Genetic Inventory of Bull Trout and Westslope Cutthroat Trout in the Pend Oreille Subbasin

Annual Report
2003 - 2004

DOE/BP-00009440-2

November 2004

This Document should be cited as follows:

Olson, Jason, Joseph Maroney, Todd Andersen, James Shaklee, Sewall Young, "Genetic Inventory of Bull Trout and Westslope Cutthroat Trout in the Pend Oreille Subbasin", 2003-2004 Annual Report, Project No. 200204300, 17 electronic pages, (BPA Report DOE/BP-00009440-2)

Bonneville Power Administration
P.O. Box 3621
Portland, OR 97208

This report was funded by the Bonneville Power Administration (BPA), U.S. Department of Energy, as part of BPA's program to protect, mitigate, and enhance fish and wildlife affected by the development and operation of hydroelectric facilities on the Columbia River and its tributaries. The views in this report are the author's and do not necessarily represent the views of BPA.

Genetic Inventory of Bull Trout and Westslope Cutthroat Trout in the Pend Oreille Subbasin

2004 Annual Progress Report

PREPARED BY:

Jason Olson, Kalispel Tribe of Indians
Joseph R. Maroney, Kalispel Tribe of Indians
Todd Andersen, Kalispel Tribe of Indians
James B. Shaklee, Washington Department of Fish and Wildlife
Sewall F. Young, Washington Department of Fish and Wildlife

PREPARED FOR:

U.S. DEPARTMENT OF ENERGY
BONNEVILLE POWER ADMINISTRATION
DIVISION OF FISH AND WILDLIFE

P.O. BOX 3621
PORTLAND, OR
97208-3621

Project No. 2002-043-00
Contract No. 00009440

Executive Summary

In 2003, the Kalispel Natural Resource Department (KNRD) collected tissue samples for genetic analysis from 209 bull trout and 1,276 westslope cutthroat. The Washington Department of Fish and Wildlife developed and applied microsatellite DNA screening protocols for the analysis of bull trout at 13 loci and 24 loci for cutthroat trout. This project will continue collection and analysis of additional samples next year. At that time, a final annual report will be compiled for the three-year study that will describe the genetic characteristics for bull trout and westslope cutthroat trout. The extent of hybridization of bull trout (with brook trout) and westslope cutthroat trout (with Yellowstone cutthroat trout and rainbow trout) in the Priest Lake and Lower Pend Oreille subbasins will also be examined.

Acknowledgments

We would like to thank Glen Nenema (Chairman, Kalispel Tribal Council), the Kalispel Tribal Council and members of the Kalispel Tribe for providing the support and the opportunity to conduct this project. We would like to thank the following individuals for their interest, cooperation, and participation in this project: Ned Horner, Joe Dupont and Matt Campbell (Idaho Department of Fish and Game), Scott Deeds (U.S. Fish and Wildlife Service), and Jill Cobb (U.S. Forest Service). Special thanks goes to Ron Morinaka (Contracting Officer Technical Representative) for ensuring smooth project implementation and needed insight. The Kalispel Natural Resource Department provided field support and equipment. Cherril Bowman, Alice Pichahchy, and Jennifer Von Bargen (WDFW Genetics Lab) processed tissue samples and generated raw genetic data. Janet Loxterman (WDFW Genetics Lab) assisted with data processing.

Table of Contents

List of Tables

List of Figures

Introduction

Genetic conservation is an important element of wild salmonid management policies. Genetic conservation focuses on two aspects of genetic variation: local adaptation and genetic diversity (Bergdahl 1998). Effective management of a species living at risk of extinction, such as bull trout (*Salvelinus confluentus*), requires an understanding of the variety of life histories, habitat requirements, and genetic descriptions of populations (Spruell et al. 1999). Past and present efforts to assess, protect and restore existing bull trout populations have been limited by the lack of basic information about bull trout ecology, life history and genetics (Howell and Buchanan, 1992; Rieman and McIntyre, 1993; Rieman and McIntyre, 1995; Buchanan et al. 1997; Spruell and Allendorf, 1997).

