

**FISHERIES BUREAU
MONTANA FISH, WILDLIFE & PARKS**

Federal Aid Job Progress Report

Montana Statewide Fisheries Management

Federal Aid Project Number: F-113

Fiscal Period: July 1, 2009 – June 30, 2011

Project Title: Statewide Fisheries Management

Job Title: Middle Clark Fork River Drainage

Project Number: 3240

Project Biologist: William Ladd Knotek

ABSTRACT

This report consists of three chapters involving adult bull trout telemetry, monitoring of bull trout spawning activity using redd count index sections, and results of *Oncorhynchus* genetic sampling in the middle Clark Fork region of west-central Montana.

Radio-telemetry results indicated that adult bull trout migrated to and entered spawning tributaries in June. Telemetered bull trout migrated to five stream segments where spawning and juvenile rearing areas had previously been identified, validating the location and timing of redd count index sections. Sub-adults and non-spawning adults did not ascend tributaries during summer, but staged primarily at stream mouths – presumably to take advantage of cooler water temperatures in these areas. This study also highlighted the vulnerability of staging and migrating bull trout to angling.

Index sections were established, refined and completed annually to monitor fluvial bull trout spawning activity and escapement on four streams in the Middle Clark Fork drainage from 2000-2009. Redd abundance was low and annually variable for all remaining fluvial populations. Initial trend data from these index reaches indicated that middle Clark Fork populations are severely depressed, but generally stable.

We systematically collected and genetically analyzed *Oncorhynchus* samples from tributary drainages of the middle Clark Fork and lower Bitterroot River systems to assist in developing conservation plans, prioritizing fisheries enhancement projects, and evaluating fish passage issues. Samples were collected and tested at 346 sites in 160 stream segments. The relative westslope cutthroat trout genetic contribution within samples and among populations was highly variable, but was generally inversely related to stream order where upstream fish passage barriers were not present. In total, hybridization was not detected in 89 population segments within the project area. These included 53 population isolates where a confirmed or suspected physical barrier segregated the population from hybridized individuals downstream.

BULL TROUT RADIO TELEMETRY IN THE MIDDLE CLARK FORK RIVER DRAINAGE

Radio telemetry has been one of the most important tools for investigating fluvial bull trout biology and population status within the upper Clark Fork Basin of western Montana. In recent studies in the Milltown area (Swanberg 1997a; Schmetterling 2003), Rock Creek (Carnefix 2002), Rattlesnake Creek and the Clark Fork River (Knotek et al. 2004) and the Blackfoot River (Swanberg 1997b; Schmetterling 2003; Pierce et al. 2004), radio telemetry has provided critical information on the timing and location of bull trout movements, identification of spawning areas, and sources of mortality. Telemetry has also been used to identify environmental and anthropogenic factors that are limiting migratory populations. Although sample size is often small, information gained from individuals over long periods of time can provide insights that are unattainable using traditional fisheries methods. This information has provided the basis for population monitoring programs, habitat and fish passage enhancements, and protective fishing regulations in the basin.

We applied many of these same concepts in the middle Clark Fork River and its tributaries, where some bull trout populations may be on the verge of extirpation. In basin-wide tributary surveys from 1999-2005, segments of only five tributaries supported viable migratory populations within the 120 mile reach from the Blackfoot River to the Flathead River confluence (Knotek 2005). Recent main stem population estimates indicate that adult bull trout densities are extremely low (1-2 per mile) in the main stem Clark Fork River (Berg 1999; Knotek 2005).

In this three year telemetry study, we focused on fluvial bull trout in the middle Clark Fork River reach (and tributaries) from the Fish Creek confluence (river mile 305) to the Flathead River confluence (river mile 245). Objectives were to identify seasonal habitat use and the timing of movements, the location of tributary spawning areas, and factors that may be limiting survival or reproduction. This information was then used to prioritize habitat and fish passage enhancement projects, evaluate fishing regulations, prioritize enforcement efforts and to help establish and corroborate population monitoring methods. Special attention was directed at identifying bull trout congregations (i.e. staging, spawning and thermal refuge areas) that are experiencing heavy angling pressure.

Methods

We captured bull trout by electrofishing the main stem Clark Fork River (river mile 265-305; Figure 1) in daylight with a boom-suspended electrofishing unit mounted on an 18 foot aluminum jet boat in 2003-2005. Sixteen 4-6 hour shocking periods were completed between May 11 and June 11 when water temperatures were 8-13 °C (generally prior to 14:00). Bull trout were selectively netted and placed in an onboard holding tank prior to being anesthetized with Fintrol (MS-222), weighed and measured. A total of 36 adult and sub-adult bull trout (268-655 mm total length (TL); see Figure 2) were captured and radio transmitters were implanted in 17 of the largest fish (415-655 mm TL) that were suspected to be adults (Figure 2). Individually coded transmitters (7.7-10.0 g, 5 s burst rate, Lotek Engineering) did not exceed 2% of each fish's weight (Winter 1996) and were implanted using standard surgery techniques described in Schmetterling (2001). Transmitter life (278 d to 839 d) varied as both continuous and half-duty models were employed. Surgeries were completed in 2-4 min and fish recovered for at least 10 min in a live car

prior to being released near their capture location. A passive integrated transponder (PIT) tag was injected into the abdominal cavity of bull trout that did not receive a transmitter. The adipose fin and a portion of the caudal fin were also clipped on all of the bull trout we handled for subsequent visual identification and genetic testing, respectively.

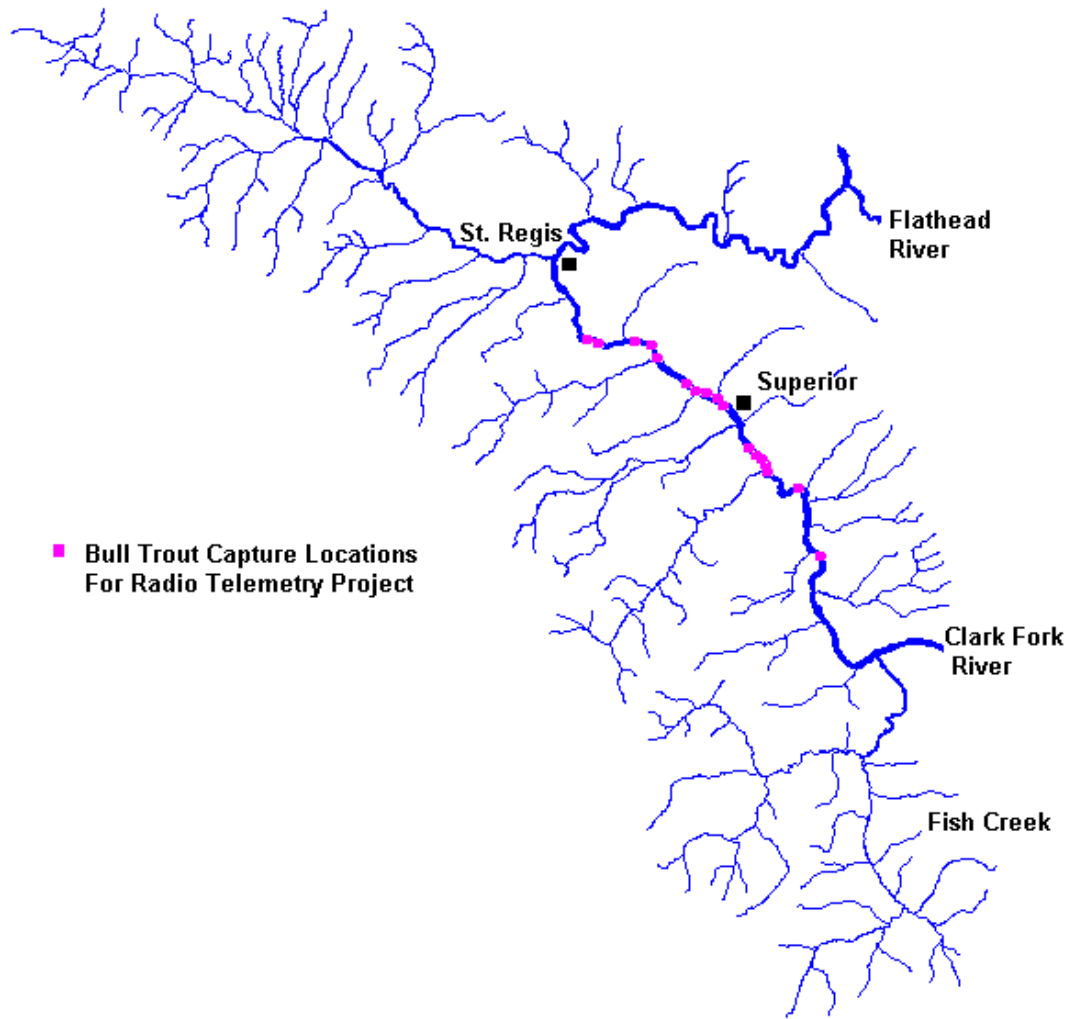


Figure 1. Capture locations for fluvial bull trout implanted with radio transmitters in the middle Clark Fork River, 2003-2005.

Telemetered fish were generally tracked weekly (March – May) with Lotek SRX 400 receivers, but were tracked more frequently during peak migration and spawning periods (June - Sept) and less frequently during late fall and winter (Oct – Feb). Tracking continued throughout the life of each transmitter or until fish mortality. Fish were tracked by truck, bicycle and on foot (depending on access and terrain) with both roof-mounted and directional antennas.

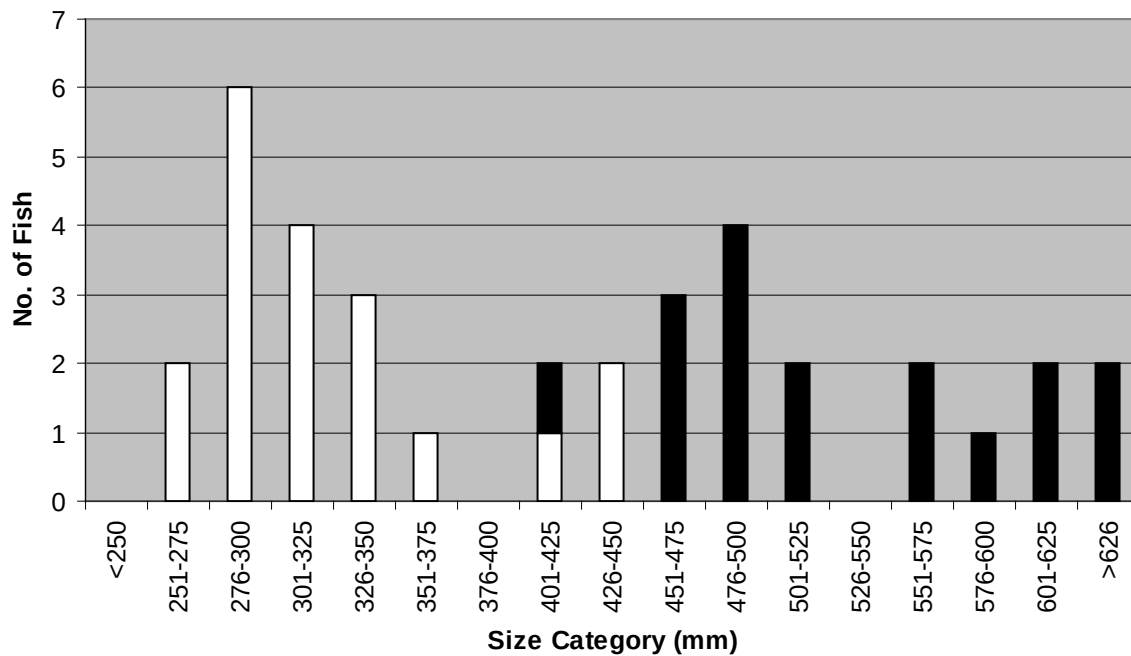


Figure 2. Size distribution of bull trout captured in the middle Clark Fork River in 2003-2005, including fish that were implanted with radio transmitters (black bars) and those that were not (white bars).

Results and Discussion

Radio transmitters were implanted in 17 bull trout (Table 1), but one fish disappeared 2 days after surgery. The remaining individuals were tracked for 76-530 days (mean 245 d), which included at least one summer and early fall (spawning period) for most fish. In the first two weeks after surgery, all fish remained in the Clark Fork River and movements were variable. However, a clear dichotomy emerged between tributary migrants (assumed spawning adults) and those that remained in the main stem river (assumed sub-adults and non-spawning adults) in June and early July. None of the bull trout we handled were re-captured in subsequent sampling runs.

Tributary Migrants

Of the 16 telemetered bull trout tracked and consistently located during the pre-spawn and spawning period (June 1 – Sept 25), seven ascended tributaries. These fish entered tributaries between June 4 and June 28 after staging near the Clark Fork River-tributary confluence areas for less than one week (see Table 2). All tributary migrants moved to upper Fish Creek (n=4), Trout Creek (n=2) or Cedar Creek (n=1). Within Fish Creek, most bull trout spawned within established redd survey index reaches in the West Fork (n=2) and the North Fork (n=1). The exception was a bull trout that ascended Cache Creek, where juveniles have been consistently detected but no redd count section has been established. In Trout Creek, both adults migrated to the mouth of a large canyon and series of cascades that apparently act as a complete barrier to upstream passage. Neither fish was observed spawning and no redds were located in the stream reach downstream of the canyon. This is consistent with stream electrofishing data

Table 1. Data summary for bull trout implanted with radio transmitters in the middle Clark Fork River in 2003-2005.

FISH ID	DATE	LENGTH	RELEASE LOCATION (RM) ¹	TRACKING DURATION ²
21-82	6/11/03	495 mm	Just below First Creek (RM 291)	404 d
21-83	6/11/03	491 mm	1.5 mi above Cedar Cr (RM 288)	409 d
21-84	6/6/03	467 mm	3 mi below Dry Cr (RM 277)	415 d
22-44	6/11/03	560 mm	Just below Trout Cr (RM 289)	104 d
22-45	5/11/04	602 mm	Mouth of Flat Cr (RM 284.5)	530 d+
22-46	5/13/04	593 mm	0.5 mi above Sloway Gul (RM 278.5)	90 d
22-47	5/25/05	490 mm	0.25 mi below Flat Cr (RM 284.25)	76 d
22-48	6/11/03	475 mm	1.5 mi above Cedar Cr (RM 288)	105 d
22-49	6/6/03	570 mm	Mouth of Dry Cr (RM 280)	485 d
22-50	6/6/03	621 mm	3 mi below Dry Cr (RM 277)	99 d
22-51	6/2/05	473 mm	0.75 mi below Flat Cr (RM 283.75)	141 d+
22-52	6/1/05	520 mm	Mouth of Trout Cr (RM 289.5)	143 d+
22-53	6/8/05	636 mm	Just below Trout Cr (RM 289.4)	2 d
22-80	6/10/03	415 mm	1.5 mi below Nemote Cr (RM 297.5)	217 d
22-81	6/6/03	522 mm	0.5 mi below Dry Cr (RM 279.5)	272 d
22-83	6/5/03	476 mm	Near Thompson Cr mouth (RM 283)	221 d
22-90	6/2/05	655 mm	0.5 mi below Flat Cr (RM 284)	201 d+

¹ RM = River mile location from the State of Montana index for the Clark Fork River (DNRC 1984).

² Tracking Duration = No. of days from transmitter implant to final recorded location (while fish presumed alive)

(Knotek 2005) as juvenile bull trout were only detected in selected reaches upstream of the canyon. The bull trout that migrated to Cedar Creek spawned in the primary redd survey index reach in lower Oregon Gulch, where juvenile densities have also been consistently highest since 1999. These streams constitute the majority of tributaries in the project area where juvenile bull trout were detected during basin-wide electrofishing surveys in 1999-2005 (Knotek 2005; MFWP unpublished data). Other nearby streams that support viable bull trout populations include Albert Creek (near Frenchtown) and Little Joe Creek (near St. Regis). Both of these populations are believed to be predominantly stream-resident based on size structure and low density of fluvial redds. Migratory bull trout may be completely excluded from Albert Creek by two anthropogenic fish passage obstructions in a lower stream reach.

