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Effective Population Size, Connectivity, and Occupancy of Bull Trout: Tools to Assist in Recovery

2005-2013 Synthesis Report

J. Michael Hudson, Brook P. Silver, Justin R. Cook, and Timothy A. Whitesel

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On the cover: *Fluvial bull trout from the NF Imnaha River. Photograph by Justin R. Cook*

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Effective Population Size, Connectivity, and Occupancy of Bull Trout: Tools to Assist in Recovery
2005-2013 SYNTHESIS REPORT

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Abstract – Achieving recovery of bull trout throughout their range will require a variety of actions targeting limiting factors in an effort to achieve minimum viable population sizes that can persist into the future. This project evaluated empirical information in an effort to relate effective population size theory to absolute abundance and population genetic variability, addressing potential limiting factors, and, ultimately, providing information toward defining minimum viable population requirements for bull trout. The objectives of the project were: 1) Determine abundance of bull trout populations above the Wallowa Valley Irrigation Canal (WVIC) as well as an area of reference unaffected by the WVIC; 2) Determine if there is connectivity (movement) between bull trout populations; 3) Determine within and among population genetic variability for five local populations of the Imnaha River core area; 4) Determine effective population size for potentially isolated populations above the WVIC as well as within a reference areas; 5) Determine bull trout occupancy throughout the Imnaha River core area using the patch analysis approach; and, 6) Determine if there is congruence between local populations identified by genetic means and patch analysis. To achieve these objectives, abundance was estimated in three local populations, connectivity evaluated using PIT technology, genetic analysis was conducted and occupancy assessed in habitat predicted to support bull trout in the Imnaha River core area. The three populations investigated appear to be stable in abundance and have varying degrees of connectivity with each other and additional populations in the core area. The genetic analysis supported the connectivity results and the identification of discrete local populations through a bull trout patch identification process using habitat metrics known to support bull trout populations. Despite low effective population size, two populations that are potentially isolated or where migratory corridors are obstructed, have persisted for over a century. The findings indicate that relatively small bull trout populations can persist with no significant evidence of genetic drift, even when potentially isolated, raising questions on interpretation of the "50/500" rule relative to recovery of this species. However, recovery actions to improve connectivity among populations will likely make populations more demographically stable and less vulnerable to stochastic events.

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Introduction

Bull trout (*Salvelinus confluentus*) were listed range wide (coterminously) as a threatened species on November 1, 1999 (64 FR 58910). Previously, the Columbia River distinct population segment (DPS) of bull trout had been listed as threatened since June 10, 1998 (63 FR 31647). Factors contributing to the listing of bull trout include range wide declines in distribution, abundance and habitat quality. Land and water uses that alter or disrupt habitat requirements of bull trout can threaten the persistence of the species. Examples of such activities include: water diversions, dams, timber extraction, mining, grazing, agriculture, nonnative fish competition and/or hybridization, poaching, past fish eradication projects, and channelization of streams. These threats are prevalent throughout the Columbia River basin (USFWS 2015a, 2015b).

Within the coterminous DPS is the Mid-Columbia Recovery Unit (MCRU). The MCRU has numerous core areas, one of which is the Imnaha River core area, which consists of at least five putative local populations (Barrows et al. 2016; USFWS 2002, 2015b). This study focused on five areas that are known to support bull trout in the Imnaha River core area: the upper Imnaha River (i.e., North Fork Imnaha River and South Fork Imnaha River), Big Sheep Creek, Lick Creek, Little Sheep Creek, and McCully Creek. Historically, these populations could have been connected by migratory individuals and functioned as one metapopulation. However, the construction of the Wallowa Valley Improvement Canal (WVIC) has potentially prevented gene flow or allowed only unidirectional movement downstream for over a century from Big Sheep Creek, Little Sheep Creek and McCully Creek. Despite the existence of these potential isolating mechanisms, bull trout populations persist in all of these streams above the WVIC. However, little previous information is available on the abundance of these populations. The resident population in Big Sheep Creek was estimated at less than 2,000 individuals, above and below the WVIC and including all tributaries (USFS 2001). The resident population in Little Sheep Creek was estimated at fewer than 500 (USFS 2003). The resident population of McCully Creek, which formerly flowed into Little Sheep Creek, was estimated at approximately 2,500 individuals (Smith and Knox as referenced in Buchanan et al. 1997).

Genetic theory indicates that an effective population size (N_e) ≥ 50 is necessary to prevent inbreeding depression, and $N_e \geq 500$ is necessary to prevent genetic drift and allow sustainability over ecological time (i.e., the "50/500" rule; Franklin 1980, Soulé 1980, Allendorf and Ryman 2002). It seems reasonable that this theory holds true for bull trout, although exceptions do exist (see Rieman et al. 1997, Whitesel et al. 2004). Whether bull trout exhibit departures from the "50/500" rule should be documented with empirical data that is robust and well described (Whitesel et al. 2004). Information that relates effective population size theory to absolute abundance and population genetic variability will provide information toward defining minimum viable population requirements for bull trout.

Bull trout distribution and occupancy throughout the study area was also examined. By implementing a habitat analysis approach that included catchment area, potential bull trout habitat patches were determined and assessed for occupancy. A patch is defined by the Bull Trout Recovery and Technical Monitoring Group as "contiguous areas within a stream network where spawning and early juvenile rearing could occur and potentially support a local population" (USFWS 2008). In general, the utility of this approach was examined to provide

guidance in determining potential bull trout distribution in core areas, specifically above and below the WVIC and throughout the Imnaha River core area.

The goal of this project was to provide empirical data toward defining minimum viable population objectives and limiting factors that can be used for restoration and recovery of bull trout across the range. The objectives toward this end were to: 1) Determine abundance of bull trout populations above WVIC as well as an area of reference unaffected by the WVIC; 2) Determine if there is connectivity (movement) between bull trout populations; 3) Determine within and among population genetic variability for the five, putative local populations of the Imnaha River core area; 4) Determine effective population size for potentially isolated populations above the WVIC as well as within a reference areas; 5) Determine bull trout distribution throughout the Imnaha River core area using the patch analysis and occupancy sampling approach; and, 6) Determine if there is congruence between local populations identified by genetic means and patch analysis.

Relationship to the Fisheries Program Strategic Plan

Implementation of this project demonstrates application of the Pacific Region's 2009-2013 Fisheries Program Strategic Plan. The following National goals (NG) and Regional objectives (RO) have been addressed by this project:

NG1 Open, interactive communication between the Fisheries Program and its partners.

- RO1.1 Develop and maintain relationships with partners throughout the Pacific Region.
- RO1.2 Implement a means of providing feedback to ensure the long-term success of partnerships.
- RO1.3 Improve data collection and management and internal and external reporting to reduce redundancy and improve access and usefulness for ourselves and our partners.

NG2 America's streams, lakes, estuaries, and wetlands are functional ecosystems that support self-sustaining communities of fish and other aquatic resources.

- RO2.1 Facilitate management of aquatic habitats on national and regional scales by working with Tribes, States, partners and other stakeholders.
- RO2.2 Develop and expand the use of its expertise to help avoid, minimize or mitigate impacts of habitat alteration on aquatic species and monitor and evaluate completed projects.

NG3 Self-sustaining populations of native fish and other aquatic resources that maintain species diversity, provide recreational opportunities for the American public, and meet the needs of tribal communities.

- RO3.1 Collaborate with Ecological Services (ES) Program, National Oceanographic and Atmospheric Administration Fisheries (NOAA Fisheries) and others, to recover fish and other aquatic resource populations protected under the ESA.

RO3.2 Maintain healthy, diverse, self-sustaining populations of fish and other aquatic resources

RO3.3 Support the research and fish culture needed to prevent listing or to recover native species listed or proposed for listing under ESA.

NG9 Science developed and used by Service employees for aquatic resource restoration and management is state-of-the-art, scientifically sound and legally defensible, and technological advances in fisheries science developed by Service employees are available to partners.

RO9.1 Develop and share state-of-the-art, scientifically sound, legally defensible scientific and technological tools, including databases, with other Service programs and in conjunction with our partners.

RO9.2 Use state-of-the-art, scientifically sound, legally defensible scientific and technological tools in formulating and executing fishery-related plans and policies.

Study Area

The Imnaha River core area is located in the Imnaha River subbasin in the northeastern corner of Oregon (Figure 1). The headwaters originate in the Eagle Cap Wilderness and drain the eastern Wallowa Mountains to the lower Snake River. The majority of the subbasin is in the Wallowa-Whitman National Forest under public ownership, and 24% of the subbasin is privately owned (USFWS 2015b).

The WVIC is a water diversion in the Imnaha River core area that has impacted bull trout and their habitat. The canal was constructed in the 1880s and diverts water to the Wallowa Valley from several Imnaha River core area streams beginning at Big Sheep Creek and continuing down to McCully Creek (Figure 1). The diverted water is primarily used for irrigation purposes.

During the construction of the WVIC, diversion structures were built, creating potential barriers for fish passage. Possible barriers are located at the diversion on Big Sheep Creek and within the canal at Salt Creek summit spillway (Figure 2a-2b). The amount of successful passage is unknown and the construction of these structures have certainly created limited mixing or potentially isolated a population of bull trout in upper Big Sheep Creek for the past century. The canal has also diverted and isolated numerous small tributaries and streams including Salt Creek, Cabin Creek, Little Sheep Creek, Redmont Creek, Canal Creek, and Ferguson Creek. At Little Sheep Creek there is a culvert approximately 200 m above the confluence with the WVIC that could impact upstream migration of bull trout and isolate a population above (Figure 2c). The WVIC does not divert McCully Creek. Instead, the WVIC is carried over the top of McCully Creek and some water from the canal is diverted into the creek (Figure 2d). Some level of immigration from fish moving down the WVIC into McCully Creek could occur through the canal structure, but it is unlikely that any fish can access the canal from McCully Creek. In addition, McCully Creek no longer drains into the Imnaha River core area. The stream bed was shifted in the past so that the creek now drains directly into the Wallowa Valley and provides another water source for irrigation. Downstream of the junction with the WVIC, McCully Creek

essentially becomes an irrigation canal and is no longer a natural creek. Many of the irrigation ditches in the upper Wallowa Valley ultimately connect to Prarie Creek on the Wallowa River. Therefore, another potential source of bull trout immigration into McCully Creek may be from the Lookingglass/Wenaha River core area. This connection seems unlikely since trout would need to navigate through a series of irrigation canals that most likely act as temperature barriers. Thus, it is reasonable to speculate that the bull trout population in McCully Creek is isolated.

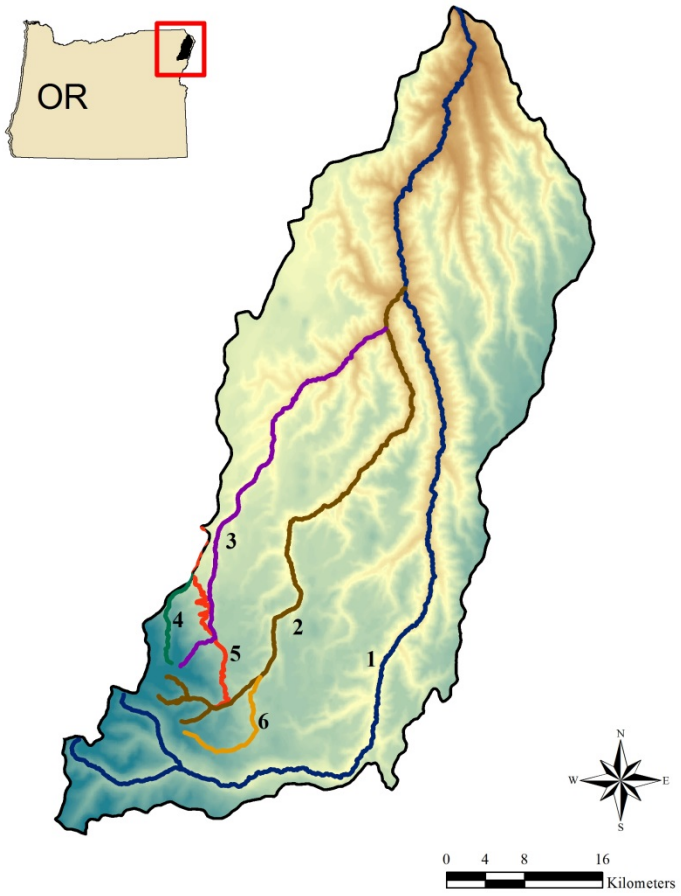
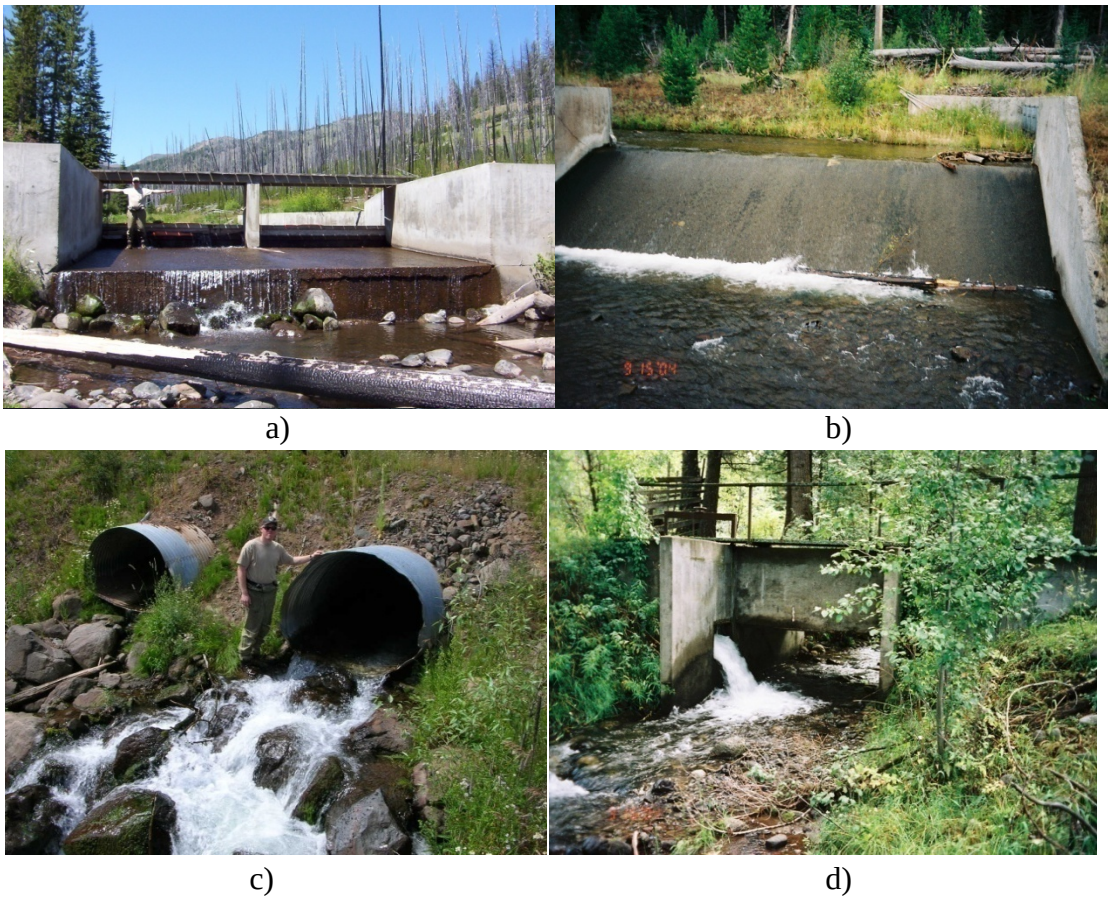


