Genetic Analysis of Bull Trout in the Jarbidge River Watershed, Nevada/Idaho

2007 Progress Report

11-29-07

Patrick DeHaan and William Ardren
U.S. Fish and Wildlife Service
Abernathy Fish Technology Center
Conservation Genetics Program
1440 Abernathy Creek Road
Longview, Washington 98632
(patrick_dehaan@fws.gov; william_ardren@fws.gov)
(360) 425-6072

Submitted to:
Allen Taylor, Todd Gilmore and Selena Werdon
U.S. Fish and Wildlife Service
Nevada Fish and Wildlife Office
1340 Financial Boulevard, Suite 234
Reno, Nevada 89502
(allen_taylor@fws.gov, todd_gilmore@fws.gov; selena_werdon@fws.gov)
(775) 861-6300

Introduction

The Jarbidge River in southwest Idaho and northern Nevada is a tributary in the Snake River basin and contains the southernmost habitat currently occupied by bull trout (*Salvelinus confluentus)*. This population segment is geographically separated from other bull trout in the Snake River basin by more than 240 kilometers (150 miles) of unsuitable habitat and several impassable dams on the mainstem Snake River and the lower Bruneau River. The Jarbidge River core area consists of the entire mainstem Jarbidge River and the East and West Forks of the Jarbidge River and their tributaries (Hydrologic Unit Codes 1705010210 to 1705010215). Local populations of bull trout currently identified within the Jarbidge River core area are the East Fork Jarbidge River (including the East Fork headwaters, Cougar Creek, Fall Creek, and Slide Creek), West Fork Jarbidge River (including Sawmill Creek), Dave Creek, Jack Creek, and Pine Creek (Figure 1). The geographic uniqueness of bull trout in the Jarbidge River, in association with their physical isolation, potentially makes this distinct population segment a high conservation priority for maintaining genetic diversity and the evolutionary potential of the species.

Spruell *et al.* (2003) conducted a range-wide genetic survey of bull trout populations and suggested that bull trout populations in the Jarbidge River basin have a shared evolutionary history with populations in the upper Columbia River Basin and upper Snake River Basin. However, this study contained a relatively small sample size (n = 37) from the Jarbidge River system and did not investigate fine scale levels of population structure present within the Jarbidge River and its tributaries. Recently more polymorphic microsatellite markers have been developed for bull trout (e.g. DeHaan and Ardren 2005) that allow for a more fine scale investigation of the population structure within the Jarbidge River system. Despite the evidence that historically there was some level of gene flow between the Jarbidge River population segment and bull trout in the Columbia River basin, bull trout in the Jarbidge River population segment have now been isolated from other populations for over 100 years (since the late 1800's; Gilbert and Evermann 1894).

The genetic consequences of population isolation and fragmentation have been previously documented for bull trout (Whitely et al. 2006; Costello et al. 2003; Nerass and Spruell 2001). Costello et al. (2003) demonstrated the effect of isolating populations

above barriers on levels of genetic variation, however, none of these populations were as geographically isolated as the Jarbidge River populations. Fragmentation of populations via dams and other habitat alterations may result in genetic bottlenecks (Yamamoto et al. 2004), increased rates of inbreeding (Rieman and Allendorf 2001), and changes in life history (Morita 2000).

Recently a broader sampling of bull trout populations within the Jarbidge River watershed has been conducted. Our objective was to use the newly developed microsatellite loci to provide a more accurate description of the genetic variation within and among bull trout populations in the Jarbidge River watershed. This detailed information on the genetic population structure of Jarbidge River bull trout will allow for a better understanding of the evolutionary relationships of these local populations within the larger, range-wide bull trout genetic landscape. This information will also be important for considering genetic rescue efforts (*e.g.*, transferring fish among isolated populations), understanding the impact of any identified hybridization with brook trout in the basin and estimating the effective number of breeders (N_e) in each local population, allowing us to quantify the rate of loss of genetic diversity in the populations and rate of inbreeding.

Methods

Sample Collections

In 2006 U.S. Geological Survey (USGS) personnel collected fin clips from 240 bull trout within seven tributaries in the Jarbidge River system: West Fork Jarbidge, Pine Cr., Dave Cr., East Fork Jarbidge, Fall Cr., Slide Cr. and Jack Cr. Individuals collected ranged in size from 95 to 330mm. All clips were stored in 100% non-denatured ethanol at room temperature. Additionally we received fin clips from 5 individuals collected in the East Fork Jarbidge River in 1999 and 10 individuals collected in Jack Creek in 1999.

Laboratory Analyses

DNA was extracted from all 255 samples using a modified chelex extraction protocol (Miller and Kapuscinski 1996). All individuals were genotyped at a suite of 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). These 16 loci were agreed upon as a standard set of loci to be used by five laboratories conducting bull trout genetic analyses so that microsatellite datasets could be combined for range-wide analyses. We are currently in the process of finalizing the standardization effort among the laboratories. PCR reactions were carried out in 15µl volumes containing 2µl template DNA, 1X polymerase buffer (10mM Tris-HCl, 50mM KCl, 0.1% Triton x-100), 1.5 or 2.0 mM $MgCl_2$, 0.2 mM of each dNTP, 0.5µM of each primer and 0.2 units of Taq DNA polymerase (Promega Co.). PCR conditions were as follows; initial denaturation at 94°C for 3 minutes, then 38 cycles of 94°C for 30 second, 30 seconds at the primer specific annealing temperature and 30 seconds primer extension at 72°C, followed by a final extension at 72°C for 7 minutes.