Table 2. Timing of significant behaviors for radio-tagged bull trout that ascended middle Clark Fork River tributaries in 2003-2005.

TIME PERIOD	BEHAVIOR
June 4 – June 28	Bull trout entered tributaries
Sept 4 – Sept 14	Spawning observed
~ Sept 10 – Oct 10	Bull trout exited tributaries*

* In some years, dewatering in lower tributary reaches inhibited downstream movement

Four of the seven bull trout that ascended tributaries were observed on spawning redds in early September. The other tributary migrants either could not reach spawning areas or died prior to spawning. Bull trout that were still alive after the spawning period returned to the Clark Fork River by early October.

Behavior of Bull Trout That Did Not Ascend Tributaries

Telemetered bull trout that did not ascend tributaries ($n=9$, mean length 482 mm, range 415-522 mm) were significantly smaller than tributary migrants ($n=7$, mean length 592 mm, range 490-655 mm). We assume fish that ascended tributaries were adults intending to spawn, while those that remained in the river throughout summer and fall were sub-adults or non-spawning adults. Bull trout that remained in the main stem displayed various upstream and downstream movements within the project area from the date of tagging through early July. However, during the warmest portion of the summer (consistently July 15-Aug 15), radio-tagged fish congregated at major tributary mouths and just downstream in the Clark Fork River (see Figure 3). One fish also entered a tributary (Trout Creek) and remained at a location ~ 0.5 mile upstream of the mouth for ~ 2 weeks before moving downstream to the river confluence.

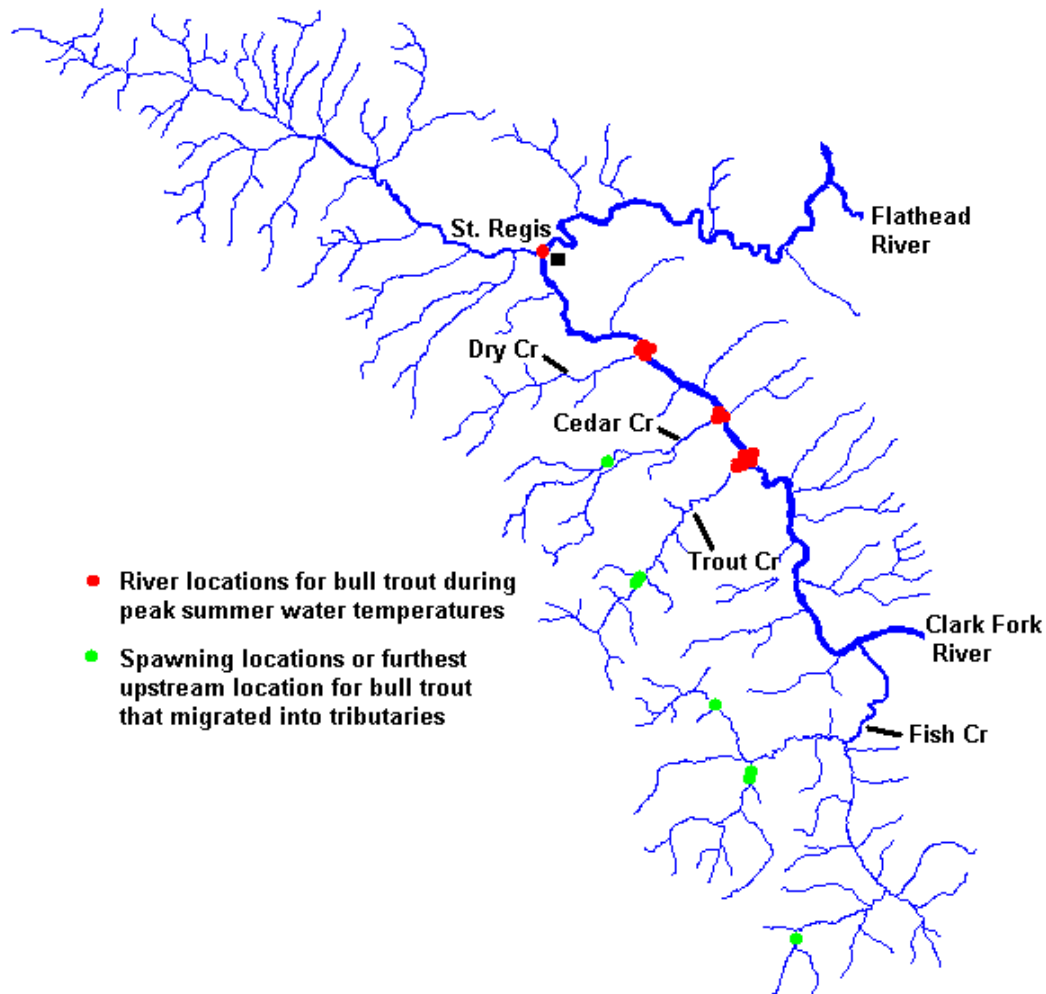


Figure 3. Location of tributary spawning sites (or upstream extent of movement) and predominant summer locations (non-spawning fish) for bull trout radio-tagged in the middle Clark Fork River system.

Occupation of tributary confluence areas coincided with the warmest Clark Fork River water temperatures in late July and early August (Figure 4). Bull trout require cold water temperatures and experience stress and high mortality when exposed to temperatures $> 20^{\circ}\text{C}$ for extended periods of time (Selong et al. 2001). Tributary mouths likely served as thermal refugia for telemetered bull trout as water from the tributaries was significantly cooler than the Clark Fork River (Figure 4). This situation may have been accentuated under the prevalent drought conditions during this study, where summer flows were low and maximum water temperatures were higher and persisted longer than long-term averages in the Clark Fork basin.

Telemetered bull trout congregated near stream mouths, where surface water from tributaries met Clark Fork River water. In some cases (e.g., Dry Creek), no surface water was present from mid-July to the following spring. At these locations, bull trout still occupied the immediate confluence area and along the river bank just downstream during summer peak water temperatures. Field investigation of these confluence areas indicated that enough groundwater seepage from the tributary drainage entered the Clark Fork River to provide small pockets of cooler water.

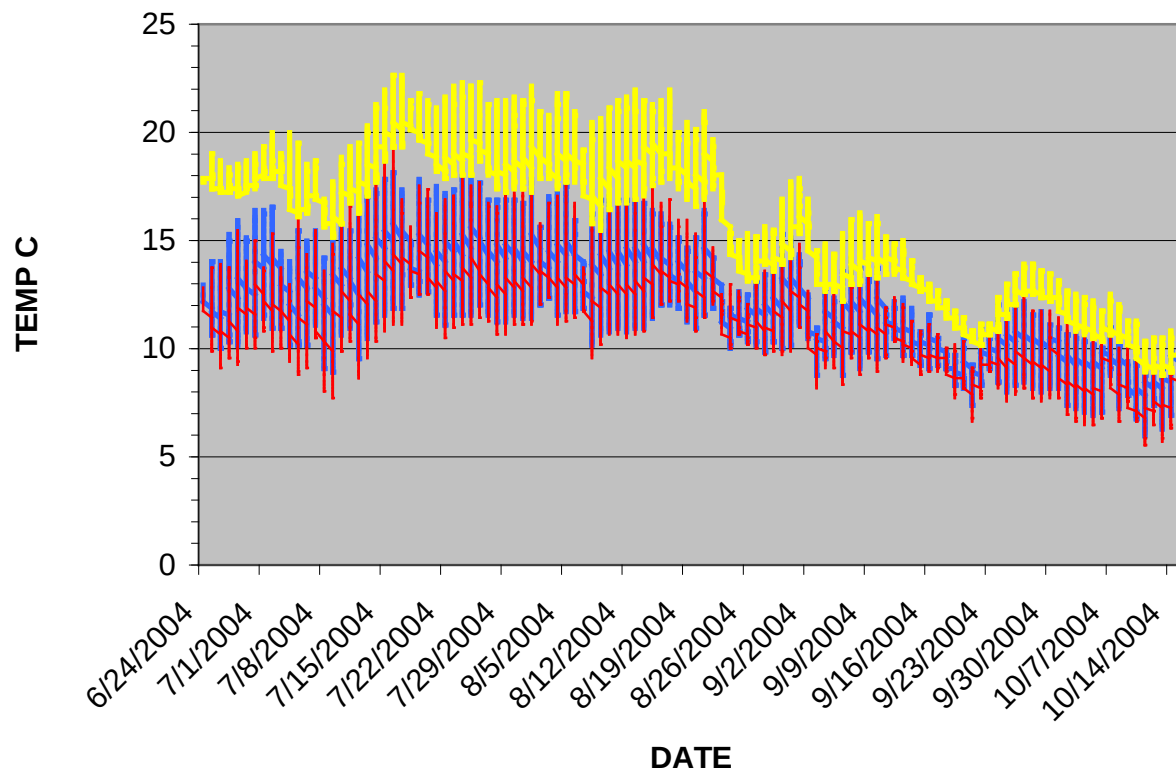


Figure 4. Summer and fall temperature regimes for lower Fish Creek (blue), lower Trout Creek (red), and the middle Clark Fork River near Superior (yellow) in 2004.

Field temperature measurements at all of the tributary confluence areas with a hand-held thermometer indicated that the thermal refuge areas for fish were small (< 0.5 hectare). Cool-water ‘pockets’ were limited because colder tributary discharges ($\sim 0.25\text{-}1.0\text{ m}^3/\text{s}$ (9-36 cfs)) were minute relative to warmer

Clark Fork River discharge ($\sim 56\text{-}84\text{ m}^3/\text{s}$ (2,000-3,000 cfs)). Triangulation with hand-held antennas indicated that telemetered bull trout occupied micro-habitats in very close proximity within the thermal refuge ‘pockets’.

Bull Trout Habitat Use in Winter and Spring

In October –June, nearly all telemetered bull trout occupied the main stem Clark Fork River within the project area. Most fish remained in the ~ 16 mile reach from First Creek (below Alberton Gorge, River Mile 291) to ~ 5 miles upstream of the St. Regis River confluence (RM 275) where bull trout were originally captured (see Figure 1). However, three fish inhabited the river reach between the St. Regis River mouth (RM 270) and the Patrick Creek confluence ($\sim$ RM 257) during winter and early spring. Movements for all fish during late fall and winter (Oct-March) were minimal (< 2 miles) and many fish were located repeatedly at the same positions for 1-3 months. High fidelity to overwintering sites has been reported in other studies within the basin (Schmetterling 2003; Swanberg 1997b).

Practical Implications for Protection and Enhancement of Bull Trout Populations

The congregation of bull trout at tributary mouths in July and August made them very vulnerable to angling. This is particularly true at the mouths of Fish Creek, Trout Creek and the St. Regis River because these locations have good public access, are generally popular for trout fishing (Knotek 2005), and anglers have difficulty distinguishing bull trout from other trout species (Schmetterling and Long 1999). Although tributary migrants generally staged at stream mouths for less than a week prior to entering tributaries, these individuals were also susceptible to angling because they occupied predictable locations (e.g., major pools) within tributaries that receive high angling pressure, they are often visible to anglers during the summer low water period, and species identification is problematic for many anglers (Schmetterling and Long 1999). Although we could not confirm the cause of mortality, we suspect that several of the telemetered bull trout in this study were harvested illegally by anglers. These concerns led to more restrictive fishing regulations in the project area designed to help protect bull trout from illegal harvest. Increased enforcement presence at key locations during appropriate time periods (based on telemetry findings) and angler education efforts were also emphasized and implemented.

This study also confirmed the importance of a few Clark Fork River tributaries as spawning and thermal refuge areas for migratory bull trout. These tributaries have been prioritized for habitat protection and restoration measures in the basin. In particular, Fish Creek and Cedar Creek should benefit from large scale land acquisition/protection projects on privately owned parcels and various habitat enhancement activities directed as fish passage and instream habitat improvements. Several of these projects are completed or in progress.

Literature Cited

Berg, R.K. 1999. A compilation of fishery survey information for the middle Clark Fork River drainage 1984-96. Montana Fish, Wildlife & Parks, Missoula, Montana.

- Carnefix, G. 2002. Movement patterns of fluvial bull trout in relation to habitat parameters in the Rock Creek Drainage, Missoula and Granite Counties, Montana. M.S. thesis. University of Montana, Missoula, Montana. 185 pp.
- Knotek, W.L. 2003. Middle Clark Fork River drainage fisheries investigations. Federal Aid Report for F-78-R6 and F-113-R1/R2. Montana Fish, Wildlife and Parks, Missoula, Montana.
- Knotek, W.L. 2005. Middle Clark Fork River and Missoula Area: 2004 Fisheries Status Report. Montana Fish, Wildlife, and Parks, Missoula, Montana. 66 pp.
- Knotek, W.L., R. Rashap and D. Schmetterling. 2004. Rattlesnake Creek: Fisheries assessment and enhancement 1999-2003. Montana Fish, Wildlife and Parks, Missoula, Montana. 49 pp.
- Pierce, R.W., R. Anderson and C. Podner. 2004. The Big Blackfoot River restoration progress report for 2002 and 2003. Montana Fish, Wildlife & Parks, Missoula, Montana.
- Schmetterling, D.A. 2001. Seasonal movements of fluvial westslope cutthroat trout in the Blackfoot River drainage, Montana. North American Journal of Fisheries Management 21:507-520.
- Schmetterling, D.A. 2003. Reconnecting a fragmented river: movements of westslope cutthroat trout and bull trout after transport upstream of Milltown Dam, Montana. North American Journal of Fisheries Management 23:721-731.
- Schmetterling, D.A. and M.H. Long. 1999. Montana anglers' inability to identify bull trout and other salmonids. Fisheries 24(7):24-27.
- Selong, J.H., T.E. McMahon, A.V. Zale, and F.T. Barrows. 2001. Effect of temperature on growth and survival of bull trout, with application of an improved method for determining thermal tolerance in fishes. Transactions of the American Fisheries Society 130:1026-1037.
- Swanberg, T.R. 1997a. Movements of Bull Trout (*Salvelinus confluentus*) in the Clark Fork River System after Transport Upstream of Milltown Dam. Northwest Science 71(4):313-317.
- Swanberg, T.R. 1997b. Movements and habitat use by fluvial bull trout in the Blackfoot River, Montana. Transactions of the American Fisheries Society 126:735-746.
- Winter, J.D. 1996. Advances in underwater biotelemetry. Pages 555-590 in B.R. Murphy and D.W. Willis, editors. Fisheries Techniques, 2nd edition. American Fisheries Society, Bethesda, Maryland.

BULL TROUT REDD COUNTS IN MIDDLE CLARK FORK RIVER TRIBUTARIES

Background

Redd counts are a common tool for monitoring escapement of adult migratory (fluvial) bull trout (Dunham et al. 2001; Spalding 1997). Redds, or nests, are excavated by spawning females and can be counted by trained personnel in consistent stream sections to serve as an index of adult spawner abundance, level of spawning activity and as an indication of anticipated recruitment in the succeeding generation. In western Montana, bull trout generally spawn during the first three weeks of September and have high fidelity to natal tributaries (Fraley and Shepard 1989).

Redd surveys are more difficult and considered less useful for monitoring bull trout populations with a stronger *stream-resident* component. Stream-resident life forms complete their life cycle in tributary systems and adults are generally smaller than those of migratory forms. In these situations, redds are often hard to differentiate because they are small or variable in size, occur in relatively small substrates and are easier to confuse with redds of other fall-spawning salmonids. For example, stream-resident brook trout (*Salvelinus fontinalis*) are abundant in many western Montana streams, with spatial and temporal overlap in spawning with bull trout. This leads to frequent hybridization, as well as difficulty in distinguishing between redds of the two species. We also consider trends in *fluvial* redd abundance a better indicator of population health because these fish have more complex life-histories and require intact habitat elements at a larger scale.

