MICROSATELLITE DNA CHARACTERIZATION OF SELECTED BULL TROUT (*SALVELINUS CONFLUENTUS*) POPULATIONS WITHIN THE PEND OREILLE RIVER BASIN

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ABSTRACT

We examined the genetic characteristics of 12 bull trout (*Salvelinus confluentus)* collections (sample size range: N = 20 to N = 70) from the Pend Oreille River Basin (Idaho, Washington, and British Columbia) using 14 newly developed homologous (i.e., primers derived directly from bull trout) microsatellite loci and one heterologous locus (i.e., developed from rainbow trout). Based on fixed allelic differences at several microsatellite loci (*Sco-102*, *Sco-104*, *Sco-107*, *Sco-110*, *Sco-202*, *Sco-215*, *Omm-1128*), hybridization between bull trout and non-native brook trout was observed to be minimal throughout the basin (N = 7 hybrids detected out of 476 fish screened). All hybrids displayed evidence of introgressed genotypes (i.e., no F1 hybrids were observed), suggesting that some F1 hybrids survive and can reproduce. However, sampling bias may have contributed to the limited number of hybrids observed, thus hybridization in the basin may be more common than the numbers indicate. Ten of 12 collections displayed deviations from Hardy-Weinberg equilibrium (HWE) proportions, which is likely the result of either ad-mixed collections (due to sampling during non-spawning time) or small effective population sizes. Collections of bull trout displayed heterozygosity levels higher than previously reported based on the screening of other loci; however they were still consistently lower than levels reported in other salmonids. Low levels of heterozygosity (H_E = 0.497 – 0.602) and allelic richness (A_O = 3.52 – 4.75) were more pronounced in upper tributary and/or small creek collections than in lower reach tributaries and larger watercourses (H_E = 0.619 – 0.726; A_O = 5.09 – 6.47) and are likely the result of smaller effective population sizes in upper tributaries and small streams and/or anthropogenic perturbations to bull trout habitat. In general, population structure followed geographic structure: collections within regions were more similar genetically than collections among regions, suggesting little or no gene flow among regional bull trout collections within the basin. Furthermore, fine-scale population genetic structure was evident among collections within regions implying a strong degree of demographic independence.

INTRODUCTION

Assessment of extinction risk among populations is one of the fundamental problems of conservation biology (Dunham et al. 1999). The development of reliable, yet sensible, methods to assess extinction risk is an important area of research (Simberloff 1988, Mace & Lande 1991; Boyce 1992; Taylor 1995). Extinction risk has been classically evaluated in terms of ecological and demographic risk factors (e.g., Caughley 1994). For example, data-rigorous procedures such as population viability analysis (Beissinger & Westphal 1998) have been found to be useful when extinction risk of only one or a small number of populations was in question. However, when species consist of several populations that may be declining on a local and/or regional basis, the extinction risk of many populations must be considered.

Genetic analysis of population structure focuses on two aspects of genetic variation – local adaptation and genetic diversity, which are imperative elements for effectively developing species/population management guidelines. In other words, understanding the distribution of genetic variation within and among geographically isolated populations is crucial for species conservation. Without an explicit definition of populations, management actions could be unfavorable to some populations as a result of initiating gene flow between historically isolated and genetically disparate populations (Spruell et al. 2003). Effective management of a species, particularly one living on the brink of extinction, requires an understanding of all life history characteristics and habitat requirements, as well as the genetic characterization of at least the populations (Spruell et al. 1999) comprising the species.

Bull trout (*Salvelinus confluentus*) are a char native to the Pacific Northwest of the United States and Canada. Historically, bull trout were found to inhabit major river drainages as far south as the McCloud River in northern California (Behnke 2002), (recognized as extinct in the 1970s – Behnke 2002), to as far north as the headwaters of the Yukon and Mackenzie River basins of Alaska and Canada (Behnke 2002). They display a diverse array of life history strategies (i.e., a polytypic species), which include: (1) a resident freshwater type that completes its life cycle in small streams; (2) an adfluvial type that migrates to and from lakes; and (3) a fluvial type that migrates from

small spawning and rearing streams to larger mainstem rivers to feed and mature (Pratt 1992 – *In* Spruell et al. 2003).

Bull trout were largely neglected as an important fish species in need of biodiversity conservation prior to the 1990s (Epifanio et al. 2003). Several current threats to bull trout viability have been directly linked to bull trout declines throughout their native range in the United States and Canada (e.g., Johnson 1987; Buchanan et al. 1997; USFWS 1998, 1999). These threats include: (1) degradation/loss of stream spawning habitat as a result of land-use practices; (2) fragmentation of migratory routes (i.e., due to hydropower dams); (3) ecological interactions with non-native fish (Epifanio et al. 2003); and (4) hybridization with non-native brook trout (Kanda et al. 2002). Due to the severe declines, bull trout have been listed as threatened by the U.S Fish and Wildlife Service (USFWS) under the U.S. Endangered Species Act (USFWS 1999). Past and current efforts to effectively develop management and recovery strategies (i.e., assess, protect, and restore) for existing bull trout populations have been limited by a lack of basic information regarding the ecology, life history, and genetics of the species (Howell and Buchanan, 1992; Rieman and McIntyre, 1995; Buchanan et al. 1997; Spruell and Allendorf, 1997).

Here, we investigate population genetic relationships of 12 bull trout collections within the lower Pend Oreille River Basin (Priest River and below its confluence), utilizing microsatellite DNA loci. To our knowledge, there have been no previous investigations of bull trout population structure in this basin. Microsatellite analyses of bull trout populations in other areas have been performed previously (e.g., Spruell et al. 1999; Neraas and Spruell 2001; Spruell et al. 2003); however, these studies were limited in the number of loci screened and their degree of polymorphism. The lack of polymorphism may be the result of the heterologous nature of these microsatellites. This could lead to a misrepresentation of the genetic variability because the regions of DNA amplified by these heterologous genetic markers (although likely variable within the species they originated – i.e., homologous markers) may not be highly variable within the bull trout species. To address these issues, this study utilizes a suite of 14 newly developed, homologous microsatellite loci (DeHaan & Ardren 2005; Bettles et al., unpubl.), along with one heterologous locus developed from rainbow trout. Microsatellite

DNA loci are arrays of short, repeated (mostly di-, tri-, and tetra-nucleotide) sequences occurring commonly in eukaryotic organisms (Wright and Bentzen, 1994). They are considered 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 these loci 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. These characteristics make microsatellites very useful markers for investigating genetic aspects of population structure.

We initially conducted an investigation for potential hybridization (and/or introgression) between non-native brook trout and native bull trout within each collection, because brook trout have been extensively stocked within the Pend Oreille River Basin (J. Maroney, pers. comm.). After identifying and subsequently removing hybrid individuals from the data set, we proceeded to investigate the population structure of bull trout among collections and among sub-basins within the basin. In conclusion, we discuss conservation implications for bull trout and stress the importance of understanding the scale at which bull trout population divergence (and presumed reproductive independence) exists.

MATERIALS AND METHODS

Pend Oreille River Basin - Geography: The Pend Oreille River originates in Lake Pend Oreille, which is fed by the Clark Fork River (Fig. 1). The headwaters of the Clark Fork River, which is the primary tributary to Lake Pend Oreille, are located in the Rocky Mountains in Montana. The Pend Oreille River flows into the Columbia River in British Columbia north of Washington State. There are several dams on the Pend Oreille - Clark Fork River system in Montana, Idaho, Washington, and British Columbia. In Washington, dams are located near the international boundary with Canada and at Box Canyon near Metaline Falls (Dames and Moore Inc. 1995). The Pend Oreille River upstream of Box Canyon Dam to Albeni Falls Dam (just east of the Washington border in

Idaho) is known as the Box Canyon Reservoir or Box Canyon Reach. This section of the river is characterized as a slow moving "run of the river" reservoir operated by the Pend Oreille County Public Utilities District (Dames and Moore Inc. 1995). Albeni Falls Dam (operated by the U.S. Corps of Engineers) is located approximately 7 river kilometers downstream from the confluence of the Pend Oreille and Priest rivers and is a complete barrier to fish passage (Dupont and Horner 2003). Pend Oreille Lake is located about 37 river kilometers upstream of the confluence of the Pend Oreille and Priest rivers with no barriers to fish migration between these locales. The Priest River, a main tributary to the Pend Oreille River, begins at the outlet of Priest Lake and joins the Pend Oreille River approximately 71 kilometers downstream. In addition, a water dam is situated at the outlet of Priest Lake and it is believed to be a barrier to fish passage (Dupont and Horner 2003). Based on reports from local residents, bull trout were regularly caught in the Priest River just downstream of Priest Lake (J. Dupont, pers. comm.), which indicates that bull trout may have historically entered the Priest River to forage or to commence spawning migrations.

Sampling: Bull trout samples (N = 20 – 70 per collection) were collected from streams within the Pend Oreille River and Priest River/Lake sub-basins located within Idaho, USA and from the Salmo River sub-basin in British Columbia, Canada (Fig. 1). Samples were collected from June through September (refer to Table 2). Tissue samples were collected from adult and juvenile fish, captured primarily by electrofishing. For streams where fish were abundant, adults were preferentially sampled over juveniles. The intent was to sample a maximum of 10 fish per 100 meters to provide a representative sample of the population and avoid collecting all samples within close proximity. In some instances, however, fish specimens were collected in closer proximity due to low numbers of fish observed throughout entire stream catchments. A small piece of fin (approximately 1 cm^2 or less) was removed and placed directly into a labeled vial containing absolute ethanol. Two separate collections were made on the Middle Fork East River, Idaho (one in 2002 and one in 2003) and these have been analyzed as separate collections. Sampling on Uleda Creek also took place in both 2002 and 2003. Since the numbers of samples collected from Uleda Creek in each year were small, we combined

samples from both years (see Table 2). Upper Priest Lake was sampled in 1998 and 1999 and, like Uleda Creek, samples from both years were combined (see Table 1). Total length (mm) was also measured for each fish. Archived tissue samples from the Priest River/Lake region were obtained from Idaho Department of Fish and Game (IDF&G).

Molecular Protocols: Genomic DNA was extracted by digesting a small piece of fin tissue using a silica membrane based kit (Machery-Nagel) using the following conditions: incubate tissue fragment 6 hours to overnight at 56^{o}C in 200 µL proteinase K solution, add 200 µL Buffer B3 and 200 µL 100% ethanol, mix and transfer the supernatant into a Tissue Binding Plate containing the silica binding membranes, centrifuge 10 minutes, add 500 µL Buffer BW, centrifuge 2 minutes, add 700 µL Buffer B5, centrifuge 4 minutes, place Tissue Binding Plate on a collection rack, incubate 10 minutes at 70^{o}C to remove residual ethanol, add 100 µL Buffer BE (elution buffer) at 70^{o}C, incubate 1 minute, centrifuge 2 minutes, dispose of Tissue Binding Plate, refrigerate eluted DNA or store at –20^{o}C.