Figure 1. Study area – 1. Imnaha River; 2. Big Sheep Creek; 3. Little Sheep Creek; 4. McCully Creek; 5. WVIC; 6. Lick Creek.



a) b)

c) d)

Figure 2. Potential barriers to upstream migration of bull trout in the Imnaha River core area – a) WVIC diversion at Big Sheep Creek; b)WVIC spillway at Salt Creek Summit; c) culverts under USFS road #130 on Little Sheep Creek, d) WVIC diversion at McCully Creek

Methods

Abundance

Electrofishing approach and data collection –

The sampling method consisted of backpack electrofishing in an upstream direction, using a Smith-Root model LR-24 shocker. Alternative approaches to capture (e.g., minnow traps, hook and line) were qualitatively evaluated, with electrofishing being the most effective and efficient means of capture. Electrofishing was conducted using a technique to reduce potential harm to the sampled population. Specifically, only areas considered holding habitat (plunge pools, overhanging banks, eddies, large woody debris, and pocket pools within riffles) were sampled, as opposed to continuous application of electricity while moving upstream. This approach included two to three netters working with one electrofisher. The electrofisher would point out the next possible holding habitat to the netters, then quietly and quickly approach and begin shocking in one fluid motion, focusing on drawing the fish back down towards the netters. This method proved effective and allowed for the capture of fish with the use of minimal electricity and impact on the fish. Fishing effort was measured by the number of seconds the electrofisher was on (electricity in the water) and remained similar among passes for the depletion and mark-recapture approaches. The LR-24 shocker used pulsed direct current set at a frequency of 20-24 Hz, 20-28% duty cycle, and voltage between 275 and 500 V. Settings were dependent upon fish response as well as current water conditions (i.e., water depth, conductivity, flow, and temperature).

At the completion of each reach, all captured fish were identified, measured (fork length), weighed, and scanned for passive integrated transponder (PIT) tags. Fish were anesthetized using 25 ppm clove oil. Scissors were used to collect approximately 4 mm^2 of tissue from the left pelvic fin of all bull trout upon initial capture. The samples were preserved in a vial of 100% ethyl alcohol and archived for future genetic analysis. For bull trout greater than 120 mm, a PIT tag (23 mm long, 3.84 mm diameter, 0.6 g, full duplex) was surgically implanted on the ventral side, posterior to the pectoral fins (Roussel et. al 2000). After full recovery within an aerated bucket, fish were released within their reach of capture.

Location and abundance estimate method employed varied across years throughout the timeframe of the project as follows:

Figure 3. Block netting sample reach.

Big Sheep Creek:

Abundance estimates for Big Sheep Creek bull trout began in 2005 with an investigation of alternative approaches to estimating population abundance. The two approaches on which this investigation was focused were multiple pass depletion and multiple pass mark-

recapture. This investigation occurred in a one-kilometer long reach of Big Sheep Creek that began approximately one kilometer above the Wilderness Area boundary (trailhead stream crossing). This reach was block netted for the duration of the study to prevent migration to and from the area (Figure 3). Three passes were completed over a period of six days through the entire one-kilometer reach to conduct a multiple pass mark-recapture estimate. Three 150 m sub-reaches (lower, middle, upper) were established within the large reach for conducting multiple pass removal depletions (Hankin and Reeves 1988; Figure 4). Each electrofishing pass attempted to represent an equal amount of fishing effort. Habitat was also fished in the same manner and sequence on each pass. A minimum of three passes and a maximum of five were completed in order to successfully deplete a sub-reach (i.e., numbers of bull trout removed in a pass were either zero or less than 10% of those removed during the previous patch; Hankin and Reeves 1988). Captured fish were stored in an in-stream holding container kept outside of the reach. Following the appropriate analysis, the most effective and efficient sampling approach was determined to estimate accurate and precise population abundances and utilized in future sampling efforts.

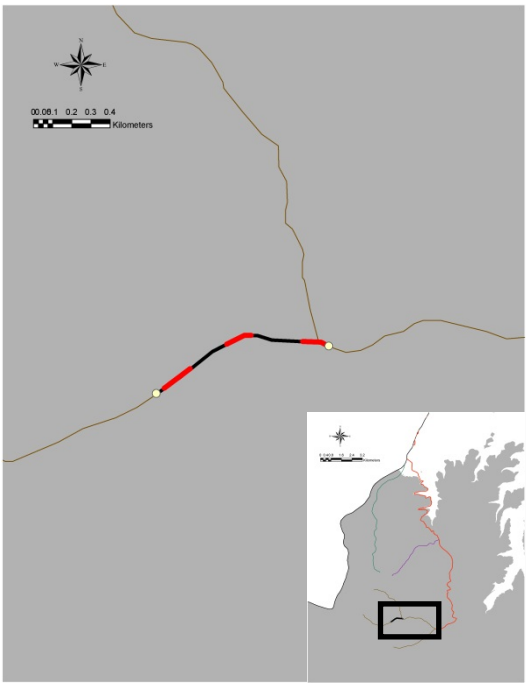


Figure 4. Big Sheep Creek study area showing reaches sampled for mark-recapture v. depletion comparison.

Abundance estimates for the Big Sheep Creek bull trout population above the WVIC were conducted in 2006, 2007, and 2011, using either a multiple pass mark-recapture approach or a single pass approach (Table 1). Sampling occurred in an 8 kilometer portion of Big Sheep Creek, beginning at the WVIC diversion structure and continuing upstream to a series of impassable natural waterfalls. The 8 kilometers were divided

Figure 5. Big Sheep Creek study area showing reaches sampled.

into 30 individual reaches including the mainstem, the south fork, the north fork, and a large side channel that circumvents a series of barrier falls (Figure 5). These reaches represented nothing other than a sampling segment. The 24 reaches on the mainstem ranged from 78 m to 310 m, averaging 230 m. There was one reach on the south fork that was 165 meters, ending at the crossing of US Forest Service Road 100. There were two reaches on the north fork that were 61 m and 232 m. There were three reaches on the side channel of the main-stem that were 311 m, 290 m and 368 m. Sampling also occurred in the first 100 meters of the un-named tributary entering Big Sheep Creek near the top of the large side channel. The north fork was not sampled after the first pass because no bull trout were captured during the first pass and habitat was not considered suitable. Due to dangerous access, the reach between the first and second major waterfall (approximately 3 rkm above the WVIC diversion), around which the side channel flows, was not sampled. However, the majority of this reach is high gradient cascades and is not accessible from downstream, both of which decrease the likelihood that there are a large number of fish in the reach and fish hold in this reach for extended periods of time.

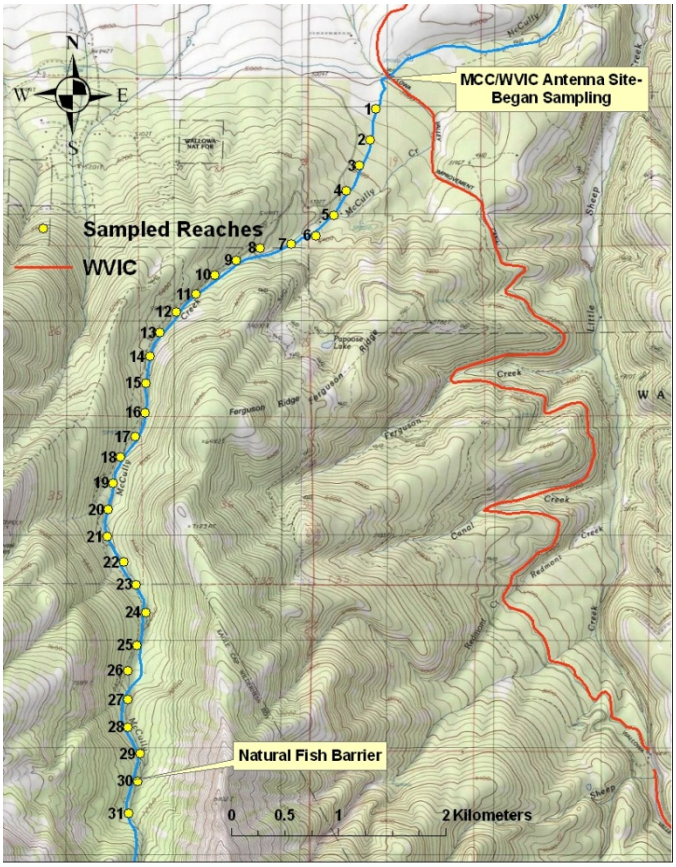


Figure 6. McCully Creek study area showing sampled reaches.

<u>McCully Creek:</u>

Abundance estimates for the McCully Creek bull trout population above the WVIC were conducted in 2007 and 2008 (Table 1). Sampling occurred in an 8.45 kilometer portion of McCully Creek beginning where the WVIC crosses and ending 0.45 kilometers above a natural barrier. The McCully Creek sampling area was then divided into 31 individual reaches measuring 250 meters each (Figure 6). These reaches represented nothing other than a sampling segment. We sampled all tributaries, springs, and side channels within the system. One additional reach above the barrier was completed on the first trip to confirm the end of fish use.

Lick Creek:

Abundance estimates for the Lick Creek bull trout population were conducted in 2008 and 2009 (Table 1). Sampling included all probable habitats within the mainstem of Lick Creek starting at an elevation of 1600 meters (the crossing of FS road 170 - river kilometer 3.34) and continuing upstream to the end of fish distribution (Figure 7). A small amount of sampling occurred within Mud Springs Creek as well. Lick Creek was divided into 41 reaches measuring approximately 250 meters each, totaling 10.3 kilometers of sampled stream (Figure 7). These reaches represented nothing other than sampling segments. During the second pass, work was also completed within Lick Creek's unnamed tributary to the north (Rkm 9.1) where 6 more reaches were sampled totaling 1.6 kilometers.

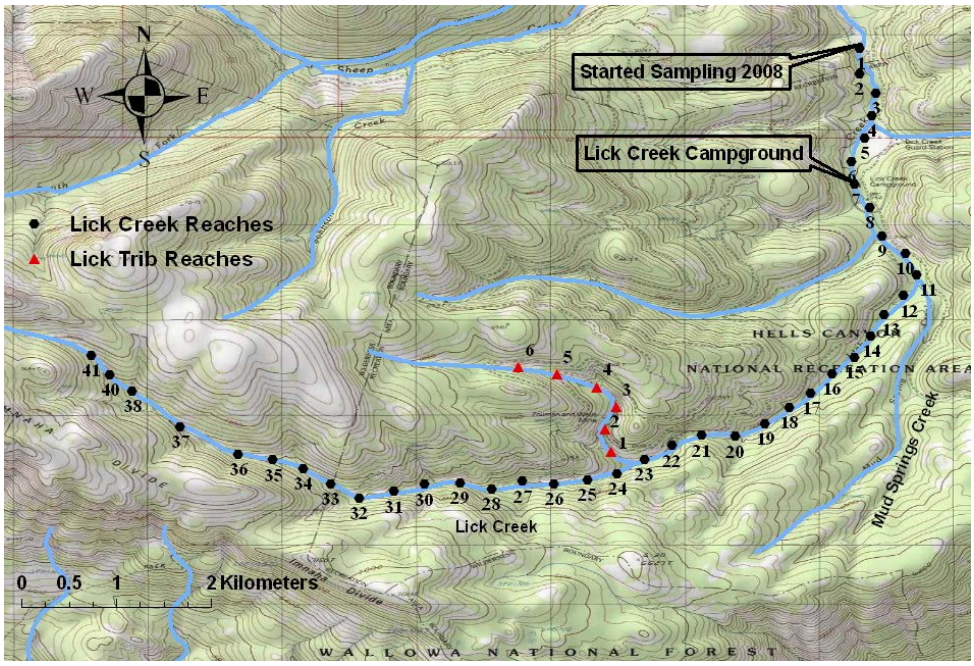


Figure 7. The study area of Lick Creek showing sampled reaches of the mainstem and tributaries.