For these reasons, we attempted to focus on bull trout populations with a predominant fluvial life history when establishing redd survey sections in middle Clark Fork River tributaries. Bull trout distribution in the Clark Fork basin is fragmented and viable populations were only detected in seven tributary drainages. Two of these populations, Grant Creek and Albert Creek, were assumed to be principally stream-resident based on limited connectivity with the Clark Fork River and population size (length) distributions. In Trout Creek, a small stream-resident population and a few fluvial adults were documented, but migratory adults apparently cannot ascend to natal spawning areas because of a natural upstream migration barrier and no fluvial redds have been located downstream of the barrier. Electrofishing assessments (MFWP, unpublished data), telemetry studies (Chapter 1; Knotek et al. 2004), angler creel surveys (Knotek 2005) and anecdotal observations indicated that tributary populations in Rattlesnake, Fish, Cedar, and Little Joe Creeks may be largely migratory.

In this Chapter, we describe the process of establishing redd survey index sections in four middle Clark Fork River tributaries and the trends in abundance observed there since 1999. These survey sections serve as an important tool for monitoring trends in bull trout abundance and the resiliency of middle Clark Fork basin populations.

Methods

Experienced field crews completed surveys between September 20 and October 5 by walking channel segments and visually searching for redds in Rattlesnake, Fish, Cedar, and Little Joe Creeks. Each

segment was walked once by personnel wearing polarized sunglasses between 10:00 and 16:00. Initial surveys (1999-2003) focused on all areas where juvenile bull trout had been detected in electrofishing surveys. Search areas were expanded to adjacent reaches and others with potential spawning habitat (Pierce and Podner 2006) in subsequent years to ensure that index reaches were representative and contained the majority of redds in each tributary. Radio-telemetry was also used to verify spawning reaches in some tributaries (Chapter 1; Knotek et al. 2004). More detailed descriptions of the process of selecting index reaches are provided below for each stream system.

Redds were identified by the presence of a pit or depression excavated in stream gravels, with an associated tail area of clean (bright) gravel relative to the surrounding substrates (Spalding 1997). Only definitive redds were included in counts. To be counted as a redd, disturbed gravels were required to (1) have a definitive ‘pit’ and ‘tail’, (2) be greater three feet in length from head of pit to end of tail, (3) be composed of uncompacted substrates in the tail that were relatively free of fine sediments (Kondolf and Wolman 1993; Fraley and Shepard 1989). In some cases (noted in text), redds were slightly smaller than three feet in length and assumed to be a combination of stream-resident and fluvial fish. Although redds were occasionally found outside of index reaches during expanded surveys, the total number of redds in index sections are the numbers reported for monitoring purposes.

Through repeated visits to spawning reaches in successive years, we attempted to schedule redd counts immediately after the termination of spawning. For most years, this corresponded to September 20–26. When redd surveys were attempted prior to this week, we often encountered spawning fish at redd sites. When redd surveys were completed later (i.e. after Oct 1), we had difficulty distinguishing redds from hydraulic features and other streambed irregularities – partially because disturbed substrates were no longer “bright” (free of algae and fine sediments).

Redd Surveys on Rattlesnake Creek

Initial redd counts were completed on the main stem of Rattlesnake Creek in 1999-2000 from the Mountain Water Company Dam (~ RM 4) upstream to near the mouth of Porcupine Creek (~ RM 16). These reaches lie almost entirely in the Rattlesnake National Recreation Area, where stream habitat and riparian corridors are largely un-impacted by human activities. None of the small tributaries to Rattlesnake Creek were surveyed as these streams are all generally intermittent and too small or steep to support spawning. The East Fork of Rattlesnake Creek was also eliminated as a potential spawning area as it is steep and apparently does not support fish above RM 0.5 (MFWP, unpublished data). Based on these surveys, two index sections were established that contained > 90% of the total redds located (Table 1 and Figure 1).

Table 1. Locations (Latitude, Longitude) of redd count sections on Rattlesnake Creek.

	Upper Boundary		Lower Boundary	
Section 1	N 46.9658	W 113.8657	N 46.9514	W 113.8392
Section II	N 47.0012	W 113.8411	N 46.9903	W 113.8351
Section III*	N 46.9767	W 113.8392	N 46.9767	W 113.8657

* Section III was established in 2005 and is not currently used as an index section

Expanded surveys were again completed in 2002 and 2005 to verify that index sections were representative and contained the majority of redds. No bull trout redds were located outside the index sections in 2002, but 5 redds were discovered in a new reach in 2005. This reach (from the East Fork mouth downstream to Section I) has been surveyed each year since 2005 and may be added as a third index section if bull trout continue to spawn there.

Although brook trout are prevalent in middle and upper Rattlesnake Creek, we have not observed them spawning concurrently with bull trout and redd sizes are typically too large to be created by stream-resident brook trout. However, infrequent hybrid bull x brook trout individuals have been visually identified in Rattlesnake Creek and their presence has been verified through genetic testing (Patrick DeHaan, USFWS Abernathy Fish Technology Center, personal communication). Brown trout are also abundant in lower Rattlesnake Creek, but this species is rare upstream of the Mountain Water Company Dam (MFWP, unpublished data) and no spawning has been observed in index reaches.

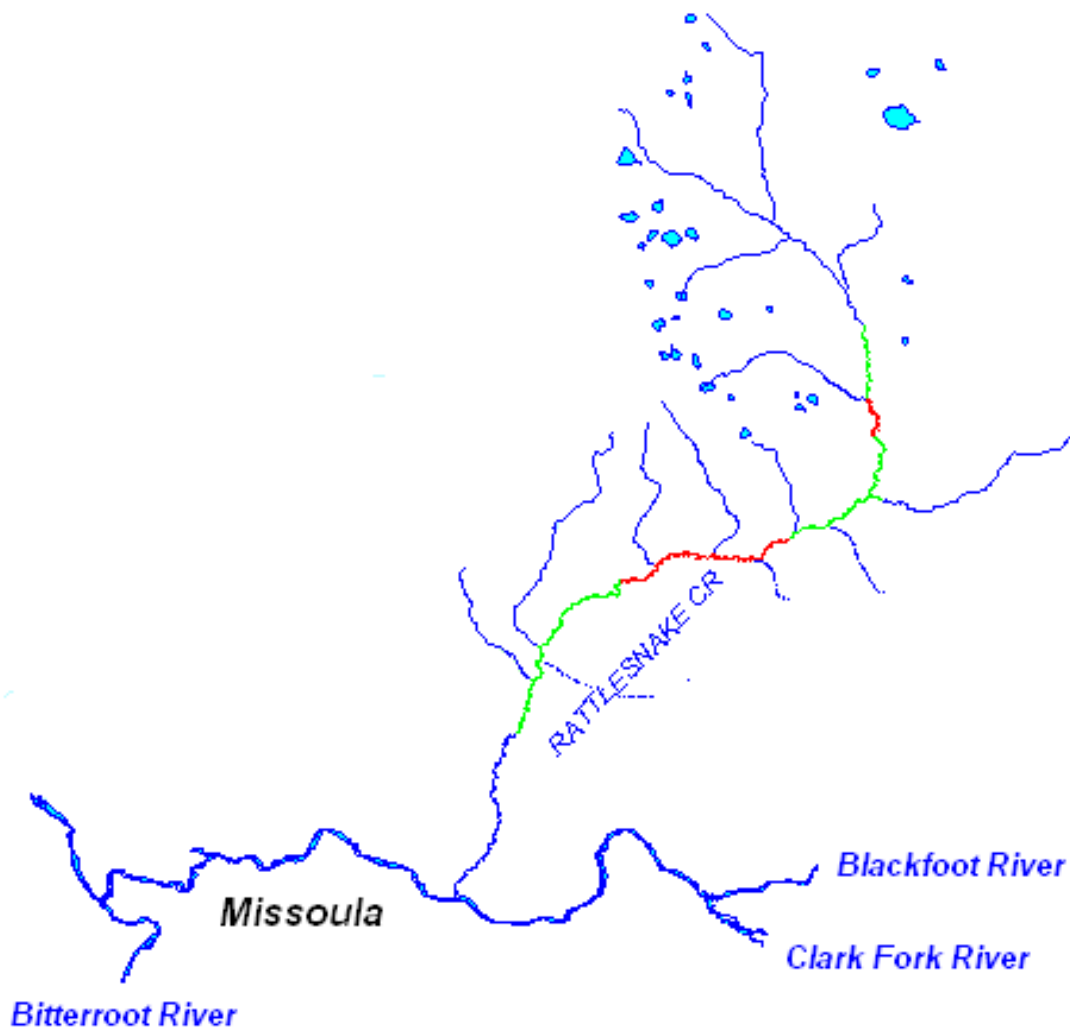


Figure 1. Location of bull trout redd count monitoring sections (red) and other areas surveyed for redds (green) in the Rattlesnake Creek drainage.

In 2001, MFWP personnel began manually transporting fluvial adult bull trout over the Mountain Water Company Dam (a complete fish barrier at ~RM 4) to enhance spawning success and recruitment. Congregations of adult bull trout had been observed below the dam for decades prior to actions that provided upstream passage. Several of the bull trout captured in 2001 and 2002 were implanted with radio-transmitters and ultimately spawned in the lower index section (Knotek et al. 2004). In 2003, permanent fish passage facilities were installed at the dam. This fish ladder, along with operational changes at the dam, presumably have provided passage of all fluvial bull trout to upstream spawning areas since 2003. These actions largely explain the increase in redds observed after 2000 in index reaches (Figure 2). Prior to 2001, redds were likely all created by stream-resident adults that reach up to 550 mm total length (Knotek et al. 2004).

Despite persistent drought conditions, bull trout redd abundance was relatively stable from 2001 through 2008. Redd counts could not be completed in 2004 due to a high flow event in early September that made redds difficult to distinguish. In 2005-2009, all redds located outside of the designated index sections were found in the reach from the East Fork confluence downstream to the top of Reach I. Prior to 2003, redds had not been observed in this section. In 2009, a significant decline in bull trout redds was observed, but the cause has not been determined.

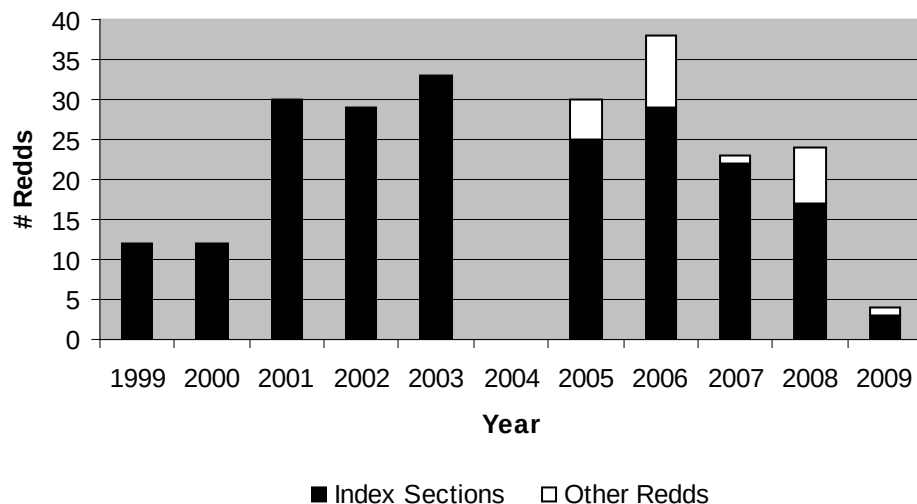


Figure 2. Annual bull trout redd counts in Rattlesnake Creek in 1999-2009. Results indicate the total count from index sections (black) and additional redds from outside index sections (white). No count was completed in 2004.

Redd Surveys on Fish Creek

Initial redd surveys were completed on the North Fork and West Fork of Fish Creek in 2001-2003, including all areas where juvenile bull trout have been documented and spawning habitat appeared suitable. Surveys included the main stem on both forks, as well as lower Indian Creek, Straight Creek and Cedar Log Creek. These portions of Fish Creek lie entirely in roadless proposed Wilderness with

relatively little habitat degradation and low human accessibility. None of the small tributaries to the West or North Forks were surveyed as these streams are either intermittent or too small and steep to support spawning. Four index reaches were selected (Table 2, Figure 3) that contained all of the redds we located. In 2004 and 2007, surveys were repeated in the upper West Fork (upstream of Indian Creek mouth) and no redds were located despite high juvenile bull trout densities in this reach. Surveys were not repeated in Indian, Straight or Cedar Log Creeks as natural barriers in the lower reaches of all of these tributaries likely prevent upstream movement of migratory adult bull trout.

Table 2. Locations (Latitude, Longitude) of redd count sections on the North and West Forks of Fish Creek.

	Upper Boundary		Lower Boundary	
N Fork - Section 1	N 46.9400	W 114.9165	N 46.9604	W 114.8754
N. Fork - Section II	N 46.9493	W 114.8518	N 46.9105	W 114.8159
W. Fork – Section I	N 46.8729	W 114.8143	N 46.9061	W 114.8056
W. Fork – Section II	N 46.8553	W 114.8358	N 46.8729	W 114.8143

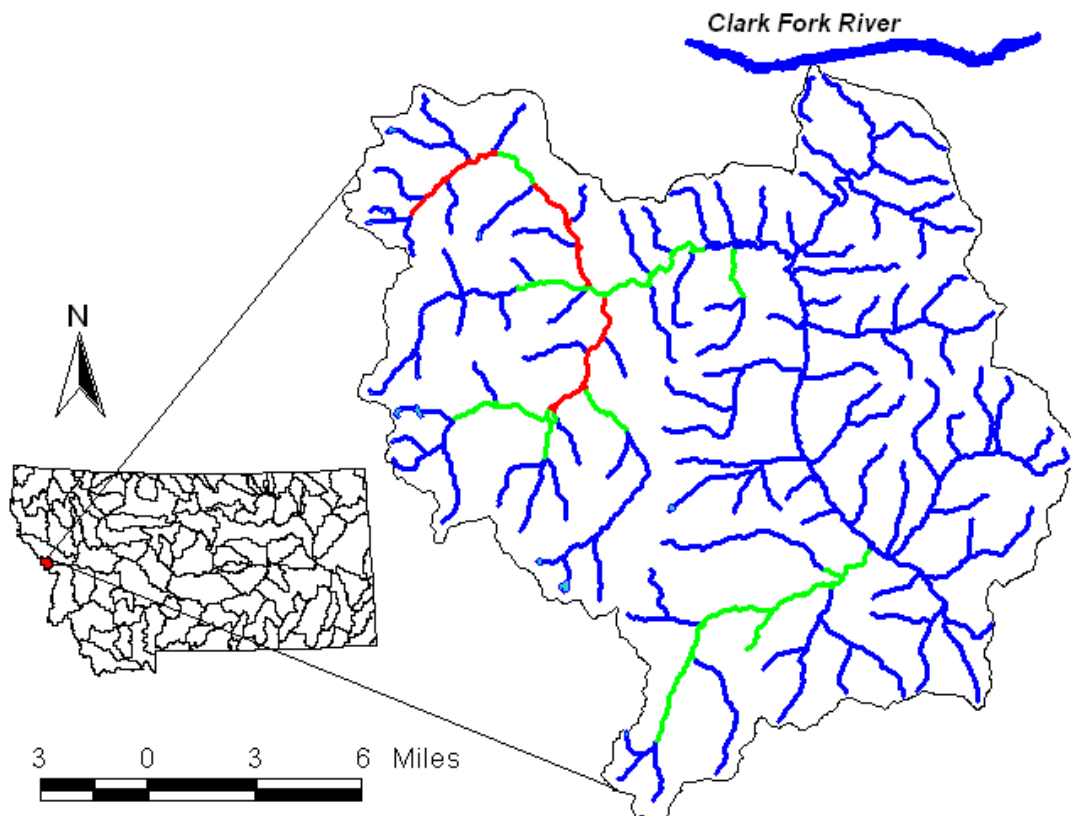


Figure 3. Location of bull trout redd count monitoring sections (red) and other areas surveyed for redds (green) in the Fish Creek drainage.