Polymerase chain reactions (PCR) were performed using a standard 10 µL reaction that contained: approximately 1 µL of template DNA, 1X Promega buffer, 1.5 mM $MgCl_2$, 200 µM each of dATP, dCTP, dGTP, and dTTP, 0.035 – 0.25 µM of each oligonucleotide primer (refer to Table 1), and 0.05 units *Taq* polymerase (Promega). Amplifications were performed using MJ Research PTC-200 thermocyclers. The thermal profile was as follows: an initial denaturation step of 3 minutes at 95^{O}C; 29 - 35 cycles of 15 seconds at 95^{O}C, 30 seconds at 47 - 60^{O}C (see Table 1), and 1 minute at 72^{O}C; plus a final extension step at 72^{O}C for 30 minutes, followed by a final indefinite holding step at 4^{O}C.

Fifteen microsatellite DNA loci (markers) were amplified via PCR using fluorescently labeled primers with 5´ vector-based tails (obtained from Applied Biosystems and Integrated DNA Technologies Inc.; see Table 1) (tailing protocol is available by request). Microsatellite data was collected using an ABI-3730 48-capillary automated DNA sequencer. Applied Biosystems software (ABI-Collection, Genemapper v.3.0) was used to collect and analyze the raw data and to estimate microsatellite sizes in

base pairs. The output tables from Genemapper were imported into MS Excel where final genotyping (allele binning and naming) was accomplished using *MicrosatelliteBinner* v.1h (S.F. Young, WDFW personal communication; available from the author). *MicrosatelliteBinner* creates groups (bins) of alleles with similar mobilities (alleles with the same number of repeat units). The upper and lower bounds of the bins were determined by identifying clusters of alleles separated by gaps (nominally 0.4 base pairs in size) in the distribution of allele sizes. The bins were then named as the mean allele size for the cluster rounded to an integer.

Data Analysis

Detection of Hybrids: Bull trout in each collection were initially tested for potential inter-specific hybridization with brook trout. Brook trout have been widely planted within the Pend Oreille River Basin (J. Maroney, pers. comm.) and have been previously identified as a contributor to the decline of native bull trout populations in some areas (see Kanda et al. 2002; Epifanio et al. 2003; Spruell et al. 2003). Several pure brook trout collections (Malheur River Oregon N = 55; South Pine Creek, Iowa N = 32; Penny Creek, Washington N = 12; were screened concomitantly with bull trout samples. Several of the microsatellite loci we screened (i.e., *Omm-1128, Sco-102, Sco-104, Sco-107, Sco-110, Sco-202, and Sco-215*) have been previously found to display fixed allelic differences between bull and brook trout; thus they are effective for identifying hybrids (P. DeHaan, pers. comm.; WDFW, unpubl. data). Not only does identifying hybrids give an indication of the genetic integrity (or lack thereof) of native bull trout populations, but it also can limit error in population structure analyses.

All fish were scored as homozygous bull trout, homozygous brook trout, or heterozygous at each of the seven diagnostic microsatellite loci. Fish that were identified as homozygous at all seven loci for one species were categorized as pure-type. First-generation (F1) hybrid fish would be expected to be heterozygous at all seven loci, while backcross and higher-order hybrid fish were those individuals having a mix of homozygous and heterozygous marker loci.

Genetic Characteristics of Bull Trout: All loci (see Table 1) were initially screened using the software program MICROCHECKER v. 2.2.0 (van Oosterhout et al. 2003) to detect for potential null alleles. Measures of within-population genetic diversity were calculated using the following statistical programs: observed and expected heterozygosity (H_O and H_E respectively) were calculated using GDA v. 1.1 (Lewis & Zaykin 2001) (see Table 2), allele frequencies for each collection were calculated using CONVERT v.1.3 (Glaubitz 2003) (see Table 2), and allelic richness (A_O) was determined using FSTAT v.2.9.3.2 (Goudet 2001). Weir and Cockerham's (1984) inbreeding coefficient (f or F_{IS}) was computed for each locus and across all loci for each collection using GENEPOP v. 3.4 (Raymond and Roussett 1995) (see Table 3). Tests for Hardy-Weinberg equilibrium (HWE) (i.e., indication of non-random mating or small population size) were performed at each locus (Table 3), and over all loci (Table 2), for each collection also using GENEPOP. The resulting estimates of the probability of departure from HWE were tested for significance using a Bonferroni correction (Rice 1989) to account for multiple simultaneous tests (12 collections x 15 loci = 180 comparisons). Linkage disequilibrium between loci in each collection was evaluated using GENEPOP. Linkage disequilibrium (i.e., gametic phase disequilibrium) between pairs of loci may be a more sensitive indicator of non-random mating or admixture (than HWE), as it requires more generations to deteriorate (Campton & Utter 1985). With 15 loci, there were 105 different two-locus combinations to evaluate. Critical significance levels for simultaneous tests were again conducted using a Bonferroni correction ($\alpha = 0.000476$).

Relationships among collections were examined utilizing pairwise tests. Collections were tested for differences in genotypic distributions (i.e., genotypic differentiation) at each locus and over all loci using a G-test implemented in GENEPOP (200 batches, 2000 iterations) with significance evaluated using a Bonferroni correction. Pairwise multi-locus F_{ST} comparisons (a measure of population divergence) were evaluated using ARLEQUIN v.2.0001 (Schneider et al. 2000). Measures of F_{ST} were also estimated for all collections and for each sub-basin using GENEPOP. A series of AMOVA (Analysis of Molecular Variance) tests (Excoffier et al. 1992) were performed using ARLEQUIN to calculate Wright's F-statistics (Wright 1978) in order to investigate regional structuring of genetic variation within the lower Pend Oreille River Basin.

Grouping of collections corresponded to their geographic locations (Salmo River (BC), Upper Priest River (ID), Lower Priest River (ID)).

Genetic relationships among collections were also explored using a dendrogram analysis. Pairwise Cavalli-Sforza & Edwards (CSE) chord distances were generated from allele frequencies using the computer software program CONVERT (Glaubitz 2003). A neighbor-joining (NJ) tree (i.e., cluster analysis) was then created utilizing tools within the PHYLIP software program (Felsenstein 1993). The program SEQBOOT was utilized to test the repeatability of 1,000 tree branch replicates (i.e., bootstraps), which were then analyzed using GENDIST. Dendrogram topologies were created for replicates using the NEIGHBOR program and a consensus tree was created in CONSENSE. Trees were visualized, along with associated bootstrap values, using the TREEVIEW v.1.4 program (Page 1996).

RESULTS

Hybridization with Brook Trout: Seven microsatellite markers (out of 15 screened) that displayed fixed allelic differences (i.e., 100% diagnostic) between bull trout and brook trout (see Fig. 2 for example) were utilized to detect hybridization. A total of seven hybrids (out of 476 fish screened) were identified from four of the 12 collections. No hybrids were detected in the remaining eight collections. Of the seven hybrids identified, five were found within the MF East River collections (four from MF East River–b (2002) and one from MF East River–a (2003) respectively), one hybrid was identified in the Upper Priest River collection, and one hybrid was identified in the Upper Priest Lake collection. None of the collections contained F1 hybrids suggesting genetically pure bull trout and brook trout have not interbred recently or is a reflection of sampling bias towards pure bull trout. The presence of more than one backcross genotype, however, indicates that introgression is likely ongoing. All seven fish identified as hybrids were excluded from further statistical analyses.

Within Collection Characteristics: Of the 15 loci screened, none displayed evidence for possible null alleles. All loci displayed polymorphism ranging from 4 (*Sco-102*) to 29 (*Sco-104*) alleles per locus (Table 1). Observed heterozygosity (H_O) estimates varied, yet

displayed low to moderate levels across collections (range: 0.465-0.719) (Table 2). Estimates of allelic richness (A_O), averaged across all loci, ranged from a low of 3.52 (Indian Creek) to a high of 6.46 (Upper Priest Lake) with a mean allelic richness of 5.23 over all collections. Tributaries located in the upper reaches of drainages as well as independent small creeks displayed much lower allelic diversities than collections from larger rivers or collections located in lower reaches of a given drainage (see Table 2). For example, Clearwater Creek exhibited lower allelic richness (A_O = 3.90) than the upper Salmo River (A_O = 4.64). Additionally, Sheep Creek (A_O = 5.09) and the South Salmo River (A_O = 5.81) (see Fig. 1) have more comparable allelic richness estimates to the lower Salmo River (A_O = 5.47) collection, yet are much higher than the allelic richness estimates observed in the upper Salmo River and Clearwater Creek. A similar pattern was observed in the lower Priest River drainage – Uleda Creek, a tributary to the MF East River (see Fig. 1) exhibited much lower allelic diversity (A_O = 4.75) than both collections from the MF East River (A_O = 5.93 & 6.17 respectively – Table 2). These patterns suggest potential small effective population sizes in upper reach tributaries and small creek drainages. Collections from lower reaches may also receive allelic contributions from strays, which may explain the increased allelic diversity in these collections.

HWE and Linkage Disequilibrium: Genotypic frequencies departed substantially from Hardy-Weinberg expectations in many cases. Significant deviations from the expected proportions ($P < 0.05$) were almost five times greater (40 of 180 tests) than the expected 9 of 180 comparisons projected to exceed an alpha level of $P < 0.05$ by chance alone. Furthermore, deviations from HWE expectations were detected in 13 of 180 locus-by-collection tests after Bonferroni adjustment, a likely indication of small effective population sizes or admixed collections. Inbreeding coefficients calculated over all loci (F_{IS} Overall – Table 3) demonstrated significant heterozygote deficiencies ($F_{IS} > 0$) in nine collections that deviated from HWE before Bonferroni adjustment. These deviations suggest that these populations may have experienced some level of inbreeding, which would likely result of small effective population sizes sustained over several generations or an admixture of gene pools. No loci were observed as the main cause for the HWE deviations as would be expected if deviations were due to null alleles.

There was no evidence of physical linkage between any loci used in this study, however significant linkage disequilibrium was detected in several collections. Most collections (lower Salmo River, South Salmo River, MF East River–b, Indian Creek, Upper Priest River, Upper Priest Lake) exhibited relatively low levels of linkage disequilibria (i.e., < 5%; see Table 2). Three collections (upper Salmo River, Clearwater Creek, and Sheep Creek) displayed no linkage disequilibria (i.e., 0%), while three collections (Uleda Creek, MF East River–a, and Gold Creek) exhibited disequilibrium >10% (Table 2). One locus pair *Sco-104*/*Sco-106* was found to be in linkage disequilibria in four collections: lower Salmo River, South Salmo River, MF East River–a, and Gold Creek. The observation that linkage disequilibria were inconsistent across collections indicates that disequilibria may be due to small effective population sizes or the result of admixed collections.