Table 1. Year, location and method of abundance estimates.

Year	Location	Abundance Estimate Approach
2005	Big Sheep Creek	3 pass mark-recapture
		Depletion
2006	Big Sheep Creek	3 pass mark-recapture
2007	McCully Creek	2 pass mark-recapture
	Big Sheep Creek	Single pass
2008	Lick Creek	2 pass mark-recapture
	McCully Creek	Single pass
2009	Lick Creek	Single pass
2011	Big Sheep Creek	Single pass

Data analysis –

Depletion:

Depletion data was analyzed using a maximum-likelihood population estimate based on a removal depletion strategy (Zippin 1958). The results were generated using MicroFish 3.0 to determine the estimate, confidence intervals around the estimate, the coefficient of variation, and the probability of capture (Vandeventer and Platts 1985).

Mark-recapture:

Mark-recapture data was analyzed using CAPTURE (Otis et al. 1978; White et al. 1982; Rexstad and Burnham 1991) within MARK (White and Burnham 1999). CAPTURE was used to help determine the most appropriate estimator (*Mo* [null estimator], Jackknife *Mh*, Darroch *Mt* , Chao *Mth*, Chao *Mt* , and Chao *Mh*), but assumptions and variables associated with the choice of the most appropriate estimator were also considered. CAPTURE was used to determine confidence intervals around the estimate, the coefficient of variation, and the probability of capture.

Single-pass:

Single-pass data was analyzed using the population estimate for single catches method (Seber and Le Cren 1967):

$$\tilde{N} = C/\tilde{p},$$

where $\tilde{N}$ is the estimated abundance, C is the number of captured individuals from the single-pass, and $\tilde{p}$ is the estimated capture probability. Capture probability in Big Sheep Creek was assumed to be the same as the capture probability that was generated from previous mark-recapture estimates conducted in this project. Confidence intervals

(95%) around the single-pass estimate were generated using the methodology of Seber and Le Cren (1967).

Connectivity

PIT tag antennae arrays (Zydlewski et al. 2006) were used to monitor the movement of PIT-tagged fish at four locations: the Big Sheep Creek/WVIC diversion (the canal origin), the Salt Creek summit spillway (5.9 kilometers down the canal), the Little Sheep Creek acclimation facility and the intersection of the WVIC and McCully Creek (21.2 kilometers down canal). These antennas provided data regarding fish migrations to and from the upper portions of Big Sheep Creek from areas below the canal as well as within the canal (Figure 8). They allowed us to assess whether bull trout left Big Sheep Creek and entered the canal, migrated up and down the canal past Salt Creek Summit, were present in Little Sheep Creek below the canal, and migrated to and from McCully Creek.

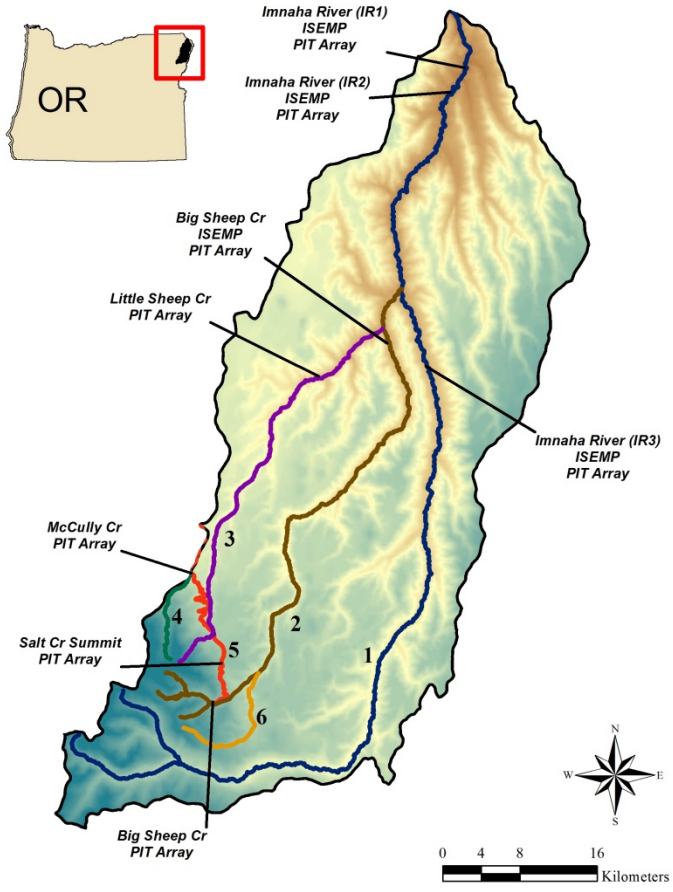


Figure 8. Study area – 1. Imnaha River; 2. Big Sheep Creek; 3. Little Sheep Creek; 4. McCully Creek; 5. WVIC; 6. Lick Creek.

Antennas were constructed as open coil inductor loops with PVC-coated multi-strand wire strung through PVC pipe, or encased within a flat panel wooden or PVC sheet design. The antennas were then connected to a Destron-Fearing reader that emits a 134.2 kHz electromagnetic energizing signal through the antenna. A computer received serial data output from the reader at each site; detected tag identification numbers, date and time of detection were recorded. The readers, batteries and/or power supplies, and computers were housed within a weather-proof box located outside of the immediate flood zone of the streams. Antennas located at Big Sheep Creek and Salt Creek Summit were powered with propane thermoelectric generators initially, which were replaced with solar power in 2009. These monitoring sites were maintained throughout the year, but operation was intermittent during winter months due to location and severe weather conditions. Antennas located at McCully Creek and Little Sheep Creek were powered by AC, facilitating more continuous operation throughout the year (Table 2; Figure 9).

Big Sheep Creek –

Antennas were initially installed at the WVIC diversion on Big Sheep Creek in October 2006 (Figure 10a). The location and severe weather conditions prevented regular access and maintenance at this remote site during the winter months of 2006-2007. As a result, the array did not operate from November 2006 – May 2007. The winter conditions also resulted in the failure of the two lower hanging antennas on the Big Sheep Creek diversion structure (Figure 10b). Operation of remaining antennas resumed in May 2007. We also installed three new antennas in July 2007 (Figure 11 – A4, A5, A6). Two flat panel antennas (A5 and A6), constructed from PVC sheeting, were installed on the structure that separates the upper and lower portions of Big Sheep Creek (Figure 12a). The third antenna (A4) was installed just downstream of the spillway structure on Big Sheep Creek. The two flat panel antennas installed on the spillway never functioned properly due to problems thought to be associated with loading interference from the diversion structure. In October 2007, one of these flat panels was replaced with a PVC pipe antenna and relocated into the canal just downstream of the Big Sheep Creek diversion structure (A3). In August 2009, the flat panel in the canal (Figure 11 – A3) was replaced with a hybrid PVC pipe antenna that is only connected to the substrate on the upstream end of the antenna, allowing the downstream end to float in the water column and move up and down with changes in flow (e.g., Figure 12d). At the same time, two additional hybrid antennas were installed in Big Sheep Creek above the diversion structure and in the SF Big Sheep Creek (Figure 13).

Table 2. Timespan of operation and total functional operational time of antenna arrays (MCC = McCully Creek, BSC = Big Sheep Creek, SCS = Salt Creek Summit, LSC = Little Sheep Creek).

Antenna Array	Timespan of Operation	Total Days	Dates not in operation (days)	Total functional operation days	Percentage of total days of functional operation
MCC	4/24/2006 – 10/11/2012	2,363	8/12-11/28/2006 (109); 12/21/2006-5/4/2007 (135); 9/1-9/12/2007 (12); 1/19-2/7/2008 (20); 4/25-5/15/2008 (21); 7/10-15/2008 (6)	2,060	87%
BSC	5/1/2007 – 10/27/2011	1,641	6/17-29/2007 (13); 7/15-8/14/2007 (31); 8/30-9/7/2007 (9); 9/26-10/1/2007 (6); 10/17-28/2007 (12); 11/13-27/2007 (15); 12/28/2007-2/7/2008 (42); 5/12-14/2008 (3); 9/10-14/2008 (5); 12/25/2008-1/5/2009 (12); 1/24-2/1/2009 (9); 10/5-6/2009 (2); 12/8/2009-2/3/2010 (58); 3/4-4/18/2010 (46); 12/16/2010-2/7/2011 (54); 2/20-4/6/2011 (46)	1,278	78%
SCS	10/18/2006 – 10/11/2012	2,186	11/7-12/2006 (6); 11/25-30/2006 (6); 1/4-3/6/2007 (62); 4/3-12/2007 (10); 12/20/2007-5/15/2008 (148); 1/2-6/2009 (5); 3/16-27/2009 (12); 6/8-14/2009 (7); 10/2-6/2009 (5); 11/17/2009-2/2/2010 (78); 12/4-15/2010 (12); 12/29/2010-1/9/2011 (12)	1,823	83%
LSC	9/6/2009 – 10/11/2012	1,132	10/1-6/2009 (6); 10/27-12/7/2009 (42)	1,084	96%

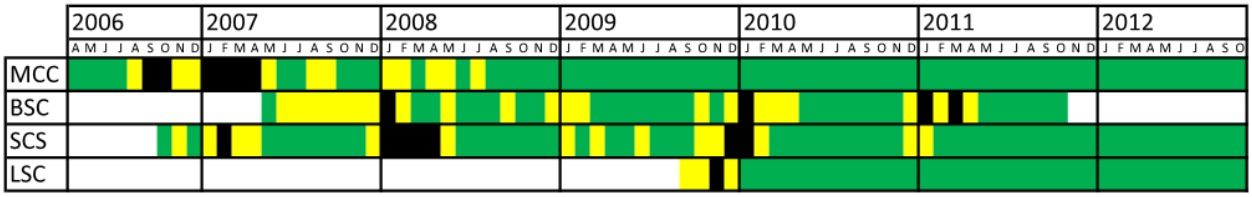


Figure 9. Timespan of operation and total functional operational time of antenna arrays by month within each year (MCC = McCully Creek, BSC = Big Sheep Creek, SCS = Salt Creek Summit, LSC = Little Sheep Creek; Green = array operational entire month, Yellow = array operational for portion of month, Black = array not operational in that month).

a)

b)

Figure 10. Antenna arrays constructed at Big Sheep Creek (a) and Salt Creek summit (b) and the subsequent damage caused by snow and ice.

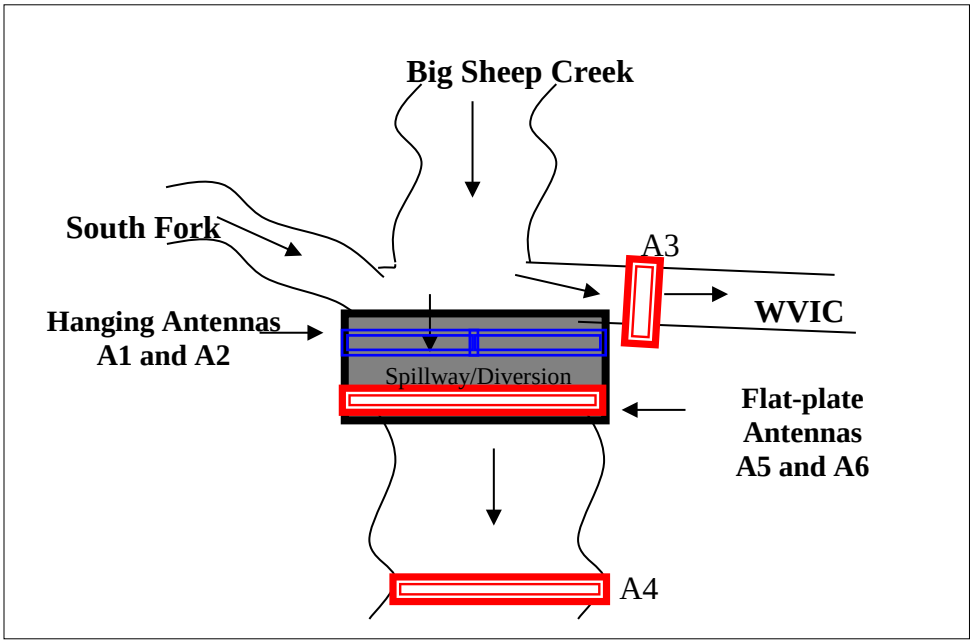


Figure 11. A diagram of the Big Sheep Creek diversion antenna site, 2006-2009. Arrows indicate water flow direction.

Figure 12. Antennas installed in 2007 - a) Big Sheep Creek diversion flat panels and upper antennas; b) upstream of Salt Creek summit flat panel (wood); c) Antenna in the WVIC just below where McCully Creek goes under the canal; d) McCully Creek antennas upstream of the WVIC.