Although brook trout and brown trout are common in the South Fork and lower main stem of Fish Creek, neither of these species has been observed in the West Fork or North Fork within or near redd count index reaches (MFWP, unpublished data). Redd size distribution and the size of individuals sampled in electrofishing surveys indicate that Fish Creek bull trout populations are predominantly fluvial.

Radio-telemetry investigations in the middle Clark Fork Basin (see Chapter 1) included four adults that migrated to Fish Creek and attempted to spawn. Three of these fish spawned or ended migrations in redd count index sections in the North Fork (Section I) and West Fork (Sections I and II) of Fish Creek. In addition, one adult bull trout migrated from the Clark Fork River to Cache Creek, where it disappeared just prior to the spawning period.

Redd surveys were also completed throughout the main stem of Cache Creek in 2000, 2001, 2007 & 2008 from the mouth to near Pebble Creek (see Figure 3) where discharges were $> \sim 5$ cfs and habitat appeared suitable for spawning. Historically, Cache Creek supported a very large run of fluvial bull trout (MFWP, unpublished data and personal communications). No definitive redds were located in 2001, 2002, or 2007, although electrofishing surveys indicated low abundance of bull trout in middle and upper portions of the main stem. In 2008, one redd and one fluvial adult bull trout were observed in the upper main stem. Because of the low abundance of bull trout and redds, no index section was established in Cache Creek.

Total redd counts were stable to increasing from 2001 to 2009 in Fish Creek index sections (Figure 4), despite persistent drought from 2001-2007. Index reach I on the upper North Fork is particularly susceptible to low water conditions as instream flows were too low for fish passage

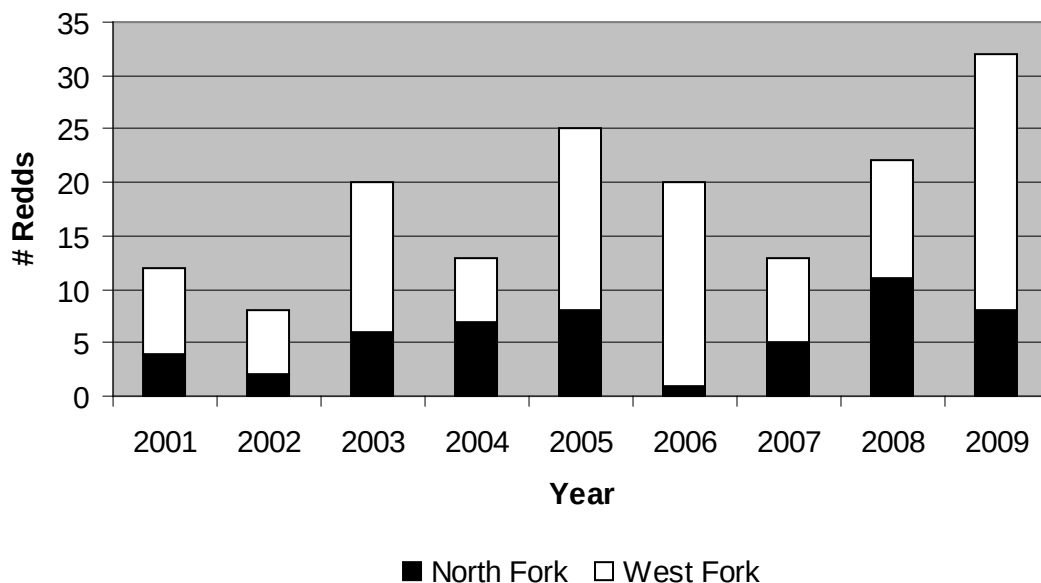


Figure 4. Annual bull trout redd counts in Fish Creek in 2001-2009. Results indicate the total count from the North Fork index sections (black) and the West Fork index sections (white).

or completely dry above the mouth of French Creek in at least four years during the survey period. Instream flows remained sufficient in the West Fork spawning reaches throughout the survey period. However, a portion of the main stem Fish Creek (West Fork near confluence with south Fork) was completely dewatered in July-September during low flow years. This likely affects the ability of some fluvial adults to reach spawning areas. Lack of instream flows and illegal harvest are two of the biggest challenges for recovery of bull trout in this drainage.

Redd Surveys on Cedar Creek

Initial redd surveys were completed throughout middle and upper Cedar Creek in 2001-2003. This included Oregon Gulch, Lost Creek and Cedar Creek downstream of Montreal Gulch. Juvenile bull trout were found in most of these reaches during electrofishing surveys (MFWP, unpublished data). No redd surveys were completed below RM 2 on the main stem as this segment supported limited surface flows in July-September in most years. Redds were found consistently in the portion of Oregon Gulch between the mouths of Lost Creek and main stem Cedar Creek; this reach was selected as the index section (Table 3; Figure 5). We also located bull trout redds on upper Cedar Creek between Montreal Gulch and Cayuse Gulch (2002, 2003, 2005) and in Lost Creek (2009). Redd abundance was low in these areas and the size of redds indicated that many were created by stream-resident fish. Overall, redd sizes in Cedar Creek indicated that the bull trout population is a mixture of stream-resident and fluvial forms.

Table 3. Location (Latitude, Longitude) of the redd count index section on Cedar Creek and locations where other redds have been observed.

	Upper Boundary		Lower Boundary	
Index Section	N 47.1280	W 115.0130	N 47.1432	W 114.9686
Other Location 1*	N 47.0784	W 115.0148	N 47.1258	W 115.0043
Other Location 2*	N 47.1328	W 115.0512	N 47.1280	W 115.0130

* Bull trout redds at locations 1&2 constructed predominantly by stream-resident fish.

Unlike most other tributaries in the middle Clark Fork Basin, the fish community of Cedar Creek is generally comprised of native fish. Although several headwater lakes contain brook trout and the main stem near the mouth supports low densities of rainbow trout and brown trout, these species have not been observed in the majority (> 90%) of the watershed. No introduced fish species have been documented in or near redd count index sections.

Total bull trout redd abundance in Cedar Creek was low and the geographic distribution of spawning was more variable than in other tributaries (Figure 6). Redd counts in 2002-2009 indicated that a concentration of spawning occurs just downstream of a large mining site near the confluence of Lost Creek and Oregon Gulch. Channel relocation around the mining site has resulted in late summer and fall dewatering in the section and likely inhibits upstream movement of bull trout in August and September in poor water years. A remediation project to correct this problem is planned for 2010-2011. Other reaches

of Cedar Creek and its tributaries have experienced similar impacts as this drainage was heavily mined in the early and mid- 1900s. Despite vast areas of physical habitat degradation, an extremely cold temperature regime in Cedar Creek has likely been key in maintaining bull trout population viability.

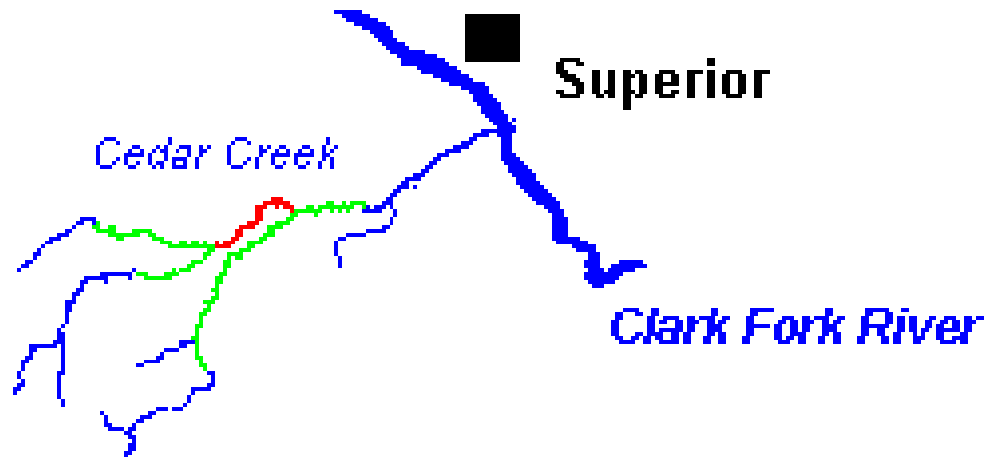


Figure 5. Location of bull trout redd count monitoring section (red) and other areas surveyed for redds (green) in the Cedar Creek drainage.

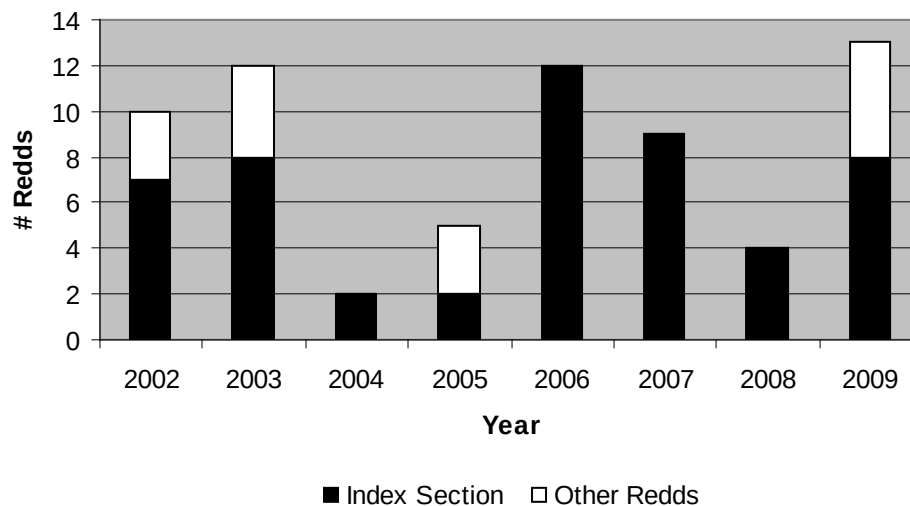


Figure 6. Annual bull trout redd counts in Cedar Creek in 2002-2009. Results indicate the total count from the index section (black) and additional redds found outside index sections (white).

Redd Surveys on Little Joe Creek

Although lower portions of Little Joe Creek are intermittent, electrofishing surveys suggested that juvenile bull trout were present throughout most of both forks (MFWP, unpublished data). Main stem sections of the South Fork and North Fork were evaluated in 2002-2004 to identify the primary spawning reaches upstream of dewatered sections (lower end of each fork and main stem below forks confluence; Figure 7). Both forks have similar hydrology and geomorphology, with high habitat complexity. Redd surveys indicated dispersed spawning activity throughout the middle and upper South Fork and more concentrated spawning in the lower half of the North Fork. The location of redd count index sections reflects this distribution (Table 4; Figure 7). Expanded redd surveys were completed throughout Little Joe Creek again in 2005 and no redds were observed outside the selected index reaches.

Table 4. Locations (Latitude, Longitude) of redd count sections on Little Joe Creek.

	Upper Boundary		Lower Boundary	
North Fork Section	N 47.2470	W 115.2198	N ~47.2701	W ~115.1554
South Fork Section	N 47.1864	W 115.2256	N ~47.2517	W ~115.1659

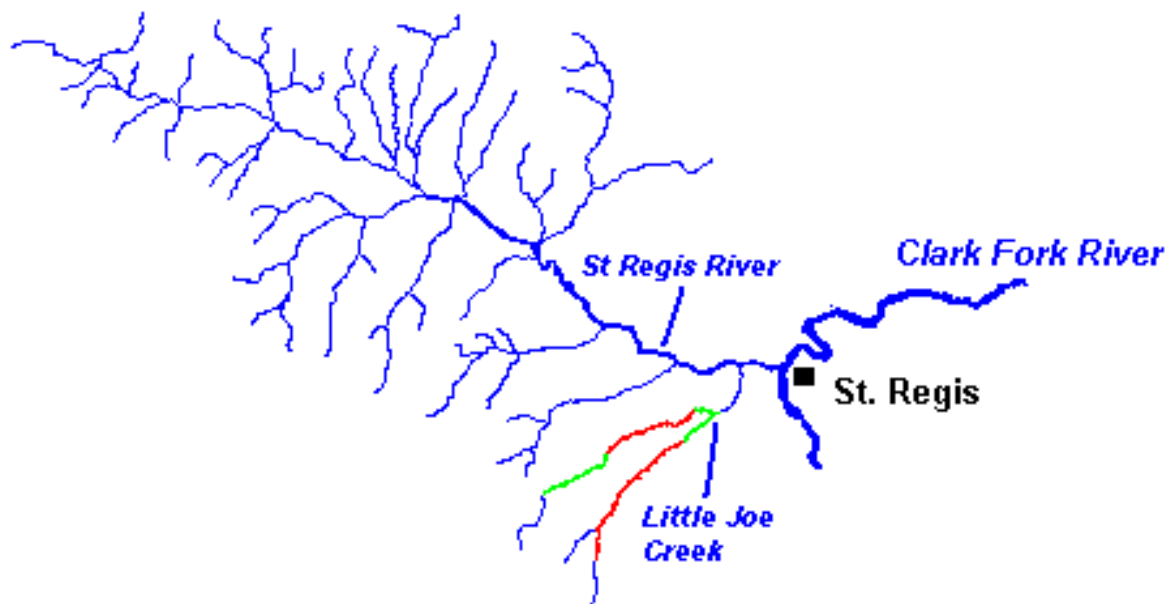


Figure 7. Location of bull trout redd count monitoring sections (red) and other areas surveyed for redds in the Fish Creek drainage (green).

Fluvial bull trout redds were more difficult to identify in Little Joe Creek relative to other tributaries in the middle Clark Fork drainage. Based on the size of redds and spawning adults observed, this bull trout population is a combination of fluvial and stream-resident forms. In addition, both forks support sympatric brook trout populations that appeared to be spawning in the same reaches (in some cases with bull trout) during redd surveys. Hybrid bull trout x brook trout have been identified visually in this stream (MFWP, unpublished data) and verified through genetic testing (Patrick DeHaan, USFWS Abernathy Fish Technology Center, personal communication).

Despite the challenges in conducting bull trout redd surveys on Little Joe Creek, counts in index sections were completed in five years from 2003-2009 (Figure 8). Redds were consistently more common in the South Fork, although the length of this index section is much longer than the North Fork section. Based on the data available, spawner numbers appear to be stable (Figure 8). However, the trend in redd abundance likely represents spawning activity by a combination of both fluvial and stream-resident fish, and is considered a less reliable index of fluvial population viability than surveys in Rattlesnake and Fish Creeks.

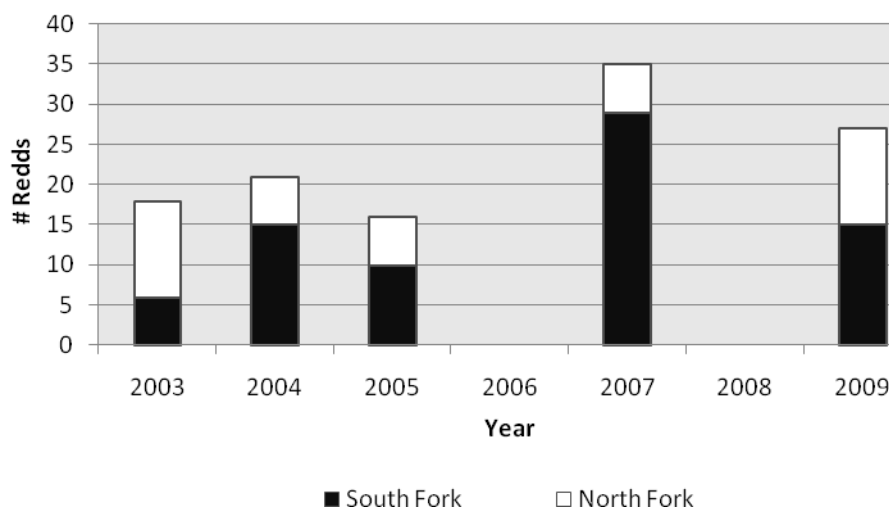


Figure 8. Annual bull trout redd counts in Little Joe Creek index sections in 2003-2009. Results from 2003 do not include the lower portion of the South Fork index section (not counted).

