

**Fine-Scale Population Structure Analysis and Genetic Population
Assignments of Wenatchee River Sub-Basin Bull Trout**

Final Report

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Summary

Defining population boundaries is an important aspect of conservation planning for threatened and endangered species. The U.S. Fish and Wildlife Service currently designates seven local populations of bull trout within the Wenatchee River Sub-basin; some of which contain a single spawning tributary and others which are comprised of multiple spawning tributaries. In this study, we were interested to know if different tributaries within two designated local populations, Icicle Creek and Peshastin Creek, contained a single or multiple spawning populations of bull trout. We collected juvenile bull trout from French Creek and Jack Creek within the Icicle Creek watershed and Ingalls Creek and Etienne Creek within the Peshastin Creek watershed. Juvenile bull trout were genotyped at 15 microsatellite loci and we used a number of analysis methods to determine the number of genetically unique spawning populations in each watershed. Based on our data, we could not definitively conclude that Jack Creek and French Creek were independent populations. Data for Peshastin Creek were much more conclusive and suggested that Ingalls Creek and Etienne Creek were genetically independent populations. This information on population structure was then incorporated into a genetic baseline dataset previously developed for upper Columbia River bull trout and we used this information to conduct genetic population assignments for bull trout collected downstream of spawning areas in Icicle Creek and Peshastin Creek. Genetic assignments for fish collected in Icicle Creek suggest that individuals from a number of different local populations utilize habitat in lower Icicle Creek and that putative barriers in Icicle Creek downstream of the Boulder Falls do not preclude upstream movement. All bull trout collected above the Boulder Falls were assigned to Icicle Creek. Genetic assignments for bull trout collected in a rotary screw trap downstream of spawning tributaries in Peshastin Creek suggested that most migratory fish originated in Ingalls Creek and that fish in Etienne Creek primarily had a resident life history. Data from this study will be useful for refining population boundaries, targeting different populations for specific management actions, and evaluating the effects of different putative barriers on bull trout movements and migratory patterns.

Introduction

Defining population boundaries is an important, and sometimes challenging, task for fish biologists and natural resource managers (Pearse and Crandall 2004; Waples and Gaggiotti 2006; Hasselman et al. 2010; DeHaan et al. 2011b). Conservation measures are generally implemented and evaluated at the population level; therefore, a clear understanding of what constitutes a population and how population boundaries vary among different watersheds is essential to the success of actions directed towards species conservation. Several types of data including information on movement and dispersal (Knights et al. 2002; Glover et al. 2008; Buckmeier et al. 2013), habitat features (Rieman and McIntyre 1995), population demographics (Koizumi 2011), and genetic data (Spruell et al. 1999; Waples and Gaggiotti 2006; Hasselman et al. 2010) have all been utilized to help define populations; however, different types of information may lead biologists to different conclusions with regards to population boundaries (Koizumi 2011).

Bull trout, *Salvelinus confluentus*, are native to northwestern North America, historically ranging from northern California to the Yukon Territory and from coastal drainages as far inland as the headwaters of the Saskatchewan River (Cavender 1978; Hass and McPhail 1991). Like many species of salmonids, bull trout have both resident and migratory life history types, and the two life history types often occur sympatrically (Dunham and Rieman 1999; Rieman and Dunham 2000). The species has declined substantially throughout its range and is currently listed as a threatened species by the U.S. Fish and Wildlife Service ([USFWS] 1999). Causes for the species decline and continued threats to persistence include: overharvest, habitat fragmentation, competition and hybridization with non-native species, and a changing climate (Rieman et al. 1997; USFWS 2002; Wenger et al. 2013). Previously a number of different studies have used genetic data to examine the population structure of bull trout at both broad (Taylor et al. 1999; Spruell et al. 2003; Ardren et al. 2011) and fine spatial scales (Spruell et al. 1999; Costello et al. 2003; Whiteley et al. 2006; DeHaan et al. 2011b; Nyce et al. 2013). A common result among these studies was that bull trout from different sub-basins/watersheds showed a strong level of genetic differentiation from one another and within sub-basins/watersheds, individual tributaries frequently contained genetically distinct populations.

Previously, DeHaan and Neibauer (2012) conducted an analysis of genetic variation within and among bull trout populations in the upper Columbia River Basin (i.e., the Wenatchee, Entiat, and Methow river sub-basins). That study demonstrated that collections of bull trout from

the three sub-basins were highly differentiated from each other (DeHaan and Neibauer 2012). For statistical analysis, the authors designated populations according to the most recent recovery planning information developed by the USFWS (USFWS 2002; USFWS 2010). All local spawning populations were genetically distinct from one another (DeHaan and Neibauer 2012), which was consistent with previous observations from other sub-basins (Whiteley et al. 2006; DeHaan et al. 2011b; Nyce et al. 2013). The baseline dataset developed for the study was then used to conduct population assignments for a number of subadult and adult bull trout collected from different locations downstream of spawning areas in the Wenatchee River Sub-basin in order to infer patterns of movement. Populations in that study consisted of individuals collected from a single spawning tributary within a watershed, as well as individuals collected from multiple spawning tributaries within a watershed (see Table 1 in DeHaan and Neibauer 2012).

For this project we were interested in exploring fine-scale population structure of bull trout in two watersheds within the Wenatchee River sub-basin: Icicle Creek and Peshastin Creek. Each of these creeks contains multiple tributaries where juvenile bull trout are frequently found. For previous genetic analysis, bull trout collected from multiple spawning tributaries in Icicle Creek and Peshastin Creek were grouped into two populations representing these two watersheds (DeHaan and Neibauer 2012). Our objective was to determine if there was a single or multiple spawning populations in each of these two watersheds. Based on this information, we also wished to use genetic assignments to examine the origins of bull trout collected in areas downstream of spawning habitat in these two watersheds and to use this information to make some general inferences regarding life history and movement patterns of bull trout in these two watersheds.

Study Area

Icicle Creek

Icicle Creek is one of seven local populations of bull trout in the Wenatchee Core Area of the draft Mid-Columbia Recovery Unit (USFWS 2002; Figures 1 and 2). Icicle Creek contains both resident and migratory bull trout. The majority of bull trout spawning in Icicle Creek is thought to occur in French Creek (Brown 1992; Nelson 2007; Nelson et al. 2009, 2011, 2012; Nelson and Sulak 2013). Low numbers of small bull trout have also been observed in lower Jack Creek (Kelly Ringel 1997; USFWS 2005), but it is uncertain if reproduction occurs there or if

these are actually migrants from French Creek. Bull trout abundance and distribution in the Icicle watershed upstream of French Creek is largely unknown (Kelly Ringel 1997). High numbers of bull trout of several size classes have been observed in lower Icicle Creek during annual snorkel surveys downstream of the Boulder Falls (rkm 9.2; Figures 2 and 3), with the majority classified as subadults (USFWS 2011, 2012, 2013). Radio-telemetry and PIT (passive integrated transponder) tagging studies indicate lower Icicle Creek is used by migratory subadult and adult bull trout from several other local populations in the upper Columbia River Basin (Nelson et al. 2009, 2011, 2012; Kelly Ringel et al. 2014; PTAGIS data from files).

Several types of putative barriers exist within the Icicle Creek watershed that may limit bull trout movements. The construction of irrigation dams and Leavenworth National Fish Hatchery (LNFH) placed several concrete structures in the stream corridor that are potential impediments to the movements of fish (Figures 2 and 3). Natural obstacles are also present in Icicle Creek including the Boulder Falls at rkm 9.2 (Figures 2 and 3). Thus, lower Icicle Creek can be partitioned into several zones based on the locations of obstacles in the stream corridor (Figure 3). For many years and for several reasons, operations of structures were not concerned with migratory fish, but within the last 10 years management of the LNFH has been modified to increase opportunities for fish passage. The Boulder Falls were long considered impassable to upstream migrants (WDF 1938), and while several lines of evidence indicate that these falls are a major impediment to upstream movements, there apparently is a narrow window of opportunity for upstream passage under certain conditions (Dominguez et al. 2013). Following modifications to how certain structures are operated, bull trout are now observed more frequently in areas where previously few were detected.

Peshastin Creek

Peshastin Creek is also one of seven local populations of bull trout in the Wenatchee Core Area (USFWS 2002; Figure 1). The main stem of Peshastin Creek, from its mouth to Etienne Creek, is considered foraging, migrating, and overwintering habitat while spawning and rearing habitat is located in Ingalls and Etienne creeks (USFWS 2010). Bull trout were documented in the migratory corridor of Peshastin Creek during trapping at irrigation diversions in 1937 (WDF 1938) and during rotary screw trapping in 2004 and 2005 (Cooper and Mallas 2004; USFWS 2006). Extensive daytime snorkeling surveys specific to bull trout were

conducted in 1995. These surveys documented resident-sized bull trout in Ingalls Creek but no bull trout were found in Etienne Creek (Kelly Ringel 1997). Bull trout were finally confirmed in Etienne Creek when observed during limited electrofishing/snorkel surveys conducted in 2005, 2006, and 2007 for the Integrated Status and Effectiveness Monitoring Program (ISEMP data from files) and during intensive surveys conducted in 2013 (USFWS 2014).

Methods

Sample collection

For the fine-scale population structure analysis, we focused on collecting juvenile bull trout from French and Jack creeks in the Icicle Creek watershed and Ingalls and Etienne creeks within the Peshastin Creek watershed (Figures 2 and 4). Collection efforts targeted juvenile bull trout (mostly individuals <200mm total length) because they presumably had not yet emigrated from natal tributaries, as opposed to sub-adult and adult bull trout which can migrate extensive distances while maturing, foraging, or between spawning periods (Mogen and Kaeding 2005; Muhlfeld and Marotz 2005; Brenkman et al. 2007). Juvenile bull trout from these four tributaries were captured primarily by hand net during night snorkeling or by electrofishing. Genetic tissue samples were taken from individuals collected from French and Jack creeks during 2005, 2006, and 2012. Genetic tissue samples were taken from individuals collected from Ingalls Creek during 2006 and Etienne Creek from 2005 to 2007 and 2013.

We also collected sub-adult and adult bull trout from areas downstream of spawning habitat in Icicle Creek and Peshastin Creek for population assignments. Population assignments for these fish were used to examine the effects of different structures or putative barriers in Icicle Creek on bull trout movements and to infer migratory patterns in the two watersheds. Sub-adult and adult bull trout were collected from four different zones in the Icicle Creek system (zones A, B, D, and E; Figure 3) from 2005 to 2013. Individuals were collected in each zone using a number of different methods including angling, electrofishing, and entrainment in the LNFH water supply system. Sub-adult bull trout were collected during downstream migrations in Peshastin Creek in 2005 at a rotary screw trap located at river kilometer 9.6 (Figure 4).