The same can be said of westslope cutthroat trout (*Oncorhynchus clarki lewisi*). Although westslope cutthroat stocks with varying degrees of genetic purity are known to occur across the subspecies' range, there is currently little definitive information on the genetic characteristics of most stocks (U.S. Fish and Wildlife Service 1999). Throughout the historic range of westslope cutthroat trout, few of the remaining stocks have been genetically classified on the basis of chromosome counts, biochemical characteristics, or molecular genetic information (USFWS 2000).

In 1999, the Washington Department of Fish and Wildlife (WDFW) collected genetic information from westslope cutthroat trout in eight tributaries to the lower Pend Oreille River, Washington (Shaklee and Young 2000). They compared these populations to two hatchery stocks of westslope cutthroat trout stocked extensively in Washington and to Yellowstone cutthroat trout (*O. clarki bouvieri*). The results indicated the occurrence of genetically distinct populations of westslope cutthroat trout in tributaries to the Pend Oreille River. Additionally, introgression with hatchery stocks failed to be detected with the single exception of Slate creek. Shaklee and Young (2000) concluded that westslope cutthroat trout in the Pend Oreille drainage are subdivided into distinct genetic stocks at least at the level of major tributaries (if not at the level of individual creeks). The management and conservation of these fish should be focused at this fine geographic scale in order to maintain natural levels of productivity and genetic diversity.

Gayeski et al (2001) collected non-lethal fin tissues in 1999 for genetic analysis from nineteen stream trout populations (fifteen initially expected to be cutthroat populations and four initially expected to be interior rainbow populations) residing in headwater tributaries of the Pend Oreille River, Kettle River, Sanpoil River, and Sherman Creek subbasins within the Colville National Forest in northeastern Washington. They also photographed representative specimens of each population for a color catalog of phenotypes. Analysis of paired interspersed nuclear DNA elements (PINEs) was used to characterize each population as to subspecies and level of hybridization, and a genetic purity rating was assigned to each using a modification of the Binns system originally developed in Wyoming to gauge the genetic purity of interior cutthroat trout populations. Within the Pend Oreille subbasin, they concluded that three streams (East Fork Smalle Creek, Upper Sullivan Creek and North Fork Sullivan Creeks) merited an A-rating. Five additional populations were given B-ratings. The ratings for the remaining three streams were either a D or an F.

To expand further on the work that Shaklee and Young (2000) and Gayeski et al (2001), the Kalispel Tribe of Indians developed this project through the Northwest Power Planning Council's Provincial Review Process, which was ultimately funded by Bonneville Power Administration. The geographic focus of the project is Priest Lake and Lower Pend Oreille subbasins.

In 2003, the KNRD collected tissue samples from 209 bull trout and 1,276 westslope cutthroat trout from 28 streams in the Priest Lake and Pend Oreille watersheds, for genetic analysis (Figure 1). The WDFW will conduct microsatellite DNA analysis on those samples. This project will continue collecting and analyzing additional samples next year. At that time, a final report will be compiled for the three-year study that will describe the genetic characteristics for bull trout and westslope cutthroat trout. Each fish will also be analyzed with regard to potential inter-specific hybridization (bull trout with brook trout (*S. fontinalis*) and cutthroat trout with rainbow trout (*O. mykiss spp.*) and between westslope and Yellowstone cutthroat).