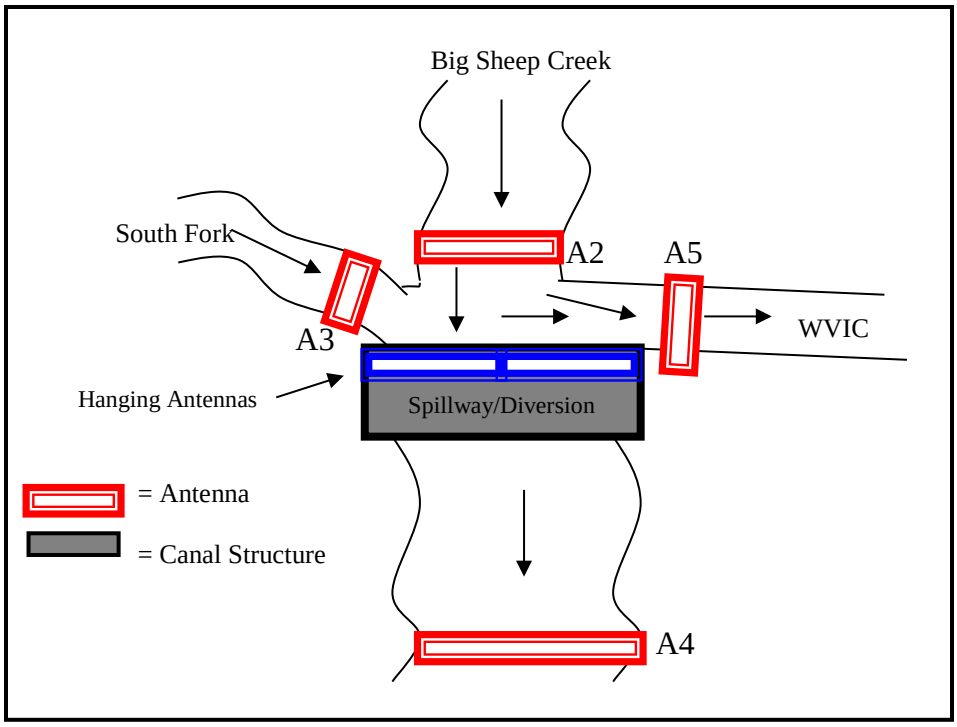


Figure 13. A diagram of the Big Sheep Creek diversion antenna site, 2009-2012. Arrows indicate water flow direction. The hanging antennas were replaced in August 2009.

Salt Creek Summit –

Antennas were initially installed at the WVIC diversion at Salt Creek Summit in October 2006 (Figure 10b). From January through April 2007, the original upper hanging antennas at Salt Creek Summit functioned properly (Figure 14 – A1, A2, A3). The lower hanging antennas failed due to winter conditions during the winter of 2006-2007 (Figure 10b). An additional wooden flat panel antenna was installed in April 2007 approximately 10 meters upstream from the diversion (Figure 12b and Figure 14 - A4). This antenna improved detection probabilities of fish that may have been missed by the remaining hanging antennas (Figure 14 – A1, A2, A3). It also provided conclusive evidence of a complete passage if a fish were to be detected on the hanging antennas followed by a detection on A4. In May 2007, a second flat panel antenna was installed approximately 40 meters downstream of diversion structure (Figure 14 – A5), allowing for detections above, below, and on the spillway. In May 2008, all existing antennas at Salt Creek Summit (Figure 14) were removed and replaced with two hybrid antennas just upstream of the spillway (Figure 15). Fish passage was determined according to where the detected fish was released and the antenna order in which it was detected (i.e., a fish tagged in Big Sheep Creek detected on A2 then A3 = downstream passage, a fish tagged below Salt Creek summit detected on A2 or A3 = upstream passage).

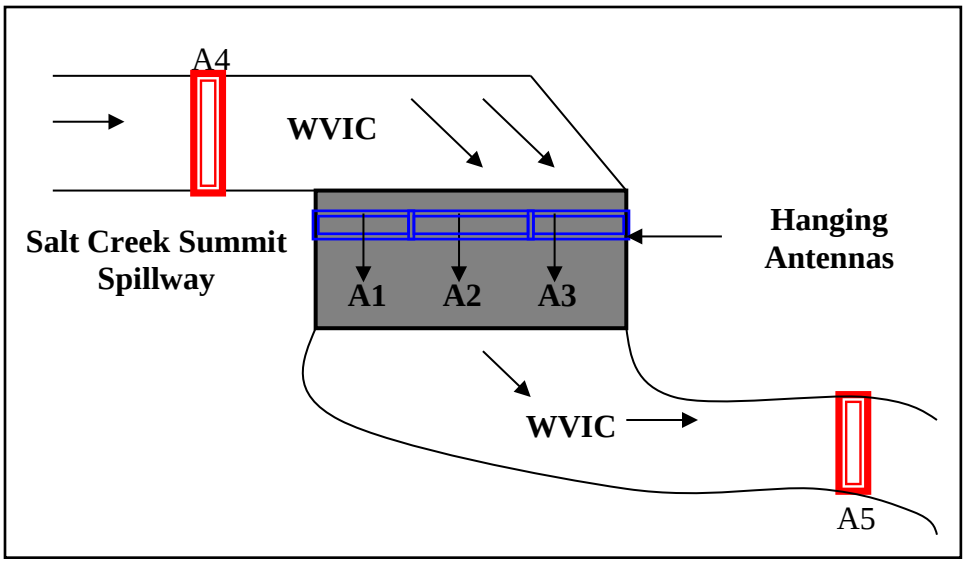


Figure 14. A diagram of the Salt Creek Summit spillway antenna site, 2006-2008. Arrows indicate water flow direction.

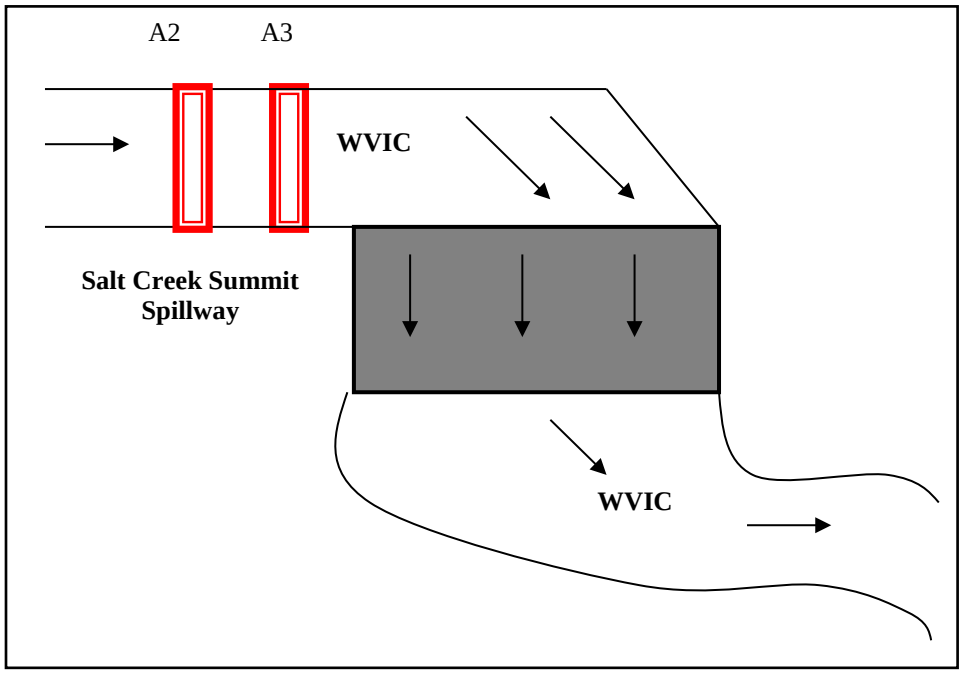


Figure 15. A diagram of the Salt Creek Summit spillway antenna site, 2008-2012. Arrows indicate water flow direction.

McCully Creek / WVIC –

The first antenna was installed at the McCully Creek/WVIC junction in April 2006. This antenna detected movement from the WVIC into McCully Creek (Figure 16 – A1). Movement back into the WVIC from McCully Creek is improbable due to the diversion structure (Figure 2d). In May 2007, two additional antennas were installed at this site. The

first was a pass-through antenna within the canal just downstream of McCully Creek (Figures 12c and 16 - A2). The second was a hybrid antenna placed in McCully Creek just upstream of the canal crossing (Figure 16 - A5). A fourth antenna (hybrid) was installed within McCully Creek, upstream of the canal (Figure 16 - A6) in June 2007 to allow for the directional movement of fish to be determined (Figure 12d). In July 2009, a fifth antenna (swim-through) was installed in the canal approximately 30 m upstream of the junction of McCully Creek with the canal outlet to Kinney Lake (Figures 16 – A3 and 17a). This antenna detected any tagged bull trout that went toward Kinney Lake instead of continuing down the canal or dropping into McCully Creek. Due to its design, fish passage back upstream through this canal outlet is unlikely.

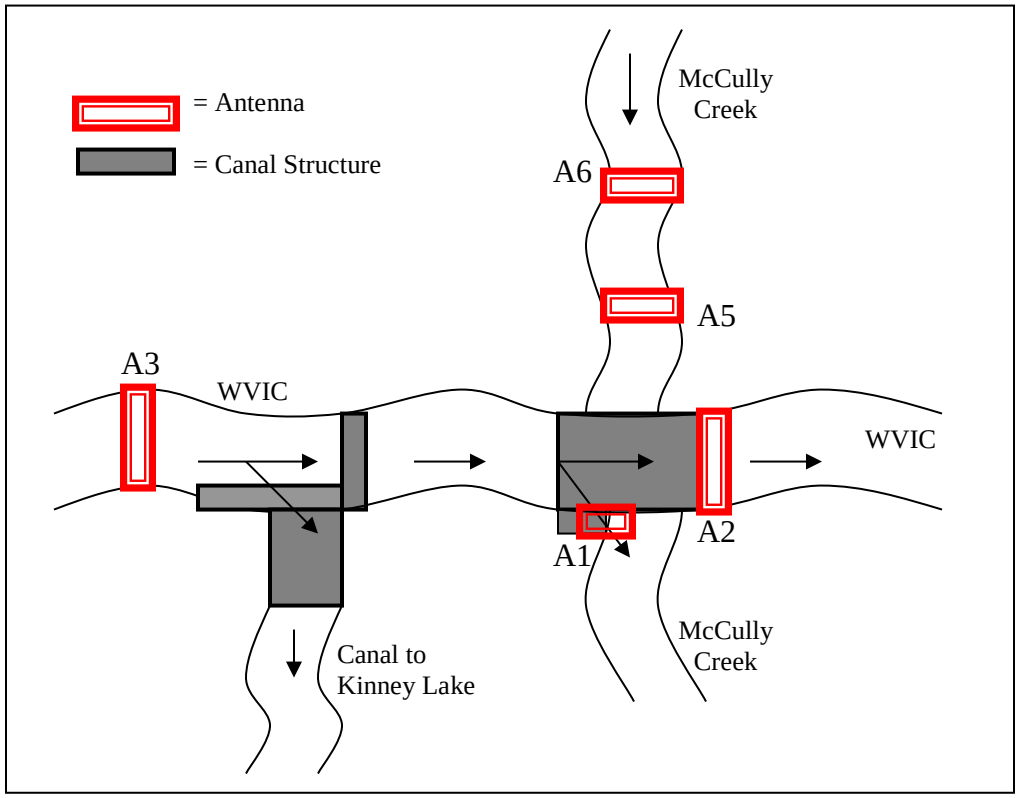


Figure 16. A diagram of the McCully Creek / WVIC antenna site, 2006-2012.

a) b)

Figure 17. Antennas installed in 2009 at a) McCully Creek / WVIC (looking downstream in canal) and b) Little Sheep Creek (looking upstream from acclimation facility).

Little Sheep Creek –

An antenna site was added in August 2009 at the ODFW fish acclamation facility on Little Sheep Creek (Figure 17b). Two hybrid antennas were installed just upstream of the facility to allow for directional movement to be determined. This site was installed to capture any tagged bull trout that make it out of the canal and down Little Sheep Creek, or fish that may come back up Little Sheep Creek.

Imnaha River / Big Sheep Creek ISEMP –

Additional opportunities to detect movement of tagged bull trout were realized beginning in 2010 with the addition of four antenna arrays elsewhere in the Imnaha River core area (Figure 8). These sites are operated by the Nez Perce Tribe and Quantitative Consultants, Inc., to collect data toward the Integrated Status and Effectiveness Monitoring Project (ISEMP). The site on Big Sheep Creek at rkm 6 (BSC) was installed in October 2010. The two lower sites on the Imnaha River at rkm 7 (IR1) and rkm 10 (IR2) were installed in November and December 2010. The final site on the Imnaha River at rkm 41 (IR3) was installed in February 2011. All arrays at these sites are comprised of flat plate antennas.

Population Genetic Variability

Genetic analysis of tissue samples collected during annual bull trout electrofishing activities was conducted by USFWS-Abernathy Fish Technology Center (Hudson et al. 2013). Samples analyzed were from Big Sheep Creek (2005, 2011 collections), McCully Creek (2007, 2012 collections), Lick Creek (2008 collection), and the upper Imnaha River (2007 collection). DNA was extracted from tissue samples using DNeasy 96 Blood & Tissue Kits (Qiagen Inc., Valencia, CA). Individuals were genotyped at 16 microsatellite loci: *Omm1128*, *Omm1130* (Rexroad et al. 2001), *Sco102*, *Sco105*, *Sco106*, *Sco107*, *Sco109*, (Washington Dept. of Fish and Wildlife unpublished), *Sco200*, *Sco202*, *Sco212*, *Sco215*, *Sco216*, *Sco218*, *Sco220* (DeHaan and Ardren 2005), *Sfo18* (Angers et al. 1995) and *Smm22* (Crane et al.

