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GENETIC POPULATION STRUCTURE AND CONSERVATION OF BULL TROUT IN THE EAST FORK BITTERROOT RIVER DRAINAGE, MONTANA

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By

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Genetic population structure and conservation of bull trout in the East Fork Bitterroot River drainage, Montana

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Bull trout *Salvelinus confluentus* are a species of conservation interest and are currently listed as threatened by the U.S. Fish and Wildlife Service. Understanding and conserving the genetic and life history diversity of bull trout populations across their range is critical as conservation, management, and recovery plans are developed. Numerous studies in different regions have shown that local bull trout populations in close geographic proximity are typically very genetically different and evidence for dispersal among neighboring tributary populations is weak. In addition to genetic diversity, maintenance of life history diversity may increase resilience of bull trout populations. The larger migratory forms have been linked to high reproductive potential and increased population persistence in unstable environments as the distribution of adults across multiple habitats may buffer them against stochastic events. Ensuring the persistence of both genetic and life history diversity are important conservation priorities.

I evaluated the genetic population structure of bull trout in the East Fork Bitterroot River, Montana and identified which tributaries produced the majority of fluvial fish using genetic assignment. My data showed that populations in tributaries are genetically distinct from each other and fish in the main stem East Fork; however, dispersal of individuals among populations was apparent suggesting a metapopulation structure. My results indicate that the scale of management for bull trout in the East Fork is the basin and that migratory fish may be important for maintaining gene flow among small populations and genetic variation within them.

Given the importance of migratory fish, I examined how well we are tracking migratory bull trout populations and threats to their existence. The evaluation of the current monitoring protocol revealed that redd count surveys are not useful. Even though mark-recapture surveys are common, there are few locations where population estimates are obtained. Improving the protocols and combining approaches may improve our inference, specifically, conducting redd counts and electrofishing population estimates in areas identified as supporting migratory fish. In general, threats such as roads, grazing allotments, and wildfire have been well tracked, although future threats to river habitat conditions (e.g., temperature and degradation) and invasions of brown trout are yet to be fully evaluated.

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INTRODUCTION AND OVERVIEW

Bull trout *Salvelinus confluentus* belong to the Salmonidae family and are native to western North America (Behnke 2002). Even though they are a species of conservation and management interest, little is known about the distribution of life history types (resident versus migratory) and genetic population structure of bull trout in the Lower Clark Fork watershed, including the Bitterroot River basin. Bull trout were once common throughout western Montana, and were documented regularly in the Bitterroot River drainage throughout the 19th and early 20th centuries (Williams 2010, unpublished data). Though Montana Fish, Wildlife & Parks (FWP) and the Bitterroot National Forest (BNF) monitor trout populations in the Bitterroot River drainage, not much is known about the overall status of migratory bull trout (C. Clancy, FWP, personal communication). There has been a documented decline of migratory bull trout in the Bitterroot River drainage (Jakober et al. 1998, Nelson et al. 2002) and this trend is a cause for concern because of the implications for population persistence.

Although the theoretical costs and benefits of diverse life history strategies have been explored, the conservation implications of the loss of different life history types is still relatively unknown (Hendry and Stearns 2004). Maintaining migratory life history forms is likely important for population persistence in unstable environments (Thorpe 1994), by maintaining gene flow among populations and allowing the possibility of re-colonization (Rieman and McIntyre 1993; Rieman et al. 1997; Rieman and Dunham 2000). Furthermore, increased size of migratory fish increases fecundity and possibly reproductive success (Jonsson and Jonsson1993). For these reasons, maintaining life history diversity is a conservation priority for many native species, including bull trout (Rieman and McIntyre 1993, Montana Bull Trout Scientific Group 1995; Montana Bull Trout Restoration Team 2000).

Even though they may be migrating long distances, studies indicate that bull trout exhibit a strong population genetic structure with nearby populations being substantially divergent (Leary et al. 1993; Spruell et al. 1999, 2003; Kanda and Allendorf 2001; Costello et al. 2003; Whiteley et al. 2004; Arden et al. 2007; Kassler and Mendel 2007). Patterns of high genetic divergence among bull trout tributary populations are expected because they spawn in headwater streams, have small breeding populations, and display strong site fidelity to natal spawning areas

(McPhail and Baxter 1996; Swanberg 1997; Neraas and Spruell 2001; Whiteley et al. 2004). The population structure of bull trout is similar to other inland salmonids that exhibit analogous breeding ecology. The results of various studies at the tributary scale show that westslope cutthroat trout *Oncorhynchus clarkii lewisi*, coastal cutthroat trout *O. c. clarki*, Lahontan cutthroat trout *O. c. henshawi* and brown trout *Salmo trutta* all have substantial population structure (Ryman 1983; see Table 5 in Allendorf and Leary 1988; Wenburg et al. 1998; Wenburg and Bentzen 2001). In contrast, Yellowstone cutthroat trout *O. c. bouvieri* exhibit population structure at larger drainage scales (Cegelski et al. 2006). Differences in the genetic population structure among tributaries versus drainages could be a function of the species life history, homing or site fidelity, and/or a function of the riverscape (e.g., available habitat, spawning habitat, and presence of barriers; Dittman and Quinn 1996; Whiteley et al. 2004).

Determining the population structure and connectedness of spawning populations within and across drainages is important for conservation and management measures to ensure persistence of existing populations. Dispersal among local populations may be particularly important for buffering against stochastic environmental risk, supporting weaker possibly sink populations, and refounding extirpated populations (Dunham and Rieman 1999; Rieman and Dunham 2000). In order to make informed conservation and management decisions for bull trout, we need to understand not just the risks to individual spawning populations but the population genetic structure among them. For example, if populations are genetically isolated, management could take a more localized approach by focusing on spawning populations at the tributary scale. In contrast, if little population structure exists, a more regional approach may be appropriate with management focused at the watershed or basin scale (Kanda and Allendorf 2001; Spruell et al. 1999, 2003). Even though studies have suggested that bull trout may function as a metapopulation and therefore should be managed at a basin scale (e.g., Rieman and Dunham 2000), there is little empirical evidence of metapopulation structure from published population genetic studies (but see, DeHaan et al. 2008a). Understanding the genetic population structure of bull trout across its range, conserving genetic diversity and allowing for genetic exchange are part of the general recovery goals for the species (USFWS 2008).

Due to the decline of bull trout and mandates for monitoring and recovery (USFWS 2008), it is important to evaluate monitoring programs periodically to determine if they are

meeting the goals, for example, detecting changes in population abundance (Allendorf et al. 1997; Al-Chokhachy et al. 2005). Conservation and management decisions are based on data from monitoring programs, it is therefore critical that we know how well we are tracking population trends (of both native and non-native species). Are we collecting information in a way that allows us to track the key components of conservation interest? Regardless of the species, it is important to make sure that we are able to identify whether abundances are decreasing as this may ultimately determine persistence (Mace and Lande 1991; Allendorf et al. 1997).

My study focused on the East Fork Bitterroot River, Montana (hereafter East Fork). This area was chosen because it contains a migratory life history component, is an open system (below Star Falls) without barriers to movement among tributaries, and is a core conservation area for bull trout (Bitterroot River Drainage Bull Trout Status Report 1995). The overarching objectives for this study were to investigate the genetic population structure of bull trout, determine the potential source tributaries contributing to the migratory life history component, and given this information examine the current monitoring protocol to evaluate whether the status and trends of migratory bull trout are being tracked.

In Chapter 2, I determined the genetic population structure of bull trout in the East Fork and explored which tributaries produced the majority of fluvial fish using genetic assignment. I used 15 microsatellite loci to determine the genetic population structure of bull trout. Even though the within-sample population genetic diversity was similar to previous studies for this species, the among-sample genetic diversity (F_{ST}) was lower. Within the East Fork basin, Tolan Creek appears to be the genetically most distinct spawning population in the basin. While there are no known barriers to movement into or out of Tolan Creek, this may suggest an unknown natural barrier or the agricultural impacts in the lower sections of the watershed are acting as a barrier to bull trout movement. Tolan Creek may be a focal tributary to ensure maintenance of genetic diversity. Additionally if it is isolated, it may be useful to monitor changes in genetic diversity over time to detect problematic changes such as loss of rare alleles or indications of inbreeding.

The relatively low differentiation among tributary populations was also supported by the results of genetic assignment that suggested bull trout in the East Fork drainage moved among tributaries, as well as between the spawning tributaries and the river. Unexpectedly, over 50% of

the individual fish captured in the main stem river (migratory fish) primarily assigned to their own group while four individuals assigned to tributaries. Although I do not know where this group is spawning, the upper East Fork is a known historical site. Thus in contrast to multiple other empirical studies that indicate very restricted gene flow among bull trout populations (Leary et al. 1993; Spruell et al. 1999, 2003; Kanda and Allendorf 2001; Costello et al. 2003; Whiteley et al. 2004, 2006; Ardren et al. 2007; DeHaan et al. 2007, 2008b, 2010a; Kassler and Mendel 2007; DeHaan and Godfrey 2009; Ardren et al. 2011), the bull trout in the East Fork appear to be functioning as a metapopulation. A metapopulation is defined as any assemblage of discrete local populations with migration among them regardless of the rate of population turnover (Hanski and Gilpin 1997, cited by Schtickzelle and Quinn 2007). Factors that may account for this apparent discrepancy could include differences in the scale and resolution of the studies or the lack of barriers and presence of the migratory life history form in the East Fork. Ultimately this is a good illustration of the importance of establishing patterns of genetic population structure across multiple drainages with different characteristics before making broad generalizations.

The objective of Chapter 3 was to qualitatively evaluate the current monitoring program in the East Fork. Specifically I addressed if the current monitoring protocol allows for trend detection of migratory bull trout. In addition, how well are we tracking potential threats to bull trout in the watershed and are data collected at an ecologically relevant temporal and spatial scale for assessing threats or tracking habitat changes? Based on the results from Chapter 2, it is important to consider the East Fork basin as a management or conservation unit and monitor across the entire basin to identify changes in population status and habitat conditions. Bull trout are notoriously difficult to monitor (Rieman and McIntyre 1993; Thurow and Schill 1996; Swanberg 1997; Peterson and Dunham 2003; Al-Chokhachy et al. 2009). Results of my evaluation of the current monitoring protocol revealed that redd counts are not useful at capturing migratory bull trout abundance. Even though mark-recapture surveys are common, there are few locations where surveys have large enough capture and recapture numbers to reliably calculate population estimates. There are only three tributaries (Meadow, Moose and Tolan) that have six to ten years of population estimates. Given the limited number of sites with substantial temporal data, any power to detect trends across the basin is weak.

The combination of existing data (genetic assignment, previous radiotelemetry studies and electrofishing surveys) can help identify the potential sources of migratory individuals. These data indicate that Warm Springs Creek, Meadow Creek, Swift Creek, and Clifford Creek may be key tributaries for migratory bull trout. Results from Chapter 2 indicated an East Fork spawning group, thus it may be useful to re-visit the upper East Fork to search for redds in an attempt to locate spawning areas. In addition, a combination of different monitoring approaches (redd counts and electrofishing population estimates) may improve our inference on migratory bull trout population status (Dunham and Rieman 1999; Dunham et al. 2001; Epifanio et al. 2003; Al-Chokhachy et al. 2005). Previous studies have indicated that redd counts performed across the scale of the tributaries (broad scale) and that are done repeatedly during the season have a decreased sampling error and increase probability of tracking the population (Rieman and McIntyre 1996; Maxell 1999; Dunham et al. 2001; Muhlfeld et al. 2006). For population estimates, examining protocols to increase capture and recapture rates and enhance the reliability of population estimates would be useful. Incorporating genetic monitoring would allow for detection of changes in genetic status, in particular the loss of rare alleles and/or signs of shifting population structure as a result of the continued loss of the migratory life history form. The largest future threats for the migratory life history form may include habitat degradation in the lower reaches of the main stem East Fork, increasing water temperatures and the presence of non-native species such as brown trout (FWP, unpublished data). These potential threats are being tracked in the current monitoring scheme, but their implications and potential solutions have yet to be determined.

CHAPTER 2

GENETIC POPULATION STRUCTURE OF BULL TROUT IN THE EAST FORK BITTERROOT RIVER DRAINAGE, MONTANA

Abstract

Investigating the genetic population structure of bull trout *Salvelinus confluentus* can be useful for developing biologically sound conservation and management strategies. We focused on the East Fork Bitterroot River (East Fork) drainage because it is a connected, core conservation area for bull trout that contains a migratory life history component. Non-lethal fin samples were collected from 17 sites: nine East Fork tributaries, the main stem East Fork, and seven other tributaries across the Bitterroot drainage. Considering all the samples, principal component analysis of allele frequencies at 15 microsatellite loci indicated the East Fork samples formed a distinct cluster compared to other tributaries sampled. Within the East Fork drainage, there was significant divergence among samples with pairwise F_{ST} ranging from 0.016 to 0.188. Based on multiple locus genotypes, most individuals assigned to their tributary of capture with over 90% probability, suggesting the tributaries contain genetically divergent populations. The main stem East Fork sample tended to form its own group, but some fish collected from it also assigned to tributaries. Four tributaries had individuals that assigned to the East Fork indicating migration from the East Fork to tributaries. Likewise, four tributaries also had individuals that assigned to tributaries different than where they were collected indicating migration from tributary to tributary. These data suggest the East Fork may contain a mixture of individuals produced from spawning in the upper main stem and migrants from different tributaries. The main stem East Fork appears to be an integral component for maintaining the migratory form of bull trout in the drainage and serves as a vehicle for potential genetic exchange among tributary populations. Thus, conservation and management efforts in the drainage need to simultaneously focus on the tributaries and the main stem East Fork.

Introduction

Determining the genetic population structure of a species allows scientists to evaluate the degree of genetic variation within and among populations, estimate amounts of gene flow (spatial and temporal) among populations, detect possible bottlenecks within populations, and obtain some understanding of the evolutionary history of the species, all of which can affect the likelihood of species persistence (Paetkau et al. 1995; Wenburg and Bentzen 2001; Scribner et al. 2003; Lowe and Allendorf 2010). Understanding the patterns of gene flow across multiple spatial scales (i.e., within and among river basins) provides information about the degree of isolation among populations which can be valuable for formulating biologically sound management and conservation programs to better ensure persistence of existing native populations (Slatkin 1987; Harrison and Hastings 1996; Dunham and Rieman 1999; Rieman and Dunham 2000; Allendorf and Luikart 2007; Lowe and Allendorf 2010). For example, dispersal that leads to gene flow among populations and the formation of a metapopulation structure may be particularly important for buffering against stochastic environmental risk, supporting sink populations, refounding extirpated populations, and promoting gene flow among populations (Harrison and Hastings 1996; Hanski 1998; Dunham and Rieman 1999; Rieman and Dunham 2000). In addition, dispersal among spawning populations helps maintain long term genetic diversity within populations potentially allowing them to adaptively respond to environmental change.

The patterns of genetic population structure for inland salmonid populations are diverse. Various studies have demonstrated substantial population structure among tributaries within the same river basin for westslope cutthroat trout *Oncorhynchus clarkii lewisi*, coastal cutthroat trout *O. c. clarkii*, Lahontan cutthroat trout *O. c. henshawi*, and brown trout *Salmo trutta* (Ryman 1983; see Table 5 in Allendorf and Leary 1988; Wenburg et al. 1998; Wenburg and Bentzen 2001; Taylor et al. 2003; Neville et al. 2006). In contrast, Yellowstone cutthroat trout *O. c. bouvieri* and mountain whitefish *Prosopium williamsoni* exhibit much reduced population structure with most divergence existing at broader drainage scales (Whiteley et al. 2004; Cegelski et al. 2006). Differences in the genetic population structure among taxa are likely a function of the species life history (e.g., migratory or resident), breeding ecology (e.g., where in the watershed they spawn and site fidelity), and the riverscape (e.g., available habitat, spawning

habitat, altered hydrology, and the presence of barriers; Dittman and Quinn 1996; Morita and Yamamoto 2002; Taylor et al. 2003; Whiteley et al. 2004; Yamamoto et al. 2004; Wofford et al. 2005; Neville et al. 2006; Winans et al. 2008; Morita et al. 2009). Given the importance of the context of the study (e.g., riverscape characteristics), it is important to consider multiple studies across different river systems before coming to any general conclusions about the population structure of a species.