Summary and Management Recommendations

Redd counts serve as a useful index of adult escapement and spawning activity for fluvial bull trout in western Montana. In the middle Clark Fork basin, we used existing sampling information and widespread ground surveys to select redd survey reaches and continue to refine these reaches to help ensure that they are representative of population trends.

Redd count index sections were established on the four tributary systems where viable fluvial bull trout populations had been identified. As redd surveys were conducted from 1999-2009, it became apparent that spawning populations in Rattlesnake and Fish Creeks were predominantly migratory fish, while populations in Cedar and Little Joe Creeks are a combination of fluvial and stream-resident forms. In addition, brook trout are prevalent in Rattlesnake and Little Joe Creeks. This is a significant complicating factor in Little Joe Creek where the size distribution of adult bull trout and redds is generally smaller and brook trout appear to be more successful in interbreeding with bull trout.

In all four tributaries, overall redd abundance and time-series trends from the past decade suggest that bull trout populations are depressed, but stable. We strongly recommend that annual redd surveys continue in Rattlesnake Creek and Fish Creek as this is currently the only reliable tool in place to monitor fluvial adult escapement. Periodic redd counts (every 2-3 years) are also recommended on Cedar and Little Joe Creeks to confirm continued spawning activity and to help monitor ongoing restoration activities in these drainages.

Juvenile population estimates and periodic longitudinal electrofishing surveys should be instituted in tributaries to help track juvenile bull trout abundance and distribution. These tools would also be important for tracking the relative abundance and distribution of non-native trout that compete and hybridize with bull trout. Given the current suppressed status and limited resiliency of Clark Fork basin bull trout populations, short-term (e.g., drought) and long-term (e.g., climate change) environmental changes may directly threaten population viability and indirectly affect interactions with introduced species.

References

- Dunham, Jason; Rieman, Bruce; Davis, Kevin. 2001. Sources and magnitude of sampling error in redd counts for bull trout. *North American Journal of Fisheries Management* 21:343-352.
- Fraley, J. J., and B. B. Shepard. 1989. Life history, ecology, and population status of migratory bull trout (*Salvelinus confluentus*) in the Flathead Lake and River system, Montana. *Northwest Science* 63:133-143.
- Kondolf, G. M., and M. G. Wolman. 1993. The sizes of salmonid spawning gravels. *Water Resources Research* 29:2275-2285.
- Pierce, R. and C. Podner. 2006. A hierarchical assessment of bull trout spawning areas in the Blackfoot River basin: a baseline for identifying potential spawning areas. Montana Fish, Wildlife & Parks, Missoula, Montana.
- Spalding, S. 1997. Montana bull trout redd survey manual – including annotated bibliography. Montana Fish, Wildlife and Parks, Helena, Montana. 8 pp.

ONCORHYNCHUS GENETIC SAMPLING:
SURVEYS AND ANALYSES TO IDENTIFY NON-HYBRIDIZED
WESTSLOPE CUTTHROAT TROUT POPULATIONS IN MIDDLE CLARK FORK AND
LOWER BITTERROOT RIVER TRIBUTARIES

Background

Hybridization with closely related, introduced salmonids is one of the greatest threats to westslope cutthroat trout (*Oncorhynchus clarki lewisi*, WCT) and other native salmonid populations (Allendorf et al. 2001). In the case of westslope cutthroat trout populations in the upper Columbia River drainage, Yellowstone cutthroat trout (*O. clarki bouvieri*, YCT) and rainbow trout (*O. mykiss*, RBT) have historically been introduced in lakes, rivers and streams to supplement sport fisheries. Hybridization of native WCT has been identified in most locations where non-native *Oncorhynchus* were stocked. Other westslope cutthroat trout populations have concurrently been isolated by various anthropogenic or natural barriers that prevent hybridization. Populations above barriers are often non-hybridized unless the drainage contains one or more headwater lakes. Although these barriers help prevent introgression of WCT populations in the short term, they often prevent genetic exchange among local native populations and suppress migratory life history expressions (Rieman and Dunham 2000). Therefore, management and conservation of westslope cutthroat trout populations requires a balance of connectivity and isolation across different drainages and spatial scales (Fausch et al. 2009).

Identification of non-hybridized WCT populations is one of the basic requirements of native fish restoration and conservation planning in upper Columbia River watersheds. In an ongoing survey, we have systematically collected and genetically analyzed *Oncorhynchus* samples from tributary drainages of the middle Clark Fork River and lower Bitterroot River systems. This information was collected to assist in developing conservation plans, prioritizing fisheries enhancement projects and evaluating fish passage issues.

Methods

Fish were collected by backpack electrofishing tributary streams of the middle Clark Fork River and lower Bitterroot River from 1999-2010. Project boundaries were Rock Creek (upstream) and Flathead River (downstream) confluences in the middle Clark Fork drainage and from Florence Bridge to the mouth of the Bitterroot River. The distribution and number of sample sites per stream varied depending on the spatial scale, access, and observed species composition based on morphological characteristics. For example, genetic samples were not collected at sites where rainbow trout were obviously the predominant species, based on morphology. At sites where fish appeared to be hybrids (i.e., WCT x RBT or WCT x YCT), we collected samples from 3-5 individuals to verify hybridization. For *Oncorhynchus* populations where only WCT characteristics were identified, we attempted to collect a series of longitudinal samples within each drainage or tributary (e.g., low, med and high in the drainage). At each site, we collected samples from multiple size classes whenever possible to represent different age classes and generations. In general, we attempted to collect samples that were representative of the geographic distribution and population structure of *Oncorhynchus* spp. within each watershed.

The total target sample size for each tributary divided among several sites within it was a minimum of 22-25 randomly selected *Oncorhynchus spp.* individuals. This sample size was based on the probability of detecting as little as 1% hybridization with 95% confidence, given a known number of markers analyzed. Because the number of diagnostic loci varied with each laboratory technique (described below), required sample sizes to reach 95% confidence of detecting as little as 1% hybridization was slightly variable. In the Clark Fork River drainage, WCT hybridization with RBT is currently much more prevalent (greater risk) than with YCT.

Laboratory analyses were conducted at the University of Montana Conservation Genetics Laboratory under the direction of Robb Leary. Early samples (~1999) were analyzed using protein electrophoresis (allozymes), while all later analyses involved evaluation of DNA using paired interspersed nuclear elements (PINES) or a combination of insertion/deletion events (Indel loci) and microsatellite loci. The number of diagnostic loci that distinguish the *Oncorhynchus* species/sub-species varies with the three techniques (Table 1),

For most samples, we collected a small portion of the anal fin from each fish for DNA analyses (PINE and Indel/Microsatellite analyses). Whole fish were collected for allozyme (protein) analysis on Marshall Creek and Deer Creeks near East Missoula. Collection of fin clips is preferred in most cases because the technique is non-lethal and samples are easier to store for long periods. Whole fish must be frozen, while fin clips are stored in 95% non-denatured ethanol.

Table 1. Number of diagnostic loci available for paired comparisons of WCT with YCT or RBT using allozyme electrophoresis, paired interspersed nuclear elements (PINEs), and insetion/deletion events (Indel) with microsatellite loci (Microsatellites).

Analysis	Comparison	Number Diagnostic Loci	Probability Detecting 1% Introgression With N=25
Allozymes	WCT : YCT	12	0.998
	WCT : RBT	6	0.951
PINEs	WCT : YCT	4	0.866
	WCT : RBT	6	0.951
Indel/Microsatellites	WCT : YCT	8	0.982
	WCT : RBT	13	0.999

In all analyses, the relative genetic contribution of WCT, RBT and YCT was evaluated. For samples where only WCT markers were detected, the probability of detecting as little as 1% hybridization was reported (based on sample size and technique). For samples where markers from > 1 species were detected (typically RBT with WCT), the distribution and frequency of markers within the population was evaluated to determine the extent of hybridization. That is, whether the sample appeared to have come from a hybrid swarm, contained a mixture of WCT and only F₁ hybrids, etc.

When genetic testing indicated discrete differences in levels of hybridization among sites in a longitudinal sample, we often returned to these sites to increase the power of detecting hybridization (by increasing sample size) in suspected non-hybridized reaches. For instance, in drainages where a low level of hybridization was detected, upstream sites often exhibited only markers characteristic of westslope cutthroat trout while lower sites contained hybrids. In these instances, we returned to upper sites to supplement sample sizes (to reach 22-25) and increase power of detection for hybridizing species.

Results and Discussion

Oncorhynchus genetic samples were collected and tested at 346 sites in 160 stream reaches throughout middle Clark Fork River and lower Bitterroot River tributaries in 1999-2010 (Appendix I). These results, when combined with previous findings, provide a picture of the genetic status for westslope cutthroat populations in these drainages (Figures 1 & 2).

The relative WCT genetic contribution within samples and populations was highly variable throughout the project area, but generally increased from main stem areas to tributary headwaters (inversely related to stream order). This trend is expected because major rivers and most larger tributary reaches (lower main stems) were historically stocked with RBT (MFWP stocking records, 1932-1988). The salmonid species composition of these reaches is currently dominated by *Oncorhynchus spp.*, which are predominantly RBT x WCT hybrids. Limited genetic testing in main stem areas, as well as fish morphological characteristics, indicate that these *Oncorhynchus* populations are composed of hybrid fish with a wide range of WCT and RBT genetic contribution, as well as some individuals (<10%) which appear to be non-hybridized WCT (Schmetterling 2001, Ardren et al. 2008, MFWP unpublished data).

Consistent with the trend described above, *Oncorhynchus* species composition was predominantly WCT in smaller tributary drainages and the headwaters of larger tributaries (see Figures 1 & 2). Stream reaches with high (>90%) WCT genetic contribution were generally (1) excluded from historic rainbow trout stocking and (2) buffered from colonization by hybrid individuals from main stem populations. 'Buffers' included changes in physical habitat conditions (i.e., stream gradient, temperature, etc.), as well as complete barriers to upstream movement (i.e., culverts, dams, waterfalls). Historic mountain lake stocking was an additional factor affecting genetic composition in some drainages (Knotek and Thabes 2008; MFWP stocking records). Non-native RBT and YCT stocked in mountain lakes from ~1950s - 1990s colonized downstream reaches via lake outlets and hybridized with native WCT populations in Fish Creek, Trout Creek, Rattlesnake Creek and possibly other tributaries. This was the only scenario where YCT genetic markers were detected in our analyses.

Non-Hybridized WCT Populations

Throughout their historic range, WCT populations have been hybridized and displaced by introduced, non-native salmonids (Shepard et al. 2005). Despite termination of non-native salmonid stocking in the project areas, wild populations with significant RBT and YCT contributions continue to threaten the genetic integrity of non-introgressed WCT populations.

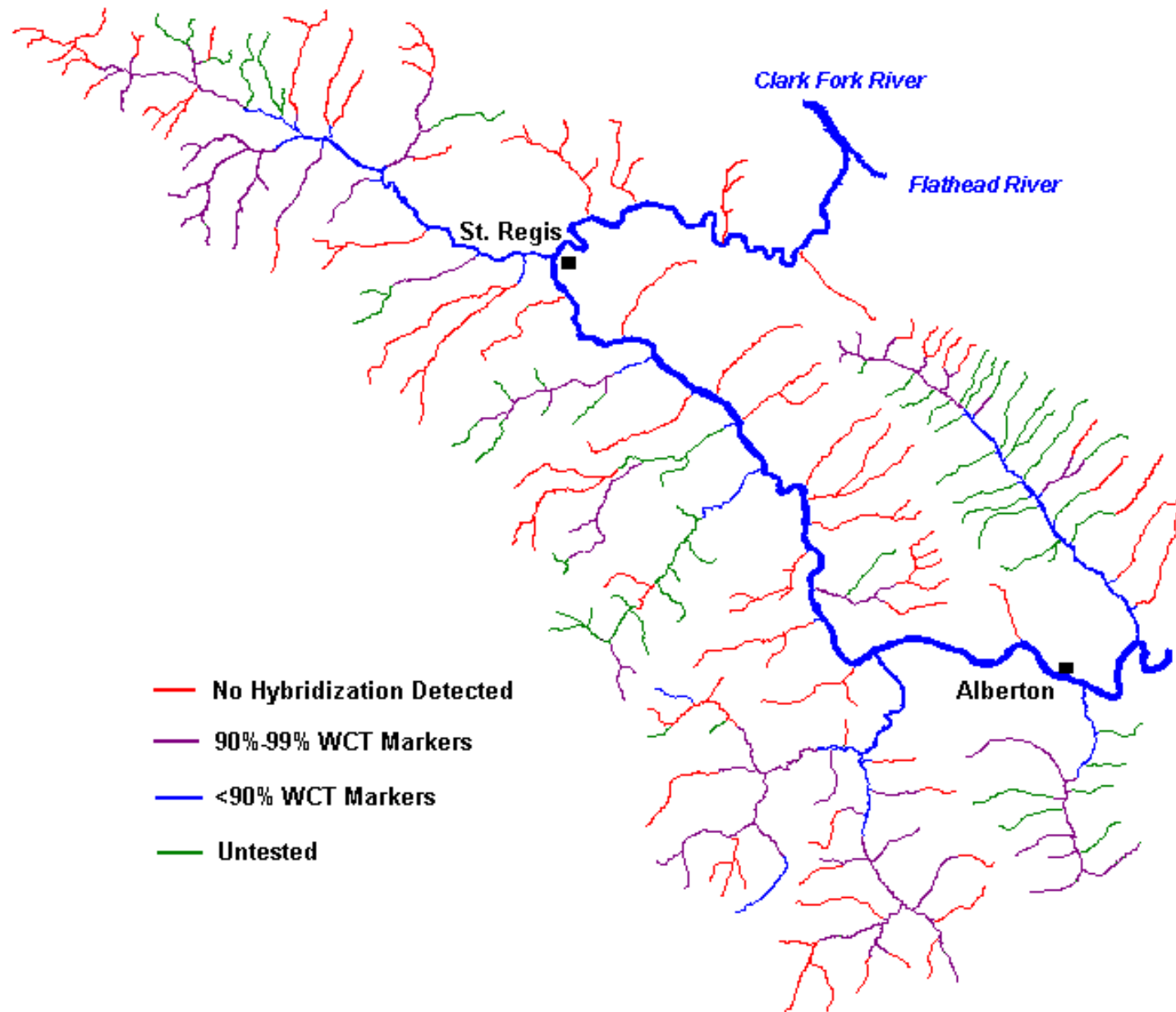


Figure 1. Geographic distribution of *Oncorhynchus* genetic testing results in the western portion of the middle Clark Fork region through 2010.