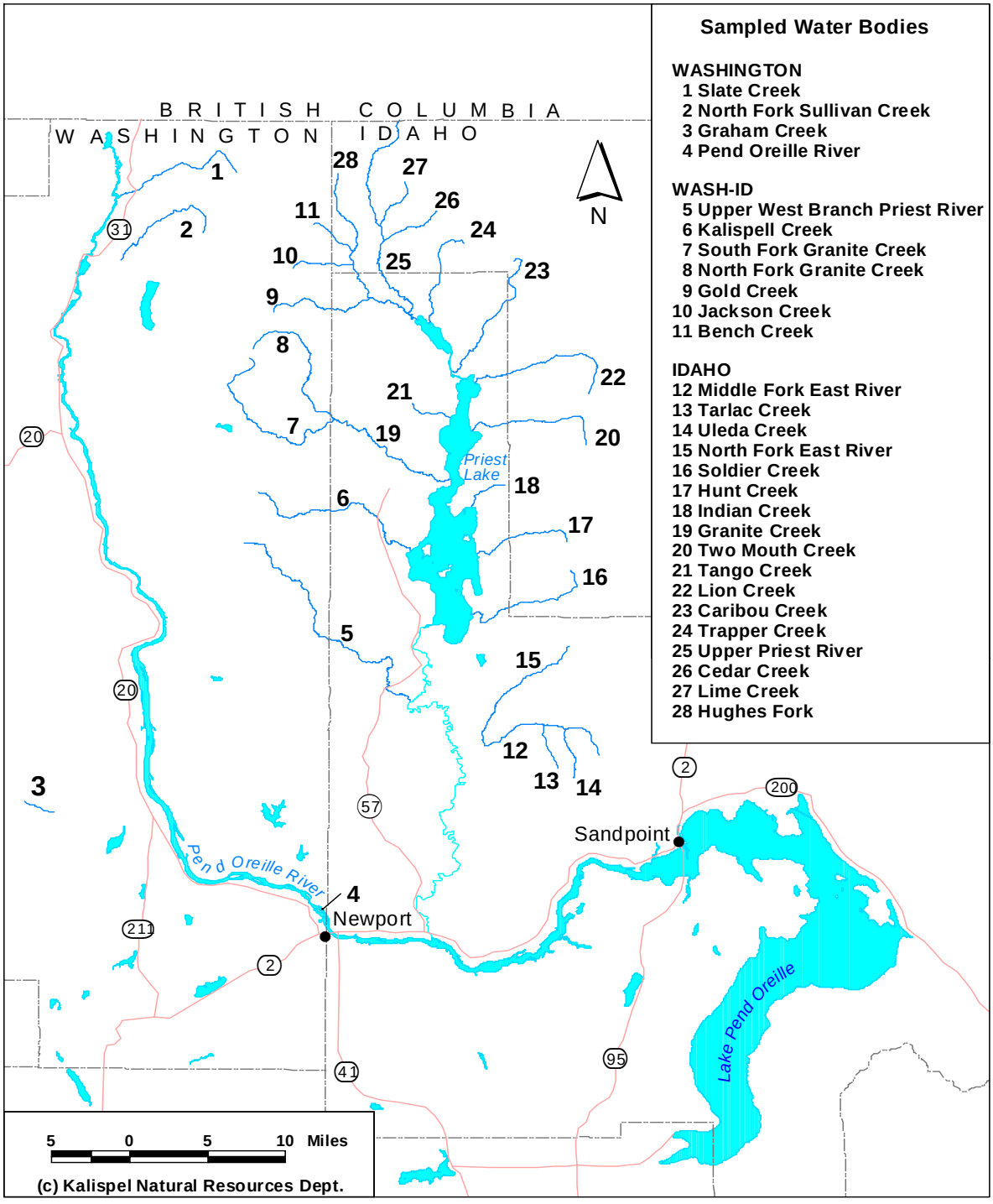

Figure 1. Streams where tissue samples were colleted in 2003.

Methods

For the three-year project, bull trout and westslope cutthroat trout will be obtained from sampling locations in the Pend Oreille and Priest Lake subbasins in Washington, Idaho, and Canada. In 2003, fish tissue samples were collected primarily through electrofishing. Both adults and juveniles were collected. In streams where fish were abundant, adults were preferred over juveniles. A maximum of 10 fish were collected per 100 meters of stream length, ensuring that the sample is representative of the population and does not contain many closely related individuals. If all samples are collected within close proximity, then the population structure may not be indicative of the entire stream. However, in some streams this was unavoidable due to the low numbers of fish collected throughout the majority of the stream. Typically in these situations, westslope cutthroat trout were isolated to headwater reaches.

Fish were collected using a Smith-Root LR-24 backpack electrofishing unit. Bull trout and westslope cutthroat trout were anesthetized with tricaine methanesulfonate (MS-222). A small piece of fin (approximately 1 mm^2) was removed and placed directly into a labeled vial containing absolute ethanol. Exact sample locations were determined using data collected with a Trimble GeoExplorer™ III. Locations were then downloaded and mapped with ArcView GIS. Total length (mm) and weight (grams) were measured for each fish.

