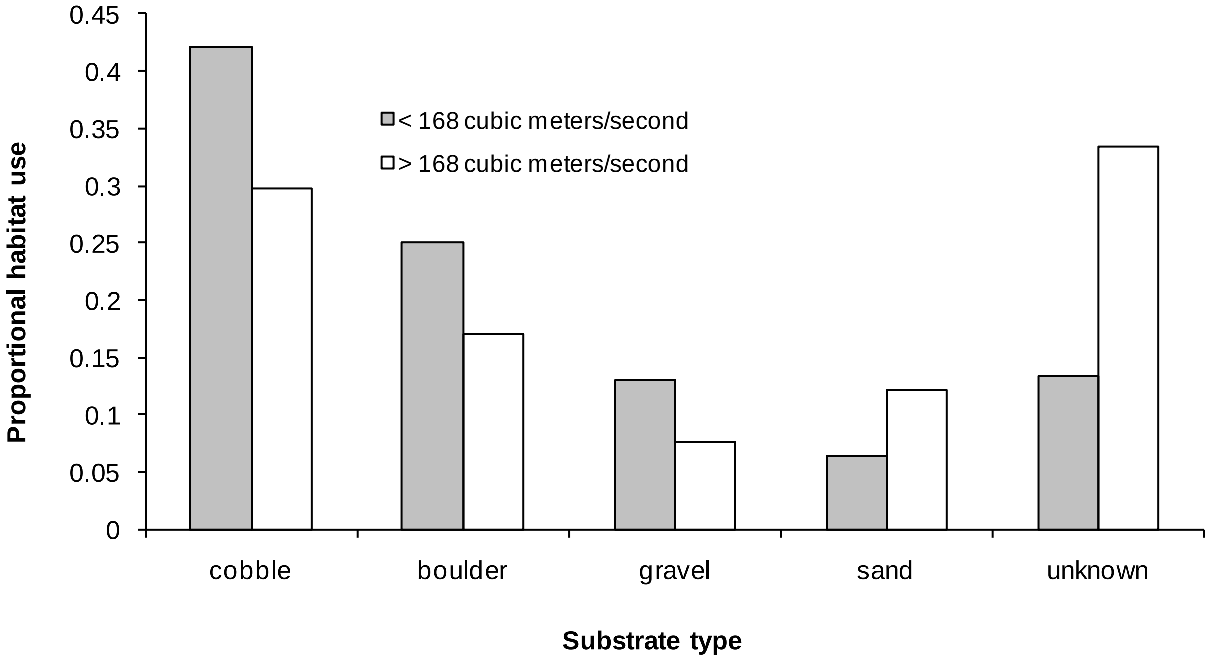

Figure 7. The dominant substrate at fish locations at high and low flow volumes.

The most common cover types measured within the FL were depth, coarse woody debris (cwd), boulder, and ice, respectively (Figure 8). There were no significant differences in cover use or preference at flows less than 168 m^3/s or flow volumes exceeding 168 m^3/s. Shelf ice was used as cover when it was available. Shelf ice only occurred at flow volumes less than 168 m^3/s and during the last week of January 2008 when water temperatures dropped below zero degrees C.

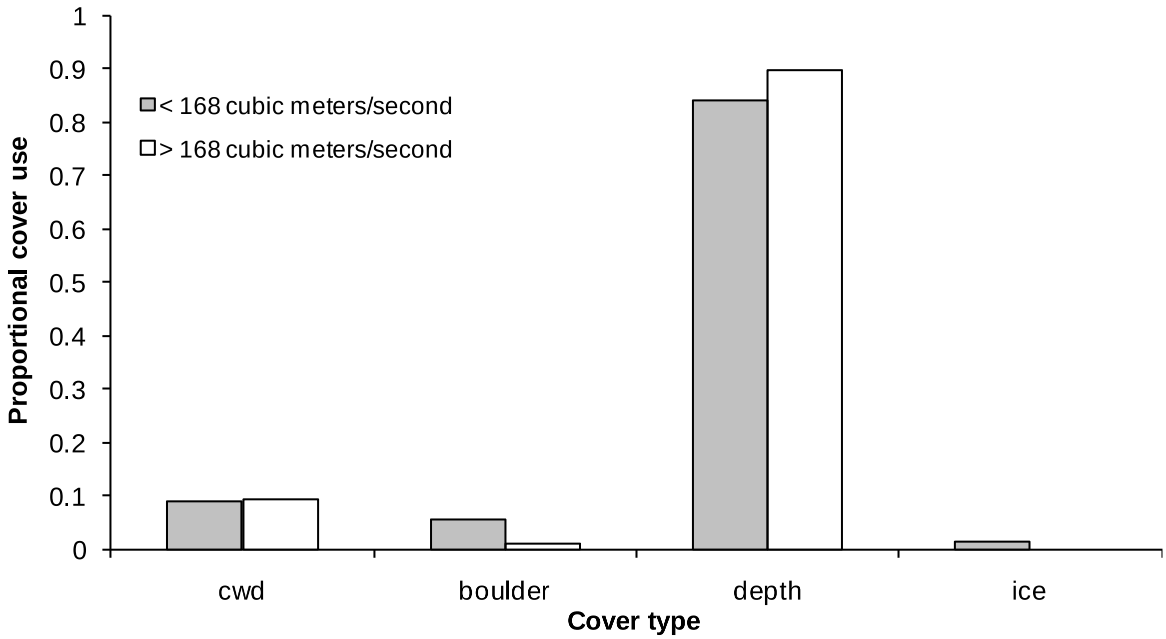

Figure 8. Cover type at fish locations at high and low flow volumes.

Twentymile Creek

Redd Surveys and Redd Measurements

We conducted 13 redd surveys and counted 44 redband trout redds from May 7 to June 3, 2007 in the 3.8 km survey reach on Twentymile Creek (Figure 2). We measured four redds to classify spawning habitat for our habitat measurements. Redds measured were located within 2 m of overhead cover and in water greater than 19 cm deep. Velocities taken at the pit and tail of the redds varied from 6 to 54 cm/s. Velocities measured at the tail of redds were equal to or greater than velocities at the pit. Dominant spawning substrate in redd tailspill was gravel and secondary tailspill substrate was sand and cobble. Redds varied from 1.10 to 2.05 meters in length and 0.63 to 0.88 m wide. The average area of a redband trout redd on Twentymile Creek was 0.94 m^2.

Habitat Measurements

We measured 60 transects with 453 sections from the Highway 95 bridge to 3.5 km upstream, the first major migration barrier, from April 4 to May 29, 2007. Of the 453 sections, 14 matched the criteria for spawning habitat. The total wetted area of the 3.5 km stream reach sampled was 15,554 m^2. Total exploited spawning habitat was 41 m^2 and total spawning habitat was 381 m^2. Total available spawning habitat was 340 m^2. We did not find spawning habitat to be limiting the recruitment in Twentymile Creek.

Barrier Surveys

Caboose Creek

Caboose Creek is located on the south side of the Kootenai River 24 km upstream of Bonners Ferry and was surveyed on June 17, 2008. The creek flows through two smooth, 14.5 m long by 1 m diameter, concrete culverts under a set of railroad tracks 34 m upstream from its confluence with the Kootenai River (Figure 2). Slopes within the culverts were 7% and 9%, with flows reaching 1.73 and 1.63 m/s at the lower end of the culverts. The downstream end of the culverts was perched 0.6 and 0.8 m above a plunge pool. We estimated that both culverts would be velocity barriers when they were measured at the end of the trout spawning season.

If these culverts were modified to allow fish passage, fish could access a maximum of 253 m of moderate to high gradient stream (6-12% slope) with some spawning gravels until they reached a 30 meter boulder cascade that would be a migration barrier to redband trout.

Caboose Creek has 34 m of stream accessible to migratory redband trout downstream of the railroad culverts. However, during low flow periods in summer the stream moves subsurface through a cobble delta that built up at its confluence with the Kootenai River.

Debt Creek

Debt Creek is on the south side of the Kootenai River 22 km upstream of Bonners Ferry and was surveyed on June 18, 2008 (Figure 2). The creek flows through two 13.7 m long by 1 m diameter smooth concrete culverts under a set of railroad tracks. The downstream ends of the culverts are perched 0.45 m and 0.2 m above a plunge pool. The culvert carrying most of the water (0.164 m^3/s) had flows of 1.84 m/s.

We believe the Debt Creek culverts were passable to redband trout at the lower volumes we sampled. However, we suspect these culverts would be barriers at the higher flows during spring run-off when redband trout migrate and during low flows due to insufficient water depth. If fish passage were improved, 546 meters of stream would be available upstream of the culverts. Stream gradients were between 3% and 8% for the first 400 meters above the culverts and from 6% to 16% from 400 meters upstream to a large barrier. We identified good spawning gravels and rearing habitat for young trout throughout this reach of stream.

There were 48 m of stream accessible to redband trout downstream of the railroad culverts when flows were high. At low summer flows, the stream moved subsurface through a cobble delta that built up at its confluence with the Kootenai River.

Curley Creek

Curley Creek is on the north side of the Kootenai River near the Idaho-Montana boarder and was surveyed on June 12, 2008 (Figure 2). This tributary had no culvert but only 50-75 meters of stream were accessible to migrating fish until they reach a series of boulder cascades and small waterfalls that would block further upstream migration for trout.