2004). Polymerase chain reaction (PCR) was carried out in 10µL reactions using 2µL of template DNA, 5µL of 2X Qiagen multiplex PCR master mix (final concentration of 3mM $MgCl_2$), and 0.2µL of oligonucleotide PCR primer mix. The reactions were conducted with an initial denaturation at 95°C for 15 minutes, then 29 cycles of 95°C for 30 seconds, 90 seconds at the multiplex specific annealing temperature, and 60 seconds primer extension at 72°C, followed by a final extension at 60°C for 20 minutes. Fragment analysis was conducted by capillary electrophoresis on an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) following the manufacturer's protocols. We analyzed electrophoresis data using Genemapper v4.0 software (Applied Biosystems Inc.). Genotypes were determined by two independent readers (double-scoring). We reanalyzed 10% of all samples following the USFWS Abernathy Fish Technology Center (AFTC) QA/QC protocol.

Characterization of genetic population structure using collections from juveniles can be biased if a relatively large portion of the collection is comprised of siblings. The maximum likelihood method implemented in the program COLONY (Wang 2004) was used to identify full-sibling families. A single run of COLONY was performed within each sample collection and removed all but two randomly chosen individuals from each putative full-sibling family to avoid overrepresentation of family groups.

Deviations from Hardy-Weinburg proportions, linkage disequilibrium, expected heterozygosity, and allelic richness were calculated using GENEPOP ver. 3.4 (Raymond and Rousset 1995) and FSTAT ver. 2.9.3.2 (Goudet 2001). Pairwise differences in allele frequencies were tested and the pairwise F_{ST} analogue, Θ_{ST}, was quantified (Weir and Cockerham 1984), using exact tests and 1,000 replicates in ARLEQUIN (Excoffier et al. 2005). Results were adjusted for multiple tests of significance with sequential Bonferroni corrections (Rice 1989).

To detect genetic patterns and obtain a multivariate analysis of the data, we performed a principal components analysis (PCA) of allele frequencies in PCA-GEN (Goudet 1999). We also performed an individual-based principal coordinate analysis (PCoA) of a covariance-standardized genetic distance matrix in GENALEX v6.0 (Peakall and Smouse 2006).

Effective Population Size

Effective population size was estimated through genetic analysis of tissue samples collected during annual bull trout electrofishing activities conducted by USFWS-Abernathy Fish Technology Center. Samples analyzed were from Big Sheep Creek, McCully Creek, Lick Creek, and the upper Imnaha River. Contemporary inbreeding N_e was estimated for each stream using LDNE (Waples and Do 2008). LDNE uses linkage disequilibrium to estimate N_e and corrects for bias using the method of Waples (2006). The program makes the following assumptions: there is no gene flow into the populations (i.e., closed populations), loci are independent and selectively neutral, and generations are discrete. In iteroperous species with overlapping generations, such as bull trout, estimates are intermediate to the number of breeders (N_b) and N_e within the generation prior to that sampled. The lowest allele frequency used was 0.01 (a larger threshold allele frequency provided similar results).

To test for evidence of bottlenecks, collections were examined for heterozygosity excess compared to that expected at mutation-drift equilibrium. The program BOTTLENECK (Cornuet and Luikart 1996) was used with the two-phase mutation model of microsatellite evolution with 10% of the infinite allele model and 90% of the stepwise mutation model (White and Searle 2007; Whiteley et al. 2010). Significance (i.e., the presence of a bottleneck) was determined using a two-tailed Wilcoxon test for heterozygosity excess.

Occupancy

Patch Identification –

The approach to describing bull trout patches in the Imnaha River core area, Oregon, follows USFWS (2008). The resulting patches were identified using temperature:elevation relationships, stream order and determining catchment areas for subwatersheds that fall within the acceptable temperature and stream size thresholds.

A maximum annual stream temperature of 16^{o}C was identified as the threshold for supporting bull trout populations. The maximum annual stream temperature for a given stream location in the Imnaha River core area was determined for the overall time period from USFS water quality monitoring data. In other words, if one year of monitoring occurred at a location, then the maximum temperature from that year was used. If several years of monitoring occurred at a location, then the highest maximum temperature achieved over all years was used. No consideration was given to the duration of the highest annual maximum temperature (e.g., one v. several days). Geographic coordinates (UTM NAD 83) were determined for all stream locations used and elevation was determined using the constructed Imnaha River core area digital elevation model (DEM).

Temperature:elevation relationships were investigated using regression analysis (SigmaStat, SPSS Inc.) and resulted in a determination of elevation above which the maximum annual stream temperature never exceeded 16^{o}C. To further improve the understanding of the Imnaha River core area and areas of the WVIC, thermographs (temperature loggers) were first deployed in the fall of 2007 and were downloaded and redeployed through 2011. Water temperature was recorded on 30 minute intervals. Thermographs were anchored under water in a low-flow area using a metal stake that was tied off to a nearby tree. Flagging, notes, and GPS coordinates were used to mark the sites. Results allowed for the modification of temperature/elevation models.

Patch delineation was conducted using ArcGIS. DEMs (10 m resolution) were acquired for each quadrangle in the Imnaha River core area from the University of Washington (GIS at Earth Space and Science, http://duff.ess.washington.edu/data). The quadrangles were appended to one another to construct a single Imnaha River core area DEM. A 1:100k resolution stream layer for the Imnaha River core area was acquired from the National Hydrography Dataset web site (http://nhd.usgs.gov). Watersheds were initially delineated by eliminating all areas that fell below the elevation threshold determined by the temperature:elevation relationship. Then all areas in which the stream size was larger than a 3rd order stream were eliminated. Finally, any patches that were smaller than 400 hectares were eliminated, resulting in the final patch delineation for the Imnaha River core area.

The design for sample sites is a random and spatially balanced design (Generalized Random-Tesselation Stratified design) developed by the Environmental Protection Agency Environmental Monitoring and Assessment Program. Sample sites were identified on a 1:100 k stream layer using Program R (Gentleman and Ihaka 1996) at a density of 1 site every 500 m. Only those sites that were identified within delineated patches were included in the sample design.

Sample Design –

Each sample site represented a 50 m reach. Up to seven sites per patch were sampled during initial implementation of the sample design. When two size classes (> 30 mm difference in fork length) of bull trout were captured within the patch, it was considered occupied (with a spawning population). If all seven sites were sampled and no bull trout were captured, the patch was considered not occupied with 80% confidence.

Sampling was conducted for occupancy and distribution assessments using backpack electrofishing. Each 50 m reach was sampled from the downstream to the upstream boundary. All fish encountered were captured, identified, and fork length and mass was documented. Distinguishing morphological features were examined when identifying *Salvelinus* species as both bull trout and brook trout may inhabit these streams and hybridization between the two could occur (e.g., dorsal fin mottling; see Markle 1992). All fish captured were released alive near the sampled reach.

Detection probability –

For each occupied bull trout patch (i.e., local population) focused on for estimating abundance, the initial seven sample sites were expanded to a maximum of 21 sites. A probability of detection (pD) for the occupancy approach (described above) was estimated specifically for Imnaha River patches. Detection probability was calculated using the ratio of sites found to contain bull trout and the number of sites sampled within the patch. The pD could then be used to create a nonlinear relationship between the estimated probability of presence (given by no detections) and the number of sites sampled (USFWS 2008).

Results

Abundance

Mark-recapture v. Depletion (2005) –

Three consecutive mark recapture passes were completed over a period of six days (every other day) within the large block-netted study reach. Sampling effort measured by seconds of electrofishing time per reach were totaled at 1588, 1360, and 1435 for passes one, two, and three, respectively. Mark-recapture electrofishing resulted in a total of 174 bull trout (≥120 mm) captured. The population abundance estimate was 161 bull trout ≥120 mm (95% CI = 143-192).

Between days of mark recapture sampling, electrofishing efforts focused on three multiple pass depletion removal sub-reaches. Each 150 meter sub-reach was blocked off within the overall one kilometer study area. Sub-reach one (SR1) required four passes to deplete, sub-reach two (SR2) required three passes, and sub-reach three (SR3) required five passes (Table 3). The abundance estimates of bull trout ≥120 mm for each reach were 3 (95% CI = 2-4) for SR1, 18 (95% CI = 17-19) for SR2, and 48 (95% CI = 37-59) for SR 3.

Table 3. Multiple pass depletion results (BT ≥120 mm) per reach and pass.

	Pass 1	Pass 2	Pass 3	Pass 4	Pass 5	Average effort per pass (sec)
Sub-reach 1	2	0	1	0	-	782
Sub-reach 2	9	5	0	-	-	1206
Sub-reach 3	13	7	3	7	2	931

Based on these results and the associated confidence intervals from the required expansion of multiple pass depletion in subreaches, it was determined that abundance estimates in subsequent years would be more precise using mark-recapture rather than depletion. Both approaches required a similar level of effort.

Mark-recapture (3-pass) v. Mark-recapture (2-pass) v. Single-pass (2006) –

The mark-recapture abundance estimate conducted in Big Sheep Creek in summer 2006 afforded the opportunity to compare estimates generated from multiple pass mark-recapture approaches and a single pass approach. Estimates of abundance for bull trout within each size classification were not significantly different among the abundance estimate approaches due to overlapping confidence intervals (Table 4). It was determined that abundance estimates in the study streams would alternate between the 2-pass mark-recapture and the single-pass approach within a given stream. Continuing to use the multiple pass mark-recapture approach allowed estimation of probability of capture that could be refined over multiple applications of this approach (i.e., improved accuracy across years) and applied to the single-pass approach. The single-pass approach reduced total effort across years and allowed abundance estimates to be generated for multiple streams within a given year.

Table 4. Abundance estimates, 95% confidence intervals, probability of capture and coefficient of variation around the mean for all bull trout ≥120 mm – 3 pass mark-recapture, 2-pass mark-recapture, and single-pass.

	Estimated Abundance	SE (±)	95% CI	Probability of Capture	Coeffecient of Variation
≥120 mm 3 pass	610	24.3	569-664	.35	4.0%
≥120 mm 2 pass	615	50.2	534-732	.33	8.2%
≥120 mm single pass	491	39.8	435-591	.34*	8.1%
≥150 mm 3 pass	388	16.0	362-425	.39	4.1%
≥150 mm 2 pass	332	26.4	291-395	.40	8.0%
≥150 mm single pass	300	29.7	262-378	.39*	9.9%
≥180 mm 3 pass	213	10.9	197-239	.41	5.1%
≥180 mm 2 pass	198	19.8	170-249	.42	10.1%
≥180 mm single pass	168	22.1	142-229	.41*	13.2%

** Probability of capture for single pass was an average of those generated from previous multiple-pass mark-recapture abundance estimates.*

Abundance Estimates (2006-2012) –

Abundance was estimated for Big Sheep Creek, McCully Creek, and Lick Creek at least twice between 2006-2011 using either a mark-recapture approach or a single-pass approach (Table 5). Abundance was consistently highest in McCully Creek for all size classifications. There were no significant differences in abundance estimates, due to overlapping confidence intervals, within Big Sheep Creek and within Lick Creek across years, with the exception of 2007 in Big Sheep Creek (Figure 18). That year's abundance estimate for McCully Creek was also significantly higher than the estimate in 2008. It is unknown what caused 2007 abundance estimates to be significantly greater for Big Sheep Creek and McCully Creek abundance estimates or if a similar increase occurred in Lick Creek.

Table 5. Abundance estimates for Big Sheep Creek, McCully Creek, and Lick Creek, 2006-2011. Standard error, 95% confidence intervals, probability of capture and coefficient of variation reported.