Following PCR, reactions were pooled for electrophoresis on an *ABI 3130xl* Genetic Analyzer (Applied Biosystems Inc., Foster City, CA). Automated electrophoresis was carried out 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 Analyses

Relatively few individuals were collected from two of the tributaries in this study; Fall Creek (n=9) and Slide Creek (n=4). Because of the low sample sizes these two populations were omitted from the statistical analysis. These populations will be targeted for sampling in 2007 therefore these populations will be added to the analysis when more samples become available.

Populations were tested for conformance to Hardy-Weinberg equilibrium (HWE) using the program GENEPOP v3.4 (Raymond and Rousset 1995). GENEPOP was also used to test each population for linkage disequilibrium. HWE tests were adjusted for multiple comparisons using a sequential Bonferroni adjustment (Rice 1989). Initial HWE tests and linkage disequilibrium tests suggested that the sample from Dave Creek included many closely related individuals, therefore we used the program Kinship v1.3.1

(Goodnight and Queller 1999) to identify full sibling individuals in Dave Creek. Where family groups were identified, we omitted all but one individual from the group for further analysis. After omitting the samples from Fall Creek and Slide Creek and omitting all but one of the related individuals from Dave Creek, our final sample size used for statistical analysis was 203 individuals (Table 1).

We used the program GDA (Lewis and Zaykin 2001) to estimate measures of genetic diversity including mean numbers of alleles and observed and expected heterozygosity. In addition we used the program F-Stat v2.9.3 (Goudet 2001) to estimate allelic richness. This program provides estimates of allelic richness that have been corrected for differences in sample size between populations. Populations were also tested for evidence of recent genetic bottlenecks using the program BOTTLENECK (Cornuet and Luikart 1996) assuming a two-phased model of mutation.

We used GDA to estimate the overall level of genetic variation among all populations (F_{st}) and the associated confidence interval based on 1000 bootstrap replicates. We used the program F-Stat v2.9.3 (Goudet 2001) to estimate pairwise levels of genetic variation (F_{st}) among all populations and test pairwise estimates for significance. Additionally we conducted a contingency test using GENEPOP to test for significant genetic differences between the five populations. The program Phylipv3.6 (Felsenstein 1993) was used to estimate Cavalli-Sforza and Edwards chord distance among the five populations. These values were used to construct a consensus neighbor-joining tree based on 1000 bootstrap replicates.

To examine movement patterns of sub-adult and adult bull trout within the Jarbidge River system we used the Jackknife option in the program WhichRun v4.1 (Banks and Eichert 2000) to assign all individuals to their most likely population of origin. With this procedure fish are removed from the baseline dataset one at a time and treated as unknowns and then assigned to their first and second most likely population of origin based on a maximum likelihood algorithm. The number of individuals that are assigned to their correct population of origin provides a means of estimating the statistical power of the baseline dataset to assign unknown individuals.

Results

Initial tests showed that Dave Creek deviated from HWE at nine of 16 loci. Further examination of the samples from Dave Creek revealed two distinct size classes of fish (Figure 2). We then split the Dave Creek sample into two groups based on size class and tested each group for HWE separately. We found that the larger Dave Creek fish conformed to HWE at all 16 loci and the smaller fish were out of HWE at six loci. Deviations from HWE at five of six loci were due to a heterozygote excess, suggesting that a small number of families had been sampled. Additionally the majority of the pairwise linkage disequilibrium tests for Dave Creek were significant, providing further evidence that this sample was comprised of several related individuals. Kinship analysis confirmed that many of the smaller fish from Dave Creek were from a single family group. We removed all but one of these individuals from further analysis and then combined the remaining juveniles from Dave Creek with the larger fish from this system. The adjusted sample size from Dave Creek was 29 individuals (Table 1).

Following adjustment of the Dave Creek sample, all populations conformed to HWE at all loci with the exception of *Sco109* which deviated from HWE in Dave Creek, East Fork Jarbidge and West Fork Jarbidge due to a heterozygote deficiency and in Jack Creek due to a heterozygote excess. *Sco200* also deviated from HWE in Jack Creek due to a heterozygote deficiency. Because Sco109 showed deviations from HWE in four of five populations it was removed from further analyses. In three of these populations deviations from HWE were due to a deficiency of heterozygotes, which can indicate the presence of a null allele. The mean number of alleles per locus ranged from 3.467 in Dave Creek to 4.800 in West Fork Jarbidge (Table 1). Allelic richness was lowest in Dave Creek (3.242) and greatest in East Fork Jarbidge (4.066) (Table 1). Observed heterozygosity ranged from 0.436 (Jack Creek) to 0.527 (East Fork Jarbidge) (Table 1). None of the five populations showed evidence of recent genetic bottleneck.

The overall estimate of genetic variation among populations (F_{st}) was 0.097 and was found to be significantly different from zero. Pairwise estimates of F_{st} ranged from 0.0068 for Pine Creek and West Fork Jarbidge to 0.1281 for Dave Creek and West Fork Jarbidge (Table 2). All pairwise estimates of F_{st} were found to be statistically significant ($\alpha = 0.05$) with the exception of the pairwise F_{st} for Pine Creek and West Fork Jarbidge. The contingency analysis showed that all populations were significantly differentiated

from one another with the exception of Pine Creek and West Fork Jarbidge. Following B-Y FDR correction (Narum 2006) these two populations were found to be significantly differentiated, however. The neighbor-joining tree showed that the two East Fork tributaries, East Fork Jarbidge and Dave Creek, clustered together and the West Fork tributaries also clustered together with Pine Creek and West Fork Jarbidge being most genetically similar (Figure 3). The nodes on the tree were highly supported.