Trout Creek

Trout Creek is located west of the Kootenai River 32 km downstream of Bonners Ferry and was surveyed on July 22–29, 2008 (Figure 2). The creek's headwaters is at Pyramid Lake and the creek flows east through a steep drainage until it splits into two channels 1 km upstream of the Westside Road. Both forks then enter diked channels and flow through the valley until they reach the Kootenai River. We found a natural barrier 301 meters upstream of the split channel that appeared to be a barrier for redband trout. This barrier was a waterfall / chute barrier 5 m long and 1.6 m in height. Another chute type barrier with a slope of 22% and length of 3.5 meters was located 30 meters upstream of the first fish barrier. We snorkeled 200 meters of stream above and below the barriers to compare fish assemblages and determine if redband trout were present upstream of the barrier. Westslope cutthroat trout were present upstream and downstream of the barriers, but no redband trout were observed. We did see one bull trout, approximately 300 mm in length, in the plunge pool of the lower barrier. No bull trout were observed upstream of the barrier in the 200 m reach we surveyed. We found no man-made barrier to fish migrations from the West Side Road upstream to the natural barriers.

Fisher Creek

Fisher Creek is located west of the Kootenai River and 39 km downstream of Bonners Ferry. We surveyed this stream on July 14–22, 2008 (Figure 2). The creek flows from a high gradient incised canyon in the Selkirk Mountains down to the Kootenai Valley where it enters a diked channel for 1 km before entering the Kootenai River. We found one man-made barrier 535 meters upstream of the Westside Road. This barrier is a low head dam used to divert water used by the Anheuser-Busch hops farm. It creates a 2.4 meter waterfall with a shallow plunge pool that is impassible to the upstream migration of fish. Fifty meters upstream of this barrier there are a series of waterfalls that exceed 3 m in height and are complete barriers to fish passage. We snorkeled 50 m of stream above the low head dam and 100 m of stream downstream of the dam. Westslope cutthroat trout were seen in both sections.

Parker Creek

Parker Creek is located north of Fisher Creek, 54 km downstream of Bonners Ferry. We surveyed Parker Creek on July 31, 2008 (Figure 2). The stream flows east through a remote incised canyon in a heavily timbered drainage in the Selkirk Mountains. The creek enters the Kootenai Valley and is confined to a diked channel for 1.2 km to its confluence with the Kootenai River. From the valley floor to the West Side Road the creek has gradients greater than 10% for the first 0.5 km. There are numerous small waterfalls that may be barriers to juvenile fish moving upstream and likely discourage spawning movements of adult trout. We surveyed 275 m of stream until we reached a steep chute type barrier with velocities and length exceeding the maximum burst speed and leaping ability for redband trout.

Long Canyon Creek

Long Canyon Creek is located north of Parker Creek, 66 km downstream of Bonners Ferry and was surveyed on July 31, 2008 (Figure 8). The creek flows out of the Selkirk Mountains into the Kootenai Valley where it enters a diked channel for 1.3 km before its confluence with the Kootenai River. We walked for 5 km upstream of the West Side Road and found no man-made or permanent geologic structures that prevent fish passage on this stream. The first potential major barrier was 1.7 km upstream of the west side road. We snorkeled between the road and this barrier and found redband trout and mountain whitefish. From this barrier upstream to where the hiking trail nears the stream (5 km upstream of Westside Road), the stream is characterized by large boulder cascades, plunge pools, and an average gradient greater than 10%. Snorkeling upstream of the first barrier to the trail (1.7-5 km upstream of the West Side Road) we found redband trout, bull trout, and brook trout. This stream was of particular interest because we have found bull trout more than five kilometers upstream of the West Side Road.

Twentymile Creek

Twentymile Creek is a tributary to Deep Creek (Figure 1). There are two railroad culverts on Twentymile Creek that are barriers to fish at extremely high and low volumes. These culverts are both at railroad crossings. The lower partial barrier is 1.3 km upstream of the confluence of Twentymile Creek and Deep Creek and the other is .5 km downstream of the Highway 95 bridge over Twentymile Creek. Each culvert is constructed of galvanized steel with 5 cm corrugations and has plunge pool depths of slightly greater than one meter. The lowest culvert is 36 meters in length and 2 meters in diameter. The lowest culvert has a gradient of 2% a falls height of 20 cm and poses the greatest barrier to fish passage. The uppermost railroad culvert is 29 meters long, 3 m in diameter, has a 1% slope, and a falls height of 10 cm. There is some cobble and gravel substrate within the upper culvert. Both culverts are passed in the spring by migrating adult redband trout but could be improved for upstream juvenile fish passage and adult upstream passage at the highest and lowest runoff conditions.

The culvert where Twentymile Creek goes under State Highway 95 is also a barrier to upstream movements of fish during summer (Figure 5). This culvert contains a set of wooden baffles to aid fish passage, but is nonfunctional during low flows.

DISCUSSION

<u>Trout Redd Counts</u>

Bull Trout Redd Counts

The 2007 bull trout redd count for the Callahan Creek drainage, a total of three, was the lowest recorded since counts began in 2002. However, the count for South Callahan Creek in 2006 decreased for the third year in a row following the high count of 10 in 2003. The highest bull trout redd counts were in North Callahan creek in 2003 with 32 while the 2006 count for the North Callahan was the second highest at 29. The estimated number of adults ranged from zero for many years to 102 in North Callahan in 2006 and 93 in 2006 while for the high years in South Callahan the estimate was 32 in 2003 and 26 in 2004. No redds have been documented in Boulder Creek with the exception of 2005 when one redd was discovered for an estimate of three adults that year.

Redband Trout Redd Surveys

Redband trout redd surveys conducted in 2007 on Twentymile Creek indicated the period of peak spawning in Twentymile Creek and set an initial index value in order to monitor trends for the next few years. If redd numbers increase significantly when the 2007 young-of-the-year mature then it may likely be due to better recruitment facilitated by fish passage improvements made by removal of the culvert under the county road.

Our habitat survey on Twentymile Creek indicated more available spawning redband trout habitat than fish were using. Our results showed spawning habitat is not limiting redband trout recruitment in Twentymile Creek. The two railroad culverts downstream of the survey reach may be migration barriers to adults during peak runoff, which coincides with their migration up Twentymile Creek. Juvenile rearing habitat could also limit recruitment in Twentymile Creek especially during the dry summer months of July and August. A low head dam located upstream of our study reach is used to divert water for the nearby town of Naples; as a result, instream flow drops significantly during dry periods from July-August most years. This problem is likely compounded by the presence of natural and man-made barriers to juvenile fish passage. Although the nearest upstream migration barrier to fish passage is natural, juvenile fish would likely benefit from removal or modification of two railroad culverts downstream of the study reach.

Redband Trout Winter Telemetry

Preliminary results showed stream morphology appeared to affect a limited number of redband trout movements during flow ramping episodes from Libby Dam. When water volume drastically changed habitat types redband moved to sites with more favorable winter habitat conditions. *Such was the case for the two fish that made regular movements as flows changed.* In fall and winter, redband trout seek out pool habitats with low water velocities adjacent to faster water that could deliver drifting aquatic insects (Simpkins and Hubert 2000). When flow volumes change, some of these microhabitats also change and fish must move to find areas with slower water velocities where they can also find food. In areas with steep banks and large substrate, fish did not need to move far to find lower velocities and cover. In areas with small substrate offering no cover from velocity and gently sloping banks and streambed, fish had to travel greater distances to find slower water and cover as flows changed. Redband trout located in areas with smaller substrate would move to areas with boulders and course woody debris when river flows increased velocities at their previous locations. This type of habitat was most common downstream of the Moyie River and upstream of Bonners Ferry in the braided reach. Upstream of the Moyie River confluence to the Montana State line the river is dominated by steep banks, deep pools, and slow runs. Fish in this section of river did not move as far or as frequently as those located in shallow habitat types with cobble and gravel substrate. Changes in volume increased velocities in run and riffle habitats but did not seem to affect velocities at most fish locations. This indicates that fish were able to find areas with lower velocities even during significant increases to volume in most areas of the Kootenai River.

Redband Trout Stock Status

Redband trout PSD values have varied widely since 1998. However, since implementation of the creel and length limit they have averaged slightly higher for PSD and QSD for pre- and post-regulation changes, 38 and 41 and 2.5 and 4.0, respectively, indicating that size structure and abundance have stabilized under the 406 mm length limit initiated in 2002 (Walters 2005). Monitoring of the redband trout population structure should continue. In addition, the Kootenai River nutrient restoration project was initiated in 2005 and may also improve the redband trout standing stock.

Nutrient restoration of the Kootenai River, Idaho was implemented in 2005 and may increase redband trout survival and densities. An average of 5,300 age-0 redband trout out-migrate to the canyon reach of the Kootenai River each year from tributary streams in Idaho (Walters et al. 2005). Little is known about the ecology of these juvenile fish, but if nutrient restoration increases available food, juvenile survival would likely increase with higher growth rates and body condition. Improved growth rates and condition of redband trout may also result in a younger age at maturity and higher fecundity rates. Monitoring of the redband population to further evaluate population densities should continue in order to assess the success of the nutrient restoration program.