The genetic population structure of bull trout *Salvelinus confluentus* is of particular conservation interest because they are currently listed as "threatened" in the USA under the Endangered Species Act (Federal Register 1998) and "at risk" in most of Canada (McCart 1997). Most studies of bull trout population structure have found relatively high genetic divergence (F_{ST}) among populations not only within large river basins (e.g, Columbia and Clark Fork River basins), but even among populations in adjacent 2nd and 3rd order tributaries (Leary et al. 1993; Spruell et al. 1999, 2003; Kanda and Allendorf 2001; Costello et al. 2003; Whiteley et al. 2004, 2006; Ardren et al. 2007; DeHaan et al. 2007, 2008b, 2010a; Kassler and Mendel 2007; DeHaan and Godfrey 2009; Ardren et al. 2011). The biological reasoning for the relatively high amounts of genetic divergence commonly observed among nearby bull trout populations include that they tend to spawn in headwater streams, display strong site fidelity to natal spawning areas leading to reduced gene flow, and have small effective population sizes (N_e) leading to increased genetic drift (McPhail and Baxter 1996; Swanberg 1997; Neraas and Spruell 2001; Whiteley et al. 2004). Amounts of genetic divergence may be affected by historical events such as debris flows caused by severe wildfires that bottleneck the population leading to increased genetic drift and the loss of rare alleles (Hallerman 2003; Allendorf and Luikart 2007). Anthropogenic activities that modify the landscape such as habitat fragmentation and altered hydrology can also lead to reduced gene flow and small N_e; therefore, increasing genetic divergence (Neraas and Spruell 2001; Morita and Yamamoto 2002; Yamamoto et al. 2004; DeHaan et al. 2007; Morita et al. 2009).

In addition to understanding genetic population structure, assessing bull trout life history diversity in core conservation areas is a conservation priority (Montana Bull Trout Restoration Team 2000). Bull trout exhibit a variety of life histories including both resident and migratory individuals (Rieman and McIntyre 1993; McPhail and Baxter 1996). Resident bull trout spend

their entire lives within their natal stream or tributary moving short distances (e.g., <2 km; Jakober et al. 1998). Migratory bull trout spend one to four years in their spawning tributaries before migrating, often 10s to 100s of kilometers to larger habitats (e.g., rivers, lakes, estuaries) for several years to forage and overwinter before returning to the tributaries to spawn (Fraley and Shepard 1989; Rieman and McIntyre 1993; McPhail and Baxter 1996; Swanberg 1997; Mogen and Kaeding 2005). Migratory bull trout are often larger in size (by two to three times) compared to resident fish which greatly increases fecundity and possibly reproductive success (Fraley and Shepard 1989; Jonsson and Jonsson 1993; McPhail and Baxter 1996; Lockard 2006). Populations may be composed of either life history type or both resident and migratory fish that coexist and breed as a single panmictic population (Jonsson and Jonsson 1993; McPhail and Baxter 1996; Jakober et al. 1998; Nelson et al. 2002; Homel et al. 2008). Maintaining life history diversity, especially migratory forms, is likely important for population persistence in unstable environments, by maintaining gene flow among populations and allowing the possibility of re-colonization after catastrophic events (Thorpe 1994; Rieman et al. 1997; Rieman and Dunham 2000; Bahr and Shrimpton 2004; Burton 2005).

Bull trout populations have declined throughout their range for a number of reasons. Threats include increasing water temperatures (Selong et al. 2001; Dunham et al. 2003), competition with non-native species (Donald and Alger 1993; Rieman and McIntyre 1993; Nelson et al. 2002; McMahon et al. 2007), hybridization with brook trout *Salvelinus fontinalis* (Leary et al. 1993; Rieman and McIntyre 1993; Kanda et al. 2002; DeHaan et al. 2010b), poaching or misidentification (Fraley and Shepard 1989; Leary et al. 1993; Swanberg 1997; Schmetterling and Long 1999), and habitat degradation and fragmentation (Rieman and McIntyre 1993; McPhail and Baxter 1996; Neraas and Spruell 2001). Even though these threats are problematic for all life history forms, many of these impacts have had disproportionately larger influences on migratory bull trout throughout their range (Swanberg 1997; Neraas and Spruell 2001).

Resident and migratory bull trout were once common throughout western Montana, and migratory fish were documented regularly in the Bitterroot River drainage throughout the 19th and early 20th centuries (Williams 2010, unpublished data). Although Montana Fish, Wildlife & Parks (FWP) and the Bitterroot National Forest (BNF) monitor trout populations in the Bitterroot

River drainage, little is known about the genetic population structure of bull trout and the status of the migratory life history (C. Clancy, FWP, personal communication). Two areas within the Bitterroot drainage known to support migratory populations of bull trout are the West Fork Bitterroot River (hereafter West Fork) and the East Fork Bitterroot River (hereafter East Fork). We focused on the East Fork because it is a connected, core conservation area for bull trout (Bitterroot River Drainage Bull Trout Status Report 1995) and has likely been experiencing decreases in the migratory life history component over the last few decades (Jakober et al. 1998; Montana Bull Trout Restoration Team 2000; Nelson et al. 2002). We had two objectives for this study: (1) describe the genetic population structure of bull trout in this core conservation area and (2) identify the potential tributary sources of migratory fish. Given results from the previously mentioned studies, we expected that neighboring tributaries would be relatively isolated and genetically distinct (high F_{ST}). We also expected our East Fork samples, presumed to be migratory fish would assign to their natal tributaries, thus identifying which tributaries are contributing to the migratory life history component.

Study Area

The Bitterroot River Basin is comprised of the main stem Bitterroot, the West Fork, and the East Fork. Located in southwest Montana the total drainage area is 7288 km^2 (Figure 2.1). The majority of the lower drainage is primarily private land ownership while higher in the drainage the major landowner is the United States Forest Service (hereafter USFS). The main stem Bitterroot supports three native salmonids: westslope cutthroat trout, bull trout and mountain whitefish. Although the East Fork was our focal area, population samples were included from four tributaries to the main stem Bitterroot (Daly, Skalkaho, Burnt Fork, and Willow Creeks) and three tributaries to the West Fork (North Fork Sheephead, Sheephead, and Slate Creeks) for comparative purposes.

The East Fork is located approximately 116 km south of Missoula, Montana. The watershed encompasses 1,057 km^2. The headwaters are approximately 73 km from the confluence with the West Fork and main stem Bitterroot River with roughly 166 km^2 lying within the East Fork Wilderness area. Much of the upper drainage is managed by the USFS with

some private lands in the valley bottom. Historical impacts in the watershed include timber harvest, forest roads, agriculture, water diversions, and wildfire. Star Falls, a natural barrier to upstream movement located at river km 64, is the upper limit for bull trout occurrence in the East Fork. There are no known barriers to movement among the tributaries within the watershed below Star Falls which enhances the opportunity of unrestricted migration allowing the full expression of life history strategies. FWP and USFS biologists have surveyed every tributary of the East Fork and 17 of the 23 main tributaries have documented bull trout occupancy. Bull trout populations occupying tributaries consist of both resident and migratory individuals whose proportions are unknown (FWP, unpublished data). Other native species present in the drainage include westslope cutthroat trout, mountain whitefish, largescale sucker *Catostomus macrocheilus*, longnose sucker *C. catostomus*, longnose dace *Rhinichthys cataractae* and slimy sculpin *Cottus cognatus*. Nonnative fishes that are found in the East Fork include rainbow trout *O. mykiss*, brown trout, and brook trout. Hybrid fishes found in the East Fork include westslope cutthroat trout x rainbow trout, westslope cutthroat trout x Yellowstone cutthroat trout x rainbow trout and bull trout x brook trout.

Methods

Sample Collection

During the summers of 2008 and 2009, we electrofished 17 East Fork tributaries where bull trout were previously encountered to obtain a sample from potential spawning populations within the basin (Figure 2.2). To capture fish we used either a bank shocking electrofishing system or backpack electroshocker and shocked 305 m (1000 ft) sections to obtain 50 bull trout tissue samples from caudal fins (hereafter fin samples). Reaches were added if 50 fin samples were not collected from the initial 305 m section. In addition, we electrofished approximately 26 km of the main stem East Fork River using a Jon boat to sample fish that were residing in the river. FWP and USFS provided additional fin samples collected from the four tributaries to the Bitterroot River and three tributaries to the West Fork to examine how the East Fork genetic diversity fits in a broader geographic scale. All captured bull trout were measured, weighed, a caudal fin sample collected and then released. We preserved all fin samples in vials with 95% ethanol.

Laboratory methods

All laboratory work was performed in the Conservation Genetics Laboratory at the University of Montana, Missoula, Montana USA. Analyses of the tissue samples included DNA extraction, polymerase chain reaction (PCR) and fragment analysis using 15 variable microsatellite loci *Omm1128, Omm1130* (Rexroad et al. 2001), *Sco102, Sco105, Sco106, Sco107* (Washington Dept. of Fish and Wildlife unpublished data), *Sco200, Sco202, Sco212, Sco215, Sco216, Sco218, Sco220* (DeHaan and Ardren 2005), *Sfo18* (Angers et al. 1995) and *Smm22* (Crane et al. 2004). Seven of these loci are diagnostic between bull trout and brook trout allowing examination of hybridization between these fishes (Table 2.1). We extracted DNA from each fin clip using a cell lysis buffer and ammonium acetate protein precipitation, followed by an isopropanol DNA precipitation. A 100 µl hydration solution (TE) was used to re-suspend the DNA. PCR reactions were conducted following the QIAGEN microsatellite protocol using the QIAGEN Multiplex PCR Kit (QIAGEN, Valencia, CA). We used three different PCR profiles: multiplex 1 and 2 used a touchdown profile with an initial annealing temperature of 63°C stepping down to 53°C, multiplex 3 used a typical PCR profile with an annealing temperature of 54°C, and multiplex 4 used a typical PCR profile with an annealing temperature of 55.4°C (Table 2.1). Samples were amplified in a PTC-200 thermocycler (MJ Research, Waltham, MA). Following PCR, fragments were visualized on an ABI3130xl Genetic Analyzer (Applied Biosystems, Foster City, CA) in the Murdock DNA Sequencing Facility at the University of Montana, Missoula, Montana, USA. Allele sizes were determined using the ABI GS600LIZ ladder and chromatogram output was viewed and analyzed using GeneMapper version 3.7 (Applied Biosystems, Foster City, CA).

Genetic Analyses

We performed exact tests for Hardy-Weinberg equilibrium (HWE) for all samples and loci with the program Genepop v4.0 (Raymond and Rousset 1995). Significant values (alpha level 0.05) for HWE in each sample were adjusted for multiple comparisons using a sequential Bonferroni adjustment (Rice 1989). We used a Fisher's exact test to test for allele frequency differences between all pairs of samples to determine if it would be appropriate to consider each independently in subsequent analyses (Genepop v4.0; Raymond and Rousset 1995).

To examine within sample genetic diversity, we calculated the number of alleles, private alleles, and observed and expected heterozygosity using GENALEX v6.4 (Peakall and Smouse 2006). The program HP Rare v1.1 (Kalinowski 2005) was used to calculate allelic richness which uses the statistical technique rarefaction to standardize the number of alleles detected in each sample to the smallest sample size. We looked for evidence of recent genetic bottlenecks in the samples using the program Bottleneck (Cornuet and Luikart 1996) assuming the two-phased model of mutation with a variance of 12.0. Tests for gametic disequilibrium between all pairs of loci in each sample were conducted using a Fisher exact test in Genepop v4.0 (Raymond and Rousset 1995).

In addition to allele frequency comparisons, we examined among sample differences with multivariate analyses and F_{ST}. We used Minitab 15 (Minitab 2007) to perform a principal component analysis (PCA) of the allele frequencies among the samples. Overall genetic divergence among the samples (global F_{ST}) was estimated in Fstat v2.9.3 (Goudet 2001) along with the associated 95% confidence level based on 1000 bootstrap replicates. To further examine genetic divergence, we computed F_{ST} estimates between all pairs of samples using Genepop v4.0 (Raymond and Rousset 1995) following standard ANOVA as in Weir and Cockerham (1984) and tests of statistical significance using the program Arlequin v3.1 (Excoffier et al. 2005). A sequential Bonferroni correction was used to adjust significance for multiple comparisons (Rice 1989). To test for a potential isolation by distance relationship between F_{ST} and fluvial distance (river km between sampling locations), we used a Mantel's test in GENALEX v6.4 (999 permutations).

We used the program ONCOR (Kalinowski 2008) to assign fish captured in the main stem East Fork to their sample of most likely origin. We first performed the leave one out test, a jackknife analysis, of the tributary samples to assess our ability to correctly assign fish to their population of origin. This procedure removes an individual from the data set and then uses a maximum likelihood algorithm to estimate the probability it came from each of the samples. Following the jackknife analysis, we used the individual assignment test in ONCOR (Kalinowski 2008) to determine the tributary of origin for each individual fish from both the East Fork tributaries and the main stem East Fork. We used an assignment probability of 90% as indicating an individual's water of origin regardless of where it was captured. If a fish assigned

elsewhere than the water of origin with high probability (≥ 90%) it may be a migrant or disperser.

Results

Each sample in the data set had ≥ 15 fin clips (Table 2.2). Of the 17 East Fork tributaries sampled, we did not detect bull trout in five tributaries and three samples were removed from further analyses due to very small sample sizes (≤ 3 fin clips). Thus, we obtained samples from nine East Fork tributaries. Sizes of individual fish in the East Fork samples ranged from 25 to 422 mm total length. In contrast, we captured 73 fish in the main stem of the East Fork whose sizes ranged from 102 to 610 mm total length. Individual fish from the three West Fork tributaries and four Bitterroot River tributaries ranged in size from 76 to 432 mm total length. Overall, the final data set was comprised of 17 samples (Table 2.2).

Within-Sample Variation

Among the 591 individuals analyzed, 41 possessed both bull trout and brook trout alleles at the diagnostic loci indicating they were hybrids (Table 2.2). No hybrid individuals were identified in the East Fork samples. All hybrid individuals occurred in samples located in the West Fork and Bitterroot River and the majority of these were collected in Willow Creek a main stem Bitterroot River tributary (Table 2.2). Hybrids were removed before further analyses as the presence of nonnative alleles can dramatically influence population structure estimates (Allendorf and Luikart 2007; Forbes and Allendorf 1991; Cegelski et al. 2006).

Among the 15 microsatellite loci analyzed, the number of alleles per locus ranged from 2 to 34 and all were polymorphic in all seventeen samples. Following a sequential Bonferroni correction (alpha level 0.05; 15 comparisons per sample; Rice 1989), there was little evidence of deviations from expected HWE in the samples. After correction, only Orphan Creek showed a significant departure from HWE and this was due to an excess of heterozygotes at *Smm22* and *Omm1130*. Although most samples did not show a significant departure from HWE, there was a strong tendency for an excess of heterozygotes among our samples (94 out 150 comparisons excess, SPSS Sign Test; P =.002). Fisher's exact comparison of allele frequencies indicated that

allele frequencies statistically differed between all pairs of samples. Furthermore, all pair-wise F_{ST} estimates were significantly different (see below and Table 2.3). Therefore, each sample was treated as a separate population for subsequent analyses. Interestingly, these results include the main stem East Fork sample which we initially assumed would be a mixture of individuals from different tributaries but this did not appear to be the case.

Among the samples, the mean number of alleles per locus (A) was 7.23 and ranged from five to eleven and mean allelic richness (A_r) was 6.12 and ranged from 4.87 to 8.46 (Table 2.2). Thirty-seven private alleles occurred in eleven different samples and the frequency ranged from 0.007 to 0.156. Averaged over all samples, expected and observed heterozygosity (H_e and H_o) were 0.66 and 0.70 while the average within-population expected heterozygosity (H_s) ranged from 0.586 to 0.703 (Table 2.2). Considering the samples from the East Fork, West Fork, and main stem Bitterroot, A, A_r, and H_e did not significantly differ among the populations from the different drainages (Kruskal-Wallis Analysis; $P > 0.05$).