Genetic Differentiation and Population Structure: General patterns of genetic divergence were apparent from the distribution of alleles throughout the Pend Oreille River system (Appendix I). Several alleles (at given loci) were either fixed or at high frequencies in particular sub-basins. For example, *Sco-106**233 (allele *233* at locus *Sco-106*) was only observes in Salmo River collections (i.e., upper Salmo River, lower Salmo River, South Salmo River, Clearwater Creek, Sheep Creek). The allelic frequency for *Sco-107**314 was substantially higher in the MF East River and Uleda Creek, than in other collections within the Priest River sub-basin (i.e., Gold Creek, Upper Priest River). Additionally, *Sco-213**189 was observed to be at relatively high frequency in collections from the Upper Priest sub-basin (Gold Creek, Indian Creek, Upper Priest River, and Upper Priest Lake).

Allelic distributions among collections within regions also exhibited fixed differences at particular loci (Appendix I). Several alleles at locus *Sco-202* (alleles *121*, *125*, *129*, and *133*) were observed in both MF East River collections, but not in Uleda Creek (a tributary to the MF East River). Alleles *271* and *275* at locus *Sco-201* were also observed in MF East River collections, but were absent from Uleda Creek. Similarly, *Sco-106*209* was observed in the lower Salmo River, South Salmo River, and Sheep

Creek collections, but was absent from the upper Salmo and Clearwater Creek collections.

Pairwise genotypic tests demonstrated heterogeneity in genotype distributions among all collections (Table 4). Interestingly, the two collections from the MF East River exhibited highly significant genetic differentiation, suggesting the samples were not drawn from a single population. Measures of genetic differentiation (pairwise F_{ST}) among collections exhibited a couple of interesting trends (Table 4). First, strong population differentiation was exhibited between regional (i.e., geographically isolated river sub-basins) collections that generally correspond to the geographic locations from which samples were collected. Pairwise F_{ST} estimates between collections from the Salmo River sub-basin and the Upper Priest River sub-basin displayed much stronger genetic differences (e.g., lower Salmo River vs. Gold Creek; $F_{ST} = 0.326$; Clearwater Creek vs. Upper Priest River; $F_{ST} = 0.293$), than pairwise F_{ST} estimates between collections from within the same sub-basin (e.g., lower Salmo River vs. Sheep Creek; $F_{ST} = 0.025$; Upper Priest River vs. Gold Creek; $F_{ST} = 0.079$), an indication of little or no gene flow between sub-basins (Table 4). Second, collections within sub-basins were identified as genetically differentiated through genotypic tests (Table 4); however, they were much more genetically similar to one another than those in different sub-basins, a possible indication of historic gene flow between collections within sub-basins. Genotypic tests differentiated the upper Salmo River and Clearwater Creek collections; however, measures of population differentiation (F_{ST} estimates) identified them as having low genetic differentiation (pairwise $F_{ST} = 0.009$), suggesting Clearwater Creek could be a recently diverged population via genetic drift. The measure of genetic differentiation between the Upper Priest Lake and the Upper Priest River collections was very low (pairwise $F_{ST} = 0.000$); however, tests of genotypic differentiation identified the collections as significantly different (see Table 4).

The estimate of F_{ST} for all 12 collections combined was 0.213; however, the genetic diversity within sub-basins varied from 0.060 in the Salmo sub-basin to 0.130 in the lower Priest River sub-basin. All F_{ST} values for each region were significantly larger than zero at the 95% confidence interval.

Significant genetic structure was exhibited using AMOVA tests (Table 6). AMOVA results displayed significant genetic structure (F_{ST} = 0.253 over all loci). AMOVA also demonstrated that while a larger proportion of the genetic variation is found within collections (~ 75%), bull trout of the Pend Oreille River Basin exhibited significant structure at the inter- and intra-sub-basin level (Table 6). Estimates of among-sub-basin (F_{CT} = 0.154) and within-region (F_{SC} = 0.110) genetic differentiation was statistically significant ($P < 0.05$) for the three sub-basin groupings.

Cavalli-Sforza and Edwards chord distance (N-J dendrogram) analysis produced three genetically distinct clusters (with 100% bootstrap support) that correspond to the geographic proximity of sample locations (see Fig. 3). Collections from the Upper Priest region (Indian Creek, Upper Priest River, Upper Priest Lake, Gold Creek) formed one discrete cluster, however displaying only moderate bootstrap support among the collections. The Salmo River collections formed a second discrete cluster (Clearwater Creek, upper Salmo River, lower Salmo River, Sheep Creek, South Salmo River) (Fig. 3). Strong bootstrap support was also observed at all branches within the cluster suggesting genetically distinct collections within this region (Fig. 3). Lower Priest River collections (MF East River-a, MF East River-b, Uleda Creek) formed the third genetically discrete cluster, which also displayed relatively strong bootstrap support among collections. The two MF East River collections were genetically distinct from Uleda Creek (66% bootstrap support). Interestingly, however, both MF East River collections also appear to be genetically distinct from one another (79% bootstrap support).

DISCUSSION

The Pend Oreille River system gave a unique opportunity to effectively address two major areas of concern that directly relate to the conservation of bull trout populations: (1) the impact of hybridization (and/or introgression) with non-native brook trout on native bull trout populations; and (2) the degree of genetic population structure among bull trout within a basin where historical habitat availability and connectivity are heavily restricted (i.e., the presence of dams).

Interspecific Hybridization: All seven hybrids detected in this study were post-F1 (i.e., backcross or higher order generation hybrids). It is likely that we have misidentified some higher-order hybrids as pure-type, since with seven co-dominant markers Boecklen & Howard (1997) estimated an approximate 12% error rate in the second backcross generation. Thus, the number of post-F1 hybrids could be higher than what our data indicate. Also, because bull trout were targeted according to phenotypic characteristics during field sampling for this project (i.e., excluding all recognized brook trout) the number of hybrids (F1 and post-F1) in these populations could be higher. Kanda et al. (2002) identified F1 hybrids from western Montana (i.e., Flathead, Swan, Bitterroot River drainages) that were produced by male bull trout and female brook trout as well as male brook trout and female bull trout, with subsequent post-F1 hybrids comprised of backcrossing to both parental species. The fact that the multilocus genotypes of the post-F1 hybrids we detected were comprised mainly of bull trout alleles, suggests that introgression is occurring, in part, with pure bull trout. Since the sampling design for this study excluded phenotypic brook trout, restricts our ability to accurately assess the true nature of introgression within the basin (i.e., extent of introgression with brook trout).

The low level of observed hybrids in this study also suggests that there could be some level of reduced fitness in the hybrids. However, the fact that some hybrids do survive to sexual maturity and are reproductively viable can have serious detrimental effects to the genetic integrity of existing, already small bull trout populations.

Utility of Microsatellites and Genetic Variation within Bull Trout Populations: Previous genetic studies have identified bull trout as generally containing low levels of genetic variation (e.g., Leary et al. 1993; Neraas & Spruell 2001; Taylor et al. 2001; Spruell et al. 2003; Costello et al. 2003). Our data displayed overall patterns of moderate genetic variation within bull trout – with values substantially higher than those reported elsewhere. However, despite our results, bull trout genetic diversity still appears to be substantially lower than that observed in other salmonids (see Small et al. 2004). Collections from the Salmo River, British Columbia sub-basin exhibited average levels of genetic variation (H_E = 0.602 – 0.712; A_O = 3.90 – 5.81). A similar pattern was also

observed in collections from the Upper Priest River sub-basin (H_E = 0.497 – 0.619; A_O = 3.52 – 6.46) and the lower Priest River sub-basin (H_E = 0.634 – 0.726; A_O = 4.75 – 6.17). The heterozygosity levels at microsatellite loci (from this study and others) could potentially result from intrinsically low mutation rates, given that microsatellites are not always necessarily hypervariable (e.g., Schug et al. 1997). Alternatively (and a more likely explanation), the low genetic variability could result from demographic processes that have reduced effective population sizes in bull trout historically following postglacial dispersal (Taylor et al. 2001). A reduction in population size, by means of bottlenecks or founder events, may have eliminated considerable genetic variation, which has not yet been recovered via mutation (cf. Schug et al. 1997) or, simply, bull trout population sizes have been reduced over the last 10,000 years as a result of increased water temperatures in inter-glacial refugia.

The increased genetic diversity observed in this study, in comparison to other bull trout genetic studies, may also be attributed to unique geographic and habitat characteristics for each region identified in the Pend Oreille River system. Spruell et al. (2003) observed consistent levels of heterozygosity (H_E – 0.241-0.372) within Pend Oreille Lake tributaries (see Fig. 1), attributing the variation to the abundance of large natural lakes within the basin that may have allowed populations to persist at elevated numbers compared to those confined to small stream networks. Not only do numerous large lakes exist throughout the lower Pend Oreille River Basin, but also there are larger rivers characteristic to the basin that may facilitate the persistence of stable bull trout populations.

It is important to consider that the discrepancies in bull trout genetic variation between our study and others (e.g., Neraas & Spruell 2001; Taylor et al. 2001; Spruell et al. 2003; Costello et al. 2003) may be largely due to the screening of different microsatellite DNA loci in the different studies. Until recently, the majority of research investigating bull trout population genetic dynamics has relied upon cross-species amplification of genetic markers (i.e., heterologous microsatellite loci). Even at heterologous loci, however, other salmonid species tend to show substantially greater microsatellite variability than do bull trout (see Brunner et al. 1998 – arctic char; Douglas et al. 1998 - whitefish). Loci used for this study were developed to meet several criteria:

(1) ability of homologous primer sets (i.e., primers developed directly from bull trout) to achieve reliable and robust amplification and minimize null alleles; (2) high level of allelic diversity and heterozygosity; (3) increased number of useful loci; and (4) ease and reliability of allele scoring. Though levels of microsatellite allelic diversity and heterozygosity in our study were higher than those reported by previous microsatellite studies of bull trout (Kanda and Allendorf 2001; H_E = 0.207 to 0.486; Neraas and Spruell 2001; H_E = 0.269 to 0.472; Taylor et al. 2001; H_E = 0.270 to 0.480), the genetic diversity was still low, in comparison to other salmonids, and likely a characteristic of bull trout as a species. Furthermore, the scale at which genetic diversity exists for bull trout in the Pend Oreille Basin appears to follow the description previously illustrated by Costello et al. (2003): intrapopulation genetic variation within bull trout is particularly low, but interpopulation genetic variation (i.e., genetic divergence) among bull trout is remarkably high. Even though previous microsatellite genetic studies of bull trout have uncovered valuable information regarding the species (utilizing limited molecular markers), we emphasize the need for a standardized approach for the use of microsatellite markers. This will allow for greater consistency across bull trout studies to effectively assess the genetic architecture and population interrelationships of the species.

Genetic Variation among Collections and Sub-basins: Despite the relatively low to moderate allelic variation at microsatellite loci in bull trout, this study generated key findings displaying substantial genetic variation at two levels: (1) among collections within sub-basins and (2) strong sub-basin disparity.