Barrier Surveys

Walters (2006) found that redband trout densities in the Kootenai River were low and stated that this was at least partly due to limited recruitment. Removing barriers on tributary streams is one approach to increasing trout densities. Even small creeks like Caboose and Debt creeks produced out-migrating redband trout in 2000, although none were found in 2001 (Walters 2002). Walters (2003) stated that redband trout spawning in tributaries upstream of Bonners Ferry are mainly fluvial fish from the Kootenai River. Thus improving spawning habitat in streams above Bonners Ferry may be more beneficial to the trout fishery in Idaho. Thus, Debt Creek and Caboose Creek may have added importance.

Deep Creek is a large tributary stream used by trout for spawning and rearing. However, mid-summer temperatures in Deep Creek may exceed 24°C at some locations (Walters 2005). Improving fish passage on Twentymile Creek (a tributary to Deep Creek) could allow juvenile trout to move upstream into areas with more suitable temperatures.

Fisher Creek has a man-made diversion dam on it. Providing fish passage past this dam would only increase trout habitat by about 50 m and would not be cost effective. Curley Creek, Trout Creek, Fisher Creek, Parker Creek, and Long Canyon Creek did not contain man-made barriers.

RECOMMENDATIONS

1. Continue annual bull trout redd surveys on index reaches of North and South Callahan creeks and Boulder Creek. This will allow construction of a time series to determine the bull trout population trend.

2. Maintain the current harvest regulations for redband trout (406 mm [16”] minimum length and two fish creel limit) and continue monitoring redband trout population statistics. Continued monitoring of redband trout population statistics will provide information necessary to determine changes due to nutrient restoration or regulation changes.

3. Continue winter telemetry of redband trout to further determine the effect of load following from Libby Dam on movement, habitat selection, and behavior.

4. We recommend having a qualified engineering firm develop options and cost estimates for the barriers on Twentymile, Debt, and Caboose creeks. Options can then be presented to the railroad company for improvement or proposed as possible future projects for mitigation for Libby Dam through the Bonneville Power Administration.

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APPENDICES

Appendix A. Redband trout catch per unit effort (CPUE) by electrofishing in the fall, Kootenai River, Idaho.

Length group (mm)	2000 CPUE (n/h)	2001 CPUE (n/h)	2002 CPUE (n/h)	2003 CPUE (n/h)	2004 CPUE (n/h)	2005 CPUE (n/h)	2006 CPUE (n/h)	*2007 CPUE (n/h)*
<= 199	7.9	3.8	7.0	3.8	4.3	4.1	4.2	*1.8*
200-305	12.7	10.5	6.5	7.0	18.1	19.8	16.7	*22.7*
306-406	7.9	3.4	7.8	7.6	7.2	7.0	7.8	*14.6*
>406	0.4	0.0	0.3	1.0	0.0	1.0	0.2	*1.5*
all	*28.8*[a]	*17.7*	*21.5*[a]	*19.3*	*29.6*	*31.9*	*29.0*	*41.4*

[a] Differs from column total due to rounding error.

Appendix B. Summary of population statistics for redband trout sampled by electrofishing during fall in the Kootenai River (rkm 250 to rkm 275), including proportional (PSD) and quality (QSD) stock densities.

Year	Population estimate	Lower 95% C. L.	Upper 95% C. L.	n/ha	n/km	PSD	PSD ± 95% CI	QSD	QSD ± 95% CI[a]	Relative weights 201-305 mm	Relative weights 306-406 mm	Relative weights >406 mm
1993	98	78	118	3.3	33	—	—	—	—	—	—	—
1994	135	114	160	4.6	45	—	—	—	—	—	—	—
1998	217	168	294	7.4	72	42	12	5	—	85	83	83
1999	217	160	332	7.4	72	47	13	3	—	95	86	81
2000	—	—	—	—	—	39	15	2	—	86	79	82
2001	—	—	—	—	—	24	22	0	—	83	80	—
2002	—	—	—	—	—	55	15	2	—	83	80	96
2003	—	—	—	—	—	55	16	6	—	84	85	83
2004	335	190	800	11.4	112	35	9	7	5	86	85	—
2005	—	—	—	—	—	29	10	4	5	89	83	84
2006	-	-	-	-	-	32	10	1	-[b]	90	84	76
2007	-	-	-	-	-	42	10	4	-	83	81	82

[a] Sample sizes were too small prior to 2004 to calculate confidence intervals for QSD.

CHAPTER TWO: AN ASSESSMENT OF HYBRIDIZATION BETWEEN ONCORHYNCHUS MYKISS (RAINBOW/REDBAND TROUT) AND WESTSLOPE CUTTHROAT TROUT IN THE KOOTENAI RIVER DRAINAGE, IDAHO

ABSTRACT

This project describes research assessing hybridization between *Oncorhynchus mykiss* (rainbow/redband trout) and westslope cutthroat trout in the Kootenai River drainage in Idaho. This research is timely given that the Endangered Species Act (ESA) status of westslope cutthroat trout is still in litigation. In total, 1,263 samples of *Oncorhynchus sp.* sampled from approximately 45 tributaries within the Kootenai River drainage were screened with seven codominant nuclear DNA markers and a mitochondrial DNA marker diagnostic between *O. mykiss* and cutthroat trout. In addition, all samples identified as *O. mykiss* were screened with five, recently developed, single nucleotide polymorphism markers that yield diagnostic allele frequency differences between native redband trout and nonnative hatchery rainbow trout. Results reveal a complicated picture of species composition and hybridization patterns throughout the drainage, which have likely been influenced by nonnative hatchery trout introductions, natural and man-made diversions, and species-specific habitat preferences. Results from this study should assist managers currently working on an updated status review for westslope cutthroat trout in Idaho.

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INTRODUCTION

Relatively more research has been conducted on native salmonids in the Kootenai River mainstem, while less is known about salmonid distribution and genetic integrity in the rest of the drainage in Idaho, including several streams with no documented surveys (Kootenai Tribe of Idaho and Montana Fish, Wildlife & Parks 2004). Redband trout are native to the Kootenai River drainage and were petitioned for listing under ESA. Recent research in the upper Kootenay River basin in British Columbia, Canada suggests that redband trout X westslope cutthroat trout hybridization is increasing and that hybrid swarms are likely to develop (Rubidge and Taylor 2005). In Idaho, westslope cutthroat trout *O. clarkii lewisi* were likely native to many Kootenai River tributaries that were inaccessible to redband trout due to upstream migration barriers. However, nonnative rainbow trout of hatchery strains were stocked into some of those tributaries and in headwater lakes (http://fishandgame.idaho.gov/apps/stocking/year.cfm?region=1). Genetically pure westslope cutthroat trout populations were recently found in four streams in the Moyie River drainage and in Mission Creek, while eight additional populations contained genetic introgression (introgression levels <3%) from redband trout (Walters 2006). Brook trout *Salvelinus fontinalis*, an introduced salmonid, also occur in the Kootenai River drainage in Idaho, but their distribution and numbers are not well documented. Identifying the distribution, abundance, and genetic integrity of salmonid species within the Kootenai River drainage in Idaho will aid in identifying future threats to these populations and in providing management direction. It will also provide information necessary to better manage for pure native stocks and reduce introgression.

Although the U.S. Fish and Wildlife Service (USFWS) determined on August 7, 2003 that listing of the westslope cutthroat trout as a threatened or endangered species under the Act was not warranted (USFWS 2003), the finding remains under litigation. The primary argument in appeals to the ruling has been that the USFWS inappropriately included hybridized populations as westslope cutthroat trout in the unit considered for listing. Much of the current legal and scientific debate is focused on how hybridization/introgression levels should be assessed (morphologically versus genetically) and whether any level of hybridization/introgression should preclude a population from ESA consideration.

In response to this ongoing litigation and a management need, genetically characterizing populations across the species' range remains a high priority for both state and federal agencies that oversee management and conservation of the species. In 2006, the Idaho Department of Fish and Game (IDFG) genetically screened 741 *Oncorhynchus sp.* samples from 11 tributaries that flow into the mainstem Moyie River, two tributaries to the mainstem Kootenai River, and a 15 mile stretch of the mainstem Kootenai River between river miles 244 and 257 (Campbell and Kozfkay In Press). This report describes results from an additional 1263 samples collected primarily from tributaries to the mainstem Kootenai River. The sample locations chosen for screening are important because many have not been previously screened for hybridization/introgression or have not been screened recently. In addition, recent research in the upper Kootenay River Basin in Canada suggests that hybridization is increasing and that hybrid swarms are likely to develop (Rubidge and Taylor 2004).

METHODS

Sample Collection and DNA extraction

During 1998-2006, IDFG personnel collected ~2,000 *Oncorhynchus sp.* fin clips from 20 tributaries to the mainstem Kootenai River and 11 tributaries to the mainstem Moyie River (Figure 8). An attempt was made to sample fish at multiple sites within each tributary (low, medium, and high in the drainage) and a sample size goal of 30 per site was attempted, although many sites had less than 30. Fish were sampled regardless of phenotypic identification and size. Fin tissue was stored in 100%, nondenatured ethanol until DNA extraction. DNA was extracted using a Nexttec Genomic DNA Isolation Kit for Fish Tissue according to the manufacturer's instructions (www.nexttec.biz).