We observed evidence of gametic disequilibrium in eight of the 17 samples. Out of 105 comparisons per sample the following samples showed significant evidence of disequilibrium: Martin Creek at two pairs of loci, Orphan Creek at three pairs, Star Creek at one pair, Swift Creek at four pairs, Tolan Creek at one pair, North Fork Sheephead Creek at one pair, Sheephead Creek at one pair, and Willow Creek at two pairs. The specific pairs of loci showing evidence of disequilibrium differed among the samples and appear to be randomly distributed among the eight tributaries with the exception of *Sco200* and *Sfo18* which were out of equilibrium in both Tolan and Willow Creeks. Therefore, disequilibrium is likely not due to linkage but some random factor such as low N_e or a recent bottleneck. The latter does not seem very likely as results of the genetic bottleneck test indicated that only one population, Star Creek, had a signal of experiencing a recent bottleneck as indicated by a significant allele frequency mode shift. However, our power to detect a signal of a recent bottleneck is weak due to the relatively small number of individuals and loci analyzed.

Among-Sample Variation

Comparisons among all the samples indicated some geographic genetic structure at the basin level with the East Fork basin samples differentiating from samples in the West Fork and

Bitterroot basin (Figure 2.3). The first two axes of the PCA explained 43% of the variation in the allele frequencies within the entire data set. All but one of the tributary samples in the East Fork basin clustered together and all but one of the tributary samples outside of the East Fork (tributaries to the West Fork and Bitterroot River) clustered together. Both Willow Creek (tributary to the Bitterroot River) and Tolan Creek (tributary to the East Fork) showed up as outliers from either cluster (Figure 2.3). Genetic structure across the basin was also evidenced by pairwise F_{ST} estimates ranging from 0.009 to 0.300 (Table 2.3). The highest pairwise F_{ST} estimates occurred between Willow Creek and all of the other samples with the exception of the pairwise F_{ST} estimate between Orphan Creek and Tolan Creek which was also about 0.20. We did not detect a significant isolation by distance relationship between genetic distance and fluvial distance across all seventeen samples distributed across the Bitterroot basin ($r^2 = 0.009$, $P = 0.25$).

Focusing on the East Fork, we found all ten samples significantly differed from each other and pairwise F_{ST} estimates ranged from 0.016 to 0.188 (Table 2.3). The greatest differences tended to be between Tolan Creek and the other East Fork samples. Global F_{ST} among the East Fork samples was 0.063 (95% C.I. = 0.057 to 0.068). We did not detect a significant isolation by distance relationship between genetic distance and fluvial distance among the tributaries within the East Fork ($r^2 = 0.0168$; $P = 0.285$).

We observed 53 to 100% correct assignment to population of origin in the leave one out assignment test. Results of the individual assignment test to determine water of origin revealed that most individuals assigned to the tributary where they were collected (67 to 100% using a $\geq$ 90% probability threshold; Table 2.4). Of the 73 East Fork samples, 58% assigned to the East Fork and four individuals assigned to the following tributaries: one to Clifford Creek, one to Warm Springs Creek and two individuals to Swift Creek. Among the tributaries, some individuals assigned to the East Fork and some assigned to tributaries other than the tributary of capture (Table 2.4). The sizes of individuals assigning to water bodies other than where they were collected (using a $\geq$ 90% probability threshold) ranged from 75 to 521 mm (Table 2.4).

Discussion

Even though it has been cited that bull trout may function as a metapopulation (Dunham and Rieman 1999; Rieman and Dunham 2000), several genetic population studies have suggested that no metapopulation structure existed and if present, metapopulation structure is weak at best (Spruell et al. 1999; Kanda and Allendorf 2001). A metapopulation is defined as any assemblage of discrete local populations with migration among them regardless of the rate of population turnover (Hanski and Gilpin 1997, cited by Schtickzelle and Quinn 2007). Numerous studies have shown that local populations in close geographic proximity (e.g., adjacent tributaries) are typically genetically very different (Spruell et al. 1999; Kanda and Allendorf 2001; Whiteley et al 2006; DeHaan et al. 2010a). In the East Fork, a connected system with a migratory life history component, our data showed that populations in tributaries are genetically distinct from each other and fish in the main stem East Fork; however, lower F_{ST} values (which may be indicative of gene flow, i.e., dispersal) were apparent suggesting a metapopulation structure.

Overall, the genetic diversity we observed within populations in the Bitterroot drainage tended to be higher than that found in other studies of bull trout. Three comparable studies using the same 15 microsatellite loci are the range-wide coterminous United States study of Ardren et al. (2011), the Warm Springs study of DeHaan et al. (2010a), and the Metolius River study of DeHaan et al. (2008a). Estimates of the mean number of alleles per locus, allelic richness, and expected and observed heterozygosity were higher in the East Fork and the West Fork/Bitterroot samples compared to any of these studies (Table 2.5).

Comparing allele frequencies among all of our samples from the East Fork, West Fork, and Bitterroot, we found that there were two major groups of samples: the East Fork and the West Fork/Bitterroot, with two outliers Tolan Creek and Willow Creek (Figure 2.3). Even though there are no known barriers between Tolan and Willow Creeks and the main stem rivers, they appeared to be more isolated than the other tributaries. It is possible that these creeks may be more isolated because of watershed disturbance, hydrology, and land use. Of all the samples in the data set, Willow Creek also had the largest proportion of bull trout and brook trout hybrids (roughly 50%) suggesting abundant brook trout and possible habitat degradation. Limited gene flow and small populations within these creeks most likely have combined to result in increased

genetic drift and relatively greater genetic divergence (Costello et al. 2003; Whiteley et al. 2006; DeHaan et al. 2007, 2010a). Although relatively isolated, because of their differences in allele frequencies Tolan and Willow Creeks constitute an important component of the genetic diversity of bull trout in the Bitterroot basin.

Many studies have investigated the genetic population structure of bull trout and have found high genetic divergence (F_{ST}) among populations (Leary et al. 1993; Spruell et al. 1999, 2003; Kanda and Allendorf 2001; Costello et al. 2003; Whiteley et al. 2004, 2006; Ardren et al. 2007, 2011; DeHaan et al. 2007, 2008b, 2010a; Kassler and Mendel 2007; DeHaan and Godfrey 2009). Even though a direct quantitative comparison cannot be made among these studies because of the different methods used, the levels of genetic divergence observed among East Fork populations are generally lower than those observed in many other studies on bull trout. The overall level of differentiation we observed was 0.063 indicating that East Fork populations have relatively low levels of genetic differentiation among them compared to amounts of genetic diversity within them.

There tended to be an excess of heterozygotes compared to HWE over all of the samples. Furthermore, there was significant linkage disequilibrium between one or more pairs of loci in eight of the 17 samples. Finally, based on the distribution of allele frequencies, the Star Creek sample showed evidence of having experienced a relatively recent bottleneck. Taken together, these data suggest that most bull trout populations in the East Fork, as well as the Bitterroot drainage as a whole, likely have relatively low N_e. This finding is not surprising based on other studies that also found low N_e (Rieman and Allendorf 2001; DeHaan et al. 2007, 2008a). In contrast, relatively low estimates of F_{ST}, especially in the East Fork, and assignment test results suggest some gene flow among populations. The existence of gene flow among populations with relatively low N_e may be very important for maintaining genetic diversity within populations and preventing the accumulation of inbreeding.

Genetic assignment was used to identify tributaries important for the production of migratory bull trout; however, results of the individual genetic assignment tests should be interpreted with caution due to the low levels of genetic divergence among samples. The power of assignment is positively correlated with genetic divergence and the methods used in ONCOR

(Kalinowski 2008) for assignment are considered robust for detecting immigration in populations with low to moderate levels of divergence (F_{ST} ~ 0.05-0.1) (Rannala and Mountain 1997; Hansen et al. 2001; Berry et al. 2004). In addition, the power of assignment is also dependent upon the number of population samples, sample sizes, the number of loci and the degree of polymorphism at each locus (Rannala and Mountain 1997; Hansen et al. 2001). Increasing the number of loci would improve the power of genetic assignment in the East Fork (Cornuet et al. 1999; Hansen et al. 2001; Berry et al. 2004). Due to the low levels of divergence (F_{ST}) we required a 90% probability for assignment. Of the East Fork samples four individuals assigned to tributaries suggesting that there is some movement of fish from tributaries to the East Fork (Table 2.4). Among the tributaries, some individuals assigned to the East Fork (Table 2.4) suggesting that there is also movement of fish from the East Fork into the tributaries. Finally, some fish from tributaries assigned to other tributaries (Table 2.4) suggesting movement of fish from tributary to tributary via the East Fork. These results from the genetic assignment tests supported information gained from radio telemetry studies of migratory fish (FWP, unpublished data; Nyce and Clancy 2008, unpublished data) further confirming the same tributaries which appear to be producing migratory fish including Warm Springs Creek, Meadow Creek, Swift Creek, Clifford Creek and the upper main stem East Fork.

Both the presence of migratory fish and dispersal among populations may be important for the persistence of the bull trout in the East Fork. Distribution of adults into multiple habitats (rivers and tributaries) can buffer populations from disturbances. For example; Sestrich (2005) and Rieman et al. (1997) determined that bull trout populations recovered rapidly after extensive wildfires. Rieman et al. (1997) concluded that two important mechanisms contributing to recovery were dispersal and varied life history (migratory fish). Conserving migratory bull trout in the East Fork is critical (USFWS 2008) the main stem East Fork appears to be an integral component for maintaining the migratory form of bull trout in the drainage and serves as a vehicle for potential genetic exchange among tributary populations. Our results like those in the Metolius River (DeHaan et al. 2008a) may be more typical of a connected system with the migratory component present. Both studies highlight the importance of considering the context and spatial scales of the specific study area versus getting stuck in the current paradigm of bull trout populations being very genetically distinct at small spatial scales.

Table 2.1. Microsatellite loci, * indicates the locus is diagnostic for bull and brook trout hybridization, PCR multiplex annealing temperatures (T_A), PCR multiplex final primer concentrations, and references.

Multiplex Group	Final Concentration (µM)	References
Multiplex 1		
T_A=55°C		
Locus		
Sco 106	0.1	Unpublished WDFW
Sfo 18*	0.16	Angers et al. 1995
Smm 22	0.15	Crane et al. 2004
Sco 216*	0.15	DeHann and Ardren 2005
Multiplex 2		
T_A=56°C		
Locus		
Sco 218*	0.1	DeHann and Ardren 2005
Sco 202	0.1	DeHann and Ardren 2005
Sco 200	0.15	DeHann and Ardren 2005
Sco 220	0.12	DeHann and Ardren 2005
Multiplex 3		
T_A=54°C		
Locus		
Sco 215*	0.075	DeHann and Ardren 2005
Omm 1128*	0.1	Rexroad et al. 2001
Sco 105	0.1	Unpublished WDFW
Multiplex 4		
T_A=55.4°C		
Locus		
Sco 102*	0.1	Unpublished WDFW
Omm 1130	0.1	Rexroad et al. 2001
Sco 107*	0.1	Unpublished WDFW
Sco 212	0.1	DeHann and Ardren 2005

Table 2.2. Sample information (sample number and location), N (sample size after excluding hybrid fish), A (mean number of alleles per locus), A_r (allelic richness), H_e (expected heterozygosity), H_o (observed heterozygosity), private alleles, and the number of hybrid individuals. East Fork refers to main stem East Fork samples.

Sample Number	Location	N	A	A_r	He	Ho	Private Alleles	Hybrid
East Fork Bitterroot								
1	Clifford	23	7.133	6.59	0.674	0.704	6	0
2	Martin	35	7.267	5.89	0.624	0.710	0	0
3	Meadow	69	8.267	6.54	0.696	0.718	1	0
4	Moose	38	7.867	6.57	0.660	0.681	0	0
5	Orphan	22	5.267	4.87	0.586	0.685	0	0
6	Star	15	6.133	6.13	0.689	0.756	0	0
7	Swift	50	7.600	5.8	0.628	0.640	0	0
8	Tolan	28	5.467	4.9	0.622	0.650	0	0
9	Warm Springs	27	7.800	6.81	0.680	0.699	1	0
10	East Fork	73	9.467	7.1	0.703	0.700	2	0
Mean		38	7.227	6.12	0.656	0.694	1	0
West Fork Bitterroot								
11	NFkSheephead	15	6.867	6.87	0.704	0.747	4	4
12	Sheephead	18	7.267	6.92	0.684	0.763	2	1
13	Slate	15	6.933	6.93	0.688	0.756	2	1
Mean		16	7.022	6.91	0.692	0.755	3	2
Main Stem Bitterroot								
14	Daly	51	11.067	8.46	0.734	0.741	3	1
15	Skalkaho	53	10.867	8.12	0.727	0.724	3	6
16	BurntFork	32	9.667	8.12	0.751	0.792	10	2
17	Willow	27	6.267	5.35	0.586	0.602	3	26
Mean		41	9.467	7.513	0.700	0.715	5	9

Table 2.3. Pairwise estimates of genetic divergence (F_{ST}) between all possible pairs of samples in the data set. All F_{ST} values are significant ($p < 0.01$).

		Population Number														
Sample Location		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16
East Fork	1 Clifford																
	2 Martin	0.071															
	3 Meadow	0.045	0.082														
	4 Moose	0.041	0.059	0.032													
	5 Orphan	0.103	0.120	0.073	0.073												
	6 Star	0.043	0.067	0.047	0.048	0.095											
	7 Swift	0.068	0.098	0.043	0.060	0.085	0.065										
	8 Tolan	0.124	0.165	0.105	0.116	0.188	0.147	0.148									
	9 Warm Springs	0.045	0.090	0.041	0.038	0.106	0.041	0.066	0.113								
	10 East Fork	0.016	0.056	0.019	0.018	0.071	0.023	0.030	0.106	0.020							
West Fork	11 NFkSheephead	0.083	0.140	0.076	0.098	0.156	0.089	0.106	0.131	0.074	0.070						
	12 Sheephead	0.087	0.122	0.074	0.102	0.161	0.097	0.091	0.126	0.083	0.071	0.041					
	13 Slate	0.088	0.114	0.078	0.089	0.139	0.089	0.100	0.122	0.071	0.068	0.046	0.050				
Bitterroot River	14 Daly	0.080	0.111	0.066	0.072	0.119	0.086	0.092	0.105	0.063	0.060	0.042	0.066	0.050			
	15 Skalkaho	0.073	0.109	0.059	0.075	0.119	0.082	0.078	0.093	0.061	0.054	0.042	0.054	0.046	0.009		
	16 BurntFk	0.097	0.133	0.095	0.096	0.136	0.102	0.130	0.143	0.093	0.081	0.063	0.098	0.079	0.030	0.057	
	17 Willow	0.240	0.284	0.212	0.244	0.300	0.227	0.262	0.212	0.239	0.224	0.215	0.208	0.221	0.195	0.179	0.209

Table 2.4. ONCOR results for genetic assignment of East Fork bull trout samples using a 90% probability criterion for water of origin. Water body of capture is the location where the sample was collected, number of individuals and percentage of individuals collected from the water body assigned to the water body of capture, the total number of fish in the sample (N), other locations where fish were assigned, probability of assignment (%), and size of the fish (mm). East Fork refers to main stem East Fork Bitterroot River.

Water body of capture	**Individuals assigned to water body of capture (%)**	**N**	**Other locations of assignment (%, size of fish (mm))**
Clifford	18 (78)	23	East Fork (99%, 380); Orphan (100%, 195)
Martin	28 (80)	35	East Fork (98%, 99); Moose (90%, 173)
Meadow	53 (77)	69	East Fork (97%, 132); Warm Spring (97%, 75)
Moose	29 (76)	38	0
Orphan	20 (91)	22	0
Star	10 (67)	15	0
Swift	36 (72)	50	4 East Fork (92%, 89; 97%, 142; 98%, 254; 99%, 147) Moose (98%, 140)
Tolan	28 (100)	28	0
Warm Springs	26 (96)	27	0
East Fork	42 (58)	73	Clifford (94%, 198); Warm Springs (100%, 183); 2 Swift (100%, 165; 100%, 521)

Table 2.5. Comparable studies of within population genetic variation for bull trout. N (sample size), A (mean number of alleles per locus), A_r (allelic richness), H_e (expected heterozygosity), and H_o (observed heterozygosity).

Location	**N**	**A**	**A_r**	**He**	**Ho**
East Fork	380	7.23	6.12	0.66	0.70
West Fork/Bitterroot	211	8.42	7.25	0.70	0.73
United States Study	2890	5.81	4.55	0.57	0.57
Warm Springs	123	3.95	3.82	0.52	0.53
Metolius	332	6.28	5.65	0.58	0.59

Figure 2.1. Map of sampling site locations within the Bitterroot River drainage. East Fork sample sites (highlighted in pink, numbers 1-10), West Fork sample sites (highlighted in orange, numbers 11-13), and Bitterroot sample sites (highlighted in green, numbers 14-17). Numbers refer to populations listed in Table 2.2.