Laboratory methods

DNA was extracted from all bull trout collected for this study using Qiagen DNeasy 96 (Qiagen Inc.) extraction kits according to the manufacturer's instructions. All individuals were genotyped at a suite of 15 microsatellite loci: *Omm1128*, *Omm1130* (Rexroad et al. 2001), *Sco102*, *Sco105*, *Sco106*, *Sco107*, *Sco109* (Washington Department of Fish and Wildlife, *personal communication*), *Sco200*, *Sco202*, *Sco212*, *Sco215*, *Sco216*, *Sco218*, *Sco220* (DeHaan and Ardren 2005), and *Smm22* (Crane et al. 2004). We conducted polymerase chain reactions (PCR) in 10 μ L volumes containing 2 μ L of template DNA, 5 μ L of 2X Qiagen multiplex PCR master mix (final concentration of 3mM MgCl₂), and 0.2 μ L of oligonucleotide PCR primer mix. Primer mix concentrations and annealing temperatures for each multiplex are given in Appendix 1. PCR conditions were as follows: 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. Following PCR, capillary electrophoresis was conducted on an ABI 3130xl Genetic Analyzer (Applied Biosystems Inc., Foster City, CA) following the manufacturer's protocols. The G5 filter set was used to produce electropherograms, and electrophoresis data was analyzed using the program GeneMapper v4.0 (Applied Biosystems Inc.).

Statistical analysis

Collections of juvenile salmonids often contain large numbers of closely related individuals (Hansen et al. 1997; Banks et al. 2000) which can lead to a biased estimate of population allele frequencies (Allendorf and Phelps 1981). Because our collections from Icicle and Peshastin creek tributaries consisted of juvenile bull trout, it was important that we identify any large groups of closely related individuals in our collections. We used the methods of Wang (2004) implemented in the program COLONY v2.0.0.1 to identify the most likely full sibling family groups from collections in each creek. For each of the four tributaries, we conducted one long and five medium runs to identify putative full sibling groups. When we consistently identified groups of full siblings across all runs, we omitted all but three individuals from each full sibling family from our analysis. Although some authors have advocated eliminating all but a single member of each family from data analysis (Banks et al. 2000), we chose to keep up to three individuals from each full sibling family in order to maintain adequate sample sizes for statistical analysis.

Juvenile bull trout were grouped according to their collection location (French, Jack, Ingalls, and Etienne creeks) for analysis. Each of the four tributary collections was tested for conformance to Hardy-Weinberg equilibrium (HWE) expectations using exact tests implemented in the program GENEPOP v4.1 (Raymond and Rousset 1995). GENEPOP was also used to test each collection for evidence of linkage disequilibrium (LD; i.e., non-random association among alleles). Alpha values for HWE and LD tests were adjusted for multiple comparisons using a sequential Bonferroni adjustment (Rice 1989). Because we were interested in determining whether collections from the different tributaries represented a single or multiple spawning populations, we also combined samples from Jack and French creeks into a single Icicle population and Ingalls and Etienne creeks into a single Peshastin population to determine if these combined collections conformed to HWE expectations (evidence that suggests both collections were from the same spawning population) and/or showed evidence of LD (evidence that suggests both collections represent independent spawning populations).

To get a preliminary idea of the relationship between the collections from Jack Creek and French Creek and the collections from Ingalls Creek and Etienne Creek, we conducted two principle components analyses (PCA) of allele frequencies; one for the two Icicle tributaries and one for the two Peshastin tributaries. PCA was conducted using the *adegenet* package (Jombart 2008) for the R statistical environment (R Development Core Team 2013). Additionally, we used the *adegenet* package to conduct a discriminant analysis of principle components (DAPC) that included the two Icicle tributaries, the two Peshastin tributaries, and four other populations previously genotyped from the Wenatchee River Sub-basin (Chiwaukum Creek, Nason Creek, Chiwawa River, and White River; see DeHaan and Neibauer 2012 for genotyping methods and results). DAPC is similar to PCA but unlike PCA, which maximizes the total variation in the dataset, DAPC maximizes the variation among different groups or clusters and minimizes variation within groups (Jombart et al. 2010). PCA and DAPC provided a preliminary means to examine how collections from the different tributaries clustered with one another.

We also used the program STRUCTURE v2.3.4 (Pritchard et al. 2000) to examine the number of genetic clusters or populations in Icicle Creek and Peshastin Creek. STRUCTURE uses a Bayesian clustering method to examine the number of populations (K) in a dataset. For each putative value of K , STRUCTURE groups individuals into clusters or populations that maximizes HWE and minimizes LD within clusters. We performed two separate STRUCTURE

analyses; one for Icicle Creek and one for Peshastin Creek. For each analysis, we performed 20 replicate runs for values of K from 1 to 5 and each replicate included 100,000 burn-in iterations followed by 200,000 data collection iterations. We used the methods of Evanno et al. (2005) to determine the most likely value of K and we also examined the graphical output from STRUCTURE to infer how individuals from the different collection areas clustered together.

We used GENEPOP to conduct contingency tests of allele frequency heterogeneity to determine if there were significant differences in allele frequencies between Jack Creek and French Creek and between Ingalls Creek and Etienne Creek and to estimate the amount of genetic variation (i.e. pairwise F_{ST}) between Jack and French creeks and Ingalls and Etienne creeks. Previous simulation analyses have demonstrated that tests of allele frequency heterogeneity and estimates of genetic variation among collections are often the most accurate methods to help determine population boundaries, particularly when the level of genetic variation among populations is relatively low (Waples and Gaggiotti 2006). Alpha values for contingency tests of allele frequency heterogeneity were adjusted for multiple comparisons following the method outlined in Rice (1989).

Population allele frequencies may change over time, particularly in small salmonid populations (Ostergaard et al. 2003; Palstra and Ruzzante 2010) and we were interested to know if there were significant differences in allele frequencies between the 2005-2006 ($n = 21$) and 2012 ($n = 31$) collections from French Creek. We used GENEPOP to conduct contingency tests of allele frequency heterogeneity to determine if there were significant differences in allele frequencies between the two temporal replicates from French Creek. We also used GENEPOP to determine the level of genetic variation (i.e. pairwise F_{ST}) between the two collections from French Creek. Sample sizes from Jack Creek, Etienne Creek, and Ingalls Creek were not sufficient to test for temporal changes in allele frequencies in these other tributaries.

We conducted leave-one-out assignment tests to examine our ability to accurately assign bull trout to their most likely population of origin within the upper Columbia River Basin. For this analysis, each fish is removed from the baseline dataset and treated as an unknown origin individual. The population allele frequencies are then re-calculated without that individual, and the individual is assigned to its most likely population of origin within the baseline dataset. The number of individuals from the baseline dataset assigned to the population they were collected from provides a measure of the accuracy of the baseline dataset for population assignments. Our

baseline dataset contained 19 local spawning populations which were defined based on the most recent bull trout recovery information for the upper Columbia River (USFWS 2002; USFWS 2010); the same criteria used in a previous genetic analysis (DeHaan and Neibauer 2012). One exception was that Ingalls Creek and Etienne Creek were split into two separate populations based on the results of the analyses described above. Bull trout from French Creek and Jack Creek were grouped as a single Icicle Creek population for genetic assignment purposes. Often times, grouping populations into ‘reporting groups’ based on genetic similarity or for management purposes can increase genetic assignment accuracy (Anderson et al. 2008). We grouped local populations from the three different sub-basins into three reporting groups (Wenatchee, Entiat, and Methow). Leave-one-out assignment tests were conducted using the program ONCOR (Kalinowski et al. 2008) and baseline individuals were assigned to their most likely population and their most likely reporting group.

Following leave-one-out tests, we used ONCOR to assign sub-adult and adult bull trout from downstream areas in Icicle Creek and the Peshastin Creek screw trap to their most likely local population and reporting group of origin. ONCOR calculated the probability that the unknown origin fish originated from their most likely population and reporting group. If an individual was assigned to its most likely population of origin with a probability less than 0.99, ONCOR also assigned the individual to its second most likely population.

Results

Fine-scale population structure analysis

Numbers of juvenile bull trout collected from each of the four tributaries were as follows: French Creek $n = 52$; Jack Creek $n = 18$; Ingalls Creek $n = 30$; Etienne Creek $n = 37$. Relatedness analysis using the program COLONY identified one full sibling family from French Creek that consisted of eight individuals. We did not observe large numbers of related individuals in the collections from the other three tributaries. When each of the four tributaries was analyzed independently, only Jack Creek deviated from HWE expectations at the locus *Sco106* due to an excess of heterozygotes. All other collections conformed to HWE expectations at all loci. Each tributary had the following number of locus pairs that showed evidence of linkage: French Creek - 12 pairs of loci out of 105 total; Jack Creek – one locus pair out of 91 total; Etienne Creek – 13 locus pairs out of 91 total; Ingalls Creek – four locus pairs out of 91 total. When we combined

collections from French and Jack creeks into a single Icicle Creek population and collections from Ingalls and Etienne creeks into a single Peshastin Creek population, the combined Icicle Creek population had no deviations from HWE expectations and 12 of 105 locus pairs that showed evidence of linkage. The combined Peshastin population had five loci that deviated from HWE expectations (two loci due to heterozygote excess, three loci due to heterozygote deficiency) and 43 locus pairs out of 91 total that showed evidence of linkage.

PCA for Icicle Creek showed that the collections from Jack Creek and French Creek clustered separately with some degree of overlap between the two collections (Figure 5a). PCA for Peshastin Creek showed that the collections from Ingalls Creek and Etienne Creek clustered separately and there was no overlap among individuals from the two collections (Figure 5b). The DAPC analysis for the entire Wenatchee River Sub-basin showed three relatively distinct clusters: one consisting of the two Icicle collections, one consisting of the two Peshastin Creek collections, and one consisting of the remaining populations (Chiwaukum, Nason, Chiwawa, and White; Figure 6). The two Icicle collections overlapped considerably on the DAPC plot and there was greater separation (but still some overlap) between the two Peshastin collections on the DAPC plot (Figure 6). One individual collected in Nason Creek clustered with the individuals from Ingalls Creek.