Diagnostic Interspecific nDNA and mtDNA Locus Amplification and Electrophoresis

All samples were screened with seven codominant nDNA markers (Occ34, Occ35, Occ36, Occ37, Occ38, Occ42, and OM55) diagnostic between *O. mykiss* and cutthroat trout (Ostberg and Rodriguez 2002; 2004). The OM55 locus is also diagnostic between westslope cutthroat trout and Yellowstone cutthroat trout. All seven loci were amplified together in one Polymerase Chain Reaction (PCR) amplification. Amplifications were performed in 10 µl reaction volumes consisting of 5 µl of QIAGEN Multiplex PCR Master Mix® (final concentration 1X), 1 µl of primer cocktail (all forward and reverse primers at 100 µM concentration combined together), 3 µl of DNase/RNase free water, and 1 µl of DNA template (varying concentrations). Amplification product was diluted by adding 1 µl of product to 40 µl of water. One µl of this dilution was then added to 0.35 µl of LIZ size standard and 30 µl of formamide, prior to electrophoresis on an ABI 3100 fragment analyzer (Applied Biosystems) and allele differentiation using GeneMapper® 3.5 software (Applied Biosystems). Reverse primer sequences, forward primer sequences with corresponding fluorescent labels, and allele sizes observed in cutthroat trout and *O. mykiss* are available from the authors upon request. All samples were also screened with one of two mitochondrial DNA (mtDNA) markers diagnostic between *O. mykiss* and cutthroat trout. Some samples were screened with a restriction fragment length polymorphism marker (mtDNA control region digested with the restriction enzyme *Rsa-I*). The mtDNA control region was amplified using primers S-phe (5'-GCTTTAGTTAAGCTACG-3') and P2 (5'-TGTTAAACCCCTAAACCAG-3'). The P2 primer was labeled with 6FAM. Amplifications were performed in 20 µl reactions consisting of 1.0 µl DNA extract (concentration unknown), 2.0 µl 10X buffer (Perkin Elmer), 2.0 µl MgCl2, 1.6 µl BSA, 2.0 µl of each primer, 1.6 µl 10.0 mM dNTPs (10mM each of dATP, dCTP, dGTP, and dTTP), 0.10 µl Taq polymerase (Perkin-Elmer), and 7.7 µl dH2O. Polymerase chain reaction conditions consisted of an initial denaturing cycle of 94°C for 1 minute and 30 seconds, followed by 39 cycles of denaturation at 94°C for 30 seconds, annealing at 52°C for 30 seconds, and extension at 72°C for 1 minute, with a final extension at 72°C for 10 minutes followed by a cool down at 4°C for 10 minutes. Following PCR, 50 µl of dH2O was added to each sample. This diluted amplification product was used for the digest. Following a 3 hour (minimum) digest with *Rsa-I*, 5 µl of this product was combined with 30 µl of formamide and 0.5 µl of LIZ size standard for electrophoresis on a 3100 fragment analyzer. Diagnostic fragments lengths are as follows: approximately 98 b.p. in Yellowstone cutthroat trout, 100 b.p. in *O. mykiss*, and 102 b.p. in westslope cutthroat trout. Samples not screened with this diagnostic RFLP marker were run with a new diagnostic mtDNA (Cytb region) single nucleotide polymorphism (SNP) taqman assay developed at Montana State University (skalinowski@montana.edu).

Diagnostic Intraspecific nDNA Locus Screening

To assess intraspecific hybridization (hybridization from nonnative hatchery rainbow trout) all samples exhibiting *O. mykiss* genotypes, following the screening described previously, were also screened with five additional nDNA SNP taqman assays (LDHBS, MYO2-111, PEPA, PROM2 and sSOD-1) and one additional mtDNA SNP taqman assay (EGL2) that exhibit diagnostic allele frequency differences between nonnative hatchery rainbow trout and native redband trout. The sSOD-1 assay, developed by Brunelli et al. (2008), identifies the single nucleotide polymorphism (SNP) difference responsible for the allozyme polymorphisms that have been used to differentiate inland redband trout and coastal rainbow trout for almost 30 years (Allendorf and Utter 1979, Knudsen et al. 2002). The remaining taqman assays were developed as part of a collaborative project with the Columbia River Tribal Fish Commission and U.C. Davis (In Prep). Information for running these assays is available from the authors upon request.

Interspecific and Intraspecific Hybrid Detection

Individual interspecific hybridization sample classification was based on composite nDNA and mtDNA genotypes following similar procedures as those outlined by Ostberg and Rodriquez (2006) and Kozfkay et al. (2007). It is important to note that although seven diagnostic, codominant nDNA loci provide sufficient power to detect very low levels of introgression within a population, the ability to detect introgression within any individual sample is limited. For example, 30 diagnostic, codominant nDNA loci would be needed to have 95% probability of detecting introgression within an individual if it were present at a frequency of 5% or greater. Keeping that caveat in mind, samples in this study were classified as "cutthroat trout" if they were homozygous for cutthroat trout alleles at all loci, "*O. mykiss*" if they were homozygous for *O. mykiss* alleles at all loci, and "hybrid" if they possessed a mixture of alleles from the two parental species. Hybrids were further classified into two categories: first-generation hybrids (F_1) if they were heterozygous at all loci, and later-generation hybrids (F_n) if they possessed a mix of heterozygous and homozygous loci. With seven codominant nDNA loci, our probability of mistaking a more advanced backcross hybrid ($>F_1$) as an F_1 hybrid is less than 1% (Boecklen and Howard 1997). Hybridization levels at each site were reported as the number of hybrids detected out of the total number of samples analyzed. Introgression levels at each site were reported in reference to the most frequent species observed in the sample. For example, if cutthroat trout were the most frequent species observed then introgression was reported as the number of *O. mykiss* alleles observed in fish classified as cutthroat trout-like and $>F_1$ hybrids out of the total alleles examined. Introgression is the actual incorporation of genes from one taxa into the population of another through hybridization and backcrossing (Kearney 2005). Therefore, alleles from fish classified as pure parental types (less frequent species) and F_1 hybrids were not included in introgression estimates. Assessments of intraspecific hybridization was accomplished subjectively by comparing allele frequencies at six diagnostic SNP assays observed at each site to allele frequencies observed in reference pure redband trout populations and reference hatchery rainbow trout populations (IDFG, unpublished data). Only sites with greater than 10 samples were evaluated for intraspecific hybridization.

RESULTS AND DISCUSSION

For the preceding results and summary discussion, we will begin with tributaries to the mainstem Kootenai River (moving from upstream to downstream sites). We will then follow this with a discussion of sites within the Moyie River drainage. The primary summary tables also follow this sequence with Kootenai River sites listed first (Table 3), followed by Moyie River sites (Table 4). Results for sites screened in 2006 are also presented in these tables for comparison purposes and at times are referred to in the text.

Kootenai River Tributaries

Three sites were sampled in the Callahan Creek drainage (South Fork, Middle, and Upper North Fork). There is no record of hatchery rainbow trout or cutthroat trout stocking in the drainage according to the IDFG historical stocking database (http://fishandgame.idaho.gov/apps/stocking/). Although numerous waterfalls exist within the Callahan Creek drainage, it is not believed that these act as complete barriers to fish movement between Callahan Creek and the mainstem Kootenai River (Knudsen et al. 2002). Callahan Creek has been described as the only stream flowing through Montana that provides spawning habitat for Kootenai River redband trout and previous allozyme analyses has indicated that redband trout populations in this drainage are pure (Muhlfeld 2003 and references therein). Our results support those previous studies for the most part, with samples from the two North Fork sites exhibiting no cutthroat trout alleles and SNP allele frequencies matching those observed in reference redband trout populations (Table 3). In contrast, results suggested that low levels of cutthroat trout and nonnative rainbow trout introgression might be present in South Fork Callahan Creek, although only a single cutthroat trout allele was observed in one sample (0.5% introgression) and SNP allele frequencies were still similar to those observed in pure reference redband populations.

Table 3. Sample sites from Kootenai River tributaries. Sample site and code, number of samples examined (N), number of samples genetically identified as cutthroat trout-like, *O. mykiss*-like, F_1 hybrid (with mtDNA lineage), and $>F_1$ hybrid (with mtDNA lineage). Total *O. mykiss* (RBT) and cutthroat trout (CUT) alleles identified, total alleles examined, % introgression (CUT or RBT, depending on dominant species at site), number of hybrids detected and % hybridization also shown. *Site not in HWE.