Fundamentally, population differentiation among bull trout appears to occur at a local geographical scale, as all intra-regional tests (except for one test) of population differentiation exhibited significantly high levels of population divergence (see Table 4). Additionally, Pend Oreille bull trout displayed a very high level of genetic differentiation (Table 5) – the estimate of F_{ST} over all twelve collections was high (0.213). The fine-scale population structure and strong genotypic differentiation among collections is consistent with the hypothesis that bull trout populations are generally small and are primarily influenced by genetic drift because of very low levels of gene flow between populations from geographically distant regions as well as limited gene flow among

populations within sub-basins. This inference is also consistent with the current existence of numerous impassable barriers (i.e. dams) throughout the basin, which can be expected to eliminate historical corridors of migration and gene flow, thereby increasing the fragmentation of bull trout population structure.

Additionally, our data indicate that populations in very close geographic proximity can be almost completely reproductively isolated (e.g., MF East River vs. Uleda Creek, South Salmo River vs. upper Salmo River) even without the presence of physical barriers. This could be attributed to strong native site fidelity by bull trout as a result of environmental cues (i.e., homing signals, stream temperature). As gene flow decreases among populations, the likelihood of bull trout adapting to local environments should increase since, over time, even relatively weak selective differences can be efficient at developing locally adaptive characteristics (Kanda & Allendorf 2001). Therefore, it is likely that bull trout populations are locally adapted, which would act as a type of reproductive isolating mechanism.

Allelic richness data indicated that small creeks, whether tributaries to larger rivers (i.e., Clearwater Creek, Gold Creek, Uleda Creek) or large lakes (i.e., Indian Creek) exhibited lower allelic diversity, compared to other collections that were located in lower reaches (i.e., Sheep Creek, South Salmo River, MF East River, Upper Priest River). This again provides further evidence that these populations may have been founded by a small number of immigrants and/or that they have low abundances and high levels of genetic drift. However, with low genetic variation (as it appears to be in bull trout), genetic drift could lead to a few populations (e.g., MF East River vs. Uleda Creek) having high frequencies of rare alleles (Kanda and Allendorf 2001). Thus, such drift could exaggerate genetic differentiation among populations and mask the effect of historical gene flow. Therefore, it is pertinent to be cautious about inferring population relationships (Kanda and Allendorf 2001).

Analysis of molecular variance (AMOVA) results demonstrates that genetic divergence is greater among sub-basins than between collections of bull trout within sub-basins (Table 6). This result is not entirely surprising, again, given the substantial number of physical migration barriers that exist in the Pend Oreille River Basin (see Fig. 1) as well as the moderate migration behavior of bull trout and rigid spawning site fidelity (see

Dupont & Horner 2003; Bahr & Shrimpton 2004). The N-J dendrogram further supports the presence of strong genetic distinctness among sub-basin bull trout collections (see Fig. 3). The presence of migration barriers throughout the basin has likely played a role in preventing potential gene flow between sub-basin bull trout populations and thereby contributed to the continuous decline of bull trout populations in the Pend Oreille River Basin as a result of habitat loss (i.e., habitat destruction and/or loss of connectivity) (see Scholz et al. 2005). Over time, habitat loss and reduced connectivity of bull trout populations would be expected to lead to the extinction of some existing populations and diminish the founding of new populations.

The strong genetic divergence observed between the two successive years of collections from the MF East River (2002 and 2003) suggests that (1) bull trout from this system has a small effective population size – more specifically – a small number of spawners per year (Waples 1998); or (2) that at least one of the two collections included fish from other locations (i.e., populations). The latter explanation is possible since bull trout collected in both 2002 and 2003 were conducted outside the non-spawning window for bull trout (see Table 2) and could simply be an admixture of fish from different populations. However, the former explanation is consistent with MF East River spawning estimates; based on the number of redds observed (a good indicator of adult bull trout; Shepard and Graham 1983), electrofishing results, and observations via radio tracking in 2002. The information gathered indicated that bull trout spawning escapement for the MF East River system was between 30 and 40 adults (Dupont and Horner 2003). This pattern of differentiation observed between collection years has been observed previously in bull trout from the upper Flathead River Basin (e.g., Kanda and Allendorf 2001), where the authors explained the small number of spawners as a potentially recent demographic decline of bull trout as opposed to an historical evolutionary event. The fact that bull trout display low genetic variation (in comparison to other salmonids), coupled with apparently small numbers of spawning adults, will have serious implications to remaining reproductively viable bull trout populations as a result of inbreeding and bottleneck effects (see Epifanio et al. 2003).

Conservation Implications: The analysis of homologous microsatellite markers in bull trout within the Pend Oreille Basin demonstrates that genetic variation is higher in this species than previously documented, but overall is still comparatively low, suggesting that low genetic variation is a characteristic of bull trout. Despite inconsistencies in the use of genetic markers coupled with the genetic patterns observed in bull trout studies to date, results suggest that significant amounts of genetic subdivision exist among populations within relatively small geographic proximity as well as across larger distances. Our data certainly imply a high degree of demographic independence among populations within sub-basins of the lower Pend Oreille River Basin, a pattern observed in other sub-basins across the native range of bull trout (e.g., Taylor et al. 2001; Kanda and Allendorf 2001; Spruell et al. 2003). This result certainly needs consideration during the development of management plans in these locations. Though we were unable to infer the precise fine-scale population structure of bull trout (due to potentially admixed sample collections), our data indicate that small populations, or populations undergoing reductions in population size, will be very difficult to recover by means of straying from other bull trout, especially without re-establishing river connectivity. Finally, bottlenecks have been reported previously in populations of bull trout (Taylor et al. 2001); the tendency for populations to go through bottlenecks, coupled with the observed low genetic variation, further indicates that bull trout are vulnerable to strong inbreeding effects, which can be deleterious and lead to continued population declines throughout their native range. The health and persistence of individual bull trout populations to the species' long-term viability is crucial and, thus, conservation and management of the species needs to start at the local level.

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Table 1 Individual locus information (i.e., repeat length, # of alleles, allele size range, source, genebank accession number) and PCR amplification conditions (annealing temp., primer concentration, and taq polymerase) for 15 microsatellite loci used for the current study. Allele size range does not include alleles from brook trout baseline samples used for identifying bull/brook trout hybridization.

Multiplex	Locus	Dye Label	Annealing temp (^{O}C)	Primer conc.(µM)	Taq (units/rxn)	Repeat Unit(s)	# of alleles	Allele size range (bp)	Source	Accession numbers
Sco-A	*Sco-104*	vic	57	0.05	0.05	$(GATA)_n$	29	335-449	Bettles et al. (2005)**	AY973578
	Sco-107	ned	57	0.05		$(TAGA)_n$	15	194-323	Bettles et al. (2005)**	AY973581
	Sco-109	6fam	57	0.25		$(TATC)_n$	27	245-431	Bettles et al. (2005)**	AY973583
Sco-B	*Sco-103*	vic	57	0.05	0.05	$(TATC)_n$	12	229-303	Bettles et al. (2005)**	AY973577
	Sco-106	6fam	57	0.06		$(GA_2CA_2)_n(TAGA)_n$	18	174-250	Bettles et al. (2005)**	AY973580
Sco-C	*Sco-102*	vic	57	0.04	0.05	$(CATC)_n$	4	167-182	Bettles et al. (2005)**	AY973576
	Sco-110	6fam	57	0.05		$(GTCA)_n(GGCA)_n(GATA)_n$	10	219-262	Bettles et al. (2005)**	AY973584
Sco-E	*Omm-1128*	vic	47	0.04	0.05	$(TCTA)_n$	21	196-345	Rexroad et al. (2001)	AF375030
	Sco-105	ned	47	0.035		$(TTGAA)_n(TAGA)_n$	11	168-208	Bettles et al. (2005)**	AY973576
Sco-G	*Sco-204*	ned	60	0.14	0.05	$(TCTA)_n$	24	164-273	DeHaan & Ardren (2005)	AY88873
	Sco-211	pet	60	0.12		$(GATA)_n$	6	221-259	DeHaan & Ardren (2005)	AY88880
Sco-H1	*Sco-201*	vic	55	0.23	0.05	$(GATA)_n(GATG)_n(GATA)_n$	21	239-324	DeHaan & Ardren (2005)	AY88870
	Sco-215	pet	55	0.1		$(GAAA)_n(GA)_n(GGGA)_n(GA)_n$	6	286-344	DeHaan & Ardren (2005)	AY88884
Sco-H2	*Sco-202*	6fam	60	0.14	0.05	$(CTAT)_n$	8	121-164	DeHaan & Ardren (2005)	AY88871
	Sco-213	ned	60	0.08		$(ATCT)_n$	14	122-244	DeHaan & Ardren (2005)	AY88882

** manuscript in prep

Table 2 Population sample, the collection dates, size range, average size, total number of fish sampled (N), observed heterozygosity (H_O), expected heterozygosity (H_E), allelic richness (A_O), overall Hardy Weinburg Equilibrium (HWE) for each population, and percentage of tests significant for pairwise linkage disequilibrium (N = 105 tests per locus pair) for 12 bull trout collections.

Population	Collection Code	Collection Year	Dates of Collection	Size Range (mm)	Average Size (mm)	N	H_O	H_E	A_O	HWE *P*-value	Linkage
Clearwater Creek	02CI	2002	Aug.13 - Sept.7	39 - 145	104	31	0.601	0.602	3.90	0.073	0.00
upper Salmo River	02CE	2002	Aug.9	36 - 116	67	33	0.633	0.646	4.64	0.005	0.00
lower Salmo River	02CF	2002	Jun.23	330 - 670	512	30	0.632	0.710	5.47	**0.0001**	0.02
South Salmo River	02CG	2002	Aug.15	32 - 125	51	50	0.713	0.712	5.81	0.015	0.01
Sheep Creek	02CH	2002	Sept.9	45 - 169	73	33	0.719	0.711	5.09	0.065	0.00
Uleda Creek	03NW 02DK	2002 2003	Aug.13 - Jul.8	75 - 550	112	30	0.580	0.634	4.75	0.008	0.11
Middle Fork East River-a	02DI	2003	Jun.16 - Jul.2	70 - 169	119	70	0.656	0.705	5.93	**0.000**	0.42
Middle Fork East River-b	02DK	2002	Aug.13 - Aug.15	46 - 762	336	40	0.677	0.726	6.17	**0.000**	0.01
Indian Creek	03DZ	2003	Jul.28 - Jul.31	92 - 176	151	20	0.465	0.497	3.52	0.011	0.01
Upper Priest River	03DN	2003	Sept.2 - Sept.3	62 - 238	150	50	0.611	0.619	6.27	0.031	0.05
Upper Priest Lake	97NA 98NA 99RJ	1997 1998 1999	Sept.22 - Sept.10	160 - 838	589	39	0.572	0.619	6.46	0.015	0.02
Gold Creek	03DM	2003	Jun.23 - Jul.1	79 - 222	132	50	0.498	0.506	4.72	0.007	0.11

underlined represents deviation from HWE before Bonferroni correction

bold-typed represents deviation from HWE after Bonferroni correction

Table 3 Inbreeding coefficient (F_{IS}) for individual loci and across all loci for each bull trout collection. Deviations from Hardy Weinberg Equilibrium (HWE) at individual loci and across all loci (F_{IS} overall) are denoted by bold-typed F_{IS} values. All deviations from HWE have been reported before Bonferroni correction (denoted by underline) as well as corrected for multiple simultaneous tests (bold-type) ($P < 0.000256$).