STRUCTURE analysis identified $K = 2$ as the most likely number of genetic clusters or populations for both Icicle Creek and Peshastin Creek. Output from the Icicle STRUCTURE analysis showed that the individuals from Jack Creek nearly all corresponded to one genetic cluster/population (i.e., nearly all individuals shaded orange in Figure 7) and individuals from French Creek corresponded to both clusters/populations (individuals shaded orange and green in Figure 7). Output from the Peshastin Creek STRUCTURE analysis showed that nearly all individuals from Etienne Creek corresponded to one genetic cluster (most Etienne individuals shaded orange in Figure 7) and individuals from Ingalls Creek all corresponded to the other cluster (all Ingalls individuals shaded green in Figure 7). There were a small number of individuals collected from Etienne Creek that looked more genetically similar to fish from Ingalls Creek (Figure 7).

Contingency tests of allele frequency heterogeneity showed that there was a significant difference in allele frequencies for all 15 loci compared between Jack and French creeks. Contingency tests showed that there was a significant difference in allele frequencies for 13 of

15 loci between Etienne and Ingalls creeks. Pairwise estimates of F_{ST} were 0.072 between French and Jack creeks and 0.149 between Ingalls and Etienne creeks. When we compared temporal replicate samples from French Creek, contingency tests of allele frequency heterogeneity showed that three loci out of 15 (*Sco212*, *Sco106*, and *Sco107*) showed a significant difference in allele frequencies between the 2005-2006 collections and the 2012 collection. The pairwise F_{ST} estimate between temporal replicate samples from French Creek was 0.035 and was significantly different from 0.0 (P value < 0.001).

Genetic population assignments

Leave-one-out tests for the updated genetic baseline (which included Ingalls and Peshastin creeks as separate baseline populations) showed relatively high assignment success. Eighteen of the 19 baseline populations had greater than 90% assignment success (i.e. individuals assigned to their collection location), and 10 populations had 100% assignment success (Figure 8). Only 64.3% of bull trout collected in Beaver Creek in the Methow sub-basin were assigned to Beaver Creek in leave-one-out tests, and 28.6% of fish collected in Beaver Creek were assigned to the geographically proximate Twisp River population. Nearly all fish were assigned to the correct reporting group in leave-one-out tests (Figure 8). A single fish each from Chiwawa River, Mad River, Entiat River, and Gold Creek ($n = 4$ total) was assigned to a reporting group that they were not collected from.

A total of 113 individuals were collected from zones A, B, D, and E of Icicle Creek from 2005 to 2013 for genetic analysis. Six individuals amplified at fewer than 12 loci, and we did not assign these fish to their most likely population of origin (Appendix 2). One fish collected in zone D in 2007 (ID# 355-002) was identified as a brook trout and one fish collected in zone D in 2005 was identified as a bull trout x brook trout hybrid (ID# 352-082). Fish collected in zone A (below LNFH) were mostly assigned to Wenatchee River tributaries with the majority of those fish (29 of 48 individuals) assigned to Chiwaukum Creek; however four fish from zone A were assigned to the Entiat River (Table 1). All fish from zone B were assigned to Wenatchee River tributaries (Table 1). Twenty of 36 bull trout collected in zone D were assigned to Icicle Creek, 14 bull trout collected from zone D were assigned to Wenatchee tributaries other than Icicle Creek, one fish from zone D was assigned to the Entiat River, and one fish from zone D was assigned to Gold Creek in the Methow River Sub-basin (Table 1). All nine bull trout collected

from zone E (above the Boulder Falls in Icicle Creek) were assigned to Icicle Creek (Table 1). Assignment probabilities for all individuals collected in Icicle Creek were greater than 0.9 and all but four fish had assignment probabilities of 1.0. Genetic assignments and the associated probabilities for each individual collected from Icicle Creek can be found in Appendix 2.

A total of 73 bull trout were collected from the Peshastin Creek screw trap in 2005 for genetic assignments. One individual (ID# 352-045) amplified at fewer than 12 loci and was omitted from genetic analysis. The majority of individuals collected in the Peshastin Creek screw trap (69 of 72 fish) were assigned to Ingalls Creek, two fish were assigned to Etienne Creek, and one fish was assigned to the Mad River, a tributary to the Entiat River (Table 2; Appendix 3). All assignment probabilities were greater than 0.95 and all but two individuals had assignment probabilities of 1.0. Genetic assignments and the associated probabilities for each individual collected in Peshastin Creek can be found in Appendix 3.

Discussion

Previous studies have documented a high degree of genetic variation among bull trout populations, including among populations within the same sub-basin and watershed (Costello et al. 2003; Whiteley et al. 2006; DeHaan et al. 2011b; Nyce et al. 2013). Our objective in this study was to examine the fine-scale population structure among bull trout in two watersheds of the Wenatchee River: Icicle Creek and Peshastin Creek. Juvenile bull trout were collected from two tributaries within each of these watersheds and the two watersheds showed slightly different patterns of genetic population structure. We incorporated this information into a genetic baseline dataset for bull trout in the upper Columbia River Basin (DeHaan and Neibauer 2012) and then used this information to examine patterns of movement and the effects of putative barriers on bull trout in each of these two watersheds.

Fine-scale population structure analysis

The USFWS currently designates seven local populations of bull trout in the Wenatchee River Sub-basin (USFWS 2002; USFWS 2010). Some of these local populations represent a single spawning tributary, and some local populations such as the Chiwawa River, Icicle Creek and Peshastin Creek are comprised of multiple bull trout spawning tributaries. Although juvenile bull trout are routinely captured and bull trout spawning redds have previously been observed in

French Creek (Brown 1992; Nelson 2007; Nelson et al. 2009, 2011, 2012; Nelson and Sulak 2013), data on juvenile bull trout density and adult spawning activity in Jack Creek are limited (e.g. Kelly Ringel 1997; USFWS 2005). Juvenile bull trout were collected from French Creek and Jack Creek to determine if these tributaries were part of a single spawning population or if each tributary represented a genetically unique spawning population. When individuals from genetically distinct populations are combined into a single population for analysis, we would expect that population to deviate from HWE expectations due to a deficiency of heterozygotes (i.e., Wahlund effect) and we would also expect a number of locus pairs to show evidence of linkage. The combined Icicle Creek population did not deviate from HWE expectations at any loci, and although we did observe some evidence of linkage, it was no greater than we observed when the two tributaries were analyzed separately. Although these results do not definitively prove that these tributaries represent a single population, the data are consistent with that pattern.

We used several methods including genetic clustering analyses, contingency tests of allele frequencies, and estimates of genetic variation, to determine the number of populations in Icicle Creek. Results of these analyses did not suggest a clear pattern of population structure. The initial PCA plot (Figure 5a) showed that French and Jack creeks clustered independently with some overlap between individuals from both tributaries, but there was greater overlap between the two Icicle tributaries in the DAPC analysis representing all of the Wenatchee sub-basin (Figure 6). Although STRUCTURE identified $K=2$ as the most likely solution for our dataset, the two clusters did not correspond exclusively to the two different tributaries similar to our results from Peshastin Creek. Simulation studies have found that contingency tests of allele frequencies and estimates of genetic variation may be more robust methods for determining the number of populations (Waples and Gaggiotti 2006). Although the pairwise F_{ST} estimate between the French and Jack creek collections ($F_{ST} = 0.072$) was lower than the estimates observed among several other upper Columbia bull trout populations (DeHaan and Neibauer 2012), we still observed a significant difference in allele frequencies between the two tributaries at all 15 loci. Overall, some of our analyses suggest that Jack and French creek are genetically distinct from one another, but clustering analyses including PCA, DAPC, and STRUCTURE also suggest some degree of gene flow among the two tributaries. Given that the data did not clearly demonstrate that French Creek and Jack Creek are genetically independent spawning populations and the fact that the collection from Jack Creek was relatively small ($n = 18$ individuals), we

cannot definitively conclude that these two tributaries represent separate populations. Future analyses to examine the number of distinct spawning populations in Icicle Creek would benefit from the collection of additional samples from both tributaries, collection of samples from areas upstream of French Creek, comparisons among collection years (similar to the temporal analysis from French Creek), as well as additional types of information such as radio telemetry, PIT tagging, and redd surveys.

Allele frequencies may change significantly in relatively short periods of time in small populations due to increased genetic drift and population extinction and recolonization events (Ostergaard et al. 2003; Walter et al. 2009; DeHaan et al. 2011a). We compared two collections from French Creek made approximately one generation apart to see if allele frequencies in this relatively small population were consistent over time. Results of allele frequency heterogeneity tests did not suggest a significant change in allele frequencies over the duration of this study and the level of genetic variation among replicate samples from French Creek was relatively low compared to previous comparisons among upper Columbia River bull trout populations (DeHaan and Neibauer 2012). Although the local population in French Creek is relatively small compared to some other upper Columbia River bull trout populations, genetic variation appears stable in this population over the generation separating the two collections that we analyzed.

Results from Peshastin Creek provided a more definitive picture of bull trout population structure in this watershed and suggested that Ingalls Creek and Etienne Creek represent two genetically distinct populations. When Ingalls Creek and Etienne Creek were analyzed independently, we did not observe any deviations from HWE expectations and very few locus pairs showed evidence of linkage. Alternatively, when collections from these two tributaries were combined into a single population for analysis, we observed several loci out of HWE and nearly half of the locus pairs showed evidence of linkage. The fact that Etienne and Ingalls creeks clustered independently on the PCA, showed roughly the same level of differentiation as other local spawning populations for the DAPC, and were distinct from one another in the STRUCTURE analysis also suggests that these two tributaries represent genetically independent populations. Finally, there were significant differences in allele frequencies at nearly all loci and the pairwise F_{ST} estimate between Ingalls and Etienne was twice as great as the estimate between French and Jack and was consistent with previous observations among other upper Columbia River bull trout populations (DeHaan and Neibauer 2012). Collectively these data suggest that

Ingalls Creek and Etienne Creek each contain a genetically unique bull trout population. Furthermore, we observed a much greater number of migratory sub-adults from Ingalls Creek (see below) and differences in life history characteristics between the two populations may also contribute to the differences we observed between these two tributaries. Although genetic data indicate that these two tributaries contain separate populations, several other types of information are typically used to define population boundaries (Rieman and McIntyre 1995; Glover et al. 2008; Koizumi et al. 2011) and additional information on bull trout in Ingalls and Etienne creeks should be considered if population boundaries in Peshastin Creek are to be refined.