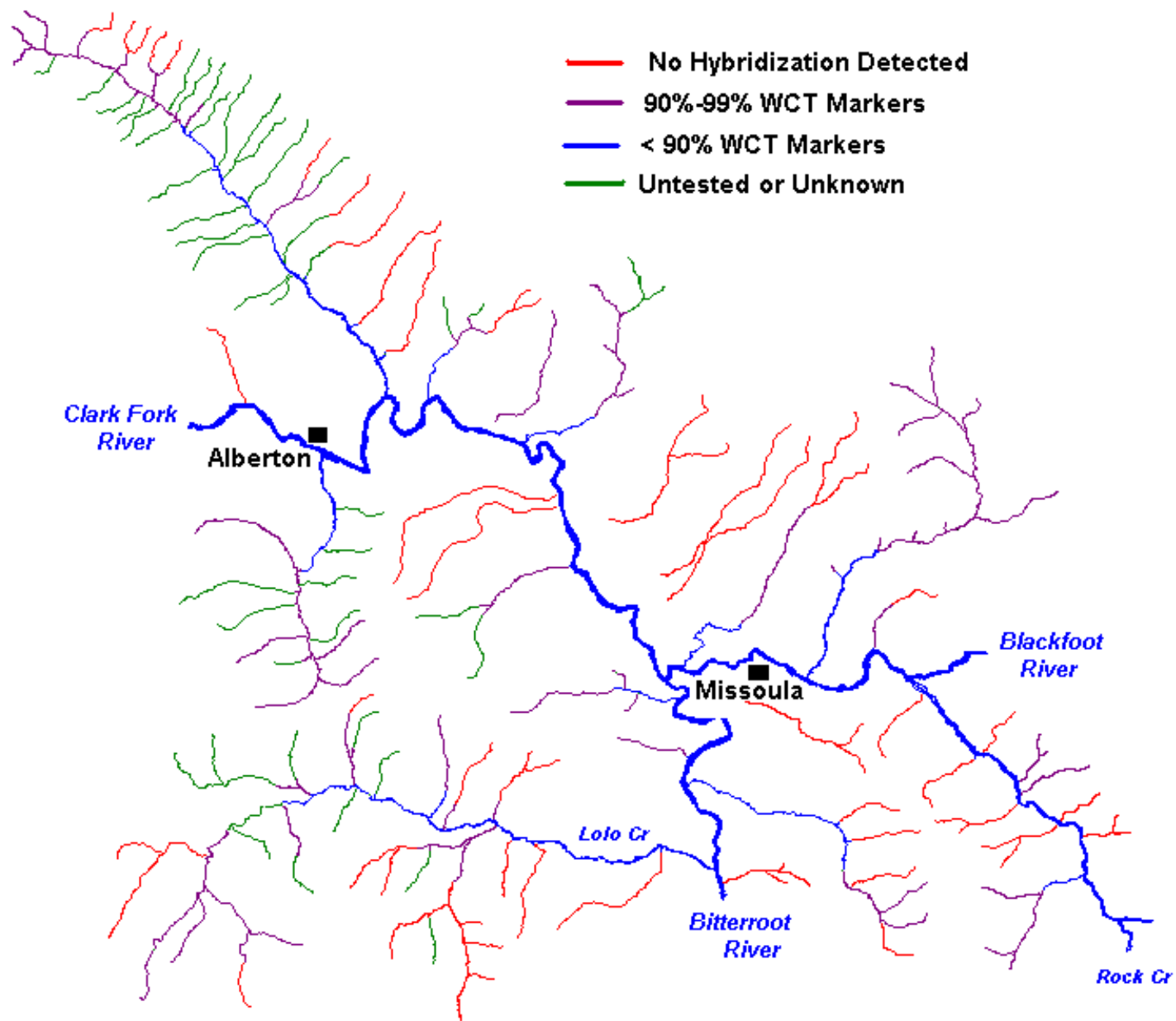


Figure 2. Geographic distribution of *Oncorhynchus* genetic testing results in the lower Bitterroot River drainage and eastern portion of the middle Clark Fork region through 2010.

In total, 89 population segments were identified in middle Clark Fork River and lower Bitterroot River tributaries where no hybridization was detected. In the majority of these samples, there was a >95% probability of detecting as little as 1% hybridization. However, sample size was inadequate in some cases, which resulted in lower power of detection (see Appendix I). Increasing sample size and power of detection will be a priority in future analyses of these populations. All populations that displayed only WCT markers were considered non-hybridized, regardless of the power of detection.

Two primary objectives of this study were to identify non-hybridized WCT populations and, if possible, document the mechanism preventing hybridization. In most cases (~53), a physical barrier or suspected barrier was identified that isolated the WCT population from hybrids downstream (Tables 2 & 3; Figures 3 & 4). Current management of these non-hybridized WCT populations includes maintenance of existing barriers and attempted confirmation of isolating mechanisms where suspected.

In some tributary systems and stream segments (~36), there was no apparent physical barrier impeding the upstream movement of hybridizing species (i.e., RBTxWCT) into the non-hybridized WCT population. The mechanisms preventing hybridization in these situations are unknown, but these populations have remained non-introgressed, despite 50-80 years of open access for RBT and their hybrids. Factors such as spawning behavior, water temperature, gradient, level of habitat degradation and discharge have been suggested as isolating mechanisms (Shepard et al. 2005). However, some maintain that hybridization is imminent when fish movement and genetic exchange are possible (Robb Leary, University of Montana Wild Trout and Salmon Genetics Laboratory, personal communication).

Future evaluations and sampling in the project area will focus on (1) increasing sample size for WCT population segments where no evidence of hybridization has been detected, but power of detection is < 95%, (2) sample collection and analysis of un-tested populations and (3) confirmation of physical upstream passage barriers where non-hybridized populations are suspected to be isolates.

Table 2. List of tributary WCT population segments in the lower Bitterroot River region with no evidence of hybridization and documented or suspected physical isolation from hybridizing species.

Population Segment	Connectivity	Isolating Mechanism
Bear Run Cr (Miller Cr)	Isolate	Culvert
Davis Creek	Isolate	Dewatering/Culvert
Dick Cr (SF Lolo Cr)	Suspected Isolate	?
Eightmile Creek	Isolate	Diversion Dam/Dewatering
Johnny Cr (SF Lolo Cr)	Suspected Isolate	?
Little Park Cr (Miller Cr)	Isolate	Culvert
Mill Cr (Lolo Cr)	Suspected Isolate	?
Mormon Creek	Suspected Isolate	?
Park Creek (Miller Cr)	Isolate	Culvert
SF Lolo Creek (upper)	Suspected Isolate	?
Tevis Cr (Lolo Cr)	Suspected Isolate	?

Table 3. List of tributary WCT population segments in the middle Clark Fork River region with no evidence of hybridization and documented or suspected physical isolation from hybridizing species.

Population Segment	Connectivity	Isolating Mechanism
Albert Creek	Isolate	Culvert/Diversion Dam
Allen Creek	Suspected Isolate	?
Butler Creek	Isolate	Irrigation Ditch
Butler Creek - Upper (Ninemile trib)	Isolate	Dam
Cold Creek	Isolate	Culvert/Dewatering
Cramer Creek	Isolate	Culverts
Crystal Creek	Isolate	Culvert
Deep Creek	Isolate	Culvert/Dewatering
Dirty Ike Creek	Isolate	Culvert/Dewatering
E. Twin Cr (St. Regis trib)	Isolate	Culvert
First Creek	Isolate	Culvert/Dewatering
Flat Creek	Isolate	Culvert
Greenough Creek	Suspected Isolate	?
Johnson Creek	Isolate	Culvert/Dewatering
LaValle Creek	Isolate	Irrigation Ditch
L. McCormick Cr. - Upper	Isolate	Check Dam
Marshall Cr - Above pond	Isolate	Dam
Meadow Creek	Isolate	Culvert/Dewatering
O'Keefe Creek	Isolate	Dewatering/Culvert
Patrick Cr	Isolate	Dewatering/Culvert
Pattee Creek	Isolate	Dam/Diversion
Quartz Creek	Suspected Isolate	Cascades/Falls
Rock Cr (near Stone-Container)	Suspected Isolate	Culvert/Dewatering
Rock Cr (Ninemile trib)	Suspected Isolate	?
Rock Creek (near Fish Cr)	Isolate	Culverts
Ryan Creek	Isolate	Irrigation Ditch/Culvert
Second Creek	Isolate	Culvert/Dewatering
Sevenmile Cr	Isolate	Culvert/Dam
Siegel Cr	Suspected Isolate	Culvert/Dewatering
Silver Creek	Isolate	Culvert
Slowey Gulch	Isolate	Culvert/Dewatering
Stony Cr (Ninemile trib)	Suspected Isolate	?
Straight Cr - Upper (Fish Cr trib)	Isolate	Series of Falls
Tamarack Cr	Isolate	Culvert
Thompson Creek	Isolate	Culvert/Dewatering
Trail Creek (Fish Cr trib)	Isolate	Culverts
Turah Creek	Isolate	Culvert/Dewatering
Upper St. Regis	Isolate	Culverts
W. Twin Cr (St. Regis trib)	Isolate	Dam
Wall Canyon Cr (Fish Cr trib)	Suspected Isolate	Culvert/Dewatering
Wallace Creek	Isolate	Culverts
West Mountain Creek	Isolate	Culvert/Dewatering

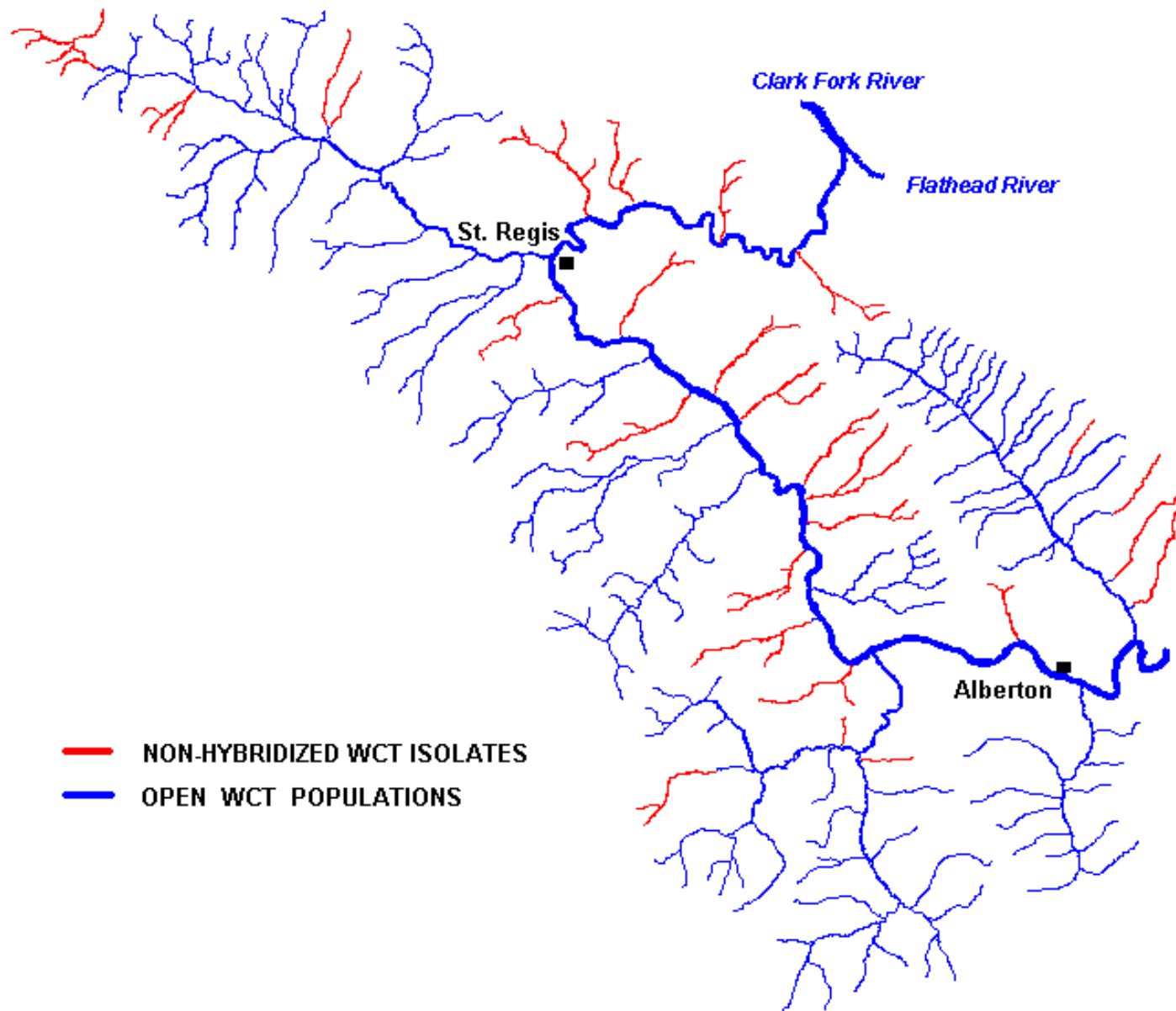


Figure 3. Geographic distribution of identified and suspected non-hybridized WCT population isolates in the western portion of the Middle Clark Fork River drainage.

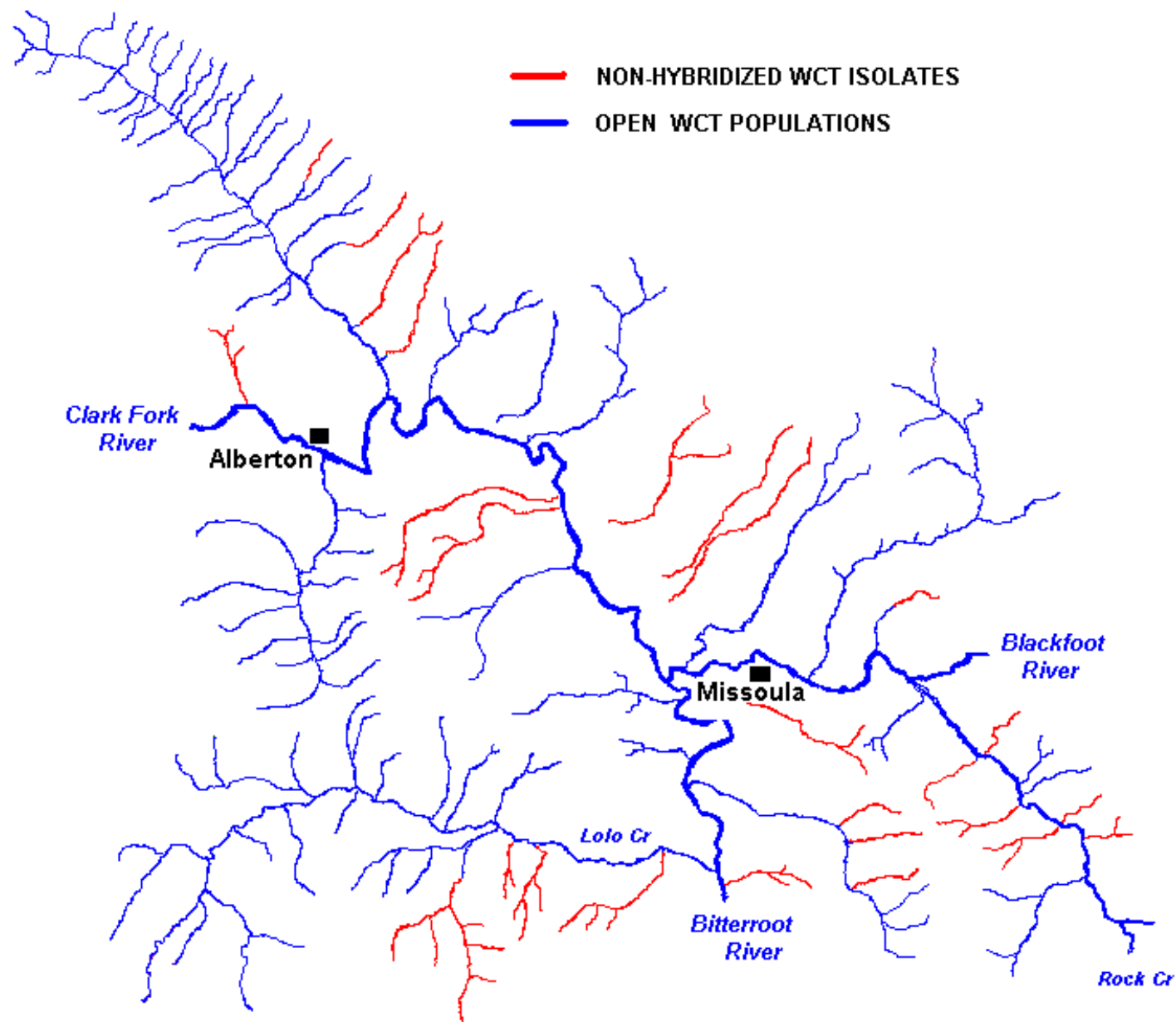


Figure 4. Geographic distribution of identified and suspected non-hybridized WCT population isolates in the lower Bitterroot River and eastern portion of the middle Clark Fork River drainages.