Populations	Upper Salmo River (BC)	Lower Salmo River (BC)	South Salmo River (BC)	Sheep Creek (BC)	Clearwater Creek (BC)	MF East River (ID)	MF East River-b (ID)	Gold Creek (ID)	Upper Priest River (ID)	Indian Creek (ID)	Uleda Creek (ID)	Upper Priest Lake (ID)
Locus												
Sco-104	-0.058	0.068	-0.134	0.020	0.050	0.003	0.212	-0.040	-0.017	-0.170	-0.126	-0.040
Sco-107	0.010	0.035	0.131	-0.042	-0.157	0.013	-0.055	-0.137	-0.068	0.000	0.339	0.198
Sco-109	0.010	0.362	**0.369**	0.055	**0.243**	0.209	0.141	**-0.190**	**0.274**	0.159	0.267	**0.409**
Sco-103	0.329	0.079	-0.046	-0.008	-0.032	0.062	0.038	-0.059	-0.027	-0.352	-0.028	0.051
Sco-106	0.127	-0.007	-0.213	-0.171	-0.101	0.012	0.112	**0.108**	-0.101	0.215	-0.136	-0.060
Sco-102	0.024	0.040	-0.153	-0.027	-0.077	0.149	-0.094	—	-0.034	—	-0.009	-0.043
Sco-110	0.110	0.196	0.004	0.149	-0.078	**-0.040**	0.043	-0.044	0.140	0.024	-0.057	-0.057
Omm-1128	-0.010	0.108	0.049	0.105	-0.051	-0.058	0.036	-0.088	0.020	-0.011	-0.217	-0.006
Sco-105	0.094	0.108	0.019	0.089	-0.087	0.053	-0.039	0.042	-0.168	—	0.151	0.126
Sco-204	-0.037	-0.013	-0.091	-0.120	-0.158	-0.037	-0.012	**0.071**	-0.066	**0.389**	0.115	0.014
Sco-211	-0.078	0.258	0.004	-0.422	-0.160	-0.046	-0.096	-0.121	0.081	-0.040	0.408	0.214
Sco-201	-0.011	0.035	0.155	0.061	0.128	**0.534**	0.341	**0.435**	**0.184**	0.401	**0.560**	0.023
Sco-215	0.026	0.001	-0.059	-0.118	0.049	0.097	0.237	—	-0.077	—	-0.182	-0.027
Sco-202	-0.030	0.190	0.028	-0.080	0.164	-0.002	0.159	-0.037	-0.091	0.451	0.129	0.110
Sco-213	-0.161	0.258	-0.092	0.229	0.123	0.114	0.075	0.028	0.081	-0.376	0.125	0.112
F_{IS} Overall	0.023	**0.115**	-0.002	-0.019	-0.010	**0.071**	**0.073**	0.016	0.013	0.067	0.087	0.068

Table 4 Genetic differentiation (pairwise F_{ST} - below diagonal; genotypic differentiation - above diagonal) for 12 bull trout collections from the lower Pend Oreille River Basin. All F_{ST} values are significant at $P < 0.05$. Non-significant P-values from pairwise genotypic differentiation have a grey square. Pairwise F_{ST} estimates and genotypic differentiation tests were averaged over all 15 loci.

	upper Salmo River (BC)	lower Salmo River (BC)	South Salmo River (BC)	Sheep Creek (BC)	Clearwater Creek (BC)	MF East River (ID)	MF East River-b (ID)	Gold Creek (ID)	Upper Priest River (ID)	Indian Creek (ID)	Uleda Creek (ID)	Upper Priest Lake (ID)
upper Salmo River (BC)	—	0.000	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
lower Salmo River (BC)	0.021	—	H.S.	0.000	0.00057	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
South Salmo River (BC)	0.096	0.032	—	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
Sheep Creek (BC)	0.068	0.025	0.066	—	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
Clearwater Creek (BC)	0.009	0.037	0.105	0.105	—	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
MF East River-a (ID)	0.187	0.148	0.129	0.145	0.216	—	H.S.	H.S.	H.S.	H.S.	H.S.	H.S.
MF East River-b (ID)	0.200	0.147	0.126	0.139	0.226	0.028	—	H.S.	H.S.	H.S.	H.S.	H.S.
Gold Creek (ID)	0.374	0.326	0.310	0.328	0.393	0.220	0.233	—	H.S.	H.S.	H.S.	H.S.
Upper Priest River (ID)	0.276	0.217	0.197	0.213	0.293	0.137	0.127	0.079	—	H.S.	H.S.	0.000
Indian Creek (ID)	0.343	0.295	0.282	0.290	0.376	0.174	0.184	0.175	0.112	—	H.S.	H.S.
Uleda Creek (ID)	0.286	0.251	0.255	0.250	0.319	0.190	0.215	0.383	0.295	0.355	—	H.S.
Upper Priest Lake (ID)	0.283	0.224	0.253	0.223	0.303	0.116	0.188	0.047	0.000	0.117	0.308	—

H.S. $P < 0.00001$

Table 5 Estimates of allelic richness (A_O), observed (H_O) and expected (H_E) heterozygosity, and overall F_{ST} values for all 12 bull trout collections (All sub-basins) and for N collections from 3 sub-basins within the lower Pend Oreille River Basin.

Group	N	A_O	H_O	H_E	F_{ST}
All sub-basins	12	5.23	0.613	0.641	0.213
Salmo R. sub-basin (BC)	5	4.98	0.660	0.676	0.060
Upper Priest R. sub-basin (ID)	4	5.24	0.537	0.560	0.070
lower Priest R. sub-basin (ID)	3	5.62	0.638	0.688	0.130

Table 6 Results of AMOVA (Analysis of Molecular Variance) for microsatellite allele variance among bull trout collections from the lower Pend Oreille River Basin.

Grouping Strategy	Source of Variation	Variance Components	% Variation	F_{ST}	F_{SC}	F_{CT}
3 Groups[a]	Among sub-basins	0.877	15.43			0.154*
	Among collections within sub-basins	0.527	9.27		0.110*	
	Within collections	4.278	75.29	0.247*		

[a] Three sub-basins - Salmo River (BC); Upper Priest River (ID); lower Priest River (ID)

* $P < 0.05$

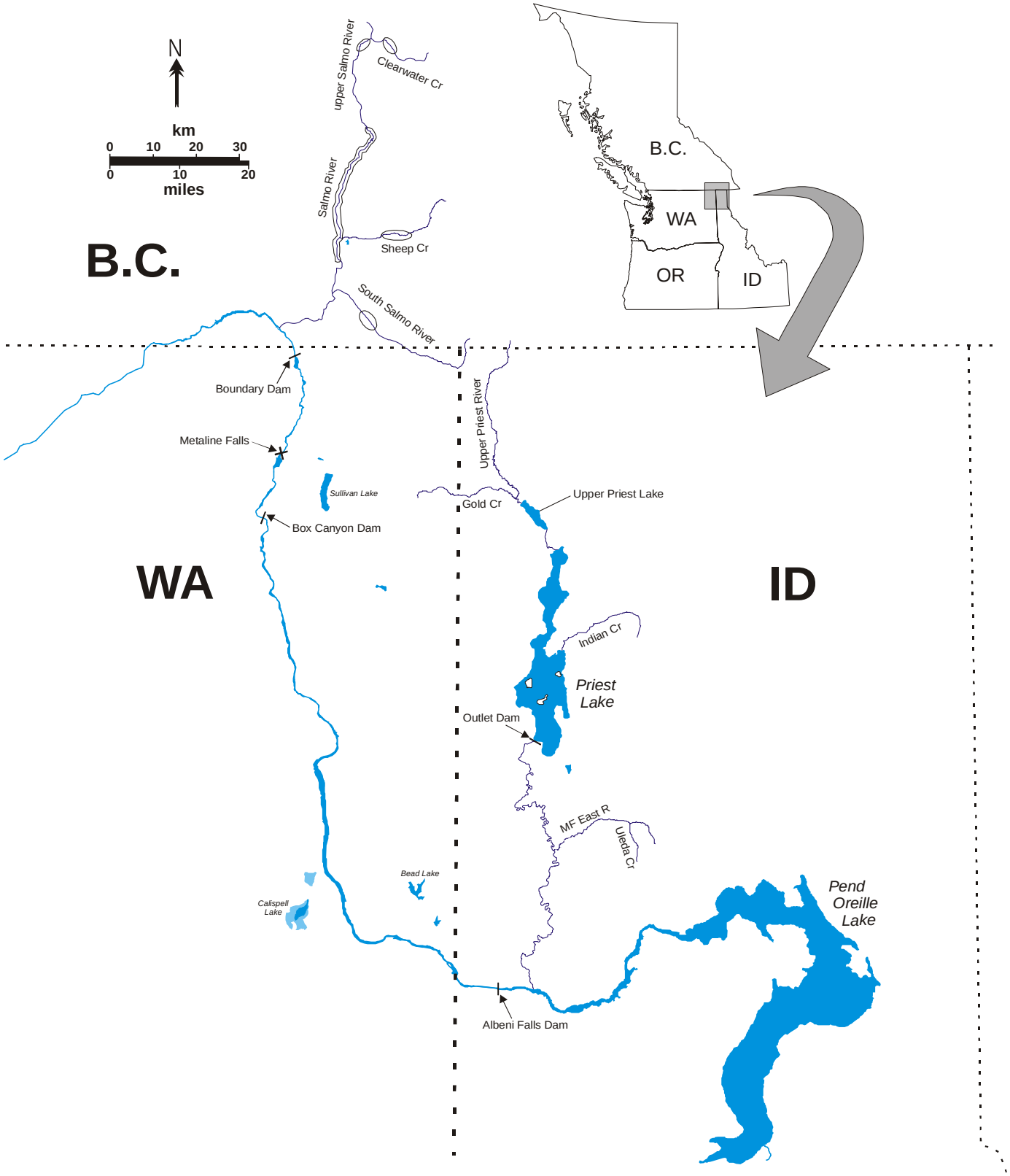
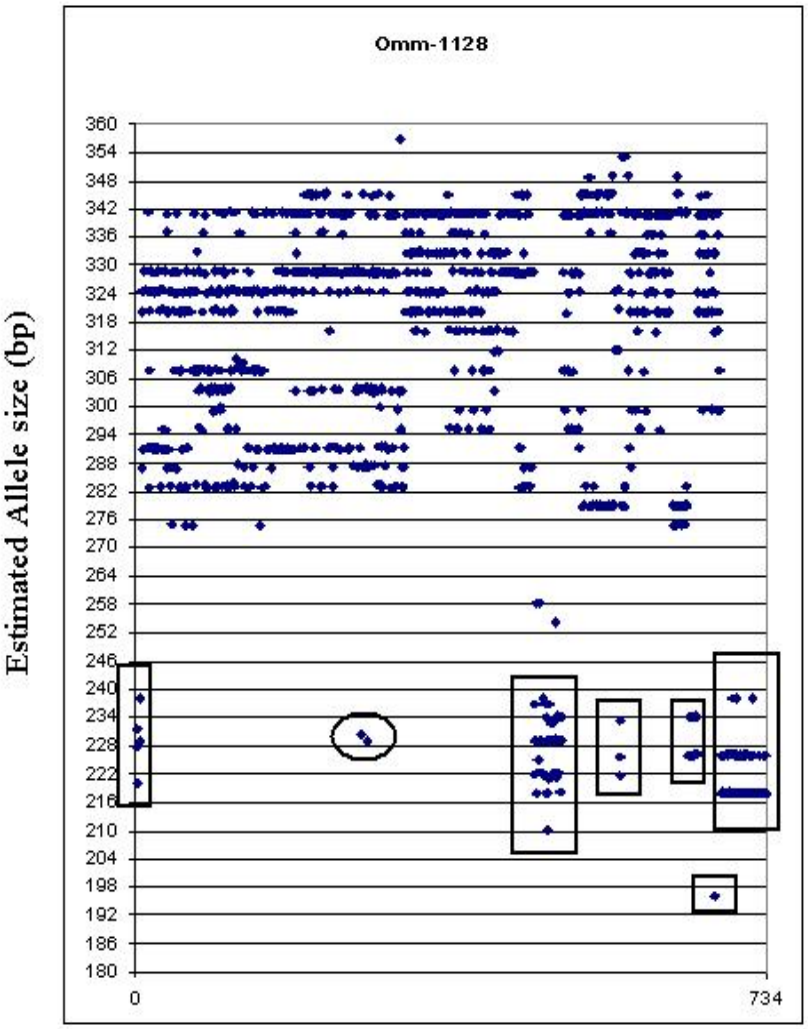