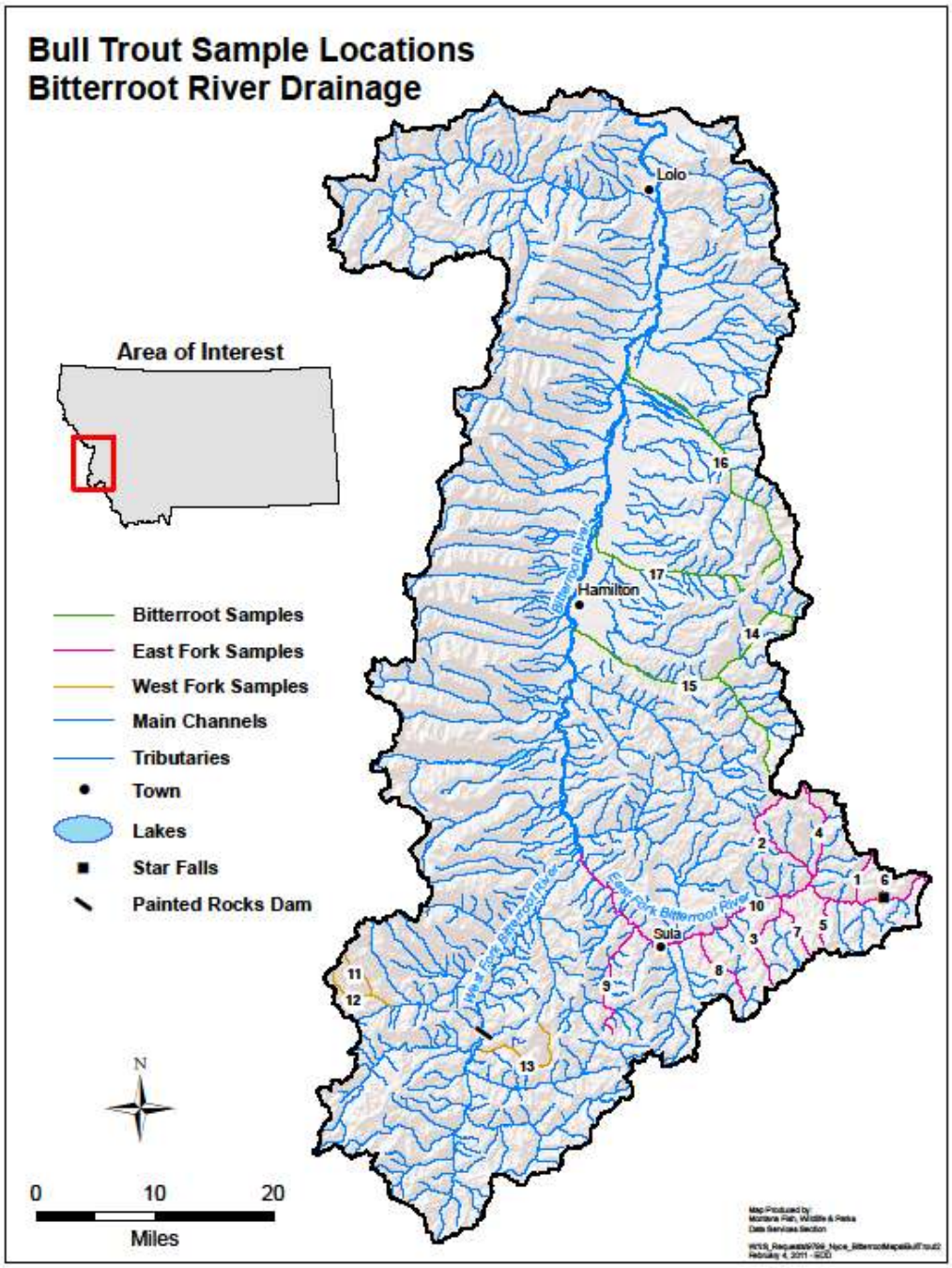


Figure 2.2. Map of the East Fork Bitterroot River drainage (focal study area), bull trout presence, and tributaries where fin samples were collected (highlighted in pink). The main stem East Fork was also sampled to collect fin samples from fluvial fish (highlighted in yellow). Tributaries highlighted in green indicate that no bull trout have been captured in these tributaries in any of the Montana Fish Wildlife & Parks or United States Forest Service sampling efforts to date. These creeks were not surveyed for this study.

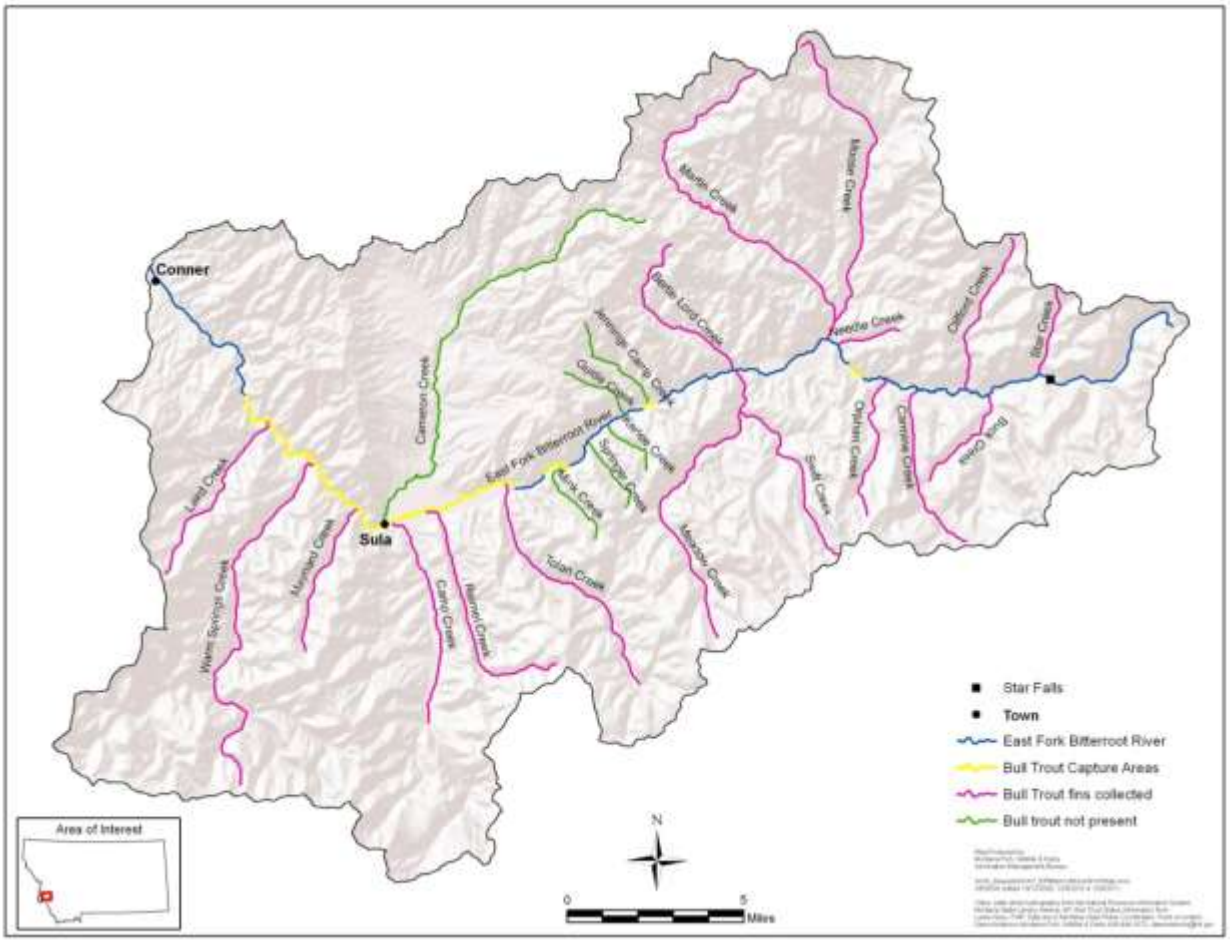
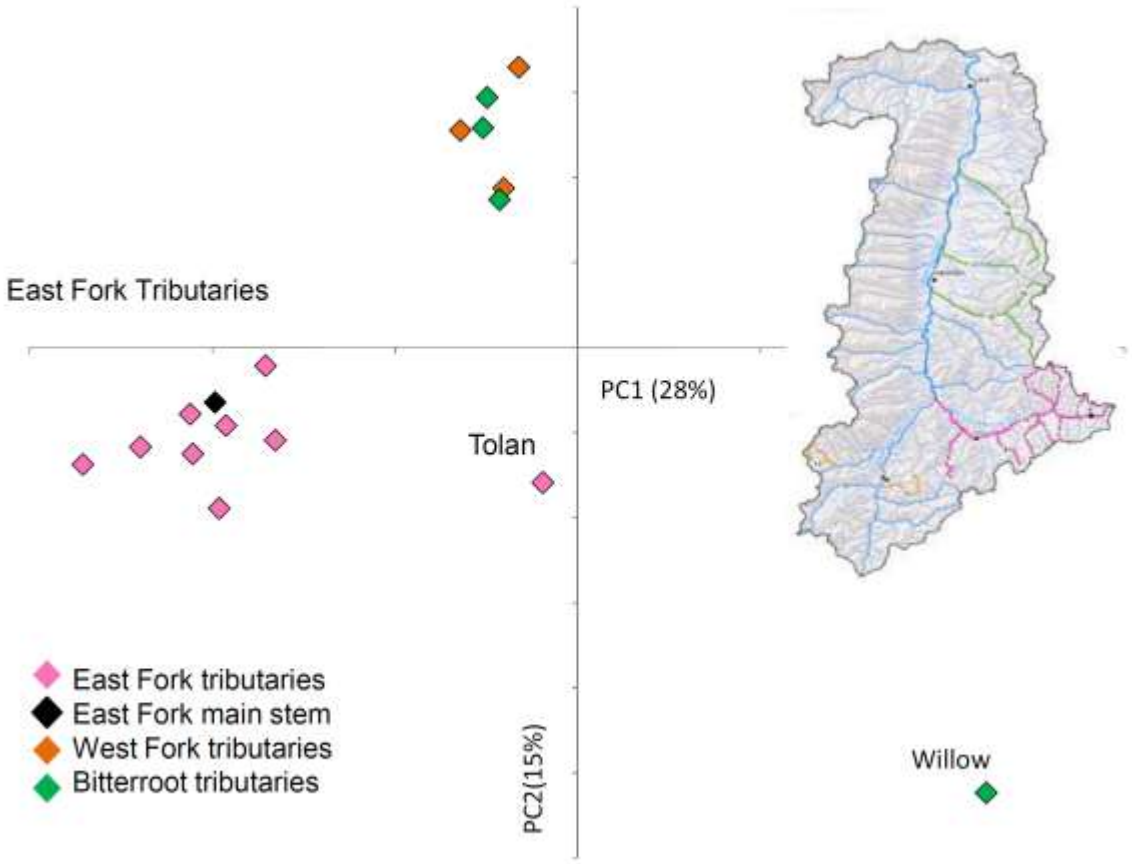


Figure 2.3. Principal components analysis based on allele frequencies of all Bitterroot population samples. The first two axes explain 43% of the variation with Tolan Creek and Willow creek as outliers.



CHAPTER 3

MONITORING POPULATION TRENDS AND THREATS TO BULL TROUT IN THE EAST FORK BITTERROOT RIVER BASIN, MT

Introduction

One common goal of fisheries monitoring programs is to detect trends of populations at the tributary scale and basin-wide scale. As decisions are based on information from these programs, it is necessary to understand our power to detect trends in abundance and how large of a change is likely to occur before detection (Peterman and Bradford 1987; Maxell 1999; Dunham et al. 2001; High et al. 2008; Al-Chokhachy et al. 2009). Regardless of the species, measures of and trends in abundance are very important for tracking populations and persistence in changing environments (Mace and Lande 1991; Allendorf et al. 1997; Al-Chokhachy et al. 2005). This is especially important for threatened and endangered species (Rieman and McIntyre 1996; Peterson and Dunham 2003; Joseph et al. 2006). Thus, it is useful to periodically evaluate how well the sampling methods and monitoring protocols are performing in capturing changes and threats to populations and specific life histories of conservation interest. One such species of conservation interest in Montana is bull trout *Salvelinus confluentus*. Bull trout are currently listed as "threatened" in the USA under the Endangered Species Act (Federal Register 1998) and the conservation of life history diversity (i.e., resident and migratory fish) is a conservation goal.

Trend detection for bull trout is a function of the sampling design, sampling method, sample size, and metric (presence/absence, relative abundance, or population estimate). Although specific sampling designs may vary by location or conservation area, many of the same general protocols are used. These protocols often involve sampling at index sites across multiple tributaries and rivers periodically through time for mark-recapture (hereafter M-R) or depletion population estimates. Additional sites may be sampled periodically associated with other studies (e.g., restoration actions, research studies, etc) with various sampling methods to determine different metrics. In Montana, the most common sampling methods include both redd counts and electrofishing surveys.

Many studies have evaluated the accuracy and precision of redd counts. While there can be substantial sampling error, redd counts are still a common metric for assessing population trends (Rieman and McIntyre 1996; Rieman and Myers 1997; Maxell 1999; Dunham et al. 2001; Al-Chokhachy et al. 2005; Muhlfeld et al. 2006). Sampling error in redd counts can result in biases such as an over or underestimate of the population. A variety of factors can cause such error, for example, interobserver omission or false identification of redds, incomplete sampling, and errors related to visibility and detection (size, age, superimposition, and stream features mistaken for redds; Maxell 1999; Dunham et al. 2001; Muhlfeld et al. 2006). Nonetheless, in several studies redd counts have been found to correlate well with the estimated number of spawning bull trout (based on comparative data using snorkel surveys and weir traps; Dunham et al. 2001; Al-Chokhachy et al. 2005). Because bull trout spawning locations vary from year to year, the use of index areas for redd counts may not provide the necessary data to detect the true trends of populations; therefore, redd counts should be conducted over a broad tributary scale if they are to be used for population trends (Rieman and McIntyre 1996; Dunham et al. 2001).

Electrofishing surveys are another common method used to evaluate fish populations in western Montana's streams and rivers. Such surveys may be used to determine occupancy or abundance. A number of studies have evaluated these methods and the results are varied, especially relating to biases in sampling methods, detection probabilities and sampling error (Thurow and Schill 1996; Peterson et al. 2004; Al-Chokhachy et al. 2009). Capture probabilities can vary with observer, weather, size and species of fish, and the type of habitat or physical stream characteristics (Peterson and Cederholm 1984; Thurow and Schill 1996). To deal with biases in fish sizes, the size classes should be separated for analyses (Ricker 1975; Bohlin et al. 1989). Because of the extensive variation in capture probabilities, population estimates are recommended to deal with different probability of detection across years, observers, and systems (Peterson et al. 2004).

All population estimators have basic assumptions associated with them, if these assumptions are violated, the results are biased. For a common closed, two sampling period M-R estimator, the assumption of a closed population could be violated if fish move into or out of the sampling reach during the survey period (Ricker 1975; Kendall 1999). Studies have investigated the use of block nets to ensure closure to address the movement of fish out of the study reach, but

the results are inconclusive (Young and Schmetterling 2004; Peterson et. al. 2005). Studies have also compared removal versus M-R approaches for population estimates and in most cases M-R is preferred because it is more robust to violations of assumptions (Peterson and Cederholm 1984; Kendall 1999; Peterson et al. 2004).

Even though many studies have promoted the advantages of population estimates for monitoring, specifically to deal with the changes in detection probability over time and space (Peterson and Cederholm 1984; Thurow and Schill 1996; Al-Chokhachy et al. 2005), using the number of unique individuals versus population estimates may be acceptable (or appropriate) when capture and recapture rates are low and capture heterogeneity is high (Slade and Blair 2000; McKelvey and Pearson 2001). Depending on the monitoring goals, effort available, and detection probability, the optimal approach may vary from occupancy (presence/absence) to population estimation (Slade and Blair 2000). Thus, with elusive species an examination of the reliability of population estimates (ability to calculate estimates, the error around those estimates, and assessment of capture heterogeneity) and how well population estimates perform versus possible indices (number of bull trout handled) is useful as we examine any monitoring program.