When we performed the jackknife analysis, the majority of the individuals were assigned to the population from which they were sampled (Table 3). All of the individuals from the East Fork tributaries were assigned to the tributary they were collected from. Approximately 87% of the individuals collected in Jack Creek were assigned to Jack Creek, 10% were assigned to other West Fork tributaries and 3% were assigned to the East Fork Jarbidge. A much lower proportion of individuals from Pine Creek and West Fork Jarbidge were assigned to the tributary they were collected from (Table 3). Of the individuals collected in Pine Creek, 50% were assigned to West Fork Jarbidge whereas only 45% were assigned to Pine Creek. In West Fork Jarbidge, approximately 70% of the individuals were assigned to West Fork and the majority of the other individuals (26%) were assigned to Pine Creek. ID numbers of the individuals assigned to tributaries other than the one they were collected from are listed in Table 4. The fish assigned to the population they were collected from ranged from 95 to 260mm with an average fork length of 161.1 mm. Fish assigned to populations other than the tributary they were collected from ranged in size from 98 to 330mm with a mean fork length of 203.5mm (Figure 4).

Discussion

Hybridization with non-native brook trout is thought to be a major threat to bull trout persistence throughout the species range (Rieman et al. 1997). While brook trout have been introduced into the Jarbidge River watershed in the past, self sustaining populations are not believed to have been established within bull trout habitat (USFWS 2004). We did not find any evidence of hybridization with brook trout in any of the fish we examined in this study.

Numbers of alleles and levels of heterozygosity that we observed in the present study were greater than those observed by Spruell et al. (2003) for a sample of bull trout from the Jarbidge River. This difference can be attributed to the different genetic markers used in the two studies; the previous study used markers developed primarily from other salmonids whereas the markers in this study were developed primarily from bull trout. Measures of genetic diversity observed in the present study were found to be lower than other bull trout populations we had examined using these same loci, however. For example, in the lower Flathead River Basin in Montana we observed an average of 7.14 alleles per locus and mean heterozygosity of 0.706 (DeHaan and Ardren 2007). The reductions we observed in genetic diversity compared to other bull trout populations can likely be attributed to the geographic location of this population as well as the fact that it has been isolated for over 100 years. The Jarbidge River is the Southern extreme of the distribution of bull trout. Previous studies have documented reduced genetic variability in fish populations at the extreme limits of species distributions (Whitely et al. 2006, Stamford and Taylor 2004). Reductions in genetic diversity have also been observed in bull trout populations isolated above barriers (Whitely et al. 2006, Costello et al. 2003). The Jarbidge River has been isolated from other bull trout populations for over 100 years due to poor habitat and the construction of dams.

In general bull trout show high levels of genetic differentiation among populations throughout their range (Spruell et al. 2003, Costello et al. 2003) often with populations in close geographic proximity showing relatively high levels of genetic differentiation (Whitely et al. 2006, Nerass and Spruell 2001, Spruell et al. 1999). The high level of genetic variation we observed among bull trout populations in the Jarbidge River was consistent with observations from other bull trout populations. For example, we observed an overall F_{st} estimate of 0.095 for bull trout in the Malheur River system in Oregon, another Snake River Tributary (DeHaan et al. 2007). Levels of genetic variation observed in this study combined with the contingency analyses suggest that levels of gene flow between local populations in the Jarbidge River are relatively low. The greatest estimates of pairwise differentiation were observed between the East Fork tributaries (Dave Creek and East Fork Jarbidge) and the West Fork tributaries (Jack Creek, Pine Creek, and West Fork Jarbidge). The East and West Fork tributaries also grouped

separately on the neighbor-joining tree. The fact that the pairwise F_{st} estimates were greatest between East and West Fork tributaries suggests that the rate of gene flow between the two forks is lower than the rate of gene flow between the tributaries within the two forks.

While the majority of the populations in this study showed a high level of differentiation from one another, the exception to this pattern appears to be the West Fork Jarbidge and Pine Creek. The pairwise F_{st} estimate for these two populations and the contingency analysis suggest that these two populations have the least amount of genetic variation among them. These two populations also grouped together with 99% bootstrap support on the neighbor-joining tree. When we assigned individuals to their most likely population of origin, 12 of 24 individuals collected in Pine Creek were assigned to the West Fork Jarbidge. While the majority of the individuals collected in the West Fork Jarbidge were assigned to the West Fork, 16 of the 19 West Fork individuals that were assigned to other populations were assigned to Pine Creek. Among the five populations that we surveyed, these two are also the closest to one another geographically. These data suggest that there is a higher rate of gene flow between these two populations compared to the other populations examined in the Jarbidge River.

The individuals that were genetically assigned to the population that they were collected from (designated as “Correctly assigned” in Fig. 4) tended to be smaller than the individuals that were genetically assigned to a population other than the one they were collected from (designated as “mis-assigned” in Fig. 4). A likely explanation for this trend is that many of these mis-assigned individuals represent migratory sub-adults sampled after they had emigrated from their natal population. Bull trout exhibit both resident and migratory life history types, often within the same river system (Brenkman et al. 2007; Fraley and Shepard 1989) and previous studies have found that sub-adult bull trout are highly migratory (Brenkman et al. 2007; Downs et al. 2006; Muhlfeld et al. 2003). Although migration into and out of the Jarbidge River is not likely to occur due to the presence of physical barriers downstream of the core area, there are no physical barriers within the study area that would prevent fluvial bull trout from moving throughout the Jarbidge River core area.

This report details the first year of work for this project and provides preliminary information important for bull trout conservation and management in the Jarbidge River system. During 2008 we plan to incorporate more populations (i.e. Fall Creek, Slide Creek) as well as add samples to populations that presently have low sample sizes (i.e. East Fork Jarbidge). By adding additional samples we hope to obtain more accurate estimates of genetic diversity for Jarbidge bull trout populations and further clarify the evolutionary relationship among these populations. We also hope to provide an idea of how many distinct spawning populations are present within the Jarbidge River system. The future combination of genetic data and data gathered on movement patterns of fish will also provide important information for bull trout management in the Jarbidge River system.