This investigation focused on the identification of remaining non-hybridized WCT populations, as well as the geographic distribution and level of hybridization for introgressed populations in the middle Clark Fork and lower Bitterroot River drainages. In addition to the threats posed by hybridization, endemic WCT also face threats created by continued mountain lake stocking and displacement by non-hybridizing species.

Although non-hybridized WCT are now the only species stocked in mountain lakes within the project area, these fish have a diverse genetic make-up which primarily originated in the South Fork Flathead River drainage (M012 Stock; MFWP Hatchery data). The impacts of stocked lake emigrants on the genetic structure of native WCT populations downstream is unknown. MFWP is currently developing sources of triploid (sterile) WCT and RBT in hatcheries, which could be used to alleviate these concerns.

Competition and displacement by non-hybridizing, introduced species (i.e., brook trout, *Salvelinus fontinalis*) is currently a larger concern in many tributaries. These populations were introduced into mountain lakes and streams in ~1940-1960 and established wild populations in many tributary systems. Eliminating or suppressing these populations is a major challenge for fisheries managers and preventing their expansion is a priority for native fish conservation.

Literature Cited

- Allendorf, F.W., R.F. Leary, P. Spruell and J.K. Wenburg. 2001. The problems with hybrids: Setting conservation guidelines. *Trends in Ecology and Evolution* 16(11): 613-622.
- Ardren, W, M. Diggs, and S. Bernall. 2008. Genetic analysis of westslope cutthroat trout at Cabinet Gorge Dam and Noxon Rapids Dam: Geographic origins, hybridization and management implications. Final Report to Avista Corporation – Agreement No. R-35343. U.S. Fish and Wildlife Service, Abernathy Fish Technology Center Conservation Genetic Program, Longview, Washington.
- Fausch, K.D., B.E. Rieman, J.B. Dunham, M.K. Young and D.P. Peterson. 2009. Invasion versus isolation: Tradeoffs in managing native salmonids with barriers to upstream movement. *Conservation Biology* 23(4):859-870.
- Knotek, W.L. and J.C. Thabes. 2008. Mountain lake surveys and management plan for the middle Clark Fork region of the Bitterroot Mountains. Montana Fish, Wildlife & Parks, Missoula, Montana.
- Rieman, B.E. and J.B. Dunham. 2000. Metapopulations and Salmonids: a synthesis of life history patterns and empirical observations. *Ecology of Freshwater Fish* 9:51-54.
- Schmetterling, D.A. 2001. Seasonal movements of fluvial westslope cutthroat trout in the Blackfoot River drainage, Montana. *North American Journal of Fisheries Management* 21:507-520.
- Shepard, B.B., B.E. May, and W. Urie. 2005. Status and conservation of westslope cutthroat trout within the western United States. *North American Journal of Fisheries Management* 25:1426-1440.

APPENDIX I

Results of *Onchorynchus* genetic testing in middle Clark Fork River and lower Bitterroot River tributaries in 1999-2010 using DNA and allozyme analyses.

**WESTSLOPE CUTTHROAT TROUT GENETIC SAMPLING
MIDDLE CLARK FORK RIVER TRIBUTARIES**

Table 1. Results of *Onchorynchus* genetic testing in middle Clark Fork River tributaries in 1999-2010 using DNA and allozyme analyses. Percent WCT denotes the contribution of westslope cutthroat trout alleles as a percentage of the entire sample.

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power [#] (%)	% WCT	Hyb. Species	Comments
Albert Creek	2283	3	22	T13N R22W S3 (headwaters)	T14N R21W S17 (intermittent reach)	93	100	-	
Allen Creek	2988	2	27	T12N R18W S26 (headwaters)	T12N R17W S18 (mouth)	96	100	-	
Butler Creek	3845-3847 1988(#237)	4	56	T15N R19W S33 (headwaters)	T14N R20W S26 (I-90)	>99	100	-	
Cedar Creek	2904	3	27	T15N R27W S20 (headwaters)	T16N R27W S27 (above Oregon G)	-	99	RBT	
Cold Creek	3094	2	24	T17N R28W S15 (headwaters)	T17N R27W S6 (intermittent reach)	94	100	-	
Cramer Creek	3985	3	24	T12N R15W S22 (headwaters)	T11N R16W S10 (I-90 Culvert)	>98	100	-	
Crystal Creek	2905	3	27	T12N R18W S16 (headwaters)	T12N R18W S11 (near mouth)	98	100	-	
Deep Creek (Huson)	2880	3	24	T13N R21W S20 (headwaters)	T13N R21W S4 (to intermittent)	-	95	RBT	
Deep Creek (Superior)	2284	2	25	T16N R24W S30 (headwaters)	T16N R25W S34 (intermittent reach)	95	100	-	
Deer Creek (E. Missoula)	2743	3	51	T12N R18W S7 (headwaters)	T13N R18W S28 Deer Cr. Rd Xing	99	100	-	
Dirty Ike Creek	3281	2	25	T12N R17W S11 (headwaters)	T12N R17W S16 (Interstate-90)	95	100	-	
Donovan Creek	3282	2	27	T12N R17W S3 (headwaters)	T12N R17W S17 (Interstate-90)	-	97	RBT	
Dry Creek (Superior)	2739	6	43	T16N R28W S9 (headwater forks)	T17N R27W S28 (dry reach)	-	98	RBT	

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power [#] (%)	% WCT	Hyb. Species	Comments
First Creek	2258	3	17	T17N R25W S36 (headwaters)	T16N R25W S16 (intermittent reach)	75	100	-	
FISH CREEK									
Bear Cr. (Fish Cr. trib)	3458	3	15	T13N R25W S11 (near headwaters)	T14N R25W S35 (below rd xings)	-	92	RBT	Hybrids at all 3 sites
Burdette Creek (Fish Cr. trib)	3455-3457	3	20	T13N R24W S25 (headwaters)	T12N R24W S9 (near FS boundary)	-	94	RBT	Upper site WCT markers; lower hybrids
Cache Creek (Fish Cr. trib)	3462	1	9	T12N R25W S24 (middle main stem)	T12N R24W S8 (near mouth)	-	>90	RBT	WCT + 3 hybrids Consist w/ #588 (1991)
Cache Cr-upper (Fish Cr. trib)	3532-3533	3	23	T11N R25W S9 (headwaters)	T12N R25W S22 (near mouth Irish)	-	>99.8	RBT?	All pure WCT with 1 questionable allele
Cedar Log Cr. (WF Fish trib)	3977-3978	2	14	T12N R25W S13 (below lakes)	T13N R25W S19 (above mouth)	-	85- 94%	YCT	Hybrid Swarm
Deer Cr- upper (Fish Cr. trib)	3466/2037	2	10	T13N R24W S11 (headwaters)	T13N R24W S10 (~ FS boundary)	>70	100	-	
Deer Creek- lower (Fish Cr. trib)	3467/2037	4	15	T13N R24W S10 (~ FS boundary)	T13N R24W S7 (mouth)	-	>95	RBT	Hybrid invasion after fire
Fletcher Gulch (N Fk Fish Cr)	3482	1	7	T14N R26W S30 (headwaters)	T14N R26W S29 (near mouth)	>68	100	-	
French Creek (N Fk Fish Cr trib)	3483	1	7	T14N R26W S18 (French Lake)	T14N R26W S21 (near mouth)	-	36	RBT	French L outlet
Greenwood Cr. (N Fk Fish Cr)	3484	1	7	T14N R26W S15 (headwaters)	T14N R26W S22 (near mouth)	>68	100	-	
Indian Creek (Fish Cr. trib)	3497, 3542-3543	4	25	T12N R26W S11 (headwaters)	T13N R26W S25 (near mouth)	>97	100	-	
Lupine Cr. (Fish Cr. trib)	3476-3478	3	20	T12N R24W S12 (headwaters)	T12N R24W S15 (near FS boundary)	>96	100	-	WCT allele freq distinct at all sites
Montana Cr (Cache Cr trib)	3459-3460, 3518	3	26	T12N R25W S10 (headwaters)	T12N R24W S18 (near mouth)	>95	100	-	
N Fork Fish Cr. (Fish Cr trib)	3480-3481	3	21	T14N R26W S29 (below Trio outlet)	T14N R26W S26 (near Crater mouth)	>96	100	-	Additional site submitted

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power [#] (%)	% WCT	Hyb. Species	Comments
FISH CR. (cont)									
NF Fish Cr - lower (Fish Cr trib)	3541	1	10	T14N R26W S25 (below Crater Cr.)	T13N R26W S1 (near mouth)	-	>95	RBT	WCT + 1 hybrid at site below Crater Cr
Oriole Cr. (Fish Cr. trib)	1490	3	26	T12N R24W S27 (headwaters)	T12N R24W S22 (below rd xing)	96	100	-	
Straight C.-upper (Fish Cr. trib)	1960-1961	4	26	T13N R26W S17 (headwaters)	T13N R26W S2 (lowest falls)	96	100	-	
Straight Cr. -lower (Fish Cr. trib)	1960-1961	1	7	T13N R26W S2 (lowest falls)	T13N R26W S1 (near mouth)	-	>90	RBT	
Surveyors Creek (Fish Cr. trib)	3071/2031	2	16	T13N R25W S36 (below rd xings)	T13N R24W S31 (near mouth)	-	>90	RBT	
Surveyors Cr.-NF (Fish Cr. trib)	3071/2031	3	25	T13N R25W S34 (near headwaters)	T13N R25W S36 (Above rd xings)	-	>90	RBT/YCT lower site	
Surveyors Cr.-SF (Fish Cr. trib)	3071/2031	2	23	T12N R25W S4 (Below Lake)	T13N R25W S36 (Above rd xings)	>90	100		
Trail Creek (Fish Cr. trib)	1515	3	25	T14N R25W S23 (headwaters)	T14N R25W S35 (below rd xings)	95	100	-	
Thompson Cr.-lower (Fish Cr.)	3487/3549	2	17	T13N R25W S23	T13N R24W S18 (near mouth)	-	>95	RBT	1 hybrid detected at mid & lower sites
Thompson Cr.-upper (Fish Cr.)	3488-3489	2	14	T13N R 25W S26 (headwaters)	T13N R25W S23	>68	100		
Wall Canyon Cr. (Fish Cr trib)	3986-3987	2	26	T14N R24W S32 (top fish-bearing)	T14N R24W S31 (just above mouth)	>98	100	-	Small population - mouth dewatered
White Creek (Cache Cr trib)	3461, 3539-3540	3	25	T12N R24W S30 (lower forks)	T12N R24W S18 (near mouth)	-	>95	RBT	All pure WCT with 1 hybrid at each site
Wig Creek (Fish Cr. trib)	3852	2	10	T13N R24W S20 (start perennial)	T13N R24W S31 (near mouth)	-	>95	RBT	Poor DNA extraction
West Fork Fish Cr - Upper (Fish Cr)	3493	2	14	T13N R26W S28 (near headwaters)	T13N R26W S25 (to Indian Cr)	-	98.4	RBT YCT	Consistent with previous sample #772
Flat Creek	3351	3	26	T17N R25W S5 (headwaters)	T17N R26W S27 (mouth)	98	100	-	

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power [#] (%)	% WCT	Hyb. Species	Comments
Greenough Creek	2907	2	25	T12N R18W S 34/35(headwaters)	T12N R17W S29 (near mouth)	97	100	-	
Johnson Creek	1957	3	25	T17N R25W S20 (headwaters)	T17N R25W S31	96	100	-	
Kendall Creek	3283	2	26	T13N R17W S32 (headwaters)	T12N R17W S7 (Interstate-90)	-	>97	RBT	
Lavalle Creek	2895	2	24	T15N R19W S32 (headwaters)	T14N R20W S26 (I-90)	94	100	-	
Marshall Creek (upper)	2741/2742	2	24	T14N R18W S33 (headwaters)	T13N R18W S6 (Moye pond)	94	100	-	WCT pure above Moye pond
Marshall Creek (lower)	2741/2742	2	31	T13N R18W S6 (Moye pond)	T13N R18W S18 (mouth)	-	95	RBT	
Mill Creek	3479	2	15	T16N R21W S25 (middle Bear G.)	T15N R20W S6 (confl w/ Mill Cr)	-	99	RBT	1 hybrid only- upper site; 1980 lower Mill Cr -79%
Nemote Cr. – Upper Forks	2113-2115 1963, 2003	4	27	T15N R24W S9/16 (headwaters)	T15N R25W S24 (forks confl.)	96	100	-	
Nemote Cr. – South Fork	4060, 2003	3	>22	T15N R24W S14 (headwaters)	T15N R24W S19 (near confluence)	>99	100	-	
Nemote Creek (lower)	2113-2115	2	15	T15N R25W S24 (forks confl.)	T15N R25W S16 (frontage road)	-	93	RBT	
NINEMILE CR									
Beecher Cr - lower (Ninemile Cr trib)	3853	1	8	T17N R24W S9 (forks confluence)	T17N R24W S16 (near mouth)	-	>95	RBT	
Beecher Cr - WF (Ninemile C trib)	3854	1	7	T17N R24W S4 (headwaters)	T17N R24W S9 (foothills Rd)	84	100	-	
Beecher Cr - EF (Ninemile C trib)	3855	1	9	T17N R24W S3 (headwaters)	T17N R24W S10 (foothills Rd)	90	100	-	
Big Blue C- lower (Ninemile Cr. trib)	3848-3849	2	23	T17N R23W S30 (Foothills Rd)	T17N R24W S36 (near mouth)	-	>95	RBT	>99% WCT @ Foothills Rd
Burnt Fk Cr -WF (Ninemile C trib)	3860	1	7	T17N R24W S10 (headwaters)	T17N R24W S15 (foothills Rd)	84	100	-	

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power [#] (%)	% WCT	Hyb. Species	Comments
NINEMILE CR	(cont)								
Burnt Fk Cr- EF (Ninemile C trib)	3861	1	8	T17N R24W S11 (headwaters)	T17N R24W S14 (foothills Rd)	88	100	-	
Burnt Fk Cr. - low (Ninemile Cr. trib)	3859	1	8	T17N R24W S15 (below Foothill Rd)	T17N R24W S22 (Ninemile Rd)	-	>95	RBT	
Butler Creek (Ninemile C trib)	3506	3	30	T16N R22W S9 (headwaters)	T16N R22W S20 (Just below dam)	>97	100	-	Above and below dam
Devil's Creek (Ninemile Cr trib)	3499	1	8	T17N R25W S10 (headwaters)	T17N R25W S13 (upper main stem)	-	96	RBT	
Eustache Creek (Ninemile Cr trib)	3500-3501	2	17	T17N R25W S2 (headwaters)	T17N R25W S13 (upper main stem)	-	>93	RBT	
Kennedy Creek – Upper (Ninemile)	3813-3814	2	22	T16N R22W S7 (headwaters)	T16N R23W S13 (upper main stem)	>99	100	-	
Lit. McCormick-Upper (Ninemile)	3824	1	14	T17N R23W S36 (headwaters)	T16N R23W S11 (check dam)	97	100	-	Above mining check dam
Little McCormick-lower (Ninemile)	3821-3823	3	24	T16N R23W S11 (check dam)	T16N R23W S15 (mouth)	-	96-99	RBT	
McCormick Creek (Ninemile Cr trib)	3825-3826	2	14	T16N R23W S1	T16N R23W S21 (near mouth)	-	>95	RBT	
Ninemile Creek (upper main stem)	3501	1	7	T17N R24W S18	T17N R24W S17	-	>95	RBT	Trouble extracting DNA -definitely hybridized
Rock Creek (Ninemile C trib)	3290	2	25	T16N R22W S34 (above culvert)	T15N R22W S16 (below culvert)	95	100	-	
U. St. Louis Cr. (Ninemile C trib)	3862	2	10	T17N R24W S5 (headwaters)	T17N R24W S8	93	100	-	
Stony Creek (Ninemile C trib)	3293, 3276	2	26	T16N R22W S22 (headwaters)	T15N R22W S5 (near FS boundary)	97	100	-	
Meadow Cr	2897	2	27	T15N R26W S26 (headwaters)	T15N R25W S19 (dewatered reach)	98	100	-	
Patrick Cr.	3096	2	25	T19N R26W S35 (headwaters)	T18N R26W S15 (near mouth)	95	100	-	