Sample Site	Code	N	Insuff. Loci	Cutthroat trout-like	O. mykiss-like	F1 Hybrid	mtDNA lineage RBT/CUT	>F1 Hybrid	mtDNA lineage RBT/CUT	RBT alleles identified	CUT alleles identified	Total alleles examined	% Intro-gression	Hybrids detected	*% Hybridi-zation*
N.F. Callahan Creek (middle)	NFA-M	27			27					378		378	CUT 0.0%		*0.0%*
N.F. Callahan Creek (upper)	NFA-U	10			10					140		140	CUT 0.0%		*0.0%*
S.F. Callahan Creek	SFA	15			14			1		209	1	210	CUT 0.5%	1	*6.7%*
Lower Boulder Creek	BOL	35			31			4	1/3	467	23	490	CUT 4.7%	4	*11.4%*
Middle Boulder Creek	BOM	56			19	5	1/4	32	24/8	579	199	778	CUT 23.3%	37	*66.1%*
Upper Boulder Creek	BOU	27		23				4	0/4	4	374	378	RBT 1.1%	4	*14.8%*
East Fork Boulder Creek	EFB	19	1		16			2	2/1	250	2	252	CUT 0.8%	2	*10.5%*
M. F. Boulder Cr. 2006 Site A	MFB06-A	12		1	1	2	1/1	8	6/2	90	78	168	N/A	10	*83.3%*
M. F. Boulder Cr. 2006 Site B	MFB06-B	38		1	13			24	21/3	403	127	530	CUT 21.9%	24	*63.2%*
Kootenai River @ Hemlock	KOT	60			44	5	2/3	11	8/3	790	47	840	CUT 1.6%	16	*26.7%*
Kootenai River 2006	KOT06	79	1	6	53	4		15	12/3	931	159	1090	CUT 4.9%*	19	*24.1%*
Upper Snow Creek-A	SNU-A	2		2							28	28	RBT 0.0%		*0.0%*
Upper Snow Creek-B	SNU-B	12		12							168	168	RBT 0.0%		*0.0%*
Upper Snow Creek-C	SNU-C	6	1	5							70	70	RBT 0.0%		*0.0%*
Middle Snow Creek	SNM	30	3	26				1	0/1	1	377	378	RBT 0.3%	1	*3.3%*
Caribou Creek	CAR	32		15		3	3/0	14	9/5	100	348	448	RBT 19.5%	17	*53.1%*
Lower Ruby Creek	RUL	25			25					350		350	CUT 0.0%		*0.0%*

Table 3. Continued.

Sample Site	Code	N	Insuff. Loci	Cutthroat trout-like	O. mykiss -like	F1 Hybrid	mtDNA lineage RBT/CUT	>F1 Hybrid	mtDNA lineage RBT/CUT	RBT alleles identified	CUT alleles identified	Total alleles examined	% Intro-gression	Hybrids detected	% Hybridi-zation
Middle Ruby Creek	RUM	16			16					224		224	CUT 0.0%		*0.0%*
Ruby Creek-3	RU3	2			2					28		28	CUT 0.0%		*0.0%*
Fall Creek-A	FAL-A	2			2					28		28	CUT 0.0%		*0.0%*
Fall Creek-B	FAL-B	4			4					56		56	CUT 0.0%		*0.0%*
Twentymile Creek	TWE	37			33			4	4/0	512	4	516	CUT 0.8%	4	*10.8%*
Deep Creek-A	DEP-A	6			6					84		84	CUT 0.0%		*0.0%*
Deep Creek-B	DEP-B	3			3					42		42	CUT 0.0%		*0.0%*
Myrtle Creek-A	MYR-A	8			8					112		112	CUT 0.0%		*0.0%*
Myrtle Creek-B	MYR-B	42			42					588		588	CUT 0.0%		*0.0%*
Lower Ball Creek	BAL	27		21	1			5	1/4	29	349	378	RBT 4.1%*	5	*18.5%*
Upper Ball Creek-A	BAU-A	40	2	35				3	0/3	3	529	532	RBT 0.6%	3	*7.5%*
Upper Ball Creek-B	BAU-B	10		10							140	140	RBT 0.0%		*0.0%*
Middle Trout Creek	TCM	26		25		1	0/1			7	357	364	RBT 0.0%	1	*3.8%*
Upper Trout Creek	TCU	24		24							336	336	RBT 0.0%		*0.0%*
Mission Creek	MIS	12		12							168	168	RBT 0.0%		*0.0%*
Mission Creek 2006-A	MIS06-A	26		26							362	362	RBT 0.0%		*0.0%*
Mission Creek 2006-B	MIS06-B	24	1	23							320	320	RBT 0.0%		*0.0%*
E. F. Mission Creek 2006-A	EFM06-A	21		21							278	278	RBT 0.0%		*0.0%*
E. F. Mission Creek 2006-B	EFM06-B	29		28				1	0/1	1	381	382	RBT 0.3%	1	*3.4%*
Brush Creek	BRU	1		1							14	14	RBT 0.0%		*0.0%*

Table 3. Continued.

Sample Site	Code	N	Insuff. Loci	Cutthroat trout-like	O. mykiss -like	F1 Hybrid	mtDNA lineage RBT/CUT	>F1 Hybrid	mtDNA lineage RBT/CUT	RBT alleles identified	CUT alleles identified	Total alleles examined	% Intro-gression	Hybrids detected	% Hybridi-zation
Fisher Creek	FIS	3						3	2/1	34	8	42	CUT 19.0%	3	*100.0%*
Long Canyon Creek	LON	44			44					616		616	CUT 0.0%		*0.0%*
Lower Cow Creek	COL-A	26		3		1	1/0	22	0/22	45	319	364	RBT 10.9%	23	*88.5%*
Lower Cow Creek B	COL-B	25		5				20	0/20	37	313	350	RBT 10.6%	20	*80.0%*
Lower Smith Creek	SML	38		8	1	3	1/2	26	14/12	206	321	530	N/A*	29	*76.3%*
Middle Smith Creek	SMM	20		13				7	0/7	9	271	280	RBT 3.2%	7	*35.0%*
Lower Beaver Creek	BEVL	40		39				1	0/1	1	559	560	RBT 0.2%	1	*2.5%*
Upper Beaver Creek	BEVU	2		2							28	28	RBT 0.0%		*0.0%*
Beaver Creek-2	BEV2	9		9							126	126	RBT 0.0%		*0.0%*
Cutoff Creek	CUT	50		50							700	700	RBT 0.0%		*0.0%*
Shorty Creek-1	SH1	4			4					56		56	CUT 0.0%		*0.0%*
Lower Shorty Creek	*SHL*	*48*	*3*		*45*					*638*		*638*	*CUT 0.0%*		*0.0%*

Table 4. Sample sites from Moyie River tributaries. Sample site and code, number of samples examined (N), number of samples genetically identified as cutthroat trout-like, *O. mykiss*-like, F_1 hybrid (with mtDNA lineage), and >F_1 hybrid (with mtDNA lineage). Total rainbow trout (RBT) and cutthroat trout (CUT) alleles identified, total alleles examined, % introgression (CUT or RBT, depending on dominant species at site), number of hybrids detected and % hybridization also shown. *Site not in HWE.

Sample Site	**Code**	**N**	**Insuff. Loci**	**Cutthroat trout-like**	**O. mykiss - like**	**F1 Hybrid**	**mtDNA lineage RBT/CUT**	**>F1 Hybrid**	**mtDNA lineage RBT/CUT**	**RBT alleles identified**	**CUT alleles identified**	**Total alleles examined**	**% Intro-gression**	**Hybrids detected**	***% Hybridi-zation***
Canuck Creek	CAN	40		40							560	560	RBT 0.0%		*0.0%*
Canuck Creek 2006-A	CAN06-A	25		25							350	350	RBT 0.0%		*0.0%*
Canuck Creek 2006-B	CAN06-B	25		25							350	350	RBT 0.0%		*0.0%*
Upper Deer Creek	DEU	31		25				6	0/6	6	428	434	RBT 1.4%	6	*19.4%*
Lower Deer Creek	DEL	19		1	3	6	2/4	9	5/4	157	107	264	N/A*	15	*78.9%*
Faro Creek	FAR	37		37							518	518	RBT 0.0%		*0.0%*
Faro Creek 2006-A	FAR06-A	23		20				3	0/3	4	280	284	RBT 1.4%	3	*13.0%*
Faro Creek 2006-B	FAR06-B	18		17				1	0/1	1	215	216	RBT 0.5%	1	*5.6%*
Unnamed Keno Trib.	UKEN	2		2							28	28	RBT 0.0%		*0.0%*
Keno Creek 2006-A	KEN06-A	20		17				3	2/1	10	258	268	RBT 3.7%	3	*15.0%*
Keno Creek 2006-B	KEN06-B	14		14							196	196	RBT 0.0%		*0.0%*
Keno Creek 2006-C	KEN06-C	15		15							210	210	RBT 0.0%		*0.0%*
Skin Creek	SKI	5		5							70	70	RBT 0.0%		*0.0%*
Skin Creek 2006-A	SKI06-A	33		33							440	440	RBT 0.0%		*0.0%*
Skin Creek 2006-B	SKI06-B	17		17							232	232	RBT 0.0%		*0.0%*
Moyie River (13 Sites)	MO1 (A-M)	204	2	3	196			3	0/3	2763	59	2822	CUT 0.6%*	3	*1.5%*
Kreist Creek 2006-A	KRI06-A	11	1	10							140	140	RBT 0.0%		*0.0%*

Table 4. Continued.