Acknowledgements

Funding for this project was provided by the U.S. Fish and Wildlife Service. We would like to thank Matt Diggs for providing laboratory assistance for this project. We would also like thank Jodi Charrier, Steven Buschback, Emily Hite, and Brien Rose with the USGS for collecting the samples used for this project and Pat Connolly also with USGS for helpful discussion regarding the Dave Creek samples. The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the U.S. Fish and Wildlife Service.

References

Angers, B., L. Bernatchez, A. Angers, and L. Desgroseillers. 1995. Specific microsatellite loci for brook char reveal strong population subdivision on a microgeographic scale. Journal of Fish Biology 47(supplement A):177-185.

Banks, M.A. and W. Eichert. 2000. WHICHRUN (version 3.2) a computer program for population assignment of individuals based on multilocus genotype data. Journal of Heredity 91:87-89.

Brenkman, S.J., S.C. Corbett and E.C. Volk. 2007. Use of otolith chemistry and radiotelemetry to determine age specific migratory patterns of anadromous bull trout in the Hoh River, Washington. Transactions of the American Fisheries Society 136:1-11.

Cornuet, J.M. and G. Luikart. 1996. Description and power analysis of two tests for detecting recent population bottlenecks from allele frequency data. Genetics 144:2001-2014.

Costello, A.B., T.E. Downs, S.M. Pollard, C.J. Pacas, and E.B. Taylor. 2003. The influence of history and contemporary stream hydrology on the evolution of genetic diversity within species: an examination of microsatellite DNA variation in bull trout, Salvelinus *confluentus* (Pices:Salmonidae). Evolution 57:328-344.

Crane, P.A., C.J. Lewis, E.J. Kretschmer, S.J. Miller, W.J. Spearman, A.L. DeCicco, M.J. Lisac and J.K. Wenberg. 2004. Characterization and inheritance of seven microsatellite loci from Dolly Varden, *Salvelinus malma*, and cross-species amplification in Arctic char, *S. alpinus*. Conservation Genetics 5:737-741.

DeHaan, P.W. and W.R. Ardren. 2007**.** Genetic Analysis of Bull Trout Populations in the Flathead Reservation Lands, Montana. Final report submitted to the Confederated Salish and Kootenai Tribes, 3-20-07. 24 pages.

DeHaan, P.W., and W.R. Ardren. 2005. Characterization of 20 highly variable tetranucleotide microsatellite loci for bull trout (*Salvelinus confluentus*) and cross-amplification in other *Salvelinus* species. Molecular Ecology Notes 5:582–585.

DeHaan, P.W., M. Diggs and W.R. Ardren. 2007. Genetic population structure of bull trout in the Malheur River basin, Oregon. Report to the Burns Paiute Tribe, Burns, OR. 9-28-07. 14 Pages.

Downs, C.C., D. Horan, E. Morgan-Harris and R. Jakubowski. 2006. Spawning demographics and juvenile dispersal of an adfluvial bull trout population in Trestle Creek, Idaho. North American Journal of Fisheries Management 26:190-200.

Felsenstein, J. 1993. Phylip: phylogeny inference package. Ver. 3.5c. Department of Genetics, University of Washington, Seattle.

Fraley, J.J. and B.S. Shepard. 1989. Life history, ecology and population status of migratory bull trout (Salvelinus confluentus) in the Flathead Lake and River system, Montana. Northwest Science 63:133-143.

Gilbert, H.G. and B.W. Evermann. 1894. A report upon investigations in the Columbia River Basin, with descriptions of four new species of fishes. Pages 169-204 in Bulletin of the United States Fish Commission, Washington, D.C.

Goudet, J. 2001. FSTAT, a program to estimate and test gene diversities and fixation indices (version 2.9.3). Available from http://www.unil.ch/izea/softwares/fstat.html. Updated from Goudet (1995).

Goodknight, K.F. and D.C. Queller. 1999. Computer software for performing likelihood tests of pedigree relationship using genetic markers. Molecular Ecology 8(7):1231-1234.

Lewis, P.O., and D. Zaykin. 2001. Genetic Data Analysis: Computer program for the analysis of allelic data. Version 1.0 (d16c). Free program distributed by the authors over the internet from http://lewis.eeb.uconn.edu/lewishome/software.html.

Miller, L.M. and A.R. Kapuscinski. 1996. Microsatellite DNA markers reveal new levels of variation in northern pike. Transactions of the American Fisheries Society 125:971-997.

Morita, K., S. Yamamoto, and N. Hoshino. 2000. Extreme life history change of white-spotted car (*Salvelinus leucomaenis*) after damming. Canadian Journal of Fisheries and Aquatic Science 57:1300-1306.

Muhlfeld, C.C., S. Glutting, R. Hunt, D. Daniels and B. Marotz. 2003. Winter diel habitat use and movement by subadult bull trout in the upper Flathead River, Montana. North American Journal of Fisheries Management 23:163-171.

Narum, S.R. 2006. Beyond Bonferroni: less conservative analyses for conservation genetics. Conservation Genetics 7:783-787.

Nerass, L.P and P.Spruell. 2001. Fragmentation of riverine systems: the genetic effects of dams on bull trout (*Salvelinus confluentus*) in the Clark Fork river system. Molecular Ecology 10:1153-1164.

Raymond, M. and F. Rousset. 1995. GENEPOP (version 1.2): population genetics software for exact tests and ecumenicism. Journal of Heredity 86, 248-249

Rexroad, C.E., R.L. Coleman, A.M. Martin, W.K. Hershberger and J. Killefer. 2001. Thirty-five polymorphic microsatellite markers for rainbow trout (*Oncorhynchus mykiss*). Animal Genetics 32: 317-319.