In order to determine genetic relationships among populations and estimate genetic variation within and among those populations, microsatellite DNA analyses are being conducted. Thirteen loci are being used for bull trout and 24 loci are being used for westslope cutthroat trout (Tables 1 and 2). These loci include many of those previously screened in WDFW's laboratory for westslope cutthroat and bull trout using procedures established in WDFW's laboratory (Shaklee and Young, 2000).

Table 1. Bull trout loci screening protocol.

	Fluorescent label and locus screened		
multiplex	**6fam**	**vic**	**ned**
Sco A	*Sco-109*	*Sco-104*	*Sco-107*
Sco B	*Sco-106*	*Sco-103*	
Sco C	*Sco-110*	*Sco-102*	*Omm-1130*
Sco D	*FGT-3*	*Omm-1070*	*Sco-105B*
Sco E	*Omm-1128*	*Sco-105*	

Table 2. Westslope cutthroat trout loci screening protocol.

	Fluorescent label and locus screened		
multiplex	**6fam**	**vic**	**ned**
Ocl D2	*Ots-101*	*Ots-107*	*Ogo-3*
Ocl E2	*Sco-110*	*Omm-1146*	*One-2*
Ocl F2	*Omm-1138*	*Sco-103*	*Omy-325*
Omy B2	*One-102*	*One-114*	*Ots-100*
Omy C2	*One-108*	*Ots-103*	*One-101*
Omy D2	*Ots-101*	*Omy-77*	*Ots-3M*
Omy E2	*Omm-1130*	*Omm-1070*	*Omy-1011*
Omy F2	*Omy-1001*	*Oki-10*	*One-18*

Microsatellite DNA loci are arrays of short, repeated (mostly di- and tetra-nucleotide) sequences occurring commonly in eukaryotic organisms (Wright and Bentzen, 1994). Microsatellites are considered to be non-coding in that they are not known to be transcribed into RNA and, therefore, do not encode proteins. For this reason, allelic variation at most microsatellite DNA loci is assumed to be selectively neutral and are, therefore, considered to be good markers for evaluating gene flow and genetic relationships among populations. Microsatellite DNA variation typically exhibits bi-parental, Mendelian inheritance and alleles are co-dominantly expressed allowing an organism's genotype to be unambiguously inferred from its DNA phenotype. Additionally, microsatellites evolve rapidly and often exhibit many alleles (10-30) and high heterozygosities (0.50 - 0.90) (Wright and Bentzen, 1994). These characteristics make microsatellites very useful markers for investigating genetic aspects of population structure.

WDFW is collecting microsatellite data using an ABI-3730 automated DNA sequencer utilizing in-lane size standards to achieve a precision in size calling of

approximately 0.2 bp. DNA template preparations for all samples are being done using chelex treatment of cellular digestions with proteinase. The DNA loci of interest are being amplified and fluorescently labeled via the polymerase chain reaction (PCR).

Raw data from the DNA sequencer are being processed using Genemapper (v.3.0) software (PE Biosystems). The output tables from Genemapper are imported into MS Excel to create input files for statistical analysis.

Programs such as GENEPOP (Raymond and Rousset, 1995), FSTAT (Goudet, 2001), MSA (Dieringer and Schlotterer, 2003), MICROSAT (Goldstein et al., 1995), and ARLEQUIN (Schneider et al., 1997) will be used to analyze the resulting data.

Each fish is also being analyzed with regard to potential inter-specific hybridization (bull trout with brook trout and cutthroat trout with rainbow trout and between westslope and Yellowstone cutthroat). The data for 24 microsatellite DNA loci for the cutthroat collections will provide robust discrimination of hybrids (in part because preliminary data we have reveals that at least four loci (*Ots-101*; Ots-107*; Ogo-3*; and Oki-10**) are informative markers for westslope vs. Yellowstone and/or cutthroat vs. rainbow.

Results and Discussion

To date this project has collected a total of 2,216 westslope cutthroat and 489 bull trout. In 2002, 940 westslope cutthroat and 280 bull trout were collected. In 2003, 1,276 westslope cutthroat and 209 bull trout were collected. In 2004, samples will be collected in the Priest Lake and Pend Oreille watersheds. In 2005, samples will be collected in previous collection sites in order to detect any genetic drift within subpopulations. WDFW developed and applied microsatellite DNA screening protocols for the analysis of bull trout samples at 12 loci, of which 8 were new microsatellite DNA markers.