Genetic population assignments

Previous studies showed that genetic stock ID is useful for examining patterns of movement for bull trout (DeHaan et al. 2011a; DeHaan et al. 2011b; Warnock et al. 2011; Nyce et al. 2013). In this study we used genetic stock ID to examine how different structures or putative barriers affected bull trout movement within Icicle Creek. Genetic stock ID of bull trout from lower Icicle Creek below the structures in the system (zone A) suggested that this area represents important habitat for bull trout from a number of different local populations throughout the upper Columbia River. For example, lower Icicle Creek may act as a thermal refuge for migratory bull trout when summer temperatures in other areas of the Upper Columbia Basin are unfavorable for these exploratory individuals (Nelson et al. 2009; USFWS 2012). Previously, it was not clear to what extent the different putative barriers in Icicle Creek affected movement of bull trout. Although sub-adult and adult bull trout have been observed between the different putative barriers, it was unclear if these fish were all from Icicle Creek or if some of these individuals originated from other local populations. Our data demonstrate that putative anthropogenic barriers downstream of the Boulder Falls do not preclude migratory bull trout from moving upstream in Icicle Creek since fish collected in zones B and D were assigned to several local populations from the Wenatchee, Entiat, and Methow sub-basins. Furthermore, our data support previous evidence from radio telemetry and genetic studies showing that both adult and subadult bull trout in the upper Columbia River Basin are highly migratory and utilize the mainstem Columbia River as an important migratory corridor between the three major river sub-basins (BioAnalysts 2004; Nelson and Nelle 2008; DeHaan and Neibauer 2012; Nelson et al. 2012; Nelson and Johnsen 2012).

Our data do not provide any evidence that bull trout move upstream of the Boulder Falls in Icicle Creek. Although we identified fish from several local populations in zones A, B, and D, all of the fish collected in zone E (above the Boulder Falls) were assigned to Icicle Creek. Furthermore, all of the Icicle Creek fish in the baseline dataset (collected in tributaries above the Boulder Falls) assigned to Icicle in the leave-one-out tests suggesting that gene flow between Icicle Creek and other Wenatchee River sub-basin local populations has been limited in recent years. However, these data should not be interpreted as evidence that fish cannot ascend the falls. The sample size from zone E was relatively small ($n = 9$ individuals) and leave-one-out tests based on analysis of juvenile bull trout reflect patterns of gene flow among populations and not necessarily patterns of individual movement. Bull trout are presumed to exhibit a high degree of local adaptation based on inferences from genetic analysis (Rieman and Dunham 2000; Whiteley et al. 2004) and it may be that Icicle Creek fish spawning above the Boulder Falls have adapted their migration timing to coincide with certain low flow conditions that permit upstream migration. Bull trout in the upper Mid-Columbia core areas show a high degree of fidelity to their natal area (Nelson and Nelle 2008; Nelson and Johnsen 2012; DeHaan and Neibauer 2012) so we would expect more individuals from the Icicle Creek population to attempt to pass the Boulder Falls than individuals from other populations. To more fully investigate upstream movement of bull trout in Icicle Creek, PIT tag studies in conjunction with genetic information will be necessary to help address these questions. It is encouraging that subadults from other populations were detected in the lower river upstream of the hatchery and just below the falls, indicating that connectivity and gene flow between local populations (and core areas) is conceivably possible in Icicle Creek.

Genetic stock ID also provided important insight into the migratory and life history pattern of Peshastin Creek bull trout. Maintaining a migratory life history is considered essential for the persistence of many bull trout populations because migratory fish can help recolonize extirpated populations, they provide gene flow among small populations, and they provide a genetic reserve in the case of stochastic events (e.g., floods, fires, landslides) that may negatively impact resident populations (Northcote 1997; Rieman and Dunham 2000). Nearly all of the fish collected in the Peshastin screw trap were assigned to Ingalls Creek, suggesting that this population produces the majority of the migratory fish from the Peshastin watershed. At the same time, the advantages of maintaining both resident and migratory populations of bull trout

has been recognized (Northcote 1997; Rieman and Dunham 2000). The low number of fish from the Peshastin screw trap assigned to Etienne Creek suggests that this population has a primarily resident life history. It will be important to consider the diversity of life history forms and which populations exhibit these different life history forms when planning conservation actions (e.g., removal of barriers, habitat restoration) in the Peshastin Creek watershed. Interestingly, one individual collected in the Peshastin screw trap was assigned to the Mad River, an Entiat River tributary. Radio telemetry and PIT tag data has documented movement of bull trout between the Entiat Core Area and Peshastin Creek (BioAnalysts 2004; Nelson and Nelle 2008; USFWS unpublished data) and previous genetic analysis suggests some level of gene flow among these systems (DeHaan and Neibauer 2012). It is important to consider that collections from the Peshastin screw trap were only made in 2005 and may not reflect long term or current patterns of movement and life history in the Peshastin Creek watershed. Additional surveys, genetic sampling, and PIT tagging in Ingalls and Etienne creeks are needed to define the range of bull trout life history patterns in the watershed.

Conclusions

Conservation actions are often implemented and evaluated at the population level; therefore it's important that populations are clearly defined for effective conservation planning. Based on our data we could not definitively conclude that bull trout in French and Jack creeks in the Icicle Creek watershed represent multiple populations whereas our data more clearly suggest that bull trout in the two Peshastin Creek tributaries, Ingalls Creek and Etienne Creek, each represent a separate population. The USFWS Draft Bull Trout Recovery Plan (USFWS 2002) highlights the need to conserve the unique genetic diversity and life history types found in different bull trout populations. Based on our data, it may not simply be enough to focus conservation efforts on bull trout in 'Peshastin Creek' but rather to focus specifically on the unique genetic diversity and multiple life history types found in different tributaries within Peshastin Creek. Data in this study also provide important information regarding the effects of different structures that have been considered barriers in Icicle Creek. Based on our results, habitat in lower Icicle Creek appears to be important for multiple local populations from the upper Columbia River Basin and conservation planning efforts should take this into account.

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Fine-Scale Structure of Wenatchee River Bull Trout

Table 1. Summary of genetic population assignments for 113 bull trout collected downstream of spawning areas in the Icicle River system. The first column represents the collection zones within Icicle Creek and subsequent columns represent the number of individuals from each zone assigned to the different baseline populations. “No assignment” refers to individuals that amplified at fewer than 12 loci and were excluded from population assignment analysis. Genetic assignments and the associated probabilities for each individual can be found in Appendix 2.

Collection Zone	Chiwaukum Creek	Chiwawa River	Etienne Creek	Icicle Creek	Ingalls Creek	Nason Creek	Entiat River	Gold Creek	Brook Trout	Hybrid	No Assignment
A	29	6	1	3	1	4	4				
B	4	3	1	3		1					
D	8	5		20	1		1	1	1	1	1
E				9							5
Total	41	14	2	35	2	5	5	1	1	1	6

Table 2. Summary of genetic population assignments for 73 bull trout collected in the Peshastin Creek screw trap in 2005. “No assignment” refers to an individual that amplified at fewer than 12 loci and was excluded from population assignment analysis. Genetic assignments and the associated probabilities for each individual can be found in Appendix 3.

Most likely population of origin	Number individuals
Ingalls Creek	69
Etienne Creek	2
Mad River	1
No Assignment	1

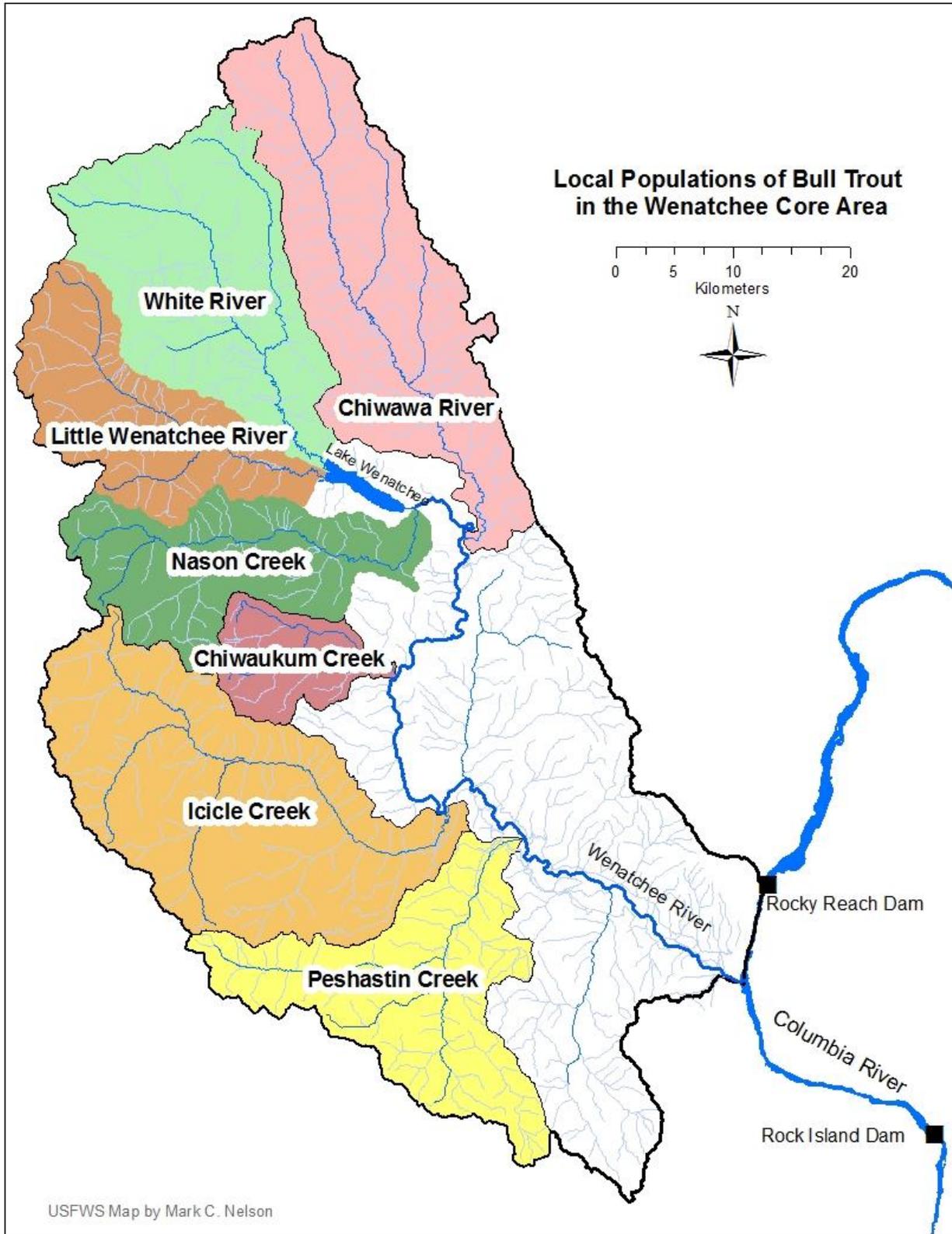


Figure 1. Map of the seven local populations of bull trout in the Wenatchee River Sub-basin.