Rice, W.R. 1989. Analyzing tables of statistical tests. Evolution 43:223-225.

Rieman, B.E. and F.W. Allendorf. 2001. Effective population size and genetic conservation criteria for bull trout. North American Journal of Fisheries Management 21:756-64.

Rieman, B., D.C. Lee, R.F. Thurow. 1997. Distribution, status and likely future trends of bull trout within the Columbia and Klamath river basins. North American Journal of Fisheries Management 17:1111-1125.

Stamford, M.D. and E.B. Taylor. 2004. Phylogeographical lineages of Arctic grayling (*Thymallus arcticus*) in North America: divergence, origins and affinities with Eurasian Thymallus. Molecular Ecology 13:1533-1549.

Spruell, P., A.R. Hemmingsen, P.J. Howell, N. Kanda, and F.W. Allendorf. 2003. Conservation genetics of bull trout: geographic distribution of variation at microsatellite loci. Conservation Genetics 4:17-29.

Spruell, P., B.E. Rieman, K.L. Knudsen, F.M. Utter and F.W. Allendorf. 1999. Genetic population structure within streams: microsatellite analysis of bull trout populations. Ecology of Freshwater Fishes 8:114-121.

U.S. Fish and Wildlife Service. 2004. Draft Recovery Plan for the Jarbidge River Distinct Population Segment of Bull Trout (*Salvelinus confluentus*). U.S. Fish and Wildlife Service, Portland, Oregon. 132 + xiii pp.

Whitely, A.R., P. Spruell, B.E. Rieman, and F.W. Allendorf. 2006. Fine-scale genetic structure of bull trout at the southern limit of their distribution. Transactions of the American Fisheries Society 135:1238-1253.

Yamamoto S, Morita K, Koizumi I, and K Maekawa. 2004. Genetic differentiation of white spotted charr (*Salvelinus leucomaenis*) populations after habitat fragmentation: spatial-temporal changes in gene frequencies. Conservation Genetics 5(4):529-538.

Table 1. Measures of genetic diversity based on 15 microsatellite loci for five bull trout populations from the Jarbidge River system, ID/NV.

	N	A	A_R	H_{exp}	H_{obs}
Dave Cr.*	29	3.467	3.242	0.449	0.421
EF Jarbidge	18	4.133	4.066	0.527	0.532
Jack Cr.	70	4.133	3.406	0.436	0.446
Pine Cr.	24	4.400	4.048	0.481	0.492
WF Jarbidge	62	4.800	3.863	0.485	0.485
	Mean	**4.1866**	**3.725**	**0.4756**	**0.4752**

A = Mean number alleles per locus
A_R = Allelic richness
H_{exp} = Expected heterozygosity
H_{obs} = Observed heterozygosity
* Sample size in Dave Creek was adjusted due to a large number of related individuals sampled

Table 2. Pairwise estimates of genetic variation among populations (F_{st}) based on 15 microsatellite loci for 5 bull trout populations in the Jarbidge River system ID/NV.

	Dave Cr.	East Fork Jarbidge	Jack Cr.	Pine Cr.	West Fork Jarbidge
Dave Cr.	---				
EF Jarbidge	0.0974*	---			
Jack Cr.	0.1156*	0.1303*	---		
Pine Cr.	0.1051*	0.0853*	0.0815*	---	
WF Jarbidge	0.1281*	0.1138*	0.0980*	0.0068	---

* Indicates a significant pairwise difference P<0.05

Table 3. Population assignments for bull trout collected in the Jarbidge River system. The first column lists the sampling location and the subsequent columns give the propotion of individuals that were assigned to each population. For example, 87% of the fish collected in Jack Creek were assigned to Jack Creek.

Location Sampled	Proportion of individuals assigned				
	Dave Cr.	East Fork Jarbidge	Jack Cr.	Pine Cr.	West Fork Jarbidge
Dave Cr.	**1.00**	0.00	0.00	0.00	0.00
EF Jarbidge	0.00	**1.00**	0.00	0.00	0.00
Jack Cr.	0.00	0.03	**0.87**	0.07	0.03
Pine Cr.	0.05	0.00	0.00	**0.45**	0.50
WF Jarbidge	0.00	0.02	0.03	0.26	**0.69**

Table 4. Individuals that were assigned to tributaries other than the one that they were collected from.