Past and ongoing genetic studies of bull trout population structure in the region have been hindered by the fact that many of the microsatellite DNA loci used by the three laboratories actively screening bull trout (Paul Spruell, Univ. of Montana; Eric Taylor, Univ. British Columbia; Sewall Young, WDFW) cannot be successfully screened in all laboratories. This situation has prevented the development of a shared, region-wide baseline. Secondly, the levels of allelic variation at many of the loci that have been

Table 3. Bull trout and westslope cutthroat samples collected in 2003.

State/Province	Subbasin	Watershed	Stream	Date	Westslope Cutt Number	Bull Trout Number	Archive Sample			
							No/Yes	Envelope	95%	Source
ID	Lower Pend Oreille	Pend Oreille River	Pend Oreille River	July-03	0	9	No			IDFG
ID	Priest Lake	Granite	North Fork Granite	July-03	50	6	No			KNRD
ID	Priest Lake	Granite	Granite	September-03	38	1	No			KNRD
ID	Priest Lake	Granite	South Fork Granite	July-03	50	0	No			KNRD
ID	Priest Lake	Granite	South Fork Granite (Lower)	August-03	35	0	No			KNRD
ID	Priest Lake	Tango	Tango	July-03	50	0	No			KNRD
ID	Priest Lake	Kalispell	Kalispell	August-03	50	0	No			KNRD
ID	East River	Uleda	Uleda	July-03	23	0	No			KNRD
ID	Priest Lake	Two Mouth	Two Mouth	July-03	50	0	No			KNRD
ID	Upper Priest Lake	Jackson	Jackson	July-03	50	0	No			KNRD
ID	Priest Lake	Lion	Lion	August-03	50	0	No			KNRD
ID	Upper Priest Lake	Gold	Gold	July-03	50	50	No			KNRD
ID	Upper Priest River	Lime	Lime	September-03	50	0	No			KNRD
ID	Upper Priest River	Cedar	Cedar	June-03	50	0	No			KNRD
ID	Priest Lake	Upper Priest River	Upper Priest River	September-03	50	50	No			KNRD
ID	Priest Lake	Indian	Indian	July-03	50	20	No			KNRD
ID	Priest River	East River	North Fork East River	July-03	50	0	No			KNRD
ID	Priest River	Priest River	Upper West Branch Priest River	August-03	50	0	No			KNRD
ID	Priest River	Tarlac	Tarlac	July-03	50	0	No			KNRD
ID	Priest Lake	Soldier	Soldier	July-03	50	0	No			KNRD
ID	Upper Priest Lake	Bench	Bench	August-03	50	0	No			KNRD
ID	Priest Lake	Hunt	Hunt	August-03	50	0	No			KNRD
ID	Priest Lake	Caribou	Caribou	August-03	50	0	No			KNRD
ID	Upper Priest Lake	Trapper	Trapper	August-03	50	0	No			KNRD
ID	Upper Priest River	Hughes Fork	Hughes Fork	July-03	50	3	No			KNRD
ID	East River	East River	Middle Fork East River	July-03	0	70	No			IDFG
WA	Pend Oreille River	Slate	Slate	August-03	50	0	No			KNRD
WA	Sullivan Lake	Sullivan	North Fork Sullivan	September-03	30	0	No			KNRD
WA	Pend Oreille River	Graham	Graham	June-03	50	0	No			KNRD

screened in bull trout have been low, providing relatively little power to discriminate populations and to identify interrelationships.

WDFW initiated the development of new microsatellite DNA markers for bull trout in an effort to provide new loci that could be routinely used by all labs in the region to develop consistent and standardized data sets and that would have increased information content. This initiative was successful in producing primers that amplify 7-8 new polymorphic loci in bull trout/Dolly Varden samples from several different regions of Washington (both east and west of the Cascade Crest) and for the collections currently available from the Pend Oreille basin (primarily Washington and Idaho, to date). In the coming year, we will be sharing these loci and the conditions for their analysis with other researchers in the region to encourage their use by all labs investigating bull trout population genetics.