Figure 1 Map of the lower Pend Oreille River Basin, depicting sample collections within British Columbia (Canada) and Idaho (USA). Circles displayed within the Salmo River collections represent approximate locations where sampling occurred.

Figure 2 Plot of microsatellite Omm-1128 alleles (by bin) observed in 734 fish screened (includes several pure brook trout collections). Each vertical column represents the two alleles in an individual fish. Diagnostic alleles for brook trout are in rectangles and presumed brook trout alleles found in sampled bull trout (i.e., hybrids) identified with a circle.



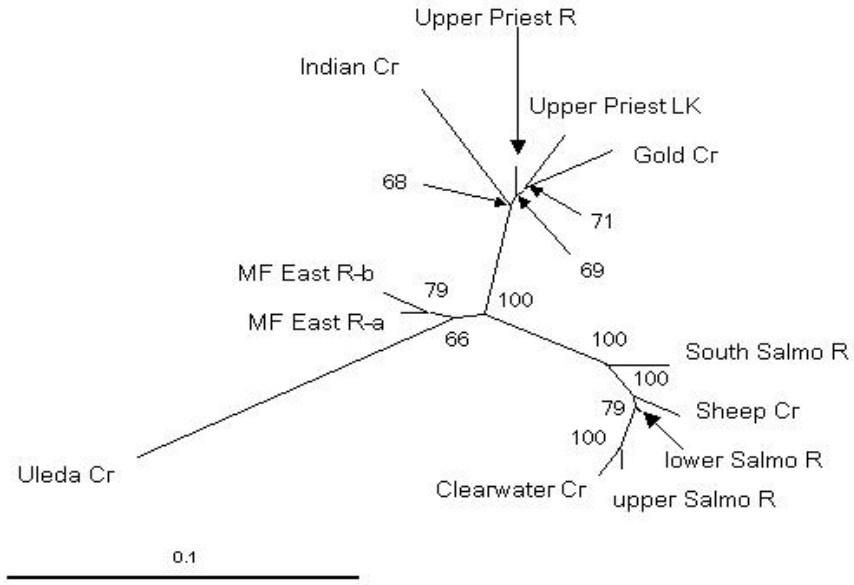


Figure 3 Cavalli-Sforza & Edwards (1967) chord distance dendrogram for 12 Pend Oreille River Basin bull trout collections. Bootstrap percentages are based on 1000 consensus dendrograms.

Appendix I Allele frequencies for bull trout from the Pend Oreille River Basin. Abbreviations correspond to the following: Up Salmo - upper Salmo River, BC; Lo Salmo - lower Salmo River, BC; S Salmo - South Salmo River, BC; Sheep - Sheep Creek, BC; Clearwater - Clearwater Creek, BC; MF East-a - Middle Fork East River-a, ID; MF East-b - Middle Fork East River-b, ID; Gold - Gold Creek, ID;, UpPriest R - Upper Priest River, ID; Indian - Indian Creek, ID; Uleda - Uleda Creek, ID; Up Priest Lk - Upper Priest Lake, ID. "Private" represents collections that displayed alleles not observed in other collections.

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-104	335	---	---	---	---	---	---	---	0.0204	---	---	---	0.0172	
Sco-104	339	---	0.05	0.0714	0.0667	---	---	---	0.4286	0.1531	---	---	0.069	
Sco-104	343	---	0.0167	---	---	---	0.125	0.2333	0.102	0.0918	---	0.0536	0.0862	
Sco-104	346	---	---	---	---	---	0.0917	0.1667	0.0204	---	---	---	0.0172	
Sco-104	351	---	---	---	---	---	---	---	---	---	---	---	0.0172	Upper Priest Lk
Sco-104	354	---	---	---	---	---	0.0833	0.1333	0.0306	0.0612	---	0.0357	0.0517	
Sco-104	358	---	---	---	---	---	0.025	0.1	---	0.0204	---	---	---	
Sco-104	362	---	0.0167	---	---	---	0.0083	---	---	---	---	---	---	
Sco-104	365	---	---	---	---	---	0.025	0.0667	---	---	---	---	---	
Sco-104	369	---	---	---	---	---	---	---	---	0.0714	---	---	0.0517	
Sco-104	376	---	---	0.0102	---	---	---	---	---	---	---	---	---	South Salmo R
Sco-104	380	---	---	0.0408	---	---	---	---	---	---	---	---	---	South Salmo R
Sco-104	385	0.0161	---	0.0306	0.0167	0.0333	---	---	---	---	---	---	---	
Sco-104	393	0.0161	0.05	0.1224	0.0167	---	0.125	0.05	---	---	---	0.1786	---	
Sco-104	397	---	0.0167	0.0918	---	---	---	---	---	---	---	---	---	
Sco-104	399	---	---	---	---	---	---	---	0.0102	---	---	0.0179	---	
Sco-104	401	---	---	0.0306	---	---	---	---	---	---	---	---	---	South Salmo R
Sco-104	404	---	---	---	---	---	---	---	---	---	---	---	0.0172	Upper Priest Lk
Sco-104	408	---	---	---	---	0.0167	---	---	0.0408	0.0816	0.2222	---	0.0862	
Sco-104	412	0.3387	0.2333	---	0.3667	0.2	---	---	0.0408	0.1224	0.1111	---	0.0345	
Sco-104	416	0.2097	0.3167	0.3265	0.2	0.3667	0.0583	0.05	0.0102	0.0612	0.0278	0.0714	0.0172	
Sco-104	420	---	0.0333	0.1939	---	0.0167	0.025	0.05	0.0918	0.0612	0.0556	---	0.1552	
Sco-104	424	0.0161	0.0833	0.051	0.1333	---	0.3083	0.0667	---	0.0102	0.3333	0.4286	---	
Sco-104	428	---	0.0167	---	---	---	0.0667	0.05	---	0.0612	---	0.0714	0.0345	
Sco-104	432	0.3871	0.15	---	0.1833	0.3667	0.0583	---	0.0102	0.0816	0.1667	0.1429	0.0862	
Sco-104	436	0.0161	---	0.0204	0.0167	---	---	0.0333	0.051	0.0612	0.0833	---	0.1552	
Sco-104	441	---	0.0167	0.0102	---	---	---	---	0.0408	0.0102	---	---	0.0345	
Sco-104	445	---	---	---	---	---	---	---	0.102	0.0306	---	---	0.0517	
Sco-104	449	---	---	---	---	---	---	---	---	0.0204	---	---	0.0172	

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-107	194	---	---	---	---	---	0.0089	---	---	---	---	---	---	MF East R
Sco-107	270	---	---	---	0.0312	---	---	---	---	---	---	---	---	Sheep Cr
Sco-107	274	0.0806	---	---	0.0312	0.1452	---	---	0.0222	0.0104	---	---	---	
Sco-107	278	---	0.0333	0.0714	0.0156	---	---	---	0.0111	0.0417	---	---	---	
Sco-107	282	0.4032	0.5333	0.5612	0.2344	0.4839	0.0982	0.125	---	0.0104	---	0.0385	0.0833	
Sco-107	286	0.3871	0.2	0.0918	0.2031	0.2742	---	0.0357	0.0889	0.0312	0.4375	---	0.1458	
Sco-107	290	0.0806	0.1667	0.0918	0.4375	0.0968	0.0804	0.25	---	0.1146	---	0.2692	0.0625	
Sco-107	294	0.0161	0.0167	0.0918	0.0156	---	0.1161	0.1071	---	0.1042	---	---	0.125	
Sco-107	298	---	---	---	---	---	0.1339	0.0357	0.3	0.1146	0.3438	0.0769	0.0417	
Sco-107	302	---	---	---	---	---	0.0089	0.0536	0.3111	0.2396	---	0.0769	0.3542	
Sco-107	306	0.0323	0.0333	0.0306	0.0312	---	0.1696	0.0714	---	0.1354	0.0938	0.0385	0.0833	
Sco-107	310	---	0.0167	0.0612	---	---	0.0179	0.0714	---	0.0521	0.125	---	0.0208	
Sco-107	314	---	---	---	---	---	0.3661	0.25	0.1556	0.1042	---	0.5	0.0833	
Sco-107	319	---	---	---	---	---	---	---	0.0556	0.0312	---	---	---	
Sco-107	323	---	---	---	---	---	---	---	0.0556	0.0104	---	---	---	