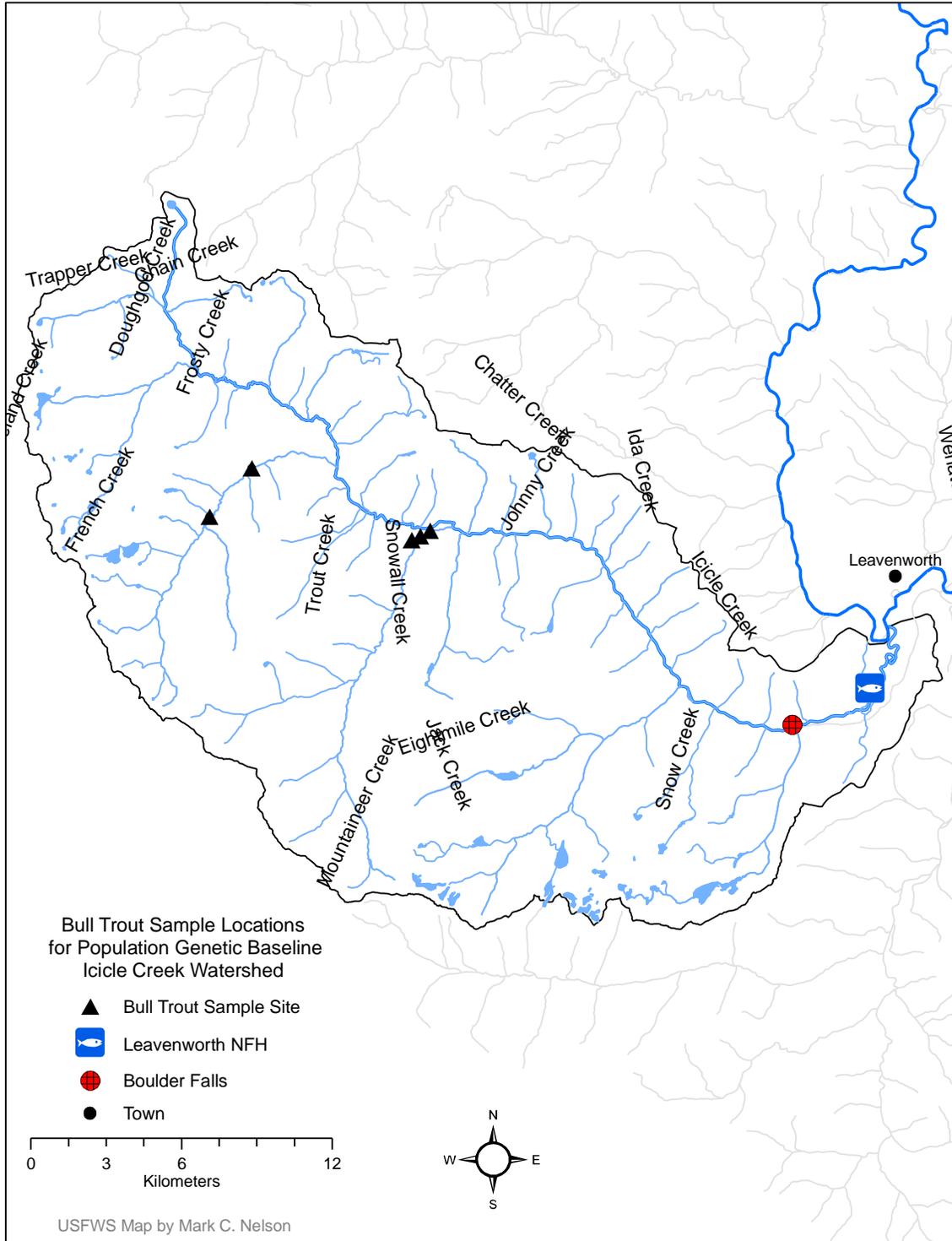


Figure 2. Map of the Icicle Creek watershed showing locations of bull trout sampling sites in French Creek and Jack Creek, the Boulder Falls, and Leavenworth National Fish Hatchery (NFH).

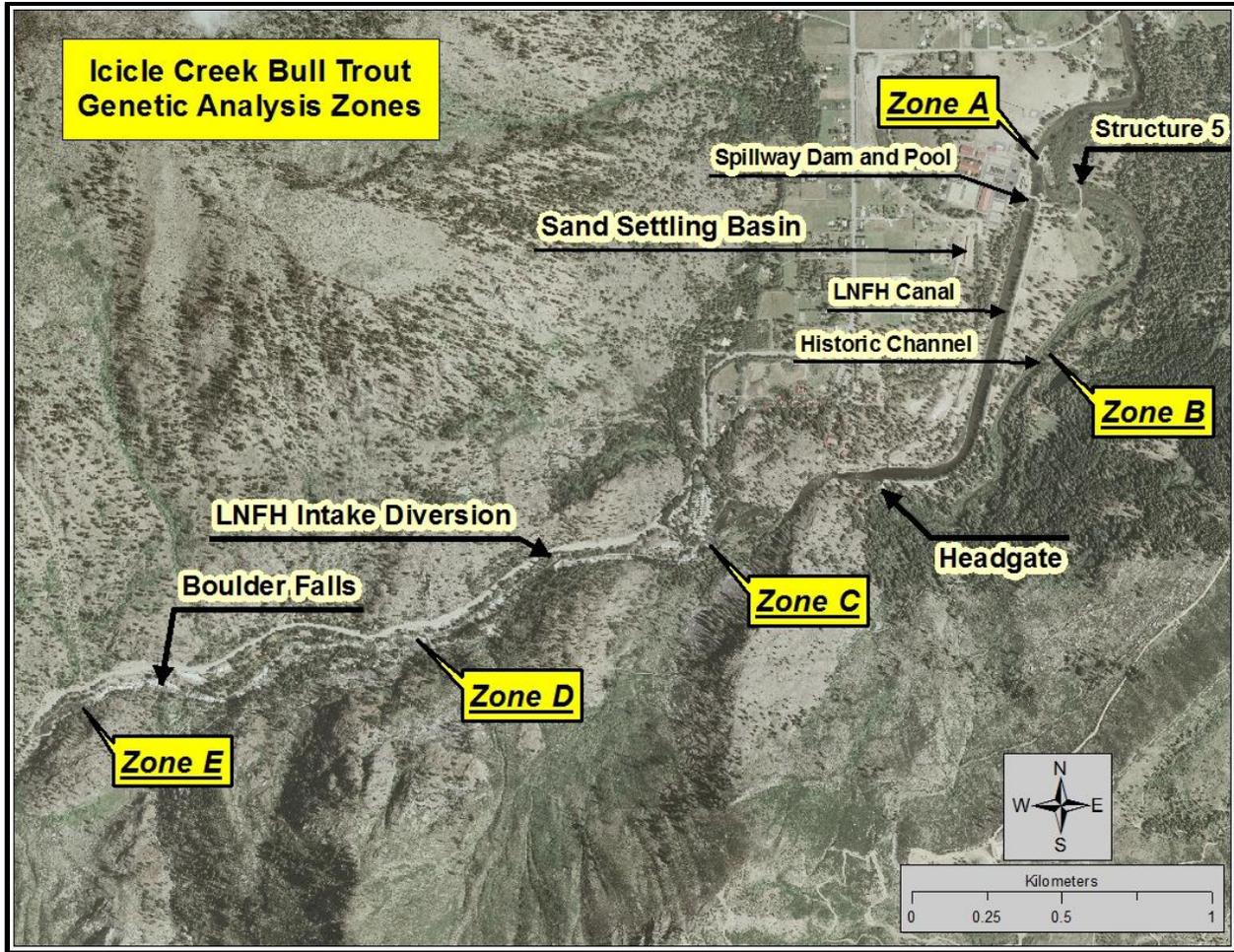


Figure 3. Locations of different in-stream structures in lower Icicle Creek and the different zones where bull trout were collected for population assignments.