Sample Location	ID	Fork Length (mm)	Most Likely Pop #1	Most Likely Pop #2	Confidence*
Jack	JARB-201	174	EF Jarbidge	Pine	4.20041
Jack	JARB-202	310	EF Jarbidge	Pine	2.52E+03
Jack	JARB-203	260	WF Jarbidge	Pine	5.98342
Jack	JARB-211	185	Pine	WF Jarbidge	2.47165
Jack	JARB-212	190	Pine	WF Jarbidge	2.60652
Jack	JARB-213	177	Pine	EF Jarbidge	5.94256
Jack	JARB-218	175	Pine	Jack	1.72904
Jack	JARB-221	221	Pine	WF Jarbidge	4.5687
Jack	JARB-223	190	WF Jarbidge	Pine	1.0339
Pine	JARB-066	187	WF Jarbidge	Pine	5.02596
Pine	JARB-069	200	WF Jarbidge	Pine	5.97489
Pine	JARB-070	187	Jack	Dave	18.306
Pine	JARB-072	201	WF Jarbidge	Pine	2.32599
Pine	JARB-074	243	WF Jarbidge	Pine	1.38555
Pine	JARB-075	195	WF Jarbidge	Pine	1.64052
Pine	JARB-076	196	WF Jarbidge	Pine	16.659
Pine	JARB-077	188	WF Jarbidge	Pine	2.04196
Pine	JARB-080	225	WF Jarbidge	Pine	5.87344
Pine	JARB-081	195	WF Jarbidge	Pine	2.35843
Pine	JARB-085	189	WF Jarbidge	Pine	1.77876
Pine	JARB-086	219	WF Jarbidge	Pine	5.0654
Pine	JARB-088	115	Dave	Jack	9.95697
Pine	JARB-090	153	Jack	Pine	85.941
WF Jarbidge	JARB-005	189	Pine	WF Jarbidge	11.664
WF Jarbidge	JARB-006	193	Pine	WF Jarbidge	4.20556
WF Jarbidge	JARB-008	229	Pine	WF Jarbidge	7.09209
WF Jarbidge	JARB-021	175	Pine	WF Jarbidge	2.5143
WF Jarbidge	JARB-024	143	Pine	WF Jarbidge	2.28533
WF Jarbidge	JARB-034	195	Pine	WF Jarbidge	12.983
WF Jarbidge	JARB-035	150	Jack	WF Jarbidge	1.31712
WF Jarbidge	JARB-041	98	Jack	Pine	2.04496
WF Jarbidge	JARB-169	205	Pine	WF Jarbidge	3.67358
WF Jarbidge	JARB-170	197	Pine	WF Jarbidge	15.65
WF Jarbidge	JARB-172	195	Pine	WF Jarbidge	4.01121
WF Jarbidge	JARB-261	276	EF Jarbidge	Pine	7.29214
WF Jarbidge	JARB-262	330	Pine	WF Jarbidge	18.96
WF Jarbidge	JARB-264	227	Pine	Jack	8.53369
WF Jarbidge	JARB-265	185	Pine	WF Jarbidge	1.27357
WF Jarbidge	JARB-266	210	Pine	WF Jarbidge	1.2696
WF Jarbidge	JARB-267	244	Pine	WF Jarbidge	7.77985
WF Jarbidge	JARB-270	310	Pine	Jack	18.392
WF Jarbidge	JARB-272	221	Pine	WF Jarbidge	1.63602

* **CONFIDENCE (X times more confident Most Likely (ML) vs 2nd Most Likely (SML)) i.e., P(ML/SML)**

Figure 1. Jarbidge River Basin in Idaho and Nevada. Sampling locations for bull trout are indicated by the shaded regions on the map.

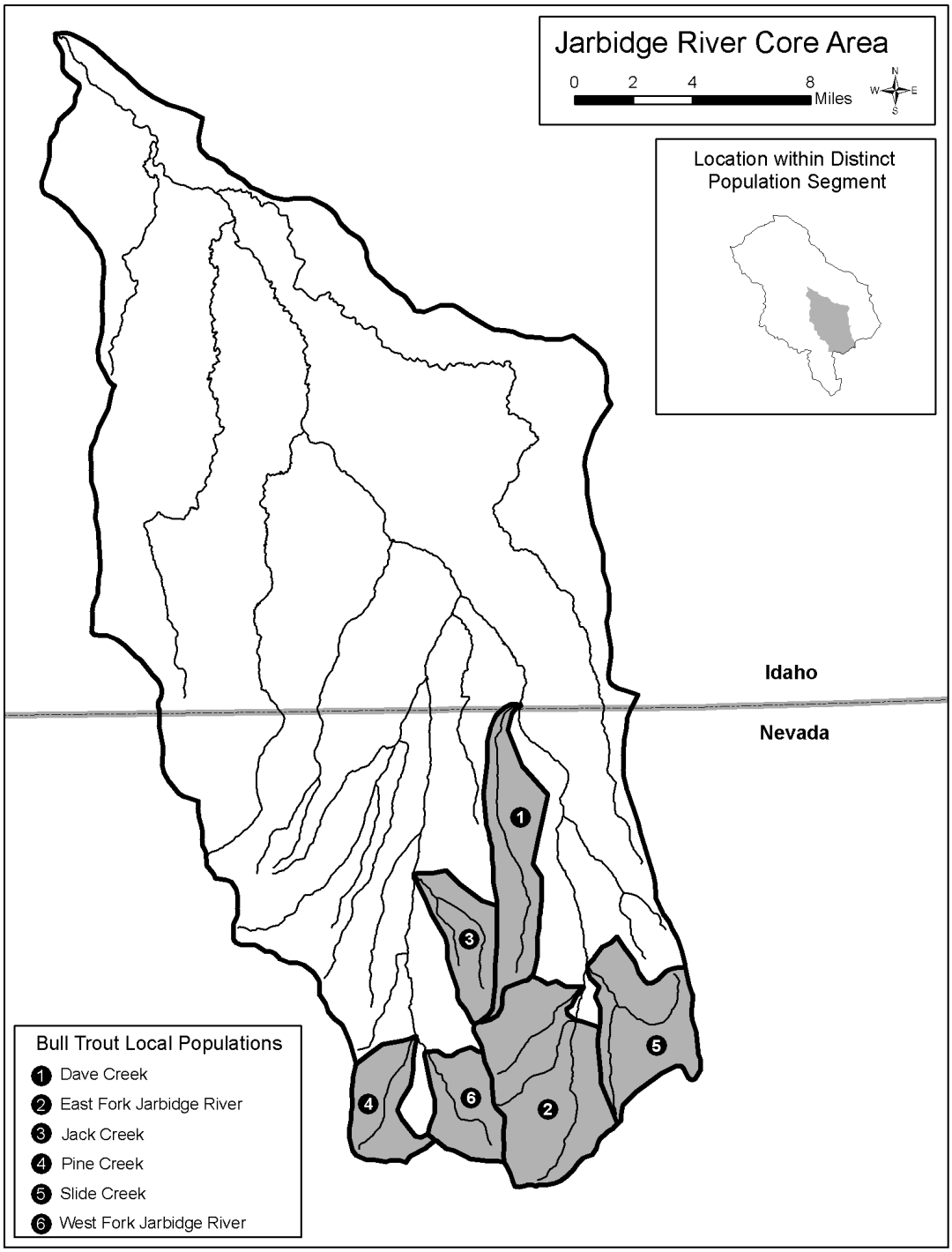

Figure 2. Length frequency histogram for bull trout collected in Dave Creek, a tributary of the East Fork Jarbidge River.