In addition to fish sampling, habitat data collection is an important part of monitoring the threats to fish populations because changes in habitat may lead to declines in abundance (Baxter et al. 1999; Dunham and Rieman 1999; Letcher et al. 2007). Both watershed (i.e., Bitterroot basin) and stream reach scale factors have been found to be associated with bull trout presence. For example, factors at the watershed scale such as temperature, roads, and geomorphology, influence the distribution of bull trout (Rieman et al. 1997; Baxter et al. 1999; Dunham and Rieman 1999; Baxter and Hauer 2000). On a stream reach scale, habitat variables such as pool frequency, large woody debris, channel width, substrate, temperature and occurrence of brook trout *S. fontinalis* are important and associated with bull trout presence (Fraley and Shepard 1989; Rieman and McIntyre 1995; McPhail and Baxter 1996; Swanberg 1997; Watson and Hillman 1997; Jakober et al. 1998, 2000; Dunham and Rieman 1999; Rich et al. 2003). Monitoring habitat changes that may affect bull trout persistence may allow for detection of potential threats and facilitate proactive conservation actions. Thus, it is important to review if we are monitoring the known threats and key habitat factors at the watershed and reach scales to

be able to detect changes in our populations and life history components of conservation interest so we can rectify them before declines occur.

The goal of monitoring in the Bitterroot River drainage, specifically in the East Fork Bitterroot River drainage (hereafter East Fork) is to capture the trends in bull trout populations (C. Clancy, Montana Fish, Wildlife & Park (FWP), personal communication). In addition, there are recent concerns about the decline in the migratory life history of bull trout (Jakober et al. 1998; Montana Bull Trout Restoration Team 2000; Nelson et al. 2002), thus there is a need to combine recent findings regarding the migratory life history stage to examine whether the current monitoring program effectively tracks changes in migratory fish. FWP and Bitterroot National Forest (BNF) employ a variety of sampling methods to examine trends in bull trout and their habitat. Using existing data, I specifically evaluated the bull trout population sampling protocol to address: *does the current monitoring protocol allow trend detection of migratory bull trout?*

Habitat information and information regarding the presence of non-native fish species from FWP and the BNF surveys were examined to address the following two questions. *First, how well are we tracking potential threats to bull trout in the watershed?* For example, are known threats to bull trout such as exotic species or a high density of roads present along an important spawning tributary recorded? *Second, are data collected at an ecologically relevant temporal and spatial scale for assessing threats or tracking habitat changes?*

Study Area and Methods

The East Fork is located in southwest Montana approximately 116 km south of Missoula, Montana. The watershed encompasses 1,057 km^2. The headwaters are approximately 73 km from the confluence with the West Fork Bitterroot River and main stem Bitterroot River with roughly 166 km^2 lying within the East Fork Wilderness area. Star Falls, a natural barrier to upstream movement located at river km 64, is the upper limit for bull trout occurrence in the East Fork. Much of the upper drainage is predominately managed by the United States Forest Service with some private lands primarily in the valley bottom. Seventeen of the 23 main tributaries of the main stem East Fork have documented bull trout occupancy (Figure 3.1). Tributaries occupied by bull trout consist of both resident and fluvial individuals whose proportions are

unknown (FWP, unpublished data). Historical impacts in the watershed include timber harvest, forest roads, agriculture, water diversions, and wildfire. Other native species present in the drainage include: westslope cutthroat trout *Oncorhynchus clarkii lewisi*, mountain whitefish *Prosopium williamsoni*, largescale sucker *Catostomus macrocheilus*, longnose sucker *C. catostomus*, longnose dace *Rhinichthys cataractae* and slimy sculpin *Cottus cognatus*. Nonnative fishes that are found in the East Fork include rainbow trout *O. mykiss*, brown trout *Salmo trutta*, and brook trout. Hybrids found in the East Fork include westslope cutthroat trout x rainbow trout, westslope cutthroat trout x Yellowstone cutthroat trout *O. c. bouvieri* x rainbow trout and bull trout x brook trout. The East Fork is a core conservation area for bull trout and there are no barriers to movement within the watershed below Star Falls (Bitterroot River Drainage Bull Trout Status Report 1995). A few tributaries to the East Fork have irrigation diversions that may be barriers to fish movement.

FWP and BNF conduct redd counts in the fall in the upper main stem East Fork (within the Wilderness Area) and in Meadow Creek. There are five reaches in the upper main stem East Fork where redd counts have been conducted (1996-2007), one of which was a reach with multiple years of surveys (index reach). The survey distance ranged from 0.8 km to 5.3 km with the index reach survey 1.8 km in length. In Meadow Creek, redd surveys have been conducted at four reaches (1994-present), one of which is an index reach. Survey distances range from 3.2 km to 5.5 km with the index reach 3.2 km in length. For all redd counts, the number of redds and probable redds are recorded by one or two individuals walking the survey reach. The disturbed area of each redd is recorded along with other information such as the specific location of redds and fish observed.

Electrofishing surveys are conducted during the summer and early fall field season. A pulsed monitoring technique is employed which monitors populations for at least three years to serve as a baseline for future population studies (Bryant 1995). Surveys for M-R population estimates involve one marking run (fish are marked by a fin clip) followed by a recapture run within seven days in a 305 m reach. Data have been collected at multiple reaches across 12 tributaries and at five reaches throughout the main stem East Fork (Table 3.1, Figure 3.1). Reaches with more than two years of data are shown. The sampling scheme was designed to have reaches distributed throughout the stream network with a low, mid and higher elevation

reach in each tributary. None of the locations are visited annually; however, some reaches have been surveyed more often than others. In addition to these reaches, there have been a variety of other locations where surveys were performed for various reasons and with various methods, including but not limited to M-R surveys, snorkeling, and single pass for fish presence/absence (Table 3.3).

To examine the question whether the current monitoring protocol allows trend detection of migratory bull trout, I first aggregated and examined the redd count and electrofishing M-R population estimate datasets. I used FA+ (FWP fisheries analysis software) which analyzes the M-R data to estimate bull trout abundance across vulnerable size classes. In many cases, population estimates could not be calculated because of the low capture and recapture numbers. If less than three individuals were recaptured, then there was not a population estimate. Where there were no population estimates, I counted the number of bull trout handled (hereafter #BTH) which is the number of fish marked (on the marking run) plus the number of fish captured (on the recapture run) minus the number recaptures. To examine the potential of #BTH for being a reliable indicator, I examined the correlation of #BTH with population estimates for seven reaches in five tributaries (SPSS v18, Pearson Correlation).

FWP and BNF have collected habitat data across the East Fork at both the watershed and reach scale. From a landscape perspective, the Forest maintains databases that include forest roads, county and state roads, road crossings, known fish barriers, grazing allotments, fire disturbances, and harvesting contracts. In addition, reach scale habitat data have been collected since the early 1990's (Table 3.4). I examined the existing habitat data qualitatively to evaluate whether we are tracking potential threats to bull trout with the appropriate response variables at relevant temporal and spatial scales.

Results

Monitoring population data summary

Redd count data revealed substantial annual variability. In the main stem East Fork redd counts varied from zero to eight; however, within the index reach from 2000 to 2007 the range was zero to five with a median of one. Four of those years recorded one probable redd and two years recorded zero redds. Redd counts were discontinued in the East Fork after the 2007 survey

because after six years of surveys no more than one probable redd was located (C. Clancy, FWP, personal communication). In Meadow Creek, redd counts varied from 1 to 21 between 1994 and 2010 including the index reach. Within the index reach, one to two larger redds are often observed (assumed made by migratory fish) while the remainder of redds appear to be constructed by resident bull trout (M. Jakober, USFS, personal communication).

M-R electrofishing surveys include information of all sizes of bull trout captured. This data represents both resident fish and juvenile migratory fish whose proportions are unknown. It is unlikely that larger (> 355 mm) migratory fish are captured and recaptured on M-R sections because they are often moving through the stream network; therefore, population estimates on larger migratory fish are not obtained. Martin, Meadow, Moose, Swift, Tolan, and Warm Springs Creeks have multiple years of M-R surveys along with five main stem East Fork reaches. Although there are data for multiple years of M-R surveys, a summary of these data reveal that there is one reach on the main stem East Fork and eight tributary reaches where trends can be evaluated using more than three years of data (Table 3.2, Figure 3.4). Some studies state that at least six to ten years of data are necessary for detecting population trends (Peterman and Bradford 1987; High et al. 2008). Five reaches in three tributaries (Meadow, Moose and Tolan Creeks) have the suggested minimum of six years of data and only two tributary reaches include at least ten years of data (Table 3.2).

Since there is capture heterogeneity and capture probabilities of bull trout are low, I examined whether an index, #BTH correlated with population estimates where both were available over the same size range of fish (Meadow, Moose, Swift, Tolan, and Warm Springs Creeks; Figure 3.4). All correlations were significant and positive, ranging from 0.82-0.99. Correlations in Meadow (reach numbers 5.6 and 7.3), Moose (reach numbers 1.4 and 3.6) and Tolan (reach number 5.1) were significant at alpha level 0.01 (2-tailed). Swift (reach number 0.7) and Warm Springs (reach number 7.4) were significant at alpha level 0.05 (2-tailed).

Overall, very little is known about the spawning locations of the migratory life history component, thus a genetic study was employed to learn about tributaries important for spawning (Nyce M.S. thesis, Chapter 2). Radio telemetry studies on migratory bull trout have been conducted in the East Fork in four different years (2000, 2005, 2007; FWP, unpublished data; Nyce and Clancy 2008, unpublished data). Overall these studies were met with limited success

due to the small number of migratory fish tagged, but the 2008 study tracked three migratory bull trout to tributaries (Martin, Orphan, and Clifford Creeks) where they had never previously been located. Results of a recent population genetics study demonstrated that many of the fish captured in the river appeared to belong to one spawning group (Nyce M.S. thesis, Chapter 2). This study indicated that there was a spawning population not associated with a known tributary (possibly spawning in the upper East Fork), but that migratory fish captured in the river also assigned to Clifford, Swift and Warm Springs Creeks. While radio telemetry studies and recent genetic results have contributed to information on the migratory component, electrofishing population surveys occasionally pick up fish large enough (> 355 mm) to be categorized as migratory but population estimates of this size class are not obtained. Migratory fish are often found in Meadow, Swift, and Warm Springs Creeks (FWP, unpublished data). Thus, the potential spawning area for the East Fork group is unknown but M-R surveys have occurred in many of the tributary populations with a migratory life history form, such as Meadow, Moose, Swift, and Warm Spring Creeks (Table 3.2).

Habitat data summary

Varieties of habitat data (at both the watershed and reach scale) have been or are currently collected across the East Fork (Table 3.4). Various surveys included the following types of watershed scale information: stream length, land and cover type, number of roads, road density, road crossings, number and types of barriers, roadless areas (wilderness), and disturbances such as fire and grazing (BNF and FWP, unpublished data). This information captures many of the known threats to bull trout, for example, road density and habitat disturbances (Rieman et al. 1997; Baxter et al. 1999; Dunham and Rieman 1999; Gregory and Gamett 2009). Road density, another predictor of bull trout presence (Baxter et al. 1999) has been recorded across the East Fork. The entire East Fork watershed encompasses approximately 1,057 km^2 and there are approximately 1960 km of roads for a total road density of roughly 1.86 km/km^2 (Table 3.5).

In addition to tracking grazing allotments across the East Fork basin, habitat data collection at the reach scale is conducted on grazing allotments and takes place every year. There are a total of five different grazing allotments across the East Fork encompassing a total of approximately 235 km^2 (BNF unpublished data). The allotments are monitored to record

riparian and stream channel conditions, such as bank erosion as a result of livestock use. This information helps track disturbances directly related to cattle presence and actions can be implemented as a result of the findings. For example, riparian fencing may be installed to inhibit cattle from denuding riparian vegetation and causing streambank erosion (BNF, unpublished data).

In addition to potential threats, reach scale habitat characteristics that link to bull trout habitat requirements have also been collected. The following types of information have been or are currently collected: stream order, slope or gradient, Rosgen channel type, reach condition, elevation, wetted width, depth, pool frequency, stream bottom material, percent fines, canopy cover, habitat type, large woody debris, temperature, and distance to a main stem source (source abundance; Table 3.4). While habitat data collection still takes place in the East Fork it is not as intense as historically and it is not specifically part of the monitoring program. A recent review of the data revealed that much of it was 10 to 20 years old with the exception of both water temperature data and more recent data on habitat characteristics collected periodically through United States Forest Service monitoring. For example, BNF conducts Inland Native Fish Strategy (Federal Register 1995) habitat surveys (collecting both watershed and reach scale data) every year across the entire Bitterroot drainage; however, those locations may not be within the East Fork.

Discussion and Recommendations

Bull trout are a difficult species to capture (and recapture) because of their low densities, elusive behavior, association with complex habitat, and cryptic coloration (Rieman and McIntyre 1993; Thurow and Schill 1996; Swanberg 1997; Peterson and Dunham 2003; Al-Chokhachy et al. 2009). In order to improve our inference, it has been suggested that managers should consider combining different monitoring methods, for example conducting redd counts, population estimates with M-R and even the use of genetics (Dunham and Rieman 1999; Dunham et al. 2001; Epifanio et al. 2003). Al-Chokhachy et al. (2005) found that within basin redd count population estimates are often very similar to M-R population estimates. Therefore, a combined approach may lead to more reliable information on population status and may provide a stronger inference for overall population trends. However, before widespread use, these

relationships between specific indicators and estimators need to be examined for the species and system of application.

Overall evaluation of the monitoring program for bull trout reflects what has been determined in previous studies; they are a difficult species to monitor. However, the data collection that takes place on bull trout abundance and occurrence (presence/absence) is captured in the East Fork and contributes to a database that allows for comparisons between streams (Clancy 2000, unpublished data).

Does the current monitoring protocol allow trend detection of migratory bull trout?

Redd count data have not been very useful for tracking bull trout abundance in the East Fork. However, in light of the results from Chapter 2, it may be worthwhile to investigate new areas where redd counts could be conducted to provide greater inference on the status of migratory fish (i.e., re-visiting the upper East Fork). It may also be useful to investigate the tributaries where migratory fish are now predicted to be based on electrofishing surveys, radiotelemetry and genetic assignment. For example, migratory individuals are often detected in Swift Creek and two individuals captured in the main stem East Fork genetically assigned to Swift Creek (Chapter 2). It seems like Swift Creek may be a good place to survey for redd counts. Based on the combination of information, other tributaries to consider would be Clifford, Meadow, Orphan and Warm Springs Creeks. If redd counts are employed to track bull trout, approaches to increase the reliability and precision of redd count surveys should be considered. It has been suggested that redd counts should be conducted over a broad tributary scale and multiple surveys during the spawning season need to take place for redd counts to be a good indicator of population size (Dunham et al. 2001). In addition, accounting for observer error will also lead to greater reliability of redd count data (Muhlfeld et al. 2006).

Even though FWP and BNF have been performing M-R surveys for bull trout throughout the basin, there are few tributaries and reaches where population estimates can be calculated over time. It appears that reliable population estimates are captured for resident and juvenile migratory bull trout in a few tributaries and reaches but not across the entire East Fork. The placement of M-R surveys is designed to capture information throughout the stream network; however, with low capture probability and densities, estimates are not always possible. M-R

methods have been shown to be precise and accurate and allow for trend detection if population estimates can be achieved (Peterson and Cederholm 1984; Slaney and Martin 1987; Al-Chokhachy et. al.2005). For the East Fork bull trout monitoring plan, it would be useful to address sources of error and different approaches for increasing capture probabilities across most of the tributaries. FWP has addressed the concern that fish may be moving during M-R surveys, thus violating the assumption of a closed population. They determined that movement was minimal and did not bias population estimates (Clancy 1996, unpublished data). In addition to working on sources of error, consideration of the desired effect size (i.e., what magnitude of decline or increase is of interest) and accepted probabilities for making statistical errors (i.e., wrongly rejecting the null hypothesis of no trend; Gibbs 2000) would be useful to evaluate (i.e., whether the monitoring plan is likely to achieve the desired goals). FWP recently had an independent consultant conduct a power analysis on population estimates for cutthroat trout and bull trout. His initial conclusions are similar to this analysis. He stated that the bull trout data are challenging for a variety of reasons: there are few locations with enough data observations to capture basin-wide trends, time series data fail to meet some of the basic assumptions for statistical analyses (i.e., data independence) and most sites do not have enough data points and statistical power to detect trends (increases or decreases of abundance). He also recommended a power analysis of population estimates, specifically addressing the accepted probability for making type I and type II error, and a priori determining the desired magnitude of detecting a decline (M. LeMoine, LeMoine Ecological Services, personal communication).