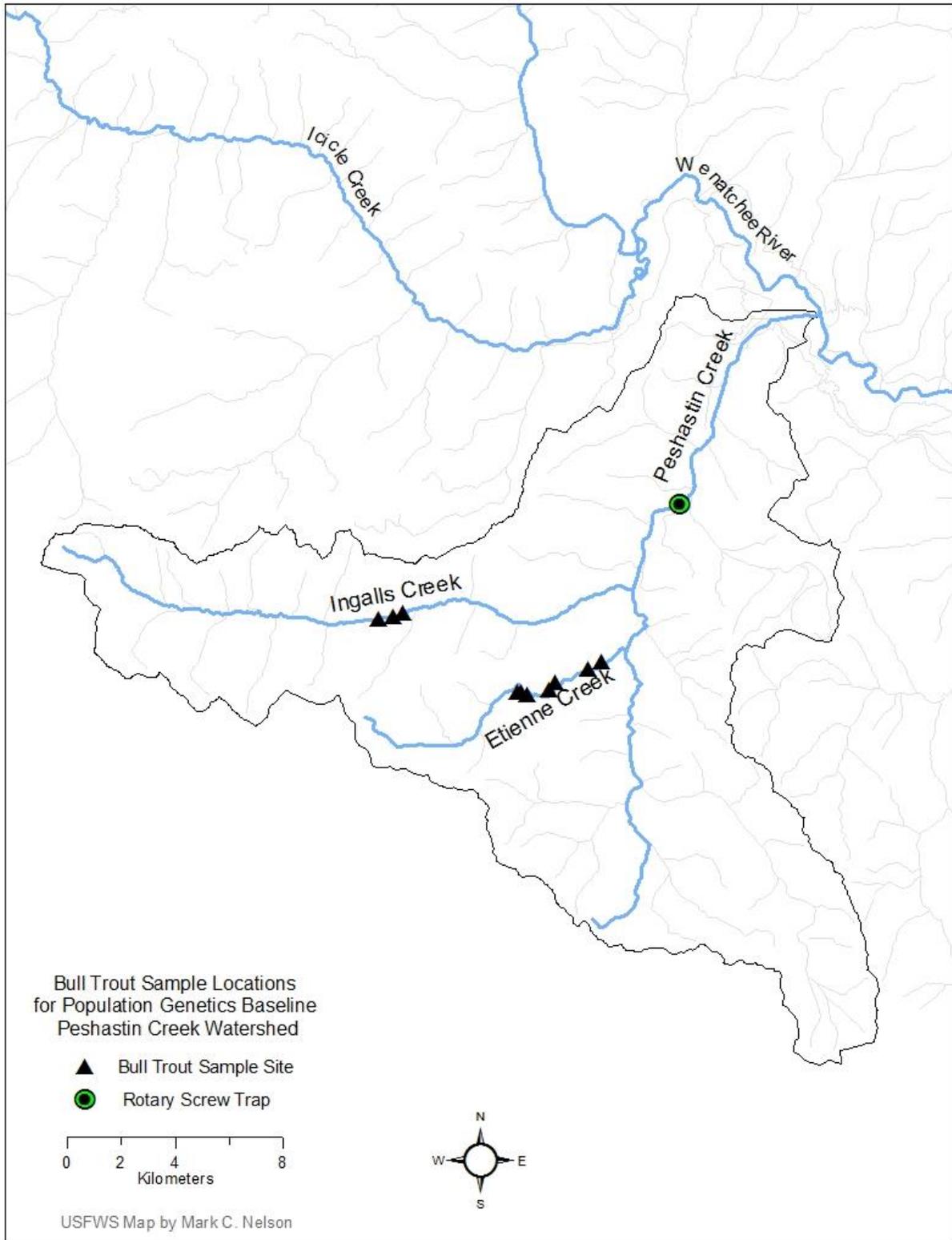


Figure 4. Map of the Peshastin Creek watershed showing locations of bull trout sampling sites and the rotary screw trap.

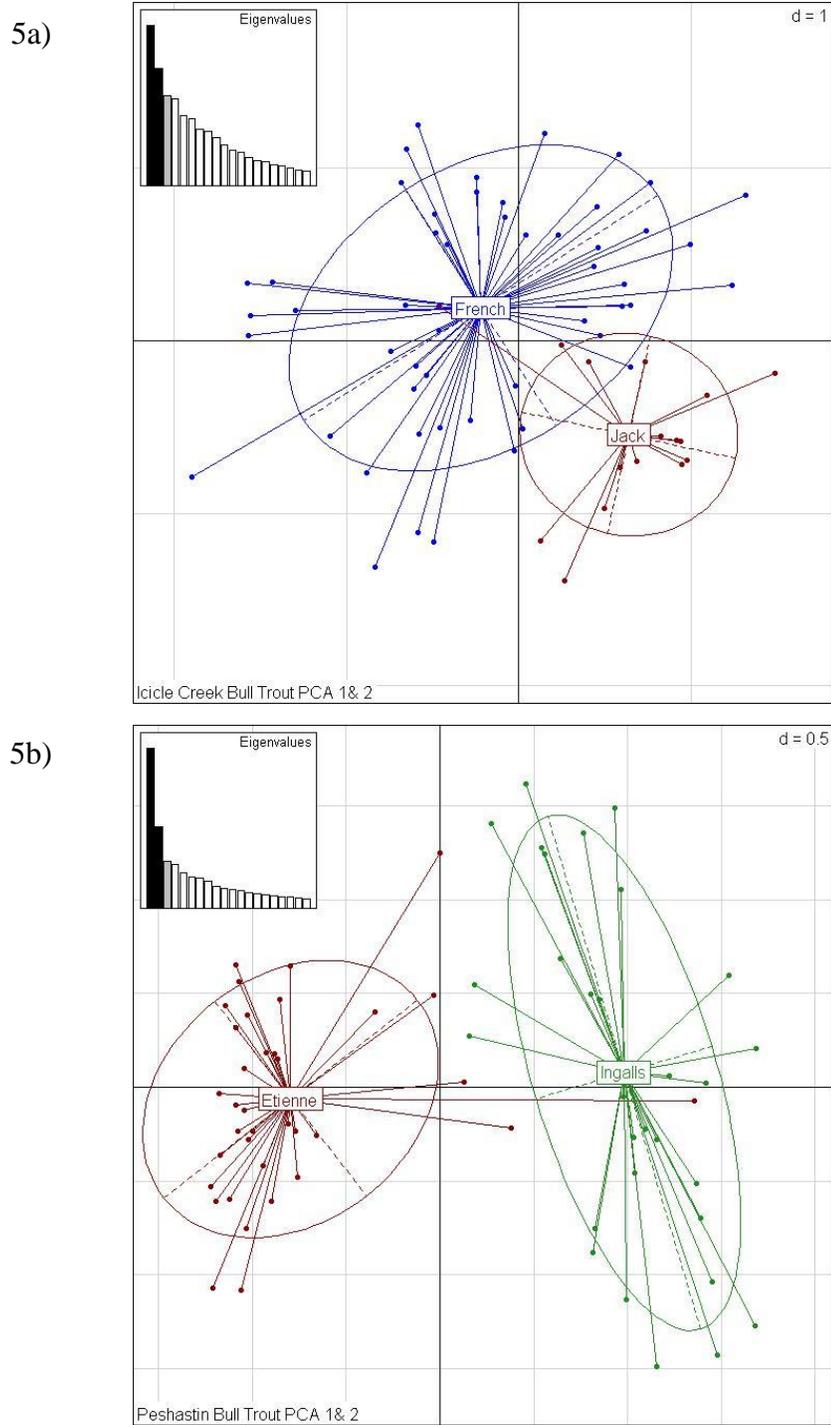


Figure 5. Plots of the first two variance components of PCA of allele frequencies for the Icicle Creek watershed (Figure 4a) and the Peshastin Creek watershed (Figure 4b). Each point on the graph represents an individual fish and colors correspond to the different tributaries from each watershed. The inset graph represents the amount of variation attributed to each principle component.

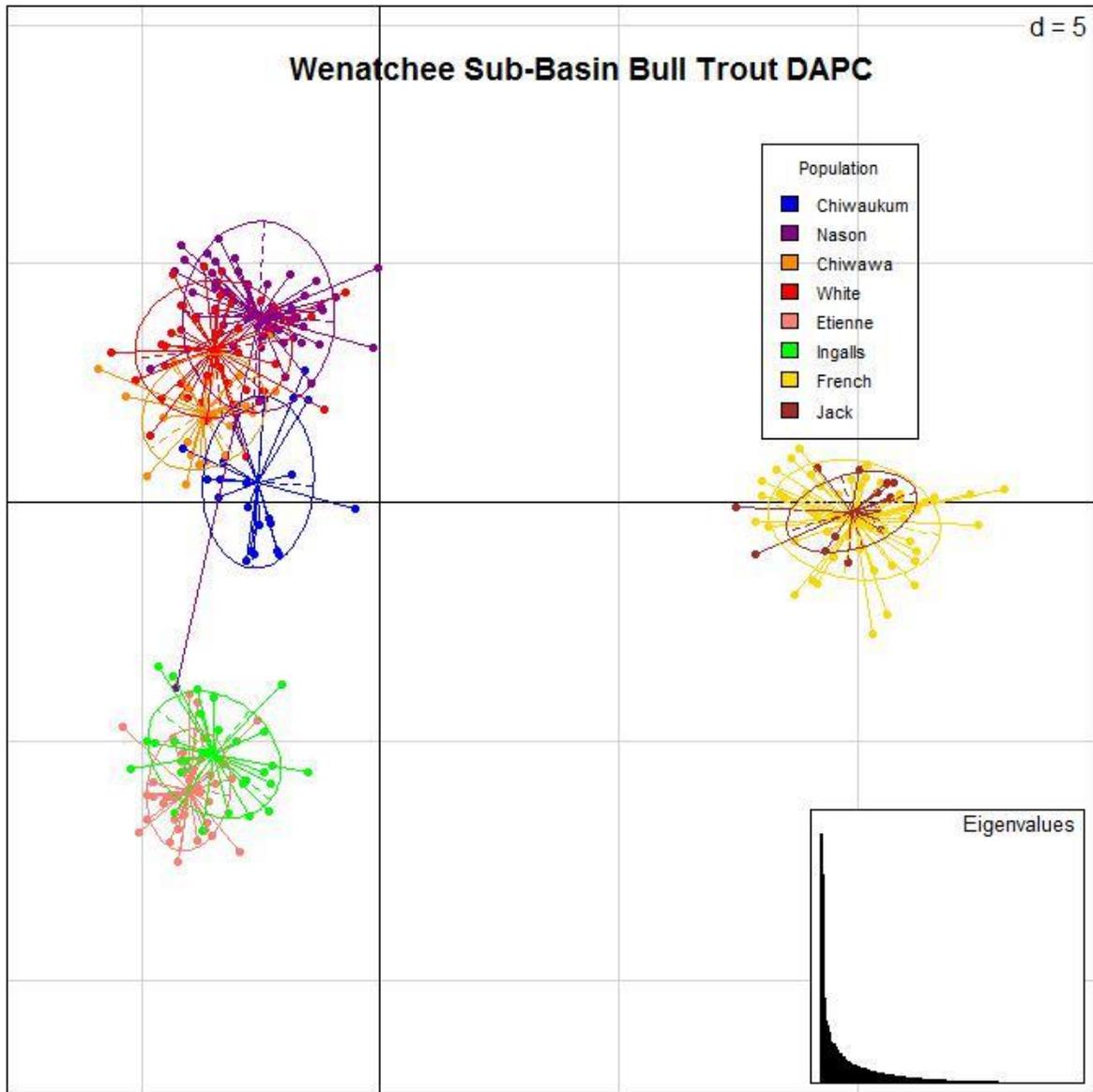


Figure 6. Plot of the first two variance components of DAPC for Wenatchee River Sub-basin bull trout collections. Each point on the graph represents an individual fish and colors correspond to the different tributaries/watersheds. The inset graph represents the amount of variation attributed to each principle component.