	Abundance Estimate	N	SE (±)	95% CI	Probability of Capture	Coeffecient of Variation
≥120 mm						
Big Sheep Creek	2006[3]	610	24.3	569-664	.35	4.0%
	2007[1]	2,137	80.8	2,019-2,336	.35*	3.8%
	2011[1]	610	43.7	549-721	.35*	7.2%
McCully Creek	2007[2]	2,188	76.8	2,051-2,352	.38	3.5%
	2008[1]	1,543	66.6	1,449-1,710	.38*	4.3%
Lick Creek	2008[2]	800	54.4	719-932	.29	6.8%
	2009[1]	982	60.0	892-1,127	.29*	6.1%
≥150 mm						
Big Sheep Creek	2006[3]	388	16.0	362-425	.39	4.1%
	2007[1]	964	52.1	893-1,097	.39*	5.4%
	2011[1]	390	33.6	346-478	.39*	8.6%
McCully Creek	2007[2]	1,368	47.6	1,285-1,472	.44	3.5%
	2008[1]	855	47.1	764-979	.44*	5.5%
Lick Creek	2008[2]	404	36.2	355-496	.34	9.0%
	2009[1]	388	35.5	339-478	.34*	9.1%
≥180 mm						
Big Sheep Creek	2006[3]	213	10.9	197-239	.41	5.1%
	2007[1]	451	35.3	406-544	.41*	7.8%
	2011[1]	165	22.0	139-225	.41*	13.3%
McCully Creek	2007[2]	573	25.1	531-630	.49	4.4%
	2008[1]	288	26.9	258-363	.49*	9.3%
Lick Creek	2008[2]	101	17.3	83-150	.43	17.1%
	2009[1]	104	17.5	85-154	.43*	16.8%

[1] single-pass
[2] 2 pass mark-recapture
[3] 3 pass mark-recapture
* Probability of capture estimated from previous mark-recapture on respective stream

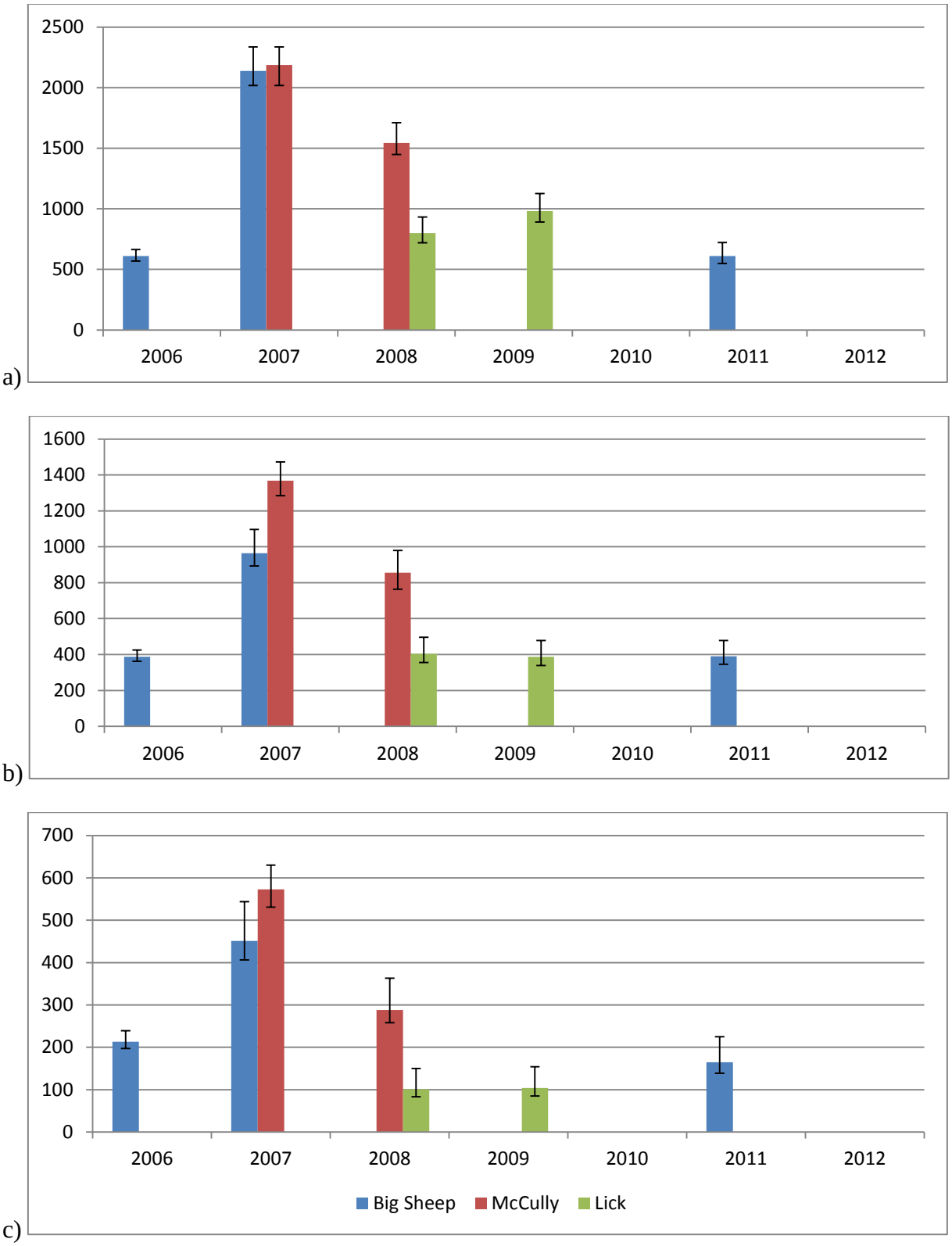


Figure 18. Estimates of abundance in Big Sheep Creek, McCully Creek, and Lick Creek for bull trout a) ≥ 120 mm, b) ≥ 150 mm, and c) ≥ 180 mm.

Connectivity

Through the duration of this project, 2,869 bull trout were tagged with PIT tags. Of those, 157 were subsequently detected at a PIT array in the Imnaha River core area (Table 6; Figure 8). Bull trout from Big Sheep Creek were detected moving upstream and downstream through both the WVIC and Big Sheep Creek. Detections at Salt Creek Summit confirm that fish can move both upstream and downstream over the spillway located there. Therefore, the potential for connectivity among all populations exists (i.e., no population is completely isolated).

The only exception to this is McCully Creek. There was no evidence of fish that moved from McCully Creek downstream past the WVIC ever returned to McCully Creek above the WVIC. In addition, there were eight fish from BSC and SCS that were detected at McCully Creek, having moved down the WVIC to this PIT array. Three of these bull trout dropped out of the WVIC into McCully Creek, however, there was no evidence that these fish ever migrated upstream in McCully Creek. Thus, suggesting that the population in McCully Creek may be isolated.

Multiple individuals (n=11) from Big Sheep Creek and Lick Creek were detected moving downstream as far as the lower PIT array near the mouth of the Imnaha River (IR1). Some of these fish were detected returning to lower Big Sheep Creek. It is unknown whether they returned to Lick Creek or upper Big Sheep Creek.

Table 6. Sample stream from which bull trout were PIT-tagged, total number of those bull trout detected, and the PIT array at which those bull trout were detected (MCC=McCully Creek, SCS=Salt Creek Summit, LSC=Little Sheep Creek, BSC=Big Sheep Creek, IR=Imnaha River).

Sample Stream	# of BT Tagged	Total # of Tagged BT Detected	Detections							
			MCC	SCS	BSC	LSC	IR1	IR2	IR3	BSC Mouth
MCC	921	47	47	0	0	0	0	0	0	0
SCS	69	12	1[1]	9	2	0	0	0	0	0
LSC	49	1	0	1	0	0	0	0	0	0
Canal Creek	32	4	0	4	0	0	0	0	0	0
BSC	1,397	85	7[1]	19	65	0	1	1	0	3
Lick Creek	394	8	0	0	0	0	7	7	0	8
Redmont Creek	7	0	0	0	0	0	0	0	0	0

[1] *These fish were detected at the McCully Creek array. Three total fish dropped out of the WVIC at McCully Creek, but were not detected moving upstream into McCully Creek*

Population Genetic Variability

COLONY identified 49 putative full-sibling families (P(inc. ≥ 70), P(exc.) ≥ 0.70; Table 7), ranging in size from two to 14 individuals (median = 2). The number of full-sib families and family sizes were fairly evenly distributed across collections. A total of 53 individuals were removed from 17 families; the other 32 families contained only two individuals and both individuals were retained for analyses. The final analysis included 241 individuals (Table 7).

Table 7. Collection information including data from before (b) and after (a) removal of putative full siblings using COLONY.

Collection ID	Location	Year	N (b/a)	Full-sib families	LD (b/a)	HWP (b/a)
BS05	Big Sheep	2005	42/27	8	48/15	3/0
BS11	Big Sheep	2011	42/36	7	29/20	3/3
UI07	Imnaha	2007	84/76	12	40/14	6/2
LI08	Lick	2008	41/23	10	75/18	6/0
MC07	McCully	2007	42/37	8	32/13	9/3
MC12	McCully	2012	43/42	4	12/9	1/1

N sample size; LD number of significant (P<0.05) pairwise linkage disequilibrium estimates; and HWP number of loci in significant (P<0.05) deviation from Hardy-Weinberg proportions.

Tests for deviation from HWP were significant in 10% of the cases (11 of 105 tests; $P < 0.05$), where approximately five were expected by chance at $\alpha = 0.05$. Significant tests were distributed across eight of 15 loci and five of seven collections. After sequential Bonferroni correction for approximately 15 tests per collection, two comparisons remained significant (one at *Sco220* in BS11and one at *Sco109* in SI07). Significant LD was detected in 15% of the tests (104 of 679 tests; $P < 0.05$). Sequential Bonferroni correction for approximately 105 locus pairs in each collection resulted in 14 significant tests, with at most three occurring in any one collection.

The mean expected heterozygosity (H_E) across collections was 0.64 (SE = 0.02) (range 0.58–0.70) and mean allelic richness (A_R) was 5.85 (SE = 0.22) (range 5.03–6.46; Table 8). *Sfo118* was monomorphic in all six collections and not included in analyses. Multi-locus observed heterozygosity generally conformed to expected heterozygosity; three heterozygote excesses were observed and four deficits (Table 8).

Table 8. Genetic summary statistics and effective population sizes (Ne) for bull trout in the upper Imnaha River drainage. Standard errors for the first three statistics are presented in parentheses. Sample ID can be found in Table 7.

Collection ID	H_O	H_E	A_R	F_{IS}	N_e (95% CI)
BS05	0.66(0.07)	0.63(0.06)	5.80(0.75)	-0.05	22 (18, 29)
BS11	0.57(0.07)	0.58(0.07)	5.03(0.69)	0.02	20 (16, 25)
UI07	0.64(0.06)	0.67(0.06)	6.61(0.84)	0.06	74 (52, 114)
LI08	0.72(0.06)	0.70(0.06)	6.45(0.76)	-0.03	20 (15, 26)
MC07	0.60(0.06)	0.64(0.06)	5.49(0.67)	0.06	21 (18, 26)
MC12	0.62(0.06)	0.61(0.06)	5.39(0.62)	-0.02	76 (54, 120)

A_R allelic richness based on sample size of 21; H_O observed heterozygosity within subpopulations; H_E unbiased expected heterozygosity within subpopulations; F_{IS} index of deviation from expected heterozygosity: 1-(H_O/H_E).

Considerable variation in allele frequencies was observed among subbasins; global F_{ST} was 0.10 (95% C.I. 0.08–0.12). Log-likelihood (G) based exact tests for differentiation among collections indicated significant differences in allele frequencies at 14 of 15 polymorphic loci ($P < 0.05$); *Sco102* was not significant. *Sco102* was monomorphic in four of seven collections and contained only three alleles, two of which were at very low frequency (< 0.05). For pairwise population differentiation (F_{ST}), 19 of 21 tests were significant before ($\alpha = 0.05$) and after correcting for multiple tests ($\alpha' = 0.05/21 = 0.002$) (Table 9). The only nonsignifcant F_{ST} comparisons occurred between temporally spaced collections from within McCully and Big Sheep Creeks (Table 9); allele frequency differences between the temporally spaced samples were significant at only 2 of 15 loci in both populations.

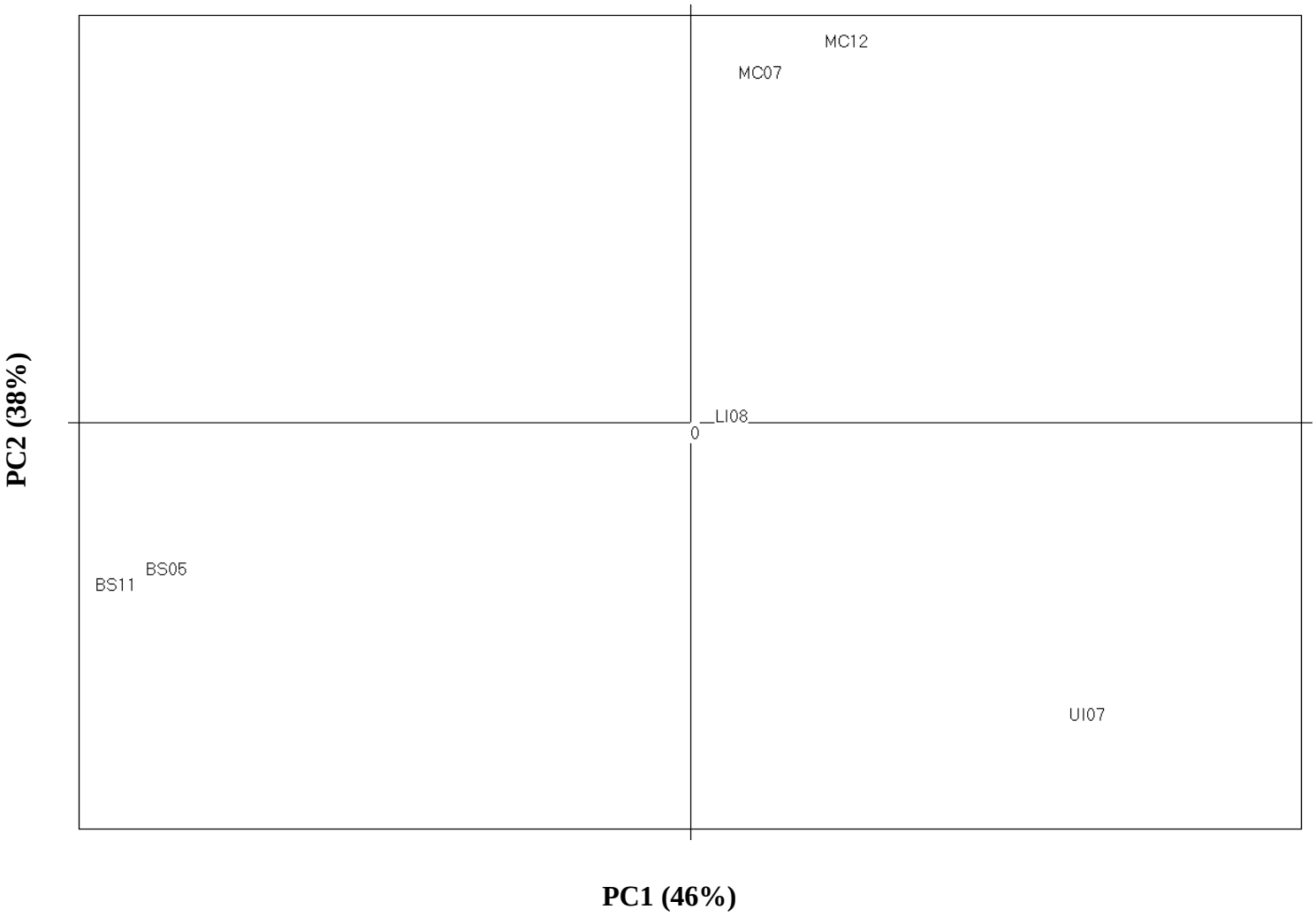
Table 9. F_{ST} estimates (below diagonal) and *P*-values (above diagonal) for bull trout in the upper Imnaha River drainage. Gray shading indicates significance (α'<0.05/21=0.002). Sample names correspond to those in Table 7.