Sample Site	Code	N	Insuff. Loci	Cutthroat trout-like	O. mykiss - like	F1 Hybrid	mtDNA lineage RBT/CUT	>F1 Hybrid	mtDNA lineage RBT/CUT	RBT alleles identified	CUT alleles identified	Total alleles examined	% Intro-gression	Hybrids detected	% Hybridi-zation
Kreist Creek 2006-B	KRI06-B	24		20				4	0/4	4	320	324	RBT 1.2%	4	*16.7%*
Mill Creek 2006-A	MIL06-A	22		22							308	308	RBT 0.0%		*0.0%*
Mill Creek 2006-B	MIL06-B	28	1	27							380	380	RBT 0.0%		*0.0%*
Hellroaring Creek 2006-A	HEL06-A	35		32				3	0/3	5	463	468	RBT 1.1%	3	*8.6%*
Hellroaring Creek 2006-B	HEL06-B	15		14				1	0/1	1	175	176	RBT 0.6%	1	*6.7%*
Spruce Creek 2006-A	SPR06-A	9		4				5	0/5	13	99	112	RBT 11.6%	5	*55.6%*
Spruce Creek 2006-B	SPR06-B	40		36				4	0/4	4	538	542	RBT 0.7%	4	*10.0%*
Bussard Creek 2006	BUS06	36		31				5	0/5	5	499	504	RBT 1.0%	5	*13.9%*
Copper Creek 2006-A	COP06-A	30		30							420	420	RBT 0.0%		*0.0%*
Copper Creek 2006-B	COP06-B	20		20							280	280	RBT 0.0%		*0.0%*
Davis Creek 2006-A	DAV06-A	26		26							364	364	RBT 0.0%		*0.0%*
Davis Creek 2006-B	DAV06-B	24		24							336	336	RBT 0.0%		*0.0%*

Four sample sites were screened in the Boulder Creek drainage as part of this present study. Only *O. mykiss* (N = 31) and >F_1 hybrids (N = 4) were observed in Lower Boulder Creek and cutthroat trout introgression was 4.7% (Table 3). Similar to the results we observed in 2006, Middle Boulder Creek exhibited high levels of hybridization, with the sample containing primarily hybrids (5 F_1 and 32 >F_1) and no cutthroat trout. Cutthroat trout introgression was calculated as 23.3%. Despite the high levels of hybridization observed at Middle Boulder Creek, relatively low levels were observed higher up in the drainage and within the East Fork Boulder Creek. The upper Boulder Creek site contained primarily cutthroat trout (N = 23), no *O. mykiss*, and only 4 >F_1 hybrids. *O. mykiss* introgression was relatively low (1.1%). No apparent physical barrier exists between middle and upper Boulder Creek (Figure 9). In contrast to upper Boulder Creek, the East Fork Boulder Creek site contained primarily *O. mykiss* (N = 16), no cutthroat trout, and 2 >F_1 hybrids. Corresponding cutthroat trout introgression was also low 0.8%). A barrier does exist on lower East Fork Boulder Creek (Figure 9). All three sites (Lower-BOL, Middle-BOM, and E.F.-EFB) containing *O. mykiss* showed evidence of extensive coastal rainbow trout introgression, with all exhibiting SNP allele frequencies more similar to reference hatchery rainbow trout populations than to native reference redband trout populations (Table 5). Although no records were found indicating that the drainage has been stocked with hatchery rainbow trout, the observation of both inter- and intraspecific hybridization in Boulder Creek is consistent with previous allozyme analyses in the drainage (USFWS 1999).

The only samples that came from the mainstem Kootenai River, as part of this current study, were collected near Hemlock Bar (Figure 1). Of the 60 samples collected and screened, 44 were identified as *O. mykiss*, 5 as F_1 hybrids, and 11 as >F_1 hybrids. No cutthroat trout were identified. Cutthroat trout introgression was 1.6%. The 44 samples identified as *O. mykiss* exhibited SNP allele frequencies more similar to reference hatchery rainbow trout populations than to native reference redband trout populations (KOT, Table 5). The only stocking record for *O. mykiss* found for the mainstem Kootenai River was 100 Kamloop fingerlings in 1972 (IDFG historical stocking database).

A total of 13 sites were sampled and screened within the Deep Creek drainage. The two tributaries sampled in the lower part of the drainage (Snow Creek and Caribou Creek) contained primarily cutthroat trout as opposed to *O. mykiss*. Although Snow Creek was stocked with hatchery rainbow trout throughout much of the 1990s (IDFG historical stocking database), only a single *O. mykiss* allele was observed in one sample (0.5% introgression) at the four sample sites (N = 45). Caribou Creek exhibited significantly higher levels of *O. mykiss* hybridization and introgression, with over half the samples identified as hybrids (3 F_1, 14 >F_1). This result is consistent with past allozyme screening efforts (USFWS 1999), although results from both studies are somewhat surprising given that only a single stocking record is noted in the Caribou Creek drainage (Caribou Lake; IDFG historical stocking database) and barriers are present at the bottom of the drainage.

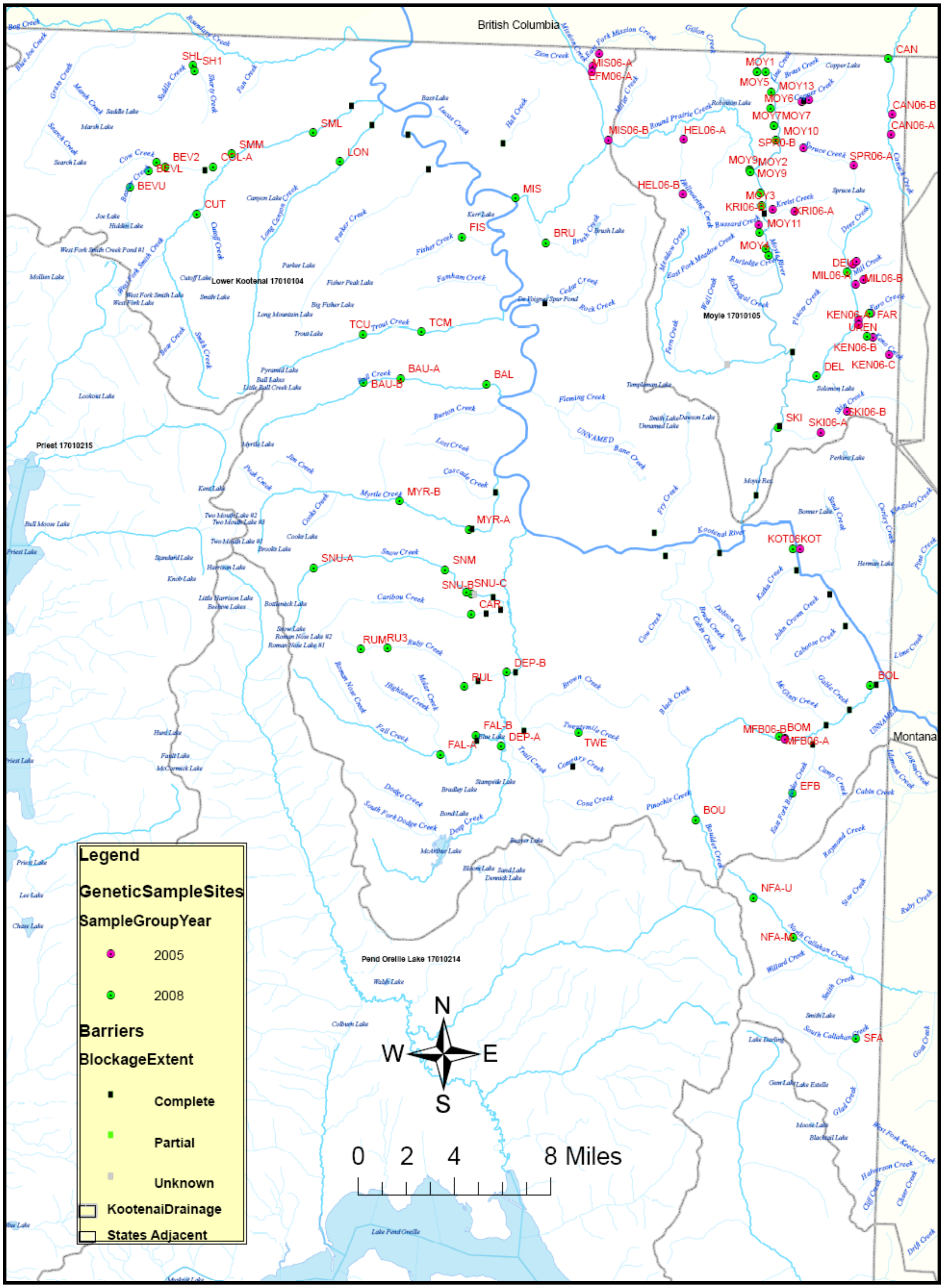

Figure 9. Sample sites from the Kootenai River drainage. Sites analyzed in 2006 are labeled with "06" in the site name.

Table 5. Diagnostic allele frequencies of reference redband trout populations, reference hatchery rainbow trout populations (Campbell et al. In Prep.), and study *O. mykiss gairdneri* populations (when sample size N > 10) for MYO211*G, PEPA*C, PROM2*T, LDHBS*T, and SOD-WSU*152.