WDFW developed and applied microsatellite DNA screening protocols for the analysis of cutthroat samples at 24 loci. WDFW believes that the data for 24 microsatellite DNA loci for the cutthroat collections will provide robust discrimination of hybrids (in part because preliminary data reveals that at least four loci (*Ots-101*; Ots-107*; Ogo-3*; and Oki-10**) are informative markers for westslope vs. Yellowstone and/or cutthroat vs. rainbow.

The WDFW is actively analyzing the resulting microsatellite DNA data to investigate population structure in the two target species. They will also be assessing each fish in each collection with regard to potential inter-specific hybridization (BT x BRT, WCT x RB, WCT x YCT). Repeat collections of both bull trout and westslope cutthroat trout from several localities may be done in the final year of this project. This is to assess the temporal stability/variability of the genetic profiles of the populations that are being investigated.

Literature Cited

Berghahl, J.C. 1998. Bull trout streams of the upper Columbia River basin of southeast British Columbia, with notes on the char's ecology and conservation biology. Northwest Biodiversity Center, Seattle, WA.

Buchanan, D.M., and S.V. Gregory. 1997. Development of water temperature standards to protect and restore habitat for bull trout and other cold water species in Oregon. Pages 1-8 *in* W.C. Mackay, M.K. Brewin, and M. Monita. Friends of the bull trout conference proceedings. Bull Trout Task Force (Alberta), c/o Trout Unlimited Calgary, Canada.

Dieringer, D. and C. Schlotterer. 2003. MICROSATELLITE ANALYSER (MSA): a platform independent analysis tool for large microsatellite data sets. Mol. Ecol. Notes 3:167-169.

Gayeski, N., B. McMillan, P.C. Trotter, P. Spruell, and A.Whiteley. 2001. Genetic And Phenotype Catalog Of Native Resident Trout Of The Interior Columbia River Basin Fy-99 Report: Populations Of The Pend Oreille, Kettle, And Sanpoil River Basins Of Colville National Forest, Report to Bonneville Power Administration, Contract No. 00004515, Project No. 199802600,

Goldstein, D.B., A. Ruiz-Linares, L.L. Cavalli-Sforza, and M.W. Feldman. 1995. An evaluation of genetic distances for use with microsatellite loci. Genetics 139:463-471.

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)

Howell, P.J., and D.V. Buchanan. 1992. Proceedings of the Gearhart Mountain bull trout workshop. Oregon Chapter of the American Fisheries Society, Corvallis, Oregon.

Raymond, M. and F. Rousset. 1995. GENEPOP (ver. 1.2): A population genetics software for exact test and ecumenicism. J. Heredity 86:248-249.

Rieman, B.E., and J.D. McIntyre. 1993. Demographic and habitat requirements for conservation of bull trout. USDA Forest Service, Intermountain Research Station. General Technical Report INT-302.

Rieman, B.E., and J. D. McIntyre. 1995. Occurrence of bull trout in naturally fragmented habitat patches of varied size. Transactions of the American Fisheries Society. 124: 285-296.

Schneider, S., J.-M. Kueffer, D. Roessli, and L. Excoffier. 1997. Arlequin ver. 1.1: A software for population genetic data analysis. Genetics and Biometry Laboratory, University of Geneva, Switzerland.

Shaklee, J.B. and S.F. Young. 2000. A microsatellite DNA-based analysis of population structure of cutthroat trout (Oncorhynchus clarki) in the Pend Oreille Basin in Washington. Unpublished WDFW report. (1pp + 7 tables and 2 figures).

Spruell, P., and F.W. Allendorf. 1997. Nuclear DNA analysis of Oregon bull trout final report. University of Montana, Missoula.

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 Fish 8:114-121.

U.S. Fish and Wildlife Service. 2000. 12-Month Finding for an Amended Petition To List the Westslope Cutthroat Trout as Threatened Throughout Its Range.

Williams, R.N., R.P. Evans, and D.K. Shiozawa. 1997. Mitochondrial DNA diversity in bull trout from the Columbia River basin. Proceedings of the friends of the bull trout conference. Calgary, Alberta.

Williams, K. 1998. Westslope cutthroat status in Washington. WDFW unpublished report.

Wright, J.M., and P. Bentzen. 1994. Microsatellites: genetic markers for the future. Rev. Fish Biol. Fish. 4:384-388.