Collection ID	BS05	BS11	LI08	MC07	MC12	UI07
BS05	-	0.14	0.00	0.00	0.00	0.00
BS11	0.00	-	0.00	0.00	0.00	0.00
LI08	0.09	0.13	-	0.00	0.00	0.00
MC07	0.11	0.13	0.08	-	0.13	0.00
MC12	0.13	0.15	0.09	0.00	-	0.00
UI07	0.13	0.14	0.09	0.10	0.11	-

Principal components analysis (PCA) of allele frequencies supports considerable genetic divergence among bull trout within patches and stability of allele frequencies within putatively isolated populations (i.e., McCully and Big Sheep Creeks). That is, temporal collections from McCully and Big Sheep Creeks cluster very closely in PCA space, indicating they display temporal stability of allele frequencies (Figure 19). PC axes 1 through 3 explained 44, 36, and 12% of the variation, respectively. PC3 most noticeably distinguishes Lick Creek (LI08) from all other samples (Figure 19).

Principal coordinates analysis (PCoA) of genetic variation among individuals revealed that individuals cluster according to the patch in which they were sampled (Figure 20). This pattern holds regardless of sample year. That is, individuals from McCully and Big Sheep Creeks, respectively, are effectively indiscernible between sampling years, which corroborates the PCA of allele frequencies. The clustering of individuals by stream of origin is consistent with the hypothesis that the streams delineate the populations present within the system. PC axes one through three explained 30, 23, and 15% of the variation, respectively.

A.



B.

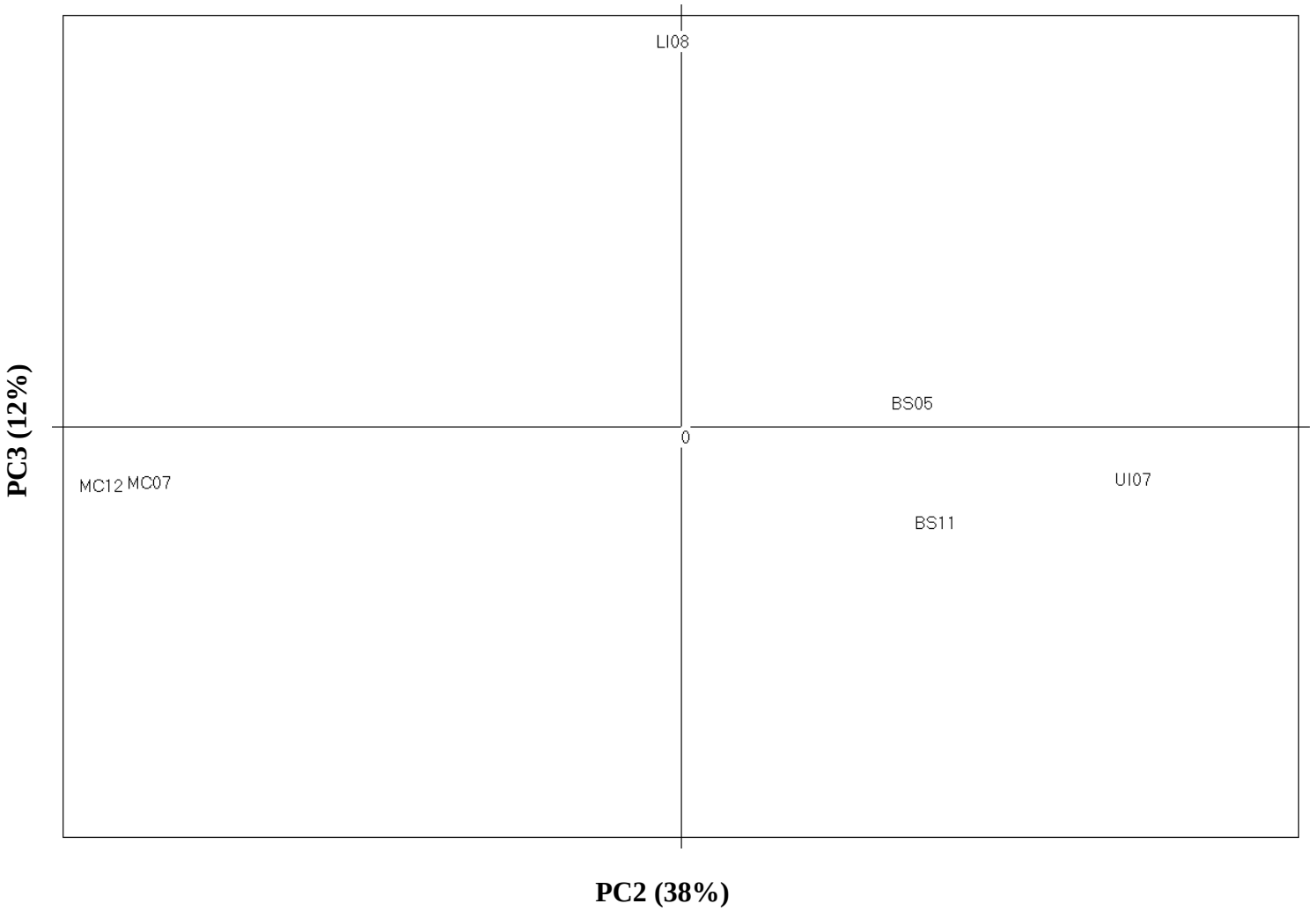


Figure 19. Plot of principal component scores 1 and 2 (A) and 2 and 3 (B) derived from allele frequencies at 15 polymorphic microsatellite loci. The percentage of variation attributable to each component is shown for each axis. Collection IDs can be found in Table 1.

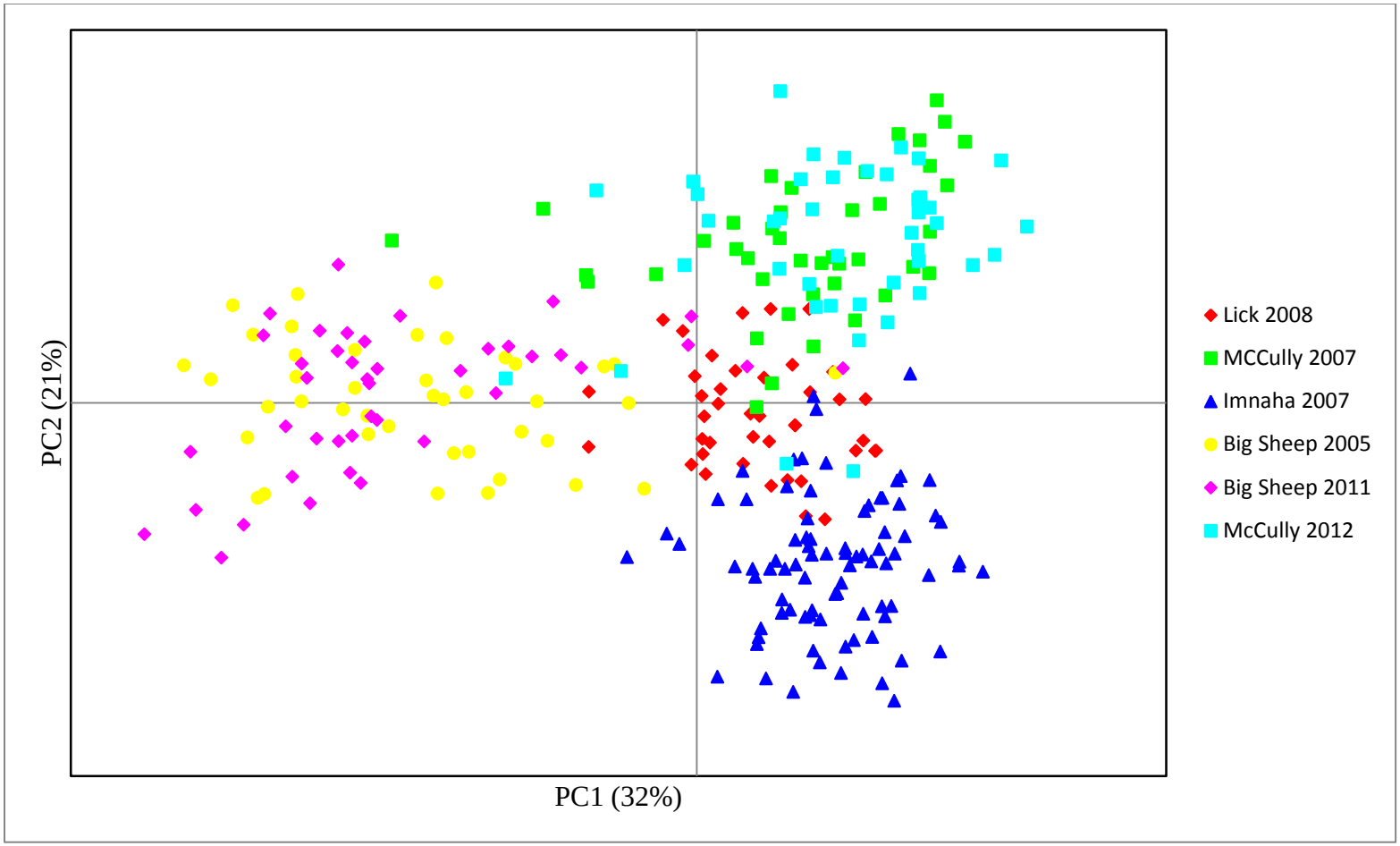


Figure 20. Plot of the first two principal coordinate scores derived from individual-based variation at 15 polymorphic microsatellite loci. The percentage of variation attributable to each component is shown for each axis. Collection ID can be found in Table 1.

Effective Population Size

Contemporary inbreeding N_e was estimated using the linkage disequilibrium method implemented in the program LDNE (Table 8). Mean N_e was 42 (range 20–88; Table 8). Confidence intervals (95% CI) for all estimates were relatively small and did not contain 0 or infinity. N_e estimates in four of six (~66%) collections were less than 50.

Recent population bottlenecks could be responsible for small estimates of contemporary N_e. Analysis in BOTTLENECK showed that the sample from McCully Creek in 2007 (MC07) had an excess of heterozygosity when compared to the expectation at mutation-drift equilibrium (Wilcoxon test, $P < 0.05$), thus indicating a potential bottleneck. This collection had an intermediate N_e estimate (21 [95% CI 18, 26]) compared to all others (Table 8). After Bonferroni correction based on seven tests ($\alpha' = 0.05/7 = 0.007$), the BOTTLENECK test was not significant.

Occupancy

Patch Delineation and Population Structure –

Using temperature/elevation relationships, catchment area, and stream orders the Imnaha River core area analysis resulted in 23 total potential bull trout patches, five of which are known to have existing bull trout populations (Figure 21). Of those, five patches were occupied with bull trout. Given our design and the requirement that bull trout from two different cohorts needed to be detected for a patch to be considered occupied, this data suggests each of these patches is occupied by a spawning population of bull trout. This interpretation is corroborated by the genetic analysis, which suggested that bull trout from McCully Creek, Lick Creek, (upper) Big Sheep Creek and the upper Imnaha River all represent distinct biological populations.

Occupancy –

Bull trout occupancy sampling was conducted in 2009 for five patches: Gumboot Creek, Skookum Creek, Owl Creek, Carrol Creek, and West Fork Carrol Creek (Figure 21). No bull trout were found to be occupying any of the sites sampled within these drainages. *O. mykiss* species were captured during efforts in Gumboot Creek and Carrol Creek drainages.

Seven sites containing water were completed within the Carrol Creek patch, no bull trout were found present, and *O. mykiss* were captured in five sites. According to the methods described to estimate the probability of presence of bull trout using a predefined value of 0.50, it is estimated with an 80% level of confidence that a population of bull trout did not occupy the Carrol Creek patch. All five sites were sampled within the Gumboot Creek patch, and only *O. mykiss* species were found present. Within the Owl Creek patch, a large portion of the patch was found to be dry, therefore reducing the size of the patch to less than 400 hectares, an area less than that predicted necessary to support a bull trout population. The West Fork Carrol Creek patch contained water in five of the seven sites visited, but no fish were found. It was suggested by local ODFW fish biologists that sampling within the

Skookum Creek patch would likely result in no fish found due to a fish barrier in the lower portion of the patch, therefore only one site was sampled and no fish were found present.