My examination of #BTH versus population estimates focusing specifically on East Fork creeks revealed that there were significant positive, linear correlations between the index #BTH and the abundance estimator (using the same size range of fish, Figure 3.6). While there is likely capture heterogeneity, substantial differences in size class estimates between years, and no fish under 127 mm total length considered it may be that #BTH could provide a reliable measure for fish of a restricted size class (possibly 127 to 254 mm total length). This index value (#BTH) is often reported by FWP for locations without population estimates (C. Clancy, FWP, personal communication). In an attempt to determine if fish are captured in similar proportions when sampling, FWP has previously compared the number of new fish handled in both sampling dates (mark run and recapture run) versus the population estimate in tributaries across the entire Bitterroot River basin (C. Clancy, FWP, personal communication). They determined that

efficiency appears to be greater for cutthroat trout compared to bull trout and there did not seem to be a good relationship between the index and population estimate. Given the low capture probabilities, it may be that presence/absence surveys would provide reliable information about species persistence across the East Fork. For a broader perspective of bull trout persistence in the East Fork basin, it may be useful to evaluate how these reaches have been surveyed (1 pass electrofishing, snorkeling, etc) and revisit reaches that have not had been surveyed in the past decade. FWP is currently reviewing their statewide monitoring program and are investigating a variety of issues including but not limited to: length of electrofishing reaches, how often to collect population estimates, what are appropriate sample sizes, what is detection probability, the use of catch per unit effort, and the optimal season to conduct sampling.

Genetic monitoring of bull trout populations in the East Fork could be a method to track changes in the genetic population structure of bull trout populations. Given the low levels of genetic divergence and apparent dispersal among the spawning tributaries, it may be possible to manage the East Fork as a metapopulation (i.e., maintaining migratory corridors and limiting habitat degradation). Genetic sampling every decade or so to monitor changes in genetic structure could be employed. This would provide insight into changes in habitat such as fragmentation of migratory corridors, the loss of the migratory component, and possible loss of dispersal among tributaries. Tolan Creek has been identified as a tributary with greater genetic differentiation compared to the rest of the East Fork. Therefore, it may be useful to watch for genetic changes such as inbreeding depression that could contribute to population declines. With the use of temporal genetic monitoring, changes in effective population size (N_e) and allelic diversity could be monitored to highlight areas that are a potential conservation risk (Newman and Pilson 1997; Soule and Mills 1998).

How well are we tracking potential threats to bull trout in the watershed? Are data collected at an ecologically relevant temporal and spatial scale for assessing threats or tracking habitat changes?

Many of the variables that have been identified as important and associated with bull trout presence have been periodically collected in the East Fork (Fraley and Shepard 1989; Rieman and McIntyre 1993; McPhail and Baxter 1996; Watson and Hillman 1997; Jakober et al. 1998, 2000; Dunham and Rieman 1999; Rich et al. 2003). Habitat monitoring is not a focus for

FWP or BNF and much of the data is dated; however, habitat data collection still occurs. At the reach scale, FWP has evaluated habitat data collection across a number of sites using regression and discriminant analyses to determine the habitat variables predicting bull trout presence (Clancy 1992, unpublished data). They concluded that elevation, wetted width and high overhead cover predicted bull trout presence. Similarly Rich et al. (2003) evaluated habitat correlates of occupancy and found positive correlation with channel width, large woody debris and the presence of a strong, neighboring main stem population. Even though basin-wide habitat surveys are rarely conducted, reach level habitat surveys are being conducted in the basin as well as around potential impacts (i.e., grazing allotments). For example, there were two recent IWALK surveys on the main stem East Fork in 2008 and 2010 (BNF, unpublished data).

At the watershed scale, grazing allotments are monitored, harvest is currently relatively low, and road density is recorded. Haynes et al. (1996) characterizes a road density of 4.4 km/km^2 as "high" and a density of greater than 12.17 km/km^2 as "extremely high". This is based on extensive analysis of road density and bull trout occurrence. While road density is not "extremely high" in the East Fork, there are areas that would be characterized as having "high" road density (Table 3.5). The highest road density is in the Bertie Lord watershed, an area of historically low numbers of bull trout and no association with the migratory life history form. Overall, grazing and road density are monitored and neither appear to be substantial threats in the East Fork.

Given the potential threats to bull trout, both the potential impact of non-native fish and warming river and stream temperatures may be of most concern. Information on the presence of non-native species is collected in all fish surveys and provides useful data on the expansion of these species into prime bull trout habitat (i.e., locations higher in the watershed). Recent sampling of tributaries in the East Fork did not indicate a large brook trout presence (Chapter 2); however, recent electrofishing surveys have noticed that an occasional brown trout is captured in tributaries and the main stem East Fork at locations higher than they were previously captured. This could be cause for concern because brown trout have been shown to have high growth rates and be superior competitors compared to native trout (Budy et al. 2008). Water temperature information is collected across the East Fork (Table 3.4) and is monitored annually. This

monitoring helps to keep track of increasing water temperatures; another known threat to bull trout (Dunham and Rieman 1999).

Overall, the monitoring program in the East Fork captures information on migratory bull trout but is weak at tracking trends in abundance using redd counts and estimators. However, the index #BTH appears to be a reliable indicator of abundance. Collection of information on habitat variables important to bull trout occupancy and tracking of threats to bull trout occurs but is not a primary focus in the East Fork.

Recommendations

- Revisit the upper main stem East Fork to find migratory bull trout spawning areas to potentially start redds counts.
- Develop a sampling protocol focused on trend detection by combining population estimates with redd counts in tributaries thought to contribute to the migratory component (Warm Springs Creek, Meadow Creek, Swift Creek and Clifford Creek).
- Conduct power analyses: what is the magnitude of decline desired for detection, what are acceptable probabilities for Type 1 and Type II errors, and minimize sampling error for M-R electrofishing surveys and redd counts.
- Evaluate the potential for monitoring occupancy across the broader basin.
- Incorporate genetic monitoring, collect new genetic sample every five to ten years to determine if there are changes in gene flow compared to data from Chapter 2
- Identify locations across the East Fork where habitat data is more than 20 years old and substantial threats exist, prioritize those locations based on bull trout occurrence and revisit to collect information on current habitat conditions.

Table 3.1. Mark-recapture population estimate locations and reach number on the main stem East Fork Bitterroot River and tributaries of the East Fork Bitterroot River. Reaches with more than two years of data are shown. ID numbers are found on Figure 3.3.

ID Number	Location/reach number
1	Main stem East Fork 2.5
2	Main stem East Fork 12.0
3	Main stem East Fork 19.1
4	Main stem East Fork 25.6
5	Main stem East Fork 31.4
6	Laird Creek 1.4
7	Laird Creek 2.3
8	Warm Springs Creek 3.5
9	Warm Springs Creek 7.4
10	Maynard Creek 0.1
11	Camp Creek, West Fork
12	Reimel Creek 3.8
13	Tolan Creek 2.1
14	Tolan Creek 5.1
15	Tolan Creek 7.3
16	Bertie Lord Creek 0.2
17	Meadow Creek 5.6
18	Meadow Creek 7.3
19	Swift Creek 0.7
20	Martin Creek 1.3
21	Martin Creek 7.5
22	Moose Creek 1.4
23	Moose Creek 3.6

Table 3.2. Mark-recapture population estimate information for the main stem East Fork Bitterroot River and tributaries of the East Fork Bitterroot River. ID number found on Figure 3.3, location and reach number, number of years of mark-recapture data collection with the date range, and number of population estimates for bull trout.

ID Number	Location/reach number	Years of Mark-recapture	Date range for Mark-recapture	# of Pop. estimates
5	Main stem East Fork 31.4	7	1992-2008	4
6	Laird Creek 1.4	12	1990-2005	0
9	Warm Springs Creek 7.4	6	1992-2008	5
10	Maynard Creek 0.1	4	2001-2004	0
11	Camp Creek, West Fork 0.3	7	1997-2007	0
12	Reimel Creek 3.8	7	1990-2003	0
14	Tolan Creek 5.1	10	1989-2007	10
15	Tolan Creek 7.3	4	1989-2003	1
16	Bertie Lord Creek 0.2	10	1990-2007	0
17	Meadow Creek 5.6	14	1989-2008	11
18	Meadow Creek 7.3	7	1989-2010	6
19	Swift Creek 0.7	4	1995-2003	3
20	Martin Creek 1.3	10	1992-2010	4
21	Martin Creek 7.5	8	1985-2003	1
22	Moose Creek 1.4	9	1991-2006	6
23	Moose Creek 3.6	8	1992-2008	8

Table 3.3. Bull trout snorkeling, electrofishing mark-recapture population estimates, and presence/absence data for the main stem East Fork Bitterroot River and tributaries of the East Fork Bitterroot River. Location, total number of presence/absence reaches for each location, number of reaches with at least three years of data, maximum number of years for any one reach, and the date range for all presence/absence data.

Location	Total # of reaches	# of reaches with at least 3 years of data	Maximum # of years for any reach	Date range
Main stem East Fork	27	4	12	1952-2010
Bertie Lord Creek	10	2	11	1990-2010
Buck Creek	3	0	1	1994-2010
Camp Creek, West	12	1	7	1993-2010
Carmine Creek	3	0	1	1994-2009
Clifford Creek	2	0	1	1994-2009
Laird Creek	12	2	8	1990-2009
Martin Creek	8	2	11	1985-2010
Maynard Creek	3	1	6	1995-2009
Meadow Creek	22	3	15	1952-2010
Moose Creek	18	2	9	1952-2010
Orphan Creek	2	0	1	1994-2010
Reimel Creek	9	3	9	1990-2010
Star Creek	1	0	1	1994-2010
Swift Creek	2	1	4	1994-2010
Tolan Creek	11	3	10	1985-2010
Warm Springs Creek	14	2	10	1990-2010

Table 3.4. Locations within the East Fork Bitterroot River basin that are known to have bull trout present, types of habitat data available with the date range, and years of temperature data available. Existing habitat data were collected by Cecil Rich (C), Montana Fish, Wildlife & Parks and United States Forest Service personnel performing IWALK (I) and R1/R4 surveys (R). These surveys collected habitat data associated with bull trout habitat needs including stream widths, habitat complexity (large wood) and habitat type (pools, riffles). An asterisk (*) indicates other habitat data are present for the location but not collected in the surveys indicated above.

Location	Types of habitat data	Date Range	Years of temperature data
Main stem East Fork	I, R, *	1992-2010	45
Bertie Lord Creek	C, I	1992-1999	11
Buck Creek	C	1994-2001	3
Camp Creek, West Fork	C,I, *	1993-1999	6
Carmine Creek	C, R	1994, 2002	1
Clifford Creek	C, R	1994, 2002	1
Laird Creek	C, I	1992-2009	11
Martin Creek	C, *	1991-1994	21
Maynard Creek	C, I	1995-2000	5
Meadow Creek	C, *	1989-1994	28
Moose Creek	C, *	1991-1996	15
Orphan Creek	C, R	1994, 2002	1
Reimel Creek	C, *	1991-1994	9
Star Creek	C	1994, 2002	1
Swift Creek	C, *	1994-1995	5
Tolan Creek	C, I, *	1989-2006	21
Warm Springs Creek	C, I, *	1992-1999	21

Table 3.5. Watersheds of the East Fork Bitterroot River drainage, watershed size, km of roads, and road density. Data provided by the Bitterroot National Forest, United States Forest Service, Hamilton, Montana.

Watershed	Watershed size (square km)	Km of Roads	Road Density (km/square km)
Laird	25.79	97.62	3.79
Warm Springs	116.47	66.64	0.57
Maynard	13.55	18.89	1.39
Camp	92.15	267.71	2.91
Reimel	23.08	8.47	0.37
Tolan	50.76	39.46	0.78
Bertie Lord	26.60	96.19	3.62
Meadow	83.24	142.41	1.71
Swift	31.29	9.93	0.32
Martin	82.57	150.97	1.82
Moose	64.54	63.75	0.99
Orphan	12.33	0.00	0.00
Carmine	17.43	0.00	0.00
Clifford	18.23	0.00	0.00
Buck	26.02	0.00	0.00
Star	11.14	0.00	0.00

Figure 3.1. Map of the East Fork Bitterroot River drainage and bull trout presence/absence. Tributaries in pink show bull trout presence and where fin samples were collected for Chapter 2. Tributaries highlighted in green indicate that no bull trout have been captured in these tributaries in any of the Montana Fish, Wildlife & Parks or United States Forest Service sampling efforts to date.

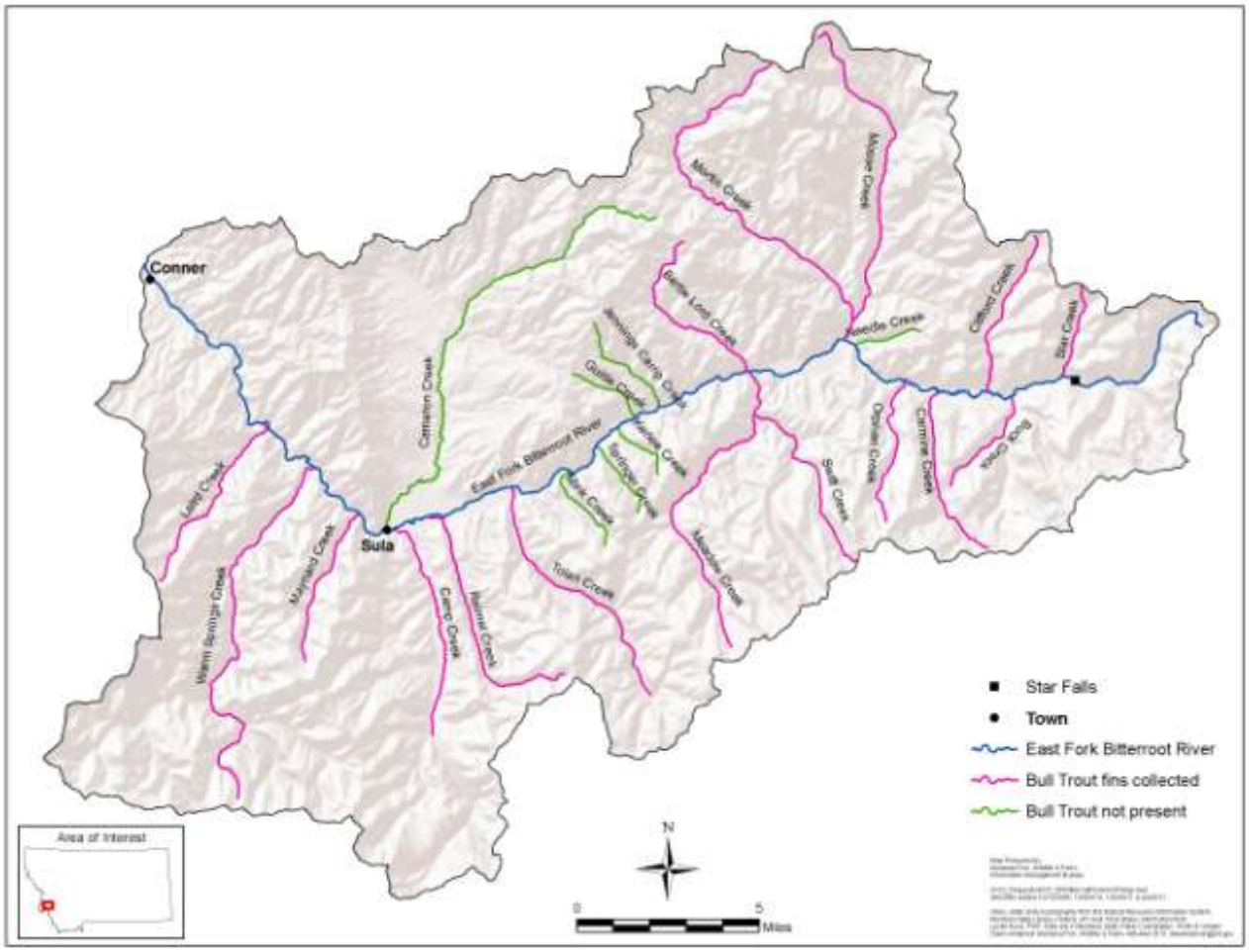


Figure 3.2. Bull trout redd count results for Meadow Creek and the main stem East Fork index reaches. Redd counts include all definite and probable bull trout redds.