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-109	245	---	---	---	---	---	0.0417	0.0484	---	---	---	---	---	
Sco-109	253	0.0484	---	---	0.0333	---	---	---	---	0.0102	---	---	---	
Sco-109	257	---	0.0345	0.0208	0.0167	---	---	---	---	---	---	---	---	
Sco-109	261	0.1452	0.1897	---	---	0.0968	0.05	---	0.3854	0.0204	---	---	0.0862	
Sco-109	265	0.129	0.1379	0.2188	---	0.371	0.025	0.0484	0.2917	0.2551	---	0.0769	0.2759	
Sco-109	269	---	0.0345	0.1354	---	---	0.55	0.5806	---	0.1837	0.1111	0.3269	0.2414	
Sco-109	273	---	0.0172	0.0938	0.05	---	0.0083	0.0161	---	0.1429	0.3333	---	0.1552	
Sco-109	277	---	---	0.0208	---	---	---	---	---	0.0408	0.1389	---	0.0517	
Sco-109	281	0.0323	---	0.1042	---	0.0161	0.1917	0.2581	---	---	---	0.2115	---	
Sco-109	285	---	---	---	---	---	0.0083	---	---	---	---	---	---	MF East R
Sco-109	331	---	---	---	---	---	0.0083	0.0323	---	---	---	0.1731	---	
Sco-109	335	---	---	---	---	---	---	---	0.0104	0.0306	0.0278	---	0.0172	
Sco-109	339	---	---	---	---	---	0.0167	---	0.0833	---	---	---	---	
Sco-109	344	---	---	---	---	---	---	---	0.0417	---	---	---	---	Gold Cr
Sco-109	347	---	---	---	---	---	0.0917	---	---	0.0204	---	0.2115	0.069	
Sco-109	386	---	---	---	---	---	---	---	0.0104	0.0306	---	---	---	
Sco-109	390	---	---	---	---	---	---	---	0.0312	0.102	---	---	---	
Sco-109	394	0.0323	---	0.0312	---	0.0645	---	---	---	0.0204	---	---	---	
Sco-109	398	0.0161	---	0.0417	---	0.0806	---	---	---	---	---	---	0.0172	
Sco-109	402	0.3548	0.3448	0.2396	0.4167	0.2903	---	---	---	0.0102	0.2778	---	0.0172	
Sco-109	406	0.1452	0.0862	---	0.1667	0.0806	---	---	0.0208	0.0612	0.1111	---	0.069	
Sco-109	410	0.0968	0.1552	0.0833	0.25	---	0.0083	0.0161	0.0938	0.0306	---	---	---	
Sco-109	415	---	---	---	---	---	---	---	0.0312	---	---	---	---	Gold Cr
Sco-109	416	---	---	0.0104	---	---	---	---	---	---	---	---	---	South Salmo R
Sco-109	419	---	---	---	---	---	---	---	---	0.0204	---	---	---	Upper Priest R
Sco-109	423	---	---	---	---	---	---	---	---	0.0204	---	---	---	Upper Priest R
Sco-109	431	---	---	---	0.0667	---	---	---	---	---	---	---	---	Sheep Cr

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-103	229	0.0625	---	---	0.0152	0.0806	0.041	0.0517	---	---	---	---	---	
Sco-103	233	---	---	0.0122	---	---	---	---	---	---	---	---	---	South Salmo R
Sco-103	237	0.0312	0.1	0.122	0.1667	---	0.1475	0.2759	0.1023	0.2551	0.4688	0.05	0.1184	
Sco-103	241	0.2031	0.4333	0.5488	0.1818	0.4355	0.3033	0.3276	0.5341	0.6633	0.5312	0.1167	0.6711	
Sco-103	245	---	0.0167	0.061	---	---	0.0492	0.1724	0.2045	0.051	---	---	0.0921	
Sco-103	249	---	---	---	---	---	0.0082	---	---	---	---	---	---	
Sco-103	253	0.6719	0.4	0.1585	0.4848	0.4677	0.2869	0.0862	0.1591	0.0102	---	0.55	0.0789	
Sco-103	257	0.0156	0.05	0.0976	0.1515	---	0.1393	0.0345	---	0.0204	---	0.1833	0.0395	
Sco-103	261	---	---	---	---	---	---	0.0172	---	---	---	---	---	MF East Rb
Sco-103	265	---	---	---	---	---	0.0246	0.0172	---	---	---	0.1	---	
Sco-103	269	0.0156	---	---	---	0.0161	---	---	---	---	---	---	---	
Sco-103	303	---	---	---	---	---	---	0.0172	---	---	---	---	---	MF East Rb

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-106	174	0.0156	---	---	---	0.0161	---	---	---	---	---	---	---	
Sco-106	182	---	---	---	---	---	0.0083	---	---	---	---	---	---	MF East R
Sco-106	185	---	---	---	---	---	0.1417	0.1379	---	0.0208	---	0.0333	---	
Sco-106	189	---	0.0167	0.0119	0.0455	---	0.0583	0.069	---	---	---	0.0167	---	
Sco-106	194	0.3438	0.2333	0.119	0.1212	0.4032	0.0417	0.0172	---	---	---	0.0167	---	
Sco-106	197	0.0625	0.15	0.119	0.2576	0.0161	0.0083	---	---	---	---	---	---	
Sco-106	201	0.0625	0.05	0.0952	0.0303	---	0.0167	0.1034	---	---	---	---	0.069	
Sco-106	205	---	---	---	---	---	0.05	0.1379	---	---	---	0.0833	---	
Sco-106	209	---	0.05	0.2381	0.1212	---	0.025	---	0.0667	0.0521	0.0294	0.1167	0.1897	
Sco-106	213	---	0.05	0.0714	0.0152	---	---	---	0.3889	0.2396	0.5588	---	0.1552	
Sco-106	217	0.2031	0.1333	0.0476	0.1364	0.2258	0.1167	0.1552	0.2778	0.3646	---	0.1	0.3448	
Sco-106	221	0.2344	0.1833	0.2857	0.1061	0.2258	0.3417	0.1034	0.0556	0.1458	---	0.5167	0.1034	
Sco-106	225	0.0625	---	---	---	---	0.1417	0.1552	0.0444	0.125	0.0588	0.0667	0.0862	
Sco-106	229	---	---	---	---	---	0.0333	0.1034	---	0.0104	0.3235	0.05	---	
Sco-106	233	0.0156	0.1333	0.0119	0.1667	0.1129	---	---	---	---	---	---	---	
Sco-106	237	---	---	---	---	---	0.0167	0.0172	0.0111	0.0312	0.0294	---	---	
Sco-106	241	---	---	---	---	---	---	---	0.1556	0.0104	---	---	0.0345	
Sco-106	250	---	---	---	---	---	---	---	---	---	---	---	0.0172	Upper Priest Lk

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-102	167	0.1094	0.1667	0.3021	0.2742	0.0167	0.7623	0.7	1	0.9574	1	0.7963	0.9459	
Sco-102	171	0.75	0.6	0.3646	0.4516	0.9	0.2377	0.3	---	0.0426	---	0.2037	0.0541	
Sco-102	178	0.0156	0.0667	0.2708	0.1774	---	---	---	---	---	---	---	---	
Sco-102	182	0.125	0.1667	0.0625	0.0968	0.0833	---	---	---	---	---	---	---	

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-110	219	0.4219	0.4167	0.2292	0.3438	0.4839	0.2097	0.35	0.05	0.0938	0.225	0.2115	0.0556	
Sco-110	223	0.1562	0.2667	0.4792	0.3125	0.0323	0.2661	0.3167	0.01	0.0208	---	0.2115	0.0417	
Sco-110	227	---	0.0167	0.1354	0.0312	---	---	0.0333	---	---	---	---	---	
Sco-110	230	---	---	---	---	---	0.0403	0.0167	---	0.0625	---	0.0577	0.0556	
Sco-110	234	---	---	---	---	---	0.2258	0.05	---	---	---	0.3846	0.0417	
Sco-110	238	---	---	---	---	---	0.0484	0.0667	0.94	0.7917	0.375	---	0.7361	
Sco-110	242	---	---	---	---	---	0.1694	0.1167	---	---	0.4	0.0769	0.0417	
Sco-110	254	0.0312	0.0333	0.0104	---	---	0.0323	0.05	---	0.0312	---	0.0577	0.0278	
Sco-110	258	0.375	0.25	0.125	0.3125	0.4839	0.0081	---	---	---	---	---	---	
Sco-110	262	0.0156	0.0167	0.0208	---	---	---	---	---	---	---	---	---	

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Omm-1128	196	---	---	---	---	---	---	---	---	---	---	---	0.0147	Upper Priest Lk
Omm-1128	229	---	---	---	---	---	---	0.0167	---	---	---	---	---	MF East Rb
Omm-1128	275	---	0.0517	---	0.0167	---	---	---	---	---	---	---	---	
Omm-1128	283	0.0345	0.0862	0.1667	0.1167	---	0.0333	0.05	---	---	---	0.1667	---	
Omm-1128	287	0.0172	0.0345	---	0.05	0.0333	0.0167	0.1167	---	---	---	0.0667	0.0147	
Omm-1128	291	0.2586	0.0517	---	0.0833	0.45	0.125	0.1	---	---	---	0.0667	0.0147	
Omm-1128	295	0.0345	---	0.0667	---	---	---	---	---	0.0714	---	---	---	
Omm-1128	299	---	---	0.0444	---	---	---	0.0167	---	0.0306	---	---	0.0735	
Omm-1128	303	---	---	---	---	---	0.0583	---	---	---	---	0.0167	---	
Omm-1128	304	---	---	0.1667	---	---	---	0.2167	---	---	---	---	---	
Omm-1128	308	0.0172	0.1724	0.1556	0.2333	---	---	---	---	0.0714	---	---	---	
Omm-1128	309	---	---	---	0.0333	---	---	---	---	---	---	---	---	Sheep Cr
Omm-1128	310	---	---	0.0111	---	---	---	---	---	---	---	---	---	South Salmo R
Omm-1128	316	---	---	---	---	---	0.0083	---	0.03	0.0918	0.075	---	0.0147	
Omm-1128	320	0.1207	0.1897	0.1111	0.0167	0.1333	---	---	0.17	0.1429	---	---	0.1618	
Omm-1128	325	0.2414	0.2759	0.1111	0.3333	0.2167	0.0583	0.0333	0.17	0.0816	---	---	0.1471	
Omm-1128	329	0.2241	0.0862	0.0889	0.05	0.0833	0.3667	0.3167	0.05	0.1122	0.6	0.4167	0.0588	
Omm-1128	333	---	---	0.0111	---	---	0.0083	---	0.23	0.0816	0.125	0.0167	0.1324	
Omm-1128	337	0.0172	---	0.0111	0.0167	---	0.0333	---	0.02	0.0612	---	---	0.0441	
Omm-1128	341	0.0345	0.0517	0.0556	0.05	0.0833	0.1667	0.0833	0.32	0.2551	0.2	0.15	0.2941	
Omm-1128	345	---	---	---	---	---	0.125	0.05	0.01	---	---	0.1	0.0294	