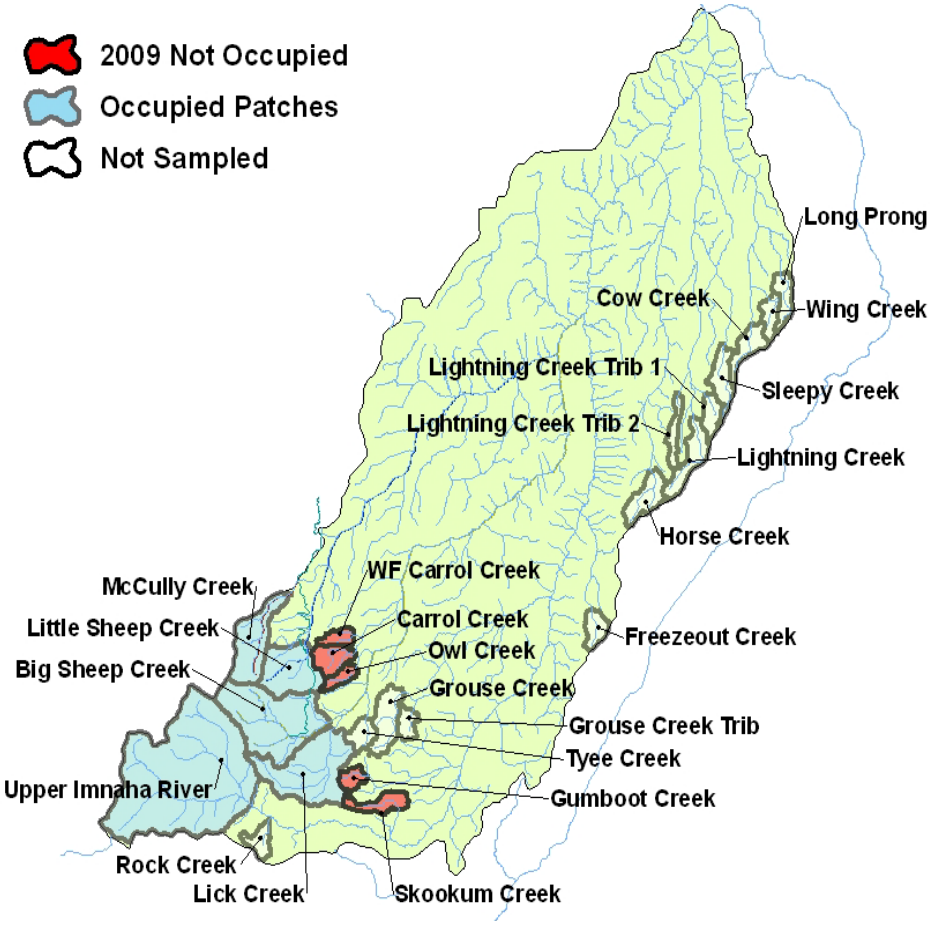


Figure 21. Imnaha River basin patches derived from temperature/elevation relationships, catchment areas, and stream order. Known bull trout occupancy patches, 2009 sampled patches, and patches not sampled.

In 2010, occupancy sampling was conducted in the Little Sheep Creek patch and the WVIC upstream to Salt Creek Summit and downstream to Redmont Creek. A total of 17 sites were sampled in Little Sheep Creek and Cabin Creek (a tributary to Little Sheep Creek above the WVIC), and an additional 12 sites were sampled in the WVIC (6 upstream and 6 downstream of Little Sheep Creek). Bull trout were captured only in the lowermost three sites of Little Sheep Creek (1 site) and Cabin Creek (2 sites) above the WVIC. The remaining 14 sites (12 above and 2 below the WVIC) did not contain bull trout. The habitat in the upper parts of the patch is degraded due to impacts from the Canal Creek fire of 1989, and possibly the Twin Lakes fire in 1994, likely affecting distribution of bull trout. Additionally, one bull trout was

captured in the WVIC upstream of Little Sheep Creek. No other bull trout were captured in the WVIC.

The remaining patches identified were not sampled for occupancy. Subsequent temperature monitoring of several of these patches suggests that higher water temperatures in these streams are likely not suitable for supporting bull trout. The initial analysis that identified these putative patches was based predominantly on temperature monitoring that occurred in the Imnaha River, Big Sheep Creek and Little Sheep Creek. All of these streams drain directly off and are fed by springs from the Eagle Cap. The patches sampled and determined not occupied and the patches that were not sampled are in watersheds that originate at lower elevations.

Detection probability –

Detection probability for occupancy sampling was determined for Big Sheep Creek in 2011, McCully Creek in 2009, and Lick Creek in 2008 (Table 10). The number of 50 m reaches sampled ranged from 12 to 16, and was restricted by distribution of bull trout in the given patch while maintaining the integrity of the sample design. Detection probability ranged from 0.676 – 0.875. These detection probabilities provide greater than 95% confidence that bull trout do not occupy a patch in the Imnaha River core area if not detected when sampling seven sites in the patch.

Table 10. Detection probability for determining occupancy in Big Sheep, McCully and Lick creeks.

Population	Number of sites sampled	Number of sites bull trout detected	Detection probability
Big Sheep Creek	12	10	0.833
McCully Creek	16	14	0.875
Lick Creek	16	11	0.676

Conclusions

Abundance was estimated in this study for bull trout ≥ 120 mm FL, ≥ 150 mm FL, and ≥ 180 mm FL. Sexual maturity was not determined for bull trout captured in this project, but the number of adults in these populations is most useful for evaluating trends in abundance and status. Relationships between length and maturation schedules are not well defined for most Oregon bull trout (Hemmingsen et al. 1996). Sankovich et al. (2004) found the majority (66%) of resident bull trout 150-159 mm FL, and all resident bull trout >159 mm FL were sexually mature (i.e., adults). This information is further supported by Fraley and Shepard (1989) and Hemmingsen et al. (2001). Therefore, we assume that the abundance estimates for all fish ≥ 180 mm FL are entirely comprised of resident adults, and the abundance estimates for all fish ≥ 150

mm FL are predominantly comprised of resident adults for each of the populations. The abundance for resident adults in each stream likely lies between the two respective estimates. In addition, the approach used in this study does not account for fluvial adults which may not have returned to the study area at the time of sampling. This did not impact the estimates for McCully Creek, for which there was no evidence of a fluvial component to the population, but does likely underestimate overall adult abundance in Big Sheep Creek and Lick Creek. However, given that few (if any) large bull trout (greater than 370 mm in fork length) were observed during this investigation, it is reasonable to suggest that fluvial adults likely comprise a relatively small proportion of these populations (see Al-Chokhachy and Budy 2008). These qualifications illustrate the difficulty and complexity of estimating the abundance of bull trout populations comprised of multiple life history strategies, and demonstrate the need to develop reliable approaches for estimating adult abundance to assess demographic response to recovery actions.

Fluctuations in abundance estimates may be part of natural cycle or episodic events. This type of uncertain population characteristic can influence the loss of genetic variation and the relationship between N_e and the numbers observed in a population (Rieman and Allendorf 2001). The genetic results indicate that Big Sheep and McCully creeks may be, at least, partially isolated due the temporal stability of allele frequencies and the least genetic diversity across all populations analyzed. We would expect that repeated or periodic demographic support from other populations would be necessary to maintain similar amounts of diversity across populations and produce temporal variation in allele frequencies (Allendorf and Luikart 2007; Harrison and Hastings 1996; Spruell et al. 1999). While allele frequencies were stable over time in Big Sheep Creek and McCully Creek during the course of this study, the significant fluctuation in abundance observed during the course of this study may be representative of past population fluctuations creating bottleneck events that could impact genetic variation, and therefore genetic N_e.

Rieman and Allendorf (2001) stated that in the absence of more detailed local population and demographic information, the best estimate of N_e is be between 0.5 and 1.0 times the mean number of adults observed annually. Our estimates of inbreeding N_e based on single sample linkage disequilibrium were small (mean=39; range 20–76), but within the range seen for bull trout across their distribution in the conterminous United States (Table 1) (Ardren et al. 2011). For example, Ardren et al. (2011) found that approximately 75% of samples had estimated effective population sizes less than 50. A commonly applied guideline is that an N_e of at least 50 individuals should be maintained to avoid the deleterious effects of inbreeding in the short term (i.e., "50/500" rule; Franklin 1980, Soulé 1980, Allendorf and Ryman 2002). An important consideration for highly structured species such as bull trout, however, is that metapopulation dynamics might maintain genetic diversity beyond that expected in the sum of the local populations (Rieman and Allendorf 2001). Consequently, special attention may be warranted in populations such as McCully and Big Sheep Creeks that have both disrupted patterns of gene flow and small N_e; especially if their N_e estimates are representative of extirpated bull trout populations that were historically present upstream of anthropogenic barriers (i.e., Little Sheep Creek).

While this project was not designed to quantify movement (or lack of movement) across the diversion structures, the data collected suggests that the diversion structures may impede direct

connectivity of these populations with the Imnaha River. For example, of the 85 fish from BSC detected moving downstream, 26 (31%) were detected moving down the canal. These fish, in addition to others tagged at SCS, LSC, Canal Creek and Redmont Creek, were not ever detected moving downstream in LSC. Therefore, the operation of diversion structure in BSC may be limiting and the diversion structure in LSC may be limiting/preventing downstream movement of bull trout to the Imnaha River. This may further suggest limitations to upstream movement.

The occupancy and distribution of bull trout in the Imnaha River core area appears to have been maximized across streams with habitat currently suitable to support bull trout. Genetic structure among bull trout support the identification of four local populations in the Imnaha River core area: upper Imnaha River, Lick Creek, Big Sheep Creek, and McCully Creek. These results are consistent with the patch identification structure, lending evidence that putative patches represent local populations. With the recovery of the upper Little Sheep Creek watershed over time, it is possible that a bull trout population will once again be present there. Providing connectivity among populations is important for this potential to be realized and ensure demographic stability over time.

The occupancy and distribution results also align with bull trout eDNA analysis that has been conducted in the Imnaha River core area to date (Young et al. 2017). However, the existing populations occupy habitat across the range of occupancy probabilities identified by the cold water climate shield for bull trout occupancy (Isaak et al. 2015, 2017). The occupancy probabilities for the upper Imnaha River (> 0.90) and Big Sheep Creek (> 0.75 to < 0.90) indicate a high likelihood bull trout should be present. The occupancy probabilities for the existing populations in McCully Creek (> 0.25 to < 0.50) and Lick Creek (< 0.25), however, indicate a relatively low likelihood that bull trout should be present. The agreement and discrepancy among these various approaches highlight the importance of recognizing the limitations of each. When local stream temperature information is available to identify putative patches in the way that we did, it can be a more accurate predictor of bull trout occupancy. It is important to consider using more than one approach to assessing, and physical verification of occupancy and distribution for bull trout.

In addition to identifying critical uncertainties relative to recovery (Appendix I), this project has established a baseline dataset for bull trout populations in the Imnaha River core area that can be used for long term monitoring and further investigation of population level effects from lack of connectivity. In the future, abundance estimates can again be coupled with genetic analysis to allow continued evaluation of inbreeding and genetic drift in small, potentially isolated bull trout populations. Furthermore, this work has supported and can continue to support ongoing range-wide bull trout eDNA analysis (Young et al. 2017) and Lower Snake River Compensation Plan work associated with the operation of the Chinook salmon weir on the mainstem Imnaha River.

The findings of this work suggest that relatively small, potentially isolated bull trout populations can persist over time. However, providing connectivity among bull trout populations will likely ensure that persistence when faced with stochastic events that impact one or more of these populations (e.g., low water year, wildfire). Frequency of these stochastic events throughout the range of bull trout is increasing and predicted to increase more in the future (Eby et al. 2014). Recovery actions for the species should consider what can be done to support the species ability

to adapt to these changes in the landscape, therefore reducing its vulnerability. The empirical evidence provided here emphasizes the importance of not only addressing limiting factors (e.g., threats) to bull trout populations in recovery efforts, but understanding the demographic parameters of those bull trout populations (e.g., population size, genetic structure, and effective population size) in assessing the response of actions taken toward recovery of the species.

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Appendix I

Outstanding Critical Questions for bull trout in the Imnaha River core area:

Abundance/Trend in Abundance

- How does the abundance of the local populations vary over time?

Connectivity

- How much of a barrier do the WVIC diversions present to existing and potential bull trout populations in the Imnaha River core area?
- How do abundance, genetic diversity, N_e, and relative proportions of life history strategies present for bull trout populations in the Imnaha River core area change with adequate fish passage provided across WVIC diversions?
- Of the known populations, the upper Imnaha River population appears to be the primary population and Lick Creek is the only other population with unimpeded connectivity to the upper Imnaha River. Do fish from Lick Creek migrate to the upper Imnaha River? Do fish from other local populations (e.g. Big Sheep) migrate to Lick Creek and/or the upper Imnaha River? Do fish from the upper Imnaha River migrate to other local populations?
- How does the WVIC influence connectivity? Specifically, if fish leave Big Sheep Creek down the canal, do they return to Big Sheep Creek? If fish leave Big Sheep Creek down Big Sheep Creek, do they return to Big Sheep Creek?
- Should the WVIC (at least at Big Sheep Creek) be screened?

Population Genetic Structure

- Especially for populations that may be all/mostly isolated (e.g. McCully Creek), and with low effective population sizes, do they exhibit signs of inbreeding depression or genetic drift over time (genetic risk)?
- Are the NF and SF of the upper Imnaha distinct populations?
- How does genetic diversity vary over time?

Effective Population Size

- How does the stability of the environment in the Imnaha River core area change over time?
- How does the Ne of a local population vary over time?

Occupancy

- What are the relative proportions of life history strategies (i.e., resident v. fluvial) that comprise bull trout populations in the Imnaha River core area?
- Is Little Sheep Creek being recolonized? (Can it be? Is culvert a barrier?)
- Are the unsampled patches occupied by bull trout (resident and/or fluvial)?

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