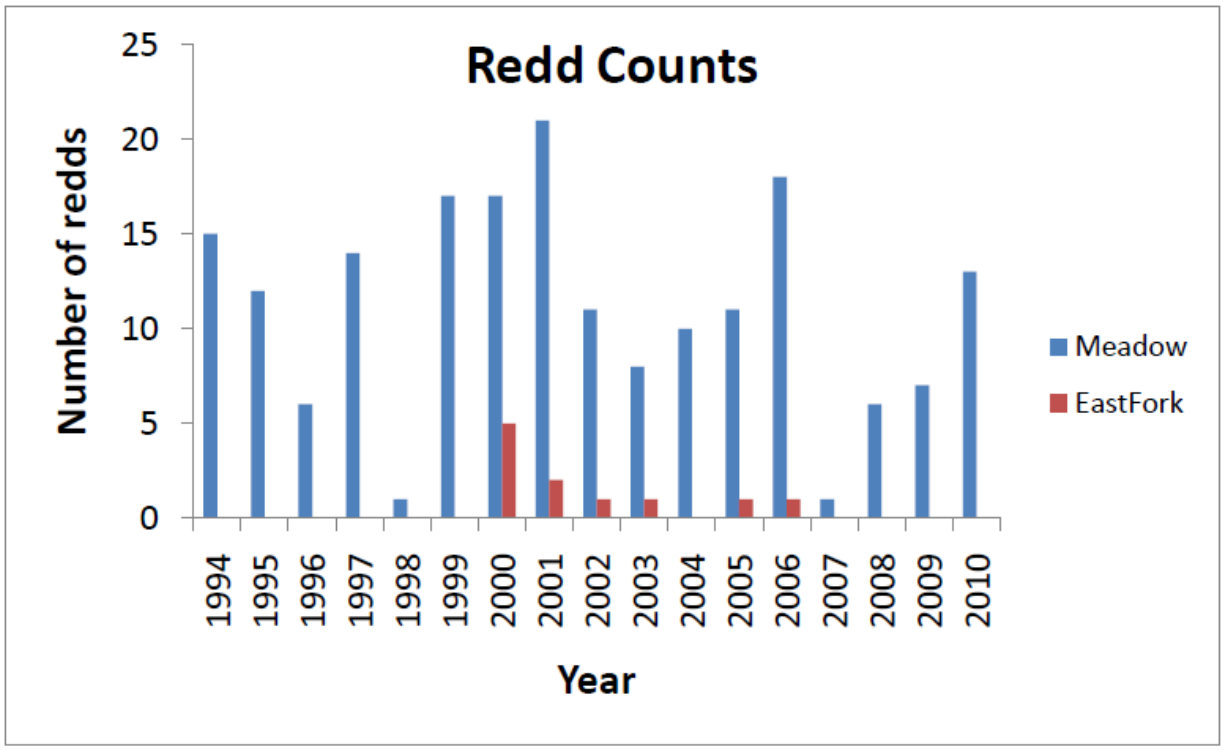


Figure 3.3. Map of mark-recapture survey reaches in the East Fork Bitterroot River basin. Yellow stars indicate sample locations, numbers refer to reaches identified in Table 3.1.

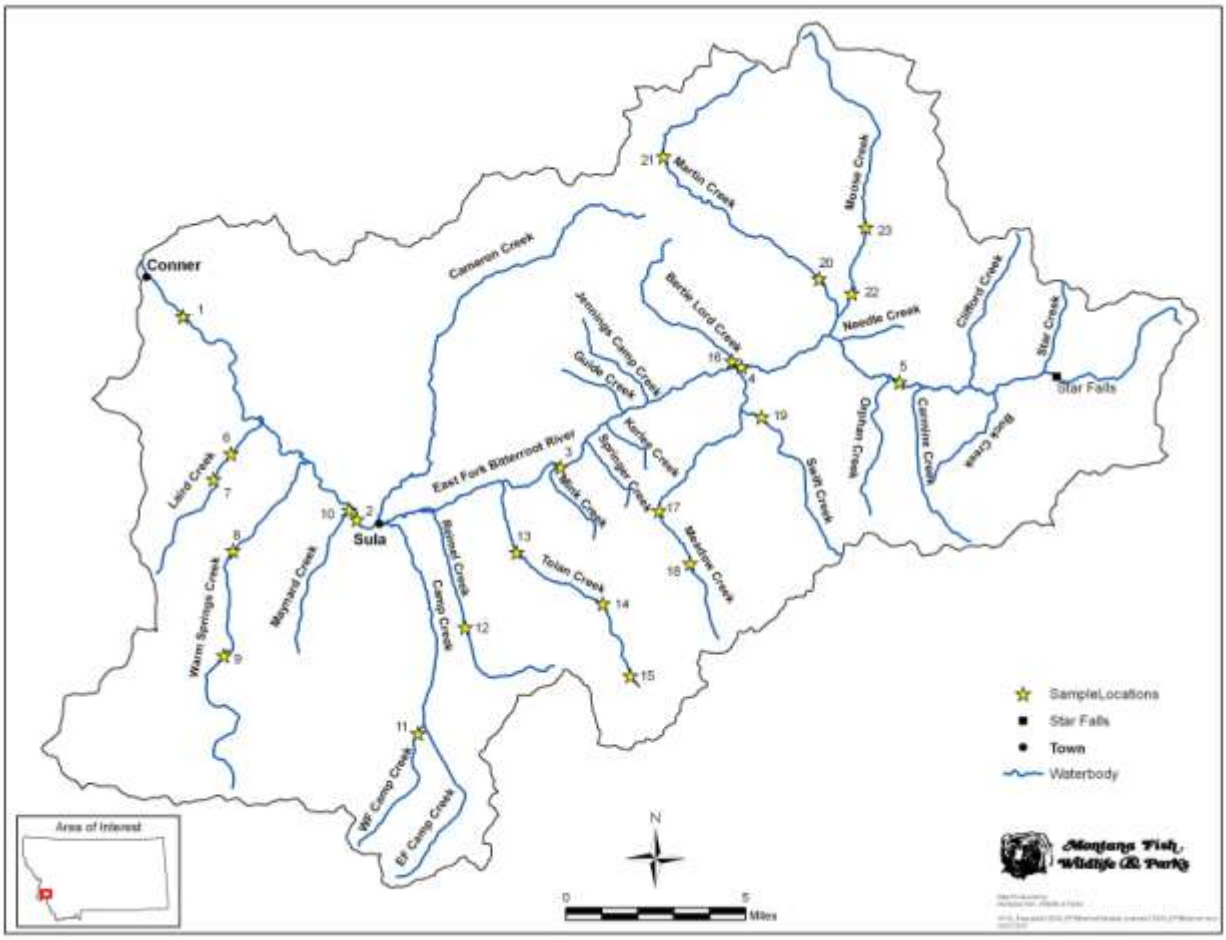
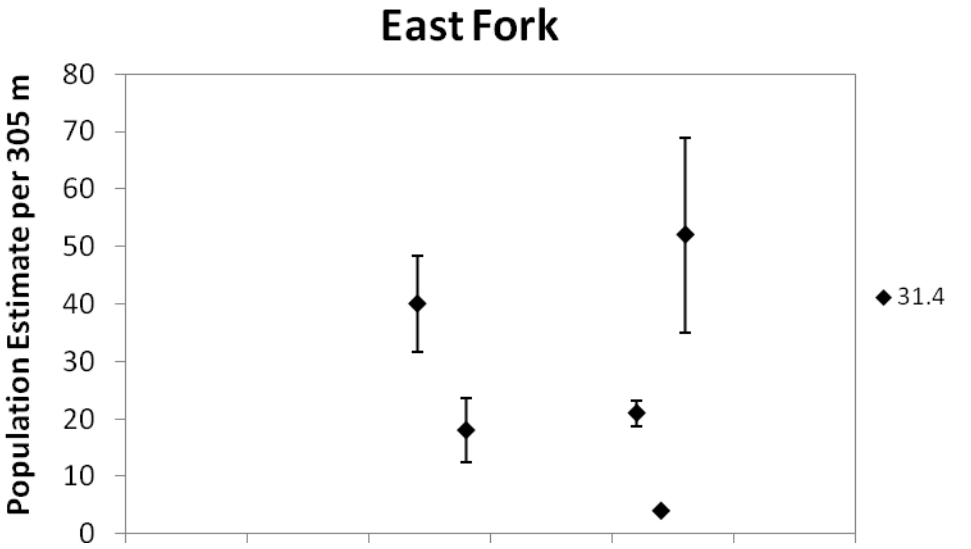
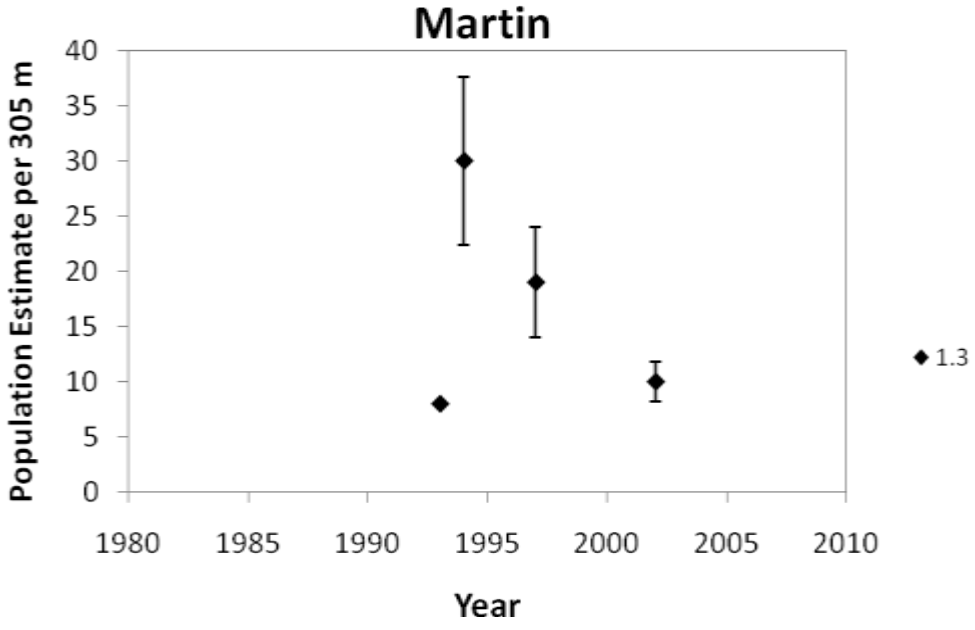


Figure 3.4. Bull trout population estimates (fish ≥ 127 mm) for a) the main stem East Fork Bitterroot River reach number 31.4, b) Martin Creek reach number 1.3, c) Meadow Creek reach numbers 5.6 and 7.3, d) Moose Creek reach numbers 1.4 and 3.6, e) Swift Creek reach number 0.7, f) Tolan Creek reach number 5.1, and g) Warm Springs Creek reach number 7.4. Error bars represent standard deviations.

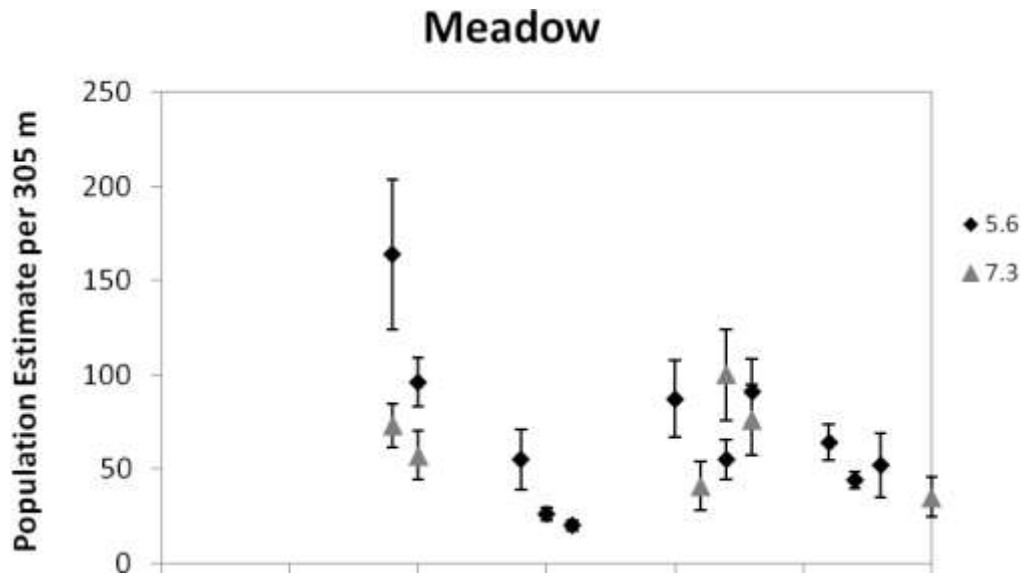
a)



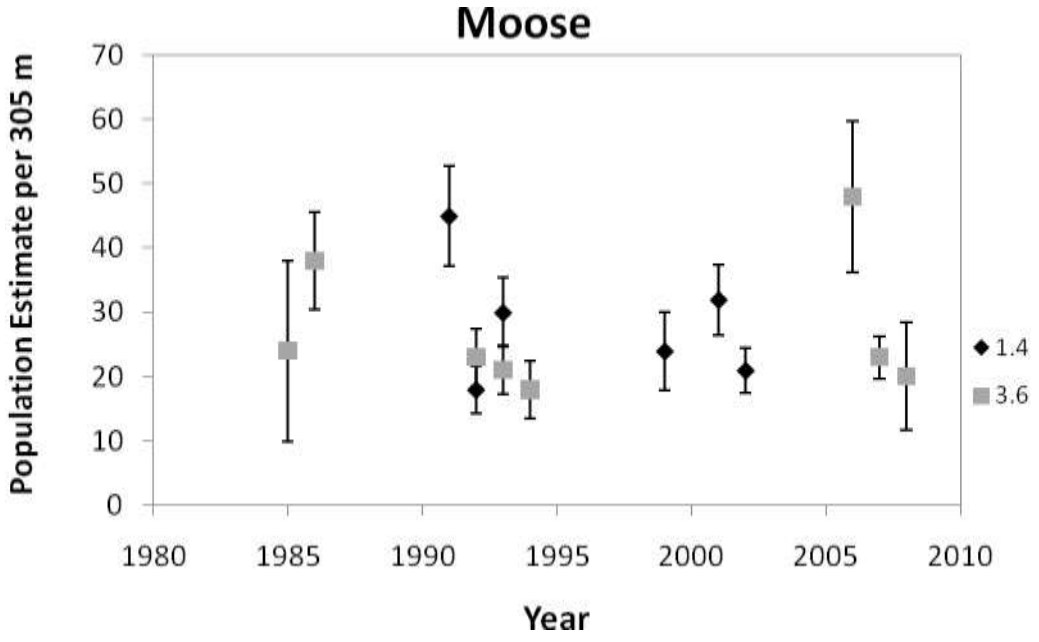
b)



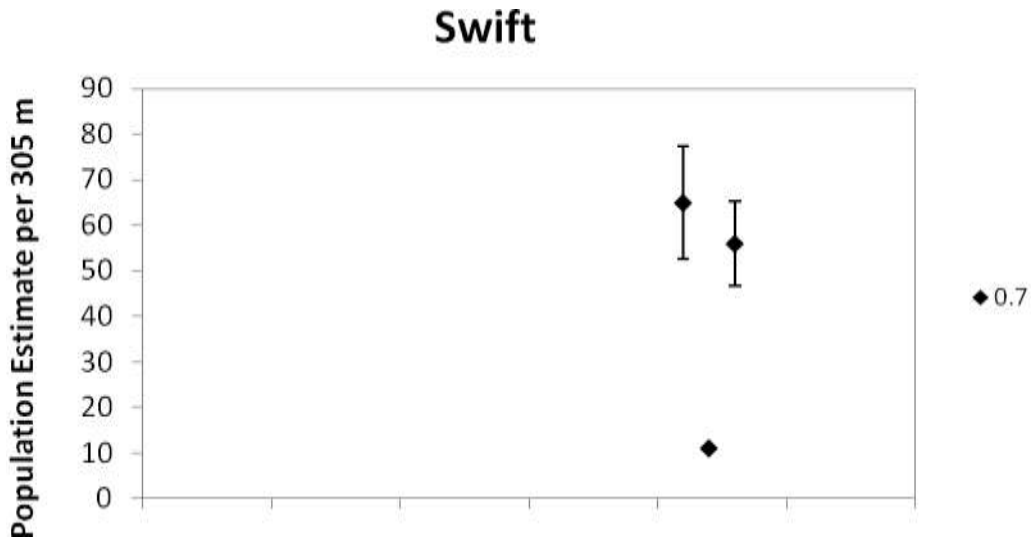
c)