									Reference Redband Populations								
LOCUS	**ALLELE**	**BENN**	**BIGJ**	**HATC**	**NFOW**	**SHAC**	**UPJE**	**UPRI**	**WOLF**	**DWOR**	**KOOT**	**UPJU**					
MYO211	G																
PEPA	C																
PROM2	T							0.033	0.017	0.056							
LDHBS	T					0.033				0.017							
SOD-WSU	152								0.017	0.017							
									Reference Hatchery Populations								
LOCUS	ALLELE	EAGL	FISH	HARD	HARR	HAY1	HAY2	ERWI	MCCO	SHAS	MTWH	MTLD	MTLH	ARLE	SHEA	SHEM	*HOFE*
MYO211	G	0.317	0.667	0.391	0.453	0.415	0.189	0.350	0.450	0.333	0.465	0.385	0.390	0.349	0.609	0.413	*0.212*
PEPA	C	0.167	0.147	0.250	0.348	0.213	0.322	0.450	0.150	0.283	0.209	0.050	0.360	0.307	0.360	0.370	*0.672*
PROM2	T	0.067	0.324	0.344	0.348	0.372	0.733	0.350	0.133	0.450	0.488	0.380	0.357	0.080	0.220	0.337	*0.061*
LDHBS	T	0.233	0.758	0.359	0.364	0.234	0.189	0.167	0.133	0.350	0.547	0.643	0.398	0.409	0.370	0.380	*0.652*
SOD-WSU	152	0.183	0.348	0.281	0.197	0.447	0.255	0.400	0.017	0.133	0.205	0.770	0.380	0.193	0.270	0.402	*0.394*
									Study O. mykiss populations								
LOCUS	ALLELE	BOL	BOM	EFB	KOT	MOY	MYR	RUL	RUM	LCAN	SHL	TWE	NFCM	NFCU	SFC		
MYO211	G	0.194	0.559	0.594	0.183	0.153	0.780	0.080	0.281	0.023							
PEPA	C	0.129	0.294	0.219	0.113	0.051	0.250	0.040	0.063	0.068					0.036		
PROM2	T	0.226	0.306	0.125	0.190	0.464	0.260	0.180	0.313	0.011		0.047			0.036		
LDHBS	T	0.258	0.250	0.344	0.159	0.481	0.240	0.080		0.045					0.036		
SOD-WSU	*152*	*0.048*		*0.031*	*0.134*	*0.312*	*0.200*	*0.042*	*0.750*	*0.091*					-		

None of the remaining tributaries sampled in the Deep Creek drainage (Ruby Creek, N = 43; Fall Creek, N = 6; Twentymile Creek, N = 37) and mainstem sample sites (N = 9) upstream of Caribou Creek contained cutthroat trout. Of these sites, only one (Twentymile Creek) exhibited *O. mykiss*/cutthroat trout hybrids (4/37). Corresponding cutthroat trout introgression levels were low (0.8%). Diagnostic SNP allele frequencies contrasted sharply between Ruby Creek (RUL and RUM) and Twentymile Creek (TWE). Ruby Creek sites exhibited SNP allele frequencies more similar to reference hatchery rainbow trout populations than to native reference redband trout populations. The opposite was observed in Twentymile Creek (Table 5). Barriers exist near the mouths on all three tributaries (Figure 10).

Moving downstream from the Deep Creek drainage, sites within Myrtle Creek also contained no cutthroat trout (N = 50). Samples of *O. mykiss* exhibited SNP allele frequencies more similar to reference hatchery rainbow trout populations than to native reference redband trout populations (MYR, Table 3). Records identified no history of stocking hatchery rainbow trout in the creek, although a single stocking of cutthroat trout occurred in 1974. Two barriers exist in the lower end of the creek (Figure 10).

In contrast to Myrtle Creek, all of the tributaries screened between Myrtle Creek and Long Canyon Creek contained primarily cutthroat trout, and most exhibited low levels of hybridization. Three sites were sampled in Ball Creek with the highest level of hybridization observed at the lower site (5 $>F_1$ hybrids, 4.1% *O. mykiss* introgression). Hybridization and introgression decreased moving upstream, with next site identified with 3 $>F_1$ hybrids and 0.6% redband trout introgression, and the highest site exhibiting no hybridization (N = 10). A similar pattern was observed in Trout Creek, with the lowest site identified with 25 cutthroat trout and 1 F_1 hybrid, and the highest site identified with 24 cutthroat trout and no hybrids. Neither of these tributaries apparently have barriers (Figure 9). Ball Creek received a single stocking of cutthroat trout in 1973.

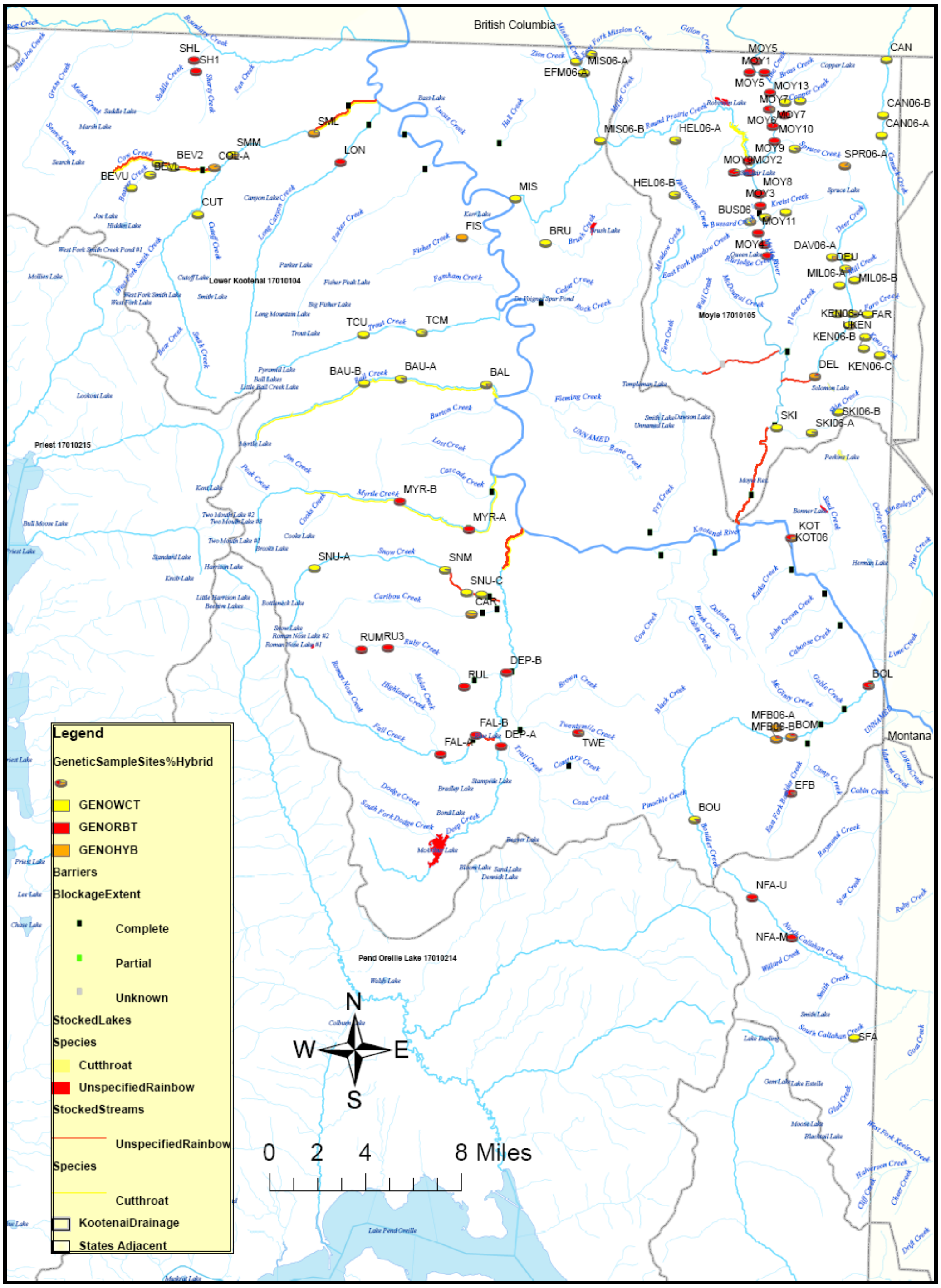

Figure 10. Results of interspecific hybridization screening within sample sites from the Kootenai River drainage. Colored pies show relative frequencies of fish identified as cutthroat trout (yellow), *O. mykiss* (red), and hybrids (orange) at each site. Highlighted streams or lakes indicate hatchery rainbow trout (red) or hatchery westslope cutthroat trout (yellow) stocking records (IDFG historical stocking database). Barriers were identified as part of westslope cutthroat trout and bull trout status assessments completed in 2002 and 2004.

A similar pattern was observed in Trout Creek, with the lowest site identified with 25 cutthroat trout and 1 F_1 hybrid, and the highest site identified with 24 cutthroat trout and no hybrids. Neither of these tributaries apparently have barriers (Figure 10). Ball Creek received a single stocking of cutthroat trout in 1973.

Four sample sites within the upper Mission Creek drainage were screened in 2006, with only a single *O. mykiss* allele observed in one sample out of 235 examined. For this study, two additional sites were included from lower in the drainage, although sample sizes for both were very low. All 12 of the samples examined from the mainstem Mission Creek site were identified as cutthroat trout. The single sample from Brush Creek was also identified as a cutthroat trout. There are no barriers identified in the Mission Creek drainage, and stocking appears limited throughout much of the drainage, except for a headwater lake to Brush Creek that has been stocked with rainbow trout (IDFG historical stocking database).

Another site with a very low sample size was Fisher Creek (N = 3). All three samples were identified as $>F_1$ hybrids with the majority of the alleles examined identified as *O. mykiss* (81.0%).

A higher number of samples was available for screening from Long Canyon Creek (N = 44), and all 44 were identified as *O. mykiss*. Williams and Jaworski (1995) reported that redband trout populations in Long Canyon Creek and Fisher Creek had been completely replaced by coastal rainbow trout as a result of hatchery rainbow trout outplantings. In this study, the frequencies of presumed diagnostic coastal alleles were generally higher in Long Canyon Creek than what has been observed in reference redband trout populations, but lower than those observed in reference hatchery rainbow trout populations (LCAN, Table 3). These results seem to support the idea that the *O. mykiss gairdneri* population in Long Canyon Creek is introgressed with coastal alleles but perhaps had not undergone complete replacement. There are no barriers identified in the drainage.