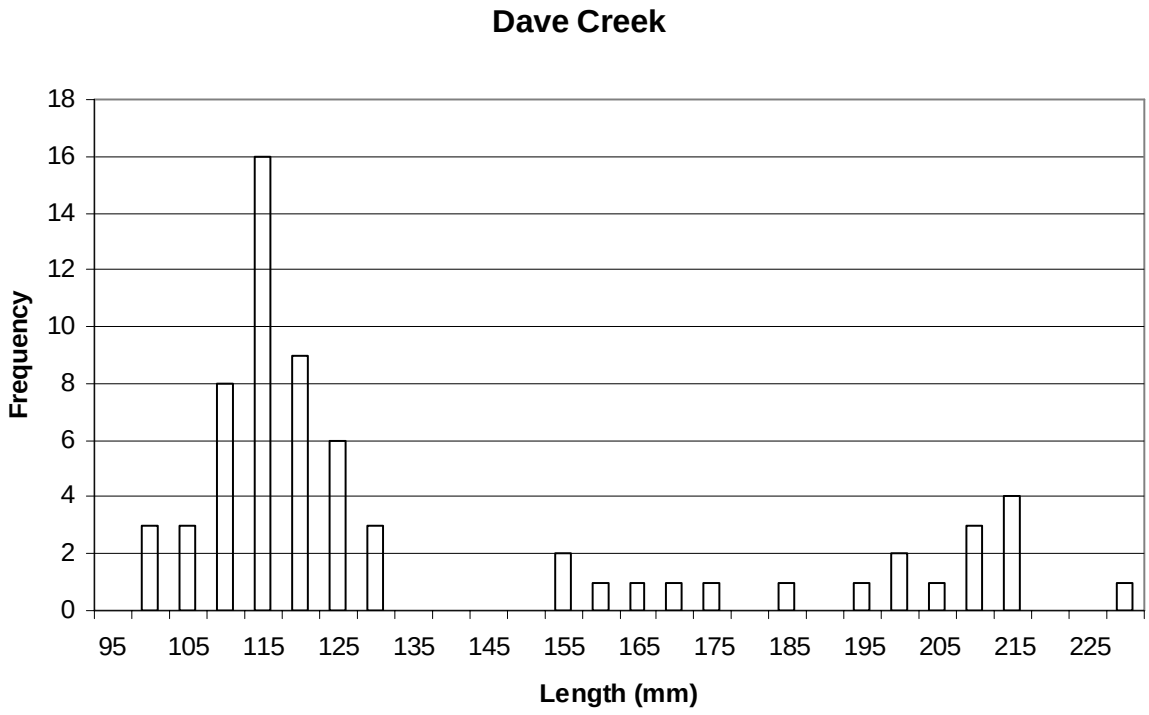

Figure 3. Consensus neighbor-joining tree based on Cavali-Sforza and Edwards chord distances for five bull trout populations in the Jarbidge River system. Values represent the number of bootstrap replicates out of 1000 that showed the arrangement.