Meadow
Population Estimate per 305 m
250
200
150
100
50
0
5.6
7.3

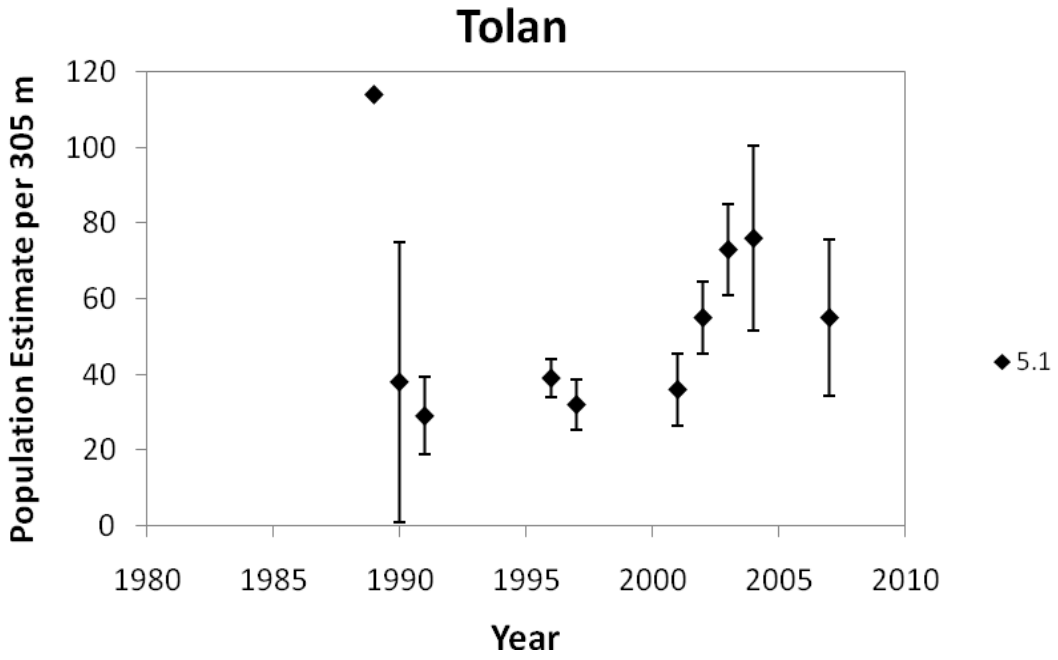
d)


Moose
Population Estimate per 305 m
70
60
50
40
30
20
10
0
1980
1985
1990
1995
2000
2005
2010
Year
1.4
3.6

e)


Swift
Population Estimate per 305 m
90
80
70
60
50
40
30
20
10
0
0.7

f)


Tolan
Population Estimate per 305 m
120
100
80
60
40
20
0
1980
1985
1990
1995
2000
2005
2010
Year
5.1

g)

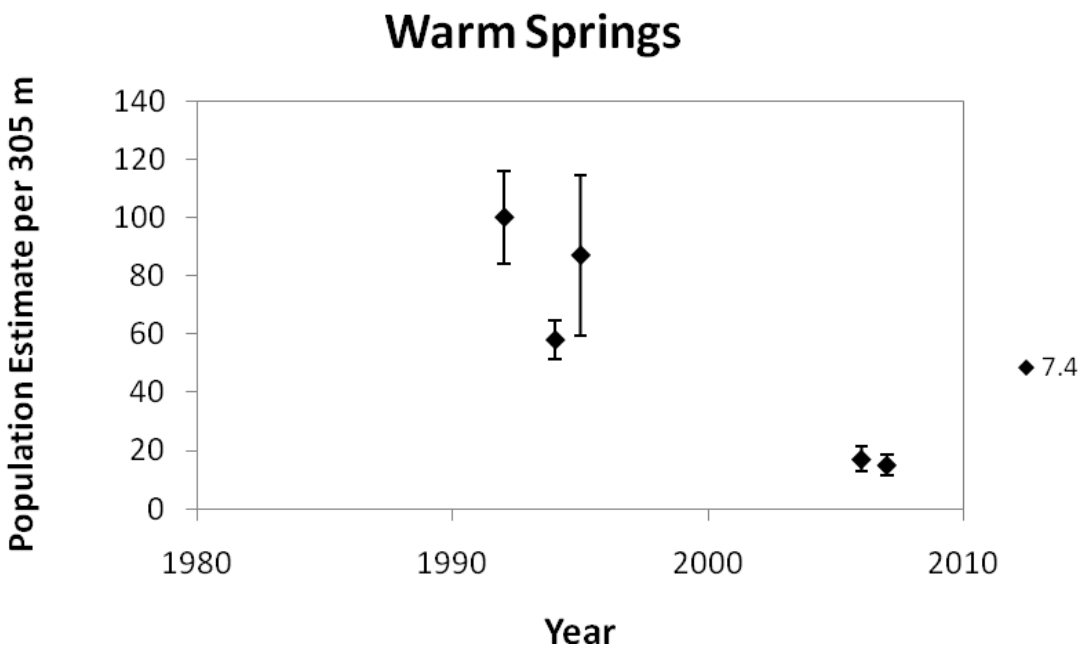
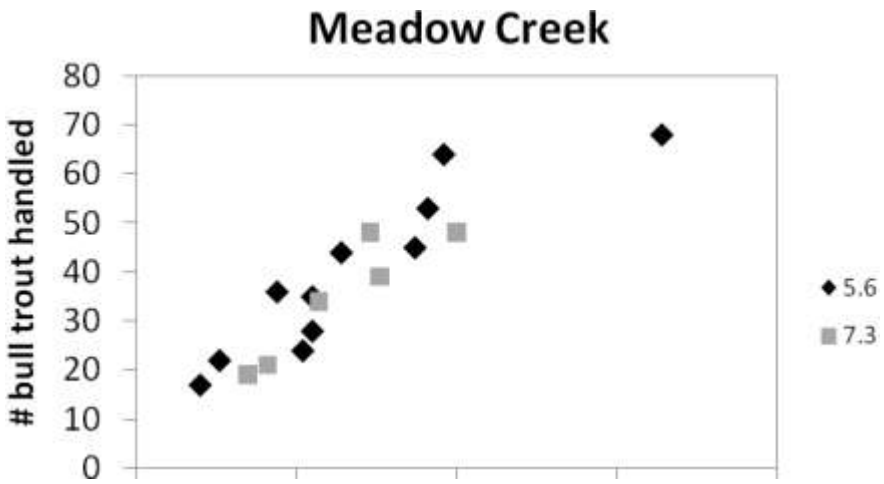
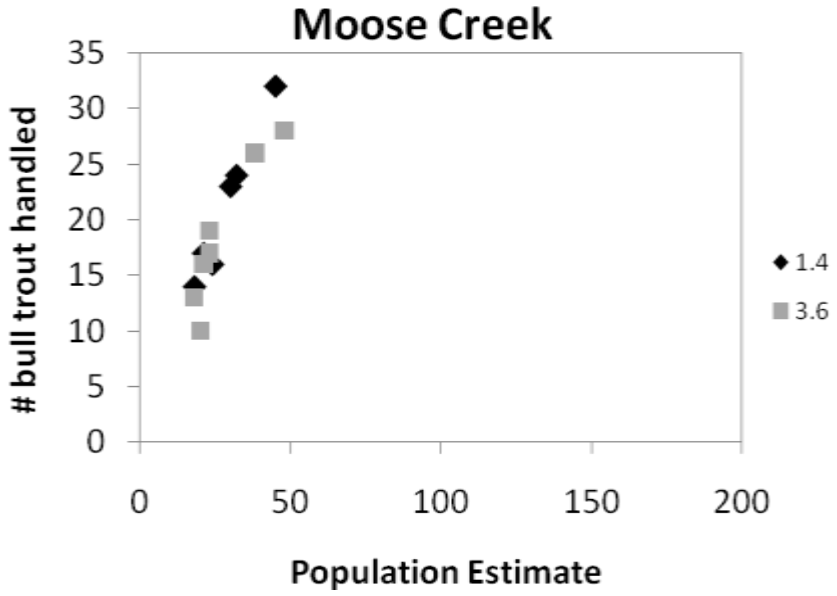

Warm Springs
Population Estimate per 305 m
140
120
100
80
60
40
20
0
1980
1990
2000
2010
Year
7.4

Figure 3.5. Number of bull trout handled (#BTH) versus population estimates for tributaries in the East Fork Bitterroot River drainage: a) Meadow Creek reach numbers 5.6 and 7.3, b) Moose Creek reach numbers 1.4 and 3.6, c) Swift Creek reach number 0.7, d) Tolan Creek reach number 5.1, and e) Warm Springs Creek reach number 7.4.

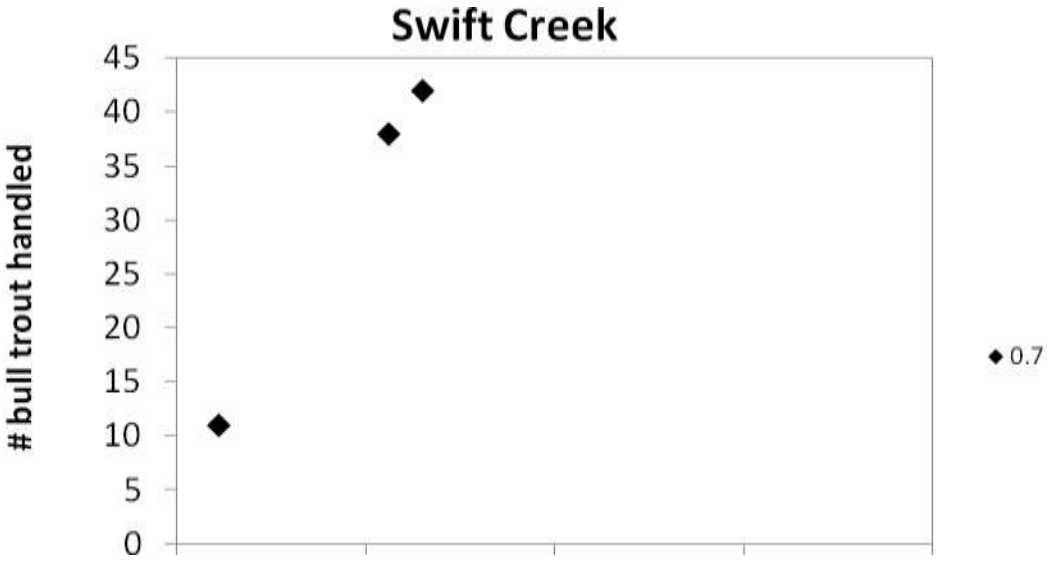
a)



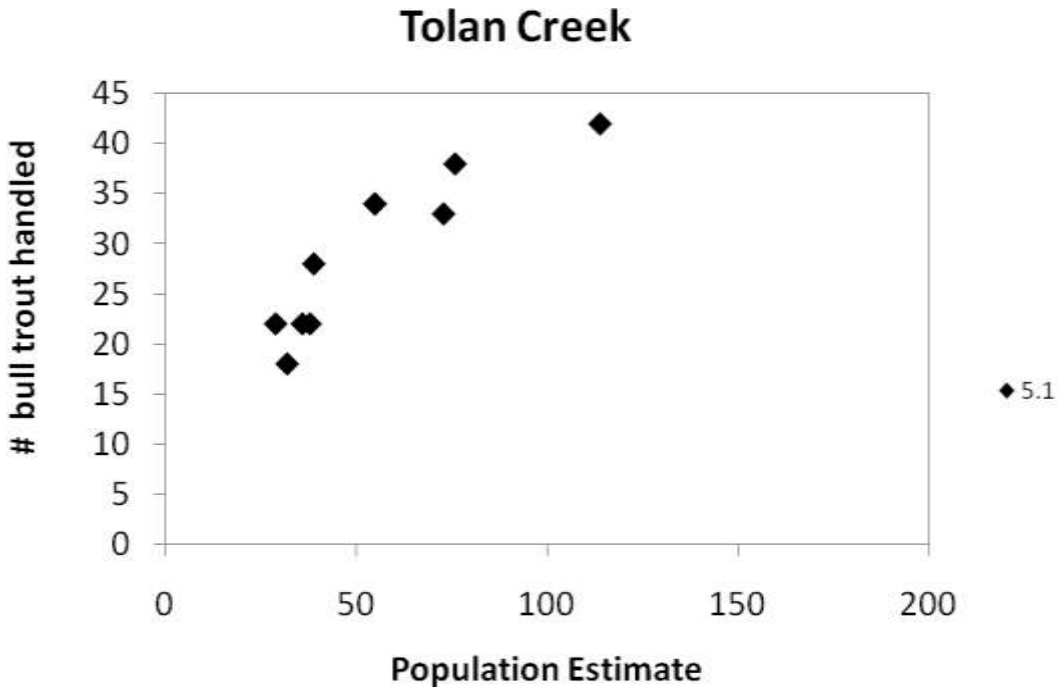
b)



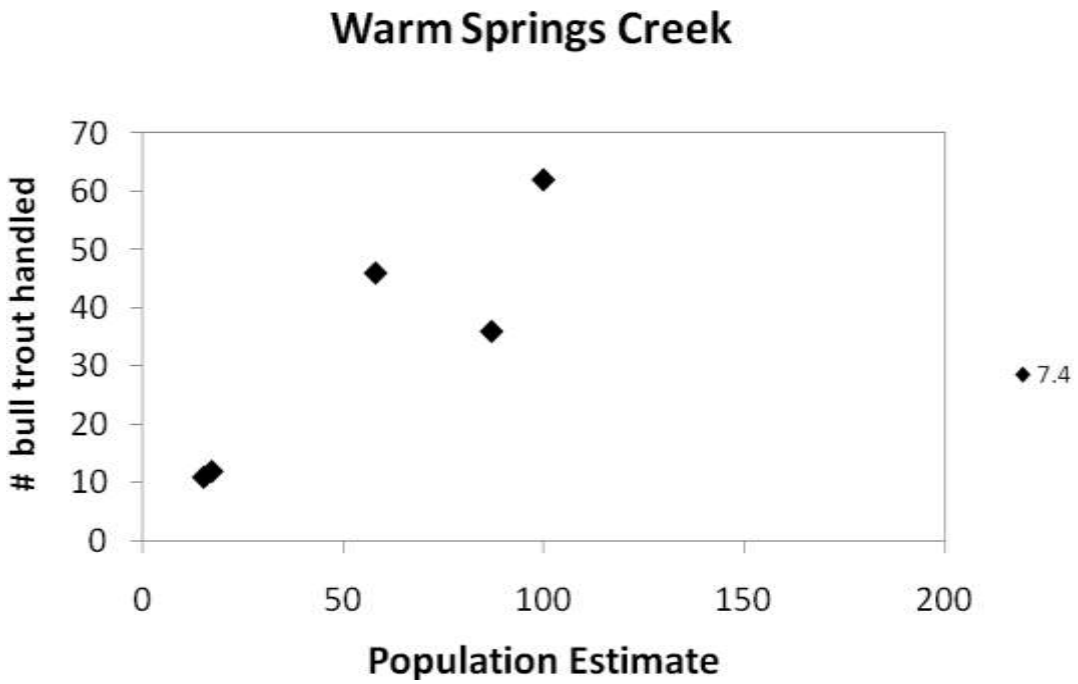
c)


Swift Creek
bull trout handled
45
40
35
30
25
20
15
10
5
0
0.7

d)


Tolan Creek
bull trout handled
45
40
35
30
25
20
15
10
5
0
0
50
100
150
200
Population Estimate
5.1

e)


Warm Springs Creek
bull trout handled
70
60
50
40
30
20
10
0
0
50
100
150
200
Population Estimate
7.4

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