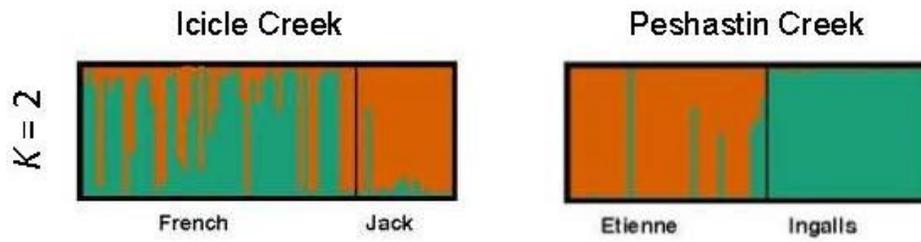


Figure 7. Results of the program STRUCTURE for Icicle Creek and Peshastin Creek assuming $K = 2$ populations. Each vertical bar on the graphs represents an individual in the analysis and the different colors represent the proportion of each individual's genotype associated with each genetic cluster or population.

Fine-Scale Structure of Wenatchee River Bull Trout

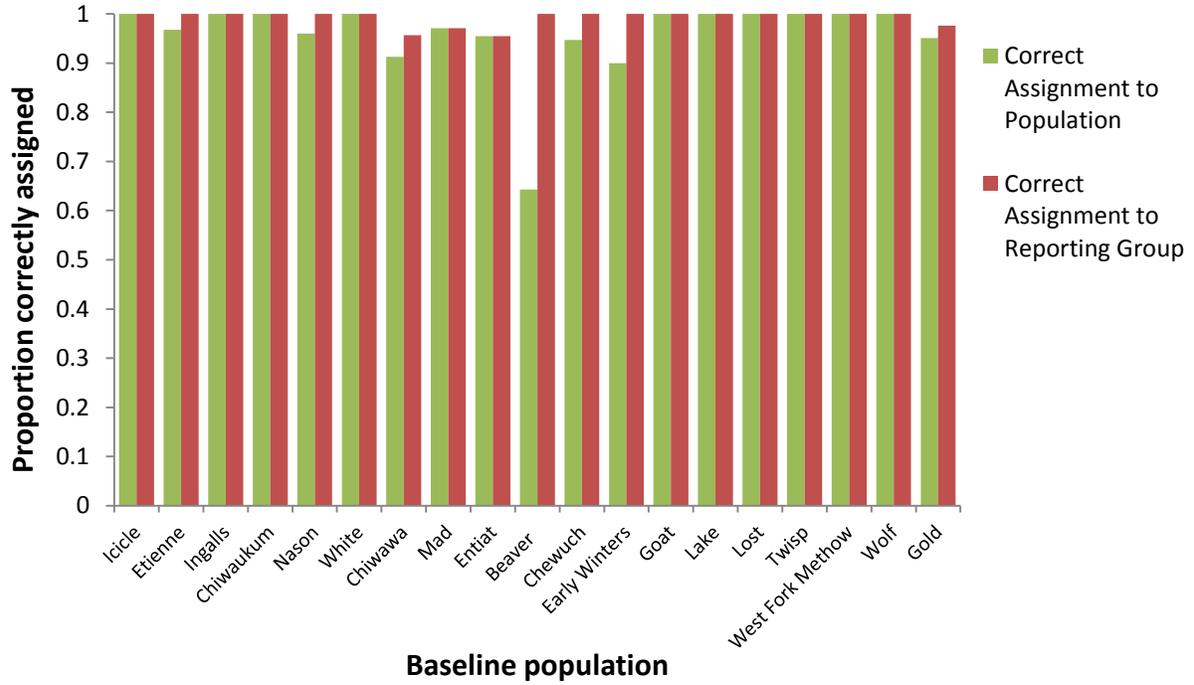


Figure 8. Proportion of individuals from each baseline population assigned to the correct population (red bars) and reporting group (green bars) in leave-one-out assignment tests of the upper Columbia River bull trout baseline.

APPENDICES

Appendix 1. Bull trout PCR multiplex primer concentrations and annealing temperatures.

Multiplex Set 1 **T_A= 54°C**

Locus Name	Dye	Final Concentration
Sfo18	6FAM	0.3µM
Sco212	VIC	1.0µM
Sco220	NED	3.3µM
Sco216	PET	4.0µM
Sco109	6FAM	6.6µM

Multiplex Set 2 **T_A= 59°C**

Locus Name	Dye	Final Concentration
Sco202	6FAM	0.6µM
Sco102	PET	1.0µM
Sco215	PET	1.3µM
Sco200	VIC	2.0µM
Omm1128	VIC	2.0µM
Sco105	NED	1.3µM
Smm22	6FAM	4.6µM

Multiplex Set 3 **T_A=56°C**

Locus Name	Dye	Final Concentration
Sco106	6FAM	1.0µM
Sco107	VIC	2.6µM
Omm1130	NED	5.3µM
Sco218	PET	3.3µM

T_A= Annealing temperature

Fine-Scale Structure of Wenatchee River Bull Trout

Appendix 2. Genetic population assignments for sub-adult and adult bull trout collected in Icicle Creek from 2005 to 2013.

Individual	Collection Date	Collection Location	Collection Zone	Most Likely Population	Probability	2nd Most Likely Population	Probability	Most Likely Reporting Group	Probability	2nd Most Likely Reporting Group	Probability
352-080	6/17/2005	LNFH adult pond	A	Icicle	1			Wenatchee	1		
352-081	11/10/2005	LNFH intake rack	D	Icicle	1			Wenatchee	1		
352-082	11/10/2005	LNFH intake rack	D	HYBRID							
352-091	7/24/2006	Lower Icicle Creek	A	Chiwaukum	1			Wenatchee	1		
352-092	7/24/2006	Lower Icicle Creek	A	Chiwaukum	1			Wenatchee	1		
355-001	6/8/2007	LNFH screen chamber	D	Icicle	1			Wenatchee	1		
355-002	10/8/2007	LNFH screen chamber	D	BROOK TROUT							
681-014	4/7/2008	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
681-016	7/25/2008	Icicle Gorge	E	Icicle	1			Wenatchee	1		
681-017	7/28/2008	Icicle Gorge	E	Icicle	1			Wenatchee	1		
681-018	7/30/2008	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
139-096	8/7/2008	LNFH headgate pool	B	Icicle	1			Wenatchee	1		
139-097	8/7/2008	LNFH headgate pool	B	Icicle	1			Wenatchee	1		
139-098	8/7/2008	LNFH headgate pool	B	Chiwaukum	1			Wenatchee	1		
139-100	8/7/2008	LNFH headgate pool	B	Nason	1			Wenatchee	1		
355-003	9/12/2008	LNFH sand basin	D	Chiwawa	1			Wenatchee	1		
355-005	9/12/2008	LNFH sand basin	D	Icicle	1			Wenatchee	1		
355-006	7/14/2009	LNFH sand basin	D	Chiwawa	1			Wenatchee	1		
355-053	7/20/2009	LNFH headgate pool	B	Etienne	1			Wenatchee	1		
355-015	7/23/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-016	7/27/2009	Icicle Gorge	E	Icicle	1			Wenatchee	1		
355-017	7/31/2009	Rock Island Camp pool	E	Icicle	1			Wenatchee	1		
355-007	8/4/2009	LNFH sand basin	D	Chiwaukum	1			Wenatchee	1		
355-018	8/5/2009	Icicle Island	D	Chiwaukum	1			Wenatchee	1		
355-019	8/7/2009	Boulder Falls pool	D	Entiat	1			Entiat	1		
355-020	8/7/2009	Icicle Island	D	Ingalls	1			Wenatchee	1		
355-021	8/7/2009	Icicle Island	D	Chiwaukum	1			Wenatchee	1		
355-022	8/10/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-023	8/10/2009	LNFH spillway pool	A	Chiwawa	1			Wenatchee	1		
355-024	8/10/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-025	8/10/2009	LNFH spillway pool	A	Chiwawa	1			Wenatchee	1		
355-026	8/10/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-027	8/10/2009	LNFH spillway pool	A	Nason	1			Wenatchee	1		
355-028	8/10/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-029	8/10/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		

Fine-Scale Structure of Wenatchee River Bull Trout

Individual	Collection Date	Collection Location	Collection Zone	Most Likely Population	Probability	2nd Most Likely Population	Probability	Most Likely Reporting Group	Probability	2nd Most Likely Reporting Group	Probability
355-030	8/10/2009	Downstream of Snow Cr.	D	Chiwaukum	1			Wenatchee	1		
355-031	8/10/2009	LNFH headgate pool	B	Chiwawa	1			Wenatchee	1		
355-033	8/10/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-034	8/10/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-055	8/10/2009	LNFH spillway pool	A	Nason	1			Wenatchee	1		
355-035	8/11/2009	LNFH spillway pool	A	Chiwawa	1			Wenatchee	1		
355-036	8/11/2009	LNFH spillway pool	A	Etienne	1			Wenatchee	1		
355-037	8/11/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-038	8/11/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-056	8/11/2009	LNFH spillway pool	A	Nason	1			Wenatchee	1		
355-057	8/11/2009	LNFH spillway pool	A	Entiat	1			Entiat	1		
355-039	8/12/2009	Foot Bridge pool	D	Gold	1			Methow	1		
355-040	8/13/2009	LNFH headgate pool	B	Icicle	1			Wenatchee	1		
355-041	8/13/2009	Historic channel	B	Chiwaukum	1			Wenatchee	1		
355-042	8/13/2009	LNFH headgate pool	B	Chiwawa	0.93	Chiwaukum	0.071	Wenatchee	1		
355-043	8/13/2009	LNFH headgate pool	B	Chiwawa	1			Wenatchee	1		
355-044	8/13/2009	Foot Bridge pool	D	Icicle	1			Wenatchee	1		
355-045	9/25/2009	LNFH spillway pool	A	Chiwawa	1			Wenatchee	1		
355-046	9/25/2009	LNFH spillway pool	A	Icicle	1			Wenatchee	1		
355-049	9/25/2009	LNFH spillway pool	A	Entiat	0.998			Entiat	1		
355-050	9/25/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-059	9/25/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-047	9/28/2009	LNFH spillway pool	A	Icicle	1			Wenatchee	1		
355-048	9/28/2009	LNFH spillway pool	A	Ingalls	1			Wenatchee	1		
355-051	9/28/2009	LNFH spillway pool	A	Nason	1			Wenatchee	1		
355-058	9/28/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-060	9/28/2009	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
355-008	10/29/2009	LNFH sand basin	D	Chiwaukum	1			Wenatchee	1		
355-009	10/29/2009	LNFH sand basin	D	Icicle	1			Wenatchee	1		
355-010	10/29/2009	LNFH sand basin	D	Chiwaukum	1			Wenatchee	1		
355-011	10/29/2009	LNFH sand basin	D	Chiwawa	1			Wenatchee	1		
355-012	10/29/2009	LNFH sand basin	D	Icicle	1			Wenatchee	1		
355-013	10/29/2009	LNFH sand basin	D	No Amplification							
355-014	10/29/2009	LNFH sand basin	D	Chiwaukum	1			Wenatchee	1		
355-052	11/23/2009	LNFH adult ladder	A	Chiwaukum	1			Wenatchee	1		
878-013	8/23/2010	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
878-014	8/23/2010	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		