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-105	168	---	---	---	---	---	---	---	---	---	---	---	0.0135	Upper Priest Lk
Sco-105	172	---	---	---	---	---	0.1532	0.3167	---	---	---	0.15	0.0135	
Sco-105	176	---	---	---	---	---	---	---	0.02	0.1429	---	---	0.027	
Sco-105	180	0.5172	0.3276	0.2222	0.2069	0.3966	0.0323	0.0333	0.02	0.0102	---	0.1167	0.0811	
Sco-105	184	---	0.0172	0.1333	---	---	0.0081	0.0167	---	---	---	0.0833	0.0135	
Sco-105	188	---	0.0172	0.1222	---	0.0172	0.1774	0.2167	0.13	0.3265	0.025	---	0.2973	
Sco-105	192	0.1034	0.069	0.0222	0.1207	0.2414	0.2823	0.2667	0.82	0.4592	0.975	0.1167	0.527	
Sco-105	196	0.1034	0.1379	0.1333	0.1552	0.0345	0.2903	0.15	0.01	0.0612	---	0.5	0.027	
Sco-105	200	---	---	0.0111	0.0172	---	---	---	---	---	---	0.0167	---	
Sco-105	204	0.2759	0.431	0.3	0.5	0.3103	0.0565	---	---	---	---	0.0167	---	
Sco-105	208	---	---	0.0556	---	---	---	---	---	---	---	---	---	South Salmo R

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-204	164	---	---	---	---	---	---	---	0.15	0.0957	---	---	0.0857	
Sco-204	168	---	---	0.0109	---	---	0.375	0.35	0.1	0.117	0.2059	---	0.1	
Sco-204	172	---	---	---	---	---	---	---	---	0.0213	---	---	0.0143	
Sco-204	176	---	---	---	---	---	---	---	0.0375	0.117	---	---	0.0429	
Sco-204	180	---	---	---	---	---	0.0714	---	---	---	0.1176	---	0.0286	
Sco-204	184	---	0.0577	---	0.1786	0.0172	---	0.0167	---	---	---	---	---	
Sco-204	193	---	0.0192	0.0109	0.0714	---	0.0268	0.05	---	0.0106	---	---	0.0143	
Sco-204	197	---	---	0.0326	---	---	0.0089	---	---	---	---	0.3167	0.0143	
Sco-204	200	0.2258	0.1538	0.0978	0.0179	0.2069	0.0357	0.0667	---	0.0106	---	---	---	
Sco-204	204	0.2258	0.2115	0.0978	0.2321	0.2759	0.0089	---	0.0375	0.0213	---	---	0.0286	
Sco-204	208	---	0.0577	0.1087	0.0357	---	0.3571	0.2667	0.275	0.0213	0.1176	0.05	0.2571	
Sco-204	212	---	---	---	---	---	---	---	0.0875	0.2021	0.4118	---	0.1571	
Sco-204	216	---	---	---	---	---	0.0804	0.1	0.025	---	---	---	0.0143	
Sco-204	220	0.0161	---	---	---	---	---	---	---	0.0319	---	---	0.0143	
Sco-204	229	---	---	---	---	---	---	---	---	0.0213	---	---	---	Upper Priest R
Sco-204	233	---	---	---	---	---	---	0.0167	---	0.0106	---	0.0333	0.0143	
Sco-204	237	---	0.0192	0.0543	---	---	---	---	0.0125	---	---	0.3833	---	
Sco-204	240	0.0161	0.0192	---	---	---	---	---	0.2375	0.2128	0.1471	---	0.1714	
Sco-204	245	0.1129	0.0769	0.0217	0.1071	0.0172	0.0357	0.1	---	0.0319	---	0.2167	0.0143	
Sco-204	248	0.0161	0.1731	0.3043	0.0893	0.069	---	---	0.0375	0.0532	---	---	0.0286	
Sco-204	252	0.3871	0.1731	0.25	0.2679	0.4138	---	---	---	---	---	---	---	
Sco-204	257	---	0.0385	---	---	---	---	0.0333	---	---	---	---	---	
Sco-204	264	---	---	---	---	---	---	---	---	0.0213	---	---	---	Upper Priest R
Sco-204	273	---	---	0.0109	---	---	---	---	---	---	---	---	---	South Salmo R

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-211	221	---	---	---	---	---	0.0085	0.0167	---	---	---	---	---	
Sco-211	228	0.8	0.66	0.5417	0.3519	0.8704	0.3814	0.2333	0.1184	0.1771	0.0714	---	0.1719	
Sco-211	232	0.15	0.3	0.4583	0.5741	0.1296	0.6102	0.75	0.8816	0.8229	0.9286	---	0.8281	
Sco-211	236	0.05	0.04	---	0.0741	---	---	---	---	---	---	---	---	
Sco-211	255	---	---	---	---	---	---	---	---	---	---	0.55	---	Uleda Cr
Sco-211	259	---	---	---	---	---	---	---	---	---	---	0.45	---	Uleda Cr

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-201	239	0.0938	0.05	---	0.0781	0.0667	---	---	---	---	---	---	---	
Sco-201	243	---	0.05	0.0761	---	---	---	---	---	0.0102	---	---	---	
Sco-201	247	---	---	---	---	---	---	---	0.0213	0.0408	---	---	0.1094	
Sco-201	251	---	---	---	---	---	---	---	---	---	0.05	---	---	Indian Cr
Sco-201	255	---	---	---	---	---	0.1346	0.0417	---	0.0408	---	---	---	
Sco-201	259	0.0312	---	0.0217	0.0469	0.05	0.0385	0.0208	0.0426	0.0102	---	---	0.0625	
Sco-201	263	0.3906	0.4833	0.4891	0.4375	0.4167	0.1154	0.1875	0.117	0.5102	0.075	---	0.1875	
Sco-201	267	0.3438	0.2333	0.1522	---	0.3667	---	0.0208	0.1809	0.0102	0.05	---	0.0312	
Sco-201	271	---	---	0.1087	---	---	0.1346	0.1667	---	0.0102	---	---	---	
Sco-201	275	0.1406	0.1667	0.0652	0.4219	0.1	0.3173	0.4375	0.0213	0.051	---	---	0.0469	
Sco-201	279	---	---	---	0.0156	---	0.0192	---	---	0.0306	0.075	---	0.0625	
Sco-201	283	---	0.0167	0.0326	---	---	---	0.0208	---	---	---	0.0185	---	
Sco-201	287	---	---	---	---	---	0.1827	---	0.2234	0.0612	0.225	---	0.1719	
Sco-201	291	---	---	---	---	---	---	---	0.1809	0.0816	0.45	---	0.1094	
Sco-201	295	---	---	---	---	---	0.0385	0.0208	0.1064	0.0408	0.075	---	0.0469	
Sco-201	299	---	---	---	---	---	0.0192	0.0833	0.0106	---	---	0.1111	---	
Sco-201	303	---	---	---	---	---	---	---	---	0.0408	---	0.5185	0.0156	
Sco-201	307	---	---	0.0543	---	---	---	---	0.0532	0.0204	---	---	0.125	
Sco-201	311	---	---	---	---	---	---	---	---	0.0408	---	0.037	0.0312	
Sco-201	316	---	---	---	---	---	---	---	0.0426	---	---	0.2407	---	
Sco-201	324	---	---	---	---	---	---	---	---	---	---	0.0741	---	Uleda Cr

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-202	121	---	---	---	---	---	0.1518	0.3462	0.01	0.0667	0.325	---	---	
Sco-202	125	0.0156	0.1	0.0312	0.1034	---	0.1161	0.0962	0.01	0.0667	---	---	0.0435	
Sco-202	129	0.0469	0.04	0.0625	0.1207	---	0.1429	0.2115	0.94	0.6667	0.675	---	0.8043	
Sco-202	133	0.7031	0.64	0.7917	0.6207	0.7333	0.5357	0.2692	0.04	0.1333	---	---	0.1304	
Sco-202	147	---	---	0.0312	---	---	---	---	---	0.0667	---	---	0.0217	
Sco-202	151	0.2344	0.22	0.0833	0.1552	0.2667	0.0536	0.0769	---	---	---	0.1167	---	
Sco-202	156	---	---	---	---	---	---	---	---	---	---	0.0333	---	Uleda Cr
Sco-202	164	---	---	---	---	---	---	---	---	---	---	0.85	---	Uleda Cr

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-213	122	---	---	---	---	---	0.0086	---	---	---	---	---	---	MF East R
Sco-213	126	---	---	---	---	---	0.0086	---	---	---	---	---	---	MF East R
Sco-213	176	0.2656	0.1833	0.1304	0.1129	0.2586	0.2931	0.06	0.0204	0.051	0.025	---	0.0588	
Sco-213	180	0.25	0.3167	0.2609	0.3871	0.1207	0.1121	0.12	0.0306	0.3776	0.45	---	0.3235	
Sco-213	184	0.1875	0.2167	0.4457	0.1613	0.2586	0.25	0.36	0.1429	0.2857	0.35	---	0.2353	
Sco-213	189	---	---	---	---	0.0172	0.0776	0.08	0.5612	0.2143	0.175	---	0.2647	
Sco-213	193	---	---	---	---	---	0.0345	0.04	0.1939	0.0102	---	---	0.0441	
Sco-213	197	---	---	---	---	---	---	---	0.051	0.0102	---	---	0.0441	
Sco-213	204	---	0.0167	---	0.0806	---	0.0517	0.02	---	---	---	0.4667	---	
Sco-213	208	---	---	---	---	---	---	---	---	---	---	0.1167	---	Uleda Cr
Sco-213	212	---	---	---	---	---	---	---	---	---	---	0.1167	---	Uleda Cr
Sco-213	216	0.2969	0.2667	0.163	0.2581	0.3448	0.1379	0.24	---	0.051	---	0.15	0.0294	
Sco-213	220	---	---	---	---	---	0.0259	0.08	---	---	---	---	---	
Sco-213	244	---	---	---	---	---	---	---	---	---	---	0.15	---	Uleda Cr

Locus	Size	Up Salmo	Lo Salmo	S Salmo	Sheep	Clearwater	MF East-a	MF East-b	Gold	Up Priest R	Indian	Uleda	Up Priest Lk	*Private*
Sco-215	286	0.4844	0.3833	0.3913	0.3906	0.5	0.0909	0.1923	---	0.0349	---	---	0.0152	
Sco-215	290	0.2344	0.25	0.2283	0.1875	0.2	0.7545	0.5769	1	0.8953	1	---	0.9545	
Sco-215	294	0.2812	0.3667	0.3804	0.4219	0.3	0.1545	0.2115	---	0.0698	---	---	0.0303	
Sco-215	317	---	---	---	---	---	---	---	---	---	---	0.6167	---	Uleda Cr
Sco-215	321	---	---	---	---	---	---	---	---	---	---	0.3833	---	Uleda Cr
Sco-215	344	---	---	---	---	---	---	0.0192	---	---	---	---	---	MF East Rb