The Smith Creek drainage was stocked with hatchery rainbow trout and cutthroat trout in the 1960s and 1970s, both in lower mainstem, as well as in Cow Creek in the upper part of the drainage (IDFG historical stocking database). Large numbers of *O. mykiss*/cutthroat trout hybrids were detected in sample sites from Lower and Middle Smith Creek. Lower Smith Creek contained predominantly hybrids (3 F_1 and 26 $>F_1$), but also contained parental types (1 redband trout and 8 cutthroat trout). Middle Smith Creek contained higher numbers of cutthroat trout (N = 13) and no *O. mykiss*, but also exhibited substantial hybridization with 7 $>F_1$ hybrids identified. Backcross hybrids ($>F_1$) were the dominant fish sampled at both of the Lower Cow Creek sites (>80%), although fish with genotypes indicative of cutthroat and F_1 hybrids were also identified. A barrier exists just upstream from the Lower Cow Creek sites, and Beaver Creek, a tributary to Cow Creek above this barrier, exhibited very little hybridization. Of the three sites screened from Beaver Creek (N = 51 total samples), only the lowest site was identified with a single *O. mykiss* allele in one sample (2.5% introgression). Cutoff Creek, which is a tributary to the West Fork of Smith Creek is not isolated from the Lower Cow Creek sites, but exhibited only cutthroat trout (N = 50).

The final tributary sampled within the mainstem Kootenai River drainage was Shorty Creek. Only *O. mykiss* were sampled at two sites within Shorty Creek (N = 49 total) and samples exhibited SNP allele frequencies more similar to reference redband trout populations than to native reference hatchery rainbow trout populations (SHL, Table 3). Shorty Creek is a tributary to Boundary Creek and previous genetic studies on Boundary Creek indicated that redband trout in the drainage had not been introgressed with coastal hatchery rainbow trout but exhibited low levels of cutthroat trout introgression (USFWS 1999).

Moyie River Mainstem and Tributaries

There is still some debate regarding whether redband trout are native to the Moyie River (Dupont and Horner 2009). One argument is that Moyie River Falls likely prevented natural invasion of redband trout, similar to other barrier falls found on the Spokane, Pend Oreille, Snake, and Upper Kootenai rivers (Behnke 1992). It is presumed that the extensive stocking of hatchery rainbow trout in Moyie Lake since the early 1920s (Fisheries Inventory Data Queries, http://srmapps.gov.bc.ca/apps/fidq/), and in the Moyie River since at least the early 1970s (http://fishandgame.idaho.gov/apps/stocking/), produced the self-reproducing rainbow trout populations that currently exist in the drainage. A counter argument is that some redband trout populations may have been present prior to the formation of present day impassable barriers (Dupont and Horner 2009, citing Muhlfeld et al. 1999). Under the assumption that *O. mykiss gairdneri* were not native to the drainage, we previously genetically screened samples from 11 tributaries that flow into the mainstem Moyie River to assess the impact of past hatchery rainbow trout introductions and existing self-sustaining rainbow trout populations (Campbell and Kozfkay In Press). Those results showed that while a majority of the tributaries sampled in the Moyie River exhibited some level of hybridization, introgression levels were low (all sites <3%). Additionally, no *O. mykiss* or F_1 hybrids were identified at any site (N = 512 total samples). For this study, we increased the number of sites in some tributaries and also screened a large number of *O. mykiss* from 13 sites in the mainstem Moyie River with the goal of providing additional information on the purity and origin of *O. mykiss* in the drainage.

Results from the tributary sampling generally yielded very similar patterns as observed in 2006. Of the six sites examined, no *O. mykiss* or F_1 hybrids were observed in five (Table 4: Canuck Creek, N = 40; Upper Deer Creek, N = 31; Faro Creek, N = 37; Keno Creek, N = 2; or Skin Creek, N = 5). Among these five, *O. mykiss* introgression was only detected at one site (Upper Deer Creek, 1.4%). The one sample site that yielded a different composition pattern to the 2006 screening was Lower Deer Creek. Of the 19 samples examined from this site, one was identified as a cutthroat, three as *O. mykiss*, six as F_1 hybrids, and nine as $>F_1$ hybrids. While most of the tributaries in the Moyie River have not received direct hatchery rainbow trout stocking, the lower end of Deer Creek has been stocked (IDFG historical stocking database) which may help explain these results.

Tissue samples were collected from 204 *Oncorhynchus sp.* sampled as part of electroshocking efforts at 13 sites on the mainstem Moyie River. Of the 202 samples that yielded complete genotypes, 196 were identified as *O. mykiss*, three as cutthroat trout, and three as $>F_1$ hybrids. Allele frequencies from a pooled sample (all 196 *O. mykiss*) were more similar to reference hatchery rainbow trout populations than to native reference redband trout populations (MOY, Table 3).

SUMMARY CONCLUSION AND MANAGEMENT RECOMMENDATIONS

Results from this study as well as the 2006 study should assist managers currently working on an updated status review for westslope cutthroat trout in Idaho. The major findings and recommendations from these studies are:

- Few sites exhibited genotypes indicative of both *O. mykiss* and cutthroat trout.

- Of the 78 total sites screened, only seven contained both *O. mykiss* and cutthroat trout. This suggests that while these species exist in sympatry in reference to the Kootenai River drainage, a more contiguously allopatric distribution pattern is observed at the tributary level.

- Interspecific hybridization (between *O. mykiss* and cutthroat trout) was identified at many sites and in many tributaries throughout the drainage.
  - Despite the fact that *O. mykiss* and cutthroat trout were seldom found together at individual sample sites as well as in individual tributaries, 13 of the 21 tributaries to the mainstem Kootenai River examined (61.9%) contained *O. mykiss*/cutthroat trout hybrids (22/49 sites, 44.9%). In the Moyie River drainage, eight of the 13 tributaries examined (61.5%) contained hybrids (12/29 sites, 41.4%).

- Interspecific introgression levels were generally low.
  - Despite the fact that hybrids were found at 22 sites in tributaries to the mainstem Kootenai River, only two sites exhibited greater than 20% introgression, and 14 exhibited less than 5% introgression. Sites with introgression >5% were limited to just three tributaries (Boulder Creek, Caribou Creek, and Lower Cow Creek). Of the 12 sites in the Moyie River drainage identified with hybrids, only two exhibited introgression >5% (Upper Spruce Creek and Lower Deer Creek).

- Many of the cutthroat trout populations examined in the Kootenai River drainage would meet the criteria proposed by the USFWS for inclusion as westslope cutthroat trout under the ESA.
  - Of the tributary populations to the mainstem Kootenai River, westslope cutthroat trout in: Snow Creek, Ball Creek, Trout Creek, Mission Creek, Beaver Creek, and Cutoff Creeks all exhibit less than 20% *O. mykiss* or YCT alleles (USFWS 2003).
  - Of the tributary populations examined in the Moyie River drainage, all but Spruce Creek and Lower Deer Creek exhibit less than 20% nonnative *O. mykiss* or YCT alleles (USFWS 2003).

- Recent, ongoing interspecific hybridization was detected in four Kootenai River tributary drainages.
  - Boulder Creek, Caribou Creek, Lower Smith Creek/Lower Cow Creek, Lower Deer Creek all contained F_1 hybrids.
  - Previous studies investigating hybridization between WCT and nonnative rainbow trout have indicated that straying of hybrids can expand hybridization and impact unstocked locations (Hitt et al. 2003; Rubidge and Taylor 2005).
  - Future research efforts should focus on monitoring these areas to assess their fate (e.g., formation of stable hybrid zones, reemergence of reproductive isolating mechanisms, or complete genetic introgression) and determine whether active management actions are needed to remove hybrids and nonnative rainbow trout.

- Evidence of intraspecific hybridization (nonnative hatchery rainbow trout/native redband trout) was identified at many sites and in many tributaries throughout the drainage.
  - Of the seven tributaries to the mainstem Kootenai River that exhibited primarily *O. mykiss*, four (Boulder Creek, Ruby Creek, Myrtle Creek, and Long Canyon) exhibited SNP allele frequencies more similar to reference hatchery trout populations than to native reference redband trout populations. Samples from the mainstem Kootenai River also showed evidence of hatchery rainbow trout introgression.

    - Only Callahan Creek, Shorty Creek, and Tenmile Creek appeared to contain pure redband trout populations with no rainbow trout hatchery influence.

- Within this limited dataset, the IDFG historical stocking database did not appear to serve as a good predictor in identifying sites that should contain pure redband trout.
    - There were no records in this database indicating that that any of the four tributaries we identified as introgressed had been stocked with hatchery rainbow trout.

- Diagnostic allele frequencies of *O. mykiss* collected from the mainstem Moyie River were more similar to reference hatchery rainbow trout populations than to native reference redband trout populations.
    - These results provide no evidence that native *O. mykiss* exist in Moyie River drainage.

- More analyses are needed to better understand the relationship between barriers (physical and ecological) and species composition and hybridization patterns in the Kootenai River drainage.

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