Fine-Scale Structure of Wenatchee River Bull Trout

Individual	Collection Date	Collection Location	Collection Zone	Most Likely Population	Probability	2nd Most Likely Population	Probability	Most Likely Reporting Group	Probability	2nd Most Likely Reporting Group	Probability
878-015	8/23/2010	LNFB spillway pool	A	Chiwaukum	1			Wenatchee	1		
878-016	8/23/2010	LNFB spillway pool	A	Chiwawa	0.991			Wenatchee	1		
878-017	8/23/2010	LNFB spillway pool	A	Chiwaukum	1			Wenatchee	1		
878-018	8/23/2010	LNFB spillway pool	A	Chiwaukum	1			Wenatchee	1		
878-019	8/23/2010	LNFB spillway pool	A	Chiwaukum	0.99	Mad	0.011	Wenatchee	0.99	Entiat	0.011
878-020	8/23/2010	LNFB spillway pool	A	Entiat	1			Entiat	1		
878-001	8/24/2010	Icicle Gorge	E	No Amplification							
878-002	8/24/2010	Icicle Gorge	E	Icicle	1			Wenatchee	1		
878-003	8/24/2010	Icicle Gorge	E	No Amplification							
878-004	8/24/2010	Icicle Gorge	E	Icicle	1			Wenatchee	1		
878-005	8/24/2010	Icicle Gorge	E	No Amplification							
878-006	8/24/2010	Icicle Gorge	E	No Amplification							
878-007	8/24/2010	Icicle Gorge	E	Icicle	1			Wenatchee	1		
878-008	8/24/2010	Icicle Gorge	E	Icicle	1			Wenatchee	1		
878-009	8/24/2010	Icicle Gorge	E	Icicle	1			Wenatchee	1		
878-010	8/24/2010	Icicle Gorge	E	No Amplification							
878-011	8/27/2010	LNFB headgate pool	B	Chiwaukum	1			Wenatchee	1		
878-012	8/27/2010	LNFB headgate pool	B	Chiwaukum	1			Wenatchee	1		
879-001	10/20/2010	LNFB sand basin	D	Icicle	1			Wenatchee	1		
879-003	10/20/2010	LNFB sand basin	D	Icicle	1			Wenatchee	1		
879-002	5/12/2011	LNFB sand basin	D	Icicle	1			Wenatchee	1		
879-004	10/20/2011	LNFB sand basin	D	Icicle	1			Wenatchee	1		
879-005	10/20/2011	LNFB sand basin	D	Icicle	1			Wenatchee	1		
879-006	10/20/2011	LNFB sand basin	D	Icicle	1			Wenatchee	1		
878-052	9/25/2012	LNFB sand basin	D	Icicle	1			Wenatchee	1		
878-053	10/2/2012	LNFB sand basin	D	Icicle	1			Wenatchee	1		
878-054	10/2/2012	LNFB sand basin	D	Icicle	1			Wenatchee	1		
878-055	11/8/2012	LNFB sand basin	D	Icicle	1			Wenatchee	1		
878-056	11/13/2012	LNFB sand basin	D	Icicle	1			Wenatchee	1		
878-057	4/29/2013	LNFB screen chamber	D	Icicle	1			Wenatchee	1		
2490-001	7/2/2013	LNFB screen chamber	D	Icicle	1			Wenatchee	1		
2490-002	7/10/2013	LNFB adult pond	A	Chiwaukum	1			Wenatchee	1		
2490-004	8/6/2013	LNFB screen chamber	D	Chiwawa	1			Wenatchee	1		
2490-003	8/9/2013	LNFB screen chamber	D	Chiwawa	1			Wenatchee	1		
2490-005	8/14/2013	LNFB sand basin	D	Icicle	1			Wenatchee	1		
2490-006	8/15/2013	LNFB sand basin	D	Chiwaukum	1			Wenatchee	1		
2491-031	9/5/2013	LNFB spillway pool	A	Entiat	1			Entiat	1		

Fine-Scale Structure of Wenatchee River Bull Trout

Individual	Collection Date	Collection Location	Collection Zone	Most Likely Population	Probability	2nd Most Likely Population	Probability	Most Likely Reporting Group	Probability	2nd Most Likely Reporting Group	Probability
2491-032	9/5/2013	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
2491-033	9/5/2013	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		
2491-034	9/5/2013	LNFH spillway pool	A	Chiwawa	1			Wenatchee	1		
2491-035	9/5/2013	LNFH spillway pool	A	Chiwaukum	1			Wenatchee	1		

Appendix 3. Genetic assignments for sub-adult bull trout collected in the Peshastin Creek screw trap in 2005.

Individual	Most Likely Population	Probability	Second Most Likely Population	Probability	Most Likely Reporting Group	Probability	2nd Most Likely Reporting Group	Probability
352-001	Ingalls	1.000			Wenatchee	1.000		
352-002	Ingalls	1.000			Wenatchee	1.000		
352-003	Ingalls	1.000			Wenatchee	1.000		
352-005	Ingalls	1.000			Wenatchee	1.000		
352-006	Ingalls	1.000			Wenatchee	1.000		
352-008	Ingalls	1.000			Wenatchee	1.000		
352-009	Ingalls	1.000			Wenatchee	1.000		
352-010	Ingalls	1.000			Wenatchee	1.000		
352-011	Ingalls	1.000			Wenatchee	1.000		
352-012	Ingalls	1.000			Wenatchee	1.000		
352-013	Ingalls	1.000			Wenatchee	1.000		
352-014	Ingalls	1.000			Wenatchee	1.000		
352-015	Ingalls	1.000			Wenatchee	1.000		
352-016	Ingalls	1.000			Wenatchee	1.000		
352-017	Ingalls	1.000			Wenatchee	1.000		
352-018	Ingalls	1.000			Wenatchee	1.000		
352-019	Ingalls	1.000			Wenatchee	1.000		
352-020	Ingalls	1.000			Wenatchee	1.000		
352-021	Ingalls	1.000			Wenatchee	1.000		
352-022	Ingalls	1.000			Wenatchee	1.000		
352-023	Ingalls	1.000			Wenatchee	1.000		
352-024	Etienne	0.964	Mad	0.036	Wenatchee	0.964	Entiat	0.036
352-025	Ingalls	1.000			Wenatchee	1.000		
352-026	Ingalls	1.000			Wenatchee	1.000		
352-027	Ingalls	1.000			Wenatchee	1.000		
352-028	Ingalls	1.000			Wenatchee	1.000		
352-029	Ingalls	1.000			Wenatchee	1.000		
352-030	Ingalls	1.000			Wenatchee	1.000		
352-031	Ingalls	1.000			Wenatchee	1.000		
352-032	Ingalls	1.000			Wenatchee	1.000		
352-033	Mad	1.000			Entiat	1.000		
352-034	Ingalls	1.000			Wenatchee	1.000		
352-035	Ingalls	0.982	Etienne	0.018	Wenatchee	1.000		

Appendix 3. Continued

Individual	Most Likely Population	Probability	Second Most Likely Population	Probability	Most Likely Reporting Group	Probability
352-036	Ingalls	1.000			Wenatchee	1.000
352-037	Ingalls	1.000			Wenatchee	1.000
352-038	Ingalls	1.000			Wenatchee	1.000
352-039	Ingalls	1.000			Wenatchee	1.000
352-040	Ingalls	1.000			Wenatchee	1.000
352-041	Ingalls	1.000			Wenatchee	1.000
352-042	Ingalls	1.000			Wenatchee	1.000
352-043	Ingalls	1.000			Wenatchee	1.000
352-044	Ingalls	1.000			Wenatchee	1.000
352-045	Not Enough Loci Amplified					
352-046	Ingalls	1.000			Wenatchee	1.000
352-047	Ingalls	1.000			Wenatchee	1.000
352-048	Ingalls	1.000			Wenatchee	1.000
352-049	Ingalls	1.000			Wenatchee	1.000
352-050	Ingalls	1.000			Wenatchee	1.000
352-051	Ingalls	1.000			Wenatchee	1.000
352-052	Ingalls	1.000			Wenatchee	1.000
352-053	Ingalls	1.000			Wenatchee	1.000
352-054	Ingalls	1.000			Wenatchee	1.000
352-055	Ingalls	1.000			Wenatchee	1.000
352-056	Ingalls	1.000			Wenatchee	1.000
352-057	Ingalls	1.000			Wenatchee	1.000
352-058	Ingalls	1.000			Wenatchee	1.000
352-059	Ingalls	1.000			Wenatchee	1.000
352-060	Ingalls	1.000			Wenatchee	1.000
352-061	Ingalls	1.000			Wenatchee	1.000
352-062	Ingalls	1.000			Wenatchee	1.000
352-063	Ingalls	1.000			Wenatchee	1.000
352-066	Ingalls	1.000			Wenatchee	1.000
352-067	Ingalls	1.000			Wenatchee	1.000
352-068	Etienne	1.000			Wenatchee	1.000
352-070	Ingalls	1.000			Wenatchee	1.000
352-071	Ingalls	1.000			Wenatchee	1.000
352-072	Ingalls	1.000			Wenatchee	1.000
352-073	Ingalls	1.000			Wenatchee	1.000
352-074	Ingalls	1.000			Wenatchee	1.000
352-075	Ingalls	1.000			Wenatchee	1.000

Appendix 3. Continued

Individual	Most Likely Population	Probability	Second Most Likely Population	Probability	Most Likely Reporting Group	Probability
352-076	Ingalls	1.000			Wenatchee	1.000
352-077	Ingalls	1.000			Wenatchee	1.000
352-078	Ingalls	1.000			Wenatchee	1.000