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power [#] (%)	% WCT	Hyb. Species	Comments
Pattee Creek	2281/2151 3989/3990	3	23	T12N R19W S2 (headwaters)	T12N R19W S4 (Higgins Pond)	>99	100	-	Hybridized below pond
PETTY CREEK									
Bills Creek (Petty Cr trib)	3693	1	8	T13N R22W S19	T13N R23W S25 (near mouth)	-	>99	RBT	Hybrid swarm
Johns Creek (Petty Cr trib)	3690	1	8	T13N R23W S23/24	T13N R23W S24 (near mouth)	-	>99	RBT	Hybrid swarm
Petty Creek – East Fork	3691	1	8	T13N R22W S28/29	T13N R22W S31 (near mouth)	-	>99	RBT	1 fish with RBT markers
Petty Creek – South Fork	3692	1	14	T12N R23W S3	T13N R22W S31 (near mouth)	-	>99	RBT	1 fish with RBT markers
Petty Creek – West Fork	3689	1	8	T14N R23W S30 (headwaters)	T14N R23W S35 (near mouth)	-	>99	RBT	
Quartz Cr	2899	3	27	T14N R26W S3 (headwaters)	T15N R25W S32 (dewatered section)	98	100	-	
Rattlesnake Creek (upper)	3368	4	28	T15N R18W S21 (confl. upper tribs)	T14N R18W S11 (just above canyon)	-	98	RBT YCT	2% RBT, <1% YCT
Rattlesnake Creek (lower)	2271	1	24	T13N R19W S2 Mtn Water Dam	T13N R19W S2 USFS Bridge	-	61	RBT	
Rock Creek (Huson)	2882	3	25	T13N R22W S2 (headwaters)	T14N R21W S21 (to intermittent)	97	100	-	
Rock Creek (Alberton)	1959	3	24	T14N R25W S17 (headwaters)	T14N R25W S12 (above barrier)	94	100	-	
Roman Creek (Huson)	2979	2	26	T15N R21W S15	T15N R21W S28 (I-90)	-	96.5	RBT	
Ryan Creek	3301	2	26	T11N R15W S5 (below reservoir)	T11N R15W S7 (mouth of canyon)	95	100	-	
Second Creek	2883	2	27	T16N R25W S13/14 (Head)	T16N R25W S22	98	100	-	

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power [#] (%)	% WCT	Hyb. Species	Comments
Sevenmile Creek	2285	2	26	T19N R27W S27 (lower USFS)	T18N R27W S2 (above barrier)	96	100	-	
Siegel Creek	2033/ 93-046	2	24	T17N R25W S1 (headwaters)	T18N R25W S27	94	100	-	Small population
Sixmile Creek (lower)	2978	2	15	T15N R22W S12	T15N R22W S26	-	<90	RBT	
Sixmile Creek (upper)	2978	1	12	T15N R21W S5 (headwaters)	T15N R21W S7	77	100	-	
Slowey Gulch	2272	3	23	T18N R26W S30 (near headwaters)	T17N R27W S1 (Little Pittsburgh)	94	100		
Swartz Creek	2700	5	51	T11N R18W S24 /25 (headwaters)	T12N R17W S34 (near mouth)	-	85	RBT	
Tamarack Cr.	1958	3	25	T19N R28W S18 /22 (headwaters)	T18N R27W S9 (above barrier)	96	100	-	
Thompson Cr.	3093	2	30	T16N R27W S16 (headwaters)	T17N R26W S32	97	100	-	
Trout Creek - (Deep Creek)	3087	2	41	T15N R27W S14 (Headwaters)	T15N R27W S24 (Rd culvert)	99	100		Consistent with Sample #422 (1990)
Tura Creek	3284	2	15	T13N R17W S31 (headwaters)	T12N R18W S2 (Interstate 90)	43	100	-	
Wallace Creek	3280	2	26	T12N R16W S18 (headwaters)	T12N R17W S23 (Interstate-90)	93	100	-	
West Mountain Creek	2902	2	27	T15N R23W S17 (headwaters)	T15N R23W S29 (near mouth)	98	100	-	
ST. REGIS R.									
Big Cr. – E Fork (St Regis R. trib)	3380	2	16	T18N R30W S17 (near headwaters)	T19N R30W S33 (forks confluence)	-	>90	RBT	4 Hybrids at upper sample site
Big Cr. – M Fork (St Regis R. trib)	3375	2	16	T18N R31W S13 (headwaters)	T19N R30W S33 (forks confluence)	-	94	RBT	2 hybrids at lower sample site only
Big Cr. – W Fork (St Regis R. trib)	2928	3	26	T19N R31W S36	T19N R30W S33 (forks confluence)	-	94	RBT	

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power* (%)	% WCT	Hyb. Species	Comments
ST. REGIS R.	(cont.)								
Brimstone Cr. (St. Regis R. trib)	90-089	1	28	T20N R32W S25 (headwaters)	T19N R32W S3 (mouth)	>95	100	-	Allozyme analysis
Deer Creek (St. Regis R. trib)	2906	3	27	T18N R30W S22 (headwaters)	T19N R30W S36 (near mouth)	-	95	RBT	
Denna Mora Cr. (St. Regis R. trib)	3843	1	8	T19N R32W S15 (headwaters)	T19N R32W S11 (mouth)	88	100	-	
Dominion Creek (St. Regis R. trib)	3359/3080	3	39	T19N R31W S19 (headwaters)	T19N R31W S18 (near mouth)	-	>98	RBT	
Hannaker Creek (St. Regis R. trib)	3842	1	10	T19N R32W S9 (headwaters)	T19N R32W S3 (mouth)	>99	100	-	
Henderson Cr (St. Regis R. trib)	2894	2	27	T18N R29W S18 (headwaters)	T18N R29W S4 (near mouth)	-	98	RBT	
Little Joe Cr - NF (St. Regis R. trib)	2898	2	26	T17N R29W S14 (headwaters)	T17N R28W S5 (above intermit.)	96	100	-	
Packer Cr-upper (St. Regis R. trib)	3526	2	14	T20N R31W S23 (below barrier)	T20N R31W S35 (power line)	97	100	-	Upper main stem
Packer Cr - lower (St. Regis R. trib)	3525	1	7	T19N R31W S11 (below forks)	T19N R31W S14 (mouth)	-	92.9	RBT	
Packer Cr -W Fork (St. Regis R. trib)	3524	1	7	T19N R31W S3 (below forks)	T19N R31W S11 (forks confl.)	-	98.9	RBT	
Rainy Cr - upper (St. Regis R. trib)	3356	1	14	T19N R32W S23 (headwaters)	T19N R32W S13	~ 88	100	-	
Rainy Cr - lower (St. Regis R. trib)	3356	1	11	T19N R32W S13	T19N R31W S7 (mouth)	-	>95	RBT	2 hybrids detected at lower site only
Randolph Creek (St. Regis R. trib)	3354	2	16	T20N R31W S30 (headwaters)	T20N R31W S32 (below Taft Sub)	>90	100	-	Upper 2 sites only WCT markers
Randolph Creek (St. Regis R. trib)	3354	1	10	T19N R31W S5 (below Taft sub)	T19N R31W S7 (mouth)	-	>90	RBT	Lower site had 2 confirmed hybrids
St. Regis River (lower mainstem)	2900	3	15	T19N R30W S27 (Haugan)	T18N R28W S25 (mouth)	-	49	RBT	
St. Regis River (upper mainstem)	3841	2	12 (37)	T19N R33W S1 (headwaters)	T19N R32W S3 (I-90 barrier)	>99	100	-	Supplements sample #699

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power [#] (%)	% WCT	Hyb. Species	Comments
ST. REGIS R.	(cont.)								
Savenac Creek (St. Regis R. trib)	3371/3382	4	27	T20N R30W S26 (near headwaters)	T19N R30W S22 (near mouth)	99	99.6	RBT	Few RBT markers found at middle site
Silver Cr -upper (St. Regis R. trib)	2032	2	25	T19N R31W S33 (headwaters)	T19N R31W S14 (above barrier)	96	100	-	
Silver Cr - lower (St. Regis R. trib)	2032	1	13	T19N R31W S14 (below barrier)	T19N R31W S14 (mouth)	-	93	RBT	
Twelvemile Creek (upper)	2279/2886	3	23	T20N R29W S28 (headwaters)	T20N R29W S36 (upper forks)	~95	100	-	
Twelvemile Creek (lower)	2279/2886	3	9	T19N R29W S1	T19N R29W S34 (mouth)	-	<90	RBT	
Twelvemile Cr – East Fork	3730-3731	2	9	T19N R28W S32 (headwaters)	T19N R29W S36	>85	100	-	DNRC sample
Twomile Cr (St. Regis R. trib)	2901	3	27	T17N R29W S10 (headwaters)	T18N R28W S29 (near mouth)	-	92	RBT	
Twin Cr– E Fork (St. Regis R. trib)	3357	2	26	T19N R29W S5 (headwaters)	T19N R29W S19 (near mouth)	98	100	-	

* Power or percentage chance of detecting 1% hybridization with RBT given sample size and number of diagnostic loci

**WESTSLOPE CUTTHROAT TROUT GENETIC SAMPLING
LOWER BITTERROOT RIVER TRIBUTARIES AND LOLO CREEK DRAINAGE**

Table 2. Results of *Onchorynchus* genetic testing in lower Bitterroot River tributaries in 1999-2009 using DNA and allozyme analyses. Percent WCT denotes the contribution of westslope cutthroat trout alleles as a percentage of the entire sample.

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power [#] (%)	% WCT	Hyb. Species	Comments
Bear Creek (Lolo Cr trib)	3675-3677	3	24	T12N R22W S13 (near FS boundary)	T12N R22W S26 (near mouth)	-	>95	RBT	Hyb + WCT
Bear Run Creek (Miller Cr trib)	3973-3974	3	22	T12N R18W S19 (headwaters)	T12N R19W S23 (near mouth)	99	100	-	
Camp Creek – Upper (Lolo Cr)	3679	1	12	T12N R21W S18 (headwaters)	T12N R22W S24	96	100	-	
Camp Creek – lower (Lolo Cr)	3678	1	12	T12N R22W S24	T12N R22W S36 (near mouth)	-	>96	RBT	Mixed hybrids
Cloudburst Cr (Lolo Cr trib)	3829-3830	2	22	T12N R23W S35 (section 2 bndry)	T12N R23W S27 (near mouth)	-	>95	RBT	WCT + hybrids
Cooper Creek (SF Lolo Cr)	3817	2	23	T11N R22W S16 (near headwaters)	T11N R22W S9 (~0.5 mi to mouth)	99	100	-	
Davis Creek	4055	2	22	T11N R19W S10 (headwaters)	T11N R19W S7 (above diversions)	99	100	-	No fish in northern forks
Eightmile Creek	3972	4	28	T11N R18W S33 (headwaters)	T10N R19W S10 (above diversion)	>99	100	-	Supplements 1998 sample (n=10)
East Fork Lolo Creek	3468	3	22	T11N R23W S25 (headwaters)	T11N R23W S17 (near mouth)	-	99	RBT	Hybrid Swarm + rare F1s
Granite Creek (Lolo Cr trib)	3469-3471	3	24	T11N R24W S33 (headwaters)	T11N R24W S1 (~ 1 mi to mouth)	>95	100	-	Consistent with 1982 samples
Grave Creek (upper)	3812	1	9	T13N R22W S34 (headwaters)	T13N R22W S32 (upper main stem)	90	100	-	
Grave Creek (lower)	3811	2	14	T12N R22W S6 (upper main stem)	T12N R22W S20 (near mouth)	-	>95	RBT	Hyb swarm + hybrids

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power [#] (%)	% WCT	Hyb. Species	Comments
Hayes Creek	2893	2	27	T12N R20W S6 (headwaters)	T12N R20W S9 (USFS boundary)	-	97	RBT	
Holloman Creek (Miller Cr trib)	3513-3514	2	19	T11N R18W S15 (headwaters)	T11N R18W S18 (culvert - mouth)	-	?	RBT	One site pure, one 76% RBT?? Sites reversed?
Howard Creek (lower)	3818-3820	3	25	T12N R23W S22	T12N R23W S25 (near mouth)	-	>95	RBT	
Lee Creek (Lolo Cr trib)	3472	2	18	T11N R23 W S30	T11N R23 W S18 (W Fork confl.)	-	90	RBT	Both sites hybridized
Little Park Cr (Miller Cr trib)	2896	1	13	T12N R18W S28 (headwaters)	T12N R19W S25 (culvert - mouth)	84	100	-	
Lost Park Cr (upper)	3475	1	11	T10N R23 W S9 (headwaters)	T10N R23 W S4	94	100	-	
Lost Park Creek (lower)	3474	1	11	T11N R23 W S33	T11N R23 W S28 (E Fork confl)	-	94	RBT	WCT + 2 hyb
Mill Creek (Lolo Cr trib)	3545-3546	3	21	T11N R21W S21 (near headwaters)	T11N R21W S3 (near mouth)	>95	100	-	
Miller Creek - Upper	3534	1	10	T11N R18W S21 (headwaters)	T11N R18W S18 (above Holloman)	93	100	-	
Mormon Creek (Lolo Cr trib)	3547-3548	3	21	T11N R21W S13 (near headwaters)	T11N R20W S4 (~ 1 mi to mouth)	>95	100	-	
O'Brien Creek	3485-3486	2	15	T13N R21W S34 (near headwaters)	T13N R20W S27 (near FS boundary)	-	93	RBT	1 hybrid at upper site More hybridized below
Park Creek (Miller Cr trib)	3088	2	22	T11N R18W S5 (headwaters)	T11N R19W S1 (culvert - mouth)	93	100	-	
Plant Creek (Miller Cr trib)	3527	1	20	T11N R18W S4 (headwaters)	T11N R18W S7 (culvert - mouth)	-	97.7	RBT	
S Fork Lolo Cr (above Dick Cr)	446,3828	3	38	T10N R22W S26 (headwaters)	T11N R22W S13 (below Dick Cr)	>99	100	-	Consistent with 1990 samples
S Fork Lolo Cr (below Dick Cr)	3827,22,24	3	64	T11N R22W S13 (below Dick Cr)	T12N R21W S32 (mouth)	-	97-98	RBT	Consistent with 1982/1990 samples

Stream	Lab Sample #	No. Sites	n	Upstream Boundary	Downstream Boundary	Power* (%)	% WCT	Hyb. Species	Comments
Tevis Creek (Lolo Cr trib)	3682-3683	2	25	T11N R21W S8 (headwaters)	T11N R21W S5 (above private)	>99	100	-	
WF Butte (Lolo Cr) - Upper	3816	2	13	T11N R22W S17 (headwaters)	T11N R22W S3 (near Marshall Cr)	97	100	-	
WF Butte (Lolo Cr) - Lower	3815	1	11	T11N R22W S3 (near Marshall Cr)	T11N R22W S1 (near mouth)	-	>95	RBT	
W Fork Lolo Cr (Above S. Falls)	3975	2	22	T10N R24W S2 (near headwaters)	T11N R24W S26 (Snowshoe Falls)	-	99.5	RBT	Could be pure?
W Fork Lolo Cr	3494-3496	3	24	T10N R24W S26 (Snowshoe Falls)	T11N R23W S18 (E Fork confl)	-	93	RBT	Hybrid swarm + rare new hybrids (hybrids:all sites)
Woodman Cr-Upper (Lolo)	3681	1	10	T12N R21W S16 (near headwaters)	T12N R21W S20	93	100	-	
Woodman Cr-Lower (Lolo)	3680	1	9	T12N R21W S20	T12N R21W S29 (near mouth)	-	>95	RBT	

* Power or percentage chance of detecting 1% hybridization with RBT given sample size and number of diagnostic loci