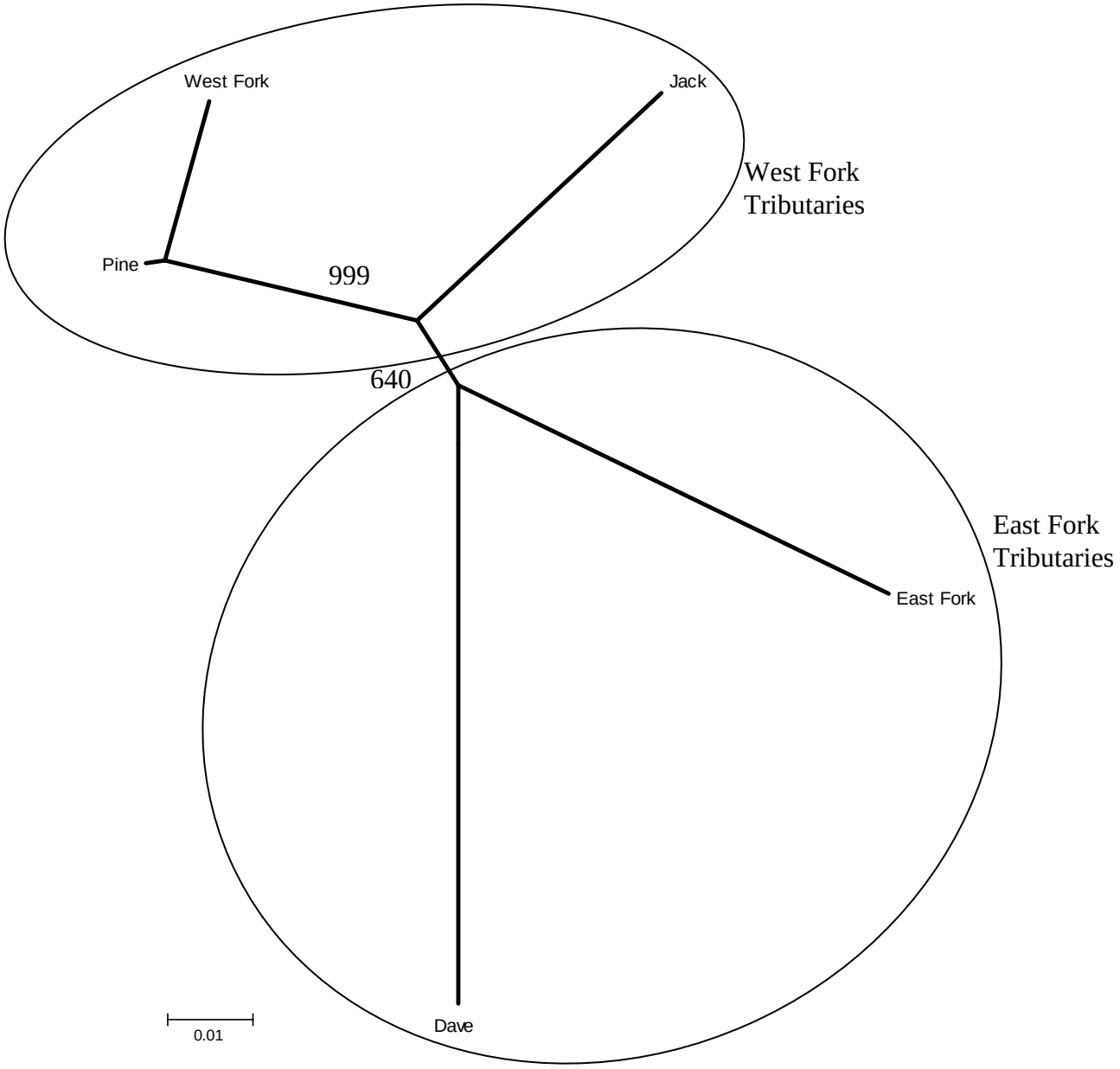
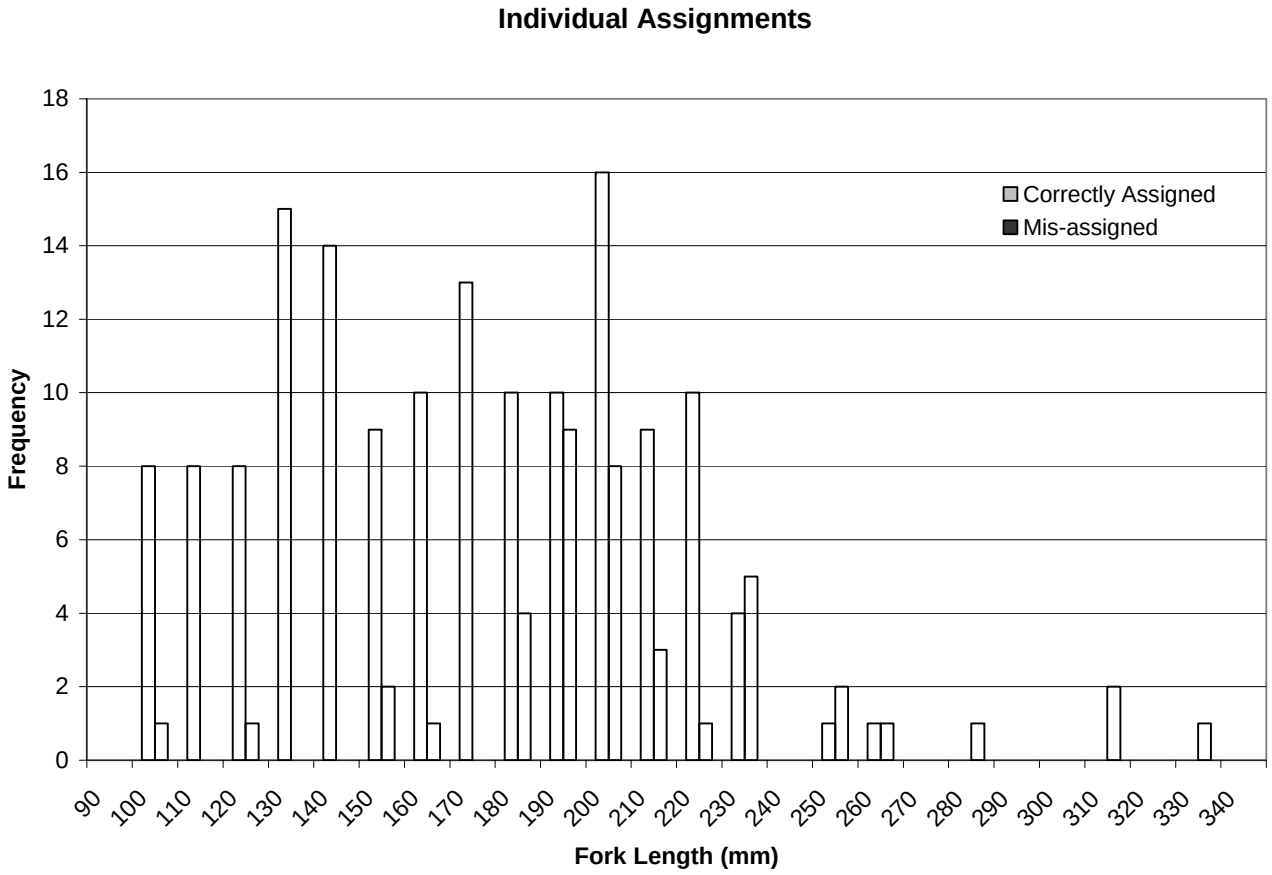
Figure 4. Length frequency histogram of bull trout population assignments. The "correctly assigned" individuals are those that were assigned to the tributary they were collected from. "Mis-assigned" individuals are those that were assigned to a tributary other than the one they were collected from.